Taurine kinetics assessed using \([1,2-^{13}\text{C}_2]\)taurine in healthy adult humans

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Rakotoambinina, Benjamin, Lisa Marks, Abdou Monem Badran, Frank Igliki, François Thuillier, Pascal Crenn, Bernard Messing, and Dominique Darmaun. Taurine kinetics assessed using \([1,2-^{13}\text{C}_2]\)taurine in healthy adult humans. Am J Physiol Endocrinol Metab 287: E255–E262, 2004. First published March 9, 2004; 10.1152/ajpendo.00333.2003.—To assess the dynamics of taurine metabolism in vivo, two sets of studies were carried out in healthy volunteers. First, pilot studies were carried out in a single human subject to determine the time course of plasma and whole blood isotope enrichment over the course of an 8-h, unprimed continuous infusion of \([1,2-^{13}\text{C}_2]\)taurine. Second, five healthy adult males received two tracer infusions on separate days and in randomized order: 1) a 6-h continuous infusion of \([1,2-^{13}\text{C}_2]\)taurine (3.1 ± 0.2 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\) and 2) a bolus injection of \([^{13}\text{C}_2]\)taurine (3.0 ± 0.1 \(\mu\text{mol/kg}\)). Isotope enrichments in plasma and whole blood taurine were determined by gas chromatography-mass spectrometry. The pilot experiments allowed us to establish that steady-state isotope enrichment was reached in plasma and whole blood by the 5th h of tracer infusion. The plateau enrichment reached in whole blood was lower than that obtained in plasma taurine (\(P < 0.02\)). In the second set of studies, the appearance rate (\(R_a\)) of plasma taurine, determined from continuous infusion studies was 31.8 ± 3.1 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\). After a bolus injection of tracer, the enrichment decay over the subsequent 2 h was best fitted by a two-exponential curve. Taurine \(R_a\) was \(\approx 85\%\) higher when determined using the bolus injection technique compared with continuous infusion of tracer. We conclude that 1) taurine \(R_a\) into plasma is very low in healthy postabsorptive humans, and, due to taurine compartmentalization between the extra- and intracellular milieus, may represent only interorgan taurine transfer and merely a small fraction of whole body taurine turnover; and 2) the bolus injection technique may overestimate taurine appearance into plasma. Further studies are warranted to determine whether alterations in bile taurine dynamics affect taurine \(R_a\).

constant infusion; bolus injection technique

TAURINE (2-ETHANEAMINOSULFONIC ACID) is, after glutamine, the second most abundant free amino acid in mammalian cells (2) and is involved in various physiological functions, including osmoregulation, defense against oxidative stress, detoxification of xenobiotics, membrane stabilization, retinal function, and bile conjugation (22). The two sources of body taurine are dietary intake and de novo taurine synthesis from methionine and cysteine, its sulfur amino acid precursors (22). Due to the limited taurine synthetic capacity of the newborn, taurine is considered a conditionally essential amino acid in infants (28). Taurine depletion has been described in adult patient populations as well (13, 50), as a result of either 1) increased utilization (11), 2) decreased intake (38), 3) decreased rate of de novo synthesis (45), or 4) various combinations of the three.

Most of the knowledge of the dynamics of taurine metabolism, however, is derived from the measurement of taurine concentration, which provides little information as to the mechanisms leading to taurine depletion and repletion (11, 35, 45, 48, 50). In theory, the use of tracer dilution techniques should provide further insight into these mechanisms and help in designing strategies for taurine supplementation as well. Although tracer methodology has been applied extensively to the exploration of various amino acids, including methionine and cysteine (37), very few data have appeared with regard to in vivo taurine kinetics. The first estimates of taurine kinetics were reported from compartmental analysis using either \(^{35}\text{S}\)taurine bolus injection in humans (44) or bolus injection of \(^{13}\text{C}\)taurine and \(^{13}\text{C}\)taurine in rhesus monkeys (33). In recent years, we developed a gas chromatography-mass spectrometry (GC-MS) assay to determine both the concentration and the stable isotope enrichment of taurine in blood and obtained preliminary estimates of taurine kinetics in human subjects by use of \([1,2-^{13}\text{C}_2]\)taurine tracer (31). The aims of the present study were therefore 1) to expand on these preliminary data and assess the turnover rate of circulating taurine and the size of taurine tracer-miscible pools in healthy adults in the fasting state and 2) to determine whether the administration of labeled taurine as an intravenous bolus injection would yield kinetic parameters comparable to those obtained with constant infusion.

