Incretin Effect after Oral Amino Acid Ingestion in Humans.

Lindgren, Ola; Pacini, Giovanni; Tura, Andrea; Holst, Jens J; Deacon, Carolyn F; Ahrén, Bo

Published in:
Journal of Clinical Endocrinology and Metabolism

DOI:
10.1210/jc.2014-3865

2015

Incretin Effect after Oral Amino Acid Ingestion in Humans

Ola Lindgren[1], Giovanni Pacini[2], Andrea Tura[2], Jens J Holst[3], Carolyn F Deacon[3] and Bo Ahrén[1]

[1]Lund University, Department of Clinical Sciences, Lund, Medicine, Sweden

[2]Metabolic Unit, Institute of Biomedical Engineering, CNR, Padova, Italy

[3]Novo Nordisk Foundation Center for Basic Metabolic Research and Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

Short title: Amino acids and the incretin effect

Key words: amino acids, insulin secretion, incretin effect, GIP, GLP-1

Word count: 2 075

Disclosure statement: The authors have nothing to disclose

Proof and correspondence:

Dr Bo Ahrén

Department of Clinical Sciences Lund

Lund University

B11 BMC, 221 84 LUND

e-mail Bo.Ahren@med.lu.se

Abstract

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

Objective: To explore insulin secretion and islet hormones after oral and intravenous amino acid administration at matched total amino acid concentrations in healthy subjects.

Design: Amino acid mixture (Vaminolac^{R}) was administered orally or intravenously at a rate resulting in matching total amino acid concentrations to twelve male volunteers with age 22.5±1.4 yr and BMI 22.4±1.4 kg/m^2, who had no history of diabetes.

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

Results: Insulin, C-peptide and glucagon levels increased after both oral and intravenous administration, but insulin secretion was 25% higher after oral than after intravenous amino acid challenges (P=0.006), whereas there was no significant difference in the glucagon response. Intact and total GIP rose after oral but not after intravenous 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 intravenous amino acid at matching amino acid levels and that this is associated with increased GIP level, suggesting that an incretin effect exists also after oral amino acids, possibly mediated by GIP.

Introduction

The incretin effect is defined as the amplification of insulin secretion after oral versus intravenous 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 ingestion of other macronutrients (3) and it is of interest whether an incretin effect exists also in relation to these other macronutrients. Along this, we recently presented evidence for an incretin effect after oral administration of pure lipids using a lipid emulsion (Intralipid^{R}) to match triglyceride levels after oral and intravenous 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 versus intravenous amino acids at matching plasma amino acids concentrations. We have therefore examined insulin and incretin hormone secretion after oral ingestion versus intravenous 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 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 yr (22.5±1.4 yr) who had no history of diabetes (HbA1c 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 had all values of liver enzymes, creatinine, eGFR, Hb, WBC and thyroid hormones within the normal range. The study was undertaken according to GCP 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 the clinicaltrial.gov data base (NCT01366768).

Study protocol. At 0800 h after an overnight fast, the subjects had a catheter inserted in an antecubital vein. They then either ingested 100 ml amino acid mixture, corresponding to 6.5g amino acids, (Vaminolac^R; Fresenius Kabi, Uppsala, Sweden) or received an intravenous infusion of Vaminolac^R (1.0 ml/min (i.e., 65 mg/min) for min 0-15 followed by 2.1 ml/min (136 mg/min) for min 15-60), corresponding to 7.0g amino acids). During the intravenous test, subjects also ingested plain water (100ml) to control for gastric distension after oral Vaminolac^R. This infusion rate was based on a preliminary experiment in two subjects, in whom Vaminolac^R was infused at either at 1.0 ml/min for 15 min followed by 2.1 ml/min for 45 min or at 1.0 ml/min for 30 min followed by 1.5 ml/min for 30 min; 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^R (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, Bagsvaerd, Denmark), were immediately centrifuged at 4°C. Glucose was measured with the glucose oxidase method. Amino acids were analysed by

the Department of Clinical Chemistry, Skåne University Hospital. Insulin levels were measured using Luminex xMAP Multiplexing technology (Millipore Corp., Billerica, MA), and glucagon and C-peptide were analyzed using double antibody RIA (Linco Research, St. Charles, MO). Blood samples for determining GIP and GLP-1 were collected into chilled tubes containing EDTA and aprotinin with addition of diprotin A (0.1 mmol/l; Bachem, Bubendorf, Switzerland). Intact GLP-1 (Linco Research), total GLP-1 (antiserum code no. 89390), intact GIP (antiserum code no. 98171) and total GIP (antiserum code no. 80867) were measured as described previously (4).

