Tracer methods underestimate short-term variations in urea production in humans

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Hamadeh, Mazen J., and L. John Hoffer. Tracer methods underestimate short-term variations in urea production in humans. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E547–E553, 1998.—Urea production rate ($R_a$) is commonly measured using a primed continuous tracer urea infusion, but the accuracy of this method has not been clearly established in humans. We used intravenous infusions of unlabelled urea to assess the accuracy of this technique in normal, postabsorptive men under the following four different conditions: 1) tracer [13C]urea was infused under basal conditions for 12 h (control); 2) tracer [13C]urea was infused for 12 h, and unlabelled urea was infused from hours 4 to 12 at a rate twice that of the endogenous $R_a$ (“step” infusion); 3) tracer [13C]urea was infused for 12 h, and unlabelled urea was infused from hours 4 to 8 (“pulse” infusion); and 4) tracer [13C]urea was infused for 9 h, and unlabelled alanine was infused at a rate of 120 mg·kg$^{-1}$·h$^{-1}$ (1.35 mmol·kg$^{-1}$·h$^{-1}$) from hours 4 to 9. Urea $R_a$ was calculated using the isotopic steady-state equation (tracer infusion rate/tracer-to-tracer ratio), Steele’s non-steady-state equation, and urinary urea excretion corrected for changes in total body urea. For each subject, endogenous urea $R_a$ was measured at hour 4 of the basal condition, and the sum of this rate plus exogenous urea input was considered as “true urea input.” Under control conditions, urea $R_a$ at hour 4 was similar to that measured at hour 12. After 8-h step and 4-h pulse unlabelled urea infusions, Steele’s non-steady-state equation underestimated true urea input by 22% (step) and 33% (pulse), whereas the nonisotopic method underestimated true urea input by 28% (step) and 10% (pulse). Similar conclusions were derived from the alanine infusion. These results indicate that, although Steele’s non-steady-state equation and the nontracer method more accurately predict total urea $R_a$ than the steady-state equation, they nevertheless seriously underestimate total urea $R_a$ for as long as 8 h after a change in true urea $R_a$.

METHODS

Subjects and protocols. Eleven healthy men with normal blood biochemistries and taking no medications participated in four study protocols, each involving six separate infusions (Table 1). No subject was studied more than two times, and, when this occurred, at least 1 wk separated infusion studies. All volunteers gave written consent for the study, which was approved by the Department of Medicine Human Ethics Committee of McGill University.

The subjects were admitted to the clinical research unit at 0900 h after an overnight fast. On this day, a low-protein breakfast (0930 h), lunch (1300 h), dinner (1800 h), and snack (2030 h) at maintenance energy were consumed to reduce and stabilize urea production by minimizing exogenous amino acid oxidation. Caloric intake was adjusted for individual basal energy expenditure as calculated using the Harris Benedict equation multiplied by a factor of 1.7 to account for activity (9). Proper hydration was maintained throughout the study period. The food consisted of protein-free muffins, low-protein wafers, butter, and apple juice. Consumption of other foods and beverages was not permitted. Total energy intake was 42 kcal/kg body wt (0.5% protein, 43% fat, and 56.5% carbohydrate). Infusion studies were carried out on the second morning with subjects in the postabsorptive state. Body weight was recorded, and TBW was measured by bioimpedance analysis (BIA-101A; RJL Systems, Mt. Clemens, MI; see Ref. 15). No food was consumed for the duration of these studies.

In the first protocol (control), only tracer urea was infused for 12 h to measure endogenous urea production rate ($R_a$) and confirm whether urea $R_a$ as measured during hour 4 of a primed continuous tracer infusion, accurately indicates urea $R_a$ over the subsequent 8 h.

Several researchers have evaluated the use of primed continuous tracer infusions for calculating urea turnover (11, 17, 25). J.ahor and Wolfe (11) reported that, after a proper priming dose, isotopic steady state is reached in 2–3 h. Others have pointed out, however, that under- or overpriming this slowly turning-over substrate pool could give rise to an “apparent” isotopic steady state when, in fact, the levels of plasma tracer enrichment would still be changing, albeit slowly (17).

