Splanchnic Utilization of Enteral Alanine in Humans

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The splanchnic bed extracts the majority of the enteral nonessential amino acids glutamine and glutamate, while extracting a much smaller proportion of essential amino acids such as leucine and phenylalanine. Alanine is an abundant nonessential amino acid that plays an important role in hepatic gluconeogenesis and ureagenesis. However, its enteral fate has not been studied. Twelve normal healthy postabsorptive adults received a 7-hour infusion of [1-^{13}C]alanine, 3.5 hours intravenously (IV) and 3.5 hours via a nasogastric tube (NG). The order of infusion was randomized among subjects. Alanine kinetics were calculated from the enrichments of plasma alanine ^{13}C and expired $^{13}CO_2$. The alanine appearance rate (R_a), measured during the IV tracer infusion, was 279 ± 17 μmol/kg/h; 92% ± 2% of the IV-infused and 86% ± 2% of the NG-infused [1-^{13}C]alanine tracer was recovered as $^{13}CO_2$. From the difference in plasma alanine ^{13}C enrichment between IV-infused and NG-infused tracers, we determined that the splanchnic bed extracted 69% ± 1% of the enterally delivered alanine tracer on the first pass during absorption. Only one third of the enteral alanine passed intact through the splanchnic bed and was made available to systemic tissues. Of the enteral alanine extracted, 83% ± 3% of the carboxyl-carbon label was recovered as CO_2, leaving only 17% of the sequestered alanine available for use in splanchnic protein synthesis. Thus, the splanchnic bed, presumably the liver, extracts and metabolizes most of the enterally delivered alanine.

ALANINE is a nonessential amino acid that is transaminated rapidly to pyruvate. Alanine is both a nitrogen (N) donor by transamination to pyruvate and a N receptor via transamination of pyruvate with a variety of amino acids. Skeletal muscle synthesizes and releases alanine in large amounts, while the liver is the primary site for alanine uptake.[1-5] Alanine is also produced by the kidney and the gut.[3,4,6] Thus, interorgan transfer is a key feature of alanine metabolism. Presumably, the source of pyruvate for alanine formation originates from glucose via glycolysis. Once alanine has been deaminated to form pyruvate, the pyruvate can be either (1) reduced further to lactate, (2) decarboxylated to form acetyl-coenzyme A, or (3) carboxylated to form oxaloacetate. The oxaloacetate entering the citric acid cycle may be oxidized to CO_2 or may provide carbon for gluconeogenesis. The net deamination of alanine leads to formation of urea in the liver, which is also the primary site for gluconeogenesis from the pyruvate carbon that is produced.

Much of our knowledge of the splanchnic metabolism of alanine has arisen from animal studies using multiple-organ catheterization techniques.[3,4,7] Studies in humans in which the balance of alanine across the splanchnic bed has been measured have demonstrated that the splanchnic bed extracts 25% to 50% of circulating alanine.[2,6,8,9] However, this figure does not account for simultaneous production of alanine by the gut and extraction of alanine by the liver.

We have previously applied stable-isotope tracer techniques to assess splanchnic disposal of the essential amino acids leucine and phenylalanine[10] and the nonessential amino acids glutamine and glutamate.[11,12] We found that the splanchnic bed retains small amounts of the essential amino acids but extracts and oxidizes almost all of the enterally delivered glutamate and about half of the enterally delivered glutamine.[10-12] This pattern has also been defined by other investigators.[13-15] Like alanine, both glutamine and glutamate play key roles in amino acid metabolism, interorgan transport of amino acid N, and gluconeogenesis.[1,4,16] Based on the animal-catheterization studies, we hypothesize that the liver should extract the majority of enteral alanine on the first pass. However, the fate of enteral alanine has not been studied in humans. We performed the present study to quantify the fraction of enteral alanine that is removed by the splanchnic bed on the first pass during absorption, and to determine whether the extracted alanine is retained as alanine or converted to pyruvate and metabolized.

SUBJECTS AND METHODS

Materials

L-[1-^{13}C]alanine, [2,3,3,3-$^{2}H_4$]alanine, and sodium [^{13}C]bicarbonate were purchased from Tracer Technologies (Somerville, MA), KOR Isotopes, and Cambridge Isotope Laboratories (Woburn, MA), respectively. Chemical and isotopic purity of [1-^{13}C]alanine was determined by gas chromatography–mass spectrometry (GCMS). [1-^{13}C]alanine was also checked for optical purity (absence of the D-stereoisomer) by GCMS. Before every infusion study, sterile solutions of [1-^{13}C]alanine and sodium [^{13}C]bicarbonate were prepared using aseptic technique. Accurately weighed amounts of the labeled compounds were dissolved in weighed volumes of sterile, pyrogen-free saline and filtered through a 0.22-μm Millipore (Bedford, MA) filter before use. An aliquot of the sterile solution was initially verified to be pyrogen-free before administration to human subjects. Solutions were prepared no more than 24 hours before use and were kept at 4°C prior to administration.