Calculations and statistics. Means ± SEM are shown unless otherwise specified. Areas under curves (AUC) were calculated by the trapezoidal rule during the 120 min study period with basal AUC subtracted. Insulin secretion was estimated by deconvolution of C-peptide data and application of population-based elimination kinetics (8). Insulin clearance (in l/min) was calculated as the ratio between total insulin secretion and the insulin concentration AUC. Paired *t* test was used for estimation of differences in response to oral *vs.* intravenous ingestion.

Results

Amino acids levels. Fig. 1A shows that total amino acid levels were well matched after the oral ingestion of Vaminolac^{R} compared to the intravenous infusion. Supplementary Table 1 shows the baseline and 60 min concentrations of individual amino acids after the oral and intravenous amino acid mixture administration. With the exception of asparagine and tyrosine, concentrations of all individual amino acids were increased at 60 min compared to baseline (all $P<0.001$). There was no significant difference in the increase in concentrations between oral and intravenous administration for all except three individual amino acids; aspartic acid, glutamic acid and phenylalanine rose to a higher value after intravenous compared to oral administration ($P<0.001$), but no amino acid concentration was higher after oral than after intravenous administration.

Glucose, insulin, C-peptide and glucagon. Glucose levels did not change after either oral or intravenous amino acid administration (Fig. 1B). However, insulin, C-peptide and glucagon all rose on both instances (Fig. 1C-E). $AUC_{C\text{-}peptide}$ increased and total insulin secretion were 25% higher after oral than after intravenous amino acid administration(Table 1). Insulin clearance was also higher after oral amino acid administration (3.35±0.45 l/min) than after intravenous amino acid administration (2.60±0.32 l/min; $P=0.006$), whereas $AUC_{glucagon}$ was similar in both tests.

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

Discussion

This study examined whether an incretin effect exists after oral ingestion of amino acids, as it has previously been shown after oral glucose and oral lipids (2,4). The main findings were: (i) insulin secretion increased by 25% more after oral than after intravenous amino acid administration at matching total amino acid concentrations, (ii) GIP levels increased only after the oral load, whereas (iii) 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 intravenous administration. GLP-1 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 intravenous amino acid administration is explained by the higher concentration of amino acids stimulating the K cells from the gut lumen after oral versus intravenous 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 intravenous 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 intravenous 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 of 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 6.5g of the mixture was given, which is less than the amount given as pure protein in a previous study which showed an increase in GLP-1 secretion (5). Similarly, when the stimulation of GLP-1 secretion by glutamine was documented in humans, 15-30g 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 sub-set of the very same cells which 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 intravenous Vaminolac^{R} , 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 intravenous administration. This confirms what was seen previously, i.e., that intraduodenal administration of an amino acid mixture yields an augmented insulin secretion compared to that when the mixture is given intravenously (17) but extends the knowledge because we have matched the levels of amino acids. Since GIP levels were increased following 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 thus that although total amino acid levels were well matched, not all of the individual amino acids were absolutely matched after oral and intravenous administration (see Supplementary Figure 1). However, the amino acids showing a different increase after oral versus intravenous administration (aspartic acid, glutamic acid and phenylalanine) had higher levels after the intravenous 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 intravenous amino acid administration to control for the oral load of Vaminolac^{R} was plain water, with other osmolality than the amino acid solution.

Insulin clearance was increased after oral compared to intravenous 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 may explain why, even though insulin secretion and the increase in circulating C-peptide were larger after oral than after intravenous administration of amino acids, the plasma concentrations of insulin were not significantly different.

Glucagon levels increased after intravenous amino acid administration, in line with previous observations, that arginine and alanine can stimulate glucagon secretion (10,15,21). However, the oral and intravenous 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 intravenous 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.