The present research was designed, first, to reassess the validity of the steady-state urea turnover model using the primed continuous infusion method under basal conditions and, second, to investigate the accuracy of estimating short-term changes in urea production, as might occur in the normal postprandial situation, by both tracer dilution and the older nonisotopic method based on urinary urea excretion with a correction for changes in total body urea. To do this, we administered a primed continuous infusion of [13C]urea and measured plasma tracer dilution during the infusion of unlabelled urea or of alanine, a precursor for urea synthesis.

UREA ACCOUNTS for >80% of urinary N on a normal diet (3). The main determinant of the rate of urea synthesis in the normal liver is immediate amino acid catabolism (10). Thus urea production and amino acid oxidation fluctuate concomitantly (6, 22), and orally and intravenously administered loads of protein or the urea precursor alanine cause a dose-dependent increase in urea production (14, 19). In addition to its urinary excretion, urea is distributed throughout total body water (TBW), including intestinal water, where it is subject to the action of bacterial urease. Because of this, urinary urea excretion, even when corrected for changes in body urea content, underestimates the actual urea synthetic rate by 20–30% according to most investigators (6, 12, 16, 24). This observation has led to the development of tracer methods to accurately measure true urea production in humans and animals (11, 17, 20).
was infused for 4 h. Only the tracer was infused from 8 h. 
was measured over the first 4 h, after which unlabeled urea 
was measured at a rate approximately two times the endogenous R for 8 h.
In the second protocol ("step" infusion), endogenous urea R was 
measured over the first 4 h, after which unlabeled urea was 
infused at a rate approximately two times the endogenous R for 8 h.
In the third protocol ("pulse" infusion), endogenous urea R was 
measured over the first 4 h, after which unlabeled urea was 
infused for 4 h. Only the tracer was infused from hours 8 to 12.
In the fourth protocol, endogenous urea R was measured over 
the first 4 h. This was followed by a 5-h infusion of 
unlabeled alanine while tracer administration continued.
In each of the four protocols, a primed-continuous infusion of 
$^{13}$C urea (Masstrace, Woburn, MA) was administered in 
which the priming dose was equal to the amount of 
tracer infused in 9.3 h (11). This priming dose was infused 
over 20 min to avoid tracer loss in the urine and was 
immediately followed by the continuous infusion of the 
tracer at 42 mg $^{13}$C urea/h (688 µmol/h) for either 12 
(protocols 1–3) or 9 (protocol 4) h. Both the priming dose and 
the continuous infusion were administered using a Harvard 
Syringe Infusion Pump 681 E (Harvard Apparatus, Milford, 
MA). For the step and pulse infusions, unlabeled urea was 
infused using a Baxter Flo-Gard volumetric infusion pump 
adjusted to administer 20 mg of urea·kg$^{-1}$·h$^{-1}$ (333 
µmol·kg$^{-1}$·h$^{-1}$), approximately two times the endogenous R, 
from a stock solution with a concentration of 36 mg/ml (600 
mmol/l).
For the control study, blood samples were drawn from an 
arterialized vein before the priming dose was given, after the 
priming dose, and every half hour thereafter. For the step 
infusion, blood was sampled at 10-min intervals during the 
first hour of unlabeled urea infusion and at 15-min intervals 
during the second hour. For the pulse infusion, blood was 
sampled at 10-min intervals during the first hour and at 
15-min intervals during the second hour after the start and 
cessation of the unlabeled urea infusion.
For the alanine infusion, a priming dose of 120 mg/kg (1.35 
mmol/kg) was administered over 15 min followed by continuous 
infusion of 120 mg·kg$^{-1}$·h$^{-1}$ (1.35 mmol·kg$^{-1}$·h$^{-1}$) for 5 h 
commencing 4 h after the start of the $^{13}$C urea tracer 
continuous infusion, as described by Wolfe, Jahoo, and Shaw 
(27). Blood was sampled at 10-min intervals during the first 
hour and at 15-min intervals during the second hour after the 
start of the alanine infusion.
In each experiment, the amount of unlabeled urea and 
alanine infused was verified by weighing the infusate con- 
tainer bags before and after each infusion. All infusates 