Subjects

Twelve healthy adults with normal weight for height were studied at either the University of Vermont (UVM) or New York Hospital-Cornell University Medical Center (NYH-CUMC) Clinical Research Centers (CRCs). Characteristics of the individual subjects are presented in Table 1. A medical history, physical examination, and biochemical laboratory screening tests were obtained to verify that each subject was free of

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Table 1. Subject Characteristics

Subject No.	Sex	Age (yr)	Weight (kg)	Height (cm)
Study 1				
A	F	23	58.2	163
B	F	23	56.8	160
C	M	36	86.8	182
D	M	25	70.8	172
E	M	22	74.0	180
F	M	22	70.3	170
Mean ± SE		25 ± 2	69.5 ± 4.5	171 ± 4
Study 2				
G	M	31	84.1	184
H	M	27	68.3	176
I	M	34	68.6	180
J	M	27	71.4	179
K	M	20	61.0	166
L	M	27	82.1	185
Mean ± SE		28 ± 2	72.6 ± 3.6	178 ± 3

chemically evident metabolic, gastrointestinal, cardiovascular, neurologic, or infectious disorders. The subjects were instructed on the purpose, benefits, and risks of the study and provided written consent in accordance with protocols approved by the UVM and NYH-CUMC Institutional Review Boards and the UVM and NYH-CUMC CRC Scientific Advisory Committees.

Infusion Protocol

Each subject was admitted to the CRC 1 day before each infusion study. Subjects consumed a liquid formula (355 kcal per container Ensure-Plus; Ross Laboratories, Columbus, OH) for their breakfast, noon, and evening meals to provide energy intake at 1.5 times their predicted metabolic rate. After 8:00 PM, subjects drank only water until completion of the infusion study the following day at 3:30 PM. An 8-Fr, 109-cm weighted nasogastric tube ([NG] Corpak, Wheeling, IL) was placed in the evening prior to the evening meal. At 6:30 AM the next morning, subjects were awakened. An intravenous (IV) catheter was placed in a forearm vein for infusion of tracers, and a catheter was inserted retrogradely into a superficial dorsal vein of the hand of the contralateral arm for blood sampling. Before obtaining any blood from the catheter, the hand was warmed in a heated-air box (air temperature, 50° to 55°C) to produce arterialized venous blood samples. The catheters were kept patent with a slow infusion of sterile saline.

Two infusion protocols were used. In study 1, [1-^{13}C]alanine was infused IV for the first 3.5 hours (period A) and via the NG tube for the second 3.5 hours (period B). Study 2 was identical except that [1-^{13}C]alanine was infused for the first 3.5 hours by the NG route, and then by the IV route for the last 3.5 hours. Six subjects were infused in study 1 and six in study 2. Just before the start of each infusion (7:30 AM), a priming dose of [1-^{13}C]alanine and [^{13}C]bicarbonate was administered as an IV bolus. Immediately thereafter, the infusion of [1-^{13}C]alanine tracer began; at 3.5 hours, the route of infusion was switched. In study 1, the priming dose was 24 and 11 μmol/kg for [1-^{13}C]alanine and [^{13}C]bicarbonate, respectively. The IV infusion rate of [1-^{13}C]alanine was 7.8 μmol/kg/h (6 mL/h) during period A; the NG infusion rate of [1-^{13}C]alanine was increased by one third to a rate of 10.4 μmol/kg/h for period B by increasing the volume infusion rate to 8 mL/h. In study 2, the priming dose was 9.6 and 20 μmol/kg for [1-^{13}C]alanine and [^{13}C]bicarbonate, respectively. The NG infusion rate of [1-^{13}C]alanine was 10.7 μmol/kg/h (8 mL/h) during period A; the IV infusion rate of [1-^{13}C]alanine was decreased by one third to a rate of 8 μmol/kg/h for period B by decreasing the volume infusion rate to 6 mL/h.

