Variations in Dietary Protein but Not in Dietary Fat Plus Cellulose or Carbohydrate Levels Affect Cysteine Metabolism in Rat Isolated Hepatocytes

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ABSTRACT To determine if previously observed effects of dietary protein on hepatic cysteine metabolism were due specifically to increases in dietary protein or to the accompanying decreases in dietary carbohydrate, two experiments were conducted. In one experiment, rats were fed diets that contained different levels of protein vs. an isocaloric mixture of fat + cellulose and a constant amount of carbohydrate. In the other, rats were fed diets that contained a constant amount of protein but different levels of carbohydrate vs. an isocaloric mixture of fat + cellulose. Diets were fed for 2-3 wk and hepatocytes were then isolated. Hepatic cysteine dioxygenase activity increased and cysteinesulfinate decarboxylase and γ-glutamylcysteine synthetase activities decreased in a stepwise manner when protein was added to the diet at the expense of fat + cellulose. Changes in cysteine dioxygenase, cysteinesulfinate decarboxylase and γ-glutamylcysteine synthetase activities were consistent with changes in rates of cysteine catabolism, taurine production and glutathione synthesis, respectively, by intact hepatocytes incubated with 0.2 mmol/L cysteine. When the carbohydrate to fat + cellulose ratio was varied, but the protein level was held constant, little or no change in enzyme activities or levels of metabolite production was observed. Regulation of the activities of enzymes involved in cysteine metabolism is predominantly due to changes in dietary protein intake and not to the associated changes in intake of other dietary macronutrients. J. Nutr. 126: 2179-2187, 1996.

INDEXING KEY WORDS:
- cysteine
- cysteinesulfinate decarboxylase
- cysteine dioxygenase
- γ-glutamylcysteine synthetase
- rats

Like most other amino acids, cysteine is required for the synthesis of proteins and of other essential non-protein compounds. Glutathione, sulfate and taurine are essential for a wide variety of critical functions in the body (Krijgheld et al. 1982, Meister 1991, Sturman and Chesney 1995). Glutathione's cellular functions include maintenance of cellular redox potential, participation in various enzymatic reactions including those catalyzed by glutathione sulfotransferases and peroxidases, and transport of amino acids. Sulfate is required for numerous sulfation reactions including the sulfation of glycosaminoglycans and the formation of sulfate esters of drugs as a detoxification mechanism. The role of taurine in bile acid conjugation is well known, whereas the specific role of taurine in other processes has not been clearly elucidated.

The activities of key enzymes of cysteine catabolism and the rates of sulfate, taurine and glutathione production have been observed to change in the liver of rats fed different levels of dietary protein (Bagley and Stipanuk 1994, Bella and Stipanuk 1995 and in press). Cysteine dioxygenase \([\text{CDO}^4; \text{EC 1.13.11.20}]\) and cysteine catabolism [sulfate + taurine production] increased and cysteinesulfinate decarboxylase \([\text{CSDC}; \text{EC 4.1.1.29}]\) and the rate of conversion of cysteinesulfinate to taurine decreased in a dose-dependent manner in response to increases in the amount of dietary protein (Bagley and

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2 The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.
3 To whom correspondence should be addressed.
4 Abbreviations used: AAT, aspartate (cysteinesulfinate): α-ketoglutarate aminotransferase; CDO, cysteine dioxygenase; CyL, cystathionine γ-lyase (cystathionase); CSDC, cysteinesulfinate decarboxylase; GCS, γ-glutamylcysteine synthetase; HC/LF, high carbohydrate (603 g carbohydrate/kg diet)/low fat; HP/LF, high protein (400 g casein/kg diet)/low fat; LC/HF, low carbohydrate [311 g carbohydrate/kg diet]/high fat; LP/HF, low protein [100 g casein/kg diet]/high fat; MC/MF, moderate carbohydrate [457.2 g carbohydrate/kg diet]/moderate fat; MP/MF, moderate protein [200 g casein/kg diet]/moderate fat.
The rate of glutathione production also decreased in response to dietary protein level, but the activity of the rate-limiting enzyme in glutathione synthesis, \( \gamma \)-glutamylcysteine synthetase (GCS; E.C.6.3.2.2), was not measured.

We have also shown that rat hepatic CDO activity increased with dietary sulfur amino acid supplementation (3 or 10 g L-methionine/kg of diet or the sulfur equivalent as cysteine) of a low protein diet (100 g casein/kg diet), whereas CSDC activity was unaffected by these levels of sulfur amino acids (Bagley and Stipanuk 1995). When a much higher level of sulfur amino acids in the diet was tested (~23 g L-methionine/kg diet), a decrease in CSDC activity in response to supplemental sulfur amino acids was observed, but the decrease was much less than that observed with an equisulfur amount of protein. This difference in responsiveness to sulfur amino acid intake suggests that CDO and CSDC activities may respond to different components of the low vs. high protein diets and that the effects of dietary protein on CSDC activity cannot be explained by the level of sulfur amino acids alone.

