Incretin Effect After Oral Amino Acid Ingestion in Humans

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Context: The incretin effect is the augmented insulin secretion by oral vs iv glucose at matching glucose levels. We previously demonstrated an augmented insulin secretion when fat is given orally rather than iv, suggesting an incretin effect also after fat. However, whether an incretin effect is also present after amino acid ingestion is not known.

Objective: The objective of the study was to explore insulin secretion and incretin hormones after oral and iv amino acid administration at matched total amino acid concentrations in healthy subjects.

Design: An amino acid mixture (Vaminolac) was administered orally or iv at a rate resulting in matching total amino acid concentrations to 12 male volunteers with age 22.5 $\pm$ 1.4 years and a body mass index 22.4 $\pm$ 1.4 kg/m^2, who had no history of diabetes.

Main Outcome Measures: Main outcome measures were area under the 120-minute curve for insulin, C-peptide, glucagon, intact and total glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), and the insulin secretory rate and insulin clearance.

Results: Insulin, C-peptide, and glucagon levels increased after both oral and iv administration, but insulin secretion was 25% higher after oral than after iv amino acid challenges ($P = .006$), whereas there was no significant difference in the glucagon response. Intact and total GIP rose after oral but not after iv amino acid administration, whereas intact and total GLP-1 levels did not change significantly in either test.

Conclusion: Oral amino acid mixture ingestion elicits a stronger insulin secretory response than iv amino acid at matching amino acid levels and this is associated with increased GIP level, suggesting that an incretin effect exists also after oral amino acids, possibly mediated by GIP. ***(J Clin Endocrinol Metab* 100: 1172–1176, 2015)**

The incretin effect is defined as the amplification of insulin secretion after oral vs iv glucose administration and is mainly explained by the insulinotropic effect of the two incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), which both are released from the gut in response to oral glucose (1). The incretin effect accounts for up to 70%–80% of the insulin secreted after oral glucose, depending on the amount of glucose ingested (2). However, the incretin hormones are released not only after carbohydrate ingestion but also after the ingestion of other macronutrients (3), and it is of interest whether an incretin effect exists also in relation to these other macronutrients. Along this line, we recently presented evidence for an incretin

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Abbreviations: AUC, area under the curve; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1.

effect after the oral administration of pure lipids using a lipid emulsion (Intralipid; Fresenius, Kabi) to match triglyceride levels after oral and iv administration (4). In this study we have proceeded to amino acids. Increased insulin secretion, along with increased incretin hormones, is indeed seen after protein ingestion (5–7), but it is not known whether insulin secretion is stimulated more potently by oral vs iv amino acids at matching plasma amino acids concentrations. We have therefore examined insulin and incretin hormone secretion after oral ingestion vs iv infusion of an amino acid mixture at matching amino acid levels in healthy humans to explore whether an incretin effect is also operative after amino acid ingestion.

Subjects and Methods

Subjects

We examined 12 male volunteers with a body mass index of 20–25 kg/m^2 (mean ± SD 22.4 ± 1.4 kg/m^2) and an age range of 21–26 years (22.5 ± 1.4 y) who had no history of diabetes [glycated hemoglobin 4.9% ± 0.3% (30 ± 3 mmol/mol) and fasting glucose 4.7 ± 0.2 mmol/L], no gastrointestinal diseases, and did not take any medication. They all had values of liver enzymes, creatinine, estimated glomerular filtration rate, hemoglobin, white blood cell count, and thyroid hormones within the normal range. The study was undertaken according to good clinical practice and approved by the Regional Ethics Committee of Lund, Sweden. All subjects gave written informed consent before entry into the study, which was monitored by an external monitor and registered at clinicaltrial.gov database (number NCT01366768).

Study protocol

At 8:00 AM after an overnight fast, the subjects had a catheter inserted into an antecubital vein. They then either ingested 100 mL amino acid mixture, corresponding to 6.5 g amino acids (Vaminolac; Fresenius Kabi) or received an iv infusion of Vaminolac [1.0 mL/min (ie, 65 mg/min) for min 0–15 followed by 2.1 mL/min (136 mg/min) for min 15–60, corresponding to 7.0 g amino acids]. During the iv test, subjects also ingested plain water (100 mL) to control for gastric distension after oral administration of Vaminolac. This infusion rate was based on a preliminary experiment in two subjects, in whom Vaminolac was infused at either 1.0 mL/min for 15 minutes followed by 2.1 mL/min for 45 minutes or at 1.0 mL/min for 30 minutes followed by 1.5 mL/min for 30 minutes; the total amino acid levels after the higher rate infusion, but not the lower rate infusion, matched the total amino acids after oral ingestion of 100 mL Vaminolac (data not shown). This infusion rate was therefore selected for the main study.