In both studies, blood and breath samples were obtained just before the start and at 15-minute intervals primarily during the last 1.5 hours of each of the two 3.5-hour tracer infusion periods. Aliquots of blood were placed in tubes containing EDTA and stored on ice until the plasma was prepared by centrifugation at 4°C. A 0.5-mL aliquot was withdrawn, a defined amount of [^{2}H$_4$]alanine was added as an internal standard for quantitation of the alanine plasma concentration, and the plasma was frozen at −60°C. Breath samples were placed into 20-mL evacuated tubes until measurement of $^{13}CO_2$ enrichment in the expired air by isotope ratio mass spectrometry. Each subject's CO_2 production rate was measured periodically for 15-minute periods using an indirect calorimeter with a flow-through canopy system (DeltaTrak; Sensormedics, Yorba Linda, CA). The CO_2 production rate was used to calculate the rate of alanine tracer oxidized to $^{13}CO_2$.

Analytical Methods

Plasma alanine concentrations and enrichments were measured by negative chemical ionization GCMS. Before derivatization, amino acids were isolated from plasma using cation-exchange columns as previously described.[17] Eluates from the columns were evaporated to dryness and derivatized to the *N*-heptafluorobutyryl, *n*-propyl (HFBP) amino acid ester derivatives. Injections of HFBP derivatives were made into the GCMS instrument (model 5988A; Hewlett-Packard, Palo Alto, CA) operated under selected ion monitoring and using negative chemical ionization. The $[M\text{-}HF]^-$ ion was monitored at *m/z* 307, 308, and 311 corresponding to unlabeled alanine, [1-^{13}C]alanine, and [^{2}H$_4$]alanine, respectively. For each measurement, the background-corrected amino acid enrichment in mole percent excess (mpe) and plasma concentration were determined as described previously for phenylalanine and glycerol.[17,18]

Calculations

The subscripts iv and ng refer to enrichments and tracer infusion rates during the IV and NG infusion periods. The appearance rate of alanine into plasma (synonymous with "flux" or "turnover" in the steady state) was calculated for the IV infusion period as

$$R_a - I_{iv}(E_i/E_{p(iv)} - 1), \quad (1)$$

where R_a is the rate of alanine appearance into plasma (micromoles per kilogram per hour), E_i and $E_{p(iv)}$ are the amino acid tracer enrichments (mpe) in the infusate and in plasma at steady state during the IV tracer infusion period, and I_{iv} is the tracer infusion rate (micromoles per kilogram per hour) during the IV portion of the infusion study. As discussed previously,[10] the fraction of NG tracer not extracted on the first pass by the splanchnic bed was determined by comparing the enrichment of the NG-administered tracer in plasma ($E_{p(ng)}$ with the enrichment of the IV-infused tracer in plasma ($E_{p(iv)}$), normalized for tracer infusion rates. The fraction of NG tracer extracted on the first pass by the splanchnic bed (f) was then calculated by difference,

$$f = 1 - (E_{p(ng)}/i_{ng})/(E_{p(iv)}/i_{iv}), \quad (2)$$

where i_{ng} and i_{iv} are the rates of NG and IV tracer infusions (micromoles per kilogram per hour) of enriched species per se. The rate of labeled-species infusion is the rate of total tracer material times the enrichment of the labeled species: $i_{iv} = I_{iv}E_i$ and $i_{ng} = I_{ng}E_i$.

The rate of oxidation of the [1-^{13}C]alanine tracer to release the ^{13}C label as $^{13}CO_2$ (F_{13C}) was calculated by multiplying the rate of CO_2 production by the breath $^{13}CO_2$ enrichment for each subject. This rate was increased by 1/0.81, assuming that only 81% of the metabolic CO_2 produced is released as exhaled CO_2, as shown by Allsop et al[19] and confirmed more recently by others.[20,21] The fraction of infused tracer that was oxidized to CO_2 was calculated by dividing F_{13C} by the rate of ^{13}C-alanine tracer infusion. For the intravenous tracer, the fraction of

