Use of sulfate production as a measure of short-term sulfur amino acid catabolism in humans

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Hamadeh, Mazen J., and L. John Hoffer. Use of sulfate production as a measure of short-term sulfur amino acid catabolism in humans. Am J Physiol Endocrinol Metab 280: E857–E866, 2001.—There is no fully satisfactory method for measuring amino acid catabolism in the nonsteady state that follows normal protein consumption. Because sulfate is the major product of sulfur amino acid catabolism, we tested whether its production can be accurately depicted using simple tracer or nontracer approaches under basal conditions and after the intravenous administration of a known amount of sulfate. In the basal postabsorptive state, serum sulfate concentration and urinary sulfate excretion remained constant for many hours, but the apparent steady-state serum sulfate rate of appearance achieved with primed continuous infusion of methionine in a mixture of other essential amino acids. Sulfate production above basal accounted for 59% over 6 h and 98% over 9 h of the administered dose when measured simply as urinary inorganic sulfate excretion corrected for changes in its extracellular fluid content. Using the latter method, we measured sulfate production after oral methionine and intravenous infusion of methionine in a mixture of other essential amino acids. Sulfate production above basal accounted for 59% over 6 h and 75% over 9 h of the oral methionine dose. Similar results were obtained with the mixed amino acid infusion, but interpretation of the latter experiment was limited by the mild protein sparing (and, hence, reduced endogenous sulfate production) induced by the amino acid infusion. We conclude that a simple nontracer method can provide an accurate measure of sulfate production and, hence, sulfur amino acid catabolism over collection periods as short as 6 h after a test meal. A significant portion of the sulfate derived from methionine appears to be retained in nonprotein compounds immediately after its ingestion.

stable isotope; amino acid oxidation; methionine; fed state

THE BODY’S ALLOCATION of dietary amino acids for catabolism or protein synthesis takes place largely in the fed state. For this reason, accurate measurement of fed-state amino acid catabolism is important for understanding the factors regulating whole body protein economy (13, 35, 38, 40, 48, 49). Both urea production and measures of essential amino acid (usually leucine) oxidation have been used for this purpose. However, the amino acid oxidation technique requires an accurate measurement of expired tracer carbon dioxide, and we have recently shown that short-term changes in urea production, as typically occur in the normal fed state, may not be reliably depicted by either tracer or nontracer methods (20). Sulfate production is a potentially useful adjunct to urea production and essential amino acid oxidation when whole body amino acid catabolism is measured.

Catabolism of the sulfur amino acids (SAA) methionine, cysteine, and cystine leads predominantly to the production of sulfate, since mercaptolactate, mercaptoacetate, thiocyanate, thiosulfate, and taurine contribute only very small amounts to the total (33, 34, 45). When measured over 24-h periods, urinary sulfate excretion closely matches both dietary SAA intake (44, 54) and N excretion (24, 28, 54). However, it has not been determined whether sulfate production can be measured accurately over periods of only a few hours, such as the hours after a typical meal. Short-term sulfate kinetics might be more accurately determined than those of urea, because unlike urea, which distributes throughout total body water (TBW), sulfate distributes in the considerably smaller extracellular fluid volume (ECF) (43); moreover, unlike urea, it is not subject to important losses in the gut (1, 7, 37, 43).

The present research was carried out to determine whether tracer and nontracer methods accurately depict sulfate production under basal steady-state conditions and after the change in sulfate production created by a sulfate infusion designed to mimic the amount of sulfate that would be produced after a typical protein meal. Specifically, we measured basal sulfate rate of appearance ($R_s$) by use of primed continuous oral administration of sodium $^{[34]S}$sulfate and compared this result with a nontracer technique based on urinary excretion. We determined the accuracy of the nontracer method to detect sulfate production after intravenous administration of a known amount of magnesium sulfate. We tested whether basal sulfate pro-
duction, as measured over a 3-h baseline period, is an accurate predictor of sulfate production over the subsequent 9 h in subjects whose recent previous protein intake was low or high. We measured sulfate production after oral administration of methionine and the intravenous infusion of a mixture of essential amino acids containing methionine under conditions in which baseline endogenous SAA catabolism would be expected to be constant or nearly constant. This was done to determine whether exogenous surfeit methionine is completely catabolized to sulfate within a short time after ingestion or whether its S is retained in a non-protein form in the body. The measurement periods were both 6 and 9 h, with the aim of finding the most convenient valid collection periods for future studies.

METHODS

Subjects and Protocols

Eleven healthy men with normal serum biochemistries and taking no medications participated in six study protocols. Their physical characteristics are presented in Table 1. When the same subject was studied more than once, at least 6 wk separated testing days. All volunteers provided written consent for the study, which was approved by the Research and Ethics Committee of the Jewish General Hospital in Montreal.