were aseptically prepared by the hospital pharmacy, and sterility 
and absence of pyrogens were confirmed.
To prevent starvation-induced amino acid catabolism and 
increased urea synthesis, 5% dextrose in 0.45% NaCl was 
infused at a rate of 80 ml/h (equivalent to 16 kcal/h) during 
the control, step, and pulse infusion studies. The subjects also 
drank 150 ml of water hourly to maintain an adequate (227 ± 
39 ml/h) but not excessive urine flow, which might alter urea 
clearance. Urine was collected hourly.
Analytical methods. Heparinized blood samples were centri-
fuged at 1,400 g for 15 min at 4°C, and plasma was stored at 
–30°C until analysis. To measure urea and amino acid 
concentration and enrichment, plasma (0.1 ml) and urine 
(0.01 ml) were mixed with 20 µg of $[^{13}$C, $^{15}$N$]_{2}$-urea (99% $^{13}$C, 
99% $^{15}$N; MSD Isotopes, Montreal, Quebec, Canada) and 2.5 
µg of norleucine and L-$[3,3,3$-$2$H$]_{3}$-alanine (99.4% $^{2}$H; MSD 
Isotopes) internal standards, acidified with 1.5 ml of 1 M 
aetic acid, and applied to 1-Ml columns of cation exchange 
resin (Dowex 50W-X8, 100–200 mesh hydrogen form; Bio-
Rad Laboratories, Richmond, CA). Urea was eluted into 
3.5-Ml flat-bottomed vials equipped with Teflon-lined caps (E.
I. du Pont de Nemours, Wilmington, DE) with three sequential 
1-Ml additions of 3 M NH$_{4}$OH. The NH$_{4}$OH fraction was 
evaporated under a gentle stream of N$_{2}$, tert-butyldimethyisilyl 
(TBDMs) derivatives of urea and amino acid were prepared 
as described by Patterson et al. (18).
Gas chromatography (GC)-mass spectrometry (MS) analyses 
were performed using an HP-5890 gas chromatograph 
(Hewlett-Packard, Palo Alto, CA) directly coupled to an HP- 
5988A quadrupole mass spectrometer. Samples were 
introduced by splitless injection (1.0 µl) from an HP-7673 
autoinjector onto a fused silica DB-1 capillary column (30 x 
0.15 mm, 0.25-µm film thickness; J & W Scientific, Folsom, 
CA) under the following GC conditions: initial column 
temperature, 110°C (maintained for 2 min); program rate, 6°C/ 
min; final column temperature, 200°C; helium carrier gas 
column head pressure, 70 kPa; and injector port and transfer 
line temperatures, 250°C. The electron impact (EI) MS 
conditions were as follows: ionizing energy, 70 eV; emission 
current, 300 µA; and source temperature, 200°C. The follow-
ing ions were monitored by selected ion monitoring: TBDMS-
CO(NH$_{2}$)$_{2}$ [mass-to-charge ratio (m/z) 231.1], TBDMS-
$^{15}$CO(NH$_{2}$)$_{2}$ (m/z 232.1), TBDMS-$^{13}$C$^{16}$O(NH$_{2}$)$_{2}$ (m/z 234.1), 
TBDMS-alanine (m/z 158.1), TBDMS-valine (m/z 268.1), 
TBDMS-leucine (m/z 200.2), TBDMS-isoleucine (m/z 202), 
TBDMS-norleucine (m/z 202.2), and TBDMS-$[3,3,3$-$2$H$_{3}$]-
alanine (m/z 161.1). TBDMS-leucine eluted at 15 min, 
TBDMS-isoleucine at 15.6 min, and TBDMS-norleucine at 
15.9 min.
The $^{13}$C urea, although 99% $^{13}$C, was also 8% $^{15}$O, $^{13}$C urea, 
a phenomenon noted by previous investigators (17). The same 
was true of the $^{13}$C,$^{15}$N$_{2}$ urea used as an internal standard, 
which was 9% $^{15}$O,$^{13}$C,$^{15}$N$_{2}$ urea. The resulting M + 5 mass 
could be used to advantage as an internal standard in 
quantitating plasma urea. Standard curves were constructed 
to measure the concentration of $^{12}$C urea and the mole ratio 
of $^{13}$C urea to $^{12}$C urea (M + 1/M). Varying amounts of 
$^{12}$C urea (0–40 µg) were added to tubes containing 20 µg of 
$^{13}$C,$^{15}$N$_{2}$ urea, which were then derivatized and analyzed by 
EI GC-MS, and an areas ratio standard curve relating the 
intensity ratio 231.1/234.1 (M/M + 3) and urea was con-
structed. Plasma and urine $^{12}$C urea concentrations were 
determined from their corresponding M/M + 3 intensity ra-
tios using the areas ratio standard curve. Similar results 
were obtained when the signal intensity at m/z 236.1 (M + 5) 
was used as an internal standard to quantitate $^{12}$C urea. 
Total urea was calculated as the sum of $^{12}$C urea and 
$^{13}$C urea.
To measure plasma urea enrichment due to the adminis-
terated tracer, a calibration curve was constructed by pre-
paring varying mole ratios of $^{13}$C urea to $^{12}$C urea, as described 
by Tseng and Kallhan (23). The tracer-to-trace ratio (TTR) 
was calculated by subtracting the baseline mole ratio from the

