Arterial KIC as marker of liver and muscle intracellular leucine pools in healthy and type 1 diabetic humans

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Barazzoni, R., S. E. Meek, K. Ekberg, J. Wahren, and K. S. Nair. Arterial KIC as marker of liver and muscle intracellular leucine pools in healthy and type 1 diabetic humans. Am. J. Physiol. Endocrinol. Metab. 40: E238–E244, 1999.—In human protein turnover studies with isotopically labeled leucine (Leu) as a tracer, plasma ketoisocaproate (KIC) enrichment is extensively used as a surrogate measure of intracellular leucine enrichment. To test how accurately arterial ketoisocaproate (A-KIC) represents leucine isotopic enrichment in the hepatic (HV) and femoral veins (FV), which drain liver and muscle beds, we measured Leu and KIC enrichments in samples collected from HV, FV, and femoral artery (A) in 24 control and 6 type I diabetic subjects after a primed, continuous infusion of L-[1-13C,15N]-Leu. Studies were performed during insulin deprivation or insulin replacement in the diabetic group, whereas the effect of normal saline or three different doses of insulin infusion (0.25, 0.50, and 1 μU·kg−1·min−1) were assessed in healthy controls. The ratios of baseline isotopic enrichments of A-KIC to HV Leu and FV Leu were 0.93 ± 0.01 and 0.94 ± 0.02, respectively, in normal subjects and 1.07 ± 0.04 and 1.05 ± 0.03, respectively, in diabetic subjects (P < 0.01, diabetic vs. normal subjects). Insulin did not change A-KIC-to-HV Leu ratios in either group, but the A-KIC-to-FV Leu ratio decreased during insulin infusion in normal subjects (P < 0.05). In conclusion, A-KIC represents a reliable surrogate measure of HV Leu enrichment at different levels of circulating insulin in humans. The present data support the use of A-KIC as a surrogate precursor pool for hepatic protein synthesis.

ketoisocaproic acid; protein synthesis; precursor pool

IN AMINO ACID TRACER studies of regional protein turnover and specific protein synthesis rates, amino acid enrichment should be accurately measured in the free pool into which it appears from protein breakdown as well as in the aminocarb-15N pool, i.e., the obligatory precursor for protein synthesis (21). Because intracellular pools are not directly measurable in most studies in humans for both practical and ethical reasons, surrogate measures are commonly employed. In particular, α-ketoisocaproate (KIC) enrichment in arterial or arterialized venous (5) blood samples, a marker of the average whole body leucine intracellular isotopic enrichment (16, 20), has also been extensively utilized as a surrogate precursor in studies of liver protein synthesis (12) and muscle protein turnover in humans (4).

However, under near steady state after primed continuous isotopic infusion, previous animal and human reports (3, 14, 28) have shown that plasma KIC enrichment may differ from tissue fluid leucine, representing the intracellular free pool, and from leucyl-tRNA enrichments in different organs. Moreover, there is considerable evidence of functional heterogeneity of the precursors of protein synthesis in different experimental settings and tissues from in vitro and ex vivo studies (1–3, 8, 10, 13–15, 22, 27, 28). On the whole, these data indicate that the accuracy of arterial KIC as a predictor of intracellular leucine in humans should be specifically confirmed in different tissues and under varying experimental conditions.

Although it may be partly influenced by leucine shunted through the capillary bed, leucine enrichment in the venous drainage from a given organ is expected to be at balance with the intracellular amino acid pool under isotopic steady state. Indeed, deep venous leucine enrichment is directly determined by intracellular leucine. In a swine model, femoral vein leucine was superimposable to tissue fluid leucine enrichment in leg skeletal muscle (3), and tissue fluid turned out to be a good predictor of leucyl-tRNA enrichment in the liver and heart in the same study. From the above observations, deep venous leucine might represent an accurate predictor of intracellular pools in a given organ, but the catheterization of deep veins is also difficult and relatively invasive and for these reasons not routinely feasible in humans.