In our previous studies, the high protein diets were much lower in carbohydrate content as well as higher in protein content compared with the basal low protein or methionine-supplemented diets. Differences in macronutrient composition may have altered the metabolic and hormonal state of the animals fed these diets and played a role in the modulation of the activities of cysteine-metabolizing enzymes, especially in the down-regulation of CSDC activity in hepatocytes of rats fed high protein, low carbohydrate diets.

To explore further the possibility that cysteine metabolism may be influenced by the macronutrient composition of the diet, two studies were conducted. In the first study, the dietary protein level was varied (100, 200 and 400 g casein/kg of diet) at the expense of an isocaloric mixture of fat + cellulose while the carbohydrate level was held constant. In the second study, the dietary protein level was held constant at 250 g/kg of diet while the proportion of carbohydrate (311, 457, and 603 g carbohydrate/kg of diet) vs. fat + cellulose was varied. Activities of key enzymes in cysteine metabolism and the production of taurine, sulfate and glutathione from cysteine by isolated hepatocytes were measured.

### MATERIALS AND METHODS

**Materials.** L-Cysteine, taurine, glutathione and the disodium salt of bathocuproine disulfonate were purchased from Sigma Chemical (St. Louis, MO). Sodium sulfate was purchased from Mallinkrodt (St. Louis, MO). L-Cysteinesulfinate was purchased from Aldrich Chemical (Milwaukee, WI), and L-cystathionine was purchased from Calbiochem (San Diego, CA). L-[35S]-Cysteine was purchased from Amersham (Product no. SJ141, Arlington Heights, IL) as the hydrochloride. L-[35S]Cysteine was purified prior to use by applying it to a 0.6 x 4 cm Dowex 1-X8 column (200–400 mesh, acetate form) and eluting it with ~20 mL of deionized water. The eluate was aliquoted into portions (0.37 MBq/tube), lyophilized and stored at ~70°C until used.

**Animals and dietary treatments.** Male Sprague-Dawley rats weighing ~150 g were purchased from Harlan Sprague Dawley (Indianapolis, IN). Rats were housed individually in stainless steel mesh cages in a room maintained at 20°C and 60–70% humidity with light from 2000 to 0800 h and fed a nonpurified diet (RHM 1000, Agway, Syracuse, NY) for 5 d prior to experimental group assignment. Rats (21 for Experiment 1 and 27 for Experiment 2) were blocked into groups of three by body weight, and those within each weight block were randomly assigned to receive one of three diets. Rats had free access to diet and water for the duration of the experiment.

Composition of the semipurified diets is given in Table 1 (Experiment 1) and Table 2 (Experiment 2). Diets were based on the AIN-76A formulation (AIN 1977). For Experiment 1, modifications were made to prepare diets that contained 100 g casein/kg diet (low protein/high fat; LP/HF), 200 g casein/kg diet (moderate protein/moderate fat; MP/MF), or 400 g casein/kg diet (high protein/low fat; HP/LF). These diets contained a constant level of 453 g carbohydrate/kg diet and variable amounts of fat and cellulose to maintain the energy density of the diet. For Experiment 2, all diets contained 250 g casein/kg diet, but the carbohydrate to fat content (g carbohydrate/g fat) for each diet was as follows: 311/180 g/kg diet (low carbohydrate/high fat; LC/HF), 457/115 g/kg diet (moderate carbohydrate/moderate fat; MC/MF) and 603/50 g/kg diet (high carbohydrate/high fat; HC/HF).

### TABLE 1
Composition of semipurified diets containing various levels of protein vs. fat + cellulose

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LP/HF</td>
</tr>
<tr>
<td>Vitamin-free casein</td>
<td>100</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>226.5</td>
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<tr>
<td>Sucrose</td>
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<td>Cellulose</td>
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<td>Corn oil</td>
<td>183.3</td>
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<tr>
<td>Vitamin mix (AIN 76A)</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
</tr>
<tr>
<td>Mineral mix (AIN 76)</td>
<td>35.0</td>
</tr>
</tbody>
</table>

1 Diets were prepared by Dyets (Bethlehem, PA) in pelleted form. LP/HF = low protein/high fat; MP/MF = moderate protein/moderate fat; HP/LF = high protein, low fat.
TABLE 2
Composition of semipurified diets containing various levels of carbohydrate vs. fat + cellulose

