Twenty-four–hour intravenous and oral tracer studies with L-[1-13C]-2-aminoadipic acid and L-[1-13C]lysine as tracers at generous nitrogen and lysine intakes in healthy adults1–3

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ABSTRACT
Background: This is a continuation of investigations of the relations between amino acid kinetics and amino acid dietary requirements in healthy adults.

Objective: The aim was to investigate the 24-h pattern and rate of the metabolism of an L-[1-13C]-2-aminoadipic acid ([13C]AAA) tracer and of whole-body L-[1-13C]lysine ([13C]lysine) oxidation and balance in healthy, young adults receiving a generous intake of lysine.

Design: Thirteen healthy adults were given an adequate, l-aminoc acid–based diet supplying 77 mg lysine · kg−1 · d−1 for 6 d before the tracer studies. Two subjects received [13C]AAA intravenously and 2 received it orally; 3 subjects received [13C]lysine intravenously and 6 received it orally. We measured 13CO2 output, plasma [13C]AAA and [13C]lysine enrichment, and urinary [13C]AAA.

Results: [13C]AAA oxidation was estimated to be higher after the orally administered than after the intravenously administered tracer; plasma [13C]AAA was similar to urinary [13C]AAA. Whole-body lysine oxidation showed a rhythm that was induced by meal feeding. The intravenous [13C]lysine tracer gave mean estimates of lysine balances (lysine intake minus oxidation) that apparently were too low (−15.7 mg · kg−1 · d−1) or too high (16.6 mg · kg−1 · d−1, P < 0.05 from zero balance) on the basis of urinary [13C]AAA or plasma [13C]lysine estimates of oxidation, respectively. For the orally administered tracer and plasma [13C]lysine enrichment, the mean balance was slightly positive (8.7 mg · kg−1 · d−1, P < 0.05 from zero).


KEY WORDS
Tracer balance, lysine oxidation, lysine balance, diurnal pattern, indispensable amino acid requirements, healthy adults, lysine kinetics, aminoadipic acid kinetics

INTRODUCTION
In continuing our investigations of whole-body (plasma) amino acid kinetics and the application of this to a quantitative assessment of the requirements for indispensable amino acids in healthy adults (1–4), we conducted a series of 24-h, tracer-infusion studies to determine directly the daily rates of leucine (5–7) and phenylalanine (8–10) oxidation. Because from a practical or international perspective (11–13) it is important to validate our proposed lysine requirements for adults (2, 3, 14), we also explored the kinetics of whole-body lysine metabolism throughout a continuous 24-h period at defined lysine intakes. Waterlow (15) concluded that although the results of our tracer studies are consistent with an increased requirement for leucine, there is less information about the other indispensable amino acids. An international group also recommended that additional studies be done to determine the indispensable amino acid requirements in both younger and older adults (16).

In previous 24-h tracer studies with [1-13C]leucine, we observed a temporal pattern in the plasma flux and whole-body rate of leucine oxidation that was modulated by the dietary intake of the amino acid and the meal intake pattern (5–7). These experiments with labeled leucine were carried out by using an intravenous infusion of the tracer because we had determined earlier that estimates of the whole-body rate of leucine oxidation were not markedly different according to whether the tracer was administered intravenously or orally (17, 18). Finally, with respect to these 24-h leucine kinetic studies, we found that, at a supramaintenance intake of dietary plus tracer leucine and when the concentration of leucine in the diet was similar to that for mixed body proteins, the measured leucine oxidation rates agreed well with those expected from the net rate of whole-body amino acid catabolism as determined from the measurement of nitrogen excretion (5, 19). This net rate was termed the irreversible protein nitrogen loss (IPNL) and it follows that it may

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be used as a reference value for helping to assess the accuracy of tracer-derived estimates of whole-body oxidation of other indispensable amino acids, such as lysine, when consumed under experimental conditions comparable with those in our earlier studies (5–7). This requires, however, that the ratio of dietary test amino acid to protein (by weight) be similar to that for mixed body proteins (5) and that the test amino acid be at a supramaintenance intake. If these experimental conditions are met, this 24-h index of amino acid catabolism (the IPNL) appears to offer a provisional gold standard for approximating a likely plasma intracellular isotope-enrichment gradient for the labeled amino acid undergoing oxidation.

In this initial exploration of whole-body \(^{13}\)C\l-
lysine oxidation over 24 h, we used both the intravenous and oral routes of tracer administration to also measure the \(^{13}\)C enrichment of aminoadipic acid in urine. This was done as a basis for using this intracellularly derived metabolite of lysine catabolism (20–22) as a possible marker of the abundance of the \(^{13}\)Clysine pool undergoing oxidation and leading to the generation of expired \(^{13}\)CO\(_2\). Hence, we report here our findings on the daily pattern and rate of metabolism of an L-\(^{1-13}\)C-2-aminoadipic acid (\(^{13}\)CAAA) tracer and the rate of whole-body \(^{13}\)Clysine oxidation in subjects receiving a generous lysine intake. Our working hypothesis was that the subjects would be in body lysine equilibrium (neutral lysine balance) or possibly at a somewhat positive balance at this generous intake of lysine, or that the daily rate of lysine oxidation would approximate the total daily lysine intake. The derived estimates of lysine oxidation were then evaluated in relation to measured \(^{13}\)CO\(_2\) excretion data as well as in association with the plasma \(^{13}\)Clysine and urinary \(^{13}\)CAAAA isotopic enrichment values. We also considered the possible effects of nonoxidative lysine losses (23) in the assessment of our findings.

SUBJECTS AND METHODS

Subjects

The subjects were students at the Massachusetts Institute of Technology (MIT) or were from the community of the Boston-Cambridge area. Three male subjects [mean (±SD) body weight, 81.2 ± 8.6 kg; mean height, 180.7 ± 5.5 cm; and mean age, 21 y] were studied with the intravenous \(^{13}\)Clysine protocol and 6 subjects (2 women: body weight, 55 and 58.9 kg; height, 171 and 160 cm; and age, 32 and 22 y; and 4 men: mean body weight, 82.3 ± 8.5 kg; mean height, 181.4 ± 7.8 cm; and mean age, 23 y) were studied with the oral \(^{13}\)Clysine tracer protocol. The subjects were all in good health as determined by medical history, physical examination, analysis for blood cell count, and routine blood biochemical profile and urinalysis. Those who smoked cigarettes, consumed ≥5 alcoholic drinks/wk, or drank > 6 cups of caffeinated beverages/d were excluded from participation. Women of childbearing age were encouraged to volunteer and the tracer experiments were conducted during the 7–10 d after the onset of menstrual bleeding. Women who were taking mild doses of contraceptive agents (such as Orthovonum 7.7.7; Ortho Pharmaceutical, Raritan, NJ) were not excluded from the study. A negative result on a pregnancy test was required 2–3 d before the study started.

The estimated daily energy intake required to maintain body weight, based on an estimate of basal metabolic rate and a diet history, was \(\approx 188 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} (45 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{d}^{-1})\) and so this was given to each of the subjects. On the infusion day the energy intake was reduced to 160 kJ \cdot kg\(^{-1} \cdot d\(^{-1}\) to account for the subjects’ lower physical activity. Subjects were asked to maintain their usual level of physical activity while avoiding excessive or competitive exercise. The purpose of the study and the risks involved were explained to each subject. The subjects signed a consent form and were paid for their participation. The experimental protocol was approved by the MIT Committee on the Use of Humans as Experimental Subjects and the Advisory Committee of the MIT Clinical Research Center. The last phase of each of the studies involved a 24-h stable-isotope-tracer protocol, which required admission of the subjects as inpatients to the MIT medical department.

\(^{13}\)C\l-Lysine tracer studies

Diet and experimental design

Each subject was given a weight-maintaining diet based on an L-amino acid mixture (Ajinomoto USA, Inc, Teaneck, NJ) for 6 d (Table 1). As shown in Table 1, the indispensable amino acid profile was close to that for hen egg protein (24). Protein-free wheat-starch cookies and flavored drinks were given as the major source of energy, exactly as described in detail previously (6). Nonprotein energy was provided in the form of lipid (\(=40%\)) and carbohydrate (\(=60%\)). Beet sugar and wheat starch were the main sources of dietary carbohydrate to maintain a low \(^{13}\)C content in the diet and, therefore, a steady background.

