Splanchnic Versus Whole-Body Production of α-Ketoisocaproate From Leucine in the Fed State

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The extent to which dietary branched-chain amino acids are deaminated by the splanchnic tissues (ie, the liver and gut) in the fed state and released as ketoacids into the systemic circulation is not known. To determine this, we combined the oral (L-[1-^{13}C]-leucine, [^{13}C]-Leu) and intravenous (L-[5,5,5-$^{2}H_3$]leucine, [$^{2}H_3$]-Leu) leucine tracer infusion with the intravenous administration of an independent isotope of the leucine ketoanalog α-ketoisocaproic acid (KIC) ([4,5-^{3}H]KIC). The study was conducted during constant administration of a complete mixed meal. We found that 26% ± 5% of the orally administered leucine was taken up by the splanchnic organs at first pass, whereas 74% ± 5% appeared in the systemic circulation. The rate of splanchnic KIC release from deamination of dietary leucine accounted for 3% ± 0.2% of the oral leucine administration rate and 13% ± 2% of leucine splanchnic uptake (fractional splanchnic deamination). The fraction of whole-body total leucine uptake that was deaminated to KIC was 41% ± 5% ($P < .05$ *v* fractional splanchnic deamination of dietary leucine uptake). We conclude that (1) the release of KIC from leucine deamination within splanchnic tissues constitutes a minimal fraction of first-pass dietary leucine uptake, and (2) splanchnic tissues are relatively less efficient than the whole body in KIC production from leucine deamination.

THE QUANTITATIVE importance of splanchnic tissues in the disposal of dietary amino acids has been recently investigated in humans by adding labeled amino acid tracers to a feeding solution during simultaneous intravenous infusion of different isotopes of the same amino acids.[1-7] Using this technique, we[1-3] and others[4-7] have shown that first-pass splanchnic uptake of dietary leucine was approximately 20% to 30% of the oral intake, and the remaining approximately 70% to 80% appeared in the systemic circulation. After entering the intracellular space, leucine is extensively deaminated to the corresponding ketoacid, α-ketoisocaproic acid (KIC).[8,9] This reversible process takes place virtually in all tissues, including the liver and gut.[8-13] As a consequence, a fraction of dietary leucine sequestered within the splanchnic organs after a meal may be deaminated and released into the systemic circulation as KIC by the combined activity of the liver and gut.[11,12] KIC may thus represent an alternative route by which dietary leucine-carbon is disposed into peripheral tissues. Through this mechanism, actual systemic availability of dietary leucine-carbon could be even higher than the reported values of 70% to 80% of the dietary intake.[1-7]

The aim of this study was to estimate first-pass splanchnic deamination of dietary leucine by combining oral (L-[1-^{13}C]-leucine, [^{13}C]-Leu) and intravenous (L-[5,5,5-$^{2}H_3$]leucine, [$^{2}H_3$]-Leu) tracer infusion[1-7] with the intravenous infusion of an independent KIC tracer ([4,5-$^{3}H_2$]KIC). This method enabled us to evaluate directly the true systemic availability of dietary leucine, ie, the amount escaping first-pass splanchnic extraction and being released into the posthepatic circulation either as leucine or KIC. Furthermore, the ability to produce KIC from leucine was compared in splanchnic organs versus the whole body.

SUBJECTS AND METHODS

Materials

[$^{2}H_3$]-Leu (~98% enriched) and [^{13}C]-Leu (~99% enriched) were purchased from Tracer Technologies (Somerville, MA). [4,5-$^{3}H_2$]KIC ([^{3}H]KIC) was prepared from L-[4,5-^{3}H]-Leu (specific activity [SA], 55 mCi/mmol; Amersham, Amersham, Buckinghamshire, UK) as described elsewhere for preparation of [1-^{14}C]KIC.[14] The purity of [^{3}H]KIC was more than 95% as analyzed by high-performance liquid chromatography (HPLC) before infusion. Isotope infusion rates were corrected accordingly. Isotopes were filtered through a 0.2-μm filter (Millipore, Molsheim, France) and proven to be sterile and pyrogen-free before use.