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Diet</th>
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<td>LC/HF</td>
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<td>Vitamin-free casein</td>
<td>250</td>
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<tr>
<td>Cornstarch</td>
<td>155.5</td>
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<tr>
<td>Sucrose</td>
<td>155.5</td>
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<tr>
<td>Cellulose</td>
<td>212</td>
</tr>
<tr>
<td>Corn oil</td>
<td>180</td>
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<tr>
<td>Vitamin mix (AIN 76A)</td>
<td>10</td>
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<td>Choline bitartrate</td>
<td>2</td>
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<tr>
<td>Mineral mix (AIN 76)</td>
<td>35</td>
</tr>
</tbody>
</table>

1 Diets were prepared by Dyets (Bethlehem, PA) in pelleted form. LC/HF = low carbohydrate/high fat; MC/MF = moderate carbohydrate/moderate fat; HC/LF = high carbohydrate/low fat.

carbohydrate/low fat; HC/LF). Because carbohydrate was replaced by fat, cellulose was added to maintain the energy density of the diet. The diets were prepared in pelleted form by Dyets (Bethlehem, PA). Rats were fed the experimental diets for 2–3 wk. Hepatocytes were isolated between 1300 and 1500 h from two rats per day, randomly selecting rats from various treatment groups within a given block and using rats from the heavier weight blocks first. Rats were anesthetized with an intraperitoneal injection of 100 mg sodium pentobarbital/kg body weight. Hepatocytes were then isolated by the method of Berry et al. (1991). The care and use of animals was approved by the Cornell University Institutional Animal Care and Use Committee.

Isolated hepatocytes were suspended in 40 mL of oxygenated Krebs-Henseleit bicarbonate buffer at 37°C and used immediately. Maintenance of cellular ATP levels at >2.0 mmol/kg wet weight of cells throughout the experimental incubation period was used as the criterion for hepatocyte viability. Hepatocytes were successfully isolated from all rats in Experiment 1 and from 24 rats in Experiment 2. In Experiment 2, two rats in the MC/MF group and one rat in the HC/LF group were lost due to problems during surgery. Data from two rats in Experiment 1 (both in the LP/HF group) and from three rats in Experiment 2 (all in the LC/HF group) were discarded due to low cellular ATP levels.

Hepatocytes (~100 mg wet weight of cells) were incubated with 0.2 mmol/L L-[35S]cysteine, and the production of [35S]sulfate, [35S]taurine (including [35S]taurocholic acid), and [35S]glutathione (including disulfide forms) and the incorporation of [35S]cysteine into protein were measured as described previously (Bella and Stipanuk 1995).

GCS activity in isolated hepatocytes was measured by incubating ~0.07 or 0.14 g (wt wt) of disrupted hepatocytes with 2.5 mmol/L L-cysteine, 20 mmol/L L-glutamate, 10 mmol/L L-glutamic acid, 0.3 mmol/L L-bathocuproine disulfonate, 5 mmol/L MgSO4, 50 mmol/L KCl, 10 mmol/L ATP, 10 mmol/L phosphocreatine, 37.5 units of creatine phosphokinase and 100 mmol/L Eppendorf buffer (pH 8.5) in a final volume of 2.5 mL for 15 or 30 min at 37°C. Reactions were terminated by addition of 0.2 mL of 2.2 mol/L trichloroacetic acid. Reactions stopped at zero-time served as blanks. Production of glutathione and γ-glutamylcysteine was quantitated using the HPLC method of Fariss and Reed (1987) as modified by Stipanuk et al. (1992).

CDO and CSDC activities in isolated hepatocytes were measured as described previously (Bella and Stipanuk 1995). Aspartate [cysteinesulfinate]:α-ketoglutarate aminotransferase (AAT; EC 2.6.1.1) activity in isolated hepatocytes was measured by the procedure described by Bergmeyer and Bernt (1974) except 20 mmol/L L-CSA was used as substrate instead of aspartate (Daniels and Stipanuk 1982). Cystathionine-γ-lyase (CγL; EC 4.4.1.1) activity in isolated hepatocytes was measured by the procedure described by Stipanuk (1979). All enzyme assays were performed under Vmax conditions.

Data were analyzed by ANOVA (Minitab 81.1, State College, PA) and Tukey’s or Tukey-Kramer’s w-procedure (Steel and Torrie 1960). Differences were considered significant at P < 0.05.

RESULTS

Experiment 1. As shown in Table 3, the daily average food intake of rats fed the diets that varied in protein vs. fat + cellulose content was similar (P > 0.05). However, the rate of weight gain and body weight at the end of the feeding period for rats fed the LP/HF diet were significantly (P < 0.05) lower than for rats fed the MP/MF or HP/LF diet. Protein concentration of hepatocytes did not differ significantly (P < 0.05) among the three groups. All hepatocyte data have been expressed on the basis of protein content of the cells.