<p>| TABLE 1 Composition of the l-amino acid mixture used to provide a generous lysine intake(^1) |
|---------------------------------|-------|</p>
<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indispensable</strong></td>
<td></td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>15.48</td>
</tr>
<tr>
<td>L-threonine</td>
<td>46.73</td>
</tr>
<tr>
<td>L-isoleucine</td>
<td>62.35</td>
</tr>
<tr>
<td>L-leucine</td>
<td>82.00</td>
</tr>
<tr>
<td>L-lysine-HCl</td>
<td>71.00</td>
</tr>
<tr>
<td>L-methionine</td>
<td>29.45</td>
</tr>
<tr>
<td>L-cystine</td>
<td>21.84</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td>54.24</td>
</tr>
<tr>
<td>L-tyrosine</td>
<td>40.42</td>
</tr>
<tr>
<td>L-valine</td>
<td>69.72</td>
</tr>
<tr>
<td>L-histidine.HCl.H(_2)O</td>
<td>30.43</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>523.66</td>
</tr>
<tr>
<td><strong>Dispensable</strong></td>
<td></td>
</tr>
<tr>
<td>L-arginine.HCl</td>
<td>75.00</td>
</tr>
<tr>
<td>L-alanine</td>
<td>190.00</td>
</tr>
<tr>
<td>L-aspartic acid</td>
<td>11.84</td>
</tr>
<tr>
<td>L-glutamic acid</td>
<td>29.28</td>
</tr>
<tr>
<td>Glycine</td>
<td>44.22</td>
</tr>
<tr>
<td>L-proline</td>
<td>40.00</td>
</tr>
<tr>
<td>L-serine</td>
<td>80.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>994.00(139)²</td>
</tr>
</tbody>
</table>

\(^1\)The mixture was patterned after hen eggs. Additional L-lysine-HCl was added to the mixture to supply \(=14.442 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}\) every day of the study to achieve a total daily intake of lysine of \(77 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}\) except in those meals given during the 24-h infusion of lysine tracer. Each subject received an amino acid intake to provide \(160 \text{ g} \cdot \text{kg} \cdot \text{d}^{-1}\) (ie, \(=1.144 \text{ g} \cdot \text{kg} \cdot \text{d}^{-1}\)).

\(^2\)Amount of nitrogen in parentheses.
enrichment of breath $^{13}$CO$_2$ over the 24-h period (5). Blood and breath $^{13}$CO$_2$ enrichments that were obtained during the lysine-tracer studies were corrected to account for the small changes in background $^{13}$CO$_2$ output that would be expected to occur without the $[^{13}$C]lysine tracer, as described previously (5).

The amino acid mix was given to the subjects to provide a daily nitrogen intake of 160 mg·kg$^{-1}$·d$^{-1}$ and an intake of L-lysine of 77 mg·kg$^{-1}$·d$^{-1}$. On the infusion day the amount of lysine given as tracer was compensated for by an equivalent decrease in lysine in the dietary amino acid mix, so that daily lysine input remained the same throughout the study. Other nutrients were given in sufficient amounts to meet or exceed dietary allowances, as described previously (5). Dietary fiber, as a preservative, measurements of respiratory exchange by indirect calorimetry, breath $^{13}$CO$_2$ background enrichment and $[^{13}$C]bicarbonate recovery were made according to a standardized procedure at 1-h intervals throughout the 24-h period (27).

Breath $^{13}$CO$_2$ background enrichment and $[^{13}$C]bicarbonate recovery

These studies were performed earlier (5) under essentially the same dietary conditions as used in the present investigation and in subjects similar to those studied here. As described below, the calculations of oxidation took into account changes in $^{13}$CO$_2$ background as well as the bicarbonate recovery factor for the present experimental conditions. As described previously (5), the...

$^{13}$CO$_2$, as the monohydrochloride, was obtained from Cambridge Isotope Laboratories (Andover, MA) and from MassTrace, Inc (Woburn, MA). The tracer was administered at a known rate of $\approx 3.1$ μmol·kg$^{-1}$·h$^{-1}$; the prime dose was $\approx 4.65$ μmol/kg. Hence, the total amount of lysine given as tracer over the entire 24-h study was $\approx 11.5$ mg·kg$^{-1}$·d$^{-1}$. The bicarbonate pool was primed with 0.8 μmol $[^{13}$C]NaHCO$_3$/L (99 atom%; Cambridge Isotope Laboratories). The oral tracer, prepared in water, was given each hour and the dose was taken in a volume of 8 mL. The intravenous tracer was prepared in sterile physiologic saline (infusion rate: 8 mL/h). Blood samples (4 mL per sample) were drawn through a 20-gauge, 3.2-cm catheter inserted into a superficial vein of the dorsal hand or the wrist on the nondominant side. The catheter was introduced in an antiflow position to facilitate blood drawing; the hand was placed into a custom-made warming box, maintained at 68°C, for 15 min before the withdrawal of each sample to achieve arterialization of venous blood. The patency of the sampling catheter was maintained by slow infusion of normal saline.

Indirect calorimetry

Total VCO$_2$ and oxygen consumption ($\dot{V}O_2$) rates were determined by using the indirect calorimeter (Deltatrac; SensorMedics, Anaheim, CA) with a ventilated-hood system. Measurements were made according to a standardized procedure at 1-h intervals throughout the 24-h period (27).

The $[^{13}$C]lysine tracer study was terminated at 1800 (day 7).

Throughout the 24-h study the subjects remained in bed in a reclined position except during sleep, when they were supine. The 24 h was divided into two 12-h metabolic periods: a 12-h fasting and a 12-h feeding period. This design helped to maintain the subject’s usual sleeping patterns and also inserted the feeding period into the second half of the 24-h study. Our purpose was to minimize the potential for recycling of the tracer (ie, its return to plasma via protein proteolysis), because the breakdown of whole-body protein increases with fasting and decreases with feeding (25, 26). Nevertheless, the recycling of the lysine tracer via protein breakdown should not markedly affect the precision of the estimation of oxidation because it is based on measurement of the ratio of $^{13}$CO$_2$ production to the isotopic enrichment of lysine in the precursor pool. This was shown in our previous leucine tracer studies (7).

The design of the 24-h $[^{13}$C]lysine tracer studies is shown in Figure 1. On the basis of comparisons made earlier (5), blood (and not breath) $^{13}$CO$_2$ enrichments were measured between 0000 and 0600 to avoid disturbing the subjects during sleep. The protocol included the collection of consecutive, complete 3-h urine samples (in 1-L plastic bottles containing 15 mL of 6 mol HCl/L as a preservative), measurements of respiratory exchange by indirect calorimetry at 1-h intervals for the measurement of carbon dioxide production (VCO$_2$) by using a ventilated-hood system, and consecutive drawing of blood and breath samples every 30 min.

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changes in $^{13}$CO$_2$ background over 24 h were not expected to exceed 1.5 atom percent excess (APE) × 1000. Bicarbonate recovery factors were taken to be 0.77 for the 12-h fasting phase and 0.85 for the 12-h feeding phase of the experiment (5).

Collection of samples

Breath samples were collected every 30 min after the collection of 3 baseline samples at $-30$, $-15$, and $-5$ min as described previously (5). Blood samples for $^{13}$CO$_2$ were collected at 30-min intervals between 0000 and 0600. Three baseline samples at $-30$, $-15$, and $-5$ min were taken before the lysine tracer was administered. Two milliliters of blood was used for $^{13}$CO$_2$ analysis. Blood samples were injected immediately via a thin needle ($0.5 \times 16$ mm) into a 15-mL capped evacuated tube coated with sodium heparin. Samples were then processed as described previously (5). Similarly, at 30-min intervals throughout the 24-h study period and at $-30$, $-15$, and $-5$ min before the tracer was given, 4-mL blood samples (for analysis of lysine isotopic abundance and lysine concentrations) were drawn into heparin-treated tubes and centrifuged for 15 min at 1200 × g in a refrigerated centrifuge (4°C). Plasma was stored at $-20^\circ$C until analyzed.

Twenty-four-hour urine output was measured daily for 5 d to help the subjects familiarize themselves with the urine collection procedure. During the 24-h tracer phase, consecutive, complete 3-h urine collections were made after a baseline sample was collected (0800–1800). The baseline sample was used to assess background $^{13}$C enrichment in urinary aminoadipic acid. The urine collection bottles contained 15 mL of 6 mol HCl/L as preservative.

