Contribution of dietary protein and inorganic sulfur to urinary sulfate: toward a biomarker of inorganic sulfur intake 1–3

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ABSTRACT

Background: Sulfiting agents are widely used as food additives. Limits are set on their use in foods because they may adversely affect health. Sulfiting agents are excreted in urine as sulfate, which is indistinguishable from sulfate derived from sulfur amino acids.

Objective: The objective was to assess the contribution of inorganic sulfur to urinary sulfate excretion and of dietary protein to urinary sulfate and nitrogen excretion with the aim of developing a urinary biomarker of inorganic sulfur intake.

Design: Nine healthy men were fed a sequence of 3 diets for 15 d (n = 7), 5 diets for 10 d (n = 6), or both. The diets contained 51–212 g protein/d (0.43–1.71 g S/d) and 0.17–0.27 g inorganic S/d; p-aminobenzoic acid–validated 24-h urine samples (n = 47) were analyzed for sulfate and nitrogen.

Results: Dietary inorganic sulfur was efficiently excreted as sulfate in urine. Urinary sulfate derived from protein correlated strongly (r² = 0.86) with urinary nitrogen. Urinary recovery of protein sulfur and nitrogen decreased from 84% at average protein intakes (72 g/d) to 70% at high protein intakes (212 g/d). The nitrogen:sulfur ratio (in g) of the protein in the study diets was 18.9, which was maintained in urine (18.4 ± 0.1) after dietary inorganic sulfur intake was subtracted from urinary sulfate. Therefore, inorganic sulfur intake (g/d) = urinary sulfur (g/d) – 0.054 × urinary nitrogen (g/d). For typical UK intakes of inorganic sulfur (0.25 g/d), this biomarker should produce mean (± SD) values of 0.24 ± 0.10 g S/d.

Conclusion: Twenty-four-hour urinary nitrogen and sulfate values can be used to predict inorganic sulfur intake. Am J Clin Nutr 2004; 80:137–42.

KEY WORDS Sulfate, sulfiting agents, inorganic sulfur, urine, biomarker, nitrogen, protein

INTRODUCTION

Sulfiting agents are food additives that control enzymic and nonenzymic browning reactions, modify the structure and functional properties of wheat proteins, and function as antimicrobial agents, enzyme inhibitors, and antioxidants (1). Their many potential uses, simplicity of use, and low cost have led to their widespread incorporation into foods such as processed meats, wines and beers, dried fruit and vegetables, fruit squashes, seafood, and biscuits. However, sulfiting agents are known to pose a risk to human health. They trigger asthma and other allergic reactions in susceptible persons and reduce the thiamine content of foods (2). Furthermore, the main oxidation product of sulfite, sulfate, can potentially be reduced to sulfide by bacteria in the large bowel (3). There is concern that sulfide may be one of the bacterial toxins that causes the inflammatory process in ulcerative colitis (4).

Sulfiting agents have GRAS (generally recognized as safe) status, although calls have been made to revoke this. The Food and Drug Administration prohibited the use of sulfiting agents on raw fruit and vegetables (excluding potatoes) in a regulation that took effect 1 January 1987 (5). In the United Kingdom and the United States, packaged sulfited foods must be labeled if sulfites are present at a concentration of ≥10 ppm. European Union legislation requiring sulfur dioxide and sulfites (≥10 ppm) to be labeled as allergens took effect 1 January 2004. A recent report from the Commission of the European Union on dietary food additives considered that sulfite is the additive most likely to exceed the Acceptable Daily Intake (6).

In terms of total sulfur intake, sulfur amino acids (SAAs) are viewed as the major source of sulfur in the diet, and protein intake is relatively easy to measure. However, research studies have shown that inorganic sulfur intakes (in the form of sulfite and sulfate) may be as high as 0.53 g S/d in the Western diet in comparison with 0.09 g S/d in a typical African rural diet (7). Intakes of sulfiting agents are much more difficult to determine because most of the sulfite added to foods oxidizes to sulfate by the time of consumption, and there is widespread and variable use of sulfiting agents. In the UK population, the average inorganic sulfur intake is 0.25 g S/d (8), whereas the amount of sulfur supplied from SAAs in an average daily protein intake of 72 g is 0.61 g.