ATP concentrations were similar (P > 0.05) for hepatocytes used in the experiment. However, the two hepatocytes preparations (out of 21) that were discarded due to cellular ATP levels <2.0 mmol/kg were both from rats fed the LP/HF diet.

The initial taurine concentration was 10 times and the initial glutathione concentration was 2.5 times greater in hepatocytes from rats fed the HP/LF diet than in hepatocytes from rats fed the LP/HF diet. The concentration of taurine in hepatocytes from rats fed the MP/MF diet was similar to that for hepatocytes from rats fed the LP/HF diet, whereas the glutathione concentration of hepatocytes from rats fed the MP/MF...
TABLE 3

**Effects of variation in the protein concentration of the diet on food intake and weight gain of rats and on protein concentration, ATP concentration and initial glutathione and taurine concentrations of isolated hepatocytes**

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>Daily diet consumption, g/d</th>
<th>Daily weight gain, g/d</th>
<th>Body weight at end of feeding period,</th>
<th>Protein concentration, mg protein/g wet hepatocytes</th>
<th>ATP concentration, µmol/g wet weight hepatocytes</th>
<th>Initial taurine concentration, µmol/g wet weight hepatocytes</th>
<th>Initial GSH concentration, µmol/g wet weight hepatocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP/HF</td>
<td>5</td>
<td>23 ± 0.3</td>
<td>2.5 ± 0.3a</td>
<td>277 ± 6.6a</td>
<td>188 ± 8.1</td>
<td>2.4 ± 0.2</td>
<td>1.3 ± 0.8a</td>
<td>2.0 ± 0.3a</td>
</tr>
<tr>
<td>MP/MF</td>
<td>7</td>
<td>19 ± 0.7</td>
<td>4.8 ± 0.4b</td>
<td>312 ± 5.2b</td>
<td>254 ± 27.1</td>
<td>2.4 ± 0.2</td>
<td>2.6 ± 0.8a</td>
<td>4.8 ± 0.6b</td>
</tr>
<tr>
<td>HP/LF</td>
<td>7</td>
<td>20 ± 1.7</td>
<td>4.8 ± 0.2b</td>
<td>316 ± 6.8b</td>
<td>249 ± 20.2</td>
<td>2.2 ± 0.2</td>
<td>13 ± 1.6b</td>
<td>5.1 ± 0.6b</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Within a row, values with different superscript letters are significantly different (P < 0.05) by Tukey-Kramer's w-procedure. LP/HF = low protein/high fat; MP/MF = moderate protein/moderate fat; HP/LF = high protein/low fat.

Consistent with previous observations for hepatocytes from rats fed diets in which protein was varied at the expense of carbohydrate rather than fat + cellulose, the activities of CDO, CSDC and GCS, but not of AAT and CyL, responded in a dose-dependent manner to increases in the level of dietary protein. Hepatic CDO specific activity for rats fed the HP/LF diet was significantly higher (P < 0.05) than that for rats fed the LP/HF diet, whereas CDO activity for rats fed the MP/MF diet was inbetween but not significantly different (P > 0.05) than that for either the LP/HF or HP/LF groups. Hepatic CSDC specific activity was significantly (P < 0.05) lower in hepatocytes from rats fed higher amounts of protein (LP/HF > MP/MF > HP/LF). GCS specific activity also underwent a stepwise decrease with increasing levels of dietary protein, with activity in hepatocytes from rats fed the HP/LF diet being significantly lower than that for rats fed the LP/HF diet, whereas GCS activity for rats fed the MP/MF diet was inbetween and not significantly different (P > 0.05) than that for either the LP/HF or HP/LF groups. Although neither AAT nor CyL activity responded in a dose-dependent manner to increases in dietary protein, CyL specific activity was significantly (P < 0.05) lower in hepatocytes from rats fed the MP/MF diet compared with hepatocytes from rats fed the LP/HF or HP/LF diet.

The metabolism of [35S]cysteine by hepatocytes from rats fed the various levels of protein is reported in Figure 2. The rate of [35S]taurine production in hepatocytes from