$[^{13}$C$]$Aminoadipic acid tracer studies

The details presented for the $[^{13}$C$]$lysine tracer studies (including the diet) also apply to the investigation of $[^{13}$C$]$AAA flux and oxidation; 4 different healthy, young adult male subjects participated in these investigations. At 1800 on day 6, 2 of these subjects received the tracer intravenously and the 2 other subjects were given the tracer orally. An unprimed, constant administration of the $[^{13}$C$]$AAA tracer (99 atom%; MassTrace, Inc) was given to both pairs of subjects at a rate of 0.7 μmol·kg$^{-1}$·h$^{-1}$. The rate of aminoadipic acid infusion was chosen on the assumption that the plasma flux of this amino acid might reflect the whole-body rate of conversion of lysine to α-aminoadipic acid via the enzymatic action of lysine-2-oxoglutarate reductase and saccharopine dehydrogenase (20). Hence, the plasma $[^{13}$C$]$AAA enrichment was expected to approach an APE of ≈10 for the anticipated rate of lysine oxidation. However, as described below, our findings revealed a different picture of $[^{13}$C$]$AAA kinetics from what we had anticipated.

The total carbon dioxide content of each tube (15 mL non-silicon-coated glass tubes) was ≈20 μmol. The samples were stored at room temperature until analyzed by method ratio mass spectrometry (MAT Delta E; Finnigan, Bremen, Germany).

The method for the isolation and analysis of urinary $[^{13}$C$]$AAA was a modification of that described by Arends and Bier (22). A set of screening columns (Whale Scientific Co, Commerce City, CO) was rinsed with 10 mL water. 2-Aminoadipic acid was isolated by adding 1 mL of a cation-exchange resin slurry (50 W-X8 hydrogen form, 100–200 mesh cation-exchange resin; BioRad AG, Hercules, CA) to the column. The slurry was washed free of acid with 20 mL water. Duplicate samples of 2 mL urine in 12 × 75 mm borosilicate glass test tubes were centrifuged for 5 min at 1500 × g at 4°C. The supernate was decanted onto the column and the eluant was discarded. To remove the excess acid in urine, the column was rinsed with 10 mL water and again the eluant was discarded. Aminoadipic acid was eluted with 1 mL of 3 mol NH$_4$OH/L followed by 1 mL water and the eluant was collected in a vial (15 × 45 mm, 4 mL capacity). The samples were dried at 80°C under a stream of nitrogen. Some of the urine samples were initially hydrolyzed at 110°C for 18 h in 6 mol HCl/L before being esterified. The results obtained for $[^{13}$C$]$AAA enrichment were the same as those for nontreated urine samples. Hence, this hydrolysis step was discontinued in the analysis of later samples. After being dried, the samples were cooled to room temperature and 0.5 mL freshly prepared propanol-HCl (5 parts anhydrous l-propanol plus 1 part acetyl chloride) was added to the vials. The vials were tightly capped with polytetrafluoroethylene-lined caps, vortex mixed, heated at 90°C for 30 min, cooled to room temperature, and dried under a stream of nitrogen at room temperature.

For derivatization of samples, 100 μL heptfluorobutyryl anhydride (HFBA) was added to the vials, which were capped tightly, vortex mixed, and heated at 60°C for 30 min. The samples were dried at room temperature under a stream of nitrogen. Dry ethyl acetate (0.5 mL) was added to the vials, which again were vortex mixed and centrifuged for 5 min at 4°C at 1500 × g, and the contents transferred to microvials and capped with crimp-top seals.

A similar approach was followed for measurement of plasma $[^{13}$C$]$AAA. To 0.5 mL plasma in 12 × 75 mm borosilicate glass test tubes, 1 mL of normal acetic acid was added and the tube was gently vortex mixed. The plasma was poured onto the column; the test tube was rinsed with 1 mL N acetic acid and vortex mixed, and again the contents were applied to the column (prepared as for analysis of aminoadipic acid in urine samples). The column was rinsed with 1 mL water and the eluate was discarded.

Aminoadipic acid in the plasma was also extracted from the column with 1 mL of 3 mol NH$_4$OH/L followed by 1 mL water and collected in vials. Esterification with prepared propanol-HCl and derivatization with HFBA were similar but not identical to the methods used for urine samples. The difference was that for plasma samples, 100 μL ethyl acetate was added after HFBA derivatization and 100-μL glass inserts were used inside the microvials.

Plasma and urinary $[^{13}$C$]$AAA enrichment were measured with the aid of a Hewlett-Packard 5890 series II gas chromatograph coupled to a 5988 quadrupole mass spectrometer with an RTE-6 data system (Hewlett-Packard, Palo Alto, CA). Thus, 1.0 μL of the derivatized urine sample (3.0 μL derivatized plasma sample) was injected into a 30-m (length) DB-WAX capillary column with an internal diameter of 32 mm and film thickness of 0.25 μm (J & W Scientific, Folsom, CA). Helium was used as the carrier gas. Mass spectrometry was performed in the negative chemical ionization mode with methane as the reagent gas. The derivatized samples were injected with an autosampler (HP 7673A; Hewlett-Packard) that used a splitless injection mode.

The temperature conditions within the gas chromatography oven were as follows: the temperature increased from 100 to 220°C in 6 min at a rate of 20°C/min, increased from 220 to 250°C in 1 min at 30°C/min, and stayed at 250°C for 5 min. The ions monitored by negative chemical ionization had mass-to-charge ratios (m/z) from 421 (m) (α-aminoadipic acid-di-n-propyl-heptfluorobutyl minus hydrogen fluoride) to 423 (m+2).
To calculate the mole fraction of the labeled aminoadipic acid in the experimental samples, a linear prediction equation was generated based on theoretical calculations (28). These calculations accounted for the distribution of masses into the unlabeled aminoadipic acid. The slope was 0.670 and the y intercept was 0.154.

For the isolation and analysis of free plasma $[^{13}C]_L$ysine, 250 $\mu$L plasma was used. The procedure was the same as for plasma $[^{13}C]_{AAA}$. Lysine was eluted with 2 mL of 3 mol NH$_4$OH/L and then with 1 mL water. The eluates were dried at 70°C under a stream of nitrogen. After the samples were cooled to room temperature, 0.5 mL freshly prepared propanol-HCl was added to the samples in vials, which were then closed with polytetrafluoroethylene-lined caps, vortex mixed, and heated for 1 h at 110°C. The samples were cooled to room temperature and evaporated to dryness at 70°C under a stream of nitrogen. Next, 100 $\mu$L HFBA was added to the vial, which was vortex mixed and heated at 60°C for 20 min with the vial tightly capped. Samples were then evaporated to dryness under a gentle stream of nitrogen at 70°C, 0.5 mL dry ethyl acetate was added, and the samples were transferred to autosampler microvials and capped with crimp-top seals. Plasma $[^{13}C]_L$ysine enrichment was measured by gas chromatography–mass spectrometry similarly to $[^{13}C]_{AAA}$enrichment. However, the temperature in the gas chromatography oven was increased from 140 to 220°C at a rate of 10°C/min and then from 220 to 265°C at 30°C/min. The ions monitored, using negative chemical ionization, were $m/z$ 560 ($m$-propyl-di-heptafluorobutyl minus hydrogen fluoride) and $m/z$ 561 ($m$ + 1) with single-ion monitoring. To compute the abundance of $^{13}C$ in plasma lysine (mole ratio) over baseline, a linear regression equation was used that was based on a calibration set of $[^{13}C]$lysine and $[^{12}C]$lysine mixtures.

For the analysis of free plasma lysine concentrations, we used a Gold high-performance liquid chromatograph with a data system (Beckman Instruments, Inc, Fullerton, CA). Norleucine was used as the internal standard. The method was as described previously (5).

**Evaluation of primary data**

**Lysine flux**

The lysine flux ($Q_1$, $\mumol \cdot kg^{-1} \cdot 30 \text{ min}^{-1}$) was calculated according to standard steady state isotope-dilution principles as follows (5, 9):

$$Q_1 = i (E_i/E_p)$$

where $i$ is the rate of tracer infusion ($\mumol \cdot kg^{-1} \cdot 30 \text{ min}^{-1}$) and $E_i$ and $E_p$ are the isotopic enrichments as mole fractions of the infused and plasma amino acid, respectively.

Although the values for the successive 30-min periods indicated change within the 24-h period, use of non–steady state kinetics was not considered important because of the limited emphasis given here to the flux values, the change in the whole-body lysine pool over a 30-min interval usually being quite small, and the uncertainties of the whole-body lysine pool size and actual volume of distribution of the lysine tracer.