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Control</th>
<th>Step</th>
<th>Pulse</th>
<th>Alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>28.0 ± 2.5</td>
<td>27.7 ± 3.3</td>
<td>26.2 ± 3.5</td>
<td>33.3 ± 2.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76.4 ± 2.8</td>
<td>73.8 ± 3.2</td>
<td>71.9 ± 3.0</td>
<td>72.5 ± 2.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>181 ± 3</td>
<td>181 ± 2</td>
<td>180 ± 3</td>
<td>180 ± 2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.4 ± 0.7</td>
<td>22.5 ± 0.9</td>
<td>22.0 ± 0.6</td>
<td>22.4 ± 0.7</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>61.7 ± 2.2</td>
<td>59.5 ± 1.5</td>
<td>58.4 ± 1.5</td>
<td>59.1 ± 1.5</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 6 subjects. BMI, body mass index; FFM, fat-free mass. Data between infusions were not significantly different, one-way analysis of variance, P < 0.05.
For the step infusion, values between hours 4 and 8 were fitted to the function

\[ y = \text{expected/observed} \times \text{true urea Ra} \]

where \( y \) is the signal intensity of m/z 231.1 and \( M+1 \) the signal intensity at m/z 232.1.

Plasma glucose (mmol/l) was analyzed using the Beckman Glucose Analyzer II.

Calculations. True urea input into the circulation was defined as the sum of exogenous urea infused and endogenous urea production. The latter was measured as the baseline urea Ra after 4 h of primed continuous tracer urea infusion. Urea Ra was measured using two methods. The first method, termed “nonisotopic,” calculated urea Ra as its urinary excretion rate corrected for changes in the amount of urea in body water (8, 14). Urinary urea excretion was normalized for urinary creatinine excretion rates to correct for any time delay in its excretion, as described by Cheema-Dhadi and Halpern (3). Briefly, assuming creatinine clearance to be constant, “expected” creatinine excretion over any hour was 1⁄12 the total creatinine excretion over the 12 h. The urea excretion for any hour was multiplied by the corresponding expected/measured creatinine excretion. The nonisotopic method does not account for urea that is hydrolyzed in the gut or leaves the body by a nonurinary route. To correct for this, we measured the recovery of [13C]urea in body water and urine, on the assumption that fractional nonurinary losses of tracer and tracee are the same. Thus “corrected” nonisotopic urea appearance was equal to nonisotopic urea appearance/divided by the fractional recovery of administered tracer in body water and urine over a specified time period. Tracer urea concentration in plasma and urine was calculated as the product of [13C]urea concentration and TTR.