Analyses

Blood samples, collected into chilled tubes containing EDTA (7.4 mmol/L) and aprotinin (500 kIU/mL; Novo Nordisk), were immediately centrifuged at 4°C. Glucose was measured with the glucose oxidase method. Amino acids were analyzed by the Department of Clinical Chemistry, Skåne University Hospital. Insulin levels were measured using Luminex xMAP Multiplexing technology (Millipore Corp), and glucagon and C-peptide were analyzed using a double-antibody RIA (Linco Research). Blood samples for determining GIP and GLP-1 were collected into chilled tubes containing EDTA and aprotinin with the addition of diprotin A (0.1 mmol/L; Bachem). Intact GLP-1 (Linco Research), total GLP-1 (antiserum code number 89390), intact GIP (antiserum code number 98171), and total GIP (antiserum code number 80867) were measured as described previously (4).

Calculations and statistics

Means ± SEM are shown unless otherwise specified. Areas under the curves (AUCs) were calculated by the trapezoidal rule during the 120-minute study period with basal AUCs subtracted. Insulin secretion was estimated by deconvolution of C-peptide data (8). Insulin clearance (in liters per minute) was calculated as the ratio between total insulin secretion and the insulin concentration AUC. A paired *t* test was used for the estimation of differences in response to oral vs iv ingestion.

Results

Amino acids levels

Figure 1A shows that total amino acid levels were well matched after the oral ingestion of Vaminolac compared with the iv infusion. Supplemental Table 1 shows the base-

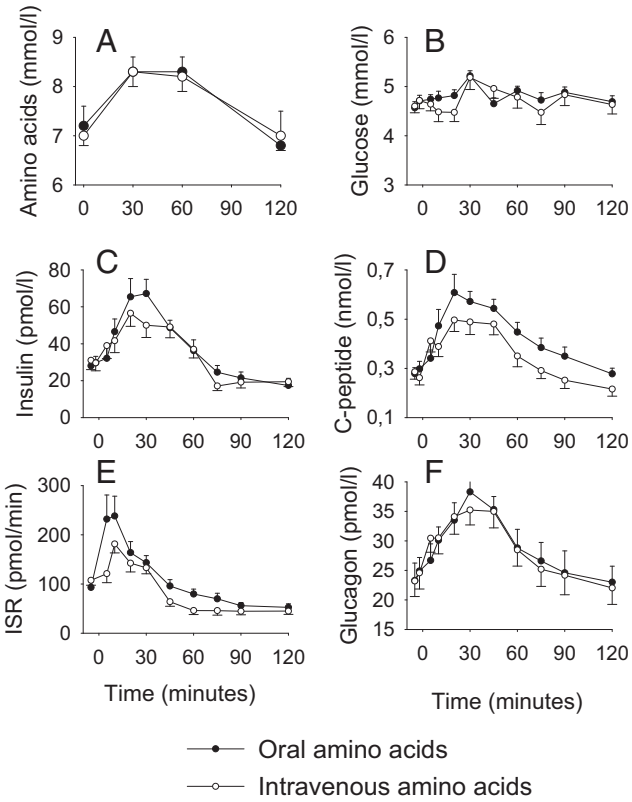

Figure 1. Plasma total amino acids (A), plasma glucose (B), plasma insulin (C), plasma C-peptide (D), insulin secretory rate (ISR; E), and plasma glucagon (F) before and during oral or iv administration of amino acid mixture Vaminolac at matching amino acid concentrations in 12 healthy subjects. Means ± SE are shown.

line and 60-minute concentrations of individual amino acids after the oral and iv amino acid mixture administration. With the exception of asparagine and tyrosine, concentrations of all individual amino acids were increased at 60 minutes compared with baseline (all $P < .001$). There was no significant difference in the increase in concentrations between oral and iv administration for all except three individual amino acids; aspartic acid, glutamic acid, and phenylalanine rose to a higher value after iv compared to oral administration ($P < .001$), but no amino acid concentration was higher after oral than after iv administration.

Glucose, insulin, C-peptide, and glucagon

Glucose levels did not change after either oral or iv amino acid administration (Figure 1B). However, insulin, C-peptide, and glucagon all rose in both instances (Figure 1, C–E). $AUC_{C\text{-peptide}}$ was 2-fold higher and total insulin secretion was 25% higher after oral than after iv amino acid administration (Table 1). Insulin clearance was also higher after oral amino acid administration (3.35 ± 0.45 L/min) than after iv amino acid administration (2.60 ± 0.32 L/min; $P = .006$), whereas $AUC_{glucagon}$ was similar in both tests.

