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Direct and indirect effects of amino acids on hepatic glucose metabolism in humans

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Abstract

Aim/hypothesis. The study was designed to examine the contribution of direct (substrate-mediated) and indirect (hormone-mediated) effects of amino acids on hepatic glucose metabolism in healthy men.
Methods. The protocols were: (i) **CON+S** (n=7): control conditions with somatostatin to inhibit endogenous hormone release resulting in fasting plasma concentrations of amino acids, insulin (~28 pmol/l) and glucagon (~65 ng/l), (ii) **AA+S** (n=7): amino acid infusion-fasting insulinaemia-fasting glucagonaemia, (iii) **GLUC+S** (n=6): fasting amino acids-fasting insulinaemia-hyperglucagonaemia (~99 ng/l) and (iv) **AA-S** (n=5): amino acid infusion without somatostatin resulting in amino acid-induced hyperinsulinaemia (~61 pmol/l)-hyperglucagonaemia (~147 ng/l). Net glycogenolysis was calculated from liver glycogen concentrations using ^{13}C nuclear magnetic resonance spectroscopy. Total gluconeogenesis (GNG) was calculated by subtracting net glycogenolysis from endogenous glucose production (EGP) which was measured with [6,6-$^2H_2$]glucose. Net GNG was assessed with the ^2H_2O method.
Results. During AA+S and GLUC+S, plasma glucose increased by about 50% (p<0.01) due to a comparable rise in EGP. This was associated with a 53-% (p<0.05) and a 65% increase (p<0.01) of total and net GNG during AA+S, whereas net glycogenolysis rose by 70% (p<0.001) during GLUC+S. During AA-S, plasma glucose remained unchanged despite nearly-doubled (p<0.01) total GNG.
Conclusion/interpretation. Conditions of postprandial amino acid elevation stimulate secretion of insulin and glucagon without affecting glycaemia despite markedly increased gluconeogenesis. Impaired insulin secretion unmasks the direct gluconeogenic effect of amino acids and increases plasma glucose. [Diabetologia (2003) 46:917–925]

Keywords Amino acids, endogenous glucose production, gluconeogenesis, glycogenolysis, liver glycogen, glucagon.

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Abbreviations: AA, amino acids; APE, atom percent excess; EGP, endogenous glucose production; GNG, gluconeogenesis; NMRS, nuclear magnetic resonance spectroscopy; S, somatostatin.

Development of Type 2 diabetes can be attributed to lifestyle in the vast majority of patients [1]. Dietary modification effectively delays or even prevents Type 2 diabetes in high-risk populations [2, 3]. This strongly supports the hypothesis that nutrient excess plays a central role in the development of this disease.

Nutrient excess not only comprises higher intake of fat, but also of proteins. Meat consumption has grown by about 33% since 1960 in industrialized countries [4]. Increased plasma amino acid (AA) concentrations are detectable in insulin resistant states such as obesity [5, 6], and protein intake is associated with higher insulin requirements in Type 1 diabetic patients [7, 8].

Thus, increased availability of AA could contribute to disturbance of carbohydrate metabolism [9]. In fact, we have shown that short-term AA infusion induces peripheral insulin resistance in healthy humans by inhibition of glucose transport/phosphorylation and subsequent reduction in glycogen synthesis [10]. Less information is available, however, concerning the effects of increased AA supply on hepatic glucose metabolism in humans.

Some data suggest that high protein intake could cause hyperglycaemia by stimulating endogenous glucose production (EGP) in the fasting state [7, 11, 12, 13, 14]. This increase would primarily depend on the altered secretion pattern of glucoregulatory hormones [15, 16, 17] and subsequent changes in the portal insulin to glucagon ratio which regulates hepatic glucose metabolism [18]. In parallel, AA per se—by acting as substrates—could stimulate gluconeogenesis (GNG) [11], which would contribute to glucose production even in the early postprandial period [19, 20]. The direct (substrate) effects of amino acids on hepatic glucose metabolism have been studied in several rodent models such as isolated rat livers [21] and canine models [22, 23]. However, the respective roles of direct and indirect (hormone-mediated) effects of amino acids on hepatic glucose and glycogen metabolism have not yet been compared in vivo in humans.

This study was therefore designed to examine the direct and indirect effects of short term elevation of plasma AA on hepatic glucose metabolism by combining isotope dilution techniques including the ^2H_2O method and in vivo ^{13}C nuclear magnetic resonance spectroscopy (NMRS) of the liver to simultaneously quantify EGP, GNG and glycogenolysis [19, 20].

Subjects and Methods

Volunteers. We included seven healthy male volunteers (age: 28±2 yearsBMI: 22.9±0.9 kg/m^2) without family history of diabetes mellitus or dyslipidaemia in this study. They were neither glucose intolerant, suffering from conditions related to insulin resistance nor taking any medication. The protocols were approved by the local ethics board, and informed consent was obtained from all subjects after the nature and possible consequences of the procedures had been explained to them.