Non-steady-state tracer kinetics. Steady-state urea Ra was calculated using the equation Ra = i/TTR, where \( i \) is the tracer infusion rate in micromoles per kilogram per hour. Non-steady-state Ra was calculated using Steele’s equation (21), in which the urea pool size (U) was calculated using the formula

\[ U = p \times TBW \times \text{plasma urea concentration} \]

where \( p \) is the pool fraction.

Plasma urea concentrations were fitted to smoothing functions to minimize the effects of small analytical errors on total body urea. Plasma urea values between hours 0 and 12 for the control infusion and hours 0 and 4 for the three other infusions were fitted to the function \( y(t) = a + bt \) (GraphPad Inplot 4.0; Graph-Pad, San Diego, CA), where \( t \) refers to time. For the step infusion, values between hours 4 and 12 were fitted to the function \( y(t) = a(1 - e^{-kt}) + b(1 - e^{-kt}) + c \). For the pulse infusion, values between hours 4 and 8 were fitted to the linear function, whereas those between hours 8 and 12 were fitted to the exponential decay equation \( y(t) = ae^{-kt} + b \). For the alanine infusion, data between hours 4 and 9 were fitted to the polynomial equation \( y(t) = a + bt + ct^2 + dt^3 + et^4 \).

Statistical analyses. Unpaired Student’s t-test and one-way analysis of variance (ANOVA) were used to determine significant differences between different infusion protocols. Paired Student’s t-test and repeated-measures ANOVA were used to determine significance between different methods within the same protocol. When ANOVA results showed significance, Newman-Keuls test was used post hoc to determine the source of difference. Differences between data were considered significant at \( P \leq 0.05 \). All results are presented as means ± SE.

RESULTS

Control infusion. Under control conditions, the plasma urea concentration demonstrated a slight, albeit statistically significant (\( P < 0.05 \)), downward trend over 12 h (Fig. 1). When calculated as i/TTR, urea Ra was similar at hour 4 (181 ± 16 µmol·kg\(^{-1}\)·h\(^{-1}\)) to that at hour 12 (175 ± 33 µmol·kg\(^{-1}\)·h\(^{-1}\); Table 2). Urea Ra values as calculated using steady-state and Steele’s non-steady-state equations were similar (Fig. 2). The mean nonisotopic urea Ra was 72 ± 6% of tracer-determined urea Ra (\( P = 0.013 \)). Because isotopic Ra measured after 4 h of primed-tracer infusion was similar to the value obtained after 12 h, this justified using the sum of tracer-determined endogenous Ra at hour 4 plus infused Ra to determine “true Ra” in the subsequent unlabeled urea and alanine infusion protocols.

Step infusion. After the urea infusion commenced, the plasma urea concentration increased (Fig. 1) and TTR decreased (Fig. 3). Nonsteady and nonisotopic urea Ra promptly increased and, after 8 h of urea infusion, were 78 and 72% respectively, of the true Ra (both \( P < 0.05 \), Table 2 and Fig. 4). The non-steady-state Ra profile shown in this figure was derived using a pool fraction of one, which provided the best fit of model-derived Ra to true Ra (Fig. 5).