Table 2. Plasma Alanine and Expired-Air Values

Subject No.	Plasma Alanine Concentration (μmol/L) Period A	Plasma Alanine Concentration (μmol/L) Period B	Plasma Alanine ^{13}C Enrichment (mpe) Period A	Plasma Alanine ^{13}C Enrichment (mpe) Period B	CO_2 Production (mmol/kg/h)	Expired-Air $^{13}CO_2$ (ape × 1,000) Period A	Expired-Air $^{13}CO_2$ (ape × 1,000) Period B
Study 1							
Route	IV	NG	IV	NG		IV	NG
A	331	234	2.38	1.09	6.99	93.3	104.8
B	233	205	3.36	1.00	6.64	92.9	111.7
C	290	272	2.90	1.33	6.32	93.4	117.8
D	331	223	1.85	0.88	8.02	82.8	102.2
E	354	337	2.25	0.83	7.52	72.7	93.7
F	279	285	3.48	1.74	6.20	94.9	120.5
Mean ± SE	303 ± 18	259 ± 20	2.70 ± 0.27	1.15 ± 0.14	6.95 ± 0.29	88.3 ± 3.6	108.5 ± 4.1
Study 2							
Route	NG	IV	NG	IV		NG	IV
G	276	247	1.24	3.09	6.46	104.4	85.7
H	256	263	1.11	2.53	7.11	100.5	72.2
I	172	157	1.23	3.44	8.07	82.7	66.9
J	190	198	1.01	2.98	7.76	75.1	72.2
K	374	346	1.26	3.10	7.33	107.5	75.3
L	415	352	1.02	2.33	8.02	93.7	74.6
Mean ± SE	281 ± 40	261 ± 32	1.15 ± 0.05	2.91 ± 0.17	7.46 ± 0.25	94.0 ± 5.2	74.5 ± 2.5

NOTE. mpe represents the fraction of plasma alanine that is [1-^{13}C]alanine tracer; ape is the fraction of CO_2 that is ^{13}C from the tracer. Alanine concentration, [^{13}C]alanine enrichment, CO_2 production, and expired-air $^{13}CO_2$ enrichments were not significantly different between study groups ($P > .05$). Alanine concentration showed a time effect (lower in period B *v* period A, $P < .05$). Plasma alanine ^{13}C enrichment was lower during NG infusion *v* IV infusion ($P < .001$). Expired $^{13}CO_2$ enrichments were higher during NG infusion *v* IV infusion ($P < .001$).

infused tracer oxidized to $^{13}CO_2$ was

$$f_{ox(iv)} = F_{13C(iv)}/i_{iv}. \quad (3)$$

The NG-infused ^{13}C tracer may be oxidized either on the first pass during absorption by the splanchnic bed or later after passing into the systemic circulation. As previously defined,[12] the fraction of NG tracer oxidized directly on the first pass is the fraction of NG ^{13}C tracer oxidized, $F_{13C(ng)}/i_{ng}$, minus the fraction of tracer that escapes first-pass extraction, 1 − f, and is subsequently oxidized to $^{13}CO_2$ ($f_{ox(iv)}$):

$$f_{ox(ng)} = F_{13C(ng)}/i_{ng} - (1 - f) \cdot f_{ox(iv)}. \quad (4)$$

This value represents the fraction of NG-infused ^{13}C tracer that is (1) sequestered on the first pass and (2) oxidized to liberate the carboxyl-carbon as $^{13}CO_2$. The fraction of sequestered tracer that underwent this fate is $f_{ox(ng)}/f$.

Statistics

Data are presented as the mean ± SE. During tracer infusions, the steady state for plasma amino acid levels and amino acid enrichments was defined as an insignificant correlation ($P > .05$) with time using standard linear regression. The alanine concentration, enrichment, and kinetic data were analyzed using a two-period crossover-design repeated-measures ANOVA to define significant effects (1) between the IV and NG infusion periods and (2) between subject groups (ie, whether there was a difference between the IV first *v* IV second infusion order). The remainder of the comparisons were for data between two conditions using an unpaired *t* test and a pooled error term (comparisons between studies 1 and 2) or paired *t* test (comparisons of IV and NG data within studies).

RESULTS

The mean plasma alanine concentration for the last 1.5 hours of each experimental period is shown in Table 2 for individual subjects. Alanine concentrations were not significantly different among groups during the respective IV and NG periods, although there was a small decrement in the concentration from the first to the second half of each study (−9% ± 4%, $P < .05$). The time course of plasma [1-^{13}C]alanine enrichment is shown in Fig 1. Tracer enrichments were in isotopic steady state during the last hour of each infusion period. Because we anticipated a significant splanchnic extraction of the NG-infused alanine tracer, we increased by one third the NG infusion of [1-^{13}C]alanine to ensure measurable alanine enrichment in the plasma. In both studies, plasma alanine enrichments were lower ($P < .001$) when the tracer was infused by the NG route, even though the rate of tracer infusion was greater with the IV route (Table 2).