METHODS

Subjects and protocol design. Six healthy, 36 ± 8-yr-old men (mean ± SD; range 29–46 yr) with normal body weight (72.5 ± 3.6 kg) and body mass index (22.8 ± 1.4 kg/m\(^2\)) were recruited (Table 1). They had taken neither vitamin (especially \(B_6\)) supplements nor any medication for ≥3 mo before the initiation of the study (44). Over the week before study, they consumed a regular Western diet that was not supplemented with taurine (2,219 ± 291 kcal/day, 1.2 ± 0.15 g/kg \(-1\) day \(^{-1}\) protein; 116 ± 16 mg taurine/day). Each subject gave informed, written consent before the study, according to protocols approved by the Lariboisière-Saint-Lazare Hospital ethics committee. All tests were performed in the morning while subjects were in the postabsorptive state as outpatients at the Institut National de la Santé et de la Recherche Médicale U290 Laboratory of Lariboisière-Saint-Lazare Hospital.

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The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
A first set of preliminary experiments (protocol A) was carried out in a single subject to determine whether an isotopic steady state could be achieved in blood taurine enrichment by using a constant intravenous infusion at rates of either 2 or 3 μmol·kg⁻¹·h⁻¹ with or without a priming dose (31).

A priming dose equivalent to 1 h of tracer infusion rate was chosen for several reasons: 1) assuming that taurine would be distributed throughout body water, taurine volume of distribution would be equivalent to total body water (0.6 liter/kg), and assuming the average taurine concentration in body tissues to be close to that measured in plasma (~50 μmol/l), we estimated body taurine pool at 0.6 × 50 = 30 μmol/kg, so that a 3 μmol/kg labeled taurine prime would enable us to instantaneously achieve a taurine enrichment ~10%; and 2) in a first set of preliminary studies using a bolus injection, we had obtained an estimate of ~2.7 mmol (37 μmol/kg) for the “rapid” taurine pool, and because we aimed at a plateau enrichment ~8% during the primed, continuous infusion, we therefore elected to use 3 μmol/kg as a priming dose.

The priming dose was injected over 1–2 min and immediately followed by a continuous infusion using a syringe pump (Perfusor V, B Braun, Melsungen, Germany) at a rate of 6 ml/h. Blood samples were drawn at 0, 10, 20, and 30 min after the bolus and every 30 min thereafter until 480 min. Expired air was collected at hourly intervals throughout the study for 13 CO₂ determination.

Blood taurine analysis. For the determination of plasma taurine enrichment, blood samples were immediately separated by centrifugation at room temperature at a minimum rotary speed of 800 g for 10 min. The residual plasma was centrifuged a second time at 300 g for 10 min to remove platelets. Platelet-free plasma samples were then stored at ~80°C until the day of analysis. The method for preparing the N-pentfluorobenzoyl-di-n-butylamine derivative of taurine has been previously described in detail (31). All solvents and reagents used in the analysis of plasma taurine were, at minimum, of HPLC grade purity.

Both taurine concentration and isotopic enrichment were determined by electron impact (70 eV) GC-MS using a Hewlett-Packard 5973 (Palo Alto, CA) instrument. Aliquots were injected in triplicate into a GC (model 6890, Hewlett-Packard, Fullerton, CA) equipped with an OVI fused silica capillary column (30 m × 0.32 mm ID, 0.25 μm film thickness). Ions at mass-to-charge ratios (m/z) = 302 and 304, representing natural and [13 C₂]taurine, respectively, were selectively monitored. A standard curve obtained by diluting natural taurine with graded amounts of [13 C₂]taurine was run before each sample series. When the observed 304-to-302 ion current ratios were plotted as a function of expected [13 C₂]taurine-to-natural taurine molar ratios, linear regression coefficients of >0.99 were consistently observed along with slopes of 0.82–0.92. Taurine concentration was assessed in the full-scan mode by monitoring masses ranging from m/z = 100–350 using methyltaurine as internal standard.