**Lysine oxidation**

Lysine oxidation was computed for consecutive 30-min intervals over the 24-h period. Because a finite but undetermined amount of time would be required for the prime and continuous tracer doses of $[^{13}C]_L$ysine to achieve an isotopic equilibrium in plasma and its connecting metabolic pools, lysine oxidation during the first four 30-min intervals was computed as being equal to lysine oxidation during the slightly later 30-min interval from 120 to 150 min. A study with L-[$^{15}N]$lysine revealed that an isotopic steady state was achieved in plasma within 1–2 h after a primed, constant tracer infusion (29), and a prime dose followed by a constant tracer infusion of $[^{13}C]_L$bicarbonate gave a steady output of $^{13}C_2O_2$ within 2 h (5), each as determined by the time–isotopic enrichment relation lacking a slope. Therefore, lysine oxidation was directly measured for 44 of the total 48, 30-min intervals. For each interval, lysine oxidation ($\mumol \cdot kg^{-1} \cdot 30 \text{ min}^{-1}$) was computed as follows:

$$\text{Oxidation} = \frac{^{13}C_2O_2 \text{ production (} \mumol \cdot kg^{-1} \cdot 30 \text{ min}^{-1} \text{)}}{\text{plasma } ^{13}C\text{lysine (APE } \times 10^{-3})}$$

where

$$^{13}C_2O_2 \text{ production (} \mumol \cdot kg^{-1} \cdot 30 \text{ min}^{-1} \text{)} = \frac{V_{C0} \text{ (} \mumol \cdot kg^{-1} \cdot 30 \text{ min}^{-1} \text{)} \times \text{1/10}^5 \times 1/R_{\text{eff}}}{\text{V_{C0} \text{ enrichment} (APE } \times 1000 \text{)} \times 1/10^5 \times 1/R_{\text{eff}}}$$

where $E$ is the average of the 2 enrichments at the 2 times defining the 30-min interval [this average was corrected for $^{13}C$ background, as described previously (5)]; $R_{\text{eff}}$ is $R_{\text{fast}}$ or $R_{\text{fed}}$, the mean $[^{13}C]$bicarbonate fraction recovered during fasting (0.766) and feeding (0.851), respectively (5); and $[^{13}C]_L$lysine enrichment is the average value for the 2 times defining the 30-min interval. Moreover, for fasting and feeding states, $VCO_2$ over the time interval when it was not measured directly because calibrations were being made, meals were being eaten, or breath was being sampled was derived as the arithmetic average of $VCO_2$ measured just before and just after the interval. $VCO_2$ was usually measured during a 30–45 min period within each consecutive hour.

When the $^{13}C$ enrichment of aminoadipic acid in urine was used as a precursor enrichment of the lysine pool undergoing oxidation, we used the 4th 3-h collection in fasting (0300–0600) as an index of the mean values for the 12 h of fasting because那天 h of constant $[^{13}C]_L$lysine infusion appeared to be needed to approach an equilibrium level of labeling in this metabolite. Results obtained from our tracer studies with $[^{13}C]_{AAA}$ support this approach. For the fed phase, all of the consecutive 3-h urinary collections were used for $[^{13}C]_{AAA}$ analysis and subsequent computation of lysine oxidation. Comparative data obtained in one subject (after an intravenous $[^{13}C]_{AAA}$ tracer) revealed that urinary and plasma $[^{13}C]_{AAA}$ enrichments were similar.

**Lysine balance and 2-aminoadipic acid kinetics**

Measured 24-h lysine balance (in $\mumol/kg$) was computed as input (dietary lysine plus tracer lysine) minus measured output (sum of the oxidation for the 48, 30-min intervals; derived from plasma $[^{13}C]_L$lysine or urinary $[^{13}C]_{AAA}$ as a precursor). For computation and data summary, we used a computer spreadsheet program (Excel 5.0 for the Macintosh; Microsoft Corp, Redmond, WA).

The fluxes of 2-aminoadipic acid and its oxidation were calculated as described for lysine. It involved similar corrections for bicarbonate recoveries.
Statistical methods and evaluation of the data

The paired t test was used to compare data within the same group of subjects, such as 24-h lysine balance using plasma [13C]lysine abundance as the precursor compared with 24-h lysine balance using urinary [13C]AAA as the precursor. The paired t test was also used to compare rates of lysine oxidation based on the same precursor abundance (plasma [13C]lysine or urinary [13C]AAA) during fasting and feeding and to compare lysine oxidation by using different 13C-labeled precursors within the same condition (ie, fasting or feeding). A one-sample, one-tailed t test was used to compare the calculated 24-h lysine balance with a zero value. A P value > 0.1 was considered not to be significant. Data analyses were done with SAS software (version 6; SAS Institute Inc, Cary, NC).

Comparison with previous 24-h leucine kinetics data

As discussed in the Introduction, we showed with [13C]leucine as a tracer that an accurate estimation of whole-body leucine oxidation can be made under the present experimental conditions. Also, the estimation agreed well with nitrogen excretion (12-h fasting, 12-h feeding, and 24 h). Hence, it is reasonable to use our earlier findings (5) together with our estimate of IPNL from indispensible amino acid oxidation (leucine or lysine) or nitrogen excretion (5) to draw further inferences about the quantitative reliability of the present estimates of lysine oxidation and balance. Therefore, we compare our present findings with some of our previous findings (5). We used one-way analysis of variance (ANOVA) to compare 3 data sets from different groups of subjects. When these data sets were generated from the same group of subjects, we performed a repeated-measures ANOVA followed by a pairwise comparison among means by using the Student-Neuman-Keuls test.

RESULTS

[13C]AAA kinetics

As depicted in Figure 2, plasma and urinary [13C]AAA enrichment values were found to be similar in the first subject we studied. For this reason, determinations of [13C]AAA abundance in the [13C]lysine tracer studies were made with urine samples to limit the amount of blood taken over the 24-h period.

The 13C enrichment of urinary aminoadipic acid in subjects given the intravenous and oral tracers increased rapidly, with equilibrium usually being reached within the first 6 h or so of the tracer-infusion period (Figure 3). Enrichment then decreased promptly, especially in the group administered the tracer orally, when the feeding period began (Figure 3). The degree of aminoadipic acid labeling was markedly less during the feeding state when the tracer [13C]AAA was given orally than when it was given intravenously.

13CO2 production (Figure 4) after the unprimed infusion of [13C]AAA increased during the initial 10 h or so. Because this curve was similar to that which would be expected after an unprimed, constant infusion of [13C]bicarbonate, it appears that the intravenous and oral tracers rapidly labeled the pools of aminoadipic acid that were undergoing oxidation.

The fluxes of aminoadipic acid for the fasting and feeding states were calculated from the urinary aminoadipic acid enrichments at the 9–12-h collection for fasting and at the 6–9-h collection for feeding. The mean fluxes for the intravenous (n = 2) and oral (n = 2) subjects were 2.9 and 3.8 μmol·kg\(^{-1}\)·h\(^{-1}\), respectively, during fasting and 3.8 and 7.4 μmol·kg\(^{-1}\)·h\(^{-1}\), respectively, during feeding (Table 2). In the intravenous tracer group, the infusion accounted for 72% (fasting) and 55% (feeding) of the calculated flux. This suggests that the mean rate of plasma appearance of endogenous aminoadipic acid arising from an endogenous source, namely lysine catabolism, during fasting was perhaps <1 μmol·kg\(^{-1}\)·h\(^{-1}\) [2.9 × (1 − 0.72) = 0.81].

With the intravenous and oral tracers, from 71% to 88% of the 13C administered [(13CO\(_2\) in breath/13C tracer given) × 100] over the 24 h was recovered in expired air; when the whole-body oxidation of the amino acid was then calculated, by using an approach similar to that for lysine oxidation (product-precursor ratio), it approximated 20.6 μmol·kg\(^{-1}\)·d\(^{-1}\) for the intravenous route. Because the urinary aminoadipic acid enrichment was lower in the oral than in the intravenous group during feeding, the mean rate of whole-body aminoadipic acid oxidation for the 2 subjects studied with the oral tracer was 38.6 μmol·kg\(^{-1}\)·d\(^{-1}\).

FIGURE 2. 13C enrichment of plasma (■) and urinary (□) aminoadipic acid for an adult receiving an unprimed, constant, intravenous infusion of [13C]aminoadipic acid for 24 h. The values for each 3-h urine collection have been positioned in the midpoint of the period as a set of identical values stretching across the respective period. APE, atom percent excess.
Oxidation after either tracer, are shown in $\text{[13 C]}$ flux (\text{[13 C]}Lysine kinetics).