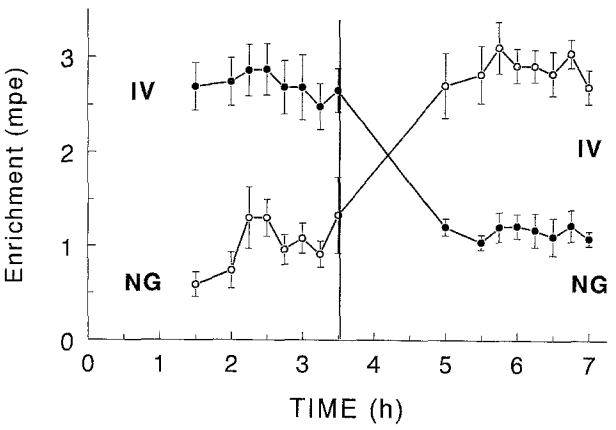

Fig 1. Time course of plasma [1-^{13}C]alanine enrichment (mpe). (●) Study 1; (○) study 2. The route of tracer delivery was switched in each study at 3.5 hours, and the tracer infusion rate was altered. Tracer infusion was one third faster during NG infusion compared with IV infusion in both studies. mpe defines the fraction of plasma alanine that is the [1-^{13}C]alanine tracer.

Plasma alanine ^{13}C enrichments were used to calculate the alanine R_a during the IV infusion periods (Table 3). The alanine R_a was similar in both studies and did not show a time effect between study 1 period A and study 2 period B. The first-pass extraction of the enteral alanine tracer, f, is also presented in Table 3 for both studies. There was no difference in the amount of enteral alanine extracted between the two studies. First-pass extraction was 69% of the enterally infused alanine tracer.

The CO_2 production rate did not change significantly with time and was not different between the two studies (Table 2). The mean values for expired $^{13}CO_2$ enrichments were different between the IV and NG infusion periods (Table 2). These enrichments were multiplied by the CO_2 production rate to calculate the rate of $^{13}CO_2$ excretion derived from the release of the alanine ^{13}C tracer as $^{13}CO_2$. The time course of $^{13}CO_2$ excretion is shown in Fig 2. The rate of $^{13}CO_2$ excretion decreased during the IV infusion period ($P < .05$) in both studies.

The time course of the fraction of ^{13}C-alanine tracer excreted as $^{13}CO_2$ is shown in Fig 2. This value normalizes for differences in the tracer infusion rate between infusion routes and studies. The fraction of the ^{13}C tracer recovered as $^{13}CO_2$ was greater when the tracer was infused by the IV route versus the NG route ($P < .05$; Table 4). These data were used to calculate for each subject the amount of NG-infused ^{13}C tracer that was released as $^{13}CO_2$ directly on the first pass by the splanchnic bed. In both studies, 57% of the enteral ^{13}C tracer was extracted and the tracer was released as $^{13}CO_2$ by the splanchnic bed on the first pass (Table 4). Dividing the fraction of tracer sequestered and oxidized ($f_{ox(ng)}$) by the fraction of tracer sequestered (f) defines what fraction of the sequestered alanine was metabolized. The fraction of NG tracer that was sequestered and metabolized was 85% ± 2% in study 1, 81% ± 5% in study 2, and 83% ± 3% overall. The primary fate of enterally sequestered alanine was therefore conversion to pyruvate with subsequent metabolism resulting in recovery of the carboxyl-carbon as CO_2.

Table 3. Alanine Kinetic Parameters Measured From Plasma Enrichments

Subject No.	Alanine R_a (μmol/kg/h)	Fraction (f) of Enteral Alanine Extracted by the Splanchnic Bed (%)
	IV Period A	NG Period B/ IV Period A
Study 1		
A	317	65.5
B	222	77.7
C	259	65.5
D	409	64.5
E	335	72.4
F	214	62.5
Mean ± SE	292 ± 31	68.0 ± 2.4
	IV Period B	NG Period A/ IV Period B
Study 2		
G	244	69.9
H	300	67.0
I	219	73.1
J	254	74.6
K	243	69.7
L	327	67.1
Mean ± SE	265 ± 17	70.2 ± 1.3
Mean ± SE of all subjects	279 ± 17	69.1 ± 1.3

NOTE. Alanine R_a and f were not significantly different ($P > .05$) between study groups.