Body composition analysis. Body composition was measured using a two-frequency (5 kHz, 1 MHz) bioelectrical impedance analyzer (IMP B01, Cachan, France) to estimate fat-free mass, i.e., lean body mass.

Calculations. The intracellular taurine concentration in “total blood cells” ([Tau]_{wb}), defined as the fraction of blood volume not accounted for by plasma, was estimated as

\[
[Tau]_{wb} = \frac{[Tau]_{wb} - (1 - Hct) \times [Tau]_b}{Hct} \tag{1}
\]

where Hct is hematocrit (expressed as a fraction of unity), and [Tau]_{wb} and [Tau]_b are the measured concentrations of taurine in whole blood and plasma, respectively. The isotopic enrichment in intracellular taurine in total blood cells (E_{wb}) was then estimated as (6, 7)

\[
E_{wb} = \frac{E_{wb}[Tau]_{wb} - (1 - Hct) \times E_b \times [Tau]_b}{Hct \times [Tau]_{wb}} \tag{2}
\]

For the continuous tracer intravenous infusion, the appearance rate (R_a, μmol·kg⁻¹·h⁻¹) of taurine into plasma in the postabsorptive state was calculated using the steady-state equation

\[
R_a = \frac{[Ei/Epss] - 1}{i} \tag{3}
\]

where i is the tracer infusion rate (μmol·kg⁻¹·h⁻¹), and Ei and Epss are taurine enrichments in infusedate and plasma at steady state, respectively. A steady state was defined as an insignificant change (i.e.,

Table 1. Characteristics of study subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Height, m</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>%FFM</th>
<th>kg</th>
<th>FM, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>1.70</td>
<td>67</td>
<td>21.4</td>
<td>82.4</td>
<td>55.2</td>
<td>17.6</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>1.75</td>
<td>70</td>
<td>22.8</td>
<td>79.2</td>
<td>55.4</td>
<td>20.8</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>1.77</td>
<td>77</td>
<td>24.6</td>
<td>82.0</td>
<td>63.1</td>
<td>18.0</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>1.83</td>
<td>74</td>
<td>22.1</td>
<td>88.9</td>
<td>65.8</td>
<td>11.1</td>
</tr>
<tr>
<td>5*</td>
<td>32</td>
<td>1.83</td>
<td>72</td>
<td>21.5</td>
<td>89.4</td>
<td>64.3</td>
<td>10.6</td>
</tr>
<tr>
<td>6*</td>
<td>46</td>
<td>1.75</td>
<td>75</td>
<td>23.9</td>
<td>80.8</td>
<td>60.6</td>
<td>19.2</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>36±8</td>
<td>1.78±0.03</td>
<td>72.5±3.6</td>
<td>22.8±1.4</td>
<td>83.8±4.4</td>
<td>60.7±4.5</td>
<td>16.2±4.3</td>
</tr>
</tbody>
</table>

BMI, body mass index; FM, fat mass; FFM, fat-free mass, %body mass, assessed by bioelectrical impedance analysis. *Shown in previously published preliminary data (31).
slope not significantly different from zero), with time in both plasma isotopic enrichment and substrate level, along with a coefficient of variation (CV) of <10% for both parameters.

For unprimed infusion, the rise of tracer enrichment [Ep, mole% excess (MPE)] to plateau was fitted to an exponential curve using a nonlinear regression

\[ Ep = Ep_{ss}(1 - e^{-kt}), \]

where \( Ep_{ss} \) is plasma taurine enrichment at plateau, and \( k \) is the rate constant (h\(^{-1}\)). The size of the tracer-miscible taurine pool (pool) was calculated as pool = \( R_a/k \) (7).