Incretin hormones

Intact and total GIP increased by oral but not iv amino acid administration, with a peak at 30 and 45 minutes, respectively (Figure 2, A and C). This resulted in significantly increased AUCs for both intact and total GIP after oral than after iv amino acid (Table 1). In contrast, intact and total GLP-1 AUCs did not change significantly after either administration (Figure 2, B and D; Table 1).

Discussion

This study examined whether an incretin effect exists after oral ingestion of amino acids because this has previously been shown after oral glucose and oral lipids (2, 4). The main findings were as follows: 1) insulin secretion increased by 25% more after oral than after iv amino acid administration at matching total amino acid concentrations, 2) GIP levels increased only after the oral load, whereas 3) GLP-1 levels did not change significantly in either test. Based on these results, we conclude that an incretin effect exists after amino acid administration and that this is mainly mediated by GIP.

The amino acid mixture clearly increased both intact and total GIP levels when given orally, whereas no change was observed during the iv administration. GIP is rapidly cleaved after secretion so total GIP levels (reflecting the sum of the intact peptide + its primary, N terminally truncated metabolite) can be used as an index of overall GIP secretion from the K cells. In contrast, intact GIP concentrations reflect circulating levels of the biologically active form. The difference in GIP levels after oral and iv amino acid administration is explained by the higher concentration of amino acids stimulating the K cells from the gut lumen after oral vs iv amino acid administration. This confirms previous results that oral protein and amino acids stimulate GIP secretion (5–7, 9) and also that intraduodenal amino acid administration stimulates GIP secretion more than iv administration (9). In contrast, however, we did not detect any significant increase in GLP-1 levels (neither intact nor total GLP-1) after oral or iv amino acid administration, suggesting that amino acids do not stimulate GLP-1 secretion when stimulating the L cells from either luminal or circulatory side at the concentrations achieved in this study.

Oral ingestion of pure protein (5), of glutamine (10) and protein-rich meal (3, 5–7) have all previously been shown to stimulate GLP-1 secretion. The finding that levels of GLP-1 did not increase after oral amino acid administration in our study is most likely related to the low dose used. In fact, in the present study, only 6.5 g of the

Table 1. The 120-minute AUCs for insulin, C-peptide, glucagon, intact and total GIP, and intact and total GLP-1 and total 120-minute insulin secretion after oral or iv administration of amino acid mixture resulting in matching plasma amino acids concentrations in 12 healthy males

120-Minute AUC	Oral Amino Acids	Intravenous Amino Acids	*P* Value
Glucose, mmol/L·min	2.1 ± 3.9	2.0 ± 4.1	.951
Insulin, pmol/L·min	718 ± 211	249 ± 326	.168
C-peptide, nmol/L·min	16.0 ± 2.3	7.4 ± 3.4	.024
Glucagon, pmol/L·min	357 ± 49	271 ± 38	.118
Total insulin secretion, nmol	14.3 ± 1.2	11.4 ± 1.2	.006
Intact GIP, nmol/L·min	62.1 ± 11.3	11.2 ± 2.8	.017
Total GIP, nmol/L·min	280 ± 64	−18 ± 50	.006
Intact GLP-1, nmol/L·min	4.5 ± 5.1	−0.39 ± 4.0	.382
Total GLP-1, nmol/L·min	−8.6 ± 24.6	−33.8 ± 11.9	.249

P indicates the probability level of random difference between the two tests by paired *t* test.

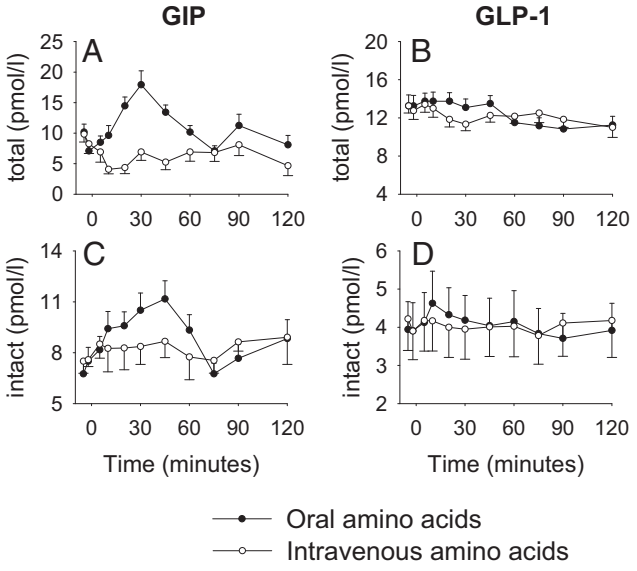

Figure 2. Plasma levels of total GIP (A), total GLP-1 (B), intact GIP (C), and intact GLP-1 (D) before and during oral or iv administration of amino acid mixture Vaminolac at matching amino acid concentrations in 12 healthy subjects. Means ± SE are shown.