Both SAAs and inorganic sulfur contribute to the common blood sulfate pool. The major pathway of excretion for sulfur consumed as both SAAs and as inorganic sulfur is via urine. The protein content of fecal bacteria also contributes to sulfur excretion. This study aims to determine the relative contributions of 1–3

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Received July 3, 2003.
Accepted for publication December 19, 2003.

SAAs and inorganic sulfur to urinary sulfate with the view to developing a biomarker of inorganic sulfur intake.

SUBJECTS AND METHODS

Subjects

Nine healthy men aged 36–58 y were recruited for this study. Each subject underwent a medical examination before the study started. The criteria for exclusion from the study included a history of established gastrointestinal disease and the use of antibiotics or other medication known to alter gastrointestinal function within the preceding 3 mo. The Ethical Committee of the Dunn Clinical Nutrition Center, Cambridge, United Kingdom, granted ethical approval (ECS 301 and 345), and all subjects were fully informed of the study protocol.

Study design and diet composition

Seven healthy male volunteers housed in a metabolic suite were fed 3 diets for 15 d each: 1) a diet low in meat and low in sulfur additives (60b), 2) a diet low in meat and high in sulfur additives (60S), and 3) a diet high in meat and high in sulfur additives (420S). Five of these volunteers and an additional 2 healthy men were also fed a sequence of 5 diets consisting of 0, 60, 240, 420, and 600 g meat/d, each of which was fed for 10 d (9). Period carryover effects were dealt with by randomly allocating subjects to one of the possible sequences of treatments. 24-h Urine samples were collected on the last 2 d of each dietary period. The subjects lived in a metabolic suite to ensure compliance and dietary control throughout each test period. Two rest days were included in the study protocol between each diet period to allow for measurements to be repeated if required.

A 3-d rotating diet containing normal foods was designed to provide an energy content, and in most cases a nutrient content, that did not vary from the mean content by >10%. The diets were isoenergetic, and the approximate 2-MJ difference in energy content between the 60 and 420S diets was balanced with Hycal (SmithKline Beecham, Brentford, United Kingdom), a low-electrolyte glucose drink. Multiples of 1-MJ increments were added or subtracted from the basic 10-MJ diet to meet the subjects’ energy requirements, which were calculated with the use of Schofield’s equations for estimating basal metabolic rate for men aged 30–59 y (10). Levels of physical activity were also taken into account.

The basal diet comprised breakfast cereal, toast, semiskim milk, low-fat spread, and marmalade. Bread, low-fat spread, chicken, tomato, relish, fresh and canned fruit, and biscuits were provided for lunch. Dinner comprised the following starters, main courses, and desserts respectively: tuna, prawns, or egg and mayonnaise; vegetable lasagna, sweet and sour vegetables with rice, or turkey with French fries and beans; and ice cream and canned fruit with cream. Meat intake was manipulated by providing chicken or beef sandwiches at lunchtime and beef lasagna, sweet and sour pork, or beefsteak for dinner. Lean meat was used throughout the study. The sulfited foods provided by the 60S and 420S diets were additional to the basal, sulfite-free diets with minor adjustments for energy content. Dried apricots (50 g) were provided as a midmorning snack, instant potatoes (120 g) and alcohol-free cider (330 mL) were consumed at lunch, and alcohol-free wine (375 mL) was consumed at the evening meal. Efforts were made to meet recommended micronutrient intakes based on dietary reference values advised by the Committee on Medical Aspects of Food Policy (11). Minerals, which are plentiful in meat, differed between the diets and were not balanced (the iron content ranged from 7 to 23 mg throughout the study). Tea and coffee made with deionized water were allowed with all meals.

Protein sulfur and nitrogen contents of the diets

The contribution each dietary protein source makes to nitrogen and sulfur intakes can be calculated by using the protein and amino acid composition database of the US Department of Agriculture (12) as follows:

\[ \text{Nitrogen (g)} = \frac{\text{protein (g)}}{6.25} \]  
\[ \text{Sulfur (g)} = 32.06 \times \left( \frac{\text{methionine (g)}}{149.2} + \frac{\text{cystine (g)} \times 2/240.3}{2} \right) \]

where 6.25 is the ratio of protein to nitrogen in a typical protein (ratio may vary depending on the protein content); 32.06, 149.2, and 240.3 are the molecular weights of sulfur, methionine, and cystine, respectively; and 2 is the number of sulfur atoms in each cystine molecule.