**FIGURE 1** Activities of cysteine dioxygenase (CDO), cysteinesulfinic acid decarboxylase (CSDC), γ-glutamylcysteine synthetase (GCS), aspartate aminotransferase (AAT), and cystathionine-γ-lyase (CyL) in isolated hepatocytes from rats fed diets with different levels of protein vs. fat + cellulose. Values are means ± SEM for five (LP/HF) or seven rats (MP/MF and HP/LF). CDO and GCS activities are one tenth and AAT activity is ten times the value read from the vertical axis. Values are expressed as specific activity in the hepatocyte homogenate (CDO and GCS) or in a 20,000 x g supernatant fraction of hepatocyte homogenate (CSDC, AAT and CyL). For each enzyme activity, bars designated by different superscript letters are significantly different (P ≤ 0.05) by Tukey-Kramer's w-procedure. CDO activity in the LP/HF group was 0.01 ± 0.01 nmol/min mg protein. LP/HF = low protein/high fat; MP/MF = moderate protein/moderate fat; HP/LF = high protein/low fat.
rats fed the HP/LF diet was significantly higher ($P < 0.05$) than in hepatocytes from rats fed the LP/HF diet and similar to that in hepatocytes from rats fed the MP/MF diet. $^{35}$S Sulfate production was significantly greater ($P < 0.05$) in hepatocytes from rats fed the LP/HF diet compared with hepatocytes from rats fed either the LP/HF or MP/MF diet. As protein intake increased, total catabolism ($[^{35}S]$taurine + $[^{35}S]$sulfate production) increased in a stepwise manner as follows (mean ± SEM): LP/HF, 76 ± 8 pmol/([min · mg protein]); MP/MF, 134 ± 28 pmol/([min · mg protein]); HP/LF, 366 ± 25 pmol/([min · mg protein]). The rate of $[^{35}S]$glutathione production (Fig. 2) was highest in hepatocytes from rats fed the LP/HF diet. The rate of $[^{35}S]$glutathione production in hepatocytes from rats fed the MP/MF and HP/LF diets was 39 and 23%, respectively, of the production in hepatocytes from rats fed the LP/HF diet. $[^{35}S]$Cysteine incorporation into protein was similar for hepatocytes isolated from rats fed the three different diets. Total cysteine utilization was significantly greater ($P < 0.05$) for the HP/LF group than for either the LP/HF or MP/MF diet group; this was due primarily to the high rate of sulfate production by hepatocytes from rats fed the HP/LF diet.

**Experiment 2.** Rats received a diet with 250 g casein/kg diet and variable amounts of carbohydrate vs. fat + cellulose. The protein was increased above the moderate level (200 g casein/kg diet) used in Experiment 1 because the highly variable hepatic CDO activity observed in the MP/MF group in Experiment 1 suggested that protein (sulfur amino acid) intake was marginal (Hosokawa et al. 1988). As shown in Table 4, the daily average food intake, the rate of weight gain and the body weight at the end of the feeding period were similar ($P > 0.05$) for rats fed diets that contained the same amount of protein but different amounts of carbohydrate vs. fat + cellulose. Protein, taurine and glutathione concentrations were similar ($P > 0.05$) for hepatocytes from all three groups. Cellular ATP content was similar for all hepatocytes used in Experiment 2, but the three hepatocyte preparations discarded due to low cellular ATP concentration (<2 mmol/kg cells) were all from rats fed the LC/HF diet.

In general, there was no effect of different carbohydrate to fat ratios in the diet on the activities of hepatic enzymes involved in cysteine metabolism (Fig. 3), except for CDO specific activity. Hepatic CDO specific activity for rats fed the MC/MF diet was significantly greater ($P < 0.05$) than that for rats fed the LC/HF or the HC/LF diet, but the magnitude of the difference was small compared with that observed in Experiment 1 when dietary protein vs. fat + cellulose was varied. In Experiment 2, hepatocytes from rats fed the MC/MF diet had only 2.4 times as much CDO activity as did hepatocytes from rats fed the LC/HF diet, whereas in Experiment 1, hepatocytes from rats fed the MP/MF diet had 60 times as much CDO as did hepatocytes from rats fed the LP/HF diet.

The metabolism of $[^{35}S]$cysteine by hepatocytes from rats fed the various diets is reported in Figure 4. The rates of $[^{35}S]$taurine production, $[^{35}S]$sulfate production, $[^{35}S]$cysteine incorporation into protein and total $[^{35}S]$cysteine utilization in hepatocytes were similar ($P > 0.05$) for all dietary treatment groups. However, the rate of glutathione production was lower in hepatocytes from rats fed the MC/MF diet than in hepatocytes from rats fed the LC/HF diet.
TABLE 4

Effects of variation in the carbohydrate vs. fat + cellulose concentrations of the diet on food intake and weight gain of rats and on protein concentration, ATP concentration and initial glutathione and taurine concentration of isolated hepatocytes1