Pulse infusion. Plasma urea increased steadily after the unlabeled urea infusion commenced (Fig. 1) and decreased slowly upon its cessation, whereas [13C]urea enrichment showed the opposite trend (Fig. 3). Nonsteady and nonisotopic Ra increased promptly upon commencement of the urea infusion and after 4 h of infusion were 67% (\( P < 0.05 \)) and 90% [not significant (NS)], respectively, of the true Ra (Table 2). Upon the cessation of the unlabeled urea infusion, i/TTR decreased at a slower rate than its increase in the previous 4 h, whereas nonsteady and nonisotopic Ra rapidly decreased (Fig. 6). Nonsteady and nonisotopic Ra values were 114 and 89% of the true Ra at hour 12, respectively (NS). As with the step urea infusion, the best non-steady-state profile was obtained using a pool fraction value of one (Fig. 7).

![Fig. 1. Plasma urea concentration (mmol/l) between hours 0 and 12 for the step (△), pulse (○), and control (●) infusions.](image-url)
Cumulative nonisotopically measured urea production. Table 3 depicts the cumulative urea production (urinary excretion corrected for changes in body pool) over the entire period of urea infusion. For the step infusion, urea production over the 4-h unlabeled urea infusion was 73% of the true urea input into the body during that period (P < 0.001). For the pulse infusion, cumulative urea production over the 8-h unlabeled urea infusion was 83% of known total urea input during that period (P = 0.012). These results ignore nonurinary losses from body water. When these were corrected for by dividing the values by fractional tracer urea recovery in urine and plasma (see METHODS), cumulative urea production during the urea infusions was 85% (P = 0.03) and 93% (NS) of the true input rate for the step and pulse infusions, respectively (Table 3).

Alanine infusion. Alanine concentrations increased from 0.24 ± 0.02 to 2.89 ± 0.23 mmol/l within 90 min of the start of the primed continuous alanine infusion and remained constant thereafter (Fig. 8). Plasma glucose remained constant at 5.0 ± 0.02 mmol/l throughout the alanine infusion. Plasma leucine, isoleucine, and valine concentrations did not change (data not shown). The alanine infusion induced a steady rise in plasma urea concentration (5.88 ± 0.40 mmol/l at 5 h of alanine infusion) similar to the slower increase observed with the step urea infusion (4.97 ± 0.40 mmol/l at 5 h of step urea infusion; Fig. 9). This steeper increase corresponded to a larger amount of urea precursor than the urea infused in the step urea infusion. Nonisotopic urea production increased in a manner similar to the step

Table 2. Urea Ra measured using different methods

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Step</th>
<th>Pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hour 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i/TTR</td>
<td>181 ± 16</td>
<td>221 ± 18</td>
<td>193 ± 22</td>
</tr>
<tr>
<td>Nonsteady</td>
<td>167 ± 33</td>
<td>189 ± 29</td>
<td>170 ± 9</td>
</tr>
<tr>
<td>Nonisotopic</td>
<td>128 ± 7</td>
<td>158 ± 30a</td>
<td>121 ± 15a</td>
</tr>
<tr>
<td>(71 ± 3)b</td>
<td>(69 ± 9)b</td>
<td>(63 ± 5)b</td>
<td></td>
</tr>
<tr>
<td>Hour 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True</td>
<td>—</td>
<td>553 ± 16</td>
<td>516 ± 18</td>
</tr>
<tr>
<td>i/TTR</td>
<td>176 ± 23</td>
<td>294 ± 18c</td>
<td>281 ± 15c</td>
</tr>
<tr>
<td>Nonsteady</td>
<td>167 ± 40</td>
<td>421 ± 15ac</td>
<td>346 ± 22ac</td>
</tr>
<tr>
<td>Nonisotopic</td>
<td>124 ± 17</td>
<td>394 ± 21ac</td>
<td>463 ± 16ac</td>
</tr>
<tr>
<td>(73 ± 10)c</td>
<td>(71 ± 4)c</td>
<td>(90 ± 5)</td>
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</tr>
<tr>
<td>Hour 12</td>
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<tr>
<td>True</td>
<td>—</td>
<td>553 ± 16</td>
<td>193 ± 22</td>
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<tr>
<td>i/TTR</td>
<td>175 ± 33</td>
<td>340 ± 14c</td>
<td>246 ± 24</td>
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<tr>
<td>Nonsteady</td>
<td>171 ± 49</td>
<td>431 ± 26ac</td>
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<tr>
<td>Nonisotopic</td>
<td>99 ± 16</td>
<td>400 ± 15ac</td>
<td>174 ± 31</td>
</tr>
<tr>
<td>(60 ± 10)b</td>
<td>(72 ± 2)c</td>
<td>(89 ± 13)</td>
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</table>