For the bolus tracer intravenous injection study, the area under the curve (AUC, \( \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{kg}^{-1} \)) sustained by plasma \([^{13}\text{C}_2] \text{taurine} \) concentration was calculated by manual trapezoidal integration or Tai’s formula (46). Taurine metabolic clearance rate (MCR, \( 1 \cdot \text{h}^{-1} \cdot \text{kg}^{-1} \)) was calculated as MCR = \( d / \text{AUC} \), where \( d \) is the amount of labeled taurine injected (\( \mu\text{mol/kg} \)), and taurine \( R_a \) was calculated as

\[ R_a = \text{MCR} \times [\text{Tau}]_{p}. \]

Taurine pool determination was performed using a two-compartment model according to Sturman (43).

**Statistics.** Data are presented as means \( \pm \) SD. Data comparisons were performed using SSSPS software (SPSS, Chicago, IL). Statistical significance was set at \( P < 0.05 \).

**RESULTS**

**Protocol A.** In this pilot study, the same individual underwent infusions of \([^{13}\text{C}_2] \text{taurine} \) on three separate occasions, either as an unprimed infusion or as a primed infusion, at 2 and 3 \( \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) (Table 2). As shown in Fig. 1, the unprimed 2 \( \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) infusion rate resulted in a slow rise in plasma enrichment, which reached an apparent plateau between 240 and 480 min of tracer infusion. The rise of plasma enrichment during the unprimed infusion was better fitted to a curve with a double exponential than it was when a single exponential curve was used, as the \( r^2 \) were 0.97 and 0.93 with the double and single exponential fits, respectively. The miscible taurine pool was determined to be 38 \( \mu\text{mol/kg} \).

The use of a priming dose equivalent to a 1-h infusion rate did not help to achieve steady state any sooner. This result suggests that the dose used resulted in “underpriming.” This is supported by the data shown in Fig. 1; as a matter of fact, the “peak” enrichment obtained after the prime appears to be significantly below the plateau enrichment subsequently achieved after 240 min of infusion. Although a larger priming dose might have solved the problem, the use of an excessive dose as a prime (“overpriming”) would have been a worse choice yet: due to the relatively slow turnover rate of taurine, plasma taurine enrichment might have declined continuously without achieving a plateau over the course of the experiment. This would have obviously resulted in erroneous \( R_a \) values, as discussed by other authors in the case of urea (32).

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**Table 2. Taurine kinetics during primed and unprimed continuous infusions of \([1,2-^{13}\text{C}_2] \text{taurine} \) in a single subject**

<table>
<thead>
<tr>
<th>Type of Infusion</th>
<th>Unprimed Continuous, 2 ( \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} )</th>
<th>Primed Continuous, 2 ( \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} )</th>
<th>Primed Continuous, 3 ( \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid</td>
<td>Plasma</td>
<td>Whole blood</td>
<td>Plasma</td>
</tr>
<tr>
<td>([^{13}\text{C}_2] \text{Tau}  )</td>
<td>6.356</td>
<td>6.753</td>
<td>1.980</td>
</tr>
<tr>
<td>CV, %</td>
<td>1.3%</td>
<td>8.7%</td>
<td>10%</td>
</tr>
<tr>
<td>Taurine, ( \mu\text{mol/l} )</td>
<td>55.9</td>
<td>56.2</td>
<td>167.7</td>
</tr>
<tr>
<td>CV, %</td>
<td>1%</td>
<td>2.3%</td>
<td>15.8%</td>
</tr>
<tr>
<td>( R_a ), ( \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1} )</td>
<td>29.4</td>
<td>27.6</td>
<td>98.7</td>
</tr>
</tbody>
</table>

Values are means of 3 or 4 sampling points for the 240– to 360- and 360- to 480-min periods, respectively; \( R_a \), rate of appearance; \([^{13}\text{C}_2] \text{Tau} \), \([1,2-^{13}\text{C}_2] \text{taurine} \) enrichment [mole percent excess (MPE)]; CV, coefficient of variation (100 \( \times \) SD/mean); [Taurine], taurine concentration in arterialized blood sample; ND, not determined.
blood cell enrichment/plasma taurine enrichment ratio was measured over the last hour of isotope infusion. The estimated total steady state was nevertheless observed in whole blood enriching the pulse dose, and for each subject, taurine concentration between 300 and 360 min was 31.8 ± 3.2 mmol/kg. Regardless of the infusion rate and prime, isotopic enrichment remained considerably lower in whole blood than in plasma taurine (P < 0.01, Table 2). As shown in Fig. 1, a steady state was nevertheless observed in whole blood enrichment over the last hour of isotope infusion. The estimated total blood cell enrichment/plasma taurine enrichment ratio was 0.15–0.29 (95% confidence limit) between 300 and 480 min.