An overall ratio of protein nitrogen to sulfur (N:S) was calculated for the study diets and for a typical British diet, taken from The Dietary and Nutritional Survey of British Adults (13), by summing the sulfur and nitrogen contributions from each dietary protein source. Nitrogen was also measured in a subset of freeze-dried study diets (0, 60a, 240, and 420) with the use of the Kjeldahl method, and regression analysis showed good agreement between measured nitrogen and nitrogen derived from database protein values (y = 1.01x; r² = 0.88). Urinary sulfur from protein was approximated as urinary sulfate minus inorganic sulfur intake.

Stool collections

During the study, the subjects took 10 radiopaque plastic pellets (Internet: Transcap.co.uk) with each meal (30/d) as balance markers and collected all stool excreted. Stools passed during each test period were weighed, X-rayed for marker determination, and stored at −20 °C for further analysis. On the last 3 d of each diet period, freshly passed stools were immediately X-rayed and then used for total sulfide and sulfate determination. The subjects were given notebooks in which they kept a daily record of the time at which stools were passed and the time at which the markers were taken. All stool samples were weighed, and a mean stool weight was calculated for the last 5 d of each test period and were corrected for marker output.

Urine collection

Twenty-four-hour urine samples were collected on the last 2 d of each diet period by using standard procedures (14). Boric acid was added to containers as a preservative. Compliance was checked by asking the volunteers to take p-aminobenzoic acid (PABA) tablets on the urine collection days, and samples were analyzed to check for completeness. The subjects were asked to report any problems with the collections, such as spillages or missed specimens during the 24-h period. Urinary volumes were recorded, and aliquots were immediately frozen at −20 °C until analyzed for urinary sulfate. Urine samples with PABA recoveries <85% were excluded from further analysis.
Measurement of sulfate, sulfite, and sulfide

Measurement of inorganic sulfur (sulfate and sulfite) in portions of homogenized duplicate diets was carried out by ion-exchange chromatography (IEC) with the use of the Transgenomic ICSep AN300B column (length: 250 mm; internal diameter: 4.6 mm) (Crawford Scientific, Strathaven, United Kingdom) with a bicarbonate mobile phase (4 mmol Na₂CO₃/L, 1.5 mmol NaHCO₃/L) at 1 mL/min (15). The mobile phase was made up in double deionized water, degassed with helium bubbled through for 30 min, and kept anaerobic by maintaining under pressure with helium. An ED40 electrochemical detector (Dionex, Camberley, United Kingdom) was used to detect anions by conductivity after suppression of background noise with an ASRS Ultra, 4-mm self-regenerating suppresser (Dionex). A range of external sulfate standards (Na₂SO₄ at 50, 100, and 200 µmol/L) was used to obtain a calibration curve. The samples were then diluted to the calibrated range. The addition of 10 µL (per 5-mL diluted sample) H₂O₂ (30% wt:vol) converted any remaining sulfite to sulfate (15). The sum of sulfite plus sulfate gave the inorganic sulfur content of the sample. 50 µL of each sample was injected in duplicate.

Measurement of total sulfate in urine and feces was carried out on acid-hydrolyzed samples by IEC as above but with an AS9-SC Dionex column (16). Acid hydrolysis of samples (1:1 with 1.6 mol HCl/L overnight at 90 °C) ensured release of any bound sulfate present. Bound sulfate accounts for ≈10% of total sulfate. Sulfide was microdistilled from zinc acetate stabilized fecal samples and quantitated by electrochemical detection (17).

Measurement of total nitrogen

Total nitrogen in 24-h urine samples from the last 2 d of each dietary period was measured by the Kjeldahl technique (18) with the use of a Markham still and pH titration. All chemicals were from Sigma (Internet: sigma-aldrich.com) or VWR (Internet: vwr.com) unless otherwise stated.

Statistical analysis

Statistical analyses were performed by using Microsoft EXCEL 98 (Macintosh version, 1998; Redmond, WA). Regression analyses were performed to investigate the relation between variables and were linear in all cases. Because the number of volunteers completing each diet period varied, equal weight was given to each person-dietary period. For example, the mean inorganic sulfur intake for all diets was calculated by multiplying the inorganic sulfur content of each diet by the number of people following that diet and then dividing by the number of person-dietary periods.