Study Protocols. All participants were instructed to ingest an isocaloric diet (25 kcal/kg per day; carbohydrate/protein/fat: 55%/15%/30%) for 3 days prior to the studies. After a 12-h overnight fast, catheters were inserted at 6:00 a.m. (–240 min) into forearm veins of both arms for blood sampling and infusions. D-[6,6-^{2}H]glucose (98% enrichment; Cambridge Isotope Laboratories, Andover, Mass., USA) was infused (bolus: 3 mg/kg; continuous infusion: 0.03 mg kg^{-1} min^{-1}) for measuring EGP from –180 min to 360 min.

Four experimental protocols were carried out. In three of them, pancreatic clamps were done to exclude indirect (hormone-mediated) AA effects resulting from AA-induced secretion of glucoregulatory hormones. Endogenous hormone secretion was inhibited by continuous infusion of somatostatin (S; 0.11 μU kg^{-1} min^{-1}; –5–360 min; UCB Pharma, Vienna, Austria). In addition, insulin (0.065 mU kg^{-1} min^{-1}; Actrapid, Novo Nordisk, Bagsvaerd, Denmark), glucagon (0.9 ng kg^{-1} min^{-1}; Novo Nordisk, Vienna, Austria) and growth hormone (2.0 ng kg^{-1} min^{-1}; Genotropin, Upjohn and Pharmacia, Vienna, Austria) [24] were replaced from 0 to 360 min to provide for their fasting peripheral concentrations. A balanced AA mixture (0.2 g kg^{-1} h^{-1}; Aminoplasmal 10% without electrolytes, Braun, Melsungen, Germany) was infused (0–360 min) in order to create postprandial conditions of increased plasma AA concentrations as described previously [10]. During control studies, normal saline was infused at identical infusion rates. To compare direct (substrate-mediated) AA effects with that of glucagon, the glucagon infusion rates were doubled (1.8 ng kg^{-1} min^{-1}) during one pancreatic clamp protocol without concomitant AA infusion.

These procedures resulted in the following protocols: (i) CON+S (n=7): control conditions with somatostatin (S) to inhibit pancreatic hormone release resulting in fasting (basal) concentrations of AA, insulin (~28 pmol/l), glucagon (~65 ng/l) and growth hormone (~0.5 μg/l), (ii) AA+S (n=7): AA infusion-fasting insulinaemia-fasting glucagonaemia-fasting plasma growth hormone, (iii) GLUC+S (n=6): fasting AA-fasting insulinaemia-hyperglucagonaemia (~99 ng/l)-fasting plasma growth hormone and (iv) AA-S (n=5): AA infusion without S resulting in hyperinsulinaemia (~61 pmol/l)-hyperglucagonaemia (~147 ng/l)-slightly increased plasma growth hormone (~1.7 μg/l).

To assess net GNG during protocols AA+S and CON+S, the participants started at –235 min to drink a total of 5 g of ^2H_2O (99.9% enrichment; Cambridge Isotope Laboratories, Andover, Mass., USA) per kilogram of body water divided into four equal doses spaced by 45-min intervals [19, 25]. Thereafter, they had free access to drinking water containing 0.5% of ^2H_2O to maintain isotopic equilibrium in body water.

In vivo 13C NMRS. During all protocols, liver glycogen concentrations were measured from –40 to –10 min, 10 to 40 min, 120 to 150 min, and from 300 to 360 min on a 3.0-T/80-cm bore NMR spectrometer (Medspec, Bruker, Ettlingen, Germany). Localized ^{13}C resonance spectra were obtained with a 10 cm circular $^{13}C/^1H$ transmitter/receiver coil placed rigidly over the lateral aspect of the liver by applying a modified one-dimensional inversion-based sequence [18, 20, 26]. Typically, one spectrum consists of 5000 scans and requires 15 min of signal averaging. The absolute liver glycogen concentration was quantified by comparing the C1 glycogen peak (100.5 ppm) integral of liver spectra with that of a glycogen standard taken under identical conditions. Correction for loading of the coil and filling of the sensitive volume were done. Magnetic resonance imaging was carried out in a clinical 1.5-T scanner (Siemens, Erlangen, Germany) in order to determine liver volume (1.54±0.02 litre) [20].

Analytical procedures. During all protocols blood samples were drawn at identically timed intervals, immediately chilled, centrifuged and the supernatants were stored at –80°C.

Plasma metabolites and hormones. Plasma glucose was measured by the glucose oxidase method (Glucose analyzer II, Beckman Instr., Fullerton, Calif., USA). Individual plasma amino acids were measured by HPLC as described previously [27]. Plasma non-esterified fatty acids (NEFA) were assayed with a microfluorimetric method (Wako Chem USA, Richmond, Va., USA). In vitro lipolysis leading to artificially increased plasma NEFA concentrations was prevented by collecting blood into vials containing orlistat and rapid centrifuga-

tion of the samples [28]. Plasma lactate and glycerol were measured enzymatically (Roche, Darmstadt, Germany). Plasma immunoreactive insulin, C-peptide, glucagon and growth hormone were measured by commercially available RIAs (insulin: Pharmacia, Uppsala Sweden; C-peptide: Cis, Gif-Sur-Yvette, France; glucagon: Linco, St. Charles, Mo, USA; growth hormone: Sorin Biomedica, Saluggia, Italy). Plasma cortisol was determined following extraction and charcoal-dextran separation by RIA [29]. Coefficients of variation for these assays were reported previously [25, 28, 30].