A chemically defined mixed meal (~11 kcal/kg body weight) containing 50% of calories as carbohydrates (sucrose and grapefruit extract), 18% as crystalline amino acids, and 32% as fats (butter and cocoa powder) was prepared on the morning of the study. The composition of the crystalline amino acid mixture (expressed as percent total grams administered) was as follows: aspartic acid 9.7, threonine 4.7, serine 4.4, glutamine 15.4, glycine 5.6, alanine 5.5, ½ cysteine 1.2, methionine 2.8, tyrosine 3.7, phenylalanine 4.3, lysine 8.8, histidine 3.1, arginine 6.5, proline 4.5, isoleucine 5.4, leucine 8.2, valine 5.7, tryptophan 1.1. Approximately 50 μmol/kg [^{13}C]-Leu was added to this solution. The final solution was mixed continuously throughout the study. Before isotope addition, samples of the meal were taken to measure natural [^{13}C]-Leu moles percent enrichment (MPE). The lipids were administered as a creamy mixture of butter, cocoa (a few milligrams), and sucrose. The meal was administered in isocaloric aliquots of both the amino acid solution and the fat/sucrose components, which were ingested every 20 minutes for 4 hours (description follows).

Experimental Design

Four healthy male volunteers (age, 42 ± 3 years; body mass index, 26 ± 1) were studied after an overnight fast. All subjects had been adapted to their usual diet (~2,000 kcal/d), containing at least 50% carbohydrates and about 18% proteins, for at least 3 months before the study. On the morning of the study, polyethylene catheters were placed in a forearm vein for isotope infusion and in a dorsal vein of the opposite hand for blood withdrawal. This hand was placed in a heated box kept at 65°C to obtain arterialized venous blood. Blood samples were taken before the start of isotope infusion in each study, to

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determine baseline natural enrichments of [$^2H_3$]-Leu, [$^2H_3$]-KIC, [^{13}C]-Leu, and [^{13}C]-KIC. At 8 AM, a primed-continuous intravenous infusion of the radioactive [^{3}H]-KIC and the stable [$^2H_3$]-Leu isotopes was initiated. Infusion rates of [$^2H_3$]-Leu and [^{3}H]-KIC were 0.061 ± 0.001 μmol/kg · min and 7,155 ± 1,866 dpm/kg · min, respectively. Priming doses of [$^2H_3$]-Leu and [^{3}H]-KIC were 30 times the continuous infusion rates per minute. After allowing 150 minutes for isotope equilibration, four baseline blood samples were obtained over 30 minutes for measurement of substrate concentrations and isotope MPE/SA. Thereafter, approximately continuous administration of the meal was started as described earlier, to attain steady-state conditions in plasma substrate concentrations and in MPE and SA of the tracers. The oral administration rate of [^{13}C]-Leu was 0.116 ± 0.005 μmol/kg · min. Blood samples were collected at 30, 90, 150, and 190 minutes after the start of meal, and thereafter at the midpoint between each meal administration (ie, 10 minutes after each aliquot).

Analytical Methods

Plasma leucine and KIC and meal leucine concentrations were determined by HPLC.[13] Plasma [^{3}H]-KIC SA was also determined by HPLC.[13] Plasma and meal enrichments of the stable isotopes of leucine and KIC were determined by gas chromatography/mass spectrometry as *t*-butyldimethylsilyl derivatives,[1] using electron-impact ionization.[1] The data were expressed as MPE, with correction for the contribution of isotopomers of small weight to the apparent enrichment of isotopomers with a greater mass.[1]