The subjects were admitted to the clinical research unit at 0800 one day before the study day. Breakfast (0830), lunch (1230), and dinner (1730) at maintenance energy (38 kcal/kg body wt) were consumed after anthropometric measurements. When used, a low-protein diet (0.3 g/kg body wt) were consumed after anthropometric measurements. When used, a low-protein diet (0.3 g/kg body wt) was administered after 1 day of fasting (fasting-HP). Such constancy would justify using the value of sulfate production measured over a 9-h baseline period as an accurate predictor of endogenous sulfate production over the subsequent 9 h in subjects with a wide range of previous habitual protein intakes. For these studies, the protein intake on the day before the study was 1.5 g/kg body wt, a customary surfeit level for healthy young men (19).

Three methionine administration studies were carried out. In a dose-finding study (high-dose methionine), 0.45 mmol/kg of methionine was taken orally. In the two subsequent studies, 0.15 mmol/kg of methionine was administered after 1 day of a normal protein diet (1.5 g·kg body wt⁻¹·day⁻¹), either as oral methionine taken alone or as intravenous methionine within a mixture of nonsulfur essential amino acids.

Model Validation Studies

Fasting-LP study. After a priming dose of 0.5 mg/kg body wt (3.47 μmol/kg), 0.05 mg·kg⁻¹·h⁻¹ of anhydrous sodium [³⁴S]sulfate (99% ³⁴S, Isoflex USA, San Francisco, CA) in water was administered by mouth every 30 min for 9 h to measure endogenous sulfate Rₚ and to determine whether steady-state sulfate Rₚ would agree with nontracer-determined sulfate production as measured using hourly urinary sulfate excretion corrected for changes in ECF sulfate.

Sulfate infusion study. Endogenous sulfate production was measured using the nontracer method for 3 h, after which 0.15 mmol/kg of MgSO₄·7H₂O (Sabex, Boucherville, QC, Canada) was infused. A Harvard Syringe Infusion Pump 681 E (Harvard Apparatus, Milford, MA) was adjusted to administer 60 μmol·kg⁻¹·h⁻¹ MgSO₄ for 2.5 h from a stock solution with a concentration of 400 μmol/ml. The amount of magne-

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Sample size, n</th>
<th>Fasting-LP</th>
<th>Fasting-HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>33 ± 5</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>73 ± 4</td>
<td>77 ± 3</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174 ± 5</td>
<td>179 ± 2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24 ± 1</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>TBW, %body wt</td>
<td>57 ± 1</td>
<td>56 ± 2</td>
</tr>
<tr>
<td>FFM, %body wt</td>
<td>78 ± 1</td>
<td>77 ± 2</td>
</tr>
<tr>
<td>CBS, mg/kg</td>
<td>210 ± 6</td>
<td>205 ± 9</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Data were not significantly different between groups. Fasting-LP, 1-day restricted protein intake; fasting-HP, nonrestricted protein intake; BMI, body mass index; TBW, total body water; FFM, fat-free mass; CBS, corrected bromide space.
sium sulfate infused was verified by weighing the infusate container before and after each infusion. Urine was collected hourly for the first 3 h and in two separate collections thereafter (hours 4–9 and 10–12).

**Fasting-HP study.** Serum sulfate concentration and urinary sulfate excretion were measured over 12 h with hourly urine collections.

**Methionine Administration Studies**

**Oral methionine.** Basal sulfate production was measured for 3 h, after which subjects consumed a “high dose” 0.45 mmol/kg (67 mg/kg) or a “low dose” 0.15 mmol/kg (22 mg/kg) of methionine in water over 30 min (Tanabe USA, San Diego, CA).

**Amino acid infusion study.** Basal sulfate production was measured for 3 h, after which 0.15 mmol/kg of methionine was administered intravenously at a constant rate as AMINOSYN-RP 5.2% (Ross Laboratories, St. Laurent, QC, Canada) over 2 h. AMINOSYN-RP 5.2% contains (mg in/100 ml): 462 isoleucine, 726 leucine, 535 lysine, 726 methionine, 726 phenylalanine, 330 threonine, 165 tryptophan, 528 valine, 600 arginine, and 429 histidine. The product was infused using a Baxter Flo-Gard volumetric infusion pump adjusted to administer 11.2 mg of methionine·kg⁻¹·h⁻¹ (75 μmol·kg⁻¹·h⁻¹) for 2 h from a stock solution with a concentration of 726 mg/100 ml (4.87 mmol/100 ml). The amount infused was verified by weighing the infusate container before and after each infusion. For the methionine administration studies, urine was collected hourly for the first 3 h and in two separate collections thereafter (hours 4–9 and 10–12).