In the current studies, arterial KIC as well as hepatic and femoral vein leucine isotopic enrichments were analyzed at isotopic near steady state under different experimental conditions to assess the reliability of KIC as an indirect measure of intracellular liver and skeletal muscle leucine pools. In particular, the ratios of arterial KIC to hepatic and femoral vein leucine enrichments, assumed to reflect liver and muscle intracellular pools, were measured in healthy subjects in the basal state and after different rates of insulin administration, as well as in type 1 diabetic patients during insulin deprivation and insulin treatment. The relationships between KIC and leucine enrichments within each sampling site were also analyzed and discussed in the present study.

METHODS

Materials. L-[1-13C,15N]leucine (99 atom percent excess) was purchased from Cambridge Isotope Laboratories (Woburn, MA). The tracer was tested for chemical, isotopic, and optical purity and was shown to be sterile and pyrogen free before use.

The current results are part of a larger protocol aimed at investigating protein turnover and its regulation by insulin in the splanchnic and skeletal muscle tissues in healthy and
diabetic subjects (17, 18). The experimental protocols were submitted to and approved by the Institutional Review Boards of Mayo Clinic and Foundation and Karolinska Hospital. The nature, purpose, and potential risks of the study were explained in detail to each participant, who gave his or her written informed consent. Part of the study data, pertaining to insulin effects on whole body and regional protein dynamics, including leucine transamination to KIC, has been published elsewhere (17, 18).

Subjects. Twenty-four healthy adults (12 males and 12 females, aged 21–38 yr, body mass index 18–27 kg/m²) and six male type 1 diabetic patients (aged 25–35 yr, body mass index 20–24 kg/m²) were studied. Except for insulin in the patients, no other medications were administered to any of the participants at the time of the study. The insulin regimen included four daily insulin injections, with regular insulin before meals and intermediate acting insulin at bedtime. Insulin requirement was <70 U/day in all patients. Duration of diabetes was 18.3 ± 2.4 yr. Four patients had background retinopathy, and one had microalbuminuria; no other diabetic complications were detected.

Protocol. The studies were performed after the overnight fast. In all subjects, catheter sheaths were inserted in the right femoral artery and vein and kept patent by a slow saline infusion. Hepatic vein catheters were inserted under fluoroscopic guidance and placed as previously reported (25), and a femoral artery catheter was placed through the arterial sheath. An antecubital vein was also cannulated and used for primed (1 mg/kg), continuous (1 mg·kg⁻¹·h⁻¹) infusion of L-[1-¹³C,¹⁵N]leucine.

In healthy volunteers, blood samples were collected from right femoral artery and femoral and hepatic veins before the start of isotope infusion and at 10-min intervals between 120–150 and 270–300 min. From 150 min, each participant was randomly assigned to receive a peripheral constant intravenous infusion of either saline or insulin at 0.25, 0.5, or 1 mU·kg⁻¹·min⁻¹ (n = 6 subjects/group). When insulin was infused, baseline blood glucose level was maintained in each subject by means of 20% dextrose intravenous infusion (7).

Type 1 diabetic patients were studied twice on two separate occasions (insulin deprivation and insulin treatment) at 2- to 3-wk intervals. On both occasions, patients were hospitalized on the day before the study and subcutaneous insulin treatment was withdrawn after the evening meal at 6:00 PM. At 9:00 PM, a peripheral intravenous infusion of either saline or insulin (insulin deprivation) or insulin to maintain a blood glucose level of 4–6 mmol/l (insulin treatment) was started. Ingestion of water was allowed. In the insulin treatment study, from 7:00 AM and until the end of the study, the insulin-infusion rate was adjusted on the basis of blood glucose monitoring every 10–15 min to clamp glycemia at 4.4 ± 0.4 mmol/l. In both studies, blood samples were obtained from femoral artery and vein, as well as from hepatic vein catheters, before the start of the isotopic infusion and at 170, 175, 180, 200, 205, 210, 230, 235, and 240 min, when the study was stopped.