Acknowledgements

The study has been reported at the ADA meeting in San Francisco, June 2014. We thank Research nurse Gustav Dahl and laboratory technician Kristina Andersson for invaluable help in this study. We thank the Swedish Research Council, Region Skåne and the Faculty of Medicine, Lund University, for financial support. Contributions: OL and BA designed and performed the study. BA wrote the manuscript. GP and AT performed the modeling of the data for analyses of insulin secretion and insulin clearance. BA analysed insulin, C-peptide, glucagon and intact GLP-1. JJH and CFD analysed intact and total GIP and total GLP-1. All authors researched data and contributed to the interpretation of the data and results; all

discussed, reviewed and edited the manuscript. BA is the guarantor of the work and takes responsibility for the contents of the article.

References

1. **Drucker DJ, Nauck MA** 2006 The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 502:1696-1705
2. **Nauck M, Stöckmann F, Ebert R, Creutzfeldt W** 1986 Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. Diabetologia 29:46-52
3. **Ahrén B, Carr RD, Deacon CF** 2010 Incretin hormone secretion over the day. Vitam Horm 84:203-220
4. **Lindgren O, Carr RD, Deacon CF, Holst JJ, Pacini G, Mari A, Ahrén B** 2011 Incretin hormone and insulin responses to oral versus intravenous lipid administration in humans. J Clin Endocrinol Metab 96:2519-2524
5. Carr RD, Larsen MO, Winzell MS, Jelic K, Lindgren O, Deacon CF, Ahrén B 2008 Incretin and islet hormonal responses to fat and protein ingestion in healthy men. **Am J Physiol Endocrinol Metab** 295:E779-E784
6. **Fieseler P, Bridenbaucgh S, Nustede R, Martell H, Ørskov C, Holst JJ, Nauck MA** 1995 Physiological augmentation of amino acid-induced insulin secretion by GIP and GLP-1 but not by CCK-8. Am J Physiol Endocrinol Metab 268:E949-E955
7. **Belza A, Ritz C, Sørensen MQ, Holst JJ, Rehfeld JF, Astrup A** 2013 Contribution of gastroenteropancreatic appetite hormones to protein-induced satiety. Am J Clin Nutr 97:980-989
8. **van Cauter F, Mestrez F, Sturis J, Polonsky KS** 1992 Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 41:368–377

9. **Thomas FB, Sinar D, Mazzaferri EL, Cataland S, Mekhijan HS, Caldwell JF, Fromkes JJ** 1978 Selective release of gastric inhibitory polypeptide by intraduodenal amino acid perfusion in man. Gastroenterology 74:1281-1285
10. **Greenfield JR, Farooqi IS, Keogh JM, Henning E, Habib AM, Blackwood A, Reimann F, Holst JJ, Gribble GM** 2009 Oral glutamine increases circulating glucagon-like peptide 1, glucagon, and insulin concentrations in lean, obese and type 2 diabetic subjects. Am J Clin Nutr 89:106-113
11. **Mortensen K, Christensen LL, Holst JJ, Ørskov C** 2003 GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. Regul Pept 114:189-196
12. **Floyd JC Jr, Fajans SS, Conn JW, Knopf RF, Rull J** 1966 Stimulation of insulin secretion by amino acids. J Clin Invest 45:1487–502
13. **Floyd JC Jr, Fajans SS, Pek S, Thiffault CA, Knopf RF, Conn JW** 1970 Synergistic effect of essential amino acids and glucose upon insulin secretion in man. Diabetes 19:109–115
14. **van Haeften TW, Voelberg GA, Gerich JE, van der Veen EA** 1989 Dose-response characteristics for arginine-stimulated insulin secretion in man and influence of hyperglycemia. J Clin Endocrinol Metab 69:1059-1064
15. **Ahrén B** 2009 ß- and α-cell dysfunction in subjects developing impaired glucose tolerance. Diabetes 58:726-731
16. **van Loon LJC, Saris WHM, Verhagen H, Wagenmakers AJM** 2000 Plasma insulin responses after ingestion of different amino acid or protein mixtures with carbohydrate. Am J Clin Nutr 72:96-105