**Analytical Methods**

**Inorganic sulfate.** Unless referred to otherwise, all sulfate measurements refer to the inorganic sulfate anion. Blood collection tubes were centrifuged at 1,400 g for 30 min, and the serum was transferred into screw-cap vials and stored at −30°C. Serum and urine were analyzed by ion exchange high-performance chromatography with conductivity detection (IEC-CD) using a Dionex 2110i chromatography system (Dionex, Sunnyvale, CA) for inorganic sulfate and bromide concentrations, as previously described (5, 21). Serum was diluted 10-fold with water and passed through a 10,000-Da molecular mass cutoff Amicon filter (Beverley, MA) by centrifugation (1,400 g), and the filtrate was injected into the IEC-CD. Urine was diluted 50-fold, filtered through 0.45-μm syringe filters, and injected into the IEC-CD. Samples were analyzed in triplicate.

**34SO4/32SO4 enrichment.** **34SO4/32SO4 enrichment** was measured by electrospray tandem mass spectrometry (ESI-MS/MS), as previously described (5). In brief, 1.0 ml of serum was mixed with 0.5 ml of water and 5.0 ml of ice-cold methanol, kept on ice for 10 min, and then centrifuged at 1,400 g for 10 min at 4°C. The supernatant was acidified with 0.1 ml of 1 M HCl to remove bicarbonate anions and then passed through an On-Guard-Ag cartridge (Dionex, Oakville, ON, Canada) to remove inorganic anion other than sulfate. The first 3 ml of filtrate were discarded, and the rest was saved for analysis.

ESI-MS/MS analyses were performed using a Quattro II triple quadrupole (Micromass, Manchester, UK) configured for negative ion analysis. Samples were introduced directly into the electrospray probe at 40 μl/min with a 1.0 mA disposable syringe under the following conditions: neutral loss mode, 17 Da; range, 92–102 Da; cone voltage, 25 V; source temperature, 120°C; sample infusion pump, 40 μl/min; nitrogen bath gas flow rate, 300 l/h; nebulizer gas flow rate, 18 l/h; collision cell energy, 23 eV; and argon pressure in the collision cell, 130 Pa. Acquisitions of 2 min each were made in triplicate in multichannel acquisition mode. The following ions were monitored: H32SO4⁻ [mass-to-charge ratio (m/z) 97], and H34SO4⁻ (m/z 99).

To measure sulfate enrichment due to the administered tracer, a calibration curve was constructed by preparing varying mole ratios of 34SO4/32SO4, as described by Tserng and Kalhan (50).

**Organic (ester) sulfate.** Inorganic sulfate was eliminated from urine samples by barium chloride precipitation, as described by Lundquist et al. (32). Three milliliters of a solution of 0.977 g BaCl2·2H2O and 4.1 ml concentrated HCl in 100 ml were mixed with 1.5 ml urine, centrifuged at 1,400 g for 10 min at 20°C, and 1.5 ml of the supernatant were applied to 1-ml columns of cation exchange resin (Dowex 50W-X8, 100–200 mesh hydrogen form, Bio-Rad Laboratories, Richmond, CA). Water (2.5 ml) was added, and the eluate was collected in screw-cap glass tubes. The sealed tubes were heated at 100°C for 30 min in a water bath to release organic sulfate esters (32), and 0.5 ml of the resulting solution was diluted 10-fold. To 1 ml of this diluted solution, 5 ml of ice-cold methanol were added, and sulfate was measured.

**Other measurements.** Plasma amino acid concentrations were measured by HPLC after derivatization with phenylisothiocyanate (42). Serum insulin was measured using a human insulin-specific radioimmunoassay kit (catalog no. HI-14K, Linco Research, St. Charles, MO). Samples were counted using a Cobra II Auto-Gamma (model D5002, Canberra-Pakard Canada, St. Laurent, QC, Canada/Pakard Instrument, Downers Grove, IL). Serum and urine urea and creatinine were analyzed using a Hitachi 917 automated analyzer (Laval, QC, Canada).

**Calculations**

**Sulfate Ra, enrichment, and recovery.** Sulfate Ra was calculated as i/TTR, where i is the rate of tracer sulfate administration in micromoles per kilogram per hour. The tracer-to-tracee ratio (TTR) was calculated by subtracting the baseline mole ratio from the sample mole ratio

\[
TTR = \left[ \frac{M}{2} + \frac{M}{2} \right]_{\text{sample}} - \left[ \frac{M}{2} + \frac{M}{2} \right]_{\text{baseline}}
\]

where \( M \) is the signal intensity of m/z 97, and \( M+2 \) is the signal intensity of m/z 99.