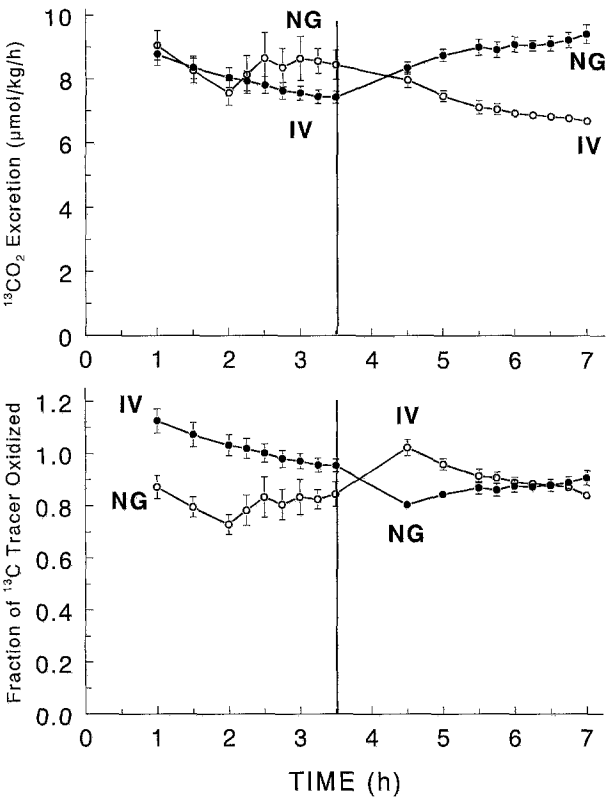

Fig 2. Time course of expired $^{13}CO_2$ excretion. (●) Study 1; (○) study 2. (A) Rate of $^{13}CO_2$ excretion; (B) rate of $^{13}CO_2$ excretion divided by ^{13}C-alanine infusion rate. The route of tracer delivery was switched in each study at 3.5 hours, and the tracer infusion rate was altered. Tracer infusion was one third faster during NG infusion compared with IV infusion in both studies. Error bars smaller than the symbols are not visible.

DISCUSSION

Several nonessential amino acids have ketoacid metabolites that play important roles in gluconeogenesis and provision of carbon for the tricarboxylic acid (TCA) cycle. These amino acids are alanine, glutamic acid, aspartic acid, and their amide derivatives glutamine and asparagine. Through transamination, these amino acids readily exchange amino-N. When these amino acids are provided in the diet, they may be used as amino acids for the synthesis of new proteins or as a source of carbon for immediate energy or gluconeogenesis. Because they are nonessential, these amino acids do not need to be conserved. We and others have found that the splanchnic bed extracts nearly all of the glutamate and half of the glutamine delivered enterally.[11,12,15,22] In contrast, between 20% and 40% of the essential amino acids leucine and phenylalanine are extracted by the splanchnic bed when delivered enterally.[10,12,23,24] Alanine, like

Table 4. Alanine Kinetic Parameters Measured From Expired $^{13}CO_2$

Subject No.	Fraction of Infused Tracer Recovered as $^{13}CO_2$ (%)		Fraction of NG-Infused Tracer Oxidized to $^{13}CO_2$ On First Pass (%)
	IV Infusion, Period A	NG Infusion, Period B	
Study 1			
A	103.2	86.9	51.3
B	97.7	88.1	66.3
C	93.5	88.4	56.1
D	105.0	97.3	60.0
E	86.6	83.7	59.8
F	93.2	88.7	53.8
Mean ± SE	96.5 ± 2.8	88.9 ± 1.8	57.9 ± 2.2
Study 2			
G	87.6	80.0	53.7
H	81.2	84.8	58.0
I	85.5	79.3	56.2
J	88.7	69.2	46.7
K	87.3	93.4	67.0
L	94.7	89.2	58.1
Mean ± SE	87.5 ± 1.8	82.7 ± 3.5	56.6 ± 2.7
Mean ± SE of all subjects	92.0 ± 2.1	85.8 ± 2.1	57.3 ± 1.7

NOTE. The fractional oxidation of IV- and NG-infused tracers was greater in study 1 *v* study 2 ($P < .05$). Significantly more IV-infused tracer was oxidized *v* NG-infused tracer ($P < .05$). The fraction of NG tracer extracted and oxidized on the first pass was not significantly different between the 2 studies.

glutamate, should be readily usable as a carbon donor for the TCA cycle. Alanine uptake by the liver may be more important for its use as a carbon source than for its requirement as an amino acid for new protein synthesis.