At all time points, whole blood taurine concentrations were higher than plasma taurine (P < 0.01), due to considerably higher intracellular taurine levels, as estimated using hematocrit (Eq. 2). Regardless of the infusion rate and prime, isotopic enrichment remained considerably lower in whole blood than in plasma taurine (P < 0.02; Table 2). As shown in Fig. 1, a steady state was nevertheless observed in whole blood enrichment over the last hour of isotope infusion. The estimated total blood cell enrichment/plasma taurine enrichment ratio was 0.15–0.29 (95% confidence limit) between 300 and 480 min.

Regardless of the infusion protocol, no detectable 13C enrichment could be found in CO₂ in expired air collected between 240 and 360 min, as attested by slopes of regression curves not different from zero when enrichment was plotted as a function of variation ranging between 6.2 and 2.1% (Table 3) in plasma enrichment.

The time course of 13C₂ enrichment in plasma taurine after a single intravenous bolus of 3.02 ± 0.07 mmol/kg [13C₂]taurine is depicted in Fig. 2 for four of the same five subjects. Because the very early sampling points were missed for subject 5, the Rₐ calculation could not be performed for that subject. There was no significant rise in the total (unlabeled + labeled) concentration of plasma taurine following the pulse dose, and for each subject, taurine concentration remained relatively stable between 2 and 120 min.

The time-dependent decline of plasma [13C₂]taurine enrichment was well fitted to a two-component exponential decay curve (Fig. 2), as the r² for the four individual curves were 0.988, 0.995, 0.996, and 0.997 (mean ± 0.994). The taurine production rates calculated from this set of data are shown in Table 4. Two taurine pool sizes were identified, the first one ranging from 2.7 to 8 mmol and the second from 51 to 88 mmol.

The noncompartmental analysis using the AUC describing the time course of the tracer-to-tracee curve between 2 and 120

Table 3. Estimates of taurine fluxes (Rₐ) calculated from different periods (240–300 and 300–360 min) during a 360-min primed continuous infusion (3 μmol·kg⁻¹·h⁻¹) of [13C₂]taurine in 6 healthy adults

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Mean Ep, MPE</th>
<th>CV, %</th>
<th>Rₐ, μmol·kg⁻¹·h⁻¹</th>
<th>Mean Ep, MPE</th>
<th>CV, %</th>
<th>Rₐ, μmol·kg⁻¹·h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.037</td>
<td>3</td>
<td>30.6</td>
<td>9.136</td>
<td>2.3</td>
<td>30.2</td>
</tr>
<tr>
<td>2</td>
<td>11.116</td>
<td>3.5</td>
<td>29.9</td>
<td>11.609</td>
<td>0.5</td>
<td>28.5</td>
</tr>
<tr>
<td>3</td>
<td>9.019</td>
<td>8.5</td>
<td>31.0</td>
<td>9.661</td>
<td>1.2</td>
<td>28.8</td>
</tr>
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<td>4</td>
<td>7.223</td>
<td>10.3</td>
<td>39.5</td>
<td>7.806</td>
<td>3.5</td>
<td>35.6</td>
</tr>
<tr>
<td>5</td>
<td>7.737</td>
<td>7.3</td>
<td>35.4</td>
<td>8.110</td>
<td>3.6</td>
<td>33.6</td>
</tr>
<tr>
<td>6</td>
<td>8.420</td>
<td>5</td>
<td>32.5</td>
<td>8.464</td>
<td>1.5</td>
<td>32.3</td>
</tr>
</tbody>
</table>

Ep, plasma enrichment (MPE).

The time-dependent decline of plasma [13C₂]taurine enrichment was well fitted to a two-component exponential decay curve (Fig. 2), as the r² for the four individual curves were 0.988, 0.995, 0.996, and 0.997 (mean ± 0.994). The taurine production rates calculated from this set of data are shown in Table 4. Two taurine pool sizes were identified, the first one ranging from 2.7 to 8 mmol and the second from 51 to 88 mmol.