Gas chromatography-mass spectrometry (GC-MS). For calculation of net GNG, hydrogens bound to carbons 2 and 5 of blood glucose were isolated in formaldehyde and derivatized as hexamethylenetetramine, which was assayed by GC-MS [19].

For measurement of EGP, glucose was isolated, derivatized and tracer-to-tracee ratios (TTR) were determined by GC-MS on a Hewlett-Packard 5,890 gas chromatograph interfaced to a mass selective detector as reported previously [10].

Calculations and statistical evaluation. At baseline, steady state rates of EGP were calculated by dividing the tracer ([6,6-$^2H_2$]-glucose) infusion rate by the TTR [31]. During the clamp tests rates of glucose appearance (R_a) and disappearance (R_d) were calculated from TTR using Steele`s non-steady state equations [32] modified for the use of stable isotopes [33, 34]. Rates of net hepatic glycogenolysis (μmol kg^{-1} min^{-1}) were calculated from the best linear fit of the liver glycogen (mmol/l liver) -time curves by the method of least squares and multiplying the slope of that line by the liver volume (in litres) divided by the body weight (in kg) [20]. Rates of net glycogenolysis were calculated for the complete time period (0–360 min) as well as separately for the first (0–180 min) and the second (120–360 min) half of the protocol. Linearity of these segments was confirmed by linear regression analysis ($p<0.05$).

Glucose-6-phosphate synthesized from gluconeogenic precursors could be either converted to glucose which is released into the circulation or it could serve as a substrate for glycogen synthesis (glyconeogenesis). Thus, gluconeogenesis (GNG) is either defined as rate of formation of both (blood-) glucose and (liver-) glycogen via glucose-6-phosphate from gluconeogenic substrates (total GNG or glucose-6-phosphoneogenesis) or as rate of only (blood-) glucose synthesis from the same gluconeogenic precursors (net GNG) [35, 36]. Rates of formation of glucose-6-phosphate, i.e. total GNG, are given as the difference between rates of EGP and net glycogenolysis (NMRS method) [20, 35]. The fractional contribution of glucose synthesized from gluconeogenic precursors to EGP is estimated from the ratio of the ^2H enrichments bound at C5 to that at C2 of blood glucose after oral ^2H_2O administration [19]. Rates of synthesis of glucose via glucose-6-phosphate, i.e. net GNG are calculated by multiplying this fraction by the rate of EGP (^2H_2O method) [19, 35].

Data are shown as means ± SE. Differences between paired experimental protocols were analyzed by the paired two-tailed Student's *t* test and between more than two groups by ANOVA and Dunnett's post hoc testing. Changes of sequential (time-dependent) data within experiments were analyzed by ANOVA for repeated measurements. Differences were considered significant at *p* values less than 0.05

Results

Amino acids. (AA) Fasting plasma AA concentrations were similar in all protocols (Fig. 1A, Table 1). During AA infusion with (AA+S) and without (AA-S) somatostatin, the total plasma AA concentrations increased by 3.2 fold ($p<0.01$) and 2.1 fold ($p<0.01$), respectively (Fig. 1A). Plasma concentrations of individual AA at baseline and during experimental conditions are presented in Table 1. Except for tyrosine all AA contributed to the rise in total plasma AA concentrations during AA infusion with somatostatin (AA+S). During AA-S, plasma AA rose to less extent than during AA+S (Fig. 1, Table 1). Serum electrolytes and osmolality were not affected by AA infusion (data not shown).