Calculations

All kinetic data were determined under steady-state conditions for substrate concentrations and tracer MPEs and SAs, attained in the final 30 minutes of the basal period and the final 80 minutes of the meal administration (Fig 1). The rate of appearance (Ra) in plasma of unlabeled leucine and KIC was calculated by conventional formulas:

$$\text{Leu Ra} = \text{r}[^2H_3]\text{-Leu}/([^2H_3]\text{-Leu MPE} \times 0.01) - \text{r}[^2H_3]\text{-Leu}, \quad (1)$$

$$\text{KIC Ra} = \text{r}[^3\text{H}]\text{-KIC}/[^3\text{H}]\text{-KIC SA}. \quad (2)$$

r[$^2H_3$]-Leu and r[^{3}H]-KIC define infusion rates of the intravenous tracers. Dilution equations of intravenous tracers were also used to calculate the systemic Ra of the orally administered [^{13}C]-Leu and the [^{13}C]-KIC and [$^2H_3$]-KIC derived from intracellular deamination of the corresponding leucine tracers. Thus, in the following equations, [$^2H_3$]-Leu and [^{3}H]-KIC behaved as tracers, whereas [^{13}C]-Leu, [^{13}C]-KIC, and [$^2H_3$]-KIC behaved as tracees:

$$[^{13}\text{C}]\text{-Leu Ra} = \text{r}[^2H_3]\text{-Leu}/([^2H_3]\text{-Leu MPE}/[^{13}\text{C}]\text{-Leu MPE}), \quad (3)$$

$$[^2H_3]\text{-KIC Ra} = \text{r}[^3\text{H}]\text{-KIC}/([^3\text{H}]\text{-KIC SA}/[^2H_3]\text{-KIC MPE}), \quad (4)$$

$$[^{13}\text{C}]\text{-KIC Ra} = \text{r}[^3\text{H}]\text{-KIC}/([^3\text{H}]\text{-KIC SA}/[^{13}\text{C}]\text{-KIC MPE}). \quad (5)$$

[^{13}C]-Leu Ra represents the portion of ingested [^{13}C]-Leu escaping first-pass splanchnic uptake and appearing in the systemic circulation. First-pass splanchnic uptake of oral [^{13}C]-Leu is therefore calculated from equation 3 and the rate of oral administration of [^{13}C]-Leu (r[^{13}C]-Leu) as follows:

$$\text{First-pass splanchnic uptake of oral } [^{13}\text{C}]\text{-Leu} = \text{r}[^{13}\text{C}]\text{-Leu} - [^{13}\text{C}]\text{-Leu Ra}. \quad (6)$$