Analytical methods. The plasma molar percent excess (MPE) of [¹³C]leucine and [¹³C]KIC were determined by gas chromatography-mass spectrometry. Ion fragments for leucine analysis were detected as tert-butylidimethylsilyl derivatives at mass-to-charge ratio (m/z) 303/302. The correspondent quinoxalinol-Trimethylsilyl derivatives for KIC analysis were detected at m/z 232/232 (18).

Blood glucose was measured with a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin concentration was measured by standard RIA. Plasma amino acid levels were determined by reverse-phase HPLC (11).

Calculations. Isotopic plateau, defined as the slopes of enrichments of plasma [¹³C]leucine and [¹³C]KIC plotted against time not being different from zero, was observed at all sampling sites in the last 30 min of the baseline and infusion periods in healthy subjects, as well as from 170 to 240 min in both studies in diabetic patients. Mean values of all samples within each experimental period were used for calculations.

The ratios of arterial KIC to hepatic and femoral vein leucine MPE were determined, as well as those of KIC to leucine MPE within each sampling site.

Statistical analysis. All values are reported as means ± SE. Student’s t-test for paired data was used to compare MPE and the MPE ratios from baseline vs. insulin-infusion periods at each dose in healthy subjects and from the insulin deprivation vs. the insulin treatment studies in type 1 diabetic subjects. Basal data from normal subjects (n = 24) were pooled for unpaired comparison with data from the insulin deprivation and insulin treatment studies in diabetic patients. An unpaired t-test was also used to compare MPE and MPE ratios in different sampling sites within groups. Linear regression analysis was performed to describe the relation.

### Table 1. [¹³C]LEU and [¹³C]KIC isotopic enrichments (MPE) in femoral artery and HV and FV under insulin deprivation and inulin treatment

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Infusion</th>
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<th>Basal</th>
<th>Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>A KIC MPE</td>
<td>5.29 ± 0.20</td>
<td>5.56 ± 0.18</td>
<td>5.42 ± 0.24</td>
<td>5.92 ± 0.11</td>
<td>5.47 ± 0.19</td>
<td>6.29 ± 0.21</td>
</tr>
<tr>
<td>HV LEU MPE</td>
<td>5.71 ± 0.27</td>
<td>5.95 ± 0.27</td>
<td>6.13 ± 0.14</td>
<td>6.77 ± 0.10</td>
<td>5.70 ± 0.27</td>
<td>6.84 ± 0.24</td>
</tr>
<tr>
<td>HV KIC MPE</td>
<td>5.51 ± 0.22</td>
<td>5.73 ± 0.19</td>
<td>5.68 ± 0.17</td>
<td>5.98 ± 0.35</td>
<td>5.54 ± 0.23</td>
<td>6.41 ± 0.21</td>
</tr>
<tr>
<td>FV LEU MPE</td>
<td>4.60 ± 0.29</td>
<td>5.93 ± 0.21</td>
<td>5.95 ± 0.14</td>
<td>6.58 ± 0.11</td>
<td>5.72 ± 0.20</td>
<td>7.14 ± 0.28*</td>
</tr>
<tr>
<td>FV KIC MPE</td>
<td>4.92 ± 0.30</td>
<td>5.19 ± 0.25</td>
<td>4.94 ± 0.12</td>
<td>5.39 ± 0.10</td>
<td>5.15 ± 0.18</td>
<td>5.75 ± 0.20</td>
</tr>
</tbody>
</table>

Values are means ± SE. LEU, leucine; KIC, ketoisocaproate; MPE, Molar percent excess; A, artery; HV, hepatic vein; FV, femoral vein; INS, insulin. *P < 0.05 vs. infusion INS 0.25; †P < 0.05 vs. infusion INS 0.50 FV KIC; all infusion values are higher (P < 0.05) than corresponding basal values.