<table>
<thead>
<tr>
<th>Diet</th>
<th>LC/HF</th>
<th>MC/MF</th>
<th>HC/LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Daily diet consumption, g/d</td>
<td>19 ± 0.7</td>
<td>20 ± 0.9</td>
<td>19 ± 0.8</td>
</tr>
<tr>
<td>Daily weight gain, g/d</td>
<td>4.7 ± 0.5</td>
<td>4.7 ± 0.4</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>Body weight at end of feeding period, g</td>
<td>285 ± 10.0</td>
<td>297 ± 9.5</td>
<td>295 ± 8.3</td>
</tr>
<tr>
<td>Protein concentration, mg protein/g wet weight hepatocytes</td>
<td>216 ± 9.9</td>
<td>213 ± 5.3</td>
<td>220 ± 11.0</td>
</tr>
<tr>
<td>ATP concentration, µmol/g wet weight hepatocytes</td>
<td>2.1 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Initial taurine concentration, µmol/g wet weight hepatocytes</td>
<td>3.4 ± 0.9</td>
<td>8.2 ± 1.4</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>Initial GSH concentration, µmol/g wet weight hepatocytes</td>
<td>3.2 ± 0.4</td>
<td>3.3 ± 0.2</td>
<td>4.2 ± 0.7</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. Values for the three dietary groups were not significantly different [P > 0.05]. LC/HF = low carbohydrate/high fat; MC/MF = moderate carbohydrate/moderate fat; HC/LF = high carbohydrate/low fat.

DISCUSSION

Effects of dietary protein vs. other macronutrients.

In Experiment 1, CDO activity increased and CSDC and GCS activities decreased in a stepwise fashion with increasing protein levels. Consistent with the higher level of CDO activity, the rate of cysteine catabolism (sulfate + taurine production) in intact hepatocytes incubated with 0.2 mmol/L cysteine was more rapid in hepatocytes isolated from rats fed higher levels of protein. The rate of taurine production did not increase in a step-wise fashion as the protein level was increased, evidently because cysteinensulfinate decarboxylation was restricted due to the lower CSDC activity that accompanied higher CDO activity. Decreases in GCS activity with higher levels of dietary protein were accompanied by decreases in the rate of glutathione synthesis by intact hepatocytes. Thus, both the levels of metabolite production and changes in enzyme activities observed in Experiment 1 corresponded with those seen in previous studies (Bagley and Stipanuk 1994, Bella and Stipanuk 1995 and in press) in which protein was added at the expense of carbohydrate instead of fat + cellulose.

Two other enzymes involved in alternative pathways of cysteine catabolism were also assayed. AAT, especially the mitochondrial isozyme, catalyzes the transamination of cysteinesulfinate with α-ketoglutarate and leads to production of sulfate and pyruvate from cysteinesulfinate (Recasens et al. 1980, Yagi et al. 1979). CyL catalyzes the β-cleavage of cyst(e)ine to yield inorganic sulfur, pyruvate and ammonium, which

FIGURE 3 Activities of cysteine dioxygenase (CDO), cysteinesulfinate decarboxylase (CSDC), γ-glutamylcysteine synthetase (GCS), aspartate aminotransferase (AAT), and cystathionine-γ-lyase (CyL) in isolated hepatocytes from rats fed diets with constant protein levels but different levels of carbohydrate vs. fat + cellulose. Values are means ± SEM for six (LC/HF), seven (MC/MF) or eight rats (HC/LF). CDO and GCS activities are one tenth and AAT activity is ten times the value read from the vertical axis. Values are expressed as specific activity in the hepatocyte homogenate (CDO and GCS) or in the 20,000 × g supernatant fraction of hepatocyte homogenate (CSDC, AAT and CyL). For each enzyme activity, bars designated by different superscript letters are significantly different [P ≤ 0.05] by Tukey-Kramer’s w-procedure. LC/HF = low carbohydrate/high fat; MC/MF = moderate carbohydrate/moderate fat; HC/LF = high carbohydrate/low fat.
FIGURE 4 Comparison of cysteine metabolism in hepatocytes from rats fed diets with a constant protein level but different levels of carbohydrate vs. fat + cellulose. Values are means ± SEM for six [LC/HF], seven [MC/MF] or eight rats [HC/LF]. Values for \([^{35}S]taurine\) are the sum of \([^{35}S]thiopurine and \([^{35}S]taurine\) production. For each metabolite, bars designated by different superscript letters are significantly different \((P < 0.05)\) by Tukey-Kramer’s \(w\)-procedure. LC/HF = low carbohydrate/high fat; MC/MF = moderate carbohydrate/moderate fat; HC/LF = high carbohydrate/low fat.

is a major route of cysteinesulfinate-independent catabolism of cyst(e)line [Drake et al. 1987, Stipanuk and Beck 1982]. As has been observed in other studies in our laboratory [Bagley and Stipanuk 1994, Stipanuk 1979], neither AAT nor CyL responded in a dose-dependent manner to increases in dietary protein.