Serum inorganic 34SO4 concentration was calculated as

\[
[34\text{SO}_4] = \frac{[\text{SO}_4] \cdot TTR}{1 + TTR}
\]

where \([34\text{SO}_4]\) is inorganic 34SO4 concentration in micromoles per liter, \([\text{SO}_4]\) is the concentration of inorganic sulfate as measured by IEC-CD in micromoles per liter, and TTR is the tracer-to-tracee ratio.

The nontracer method calculates cumulative inorganic sulfate production between time \( t_0 \) and \( t \) as its urinary excretion rate corrected for a change in ECF sulfate over this period cumulative SO4 production between times \( t_0 \) and \( t \)

\[
\text{urinary SO}_4\text{ excretion over that period} + ([\text{SO}_4]_t - [\text{SO}_4]_{t_0}) \cdot \text{ECF}
\]

When measured hourly, urinary sulfate excretion was normalized for urinary creatinine excretion to correct for incomplete bladder emptying, as described by Cheema-Dhadli and Halperin (8). The studies to measure the recovery of infused intravenous magnesium sulfate as sulfate production and the
increase in sulfate production after methionine administration had to take into account on-going sulfate production from the catabolism of endogenous SAA. Thus sulfate production due to exogenous methionine equals total sulfate production minus sulfate production due to endogenous SAA catabolism occurring over the same period. To do this, we measured endogenous sulfate production over a 3-h baseline period before administering the test dose and extrapolated that value to the subsequent 6 or 9 h. This assumes that 1) basal sulfate production is constant over a 6- or 9-h collection period, and 2) the test substrate does not alter endogenous sulfate production by increasing or decreasing proteolysis. The first assumption was tested in the fasting studies in which basal sulfate production was monitored for 9 or 12 h the morning after consumption of low- or conventional-protein diets; the second was indirectly tested by measuring urea N balance.

Urea production. Urea production was calculated as its urinary excretion rate corrected for changes in total body urea, as in the aforementioned equation for sulfate production (8, 18, 20, 25).

TBW and CBS. Body electrical resistance was measured after the patient voided and before placement of the intravenous catheters. TBW was then calculated using the equation of Kushner and Schoeller (27). For the fasting-LP study, sodium bromide was given orally at time 0. For the other studies, it was given at hour 6 and serum and urinary bromide measured at hour 9. The CBS was calculated as described by Vaisman et al. (51)

$$\text{CBS} = \frac{[\text{Br dose} - \text{Br excreted}] \cdot 0.90 \cdot 0.95 \cdot 0.94}{[\text{Br}]_{hs} - [\text{Br}]_{hs}}$$

where CBS is in liters, the Br dose is in millimoles, Br excreted is millimoles of Br excreted over 3 h, [Br]_{hs} is the serum Br concentration at hour 9 in millimoles per liter, [Br]_{hs} is the baseline serum Br concentration in millimoles per liter, 0.90 is the correction factor for the nonextracellular distribution of Br, 0.95 is the correction factor for the Donnan equilibrium, and 0.94 is the correction factor for the water content of serum.

Statistical Analyses

One-way analysis of variance (ANOVA) was used to determine significant difference in subject characteristics between different protocols (Table 1). One-way repeated-measures ANOVA was used to determine significant difference over time within the same protocol (Figs. 1, 2, 3, and 6). Two-way repeated-measures ANOVA was used to determine significant difference in sulfate production rate (time vs. method of measurement) for the fasting-LP study (Fig. 1) and in serum sulfate and insulin (time vs. method of methionine administration) between the oral methionine and amino acid infusion studies (Figs. 5 and 7). When ANOVA results showed significance, the Newman-Keuls test was used post hoc to determine the source of difference. The unpaired t-test was used to compare data between the oral methionine and amino acid infusion studies (Tables 3 and 4). Differences between data were considered significant at $P < 0.05$. All results are presented as means ± SE.

RESULTS

Average postabsorptive serum sulfate concentrations ranged between 250 and 330 μmol/l for the different protocols, similar to previously reported values for healthy normal humans (8, 11, 22).

Model Validation Studies

Fasting-LP study. When studied in the postabsorptive state and having consumed a low-protein diet the previous day, normal subjects maintained a constant serum sulfate concentration (Fig. 1). Urinary sulfate excretion over the final 6 h was $8.3 ± 5.8\%$ less than during the initial 3 h, but this difference was not statistically significant ($P = 0.23$).