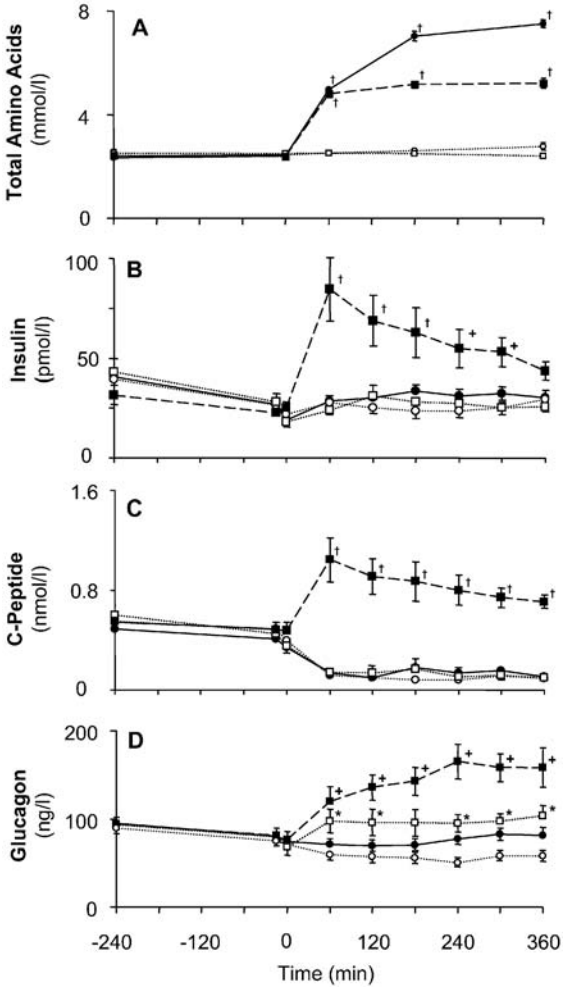

Fig. 1A–D. Plasma concentrations of total amino acids (**A**), insulin (**B**), C-peptide (**C**), and glucagon (**D**) during infusion of saline (CON+S, empty circles, *n*=7) or amino acids (AA+S, full circles, *n*=7) and selective glucagon elevation (GLUC+S, empty squares, *n*=6) together with somatostatin (pancreatic clamps) as well as infusion of amino acids without somatostatin (AA-S, full squares, *n*=5). Data are given as means±SEM. * $p<0.05$; + $p<0.01$, † $p<0.001$ vs CON+S

Hormones. Baseline plasma insulin and C-peptide concentrations were not different between the protocols (Fig. 1). During AA infusion without somatostatin (AA-S), plasma insulin and C-peptide concentra-

Table 1. Plasma amino acid concentrations (μmol/l) at 0 and 360 min during infusion of saline (CON+S, *n*=7) or amino acid (AA+S, *n*=7) and selective glucagon elevation (GLUC+S, *n*=6) together with somatostatin (pancreatic clamps) as well as infusion of amino acids without somatostatin (AA-S, *n*=5). Data are given as means±SEM

Amino Acids	Time (min)	CON+S	AA+S	GLUC+S	AA-S
Alanine	0	357±32	305±21	346±34	342±51
	360	450±40	1393±77**	364±38	849±21**,****
Arginine	0	95±7	100±9	97±9	98±8
	360	126±8	523±26**	94±6	318±53**,****
Asparagine	0	46±1	45±2	48±1	48±2
	360	53±2	192±6**	47±1	118±5**,****
Citrulline	0	33±1	33±1	33±2	33±2
	360	33±2	78±4**	26±2	51±3**,****
Glutamine	0	563±26	558±33	575±12	602±16
	360	638±33	892±38**	600±18	713±26****
Glutamate	0	54±7	56±4	45±6	33±6*,***
	360	43±8	113±11**	26±3	57±4****
Glycine	0	255±13	246±16	243±22	234±12
	360	303±16	980±41**	228±17	591±15**,****
Histidine	0	82±2	84±3	77±3	74±3
	360	89±3	293±5**	83±2	197±7**,****
Isoleucine	0	64±4	66±4	70±4	70±3
	360	63±4	255±13**	61±3	237±19**
Leucine	0	130±5	136±5	133±7	126±6
	360	189±45	532±21**	138±5	462±34**
Methionine	0	34±2	34±2	38±3	34±2
	360	40±2	267±13**	38±3	194±15**,****
Phenylalanine	0	53±2	52±2	55±3	50±2
	360	55±3	210±5**	53±2	174±6**,****
Serine	0	125±7	133±9	116±8	113±8
	360	140±9	342±20**	105±4	184±18****
Taurine	0	51±9	42±2	45±3	41±1
	360	42±2	67±4**	40±3	47±3****
Threonine	0	129±6	128±9	120±6	116±4
	360	146±7	468±20**	114±6	271±13**,****
Tryptophan	0	47±2	44±2	52±4	48±2
	360	42±2	115±6**	50±3	111±7**
Tyrosine	0	56±4	56±4	60±5	55±3
	360	66±5	79±4	55±4	51±3****
Valine	0	231±10	235±12	235±12	231±10
	360	241±10	604±20**	237±10	534±31**,***

* $p<0.05$, ** $p<0.01$, vs. CON+S; *** $p<0.05$, **** $p<0.01$ vs AA+S

tions rapidly increased ($p<0.001$ vs baseline; Fig. 1B, Fig. 1C). During pancreatic clamp tests (CON+S, AA+S, GLUC+S), plasma insulin (Fig. 1B) remained at baseline and plasma C-peptide concentrations (Fig. 1C) were equally suppressed.

Plasma glucagon concentrations were similar at baseline and remained close to baseline during CON+S and AA+S. During AA infusion without somatostatin (AA-S) and during doubled rates of glucagon infusion (GLUC+S), plasma glucagon increased by about 162% ($p<0.01$) and about 74% ($p<0.05$) within 60 min and remained higher until 360 min compared to CON+S and baseline (Fig. 1D).