In contrast to the response of cysteine metabolism to changes in dietary protein vs. fat + cellulose, there was little change in enzyme activities or levels of metabolite production when dietary carbohydrate vs. fat + cellulose was varied with the protein level held constant. The magnitude of the variations in fat + cellulose levels [vs. protein or carbohydrate] was similar in the two experiments. CDO activity was slightly higher in the MC/MF group than in the other two dietary treatment groups, but the higher CDO activity was not accompanied by an increase in cysteine catabolism by intact hepatocytes. Although there was a trend for both GCS activity and the rate of glutathione production to decrease in rats fed the MC/MF diets vs. the LC/HF diet in Experiment 2, a dose-response effect such as that observed for protein in Experiment 1 was not seen. Additionally, the changes in GCS activity in response to carbohydrate vs. fat + cellulose variations were very small relative to those observed in response to dietary protein. Given the similar effects of dietary protein regardless of whether it was added at the expense of fat + cellulose [Experiment 1] or at the expense of carbohydrate as in the studies of Bagley and Stipanuk (1994), we conclude that the regulation of the activities of enzymes involved in cysteine metabolism is predominantly due to changes in dietary protein intake and not to accompanying changes in the levels of other dietary macronutrients.

The regulation of hepatic CSDC by specific dietary components is of particular interest. CDO activity and glutathione synthesis consistently respond to changes in either dietary protein or sulfur amino acid levels. However, in previous studies, CSDC activity in isolated hepatocytes decreased in response to dietary protein levels [Bagley and Stipanuk 1995, Bella and Stipanuk 1995 and in press] but did not decrease in response to dietary methionine levels unless methionine was added in amounts exceeding 10 g/kg diet [Bagley and Stipanuk 1995, Bella and Stipanuk 1995 and in press]. Furthermore, CSDC activity in cultured hepatocytes [Ohta and Stipanuk, in press] did not change with the addition of methionine to the culture medium, whereas activities of CDO and GCS did change. Results of the present study show that CSDC activity decreased as protein intake was increased at the expense of fat + cellulose and suggest that hepatic CSDC activity changes in response to protein regardless of the carbohydrate to fat ratio of the diet. These observations collectively suggest that the hepatic signal for regulation of CSDC is related to the protein content of the diet and not solely to changes in methionine level or dietary carbohydrate, fat or fiber content. It is possible that CSDC activity may be down-regulated in response to excess amounts of certain amino acids or to mixtures of amino acids (with or without involvement of sulfur amino acids).

Reciprocal regulation of GCS and CDO by sulfur amino acids or protein. In studies in our laboratory in
which both the rates of cysteine catabolism and glutathione synthesis have been measured in rat hepatocytes (Bagley and Stipanuk 1995, Bella and Stipanuk 1995 and in press), increases in cysteine catabolism have always occurred in association with decreases in cysteine utilization for glutathione synthesis. This observed relationship suggests that cysteine catabolism and glutathione synthesis may be reciprocally and coordinately regulated, at least in response to sulfur amino acid availability. We have also observed the same reciprocal pattern of change between cysteine catabolism and glutathione synthesis in hepatocytes cultured in media with different levels of methionine (Ohta and Stipanuk, in press). In the present study, we demonstrated that CDO activity increased and GCS activity decreased in a stepwise manner with increasing protein levels, giving further support to a pattern of reciprocal and coordinated regulation of the rate-limiting enzyme activities for cysteine catabolism and glutathione synthesis. This is also the first direct evidence we have obtained for a dose-dependent regulation of GCS by dietary protein.

Although CSDC activity decreases in response to increased protein intake, changes in CSDC activity are not closely associated with changes in CDO activity as previously reported (Bagley and Stipanuk 1995, Bella and Stipanuk 1995 and in press) and, as noted above, cannot be explained entirely by the sulfur amino acid content of dietary protein.

**Relationship between hepatic glutathione and taurine levels and the capacity for glutathione and taurine synthesis.** Hepatic tissue glutathione levels have often been used as indicators of the body’s capacity for glutathione synthesis. In actuality, hepatic glutathione levels depend upon 1) cysteine availability, 2) the amount of GCS activity, 3) cellular glutathione concentration as a result of feedback inhibition of GCS, and 4) export of glutathione or of glutathione-conjugates in plasma or bile. The initial glutathione content of hepatocytes reported in Table 4 reflects the influence of all four factors as well as the probable washout of some glutathione during the hepatocyte isolation procedure. The higher level of glutathione in hepatocytes from rats fed the higher levels of protein reflects the role of sulfur amino acid availability as a major determinant of the rate of glutathione synthesis in vivo. However, our data indicate that the tissue glutathione level is not a good indicator of the tissue’s capacity to synthesize glutathione at a given cysteine concentration. The rate of glutathione production (including that retained in the cell and that exported in the medium) by hepatocytes incubated with 0.2 mmol/L cysteine was lower in hepatocytes from rats fed higher levels of protein and was consistent with their lower levels of GCS activity (measured by V_max assay of homogenate) and the presumed short-term, feedback regulation of glutathione production by glutathione. If rats adapted to a high protein diet and rats adapted to a low protein diet were to be given an equivalent load of cysteine or cysteine precursor, we would expect the liver of animals adapted to the low protein diet to respond with much greater net synthesis of glutathione. Hence, utilization of cysteine for GSH synthesis is restricted in rats fed high protein diets, even though the absolute rate of synthesis may remain high due to high substrate (cysteine) supply.