Serum $^{34}\text{SO}_4$ enrichment reached apparent steady state very soon after the primed continuous tracer administration commenced (Fig. 1). However, the sulfate Rp calculated from these enrichments was $20 ± 2\%$ higher than that indicated by urinary sulfate excretion ($P = 0.006$).

To investigate the reason for this, we measured the $^{34}\text{SO}_4$ enrichment in organic sulfate esters in the urine.
and found that it appeared rapidly in this fraction and increased over subsequent hours but did not reach equilibrium with the inorganic sulfate pool over the time course of the study (Fig. 2). Sulfate ester excretion was constant (0.99 ± 0.05 μmol·kg⁻¹·h⁻¹) as measured during hours 3, 6, and 9, representing 15% of total urinary sulfate excretion.

**Sulfate infusion study.** The serum sulfate concentration increased 2.4-fold during the magnesium sulfate infusion and then returned to near baseline by hour 12 (Fig. 3). If it is assumed that basal sulfate production over the 3-h baseline period remained constant over the subsequent 9 h, recovery of the infused sulfate in urine and ECF accounted for 95.4% over 6 h and 97.9% over 9 h after the start of the infusion [both nonsignificant (NS) from 100%; see Table 2]. Going by the results of the fasting-LP study, we could not rule out a slight decrease in endogenous sulfate production over this time period. If correction is made for this, however, recovery of the infused sulfate improves to 97.3% over the 6-h and 100.6% over the 9-h collection periods.

**Fasting-HP study.** Serum sulfate concentrations and urinary sulfate excretion remained constant over the 12-h observation period when the previous diet was high in protein (Fig. 4). Urinary sulfate excretion over the final 6 h (3.6 ± 0.4 mmol) or 9 h (5.3 ± 0.6 mmol) was closely similar to the value predicted from sulfate excretion over the initial 3 h (3.6 ± 0.5 mmol/6 h; 5.4 ± 0.7 mmol/9 h).

**Table 2. Cumulative sulfate production over 6 and 9 h after administration of sulfate or methionine**

<table>
<thead>
<tr>
<th></th>
<th>Sulfate Infusion</th>
<th>Oral Methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td>S input (A)</td>
<td>141.5 ± 6.8</td>
<td>150.3 ± 0.8</td>
</tr>
<tr>
<td>Basal sulfate production normalized (B)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>to 6 h</td>
<td>33.9 ± 1.9</td>
<td>50.8 ± 4.0</td>
</tr>
<tr>
<td>to 9 h</td>
<td>50.8 ± 2.8</td>
<td>76.2 ± 6.0</td>
</tr>
<tr>
<td>Total sulfate produced (C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>per 6 h</td>
<td>168.9 ± 7.6</td>
<td>140.2 ± 9.4</td>
</tr>
<tr>
<td>per 9 h</td>
<td>189.2 ± 7.5</td>
<td>189.3 ± 10.9</td>
</tr>
<tr>
<td>Sulfate production above basal (C - B - D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>per 6 h</td>
<td>135.0 ± 7.9</td>
<td>89.4 ± 6.4</td>
</tr>
<tr>
<td>per 9 h</td>
<td>138.4 ± 7.1</td>
<td>113.1 ± 6.4</td>
</tr>
<tr>
<td>Exogenous S retained (A - D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>per 6 h</td>
<td>6.5 ± 3.4</td>
<td>60.9 ± 5.6</td>
</tr>
<tr>
<td>per 9 h</td>
<td>3.1 ± 2.6</td>
<td>37.2 ± 5.7</td>
</tr>
<tr>
<td>Sulfate recovery, % (D/A) x 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>per 6 h</td>
<td>95.4 ± 2.4</td>
<td>59.4 ± 3.9*</td>
</tr>
<tr>
<td>per 9 h</td>
<td>97.9 ± 1.8</td>
<td>75.2 ± 3.9*</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE (in μmol/kg). *Statistically different from 100%.

**Methionine Administration Studies**

**High-dose (0.45 mmollkg) oral methionine.** The serum methionine concentration increased 29-fold (P < 0.0001) within 1 h after methionine administration and was still about sixfold higher than baseline at hour 12, indicating incomplete methionine absorption by the gut and/or incomplete tissue metabolism during the 9 h after its consumption. The serum sulfate concentration increased about twofold over the 7 h after methionine administration and was still at approximately this level (Fig. 5).
level at hour 12 ($P < 0.0001$). The increase in sulfate production above its endogenous level accounted for 36.8 ± 6.4% (at 6 h) and 54.9 ± 6.4% (at 9 h) of the S in the administered methionine. In subsequent studies, the methionine dose was reduced to 0.15 mmol/kg.