At baseline, plasma growth hormone concentration varied without statistical significance (–240 min: CON+S: 0.80±0.56, AA+S: 0.25±0.08, GLUC+S: 0.17±0.03, AA-S: 0.19±0.04 μg/l; N.S.) because of its spontaneous pulsatile secretion pattern [37]. During the pancreatic clamp tests (CON+S, AA+S, GLUC+S), plasma growth hormone concentrations were comparable between the protocols. In the absence of somatostatin (AA-S), mean growth hormone secretion peaked at 180 min, but again was not statistically different compared with the other protocols (180 min: CON+S: 0.52±0.18, AA+S: 0.49±0.21, GLUC+S: 0.14±0.02, AA-S: 3.86±2.37 μg/l; N.S.).

Plasma cortisol concentrations were comparable between all protocols (0 min: CON+S: 350±46, AA+S: 343±40,GLUC+S: 342±40, AA-S: 328±58 nmol/l; 360 min: CON+S: 214±32, AA+S: 281±34, GLUC+S: 198±26 AA-S: 191±10 nmol/l).

Metabolites. Plasma NEFA concentrations were similar in all protocols (–240 min; CON+S: 0.49±0.13, AA+S: 0.58±0.06, GLUC+S: 0.46±0.12, AA-S: 0.48±0.05 mmol/l; 360 min: CON+S: 0.24±0.04,

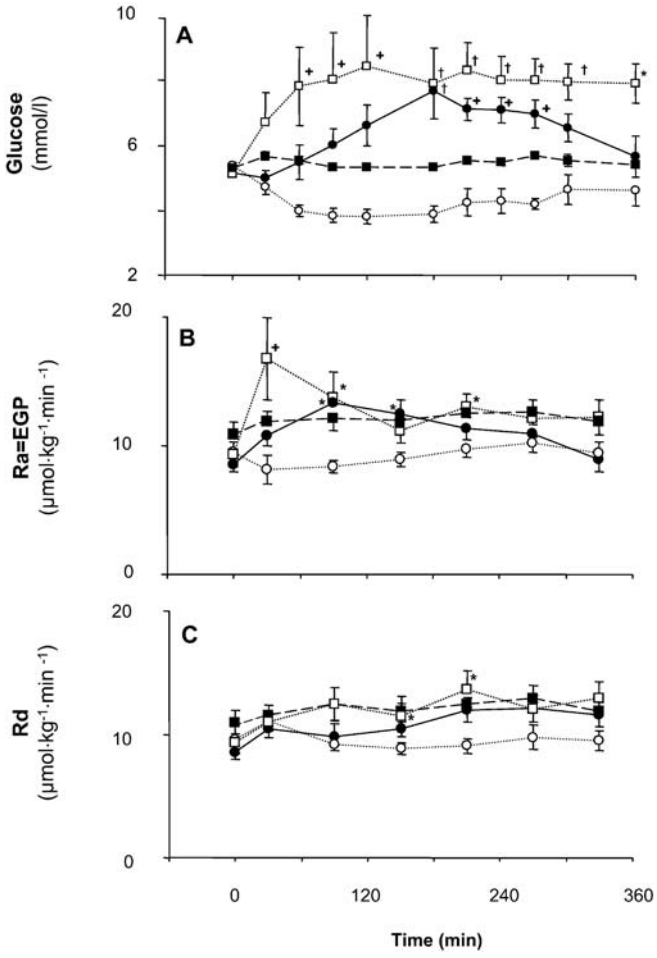

Fig. 2A–C. Plasma concentrations of glucose (**A**), rates of glucose appearance (endogenous glucose production) (**B**) and rates of glucose disappearance (**C**) during infusion of saline (CON+S, empty circles, *n*=7) or amino acids (AA+S, full circles, *n*=7) and selective glucagon elevation (GLUC+S, empty squares, *n*=6) together with somatostatin (pancreatic clamps) as well as infusion of amino acids without somatostatin (AA-S, full squares, *n*=5). Data are given as means±SEM. * $p<0.05$; + $p<0.01$, † $p<0.001$ vs. CON+S

AA+S: 0.15±0.04, GLUC+S: 0.12±0.03, AA-S: 0.22±0.03 mmol/l). Plasma glycerol and lactate concentrations neither changed from baseline values nor differed between the four protocols (individual data not shown).

Glucose metabolism. Fasting plasma glucose concentrations were comparable between the protocols (Fig. 2A). During AA-S, plasma glucose remained at baseline and did not change throughout the study. When AA-mediated hormone secretion was inhibited by somatostatin, plasma glucose increased by about 50% ($p<0.05$) during AA infusion (AA+S) between 180 min and 270 min (Fig. 2A). The selective rise in plasma glucagon concentrations during GLUC+S also resulted in an increase in plasma glucose ($p<0.05$ vs baseline from 60 to 360 min). During CON+S, plasma glucose slightly decreased ($p<0.01$) from baseline values between 60 and 270 min.