Hepatic taurine content has also been used as a proxy for taurine synthetic capacity. Hepatic taurine levels can be considered to reflect 1) cysteine availability, 2) CDO activity or capacity for cysteinesulfinate production, 3) CSDC activity or the capacity for taurine synthesis from cysteinesulfinate, and 4) efflux of taurine or taurine conjugates into plasma or bile. In vivo, high hepatic taurine in rats fed high protein diets reflects greater cysteine availability as well as a greater capacity for cysteinesulfinate production. Thus, up-regulation of CDO accompanies an increase in cysteine availability, in contrast to the inverse relationship noted between hepatic glutathione content and hepatic glutathione synthetic capacity. However, this up-regulation of cysteinesulfinate synthetic capacity in rats fed higher levels of protein may not lead to increased taurine synthesis. CSDC is down-regulated in liver of rats fed higher levels of protein, and this may limit the conversion of cysteinesulfinate to taurine and favor transamination to yield pyruvate and sulfate as we have discussed previously (Bagley and Stipanuk 1994 and 1995; Bella and Stipanuk 1995 and in press).

Given a specific cysteine concentration, the capacity for hepatic taurine synthesis will depend upon the balance of CDO and CSDC activities. In Experiment 1, hepatocytes from rats fed 200 g casein/kg diet had higher CDO activity and higher rates of taurine synthesis than did hepatocytes from rats fed 100 g casein/kg diet. However, the capacity of intact hepatocytes to synthesize taurine did not increase further as dietary protein was increased from 200 to 400 g/kg diet, despite a further increase in CDO activity, due to a marked decrease in CSDC activity. Hence, the rate of taurine production by hepatocytes incubated with 0.2 mmol/L cysteine reflects the balance of CDO and CSDC activities, with higher levels of CDO activity promoting taurine synthesis and lower levels of CSDC activity restricting taurine synthesis in hepatocytes from rats fed high protein diets. The hepatic taurine content under in vivo conditions reflects not only taurine synthetic capacity but also the role of sulfur amino acid availability as demonstrated by the much greater hepatic taurine levels of rats fed 400 g casein/kg diet than of rats fed 200 g casein/kg diet (Table 3) despite their similar in vitro capacities for taurine synthesis [Fig. 2].

Diet-induced changes in taurine synthetic capacity play an important role in vivo as indicated by changes in urinary excretion of taurine and sulfate by rats fed diets with equivalent levels of sulfur amino acids provided as either protein or methionine (Bella and Stipa-
nuk 1995). Hepatocytes of rats fed diets with excess methionine had high CDO and CSDC activities and a high taurine synthetic capacity, whereas hepatocytes of rats fed a high protein diet with an equivalent amount of sulfur amino acids had lower CDO and CSDC activities and a lower taurine synthetic capacity. Intact rats fed the diet with excess methionine excreted much higher amounts of taurine in their urine, both on an absolute basis and as a percentage of total sulfur excretion, compared with the rats fed the high protein diet. This increased synthesis of taurine was not accompanied by a significantly greater taurine concentration of liver of rats fed the high methionine diet (11 vs. 9 µmol/g), suggesting that increased synthesis was associated with increased efflux of taurine from the liver and that hepatic taurine levels may plateau at about 10 µmol/g wet weight. These observations further emphasize the fact that hepatic taurine levels are not a valid indicator of synthetic capacity.

Conclusions. Changes in activities of enzymes involved in cysteine metabolism and in the distribution of cysteine metabolism among various pathways are due to changes in dietary protein intake and not to associated changes in the levels of other dietary macronutrients. CDO and GCS appear to be reciprocally and coordinately regulated in response to sulfur amino acid availability. The response of CDO and CSDC to protein is reciprocal as shown in this study, but the limited response of CSDC to sulfur amino acids in previous studies suggests that CSDC regulation may be in response to amino acids other than or in addition to sulfur amino acids. In addition, our results demonstrate that hepatic levels of glutathione and taurine are not necessarily valid indicators of hepatic capacity for synthesis of these essential metabolites. Further study is required for full understanding of the cellular and molecular mechanisms of diet-induced regulation of CDO, CSDC and GCS and of metabolic flux of cysteine to essential metabolites.

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