mixture was given, which is less than the amount given as pure protein in a previous study that showed an increase in GLP-1 secretion (5). Similarly, when the stimulation of GLP-1 secretion by glutamine was documented in humans, 15–30 g of glutamine was given (10). Another possibility could be that L cells are in general located more distally than the K cells, and therefore, although ingested amino acids will reach the proximally located K cells to stimulate GIP release, they may not reach the distal part of the small intestine. However, arguing against this, GLP-1 has been shown to be produced also in the proximal part of the small intestine in a subset of the very same cells that produce GIP (11). A third possibility is that there was a slight stimulation of GLP-1 secretion, as indicated by a higher mean value after oral than after iv Vaminolac, but that this study did not have sufficient power to detect a statistically significant difference. In any case, our data show that the oral amino acid load dissociates the two incretin hormones, suggesting a more sensitive response of GIP than of GLP-1 secretion.

It is known that arginine, leucine, lysine, and phenylalanine simulate insulin secretion when administered to humans (12–17). We also found that insulin secretion was increased by the amino acid mixture, with the oral administration being more potent than the iv administration. This confirms what was seen previously, ie, that intraduodenal administration of an amino acid mixture yields an augmented insulin secretion compared with that when the mixture is given iv (17) but extends the knowledge because we have matched the levels of amino acids. Because GIP levels were increased after the oral load, the resulting insulin secretion may be a consequence of a GIP-mediated incretin effect. Previous studies have shown that GIP can augment amino acid-induced insulin secretion (6) and stimulate insulin secretion at normoglycemia (18, 19). Other mediators, such as other gut hormones or the autonomic nerves, cannot be excluded, but the clear increase in GIP together with its well-known effect to stimulate insulin secretion (1) suggests that GIP may be the main mediator of this incretin effect.

A limitation in our study is that although total amino acid levels were well matched, not all of the individual amino acids were absolutely matched after oral and iv administration (see Supplemental Figure 1). However, the amino acids showing a different increase after oral vs iv administration (aspartic acid, glutamic acid, and phenylalanine) had higher levels after the iv administration, when insulin secretion was smaller, than after the oral administration. This difference is thus not likely to confound the interpretation that the higher insulin secretion seen after oral administration is due to an incretin effect. Another potential limitation is that the control oral ingestion during the iv amino acid administration to control for the oral load of Vaminolac was plain water, with another osmolality than the amino acid solution.

Insulin clearance was increased after oral compared with iv amino acid administration. This may be achieved by the amino acids entering through the oral route, perhaps through a direct action in the liver, or might be secondary to the stimulated insulin secretion, which is associated to increased clearance (20). This may in turn explain why, even though insulin secretion and the increase in circulating C-peptide were larger after oral than after iv administration of amino acids, the plasma concentrations of insulin were not significantly different.

Glucagon levels increased after iv amino acid administration, in line with previous observations that arginine and alanine can stimulate glucagon secretion (10, 15, 21). However, the oral and iv loads elicited similar responses, suggesting that glucagon secretion after amino acid stimulation at normoglycemia may occur independently of gut hormones. This also is consistent with results that GIP mainly stimulates glucagon secretion in humans at reduced glucose levels (18) and does not affect glucagon secretion when given together with a meal (22).

In conclusion, we have shown that an incretin effect exists also after oral amino acids at matching amino acids levels after oral and iv administration and therefore that an incretin effect exists not only after oral glucose and lipid. Our results also suggest that the incretin effect after oral amino acids seems mainly mediated by GIP.

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Contributions of the authors include the following: O.L. and B.A. designed and performed the study. B.A. wrote the manuscript. G.P. and A.T. performed the modeling of the data for the analyses of insulin secretion and insulin clearance. B.A. analyzed insulin, C-peptide, glucagon, and intact GLP-1. J.J.H. and C.F.D. analyzed the intact and total GIP and total GLP-1. All authors researched the data and contributed to the interpretation of the data and results; all of the authors discussed, reviewed, and edited the manuscript. B.A. is the guarantor of the work and takes responsibility for the contents of the article.

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