17. **Raptis S, Dollinger HC, Schröder KE, Schleyer M, Rothenbuchner G, Pfeiffer EF** 1973 Differences in insulin, growth hormone and pancreatic enzyme secretion after intravenous and intraduodenal administration of mixed amino acids in man. N Engl J Med 1973;288:1199-1202
18. **Christensen M, Vedtofte L, Holst JJ, Vilsbøll T, Knop FK** 2011 Glucose-dependent insulinotropic polypeptide: a bifunctional glucose-dependent regulator of glucagon and insulin secretion in humans. Diabetes 60:3103-3109
19. **Vilsbøll T, Krarup T, Madsbad S, Holst JJ** 2003 Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. Regul Pept 114:115-121
20. **Tillil H, Shapiro ET, Miller AN, Karrison T, Krank BH, Galloway JA, Rubenstein AH, Polonsky KS** 1988 Dose-dependent effects of oral and intravenous glucose on insulin secretion and clearance in normal humans. Am J Physiol Endocrinol Metab 254:E349-E357
21. **Wise JK, Hendler R, Felig P** 1973 Evaluation of alpha-cell function by infusion of alanine in normal, diabetic and obese subjects. N Engl J Med 288:487-490
22. **Ahrén B, Pettersson M, Uvnäs-Moberg K, Gutniak M, Efendic S** 1991 Effects of cholecystokinin (CCK)-8, CCK-33, and gastric inhibitory polypeptide (GIP) on basal and meal-stimulated pancreatic hormone secretion in man. Diabetes Res Clin Pract 13:153-161

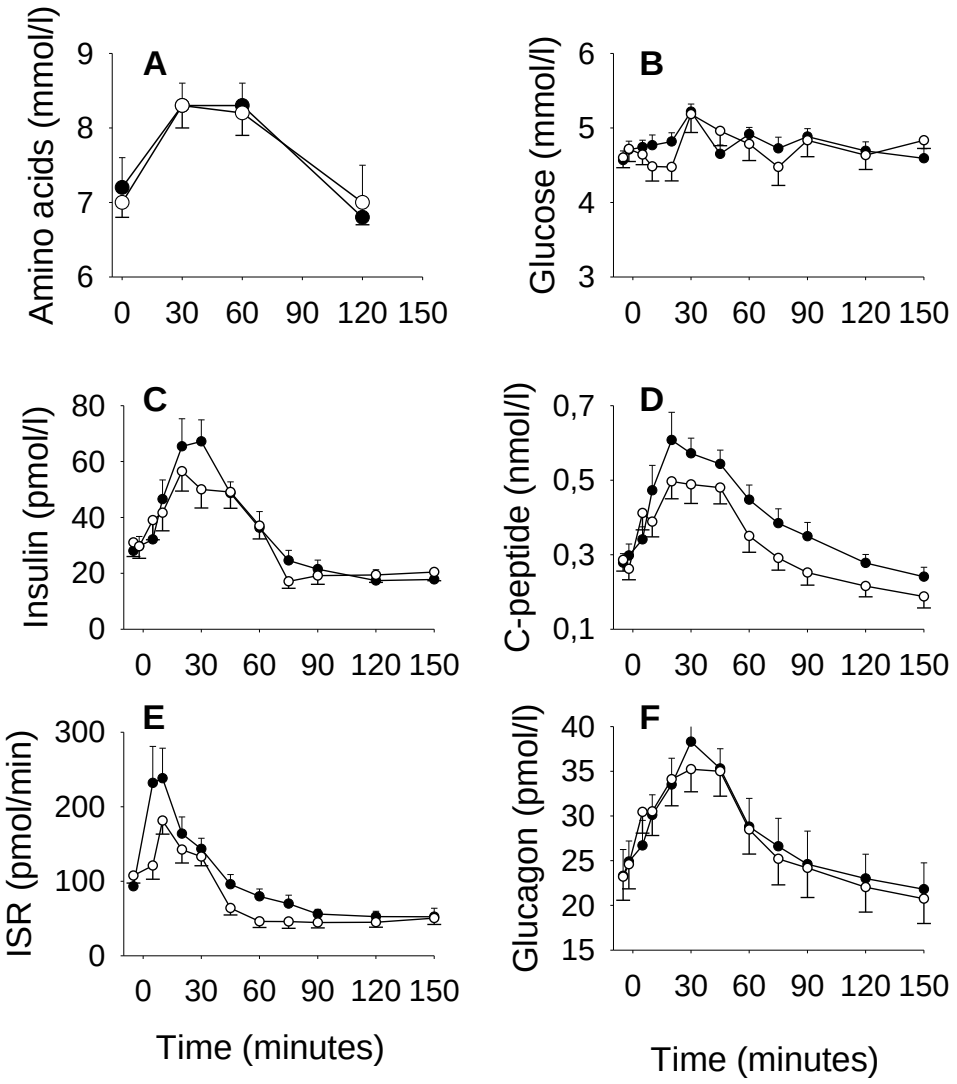
Table 1 The 120 min areas under the curve (AUC) for insulin, C-peptide, glucagon, intact GIP and intact GLP-1 and total 120 min insulin secretion following oral or intravenous administration of amino acids mixture resulting in matching plasma amino acids concentrations in 12 healthy males. P indicates the probability level of random difference between the two tests by paired t-test.