Using a [^{15}N]alanine tracer, we previously determined that alanine turnover in the body correlated with dietary carbohydrate intake and was unaffected by alterations in protein intake when dietary protein and carbohydrate intakes were manipulated.[25] We concluded that alanine is treated as an extension of pyruvate rather than as an amino acid. Pyruvate is produced during glycolysis and is used through the TCA cycle for energy and gluconeogenesis.[25] The present study was performed to explore the metabolism of alanine in the splanchnic bed. Meal feeding will deliver an influx of carbohydrate, amino acids, and/or fat and generally produce alterations in the secretion of insulin and glucagon. Because any or all of these factors may perturb alanine metabolism, we provided the ^{13}C-alanine tracer enterally to subjects in the postabsorptive state. Although this scheme does not define the response of the splanchnic bed to meal-feeding conditions, it does allow study of alanine utilization without the confounding perturbations of other variables.

The present view of alanine metabolism is that alanine is produced and released by the muscle and secondarily by the gut and is extracted by the liver.[2,4,6,7] Thus, we expected that the majority of enterally delivered alanine tracer would be extracted on the first pass by the splanchnic bed. This result was found: 69% ± 1% of enterally delivered alanine was sequestered by the splanchnic bed. The fraction of the enteral alanine tracer that was extracted by the splanchnic bed was less than what we found under the same metabolic circumstances for glutamate (≈90%) and greater than that found for glutamine (≈54%).[11,12]

Although plasma alanine enrichment was in steady state during both studies, the $^{13}CO_2$ excretion rate slowly decreased (≈4% per hour) during infusion of the IV tracer and tended to increase during the NG tracer infusion. The initial decrease in $^{13}CO_2$ during the IV infusion in study 1 could have been due to a possible overpriming of the bicarbonate pool. The same problem appeared in study 2 after decreasing the infusion rate of ^{13}C-alanine to switch the infusion from NG to IV. Because the calculations of oxidation were performed on the data at the end of each infusion period at a time when the decrease in $^{13}CO_2$ excretion was leveling, the effect of the decrease in $^{13}CO_2$ over time is minimized in the calculation of alanine ^{13}C tracer recovery as CO_2. However, there were significant differences (5% to 10%) in the measurement of the fraction of alanine ^{13}C tracer recovered as CO_2 for both the IV and NG tracers, depending on whether the tracers were infused during the first or second 3.5-hour period. These results suggest a slow response of the $^{13}CO_2$ in attaining a plateau and that the alanine carbon passes through intermediate side pools of significant mass that affect the $^{13}CO_2$ time course.

The data we obtained concerning recovery of the enteral alanine tracer are based on measurement of the infused alanine ^{13}C tracer released as $^{13}CO_2$. This parameter requires no assumption concerning compartmentation of the tracer within the system or assumptions concerning a model. It is simply a measurement of the fraction of infused ^{13}C tracer recovered as exhaled $^{13}CO_2$. The combined results of studies 1 and 2 show that 92% ± 2% of the IV-infused ^{13}C-alanine tracer was recovered as $^{13}CO_2$, while 86% ± 2% of the NG-infused ^{13}C-alanine was recovered as $^{13}CO_2$ (Table 4). These values were then used to determine the fraction of enteral alanine tracer sequestered and converted to $^{13}CO_2$ on the first pass (Eq 4). The fraction of NG-infused tracer that was sequestered and oxidized was 57% ± 2%. There was no difference in this value between studies 1 and 2 (Table 4). The fraction of sequestered ^{13}C tracer recovered as $^{13}CO_2$ was $f_{ox(ng)}/f = 0.57/0.69 = 83\%$. Thus, the primary fate of the sequestered alanine tracer was through pathways resulting in production of CO_2, not alanine use as an amino acid for splanchnic-bed protein synthesis.

The recovery of the alanine ^{13}C label in CO_2 does not demonstrate direct oxidation of the pyruvate, but rather degradation of the pyruvate through one of several pathways. The present study cannot directly quantify net alanine oxidation, but can only discern between alanine sequestration for use as an amino acid for protein synthesis versus alanine use for pathways of carbohydrate metabolism. The present results clearly show that a large fraction (69%) of the enteral alanine tracer was removed on the first pass by the splanchnic bed and that only 17% of the sequestered alanine was used in new protein synthesis. The prominent pathway for enteral alanine was through conversion to pyruvate leading to the release of the ^{13}C tracer as $^{13}CO_2$.