Oral methionine and methionine plus mixed amino acid infusion. Oral and intravenous administration of 0.15 mmol/kg methionine resulted in a similar serum sulfate response, with an initial 1.5-fold increase followed by a gradual decrease toward baseline (Fig. 5). Oral methionine increased serum methionine 7.6-fold 1 h after its administration (Fig. 6); alanine, aspartate, glutamate, serine, threonine, leucine, isoleucine, and valine concentrations changed minimally. The mixed amino acid infusion transiently increased serum methionine, threonine, leucine, isoleucine, and valine concentrations (Fig. 6), whereas alanine, aspartate, glutamate, and serine concentrations did not increase.

The increase in sulfate production above baseline accounted for 59% (at 6 h) and 75% (at 9 h) of the S in the administered methionine (Table 2). Whole body S balances were insignificantly different for both methionine administration methods (Table 3), but urea N balances were slightly less negative for the mixed amino acid infusion (Table 4). Unlike methionine alone, the intravenous amino acids stimulated insulin secretion (Fig. 7). Figure 8 illustrates directional similarity between 9-h urea N and S balances in individual subjects.

**DISCUSSION**

This study evaluated the use of sulfate production as an indicator of whole body SAA catabolism so as to be able to examine nutritional factors regulating it with short, clinically practical protocols. We measured sulfate R in under basal conditions by use of a primed continuous oral administration of sodium $^{34}$S sulfate; we also measured nontracer sulfate production after administering known amounts of intravenous sulfate, oral methionine, and an intravenous mixture of essential amino acids including methionine.

To test whether the tracer method accurately depicts sulfate production, we compared the serum sulfate R in indicated from a primed continuous oral administration of sodium $^{34}$S sulfate; we also measured nontracer sulfate production after administering known amounts of intravenous sulfate, oral methionine, and an intravenous mixture of essential amino acids including methionine.

Data are presented as means ± SE. S in, sulfur input; S out, sulfur output. S out was measured as sulfate production. There were no significant differences between corresponding mean values in the oral methionine and amino acid infusion studies.

**Table 3. Cumulative sulfur balance over 6 and 9 h after administration of methionine**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Oral Methionine</th>
<th>Amino Acid Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>S in (A) 150.3 ± 0.8</td>
<td>158.2 ± 3.9</td>
<td></td>
</tr>
<tr>
<td>S out (B) 140.2 ± 9.4</td>
<td>134.7 ± 22.6</td>
<td></td>
</tr>
<tr>
<td>per 6 h 189.3 ± 10.9</td>
<td>181.8 ± 22.4</td>
<td></td>
</tr>
<tr>
<td>per 9 h 10.1 ± 8.8</td>
<td>23.5 ± 7.2</td>
<td></td>
</tr>
<tr>
<td>S balance (A − B) 110.1 ± 8.8</td>
<td>23.5 ± 7.2</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Cumulative sulfur balance over 6 and 9 h after administration of methionine
fate production after intravenous administration of a known, physiologically relevant amount of magnesium sulfate. Baseline sulfate production, as measured over a 3-h baseline period, accurately predicted basal production over the subsequent 6 or 9 h when the preceding diet was either normal or low in protein. Using this assumption, we calculated sulfate production above basal after methionine administration. If it can be assumed that this treatment has little effect to increase or decrease net body protein balance, then a considerable portion of the S in the administered methionine appeared to be retained in a nonprotein form.

Sulfate production could provide a useful measure of short-term SAA catabolism. Urea production and tracer-determined amino acid oxidation are commonly used to measure whole body amino acid catabolism over short periods of time (3, 6, 13, 16, 14, 40, 53), but these methods have disadvantages. First, 15–30% of synthesized urea is not excreted directly into the urine but is hydrolyzed in the gut with partial recycling of the resulting ammonia for new urea synthesis (23, 30, 52). Second, the plasma urea concentration tends to change under basal and fed conditions, and because body water is a large pool, small analytical errors in the plasma urea measurement can lead to large errors in apparent urea retention or excretion. Under steady-state conditions, plasma urea turnover can be accurately measured using the technique of primed continuous tracer infusion, but both this method and the nontracer approach are unreliable during non-steady-state conditions that are of particular physiological interest (20, 47). There are theoretical advantages to using sulfate to measure SAA catabolism in non-steady-state situations. Like urea, sulfate is excreted in the urine, but unlike urea, it distributes in a smaller body compartment (the ECF) and does not appear to be subject to important losses in the gut (1, 7, 37, 43).