The noncompartmental analysis using the AUC describing the time course of the tracer-to-tracee curve between 2 and 120 min.
min yields a mean value for taurine Ra of 58.0 ± 17.8 μmol·kg⁻¹·h⁻¹. The Ra determined by bolus injection was, on average, equal to 1.85 times the value obtained when continuous infusion was used (Table 4), and the difference was significant (P = 0.03) with a two-tailed paired t-test and close to statistical significance (P = 0.06) with a nonparametric Wilcoxon paired test.

DISCUSSION

The present study demonstrates that the Ra of taurine into plasma can be assessed using a 6-h continuous intravenous infusion of [1,2-¹³C₂]taurine in humans and ranks among the lowest turnover rates of all amino acids measured to date. The lack of equilibration of labeled taurine between plasma and circulating cells, along with the slow Ra observed, further implies that taurine metabolism is highly compartmentalized and suggests that taurine Ra mostly reflects the interorgan exchange of taurine. Finally, the data suggest that the use of a single bolus injection of labeled taurine overestimates taurine Ra.

Little is known about taurine kinetics in humans. Using [1,2-¹³C₂]taurine, Vinton et al. (50) reported a taurine Ra of 5.6 μmol·kg⁻¹·h⁻¹ in five healthy volunteers, one-sixth of the taurine flux measured in the current study. Differences in study design could have contributed to the discrepancy. Whereas Vinton et al. studied fed subjects, we kept our subjects in the postabsorptive state to avoid any potential effect of exogenous nutrients in detecting isotope enrichments below 0.1 MPE by GC-MS quantitation, albeit for a different substrate. Unlike the tracer cycling observed in the current report (32 min, MPE = 2.7%), arteriovenous (a-v) gradients assessed across various vascular beds (9, 10, 16, 36) yield much lower estimates of taurine interorgan exchange: muscle releases 2.2 μmol·kg⁻¹·h⁻¹ taurine (36), and splanchnic bed (9) and brain (10) take up 1.9 and 1.3 μmol·kg⁻¹·h⁻¹ taurine, respectively, assuming blood flows of 1.200 ml/min in liver (9) and 2.2 and 60 ml·min⁻¹·100 g⁻¹ in muscle (36) and brain (39), respectively. Yet both approaches have intrinsic limitations. The a-v concentration differences are often of the same magnitude as measurement error, and determination of blood flow across organ beds is technically difficult. Moreover, a-v gradient methods measure net organ balance, so that simultaneous release and uptake in the same tissue can be missed. On the other hand, isotope dilution relies on enrichments measured in circulating taurine, although taurine may be produced and utilized inside cells without exchanging with plasma. The Ra obtained may thus reflect interorgan taurine exchange rather than whole body taurine production rate.

In the current study, estimates of taurine Ra derived from constant tracer infusion were consistently lower than those provided by bolus injection (Table 4), using the AUC describing the time course of [¹³C₂]taurine level between 2 and 120 min. These cut-off times were chosen because of the difficulty in detecting isotope enrichments below 0.1 MPE by GC-MS and may have led to an underestimation of the AUC, resulting in higher estimates of taurine Ra. In addition, as the same intravenous line was used for bolus injection and for sampling, this technique could lead to “contamination” of initial blood samples by excess amounts of tracers. This source of error was quantitated in a recent study, albeit for a different substrate. When Boles Ponto et al. (Ponto et al. (35a)) infused 2-deoxy-2-[¹⁸F]fluoroglucose ([¹⁸F]-DFG) through a forearm vein and sampled blood from the same line and a contralateral forearm vein, they found the [¹⁸F]-DFG level to be, on average, 2% higher when sampling on the infused line, i.e., when using a 10-ml saline flush and discarding the initial 1-ml blood sample. We believe the level of “contamination” was still less in the present studies, because 1) samples were obtained after a larger, 25-ml saline flush, and we discarded the initial 5 ml of