RESULTS

Subject compliance

In general, subject compliance was very good, and the subjects were extremely cooperative. One subject had to postpone the first study period for 3 mo because of illness that required treatment with antibiotics. Another subject withdrew in the early stages of the high-meat diet. A third volunteer was involved with only 2 dietary periods (60a and 600). The total number of person dietary periods was 47. Overall, 98% of the fecal markers taken were recovered in the subjects’ stool samples. All 24-h urine samples were checked for PABA recoveries and were >85% in all cases.

Dietary sulfur sources

The inorganic sulfur and protein contents of each diet (n = 8 diets, means of each 3-d rotating dietary period) as well as the protein nitrogen (g), protein sulfur (g), and N:S (g) values are shown in Table 1. The protein, protein nitrogen, and protein sulfur contents of a range of commonly consumed foods are shown in Table 2. Major protein sources, such as red and white meat and fish, have protein nitrogen to sulfur ratios of ≈18. Other foods, such as peas and grapes, have higher (41.3) and lower (13.8) N:S ratios, respectively, but do not affect the overall protein N:S of a typical diet, because they do not contain much protein or are only eaten in small quantities. For a typical diet (as defined by The National Diet and Nutritional Survey of British Adults), the protein nitrogen and protein sulfur (g) contents of each constituent food item was used to calculate an overall N:S ratio, which resulted in an average value of 18.9 (13). This value agrees with that calculated for the diets used in the current study (± SD: 18.9 ± 0.4).

Protein intake and urinary nitrogen

Protein intakes ranged from 51 to 212 g/d. The mean CV (for each subject and diet) for urinary nitrogen values between the last 2 d of each dietary period was 9.9%. The correlation (r²) between protein intake and urinary nitrogen (g/d) was 0.91 (Figure 1).
The mean efficiency of urinary nitrogen excretion from protein ranged from 83% at typical intakes of 72 g/d protein to 70% at protein intakes of 212 g/d.

Urinary sulfate from protein and protein intake

A plot of urinary sulfate from protein (expressed as g sulfur/d) against protein intake (g/d) for all subjects and diets ($n = 475$) is shown in Figure 2. A linear trend line provided a good fit for the data ($r^2 = 0.80$). At higher protein intakes, the proportion of protein sulfur excreted as sulfate in urine was reduced (85% recovery at the average protein intake of 72 g/d and 70% at 212 g protein/d).

Urinary nitrogen and urinary sulfate from protein

A plot of urinary nitrogen against urinary sulfate from protein (expressed as g sulfur/d) is shown in Figure 3 ($n = 47$); the