### Table 2. [¹³C]LEU and [¹³C]KIC isotopic enrichments (MPE) in femoral artery and HV and FV under insulin deprivation and insulin treatment in type 1 diabetic subjects

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Infusion</th>
<th>Basal</th>
<th>Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>A KIC MPE</td>
<td>4.66 ± 0.25</td>
<td>6.05 ± 0.14</td>
<td>4.37 ± 0.24</td>
<td>6.17 ± 0.14</td>
</tr>
<tr>
<td>HV LEU MPE</td>
<td>4.68 ± 0.24</td>
<td>6.39 ± 0.19*</td>
<td>4.44 ± 0.18</td>
<td>5.94 ± 0.14</td>
</tr>
<tr>
<td>HV KIC MPE</td>
<td>4.53 ± 0.20</td>
<td>6.60 ± 0.21</td>
<td>4.53 ± 0.20</td>
<td>6.60 ± 0.21</td>
</tr>
</tbody>
</table>

Values are means ± SE. I−, insulin treatment; I+, insulin deprivation. *P < 0.05 vs. I+ FV KIC; all I+ values are higher (P < 0.05) than the corresponding I− values.
ship between arterial KIC and hepatic vein leucine MPE. P values < 0.05 were considered statistically significant.

RESULTS

Plasma insulin concentration in the different study groups rose in keeping with the exogenous infusion rates, and circulating amino acid levels were reduced in a dose-dependent fashion (17, 18). Pooled plasma insulin concentrations in healthy subjects in the basal period were comparable with those in insulin-treated diabetic patients (7 ± 2 vs. 12 ± 2 µU/ml, respectively).

Arterial [13C]KIC and both hepatic and femoral vein [13C]leucine and [13C]KIC MPEs significantly increased during saline or insulin infusion in normal subjects (Table 1), and they were higher during insulin treatment than during insulin deprivation in diabetic patients (Table 2). The femoral vein leucine MPE significantly increased at the two higher insulin-infusion rates compared with the saline or low insulin dose values in normal subjects (Table 1). [13C]KIC MPE was significantly higher in both the artery and the hepatic vein than in the femoral vein in normal subjects in the basal period (n = 24; right femoral artery = 5.39 ± 0.09, hepatic vein = 5.58 ± 0.09, and femoral vein = 5.01 ± 0.09, P < 0.01 vs. right femoral artery and hepatic vein) as well as during the intermediate insulin dose infusion (Table 1). In diabetic patients, hepatic vein [13C]KIC MPE was higher than the femoral vein value in the insulin-treated state but not in the insulin-deprived state (Table 2).

The ratios of arterial KIC to hepatic and femoral vein leucine MPEs were not statistically different from 1.0 in the basal state in controls (0.93 ± 0.01 and 0.94 ± 0.02; Fig. 1), as well as in the insulin-deprivation study in diabetic patients (1.07 ± 0.04 and 1.05 ± 0.03, P ≥ 0.01 vs. normal subjects; Fig. 2). Insulin infusion at different rates in both groups did not significantly alter the arterial KIC-to-hepatic vein leucine ratio, whereas the arterial KIC-to-femoral vein leucine ratio was reduced by 8–10% (P < 0.05) at the higher insulin-infusion rates in healthy subjects (Fig. 1). Arterial KIC and hepatic vein leucine MPEs were significantly and positively related in both groups; the corresponding linear regression analysis is shown in Fig. 3.

The KIC-to-leucine MPE ratio was significantly lower in right femoral artery than in both hepatic vein and femoral vein in all experimental settings (data not shown). In normal subjects, the same ratio was higher in the hepatic than in the femoral vein in the basal as well as during the infusion periods, except for the lowest insulin dose (Table 3; Fig. 4). In the patients, the KIC-to-leucine ratio was significantly higher in the hepatic than in the femoral vein during insulin treatment but not during insulin deprivation (Table 3). All KIC-to-leucine MPE ratios within each sampling site were significantly higher in insulin-deprived diabetic patients compared with nondiabetic subjects under basal conditions (Fig. 5). During insulin replacement, the arterial KIC-to-leucine ratio was significantly reduced and close to normal values in the patients (Fig. 5), whereas the same ratio tended to remain higher than in nondiabetic subjects in the hepatic and femoral veins (Fig. 5).