Table 4. Plasma [¹³C₂]taurine kinetics in 4 fasted healthy adults

<table>
<thead>
<tr>
<th>Subject</th>
<th>Ra infused</th>
<th>Steady-State Plasma [¹³C₂], μmol/l</th>
<th>EP at 2 min, MPE</th>
<th>Ep at 120 min, MPE</th>
<th>Pools, μmol</th>
<th>Plasma [¹³C₂] at 2 min, μmol/l</th>
<th>Ra bolus, μmol·kg⁻¹·h⁻¹</th>
<th>Δ%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30.2</td>
<td>79.6</td>
<td>14.6</td>
<td>0.49</td>
<td>3.1</td>
<td>58</td>
<td>61.7</td>
<td>40.9</td>
</tr>
<tr>
<td>2</td>
<td>28.5</td>
<td>70.5</td>
<td>7.61</td>
<td>1.08</td>
<td>2.7</td>
<td>84</td>
<td>72.8</td>
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<td>3</td>
<td>30.1</td>
<td>97.2</td>
<td>12.51</td>
<td>0.76</td>
<td>5.4</td>
<td>88</td>
<td>58.7</td>
<td>63.9</td>
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<tr>
<td>4</td>
<td>36.34</td>
<td>57.2</td>
<td>10.16</td>
<td>0.02</td>
<td>8.0</td>
<td>51</td>
<td>60.5</td>
<td>80.4</td>
</tr>
</tbody>
</table>

Mean ± SD 31.3 ± 3.4 76.1 ± 16.8 11.2 ± 3.0 0.68 ± 0.46 4.8 ± 2.4 70.2 ± 18.5 63.4 ± 6.4 58.0 ± 17.8 83.0 ± 40.6

Comparison of primed continuous infusion (3 μmol/kg·h⁻¹) vs. single bolus injection technique (3 μmol/kg). Ra infused, taurine Ra, as determined from continuous infusion and bolus infusion, respectively. Δ, relative difference between Ra bolus and Ra infused: Δ = 100 × ([Ra bolus - Ra infused]/Ra infused). Ra infused vs. Ra bolus: *P = 0.03 using a 2-tailed paired t-test; P = 0.06 using a nonparametric Wilcoxon paired test.
cially decreased taurine R_a. This was obviously not the case, since the bolus injection technique yielded higher flux values than did the continuous infusion technique. Taurine R_a values obtained from [13C]taurine continuous infusion are nevertheless close to the ~20–25 µmol·kg⁻¹·h⁻¹ obtained by monitoring the decay of taurine specific activity for 48 h after a bolus of [35S]taurine in humans (44) or monkeys (33). Taken together, these studies suggest that, whereas bolus injection may be appropriate for radioactive taurine, a continuous-infusion approach is preferable when stable isotopes are used.

Regardless of the methodological uncertainties, taurine R_a is lower than most amino acid R_a values assessed to date (3). The appearance of [18O]taurine after inhalation of 16O₂ established the ability of humans to synthesize taurine de novo (24). Conversion of cysteine to taurine is the sole source of endogenous taurine and is, however, limited, due to the low activity of cysteine sulfinate decarboxylase, the rate-limiting step in taurine synthesis (8, 26, 27); 2) the low R_a of cysteine (~45 µmol·kg⁻¹·h⁻¹); and 3) the fact that two other major pathways, cysteine incorporation into body protein (22) and glutathione (12), compete for cysteine utilization (37, 42).

Regarding taurine disposal (R_d), irreversible taurine losses of 0.1 and 5.4–28.5 µmol·kg⁻¹·day⁻¹ have been reported in feces (47) and urine (38), respectively. The quantitative contribution of hepatic taurine conjugation to taurine utilization can be extrapolated from published data (15, 19, 41). Glycine is another amino acid moiety used for bile acid conjugation (18). Because 1) overall glycine R_a approximates 150 µmol·kg⁻¹·h⁻¹ (7) and 2) 2.3% of glycine flux is turning over for bile acid, then ~3.4 µmol·kg⁻¹·h⁻¹ glycine are used for bile conjugation in an adult human (17–19). Assuming a ratio of glycine to taurine of ~3.5 in bile acids (17, 19, 21), bile acid conjugation may therefore only account for ~1 µmol·kg⁻¹·h⁻¹, or <2% the taurine R_d measured in the current study. The fate of the bulk of taurine therefore remains unresolved.