### TABLE 2

Protein and protein nitrogen and sulfur contents of the study foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Protein</th>
<th>Nitrogen$^2$</th>
<th>Met</th>
<th>Cys</th>
<th>Sulfur in Met</th>
<th>Sulfur in Cys</th>
<th>Total sulfur$^3$</th>
<th>Nitrogen:sulfur</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
</tr>
<tr>
<td>Chicken</td>
<td>31.02</td>
<td>4.96</td>
<td>0.859</td>
<td>0.397</td>
<td>0.185</td>
<td>0.106</td>
<td>0.291</td>
<td>17.1</td>
</tr>
<tr>
<td>Turkey</td>
<td>29.90</td>
<td>4.78</td>
<td>0.866</td>
<td>0.311</td>
<td>0.186</td>
<td>0.083</td>
<td>0.269</td>
<td>17.8</td>
</tr>
<tr>
<td>Tuna</td>
<td>29.13</td>
<td>4.66</td>
<td>0.860</td>
<td>0.310</td>
<td>0.185</td>
<td>0.083</td>
<td>0.268</td>
<td>17.4</td>
</tr>
<tr>
<td>Pork</td>
<td>28.57</td>
<td>4.57</td>
<td>0.756</td>
<td>0.364</td>
<td>0.162</td>
<td>0.097</td>
<td>0.260</td>
<td>17.6</td>
</tr>
<tr>
<td>Beef</td>
<td>28.11</td>
<td>4.50</td>
<td>0.720</td>
<td>0.315</td>
<td>0.155</td>
<td>0.084</td>
<td>0.239</td>
<td>18.8</td>
</tr>
<tr>
<td>Cheese, hard</td>
<td>24.90</td>
<td>3.98</td>
<td>0.652</td>
<td>0.125</td>
<td>0.140</td>
<td>0.033</td>
<td>0.174</td>
<td>23.0</td>
</tr>
<tr>
<td>Prawns</td>
<td>20.91</td>
<td>3.34</td>
<td>0.589</td>
<td>0.234</td>
<td>0.126</td>
<td>0.062</td>
<td>0.189</td>
<td>17.7</td>
</tr>
<tr>
<td>Eggs</td>
<td>12.58</td>
<td>2.01</td>
<td>0.392</td>
<td>0.199</td>
<td>0.030</td>
<td>0.026</td>
<td>0.055</td>
<td>23.7</td>
</tr>
<tr>
<td>Bread, white</td>
<td>8.20</td>
<td>1.31</td>
<td>0.139</td>
<td>0.096</td>
<td>0.031</td>
<td>0.046</td>
<td>0.077</td>
<td>16.9</td>
</tr>
<tr>
<td>Apricots, dried</td>
<td>3.39</td>
<td>0.542</td>
<td>0.015</td>
<td>0.019</td>
<td>0.003</td>
<td>0.005</td>
<td>0.008</td>
<td>65.4</td>
</tr>
<tr>
<td>Eggs</td>
<td>2.02</td>
<td>0.034</td>
<td>0.047</td>
<td>0.041</td>
<td>0.010</td>
<td>0.011</td>
<td>0.021</td>
<td>16.1</td>
</tr>
<tr>
<td>Tomato</td>
<td>0.85</td>
<td>0.136</td>
<td>0.007</td>
<td>0.011</td>
<td>0.001</td>
<td>0.003</td>
<td>0.004</td>
<td>30.6</td>
</tr>
<tr>
<td>Margarine</td>
<td>0.50</td>
<td>0.080</td>
<td>0.013</td>
<td>0.005</td>
<td>0.003</td>
<td>0.001</td>
<td>0.004</td>
<td>19.4</td>
</tr>
</tbody>
</table>

$^1$ From reference 12. Met, methionine; Cys, cystine.

$^2$ Nitrogen (g) = protein (g)/6.25. A conversion factor of 6.25 was used for all foods except bread (5.70), milk (6.38), and rice (5.95).

$^3$ Sulfur (g) = sulfur in Met × [(methionine (g)/149.2) + (cystine (g) × 2/240.3)].

The mean efficiency of urinary nitrogen excretion from protein ranged from 83% at typical intakes of 72 g/d protein to 70% at protein intakes of 212 g/d.

### FIGURE 1

Protein intake versus urinary nitrogen determined by Kjeldahl analysis after the subjects consumed a diet low in meat and low in sulfur additives (60b), a diet low in meat and high in sulfur additives (60S), and a diet high in meat and high in sulfur additives (420S) for 10 d and diets consisting of 0, 60, 240, 420, or 600 g meat/d for 10 d. Each subject is represented by a different symbol: $E_0$, $E_60$, $E_60b$, $E_60S$, $E_240$, $E_420$, $E_420S$, and $E_600$.

### FIGURE 2

Protein intake versus urinary sulfur from protein (urinary sulfate/inorganic sulfur intake) after the subjects consumed a diet low in meat and low in sulfur additives (60b), a diet low in meat and high in sulfur additives (60S), and a diet high in meat and high in sulfur additives (420S) for 10 d and diets consisting of 0, 60, 240, 420, or 600 g meat/d for 10 d. Each subject is represented by a different symbol: $E_0$, $E_60$, $E_60b$, $E_60S$, $E_240$, $E_420$, $E_420S$, and $E_600$.