DISCUSSION

Reliable surrogate measures of leucine intracellular enrichment are needed for isotopic studies involving the measurement of regional protein turnover and of the synthesis rates of specific proteins in liver and muscle. In the current study, we evaluated the reliability of arterial KIC as a predictor of leucine enrichment in the hepatic and femoral veins, assumed to be at
balance with liver and muscle intracellular leucine enrichment, in both healthy and type 1 diabetic people at different levels of circulating insulin. Under the near steady-state conditions of the present study, arterial KIC was close to both hepatic and femoral vein leucine MPE. After insulin infusion, only the arterial KIC-to-hepatic vein leucine ratio remained stable in both groups, whereas the ratio of arterial KIC to femoral vein leucine significantly decreased at higher insulin-infusion rates in healthy subjects.

The stability of the ratio of arterial KIC to hepatic venous leucine enrichment at different insulin levels suggests that the former is a reliable predictor of leucine enrichment in the hepatic vein and, indirectly, in the hepatic intracellular pool in humans. Furthermore, unlike in muscle (14), in vivo studies indicate that the free and tRNA-bound liver leucine pools have similar isotopic enrichments after continuous isotopic infusion (3), probably due to the faster hepatic protein turnover (26). Venous-arterialized KIC enrichment was previously reported to be similar to apolipoprotein B100-bound leucine, another potential marker of the hepatic precursor pool of protein synthesis, under different experimental conditions, i.e., in healthy subjects after meal absorption (24). The current findings are in agreement with the quoted study in supporting the use of arterial KIC as surrogate measure of the precursor pool for hepatic protein synthesis under different experimental conditions, with particular regard to varying circulating insulin levels.

In diabetic patients during insulin deprivation, the ratio of arterial KIC to hepatic vein leucine was significantly higher (by ~15%) than in nondiabetic subjects in the basal state. The changes in whole body as well as splanchnic and muscle protein turnover during insulin deprivation (18, 19) are likely to account for this difference. In particular, increased muscle and splanchnic protein breakdown leads to higher entry of unlabelled leucine into the free leucine pool, whereas higher leucine transamination increases labeled KIC (18). Both these alterations may contribute to increase KIC-to-leucine ratios as observed in the current analysis. Because insulin replacement did not change this ratio in the patients, the current results support the use of arterial KIC enrichment as a surrogate measure of hepatic venous-intracellular leucine enrichment in studies investigating the effect of insulin deprivation on liver protein metabolism (6). At the same time, the current data suggest that caution should be exerted when arterial KIC is employed as precursor pool in comparing the synthesis rates of liver proteins in diabetic and nondiabetic subjects, in particular when relatively small differences are expected or detected among groups.

The significant decrease of the arterial KIC-to-femoral vein leucine ratio at higher insulin levels in normal subjects indicates that KIC is not a reliable

Table 3. Ratios of $^{13}$C Leu and $^{13}$C KIC MPEs in HV and FV in normal subjects under basal conditions and different rates of insulin infusion as well as in type 1 diabetic subjects under insulin deprivation and insulin treatment