One might argue that taurine is a “metabolic dead end,” implying that, once it enters cells, it remains there, and the large intracellular taurine pool may be a vast “taurine sink.” Taurine residence time in muscle was estimated at 427 h in healthy men, nine times that of glutamine (29). The low fractional turnover of taurine might account for the slow isotopic equilibration, as it took 4 h of a constant infusion of [13C₂]taurine to reach isotopic equilibrium. A delay in tracer equilibration across cell membranes invariably occurs when a substrate has a large intracellular pool, such as was observed with [15N]glutamine in the skeletal muscle glutamine pool (49).

The two-component taurine pools obtained from the current data agree with previous reports (44, 50), and the small component pool size found in four subjects explored by bolus injection (2.7–8.0 mmol; Table 5) is in the same range as the tracer-miscible pool determined using continuous infusion (38 µmol/kg body wt, i.e., ~2.6 mmol). Assuming extracellular fluid to be 0.2 l/kg with a taurine content of 50 µmol/l, we can estimate extracellular taurine pool at ~10 µmol/kg. The extracellular taurine pool thus accounts for only 26% of tracer-miscible pool (10/38), and the rest of the taurine tracer-miscible pool must be accounted for by a fraction of intracellular taurine that exchanges with plasma. These pool sizes are far from whole body taurine content, since muscle alone contains >287 mmol (2), and large amounts of taurine are found in retina, interstitial spaces, or bound with intracellular peptides (22, 30). It is therefore safe to conclude that both taurine R_a and tracer-miscible pools represent a tiny fraction of whole body taurine.

Finally, no detectable 13CO₂ appeared in breath over the course of an 8-h infusion of [13C₂]taurine. In theory, a fraction of taurine released into bile and, subsequently, duodenum could be deconjugated from bile acids and oxidized by bacterial enzymes in the large intestine. The resulting CO₂ could diffuse across the colonic wall and appear in breath, which could be an index of exposure of bile acids to bacterial deconjugating enzymes (21, 23). Although [35S]taurine is oxidized to inorganic [35S]sulfate by colonic flora (23), no exhaled 13CO₂ was detected when [13C]taurine was used. This implies that either 1) there was no bacterial overgrowth in the healthy subjects enrolled in the current study or 2) the dose or duration of [13C₂]taurine infusion was insufficient for bacterially produced 13CO₂ to appear in breath since <1% of labeled taurine administered could be converted into [13C]cholyl taurine (19).

Taken in aggregate, data from the current studies using [13C]taurine, and from earlier studies, shed some light into the peculiar nature of taurine metabolism in humans. Like glutamine, taurine is highly concentrated in intracellular space (2, 7) and compartmentalized between extra- and intracellular milieus. Yet, contrary to glutamine, taurine has a slow turnover and exchanges very little with plasma (7, 22, 26, 44). In addition, whereas the active interorgan exchange of glutamine is known to serve as a shuttle of carbon and nitrogen between skeletal muscle and splanchnic bed (34), the physiological function of interorgan taurine exchange, if any, remains to be

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**Table 5. Estimated taurine pool sizes (mmol) derived from in vivo isotope dilution studies**

<table>
<thead>
<tr>
<th>Taurine Pool</th>
<th>First Component</th>
<th>Second Component</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>[35S]taurine bolus</td>
<td>68-135</td>
<td>1.2-3.1</td>
<td>65-133</td>
</tr>
<tr>
<td>[13C₂]taurine infusion</td>
<td>70-92</td>
<td>2.7-8</td>
<td>51-88</td>
</tr>
<tr>
<td>[13C₂]taurine bolus and infusion</td>
<td>59-93</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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found. As a matter of fact, although taurine has major physiological functions in brain, retina, liver, and other tissues, the taurine needed for these functions may arise from de novo synthesis in situ rather than taurine taken up from systemic circulation. The precise significance of interorgan taurine exchange, measured using isotope dilution methods, therefore remains to be determined.

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