### FIGURE 3

A plot of urinary nitrogen against urinary sulfate from protein (expressed as g sulfur/d) is shown in Figure 3 ($n = 47$); the
for 10 d. Each subject is represented by a different symbol: high in sulfur additives (420S) for 10 d and diets consisting of 0, 60, 240, 420, the subjects consumed a diet low in meat and low in sulfur additives (60b), a diet high in meat and sulfur intake) versus urinary nitrogen determined by Kjeldahl analysis after the subjects consumed a diet low in meat and high in sulfur additives (420b), a diet low in meat and high in sulfur additives (420S) for 10 d and diets consisting of 0, 60, 240, 420, or 600 g meat/d for 10 d. Each subject is represented by a different symbol: ○ (0, 60a, 60b, 60S, 240, 420, 420S, and 600), + (0, 60a, 240, and 420), □ (60b, 60S, and 420S), ◆ (60a and 60b), × (60b, 60S, and 420S), x (0, 60a, 60b, 60S, 240, 420, 420S, and 600), ▲ (0, 60a, 60b, 60S, 240, 420, 420S, and 600), ■ (0, 60a, 60b, 60S, 240, 420, 420S, and 600), and * (60b, 60S, and 420S). n = 47, y = 18.4x; r² = 0.86.

correlation coefficient was 0.86. The gradient (SE) of the regression line was 18.4 ± 1.1, which compares favorably with the N:S for dietary protein in the study diets of 18.9.

Fecal sulfate and sulfide

Fecal sulfate in the study ranged from 0.001 to 0.006 g S/d. Fecal sulfide ranged from 0.001 to 0.010 g S/d; the higher range being in the high-meat diets. Large subject-related differences were observed in fecal sulfate and sulfide outputs throughout the study. The subjects with the lower urinary sulfate values tended to have higher fecal sulfate values than did those in the same dietary group. Thus, part of the variation in urinary sulfate values between people consuming identical diets can be attributed to differences in fecal sulfate.

DISCUSSION

This study assessed for the first time the contribution of inorganic sulfur, principally from food additives, and organic sulfur from the SAAs methionine and cysteine to urinary sulfate. The aim of this work was to develop a biomarker of inorganic sulfur intake. In the past, inorganic sulfur intake has been difficult to quantify from dietary data because of the wide range of foods that are sulfited and because of the variations in sulfiting protocols even for the same food type. To take the example of wine, the range of inorganic sulfur concentrations in 63 different wines (red and white) varied from 0.07 to 0.37 g/L (15). The development of a biomarker of inorganic sulfur intake is an attempt to circumvent the problems of using conventional dietary intake data.

Urinary sulfate from protein was approximated as urinary sulfate minus measured inorganic sulfur intake. This assumes that the efficiency of urinary excretion of dietary inorganic sulfur for the range of intakes used was 100%. The justification for this assumption is derived from a plot of urinary nitrogen against urinary sulfate. The linear regression line for this graph intersected the y axis at 0.24 g S/d, which is the amount of sulfate in urine predicted if urinary nitrogen excretion is 0. This compares with an average inorganic sulfur intake for the corresponding diets of 0.22 g S/d.

Urinary sulfate was previously shown to reflect protein intake (19, 20). It is not an ideal biomarker of protein intake because inorganic sulfur intake also affects urinary sulfate concentrations. In this study, with a limited range of inorganic sulfur intakes, the correlation (r²) between urinary sulfate (including urinary sulfate from protein) and protein intake was 0.82. A better biomarker of protein intake is urinary nitrogen (r² = 0.91; Figure 1).

The factors used in this study were 1/6.25 for converting protein (g) to nitrogen (g) and 1/18.9 for converting nitrogen (g) to sulfur (g); 18.9, calculated theoretically from the SAA composition of the proteins contained in the study diets (Table 2), compares well with 18.4—the gradient of the graph of total urinary nitrogen (g/d) against urinary sulfate (expressed as g sulfur/d) from protein (Figure 2). A linear regression line through the origin for urinary sulfate from protein against total urinary nitrogen has an r² = 0.86 (Figure 3), which suggests that the percentages of sulfur and nitrogen from protein excreted in urine are very similar for different people over a wide range of protein intakes. Both sulfide produced in the gut (a proportion of which ends up as urine sulfate) and sulfate being an underestimate of urine total sulfur (88%) (21) could affect the N:S ratio in urine. In the current study, these factors appear to balance closely.