The calculated rates of aminoacidic acid oxidation were higher for the feeding than for the fasting state; the mean fasting-to-feeding ratio was 0.72 for 2 subjects given the tracer intravenously and 0.62 for the 2 subjects given the tracer orally. As discussed below in relation to the lysine kinetics data, these estimates of fluxes and oxidation rates do not appear to reproduce the quantitative status of whole-body lysine catabolism. However, they help to clarify the use of aminoacidic acid labeling for interpretation of the \([\text{[13 C]}]\)lysine tracer studies.

**[13 C]Lysine kinetics**

The patterns of $^{13}$CO$_2$ production and plasma \([\text{[13 C]}]\)lysine enrichment throughout the 24-h \([\text{[13 C]}]\)lysine tracer period for subjects receiving the generous intake of lysine (77 mg·kg$^{-1}$·d$^{-1}$), after either tracer, are shown in **Figure 5**. From these isotopic data the various parameters of whole-body lysine metabolism were derived. Mean (±SD) lysine fluxes, as calculated directly from the plasma \([\text{[13 C]}]\)lysine values, were 85 ± 2 and 96 ± 5 μmol·kg$^{-1}$·h$^{-1}$ for fasting and feeding with the intravenously administered tracer and 119 ± 21 and 117 ± 14 μmol·kg$^{-1}$·h$^{-1}$ for fasting and feeding with the orally administered tracer,

respectively. As expected, the calculated fluxes were ≈40% and ≈22% higher ($P < 0.05$) for the oral than for the intravenous tracer group for fasting and feeding, respectively. This is thought to be principally due to the first-pass disappearance of the orally administered \([\text{[13 C]}]\)lysine tracer within the splanchnic region (30). From these data and by use of the method of calculation described previously (16–18), the approximate mean fraction of lysine tracer (or of unlabeled dietary lysine) that disappeared during a first pass through the splanchnic bed can be estimated to be 0.22 for the feeding state under these specific dietary conditions.

The pattern and rate of whole-body lysine oxidation throughout the 24-h period are depicted in **Figure 6**. For these feeding conditions, lysine oxidation increased when the first small meal was consumed and remained at a relatively constant level until within ≈30 min after ingestion of the last small meal, which was at 1500.

The pattern of lysine oxidation broadly tracked the change in plasma lysine concentration, at least in reference to the lower plasma lysine concentration (see the statistical data noted in the legend of **Figure 7** and rate of lysine oxidation in the fasting state compared with the feeding state (compare Figures 6 and 7). This type of association extends that previously noted for leucine oxidation and plasma leucine concentrations in young adults receiving different amounts of dietary leucine (5–7, 30).

The measured rates of lysine oxidation (based on \([\text{[13 C]}]\)lysine enrichment in plasma) for the 12 h of fasting, the 12 h of feeding, the last hour of fasting, and the fifth hour of feeding are summarized in **Table 3** for both groups. From these results the total daily lysine oxidation rate, obtained by summation of the measured rates, was found to equal 69.6 ± 13.4 and 60.7 ± 10.3 mg lysine·kg$^{-1}$·d$^{-1}$ for the oral and intravenous tracer groups, respectively (NS), and ≈63% and ≈67%, respectively, of this daily oxidative loss occurred during the 12-h feeding period. For both groups, measured lysine oxidation for the fasting period was different from that measured for the feeding period ($P < 0.05$ and $P < 0.001$ for the intravenous and oral tracer groups, respectively). The measured rates for the 12th and 15th hours of fasting and the 5th hour of feeding are also given here for the possible interest of and use by others, where only brief periods within each 12-h period are actually used for measurement of amino acid oxidation.

The pattern of change in the \([\text{13 C]}\) enrichment of aminoacidic acid in consecutive 3-h urine collections after the primed, constant intravenous administration of \([\text{[13 C]}]\)lysine was examined (Fig. 8). From these data it is evident that the slopes of the change in the \([\text{13 C]}\) enrichment of nonessential amino acids (cysteine and glutamine) and their nitrogen are positive in the fasting period and negative in the feeding period, as shown in **Figure 9**. These observations suggest that the fraction of total lysine consumed and remained at a relatively constant level until within 30 min after ingestion of the last small meal, which was at 1500.

### TABLE 2

Summary of \([\text{[13 C]}]\)aminoadipic acid 24-h kinetics in 4 young men

<table>
<thead>
<tr>
<th></th>
<th>Intravenous</th>
<th>Oral tracer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subject 1</td>
<td>Subject 2</td>
</tr>
<tr>
<td>Flux (μmol·kg$^{-1}$·3 h$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9–12-h Fasting</td>
<td>2.96</td>
<td>2.77</td>
</tr>
<tr>
<td>6–9-h Feeding</td>
<td>3.98</td>
<td>3.64</td>
</tr>
<tr>
<td>$^{13}$CO$_2$ output (μmol·kg$^{-1}$·12 h$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>5.49</td>
<td>5.26</td>
</tr>
<tr>
<td>Feeding</td>
<td>6.96</td>
<td>6.26</td>
</tr>
<tr>
<td>Ratio of $^{13}$CO$_2$ output to $^{13}$C tracer input</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>Feeding</td>
<td>0.83</td>
<td>0.78</td>
</tr>
<tr>
<td>24 h</td>
<td>0.74</td>
<td>0.72</td>
</tr>
<tr>
<td>Oxidation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting (μmol·kg$^{-1}$·12 h$^{-1}$)</td>
<td>9.0</td>
<td>8.3</td>
</tr>
<tr>
<td>Feeding (μmol·kg$^{-1}$·12 h$^{-1}$)</td>
<td>13.0</td>
<td>11.0</td>
</tr>
<tr>
<td>24 h (μmol/kg)</td>
<td>22.0</td>
<td>19.3</td>
</tr>
</tbody>
</table>
infusion of $^{13}\text{C}$lysine for the oral and intravenous tracer groups is shown in Figure 8. The $^{13}\text{C}$ abundance increased during fasting, reaching or approaching an apparent plateau (oral group) during a period $\approx 9–12$ h after $^{13}\text{C}$lysine administration began. With feeding of the small meals containing generous lysine intakes, the $^{13}\text{C}$ enrichment of aminoadipic acid declined, reaching a lower plateau within $\approx 6$ h after the first small meal; this level was then essentially maintained for the remainder of the feeding period.

The relations between plasma $^{13}\text{C}$lysine enrichment and the enrichment of urinary aminoadipic acid for the various times during the 24-h period are summarized in Table 4; the mean ratio of $^{13}\text{C}$ enrichment in urinary aminoadipic acid to plasma lysine, although apparently lower for the intravenous than for the oral group, was not significantly different between groups. In the oral tracer group, the mean $^{13}\text{C}$AAA enrichment by the end of fasting rapidly declined to $\approx 70–75\%$ of the plasma $^{13}\text{C}$lysine enrichment when meals providing the generous amount of lysine were consumed. Motil et al (31) reported a comparable relation between the $^2\text{H}_4$ enrichments of free lysine in plasma and VLDL–apolipoprotein B-100 in fed, nulliparous women.

From these findings for aminoadipic acid labeling in urine, and in further reference to the results above for aminoadipic acid kinetics, we estimated the rate of lysine oxidation during the 12-h fast from the $^{13}\text{C}$AAA enrichment values obtained for the 9–12-h (3 h) urine collection and that during the 12-h feeding period by using all values for urinary enrichment over this 12-h phase. These estimates are presented in Table 5. Thus, for the intravenous tracer with use of urinary $^{13}\text{C}$AAA for computation, lysine oxidation amounted to $\approx 93$ mg·kg$^{-1}$·d$^{-1}$. Although the mean lysine balance was negative ($\approx 16$ mg·kg$^{-1}$·d$^{-1}$), the large SD of 28 mg·kg$^{-1}$·d$^{-1}$ indicated that it was not significantly different from a zero value. Clearly, it is important to seek ways to minimize the experimental component of this variation in determined balance values. However, as also shown in Table 5, when plasma $^{13}\text{C}$lysine was used to compute oxidation for the intravenous tracer group, the daily lysine oxidation was estimated to be 61 mg·kg$^{-1}$·d$^{-1}$ and mean balance was 17

**FIGURE 5.** Pattern of change in $^{13}\text{CO}_2$ production (corrected for bicarbonate recoveries) and enrichment of plasma $^{13}\text{C}$lysine throughout the 24-h period for subjects receiving tracer $^{13}\text{C}$lysine either intravenously (○) or orally (●). Bars represent either $+1$ or $-1$ SEM.