[$^2H_3$]-KIC Ra (Eq 4) is the portion of intravenously infused

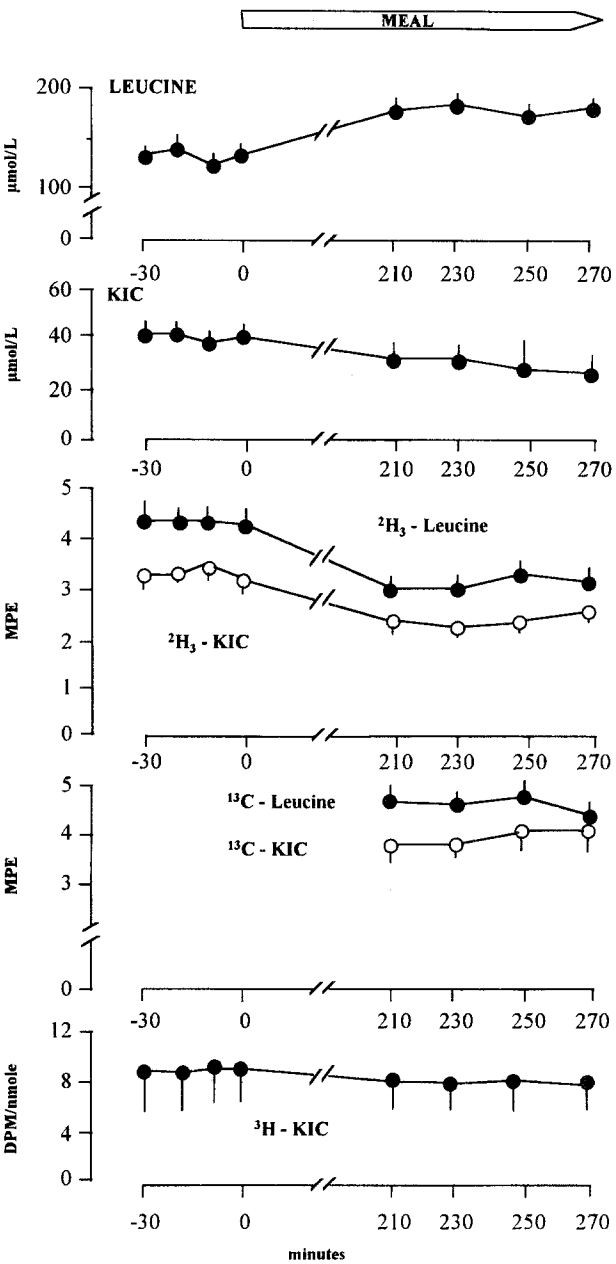

Fig 1. Plasma leucine and KIC concentrations and MPEs and SAs in the basal state and during meal absorption.

[$^2H_3$]-Leu that is deaminated and released as [$^2H_3$]-KIC at the whole-body level. Thus, the fraction of whole-body leucine disposal that is deaminated intracellularly and released as KIC is defined as follows:

$$\text{Fractional whole-body KIC production from leucine} = [^2H_3]\text{-KIC Ra}/\text{r}[^2H_3]\text{-Leu}. \quad (7)$$

[^{13}C]-KIC Ra (Eq 5) derives from intracellular deamination of ingested [^{13}C]-Leu. [^{13}C]-KIC Ra is the sum of two different components: (1) first-pass splanchnic KIC production from deamination of oral [^{13}C]-Leu taken up by the splanchnic organs, and (2) whole-body KIC production from deamination of [^{13}C]-Leu appearing in the systemic circulation. Since [^{13}C]-Leu appearing in the systemic circulation will be intracellularly deaminated to produce [^{13}C]-KIC at the same

extent of the intravenously infused ^{2}H-Leu, whole-body KIC production from deamination of [^{13}C]-Leu appearing in the systemic circulation can be calculated from equations 3 and 7 as follows:

Whole-body KIC production from [^{13}C]-Leu

$$= \text{fractional whole-body KIC production from leucine} \times [^{13}\text{C}]\text{-Leu Ra.} \quad (8)$$

First-pass splanchnic KIC production from deamination of oral [^{13}C]-Leu taken up by the splanchnic organs is then derived from equations 5 and 8 as follows:

First-pass splanchnic KIC production from oral[^{13}C]-Leu

$$= [^{13}\text{C}]\text{-KIC Ra} - \text{whole-body KIC production from oral } [^{13}\text{C}]\text{-Leu.} \quad (9)$$

Thus, fractional splanchnic KIC production from deamination of oral labeled leucine taken up by splanchnic organs at first pass can be calculated as follows:

Fractional splanchnic KIC production from oral Leu

$$= \text{first-pass splanchnic KIC production from oral } [^{13}\text{C}]\text{-leu first-pass splanchnic uptake of oral}[^{13}\text{C}]\text{-Leu.} \quad (10)$$

This figure represents the fraction of the initial splanchnic uptake of dietary leucine that is deaminated and released as KIC from splanchnic organs.

Since the unlabeled leucine administered orally has the same fate as the oral leucine tracer, the rates of first-pass splanchnic uptake of dietary leucine, first-pass splanchnic deamination to KIC, and systemic appearance (systemic Ra of oral Leu) of oral unlabeled leucine can be calculated as follows:

first-pass splanchnic uptake of oral Leu

$$= \text{first-pass splanchnic uptake of oral } [^{13}\text{C}]\text{-Leu/meal}[^{13}\text{C}]\text{-Leu MPE} \times 100, \quad (11)$$

first-pass splanchnic KIC production from oral Leu

$$= \text{first-pass splanchnic KIC production from oral } [^{13}\text{C}]\text{ Leu/meal } [^{13}\text{C}]\text{-Leu MPE} \times 100, \quad (12)$$

and systemic Ra of oral Leu

$$= ([^{13}\text{C}]\text{-Leu Ra/meal } [^{13}\text{C}]\text{-Leu MPE}) \times 100. \quad (13)$$

All data are expressed as the mean ± SE. The two-tailed Student's *t* test for paired data was used for statistical comparisons.