Sulfate production could provide a useful measure of short-term SAA catabolism. Urea production and tracer-determined amino acid oxidation are commonly used to measure whole body amino acid catabolism over short periods of time (3, 6, 13, 14, 40, 53), but these methods have disadvantages. First, 15–30% of synthesized urea is not excreted directly into the urine but is hydrolyzed in the gut with partial recycling of the resulting ammonia for new urea synthesis (23, 30, 52). Second, the plasma urea concentration tends to change under basal and fed conditions, and because body water is a large pool, small analytical errors in the plasma urea measurement can lead to large errors in apparent urea retention or excretion. Under steady-state conditions, plasma urea turnover can be accurately measured using the technique of primed continuous tracer infusion, but both this method and the nontracer approach are unreliable during non-steady-state conditions that are of particular physiological interest (20, 47). There are theoretical advantages to using sulfate to measure SAA catabolism in non-steady-state situations. Like urea, sulfate is excreted in the urine, but unlike urea, it distributes in a smaller body compartment (the ECF) and does not appear to be subject to important losses in the gut (1, 7, 37, 43).

The tracer approach that we adopted for this study was modeled after the primed continuous infusion urea tracer method and appeared to indicate rapid attainment of steady state (Fig. 1). However, the resulting sulfate Ra was significantly higher than the sulfate production rate indicated by the nontracer method. More than one factor could account for this. First, in the interest of developing the most practical and convenient model, we administered the tracer orally, and it could have been incompletely absorbed from the gut. We consider this unlikely, because small oral doses of sodium sulfate are well absorbed (10, 15, 26). In light of the present findings, however, future studies are indicated using intravenous tracer.

We detected $^{34}$SO$_4$ enrichment in urinary sulfate esters within a short time after commencing tracer administration (Fig. 2). Sulfate esters make up a relatively constant and small (9–15%) fraction of total sulfate excretion (2, 34, 33); they have been thought to arise mainly in the large intestine from conjugation of sulfate with phenolic compounds formed by bacterial action on aromatic amino acids (32). This last assumption warrants reexamination. The $^{34}$S-enriched ester sulfate detected in our study is unlikely to have arisen as a result of colonic bacterial action because of its rapid appearance and in light of recent data indicating that sulfate-metabolizing intestinal bacteria target sulfomucins rather than exogenous sulfate (12).

Partial first-pass splanchnic extraction of the tracer (with release into the circulation as sulfate esters)
could explain the present findings. Another possibility is uptake of sulfate in the general circulation into the liver and other tissues with concurrent release of some of it into the ECF in the form of sulfate esters. The last possibility implies that the apparent steady-state enrichment shown in Fig. 2 is a pseudo-plateau and that it will overestimate sulfate \( R_\text{s} \) until the esterified and inorganic sulfate pools come into isotopic equilibrium, which they clearly did not do over the time course of the present study. Ryan et al. (43) reported that, after intravenous \([35S]\) radiosulfate injection, radioactivity disappears from the plasma faster than it appears in the urine, suggesting the existence of an intracellular sulfate compartment that dynamically exchanges its sulfate with the ECF. The present observation of the prompt appearance of label in sulfate esters supports this possibility.

To determine whether the nontracer method predicts short-term acute changes in sulfate production, an amount of magnesium sulfate corresponding to the amount of amino acid S in a normal meal was administered intravenously after a 3-h baseline period (Fig. 3). Sulfate production after the sulfate infusion was corrected for endogenous sulfate production as extrapolated from sulfate production over this baseline period. Sulfate production above basal accounted for 95% (at 6 h) and 98% (at 9 h) of the sulfate infused (Table 2). This agrees with the 90–100% urinary recovery of sulfate reported by Chakmakjian and Bethune (7) 24 h after intravenous administration of an extremely large dose (362 mmol) of sodium sulfate.

Sulfate production over a 3-h baseline period accurately predicted sulfate production over the subsequent 9 h in men adapted to a normal protein intake (fasting-HP study). Hourly sulfate production was strongly correlated with hourly urinary sulfate excretion \((r = 0.96, \text{slope} = 0.95, P < 0.0001)\), as expected when the serum sulfate concentration is unchanging. Thus, in the subsequent methionine administration studies, baseline sulfate production was used to extrapolate endogenous cumulative sulfate production over the subsequent 6 or 9 h.

Whether or not subjects consumed a low- or high-protein diet the day before they were studied, their serum sulfate concentrations were unchanging over the 9- to 12-h observation period, so there was no need to account for changes in ECF sulfate (Figs. 1 and 4). This suggests that simple urinary sulfate excretion could be used in many settings as a convenient measure of baseline sulfate production. Furthermore, results obtained at hour 6 corresponded well with those obtained at hour 9. Thus acceptably accurate results can be obtained after acute short-term changes in sulfate production in study protocols that last only 6 h, an important practical advantage when carrying out metabolic studies in the clinical setting.