**FIGURE 6.** Pattern and rate of lysine oxidation throughout the 24-h period for subjects receiving tracer $^{13}\text{C}$lysine either intravenously (○) or orally (●). Bars represent either $+1$ or $-1$ SEM.

**FIGURE 7.** Pattern of change in plasma lysine concentration throughout the 24-h period for subjects receiving tracer $^{13}\text{C}$lysine either intravenously (○) or orally (●). Bars represent either $+1$ or $-1$ SEM. *Significant difference between intravenous and oral groups, $0.05 < P < 0.1$. †Significantly different from all fasting values, $P < 0.01$. ‡Significantly different from 180 and 360 min, $P < 0.01$. §Significantly different from 180, 360, and 540 min, $P < 0.01$. 

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TABLE 3
Measured rates of lysine oxidation at different periods when plasma [13C]lysine was used as a precursor1

<table>
<thead>
<tr>
<th>Tracer route</th>
<th>Fasting period</th>
<th>Feeding period</th>
<th>12-h fasting to 12-h feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15th h</td>
<td>12th h</td>
<td>12-h Total</td>
</tr>
<tr>
<td>Intravenous (n = 3)</td>
<td>1.70 ± 0.14</td>
<td>1.43 ± 0.04</td>
<td>19.6 ± 1.82 (25.4 ± 2.4)3</td>
</tr>
<tr>
<td>Oral (n = 6)</td>
<td>1.80 ± 1.04</td>
<td>1.63 ± 0.53</td>
<td>25.9 ± 5.64 (33.2 ± 7.4)</td>
</tr>
</tbody>
</table>

1x ± SD. There were no significant differences between the intravenous and oral groups.
2Specifically different from feeding: P < 0.05, 3P < 0.001.
4Percentage of 24-h lysine intake (diet + tracer) in parentheses.

mg·kg⁻¹·d⁻¹ (P < 0.05 from a zero balance). This assumes that nonoxidative losses and their possible replacement via microbial lysine synthesis in the gastrointestinal tract do not seriously confound the present tracer-based determination of lysine balance.

For the oral [13C]lysine route, the comparison of 24-h lysine balances with zero was statistically similar to that of the intravenous tracer group (with use of [13C]AAA, P > 0.1; with use of [13C]lysine, P < 0.05). The route of [13C]lysine tracer administration did not seem to significantly affect the balance values whether [13C]AAA or [13C]lysine was used as a precursor.

However, considering the fasted and fed states separately, for both the intravenous and oral groups there was a clear trend (P = 0.064 for intravenous; P = 0.098 for oral) for a difference in the 12-h lysine oxidation in the fed state between the precursor enrichment groups. No difference was found for the fasted state. In addition, within the same group, lysine oxidation was different from that in feeding (P < 0.05 for the intravenous route, P < 0.01 for the oral route).

Finally, estimates of IPNL were derived for fasting, feeding, and the entire 24-h period (Table 6). Furthermore, these lysine-tracer-based estimates of IPNL were compared with those reported previously for leucine, and for which we have validated this IPNL approach for the experimental conditions used in the present study (5). Note that the percentages of leucine and lysine in the experimental diets, when expressed per unit of total protein intake, matched values for mixed body proteins (32). Hence, because our subjects would be expected to be in protein balance, this makes it possible to compare protein intake with IPNL predicted from estimates of lysine oxidation and with IPNL predicted from nitrogen excretion.

As shown in Table 6, 24-h IPNL data were analyzed within each group. We compared 24-h IPNL (from indispensable amino acid oxidation) with 24-h IPNL (from nitrogen excretion) and with protein intake by using a repeated-measures ANOVA. These values were not significantly different except for the [13C]lysine intravenous route, for which 24-h IPNL (indispensable amino acid) was different (P = 0.02) from 24-h IPNL (nitrogen) and from the protein intake of 1000 mg protein·kg⁻¹·d⁻¹.

A comparison of 24-h IPNL (indispensable amino acid) data between groups by using a one-way ANOVA revealed a P value of 0.076, again suggesting that 24-h IPNL with use of the intravenous [13C]lysine tracer route was lower than the other 2 values. Substituting the more variable oral aminoadipic acid data for the oral lysine data in the statistical analysis led to a disappearance of the near statistical significance observed. Hence, one may argue for the use of the oral route using [13C]lysine or [13C]AAA as precursors. However, comparison of the fasting-feeding IPNL ratios between groups (Table 6) revealed a significantly lower ratio for the oral route using [13C]AAA as a precursor (P < 0.01) and this may possibly play against using [13C]AAA as a precursor in this case. Finally, as shown in Table 6, the means for 12-h fasting and 12-h feeding IPNL with this approach were ≈10% lower than those of the leucine-derived data, which was used here as a reference.

TABLE 4
Ratio of mean plasma [13C]lysine to 3-h urinary [13C]aminoadipic acid during the 24-h period1

<table>
<thead>
<tr>
<th>Condition</th>
<th>Intravenous tracer (n = 3)</th>
<th>Oral tracer (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Last 3 h of fasting</td>
<td>0.83 ± 0.21</td>
<td>1.11 ± 0.23</td>
</tr>
<tr>
<td>1st 3 h of feeding</td>
<td>0.79 ± 0.20</td>
<td>0.89 ± 0.26</td>
</tr>
<tr>
<td>2nd 3 h of feeding</td>
<td>0.53 ± 0.04</td>
<td>0.70 ± 0.24</td>
</tr>
<tr>
<td>3rd 3 h of feeding</td>
<td>0.56 ± 0.11</td>
<td>0.72 ± 0.22</td>
</tr>
<tr>
<td>4th 3 h of feeding</td>
<td>0.54 ± 0.11</td>
<td>0.75 ± 0.15</td>
</tr>
</tbody>
</table>

1x ± SD. There were no significant differences between the intravenous and oral groups.
catabolism might serve as a probe for assessing the \(^{13}\text{C}\) enrichment of the intracellular lysine pool that is undergoing oxidation. We had also speculated on the exciting possibility that measurement of the plasma aminoacidic acid flux and the rate of aminoacidic acid oxidation might serve as an estimate of the whole-body rate of lysine oxidation; aminoacidic acid is principally a product of lysine catabolism in the liver except for a contribution made via the reversible transamination of any \(\alpha\)-ketoacidic acid arising from tryptophan degradation. Furthermore, we recognize that aminoacidic acid is oxidized by leukocytes (37) and that 2-aminoacidic acid transaminase is present in the kidney (38). Nevertheless, although the present findings do not support our earlier speculation, they have provided us with guidance in the interpretation of the data emerging from our \(^{13}\text{C}\)lysine experiments.

The 24-h pattern of lysine oxidation (Figure 6) showed a prompt, sustained rise in the rate of oxidation when small, hourly meals were given beginning at \(\approx 0600\). This pattern of change is similar to that described for leucine (5) and phenylalanine (9) in

**TABLE 5**

Rates of lysine oxidation and the estimated daily lysine balance based on urinary \(^{13}\text{C}\)aminoadipic acid and on plasma \(^{13}\text{C}\)lysine enrichments in subjects receiving the lysine tracer intravenously or orally\(^4\)

<table>
<thead>
<tr>
<th>Tracer route and precursor</th>
<th>12-h Fasting oxidation</th>
<th>12-h Feeding oxidation</th>
<th>Daily lysine balance(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg·kg(^{-1})·12 h(^{-1})</td>
<td>mg·kg(^{-1})·12 h(^{-1})</td>
<td>mg·kg(^{-1})·d(^{-1})</td>
</tr>
<tr>
<td>Intravenous (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary aminoadipic acid</td>
<td>22.03 ± 6.92(^2)</td>
<td>70.99 ± 21.59</td>
<td>−15.75 ± 28.18</td>
</tr>
<tr>
<td>Plasma lysine</td>
<td>19.61 ± 1.87(^2)</td>
<td>41.09 ± 8.06(^1)</td>
<td>+16.57 ± 10.25(^4)</td>
</tr>
<tr>
<td>Oral (n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary aminoadipic acid</td>
<td>21.02 ± 3.67(^3)</td>
<td>60.72 ± 20.08</td>
<td>−3.43 ± 23.03</td>
</tr>
<tr>
<td>Plasma lysine</td>
<td>25.92 ± 5.66(^3)</td>
<td>43.70 ± 8.76(^3)</td>
<td>+8.68 ± 13.74(^4)</td>
</tr>
</tbody>
</table>

\(^{1}\bar{x} ± SD.\)

\(^{2}\)Significantly different from feeding (paired \(t\) test): \(P < 0.05, \ P < 0.01.