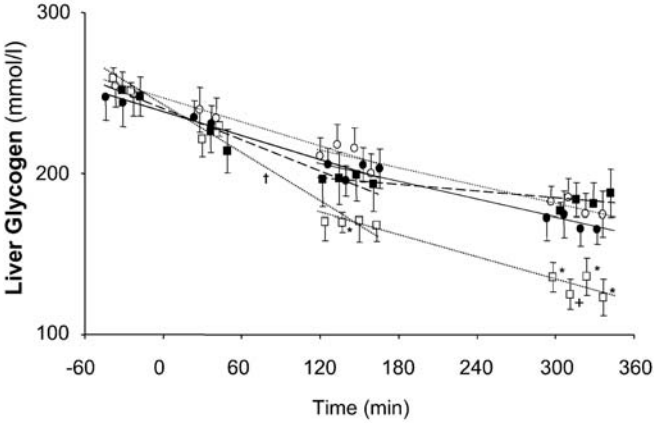

Fig. 3. Time course of liver glycogen concentrations and rates of net glycogenolysis during infusion of saline (CON+S, empty circles, *n*=7) or amino acids (AA+S, full circles, *n*=7) and selective glucagon elevation (GLUC+S, empty squares, *n*=6) together with somatostatin (pancreatic clamps) as well as infusion of amino acids without somatostatin (AA-S, full squares, *n*=5). Data are given as means±SEM. Glycogen concentrations: * $p<0.05$; + $p<0.01$ vs CON+S. Rates of net glycogenolysis: † $p<0.001$ vs CON+S

EGP was comparable between all protocols at zero time (CON+S: 9.6±0.7, AA+S: 8.6±0.6, GLUC+S: 9.4±0.9, AA-S: 10.6±1.0 µmol kg^{-1} min^{-1}). During AA-S, EGP only slightly increased ($p<0.05$) above baseline between 60 min and 300 min. In contrast, EGP rose by about 33% and about 42% during AA+S and GLUC+S, respectively and was higher ($p<0.05$) than under fasting and control conditions (CON+S; Fig. 2B). This rise in EGP preceded the increase in plasma glucose concentrations (Fig. 2A). As expected, EGP remained close to baseline during CON+S. Subsequent to the rise in plasma glucose, Rd gradually increased by about 35% and about 37% during AA+S ($p<0.05$ from 180 to 300 min) and GLUC+S ($p<0.01$ from 60 to 360 min), respectively. Rd was also slightly higher during AA-S compared to baseline ($p<0.05$) from 180 to 300 min (Fig. 2C). Under control conditions (CON+S) Rd did not change from baseline (Fig. 2C).

Hepatic glycogen metabolism. Baseline liver glycogen concentrations were similar in all protocols (CON+S: 254±13, AA+S: 247±15, GLUC+S: 259±7, AA-S: 251±11 mmol/l liver) (Fig. 3). AA infusion with (AA+S) or without somatostatin (AA-S) neither affected the glycogen concentration-time course nor net glycogenolysis. Only by doubling the glucagon infusion rate (GLUC+S), net glycogenolysis was about two-fold higher than during CON+S in the first half of the study (10.4±1.3 vs 5.0±0.4 µmol kg^{-1} min^{-1}, $p<0.001$). Net glycogenolysis was also higher when calculated for the complete time period of 360 min

Table 2. Rates of net glycogenolysis, total GNG calculated by subtracting rates of net glycogenolysis (0–360 min) from mean rates of EGP (0–360 min) (NMRS method) and rates of net GNG calculated from mean rates of EGP (0–360 min) and mean ^2H enrichments in carbons 2 and 5 of blood glucose drawn at 180 and 360 min (^2H_2O method) during the following protocols: infusion of saline (CON+S, n=7) or amino acids (AA+S, n=7) and selective glucagon elevation (GLUC+S, n=6) together with somatostatin (pancreatic clamps) as well as infusion of amino acids without somatostatin (AA-S, n=5). Data are given as means±SEM

Protocol	NMRS method		^2H_2O method
	Rates of net glycogenolysis (μmol kg^{-1} min^{-1})	Rates of total GNG (μmol kg^{-1} min^{-1})	Rates of net GNG (μmol kg^{-1} min^{-1})
CON+S	4.3±0.3	4.5±0.5	4.0±0.3
AA+S	4.4±0.2	6.9±0.5*	6.6±0.3**
GLUC+S	7.3±0.5***	5.9±0.9	n.d.
AA-S	3.4±0.3	8.8±0.9**	n.d.

* p <0.05; ** p <0.01, *** p<0.001 vs CON+S; n.d. not determined

(Table 2). Furthermore, net glycogenolysis was higher in the first (0–180 min; GLUC+S: 10.4±1.3, AA-S: 6.1±0.7 μmol kg^{-1} min^{-1}) than in the second half of the GLUC+S and AA-S protocols (120–360 min; GLUC+S: 4.7±0.6, AA-S: 1.8±0.7 μmol kg^{-1} min^{-1}; p<0.05 vs. 0–180 min). During control conditions (CON+S) and AA+S, net glycogenolysis was similar in both periods of these protocols (0–180 min; CON+S: 5.0±0.4, AA+S: 4.9±0.7 μmol kg^{-1} min^{-1} and 120–360 min; CON+S: 3.7±0.6, AA+S: 4.0± 0.5 μmol kg^{-1} min^{-1}, N.S.).