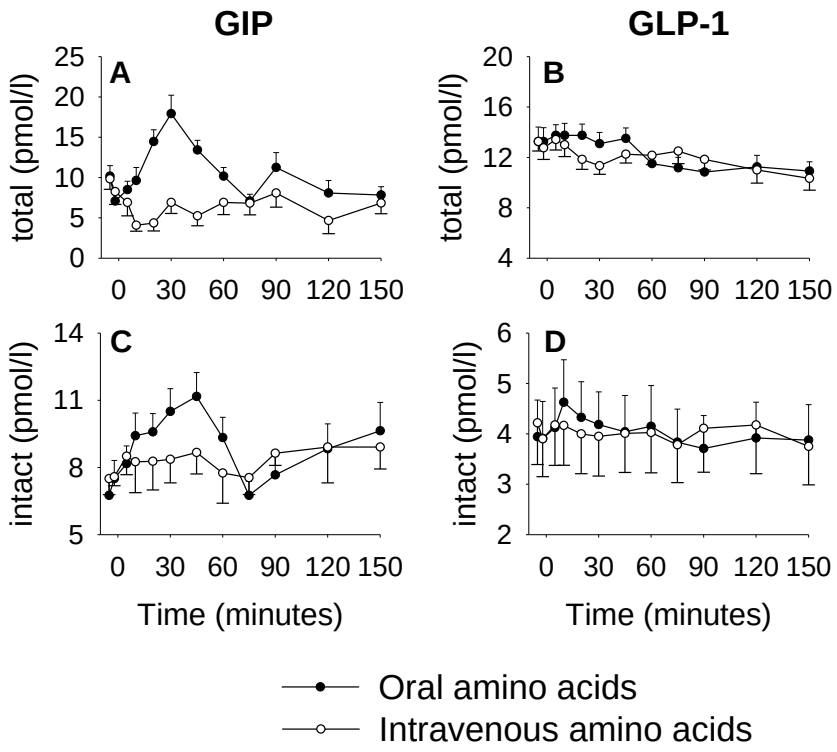
120 min AUC	Oral amino acids	Intravenous amino acids	P
Glucose (mmol/l min)	2.1±3.9	2.0±4.1	0.951
Insulin (pmol/l min)	718±211	249±326	0.168
C-peptide (nmol/l min)	16.0±2.3	7.4±3.4	0.024
Glucagon (pmol/l min)	357±49	271±38	0.118
Total insulin secretion (nmol)	14.3±1.2	11.4±1.2	0.006
Intact GIP (nmol/l min)	62.1±11.3	11.2±2.8	0.017
Total GIP (nmol/l min)	280±64	-18±50	0.006
Intact GLP-1 (nmol/l min)	4.5±5.1	-0.39±4.0	0.382
Total GLP-1 (nmol/l min)	-8.6±24.6	-33.8±11.9	0.249

Legends to the Figures

Fig. 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 intravenous administration of amino acid mixture Vaminolac^{R} at matching amino acid concentrations in 12 healthy subjects. Means±SE are shown.

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


A
Amino acids (mmol/l)
9
8
7
6
0 30 60 90 120 150
B
Glucose (mmol/l)
6
5
4
3
0 30 60 90 120 150
C
Insulin (pmol/l)
80
60
40
20
0
0 30 60 90 120 150
D
C-peptide (nmol/l)
0,7
0,5
0,3
0,1
0 30 60 90 120 150
E
ISR (pmol/min)
300
200
100
0
0 30 60 90 120 150
Time (minutes)
F
Glucagon (pmol/l)
40
35
30
25
20
15
0 30 60 90 120 150
Time (minutes)
Oral amino acids
Intravenous amino acids


GIP
GLP-1
A
B
C
D
total (pmol/l)
intact (pmol/l)
Time (minutes)
Time (minutes)
Oral amino acids
Intravenous amino acids