Data are presented as means ± SE; n = 6 subjects. Units for urea production rate (Ra) are µmol·kg⁻¹·h⁻¹. Urea was infused at 333 µmol·kg⁻¹·h⁻¹ for 8 h (Step) or 4 h (Pulse). "True" urea Ra is the sum of urea infused plus steady-state Ra determined at hour 4 of the basal condition [tracer infusion rate (i) divided by tracer-to-tracee ratio (TTR)]. Data in parentheses are % of true urea Ra. P < 0.05: a significantly different from i/TTR; b significantly different from 100%; c significantly different from true; d significantly different from control; e significantly different from nonsteady; f significantly different from step.

Cumulative nonisotopically measured urea production. Table 3 depicts the cumulative urea production (urinary excretion corrected for changes in body pool) over the entire period of urea infusion. For the step infusion, urea production over the 4 h of unlabeled urea infusion was 73% of the true urea input into the body during that period (P < 0.001). For the pulse infusion, cumulative urea production over the 8 h of unlabeled urea infusion was 83% of known total urea input during that period (P = 0.012). These results ignore nonurinary losses from body water. When these were corrected for by dividing the values by fractional tracer urea recovery in urine and plasma (see METHODS), cumulative urea production during the urea infusions was 85% (P = 0.03) and 93% (NS) of the true input rate for the step and pulse infusions, respectively (Table 3).

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urea infusion, with i/TTR slowly increasing over the 5-h unlabeled alanine infusion (Fig. 10). Both the nonisotopic and Steele's non-steady-state calculations depicted an abrupt increase in urea appearance rate and an apparently constant urea Ra within 2 h of alanine infusion, as previously described (27).

DISCUSSION

We tested the accuracy of measuring steady-state urea Ra after a 4-h primed continuous tracer infusion as well as the accuracy of this method to determine changes in whole body urea appearance produced by intravenous urea infusions that simulated postprandial changes in urea production. We also measured the short-term effect on urea Ra of infusing alanine, a precursor for urea synthesis.

When only tracer urea was infused, there was no difference between the steady and nonsteady tracer calculations of urea Ra as observed over 12 h. The steady-state Ra at hour 4 was therefore accepted as a valid measure of endogenous urea Ra when no perturbations in urea metabolism occur, in agreement with an earlier conclusion by Jahoor and Wolfe (11). The nonisotopic Ra was 28% less than the true Ra, also as reported by other researchers (13, 16).

When urea was infused to simulate a single meal (pulse infusion) or nibbling for 8 h (step infusion), i/TTR increased only slowly so that the Ra indicated by the steady-state calculation was only 61 and 54% of the true Ra after 8 h (step) and 4 h (pulse) of true urea input, respectively (both \( P < 0.05 \)). This failure to observe an isotopic steady state even 8 h after urea input increased can be explained by urea's large pool size and slow fractional turnover rate, \( \sim 9\%/h \) (4, 24).

However, Steele's non-steady-state equation and the nontracer method also underestimated the true urea rate of appearance, although to a lesser degree (Table 2).