Theoretically, the sulfate space would most accurately depict sulfate distribution. This space is \(\approx 20\%\) smaller than the CBS (21). However, sulfate recovery after magnesium sulfate infusion was most accurate when the CBS was used to estimate the ECF volume. We conclude that the CBS (or even body wt \(\times 0.2\)) (21) provides a suitable and convenient measure of ECF for determining changes in body sulfate content.

Having determined that the nontracer approach can provide accurate short-term information about sulfate production, we investigated what fraction of the S in a dose of methionine can be accounted for as increased sulfate production over the hours after its administration and under conditions in which baseline endogenous SAA catabolism would be expected to be constant or nearly constant. Because sulfate production is a measure of the sum of the catabolism of all of the SAA, any inference about the catabolism of an exogenous dose of methionine requires that endogenous SAA catabolism not be affected by this treatment. A methionine dose of 0.15 mmol/kg was administered after it was found that a 0.45 mmol/kg dose was associated with incomplete absorption and/or tissue metabolism 9 h after consumption. By use of the lower methionine dose, serum methionine concentration returned to the baseline level 9 h after its administration (Fig. 6). The resulting increase in sulfate production accounted for only 59% (at 6 h) and 75% (at 9 h) of the S in the administered methionine (Table 2).

The amount of methionine that remained as methionine after its administration can be presumed to be small. Intracellular and plasma methionine concentrations are not greatly different (4), and if serum methionine is extrapolated to TBW, changes in serum methionine amount to 4.8% of oral methionine over 6 h and 1.7% over 9 h. Because oral methionine was administered alone and did not stimulate insulin secretion, we presume that it effected no change in the negative net body protein balance typical of the postabsorptive state. It is therefore likely that the S retained in the body after oral methionine was stored in nonprotein sulfur-containing compounds such as glutathione, an important cysteine reservoir (9, 17, 46).

We also measured the effect of an infusion of methionine plus other essential amino acids on sulfate production. The resulting serum sulfate and methionine concentrations were similar to those observed after oral methionine (Figs. 5 and 6). If it is assumed that basal sulfate production was unchanged by this treatment, then 58% of the administered methionine S was accounted for by the increase in sulfate production above baseline after 6 h and 74% after 9 h. These values are similar to those observed with oral methionine alone. In this case, however, endogenous sulfate production must have been affected by the mixed amino acid infusion. This treatment increased serum methionine, threonine, leucine, isoleucine, valine, and insulin concentrations and was associated with a more positive S balance and a less negative urea N balance than occurred with oral methionine alone (Figs. 6, 7, and 8 and Tables 3 and 4). Hypocaloric infusions of leucine or mixtures of branched-chain or essential amino acids are mildly protein sparing (31, 36, 39) and, hence, will reduce net proteolysis and endogenous SAA catabolism and sulfate production. The extent of pro-
tein sparing is minor, however. This can be illustrated by comparing 9-h urea N balances after oral methionine (~2.71 mmol/kg) and amino acid infusion (~2.19 mmol/kg). This suggests a relative retention of body proteins of 0.52 mmol N/kg (46 mg whole body protein/kg body wt) due to the amino acid infusion. If it is assumed that the body protein spared by the amino acid infusion had a N/S molar ratio of 40 (16, 41), then baseline sulfate production was reduced by 13 μmol/kg over 9 h, equivalent to 8.2% of the S administered in the mixed amino acid infusion. Because 25% of the S in the oral methionine dose was retained in the body over the same period of time, we conclude, as with the administration of methionine alone, that most of the S retained in the body after the mixed amino acid infusion must have been in a nonprotein form.

The conclusion that a significant fraction of the S in an oral methionine dose or an intravenous mixture of methionine and essential amino acids is retained in nonprotein-bound form is further supported by comparing N and S balances, calculated on the assumption that short-term changes in N and S production are approximately captured by urea N and inorganic sulfate production, respectively. Positive cumulative S balances over 6 h for both the oral methionine and amino acid infusion studies indicated S retention over this period, whereas urea N balances indicated net N loss (Tables 3 and 4). Dividing the 9-h urea N balance by the S balance results in a N/S molar ratio of 69.3 after oral methionine and 92.4 after amino acid infusion, higher than in body proteins. These results suggest a greater retention of S than N in the postprandial period. This temporal offset between sulfate and urea production has been described after whole meal ingestion of methionine and essential amino acids, a significant fraction of the methionine S appears to be stored in the body in a non-protein-bound form, presumably as glutathione.

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