The relation between SAA intake and urinary sulfate (from protein) excretion and also between protein intake and urinary nitrogen indicated a reduced efficiency of excretion in urine at higher intakes. For an average protein intake of 72 g/d (0.61 g S/d) the amounts of sulfate and total nitrogen excreted in urine were 85% and 83%, respectively. At an intake of 212 g protein/d, the amounts of sulfate and total nitrogen excreted both decreased to 70%. So despite the different efficiencies in the urinary excretion of protein nitrogen and sulfur, the ratio remains the same. Consistent differences in the percentage of sulfur from protein excreted as sulfate in the urine were observed between subjects. This difference was partly explained by the differences in fecal sulfate between subjects. Fecal nitrogen was not measured.

The findings of the current study suggest that fecal sulfate was not a major pathway of sulfur excretion (<0.003 g S/d). A study of sulfate absorption by the small intestine in ileostomists showed a threshold for absorption of dietary sulfate of ~0.22 g S/d, above which significant amounts spill over into the colonic sulfate pool (16). This will mostly be converted to sulfide, absorbed, and converted back to sulfate. Dietary inorganic sulfur, in the form of sulfate and sulfiting agents, may therefore be important as regulating factors for dissimilatory sulfate reduction in the colon at higher intakes. For the range of inorganic sulfur intakes studied, a high percentage appeared to be excreted as sulfate in the urine. The range of inorganic sulfur intakes was too small to draw further conclusions.

The high correlations between protein intake and total urinary nitrogen and between protein intake and urinary sulfate derived from protein (r² = 0.86, Figure 3; r² = 0.80, Figure 2), enable a prediction of inorganic sulfur (IS) intakes from urine sulfate (US)
values if either 24-h urinary nitrogen (UN) or protein intake (PI) data are available:

\[
\text{IS (g/d)} = \text{US (g/d)} - 0.054 \times \text{UN (g/d)} \quad (3)
\]

or

\[
\text{IS (g/d)} = \text{US (g/d)} - \{[0.00523 \times \text{PI (g/d)}} + 0.144\}
\]

This study proposes that inorganic sulfur (g/d) can be calculated from the difference between total urinary sulfur (g/d) and urinary nitrogen (g/d)/18.5. The error for this biomarker is the sum of the errors for the 2 measurements: urinary nitrogen and urinary sulfur. These measurements are both similarly reproducible; thus, for a given total urinary sulfur value, the absolute error is independent of the relative contributions from inorganic and protein sulfur sources. The percentage of error in inorganic sulfur is directly proportional to the contribution of inorganic sulfur to total sulfate. An estimate of the accuracy of the proposed inorganic sulfur biomarker equation can be made by using the urinary nitrogen and sulfur values from the current study. The mean of the predicted inorganic sulfur intakes expressed as a percentage of the actual intakes was 97% ± 64% (± SD).

The average protein and inorganic sulfur intakes in the United Kingdom contribute 0.61 and 0.25 g S/d, respectively, which compares with the average protein and inorganic sulfur intakes in the current study of 0.94 and 0.22 g S/d, respectively. Thus, the contribution of inorganic sulfur to total urinary sulfate is 29% for a typical UK diet compared with 19% for these study diets. Inorganic sulfur intakes can reasonably be expected to vary between 0.06 and 0.6 g/d. For typical inorganic sulfur intakes in the United Kingdom of 0.25 g/d, the biomarker should produce mean (± SD) values of 0.24 ± 0.10 g/d. This accuracy of inorganic sulfur intake prediction compares with the accuracy of urinary nitrogen at predicting a protein intake of 112 ± 19 g/d.

The use of Equation 4 rather than Equation 3 to predict inorganic sulfur intake, although only slightly less accurate in this study, is not recommended in free-living populations because additional errors will arise from establishing protein intakes.

The abovementioned methods for quantifying inorganic sulfur intake provide a means of obtaining a value that, to date, has been difficult to establish. Further work is required to test a wider range of inorganic sulfur intakes in free-living subjects. A biomarker of inorganic sulfur intake could provide a valuable tool for establishing links between sulfitifying agents and disease. This biomarker may also reflect intakes of processed foods and beverages.

EAM, LME, and JHC were involved in the design of the study, analysis of the data, and writing of the manuscript. RC was involved with the collection and analysis of the data. None of the authors had any conflicts of financial or personal interest with the financial sponsors of this research.

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