\(^{3}\)Significance of the difference from urinary \(^{13}\text{C}\)aminoadipic acid enrichment (paired \(t\) test): \(P = 0.064, \bar{P} = 0.098.

\(^{4}\)Significantly different from zero, \(P < 0.05\) (one-sample, one-tailed \(t\) test).

**DISCUSSION**

From \(^{13}\text{C}\)-labeled amino acid tracer studies as well as on theoretical grounds (2, 4, 14, 33–35), we concluded that the estimates of the minimum physiologic requirements for the indispensable amino acids in healthy adults as proposed by the 1985 FAO/WHO/UNU (24) expert consultation are not acceptable. A major portion of the experimental data used to support our position is based on experiments with leucine as a test amino acid. Other investigators have critically and extensively reviewed our studies and found that our interpretations of the nutritional significance of the leucine kinetic data are reasonable (15, 36). The present experiment is the first in our investigation of the interrelations among whole-body 24-h lysine kinetics, amino acid balance, and dietary lysine intakes over the submaintenance to nearmaintenance range of intake for this indispensable amino acid.

The present, although limited, investigation with \(^{13}\text{C}\)AAA as a tracer was conducted to learn more about the kinetics and labeling pattern of urinary \(\alpha\)-aminoadipic acid. The study by Arends and Bier (22) led us to believe that this product of lysine metabolism might serve as a probe for assessing the \(^{13}\text{C}\) enrichment of the intracellular lysine pool that is undergoing oxidation. We had also speculated on the exciting possibility that measurement of the plasma aminoacidic acid flux and the rate of aminoacidic acid oxidation might serve as an estimate of the whole-body rate of lysine oxidation; aminoacidic acid is principally a product of lysine catabolism in the liver except for a contribution made via the reversible transamination of any \(\alpha\)-ketoacidic acid arising from tryptophan degradation. Furthermore, we recognize that aminoacidic acid is oxidized by leukocytes (37) and that 2-aminoacidic acid transaminase is present in the kidney (38). Nevertheless, although the present findings do not support our earlier speculation, they have provided us with guidance in the interpretation of the data emerging from our \(^{13}\text{C}\)lysine experiments.

The 24-h pattern of lysine oxidation (Figure 6) showed a prompt, sustained rise in the rate of oxidation when small, hourly meals were given beginning at \(\approx 0600\). This pattern of change is similar to that described for leucine (5) and phenylalanine (9) in

**TABLE 6**

Comparison of estimates of irreversible protein nitrogen loss (IPNL) derived from 24-h lysine studies with estimates from previous leucine oxidation studies\(^3\)

<table>
<thead>
<tr>
<th></th>
<th>[^{13}\text{C}]Leucine:</th>
<th>[^{13}\text{C}]Lysine:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IV KIC (n = 7)</td>
<td>IV lysine (n = 3)</td>
</tr>
<tr>
<td>Body protein content of leucine or lysine (wt/wt%)(^4)</td>
<td>8.1</td>
<td>7.7</td>
</tr>
<tr>
<td>Intake (% protein)</td>
<td>8.0</td>
<td>7.7</td>
</tr>
<tr>
<td>IPNL (IAA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-h Fasting (mg protein/kg)(^4)</td>
<td>377</td>
<td>255 (0.68)(^4)</td>
</tr>
<tr>
<td>12-h Feeding (mg protein/kg)(^4)</td>
<td>621</td>
<td>534 (0.86)</td>
</tr>
<tr>
<td>Ratio of fasting to feeding</td>
<td>0.61 ± 0.12(^5,6)</td>
<td>0.48 ± 0.06(^6)</td>
</tr>
<tr>
<td>24-h Total (mg protein·kg(^{-1})·d(^{-1}))</td>
<td>998 ± 38</td>
<td>789 ± 134(^7)</td>
</tr>
<tr>
<td>IPNL (N), 24-h total (mg protein·kg(^{-1})·d(^{-1}))(^8)</td>
<td>959 ± 111</td>
<td>1067 ± 43</td>
</tr>
<tr>
<td>Protein intake (mg protein·kg(^{-1})·d(^{-1}))</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

\(^{1}\)Leucine data summarized from El-Khoury et al (5). IV, intravenous; KIC, ketoisocaproate; IG, oral; IAA, indispensable amino acid; N, nitrogen.

\(^{2}\)Leucine or lysine content of body mixed proteins (% protein) (32).

\(^{3}\)Mean values expressed as mg protein (N × 6.25)/kg.

\(^{4}\)Percentage of the leucine value in parentheses.

\(^{5}\)\(\bar{x} ± SD.\)

\(^{6}\)Significantly different from IG aminoadipic acid, \(P < 0.01\) (one-way ANOVA).

\(^{7}\)Significance of the difference from IV KIC and IG lysine, \(P = 0.076\) (one-way ANOVA).

\(^{8}\)Computed from total N excretions [(total urinary N + 8) × 6.25] as described previously (5).

\(^{9}\)Significantly different from 24-h IPNL, \(P = 0.02\) (repeated-measures ANOVA followed by pairwise comparison between means with Student-Newman-Keuls test).

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subjects receiving generous intakes of each of these test amino acids. It is also similar to that reported by Garlick et al (39) in their 24-h tracer studies with [13C]leucine, where the fed state was achieved by giving a 12-h infusion of an adequate liquid diet beginning at =0800. In all of these studies, the intake of the tracer plus dietary and test amino acid exceeded considerably the minimum physiologic requirements, either as proposed by FAO/WHO/UNU (24) or by us (3, 14).

We had 2 main objectives in measuring lysine oxidation continuously over 24 h in this initial phase of our investigation of the lysine requirements in healthy adults. The first was to determine whether the route of [13C]lysine tracer administration (oral and intravenous) was important for making an accurate quantitative assessment of the whole-body lysine oxidation rate. The second was to evaluate whether measurement of enrichment of urinary [13C]AAA would provide a substantially better basis for determining the whole-body rate of lysine oxidation than would measurement of plasma [13C]lysine enrichment. Again, we had anticipated that this could be the case because Arends and Bier (22) showed similarity in the 13C abundances in urinary aminoadipic acid and in lysine isolated from plasma apolipoprotein B-100 after a prolonged intravenous infusion of [13C]lysine. Furthermore, the level of isotope labeling in this liver-derived protein is considered to be a useful index of the isotopic abundance in the precursor pool for liver protein synthesis (40). By extension, this might also be the case for determination of lysine oxidation, although this issue has not yet been resolved.

With respect to the route of tracer administration, and as shown in Table 6, when the intravenous tracer was coupled with use of plasma [13C]lysine for estimating oxidation, some overestimation of balance (ie, underestimation of oxidation) occurred at this generous lysine intake. Because of the few subjects studied with the intravenous protocol and the large interindividual variation, the differences between the intravenous and oral routes were not statistically significant. Nevertheless, it seems reasonable to conclude that an accurate determination of a group mean daily rate of body lysine oxidation, or of total-body lysine loss, might well be based on the oral administration of the [13C]lysine tracer. When this tracer approach is used in combination with determination of lysine oxidation from 13CO2 output and plasma [13C]lysine enrichment, the body lysine balance estimate is slightly positive (P < 0.05), or the equivalent of ≈11% of the intake. This estimate assumes that other routes of lysine loss are minor and might, therefore, be disregarded; however, from a study in subjects with terminal ileum ileostomies, Fuller et al (23) estimated that the losses of lysine and leucine via the gastrointestinal tract amount to 4 and 3 mg·kg−1·d−1, respectively. If these losses are not compensated for by the uptake of these amino acids from microbial sources, for which there is some limited evidence (41), the balance would be less positive (and more negative) than the values recorded herein. To fully resolve this issue, quantitative estimates of the metabolic fates of indispensable amino acids entering the gastrointestinal tract and the contribution made by the microbial flora to host tissue amino acid metabolism will be required.