<table>
<thead>
<tr>
<th></th>
<th>HV</th>
<th>FV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.96±0.01*</td>
<td>0.88±0.02</td>
</tr>
<tr>
<td>Saline</td>
<td>0.97±0.03*</td>
<td>0.83±0.03</td>
</tr>
<tr>
<td>INS (0.25)</td>
<td>0.89±0.06</td>
<td>0.82±0.02</td>
</tr>
<tr>
<td>INS (0.50)</td>
<td>0.94±0.03*</td>
<td>0.81±0.02</td>
</tr>
<tr>
<td>INS (1.00)</td>
<td>0.96±0.03*</td>
<td>0.82±0.03</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
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</tr>
<tr>
<td>I–</td>
<td>1.08±0.02</td>
<td>1.02±0.04</td>
</tr>
<tr>
<td>I+</td>
<td>1.04±0.04*</td>
<td>0.94±0.03</td>
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Values are means ± SE; n = 24. *P < 0.05 vs. FV; I– values are higher (P < 0.01) than normal basal values.
indicator of muscle intracellular leucine pools after insulin-induced modifications of protein turnover. Indeed, insulin inhibits muscle protein breakdown (10, 18, 23), thus decreasing isotope dilution and resulting in higher tissue leucine enrichment. In fact, femoral vein leucine enrichment significantly increased during the two higher doses of insulin in comparison with both saline or low-dose insulin in normal subjects (Table 1). In diabetic patients, the ratio of arterial KIC to femoral vein leucine MPEs was not modified by insulin treatment compared with the insulin-deprived period. However, it is likely that higher insulin doses would have further increased femoral vein leucine MPE and changed its ratio to arterial KIC. These observations suggest that the use of arterial KIC as a surrogate measure of the precursor pool in studies of muscle protein turnover and synthesis has limitations. Although it may lead to underestimations of the absolute synthesis rates of muscle proteins (14), plasma KIC enrichment is acceptable for cross-sectional comparisons in studies performed under basal postabsorptive conditions. On the other hand, the current analysis indicates that its use may result in erroneous measurements of muscle leucine turnover rates and therefore affect the study conclusions after changes in circulating insulin levels. It is therefore important to resort to alternative markers of intracellular leucine pools for most experimental settings involving manipulations of hormone concentrations.

**Fig. 5.** Percent ratios of [13C]KIC to [13C]leucine MPEs in the femoral artery and hepatic and femoral veins in insulin-deprived and insulin-treated diabetics and in normal subjects under basal conditions (n = 24). #P < 0.001 vs. I- and normal subjects; *P < 0.01 vs. normal subjects.
This study also provides an opportunity to compare the ratios of KIC to leucine isotopic enrichments in different sampling sites. In healthy subjects, arterial KIC was ≈75% of leucine arterial MPE, reflecting average whole body intracellular leucine tracer dilution, in agreement with previous findings (16, 20). Increasing insulin doses tended to reduce KIC-to-leucine ratios in the femoral artery and vein, probably because of a suppression of whole body and muscle proteolysis with subsequent increments of leucine MPE (9, 23). The KIC-to-leucine ratio was higher in the hepatic than in the femoral vein in most experimental settings (Table 3). In nondiabetic subjects, this difference was mainly accounted for by higher hepatic vein KIC enrichments under basal conditions (RESULTS and Table 1). During insulin infusions, the same difference was mainly related to smaller increments of hepatic vein leucine enrichment (Table 1), in keeping with a limited effect of the hormone on splanchnic protein breakdown (17). Overall, the consistently similar hepatic vein KIC and leucine enrichments suggest that intracellular leucine pools are more homogeneous in the liver than in other tissue beds, including skeletal muscle (14).

In insulin-deprived diabetic patients, all ratios within each sampling site were significantly higher than in normal subjects under basal conditions. Insulin treatment tended to reduce these differences, although the hepatic vein KIC-to-leucine MPE ratio remained significantly higher. As a possible explanation, the peripheral route of insulin administration might have prevented the complete normalization of portal insulin levels and of splanchnic leucine kinetics.

In conclusion, arterial KIC enrichment is close to that of leucine in the hepatic and femoral veins under basal physiological conditions in healthy subjects and in people with type 1 diabetes. High insulin concentrations do not affect the KIC-to-hepatic vein leucine ratio in either group, but the KIC-to-femoral vein leucine ratio is reduced at higher insulin doses in normal subjects. These studies indicate that arterial KIC is a reliable predictor of hepatic vein leucine enrichment in humans at different levels of plasma insulin concentration. The use of plasma KIC as surrogate precursor pool for hepatic protein synthesis and turnover is supported by these results.

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