RESULTS

Plasma leucine and KIC concentrations and tracer MPEs and SAs were at steady state in the final 30 minutes of the basal period and the final 80 minutes of the meal administration (Fig 1). Table 1 shows the mean steady-state plasma leucine and KIC concentrations and tracer MPEs and SAs in the postabsorptive state and during meal administration. As expected, plasma leucine increased during meal absorption. Plasma [$^{2}H_3$]-Leu and [$^{2}H_3$]-KIC MPEs and [^{3}H]-KIC SA were lower during the meal than in the postabsorptive state, because of dilution by unlabeled dietary leucine and by the KIC derived from deamination of the ingested leucine of the intravenous tracers. During meal absorption, the ratio between [^{13}C]-KIC and [^{13}C]-Leu (0.88 ± 0.02) was greater ($P < .05$) than the ratio between [$^{2}H_3$]-KIC and [$^{2}H_3$]-Leu (0.76 ± 0.02), indicating that a portion of the circulating [^{13}C]-KIC was derived from first-pass transamination of oral [^{13}C]-Leu within the splanchnic organs. In the basal postabsorptive state, values for leucine and KIC Ra were 1.24 ± 0.02 and 0.75 ± 0.05 μmol/kg/min, respectively. These values increased during meal ingestion ($P < .05$) to 1.69 ± 0.05 and 0.89 ± 0.08 μmol/kg/min, respectively. In Table 2, the calculated parameters of dietary leucine kinetics are reported. During meal absorption, 26% ± 5% of orally administered leucine was taken up by the splanchnic organs at first pass; 13% ± 2% of leucine splanchnic uptake was deaminated and released into the systemic circulation as KIC (here defined as fractional splanchnic KIC production from oral leucine, Eq 10). Thus, total systemic availability of dietary leucine accounted for 77% ± 5% of the oral administration rate of leucine, ie, 74% ± 5% as the systemic Ra of oral leucine, and 3% ± 0.2% first-pass KIC production from oral leucine.

Equations 7 and 10 allow for the calculation of the relative ability of the whole body versus the splanchnic tissues in terms of leucine deamination and release as KIC in the fed state. At the whole-body level, KIC appearance from intracellular leucine deamination accounted for 41% ± 3% of leucine disposal during meal absorption (here defined as fractional whole-body KIC production from leucine, Eq 7). In contrast, only 13% ± 2% of the oral leucine taken up by splanchnic tissues was deaminated and released as KIC (here defined as fractional splanchnic fractional KIC production from oral leucine; $P <$ .05, splanchnic *v* whole body). In the basal postabsorptive state,

Table 1. Plasma Leucine and KIC Concentrations, MPEs, and SAs in the Postabsorptive and Fed States

Parameter	Postabsorptive	Fed
Plasma concentration (μmol/L)		
Leucine	136 ± 6	190 ± 14*
KIC	33 ± 4	40 ± 6
Meal enrichment (MPE)		
^{13}C-Leu		11.71 ± 1.36
Plasma enrichment (MPE)		
$^{2}H_3$-Leu	4.40 ± 0.23	3.23 ± 0.24*
$^{2}H_3$-KIC	3.48 ± 0.28	2.45 ± 0.10*
^{13}C-Leu		4.53 ± 0.15
^{13}C-KIC		3.99 ± 0.19
Plasma SA (dpm/nmol)		
^{3}H-KIC	9.58 ± 2.41	8.28 ± 2.20*

NOTE. Values are the mean ± SE.
*$P \leq .05$ *v* postabsorptive state (paired *t* test).