Net and total gluconeogenesis. At baseline, rates of net GNG as obtained by the ^2H_2O method were comparable between AA+S and CON+S (4.7±0.3 and 4.2±0.3 μmol kg^{-1} min^{-1}). During AA infusion (AA+S), net GNG increased by about 40% (p<0.01) over baseline and was about 65% greater (p<0.01) than in CON+S, but remained at baseline values during CON+S. Rates of total GNG as obtained by NMRS were not different from rates of net GNG (Table 2).

The respective contribution of total GNG and net glycogenolysis to EGP was analyzed separately for the first (0–180 min) and the second half (120–360 min) of all protocols (Fig. 4). During AA+S, EGP increased by about 50% (p<0.05 vs CON+S) which was associated with an about 131% rise of total GNG in the first 180 min (p<0.05 vs respective time period during CON+S).

During GLUC+S, EGP increased by about 71% compared with CON+S (p<0.01), which was accompanied by doubling the rates of net glycogenolysis in the first 180 min. In contrast, the rise in EGP by about 33% (p<0.05) during the second half of GLUC+S was associated with slightly but not significantly augmented total GNG. During AA infusion without somatostatin (AA-S), EGP was about 48% (p<0.05) and about 34% (p<0.05) higher compared to CON+S in the first and second half of the protocol. Elevation of EGP was almost exclusively accounted for by an increased (~96%, p<0.01) total GNG in the second half of the study.

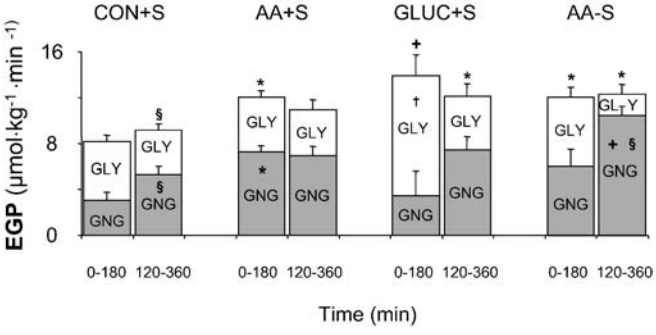

Fig. 4. Contributions of total gluconeogenesis (GNG) and net glycogenolysis (GLY; NMR method) to tracer determined rates of endogenous glucose production (EGP) in the first (0–180 min) and second (120–360 min) halves of the following studies; Saline (CON+S, n=7) or amino acid (AA+S, n=7) infusion and selective glucagon elevation (GLUC+S, n=6) together with somatostatin (pancreatic clamps) as well as infusion of amino acids without somatostatin (AA-S, n=5). Data are given as means±SEM. * p<0.05; + p<0.01, † p<0.001 vs corresponding time frame during CON+S. & p<0.05 vs. first half of the respective study

Discussion

This study shows that in the presence of fasting peripheral insulin and glucagon concentrations, hyperglycaemia in response to a short term rise in plasma AA to postprandial portal levels [38] is secondary to increased GNG and EGP. This direct (substrate-induced) effect can be clearly separated from the indirect (hormone-meditated) AA action which depends on an increase in the glucagon-to-insulin ratio and does not affect plasma glucose concentration.

Direct AA effects. In protocol AA+S, the rise in plasma glucose in response to the plasma AA elevation was observed under conditions of fasting peripheral concentrations of glucoregulatory hormones. Thus, it has to be attributed to a direct metabolic AA action which could be: (i) reduced peripheral glucose disposal (Rd) and/or (ii) increased EGP due to augmented

glycogenolysis or GNG. We have recently shown that a comparable AA infusion decreases insulin-stimulated peripheral glucose disposal by about 25% primarily by interfering with skeletal muscle glucose transport/phosphorylation in healthy humans [10]. Our study indicates that under conditions of fasting insulinaemia plasma AA elevation does not decrease Rd, which rather tended to increase during hyperglycaemia independent of insulin and is therefore most likely due to the mass action of glucose [39, 40, 41]. Consequently, insulin-dependent glucose uptake into skeletal muscle did not play a major role under the experimental conditions of this study [42]. The fall of plasma glucose towards baseline values during the second period of the AA+S protocol can be explained by the decrease of EGP which likely resulted from inhibition by hyperglycaemia [43] during the preceding 180 min. In addition, glucose-stimulated glucose disposal accounted for the gradually increased Rd [39, 40, 41], which in turn could have caused the fall in plasma glucose concentrations during the second period of AA+S.

On the other hand, EGP markedly increased in the AA+S protocol, although net glycogenolysis was comparable to that of control studies (CON+S). Thus, the augmented total and net GNG during the first 180 min primarily accounted for the observed rise in EGP. Moreover, combining ^{13}C NMRS with the $^{2}H_2O$ method made it possible to assess simultaneous glycogen synthesis during active glycogen breakdown from knowledge of rates of EGP, total and net GNG [35]. Total and net GNG were not different suggesting that under the conditions of the study there was little if any glycogen cycling [35].