The estimate of daily lysine oxidation might be improved with the aid of an intracellular marker of the 13C enrichment of the free lysine pool that is undergoing oxidation. When we used the 13C enrichment of urinary aminoadipic acid in this context, the mean absolute value for the lysine balance (oral tracer) was lower than that based on plasma [13C]lysine. However, the differences were not significant. Thus, under the present experimental conditions, use of [13C]AAA values did not significantly improve the estimation of body lysine balance when the [13C]lysine tracer was given by the oral route. Furthermore, it generated an even larger variation in the 24-h balance data (Table 5). Measurement of the appearance of the labeled lysine at the intramitochondrial site of its oxidation would permit a more secure estimate of whole-body lysine oxidation, but this would not be feasible in human subjects and would even be extremely difficult to accomplish in experimental animals. The best that can be done at present is to compare the isotopically derived estimate of lysine oxidation with the estimate that can be reasonably predicted to be equivalent to the generous intake of lysine and also equivalent to the derived value from nitrogen excretion data.

Several additional points can be made with respect to the use of [13C]AAA data for estimation of lysine oxidation. First, for the fasting state, the enrichment of aminoadipic acid appeared to take ≈9 h to approach equilibrium. This relatively slow rate of labeling of the urinary aminoadipic acid pool presumably reflects not only the limited rate of release of aminoadipic acid from the mitochondria, where it is produced during the oxidation of lysine, but also the rate of rise in the 13C enrichment of the free lysine pool in the liver. Thus, it is also worth noting that after 9–12 h of oral [13C]lysine administration, mean values for 13C enrichments of plasma lysine and urinary aminoadipic acid were similar. Furthermore, it appears that there might have been a difference in the rate of rise to a plateau value of urinary [13C]AAA enrichment after the administration of labeled aminoadipic acid (6–9 h) compared with labeled lysine (9–12 h). Because of the limited data it is not possible to be certain that a true difference existed, but if it did it might reflect a relatively slow rise in the enrichment of the liver free lysine compartment.

Second, the 13C-labeling of aminoadipic acid from both intravenous and oral [13C]lysine tracer administration declined immediately with ingestion of the lysine-containing meals, and by ≈3 h into the feeding phase the labeling of aminoadipic acid relative to that for plasma lysine (oral group) approached a ratio of ≈0.70. Thus, the mean estimated rate of lysine oxidation for the feeding period (oral tracer) was ≈40% higher when based on use of [13C]AAA than when based on plasma [13C]lysine (60.7 compared with 43.7 mg lysine·kg−1·12 h−1, P = 0.09) (Table 5). For the fasting period, the mean difference was ≈20% but was in the opposite direction (NS).

Because aminoadipic acid could arise via the reversible transamination of 2-ketoacidic acid, which is also a product of tryptophan catabolism, the 13C enrichment of aminoadipic acid may be diluted by unlabeled aminoadipic acid and may underestimate the 13C enrichment of the lysine precursor pool, leading to an overestimation of lysine oxidation. This may not be a major problem in studies involving generous lysine intakes or for usual diets, in which lysine is far more abundant in comparison with the amount of tryptophan. However, this problem could limit the use of 13C AAA labeling for determination of lysine oxidation when the dietary lysine intake is low and inadequate. Hence, it is difficult to know whether estimates of lysine oxidation derived from aminoadipic acid are any more reliable than those derived from plasma [13C]lysine enrichment. Without additional studies at various lysine intakes, it appears that use of plasma [13C]lysine enrichment values after oral administration of the tracer is currently the best way to estimate whole-body lysine oxidation.

This view is supported in reference to our previous studies
with [13C]leucine, in which we found that the rate of leucine oxidation predicted accurately the irreversible whole-body protein nitrogen loss during the 12-h fasting and 12-h feeding phases of the 24-h tracer protocol (5). Under these earlier conditions, which were essentially replicated in the present study except for the [13C]amino acid tracer used, we found that the fasted-to-fed ratio of leucine oxidation, or IPNL, was ~0.61. Hence, 62% of the daily irreversible loss of leucine occurred during the 12-h fed period. It is reasonable to expect that the present subjects would also have shown a comparable pattern of fasted-to-fed ratio for leucine loss, although this was not actually measured. The important issue then becomes whether the relative changes in lysine oxidation between the fasting and feeding periods of the 24 h for these same dietary and experimental conditions were the same as those for leucine.

The qualitative pattern of lysine oxidation throughout the 24-h period was similar to that for leucine; it needs to be determined whether the quantitative aspects of the daily lysine oxidation pattern are also comparable with those for leucine when intakes are generous. Hence, we might further examine the present values for both lysine and aminoadipic acid oxidation. The mean fasting-to-feeding ratio of [13C]AAA oxidation was 0.63 with oral administration of the tracer and 0.72 with intravenous administration of the tracer (Table 2). These ratios may reflect the comparative rates of lysine oxidation under fasted and fed conditions at these generous intakes of lysine. As presented in Table 6, the fasting-to-feeding ratio of lysine oxidation with use of oral tracers was 0.59 and 0.35 for estimates derived with use of plasma [13C]lysine and urinary [13C]AAA as precursors, respectively. The former ratio is close to that for both leucine and the α-aminoadipic acid kinetics, whereas the latter ratio is lower than that for leucine. However, the fasting-feeding ratio of lysine oxidation, at a generous intake, may be less than that for leucine if a significantly greater proportion of the lysine than leucine released from protein breakdown during fasting is retained within the free lysine pool. This retention might occur particularly in skeletal muscle and perhaps in other tissues and organs (42, 43). The free lysine pool of muscle is relatively large and shows changes in size in response to ingestion of protein-free and protein-containing meals (43). This implies a capacity to store some free lysine that is liberated via proteolysis during the fasting period. However, for a daily balance to be achieved, this retained lysine would then contribute, in effect, to the total dietary intake during the feeding period and would have to be lost via oxidation during this metabolic phase. A key issue, therefore, is the possible change in size of the free lysine pool with feeding and fasting under various conditions of lysine intake.

Unfortunately, few direct experimental data have been published on changes in concentrations of free lysine and of leucine in the metabolic pool that we might use for assessing changes in the free lysine pool in skeletal muscle. Bergstrom et al (43) showed that a protein-rich meal increased free lysine and leucine concentrations in muscle by 30% and 60%, respectively, 3 h after the beginning of the meal. When a protein-free meal was given, lysine and leucine concentrations declined by ~30–40%. Limited as these data may be, they help us to assess the foregoing estimates of lysine oxidation after oral administration of the tracer as follows: for the plasma [13C]lysine-derived values of lysine oxidation, in comparison with leucine oxidation and IPNL-derived values, it appears that the measured rate of lysine oxidation during the 12-h fast was close to 89% of that predicted from leucine kinetics (Table 6). If this is true, this reflects a relatively small retention of lysine, which can be calculated to cause a rise in the free lysine concentration in muscle that falls well within the concentration changes observed by Bergstrom et al (43). In comparison, the measured rate of lysine oxidation when based on [13C]AAA was 72% of that which might be predicted from our leucine studies. Again, this implies an increase in the free lysine content of muscle, which could also be achieved according to the data of Bergstrom et al (43). However, to maintain daily mass balance of lysine within the body free lysine pool, an increase in muscle free lysine during the fasting period would have to be followed by an equivalent fall during the feeding period. This pattern of change is inconsistent with the data of Bergstrom et al (43).

For these multiple reasons, it is our tentative recommendation that the estimate of daily lysine oxidation be based on use of plasma [13C]lysine enrichment with a 24-h, hourly oral administration of [13C]lysine. Even so, it seems that this oxidation rate may be somewhat lower than the true rate of lysine loss. As a result, the derived balance value would be slightly overestimated; our data suggest that this error might average ~10%. Supporting this interpretation are the findings of Wolfe and Jahan (44) showing there was an 81% recovery of infused [13C]acetate from labeled acetate in expired 13CO2 after a correction was made for a bicarbonate recovery of 0.8. Similarly, Pouteau et al (45) reported a 13C recovery in CO2 of ~0.7 after a constant infusion of [1-13C]acetate in postabsorptive subjects. Therefore, because an intermediate product of [1-13C]lysine oxidation is [1-13C]acetate, it is likely that the present estimates of lysine oxidation represent minimal values in this context. However, this should not restrict our ability to confirm and extend our previous estimates of the minimum physiologic requirements for lysine in healthy adults (2, 14, 46) by conducting a series of 24-h [1-13C]lysine tracer-infusion studies at various test intakes of lysine.

REFERENCES