Table 2. Metabolic Fate of Oral Leucine in the Fed States

Parameter	Value
Oral administration rate	1.08 ± 0.01
First-pass splanchnic uptake	0.28 ± 0.05
First-pass splanchnic KIC production from oral leucine	0.03 ± 0.002
Systemic Ra of oral leucine	0.80 ± 0.05

NOTE. Units are μmol/kg/min; values are the mean ± SE.

fractional whole-body KIC production from leucine (43% ± 2%) was similar to value for the fed state (41% ± 3%).

DISCUSSION

During meal ingestion, about one fourth of absorbed leucine is metabolized at first pass in the splanchnic bed by the combined activities of the liver and gut, the remainder being directly released into the systemic circulation.[1-7] The present study was designed to quantify the fraction of dietary leucine taken up by the splanchnic bed that is deaminated and released as KIC into the posthepatic circulation. We found that splanchnic KIC production from oral leucine accounted for 13% of total leucine splanchnic uptake at first pass. This figure represented only 3% of the rate of oral leucine administration. Thus, we conclude that first-pass splanchnic deamination of dietary leucine and release as KIC contribute minimally to the postprandial flux of dietary leucine-carbon from the splanchnic bed to the peripheral tissues.

In a recent study, Matthews et al[7] used the combined oral-intravenous tracer approach to determine splanchnic leucine metabolism in the postabsorptive state. However, in that study, an independent tracer of KIC was not infused. Thus, the rate of leucine conversion to KIC in the splanchnic bed was estimated by using a theoretical value for total KIC Ra. In their study, fractional splanchnic KIC production from leucine in the postabsorptive state was three times greater than the value we measured in the fed state. One reason for this apparent discrepancy could be the different KIC to leucine enrichment ratio of the oral tracer and the value we measured in the fed state (~0.88) as compared with the same ratio measured in the fasting state (~1).[7] However, our ratio was close to the value measured by Hoerr et al[6] in the fed state (~0.94). Another reason could be the slightly different initial splanchnic uptake of oral leucine (~25% in our study; ~21 in study by Matthews et al).

Simultaneous administration of independent tracers of leucine and KIC via systemic and oral routes allowed us to assess the relative ability of splanchnic tissues versus the whole body to deaminate leucine and release it systemically in the form of its ketoanalog, KIC. Our data show that about 13% of dietary leucine disposed of by the splanchnic organs was deaminated and released as KIC into the systemic circulation. In contrast, at the whole-body level, greater than 40% of leucine disposal appeared in the systemic circulation as KIC. Values for fractional KIC production from leucine at the whole-body level and in the splanchnic bed are not directly comparable, because the whole body also includes splanchnic tissues. However, the fact that the ability of the splanchnic tissues to produce KIC from leucine was less than one third of the ability of the total body clearly indicates that in some extrasplanchnic tissues (eg, skeletal muscle, kidneys, or other tissues) the fractional KIC production from leucine must be greater than both the whole-body and the splanchnic value.

The reversible deamination of leucine to KIC is catalyzed by branched-chain amino acid transaminases.[9] KIC can then undergo an irreversible decarboxylation to isovaleric acid (catalyzed by the α-keto acid dehydrogenase) or be released into the plasma space.[8]

Early studies on the distribution of branched-chain transaminases in body tissues[9,10] showed that the total amount of enzyme activity in muscle is higher than in any other tissue. In contrast, the liver had a much higher activity of the α-keto acid dehydrogenase than muscle.[15] We are not aware of any study of similar measurements in human tissues. However, in agreement with these in vitro animal data, the relatively low rate of KIC appearance from leucine in the splanchnic organs shown in our study can be explained by either a low rate of leucine transamination or a high rate of local KIC decarboxylation, or both.

In conclusion, this study provides an example of the usefulness of multiple tracer administration via different routes in the direct assessment of metabolic pathways in vivo.

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