It is of note that AA, mainly alanine and glutamine, derived from exogenous or endogenous proteins are the major source of de novo glucose formation, because lactate is derived from glucose and GNG from glycerol becomes quantitatively important only in the presence of accelerated lipolysis [11]. In the present study an AA mixture containing all gluconeogenic AA was infused so that one cannot discriminate between the impact of individual AA or a certain combination of AA on the obtained results.

These results are in line with studies in dogs that have shown that infusion of AA, which were disposed by about 90% into the splanchnic tissues, inducing a rise in hepatic glucose production [44]. Thus, direct effects of AA on GNG are likely explained by increased supply of gluconeogenic substrates. AA could also substitute as an oxidative fuel for other substrates and shunt them into the gluconeogenic pathway. Alternatively AA might induce genes of key gluconeogenic enzymes [45] similar to the action of glucagon [46].

Indirect AA effects. Amino acids are well-known to simultaneously stimulate endogenous insulin and glucagon release [15, 16, 17]. Thus, the indirect (hormone-mediated) AA effects need to be considered in addition to their direct metabolic effects. One previous report suggested that AA infusion could increase EGP by stimulating GNG in humans [47]. However, plasma glucagon concentrations were not controlled and remained consistently high during AA delivery in that study [47, 48] so it cannot be ruled out that stimulation of GNG by glucagon was responsible or at least contributed to the metabolic AA effect. Our study also aimed to compare the direct AA action on EGP and GNG with that of a selective rise in plasma glucagon concentrations (GLUC+S). We attempted to induce a similar increase in plasma glucose concentrations and rates of EGP during GLUC+S compared with the protocol testing direct AA effects (AA+S). This was achieved by doubling the glucagon infusion rate (GLUC+S) and this resulted in a rapid, but evanescent [49] rise in EGP secondary to stimulated glycogenolysis [42]. The observed effect is in contrast to the direct AA action leading to prompt stimulation of GNG. During glucagon administration, augmented net glycogenolysis explained 74% of EGP in the first half, but only 39% of EGP in the second half of the study indicating that only prolonged stimulation by glucagon increases GNG. These results confirm data obtained in dogs showing glucagon stimulated glycogenolysis first and thereafter GNG which progressively increased over time [50].

To test the combined (direct and indirect) effects of AA on hepatic glucose metabolism, AA were also infused in the absence of somatostatin (AA-S). This procedure did not affect plasma glucose concentrations despite elevated EGP. In line with previous observations [15, 16, 17] AA simultaneously-stimulated endogenous insulin and glucagon release. Consistent with arginine-induced growth hormone secretion [51], plasma growth hormone concentrations also tended to be higher under these conditions. Both direct (substrate) and indirect (glucagon mediated) AA effects therefore contributed to the observed increase in EGP and net glycogenolysis**.** The latter was more pronounced during the first half of this protocol than during control conditions. This effect is probably due to stimulation of glucagon secretion. However, in the second half of the experiment a marked increase in GNG was observed which primarily related to increased AA (substrate) supply and glucagon-mediated upregulation of gluconeogenic enzymes [11, 52]. Furthermore, insulin- and glucagon-stimulated (hepatic) AA uptake [11, 53] could have not only contributed to the up-regulation of GNG but also to the lower plasma AA concentrations during AA-S compared to AA+S. However, the rise in EGP was obviously counterbalanced by an insulin-stimulated increase in Rd, which thereby served to maintain fasting plasma glucose concentrations.

The effect of increased AA supply on hepatic glycogen metabolism has been controversial. In rats, high

dietary protein intake did not affect rates of hepatic glycogen synthesis during intraduodenal glucose infusion [13], whereas a protein meal led to glycogen depletion, secondary to increased glucagon concentrations [54]. When glucagon was kept at its basal levels during pancreatic clamps, intraportal gluconeogenic AA decreased [23], but peripheral AA delivery increased [22] net hepatic glucose uptake and glycogen synthesis in dogs. This indicates that intraportal AA delivery could generate a signal that suppresses hepatic glucose uptake. In our study carried out in humans, rates of net glycogenolysis were identical suggesting that AA do not directly stimulate glycogen synthesis.

It is of note that high protein intake is associated with insulin resistance and glucose intolerance in humans [12, 14] and rodents [13]. Moreover, in insulin-dependent diabetes splanchnic AA uptake is elevated [55] and protein ingestion increases plasma glucose and insulin requirements in these patients [7, 8]. The plasma AA concentrations observed in this study are within the range of those reached in the portal vein after ingestion of a protein meal [38]. Furthermore, the time course of plasma AA during the AA infusion is comparable to the pattern of plasma AA concentrations after ingestion of beef protein [56]. This indicates that changes in plasma AA concentrations within the physiologic range are sufficient to affect hepatic glucose metabolism. However, it cannot be excluded that incretins released from the gut in response to oral protein intake [57] or the portal route of AA supply to the liver [22] modulate the described effects of parenteral AA infusion under physiological conditions.

In conclusion, conditions creating postprandial amino acid elevation stimulate secretion of insulin and glucagon, but do not affect glycaemia despite markedly increased gluconeogenesis. However, impaired insulin secretion unmasks this direct gluconeogenic effect of amino acids and results in overt hyperglycaemia.

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