We chose the [1-^{13}C]alanine tracer instead of a [2-^{13}C]alanine or [3-^{13}C]alanine because the [1-^{13}C]alanine label is most likely removed with metabolism of the pyruvate. Several pathways exist for pyruvate metabolism: (1) conversion to lactate, but lactate only converts back to pyruvate with no net removal of ^{13}C tracer; (2) decarboxylation to form acetyl coenzyme A, releasing the ^{13}C tracer as a $^{13}CO_2$; however, this pathway is not likely a quantitatively important process in normal fasting

people[26,27]; and (3) carboxylation of pyruvate to form oxaloacetate, with the majority of oxaloacetate entering the TCA cycle. Diraison et al[28] have shown in normal postabsorptive subjects that the rate of carboxylation of pyruvate is 12 times greater than the rate of pyruvate decarboxylation to acetate. Thus, this pathway is prominent. When oxaloacetate enters the TCA cycle, the first carbon is eliminated as CO_2 with the first turn of the TCA cycle.[29] At least two passes through the TCA cycle are required to lose the second and third carbons. Carbon may be siphoned off from the TCA cycle to form phosphoenolpyruvate for glucose synthesis. The second and third carbons are retained, while the first carbon is released as CO_2. Thus, the first carbon of alanine is largely recovered as CO_2 when pyruvate is metabolized, while the second and third carbons are largely retained.

The choice of carbon label does not affect the measurement of alanine R_a because differences in tracer metabolism of pyruvate are distal to the dilution of alanine tracer in blood, which forms the basis of the R_a measurement. We have previously compared the kinetics of [1-^{13}C]alanine and [3-^{13}C]alanine tracers measured in the same subjects simultaneously, and found no significant difference with respect to alanine turnover.[29] There was no significant difference in the alanine R_a in this study (279 ± 17 μmol/kg/h; Table 3) compared with the alanine turnover determined previously.[29] Furthermore, the values for alanine turnover reported in the literature are comparable for different alanine tracers: [1-^{13}C]alanine,[25,29] [2,3-$^{13}C_2$]alanine,[30] and [3-^{13}C]alanine.[5,31-33] Thus, the measurement of the fraction of enteral alanine tracer extracted based on plasma alanine ^{13}C measurements should be the same regardless of which alanine carbon label is chosen.

The primary difference in the different carbon tracers of alanine is the differential fate of release as CO_2 versus incorporation into other compounds such as glucose. We determined that about 90% of the [1-^{13}C]alanine tracer was recovered as $^{13}CO_2$ regardless of whether the tracer was administered by IV or NG routes. Kalhan et al[30] found that only 30% of [2,3-$^{13}C_2$]alanine was recovered as $^{13}CO_2$ in normal women. Consoli et al[32] measured a recovery of only 31% of infused [3-^{13}C]alanine as $^{13}CO_2$ in normal subjects. Thus, there appears to be an approximately threefold difference in the recovery of the first carbon of pyruvate via oxidation versus retention of the second and third carbon labels for gluconeogenesis.

We used the [1-^{13}C]alanine label to differentiate enteral alanine uptake between use as an amino acid and use as a carbon source (irrespective of whether it is decarboxylated directly, after entrance in the TCA cycle, or after biotransformation in substrates that are oxidized elsewhere). Recovering most of the label in CO_2 confirmed the fate of the hepatic extraction but raised additional considerations. For example, at the same time the tracer ^{13}C-alanine was converted to pyruvate and metabolized to release $^{13}CO_2$, other pyruvate may have been formed and aminated to produce a new alanine. Our tracer measurements provide a measurement of the absolute rate of alanine metabolism, but do not address the issue of net utilization.

Splanchnic utilization of alanine has been previously measured in humans from arterial-venous differences across the splanchnic bed.[2,6,8,9] These studies have shown that the splanchnic bed extracts 25% to 50% of the alanine from the splanchnic circulation, accounting for about 70 to 100 μmol/kg/h of alanine. Chiasson et al[34] infused IV ^{14}C-alanine and measured the plasma alanine concentration and ^{14}C specific activity across the splanchnic bed. Although they did not present explicit calculations, their data indicate that 77% of endogenous plasma alanine was extracted in the splanchnic bed, a value comparable to the 69% extraction of enteral alanine in the present study.

In summary, this study in humans measured the uptake and sequestration of enteral alanine by the splanchnic bed. We determined that the splanchnic bed extracted about two thirds of the enteral alanine delivered, and that only one third of the enteral alanine was available for systemic tissues. Very little of the alanine sequestered by the splanchnic bed was used for new protein synthesis. The majority of the alanine was metabolized through pyruvate. Although this study could not differentiate between metabolism in the gut versus the liver, we presume, based on previous studies in animals, that the primary site of alanine sequestration and metabolism was the liver.

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