It should be noted that our calculations assume that the unlabeled urea infusion did not change endogenous urea Ra. It is possible that the urea infusion increased endogenous urea synthesis by increasing urea recycling. If this occurred, it would mean that the tracer and nonisotopic methods underestimate changes in

| Table 3. Cumulative nonisotopic urea production for the step and pulse urea infusions |
|-----------------|-----------------|
|                 | Step, mmol·kg\(^{-1}\)·8 h\(^{-1}\) | Pulse, mmol·kg\(^{-1}\)·4 h\(^{-1}\) |
| Urea input      | 4.50 ± 0.13     | 2.10 ± 0.07     |
| Urea produced   | 3.29 ± 0.18\(^a\) | 1.73 ± 0.07\(^a\) | 
| (73.2 ± 3.8)\(^b\) | (82.8 ± 4.5)\(^b\)  |
| Corrected urea produced | 3.81 ± 0.23\(^a\) | 1.95 ± 0.17 \|
| (84.9 ± 5.3)\(^b\) | (92.8 ± 8.0)\(^b\) |

Data are presented as means ± SE; \( n = 6 \) subjects. Urea input is the sum of endogenous and exogenous sources. Urea production is the sum of urinary excretion and increase in urea in body water. Corrected urea production is urea produced divided by the fraction of \(^{13}\)C urea infused over the measurement period that was recovered in urine and body water. Data in parentheses are % of input. \( P < 0.05: \(^a\) significantly different from urea input; \(^b\) significantly different from 100%.


urea production to an even greater extent than the present results indicate.

Cumulative urea production, measured nonisotopically as urinary excretion plus increase in body urea, was 73% (P < 0.05) of the true urea input over the 8-h step urea infusion and 83% (P < 0.05) of true urea input over the 4-h pulse urea infusion. However, this calculation ignores nonurinary urea losses. Correcting cumulative urea productions for their corresponding tracer urea recoveries improved these estimates to 85% (P < 0.05) and 93% (NS) of true urea input for the step and pulse infusions, respectively (Table 3).

During the alanine infusion, plasma alanine levels were constant over the ninth hour of alanine infusion, indicating that alanine was being disposed of at the rate it was being administered. When given alone, alanine would not be expected to stimulate protein synthesis (as confirmed by the unchanging branched-chain amino acid concentrations), so it may be assumed that alanine N was also being eliminated at the rate it was being administered. If all alanine N was converted to urea, the nonisotopic and non-steady-state calculations at hour 9 underestimated true urea $R_a$ by 40 and 39% (both P < 0.001), respectively. This is close to the extent by which these methods underestimated true urea $R_a$ during the urea infusions, strongly suggesting that urea production after alanine administration is also underestimated when both isotopic and nonisotopic approaches are used (27). In an earlier study in which alanine was infused at the same rate as in this study, urea $R_a$ accounted for an even smaller fraction of the total alanine N infused than we found (27). We therefore conclude that true urea $R_a$ is significantly underestimated even by the non-steady-state tracer method during acute administration of a urea precursor.

The present conclusions are important for interpreting the results of studies in which urea $R_a$ was measured to determine the effect of an acute intervention, such as exercise, feeding, or hormone administration (1, 2, 5–7, 26, 27). The failure to observe an increase in urea $R_a$ during acute exercise, despite increased amino acid oxidation, is consistent with insensitivity of the model to detect acute increases in urea $R_a$ (2, 26). In feeding studies (5, 6), the increase in urea $R_a$ reported to occur in the fed state using continuous [15N2]urea infusion might have underestimated a true fed state increase in urea $R_a$, resulting in an underestimate of urea recycling.

We conclude that the primed continuous tracer urea infusion method can provide a valid measure of urea $R_a$ in a 4-h study, but only under basal conditions. The method is insensitive for detecting changes in urea $R_a$, even 8 h after urea $R_a$ increases, when either non-steady-state kinetics or nonisotopically measured urea excretion is used to measure it. The accuracy of the nonisotopic measurement is improved if corrections are made to account for nonurinary urea losses by measuring recovery in urine and plasma of the dose of tracer urea introduced into the body over the period of analysis.

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