Cysteine supplementation reverses methionine restriction effects on rat adiposity: significance of stearoyl-coenzyme A desaturase

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Abstract

Stearoyl-CoA desaturase-1 (SCD1) is a key enzyme in fatty acid and energy metabolism, but little is known about its nutritional regulation. Dietary methionine restriction in rats decreases hepatic Scd1 mRNA and protein, increases energy expenditure, and decreases fat-pad mass/body-weight% (FM/BW%). In humans, plasma concentrations of the methionine product, cysteine, are associated with obesity. To determine which consequences of methionine-restriction are mediated by decreased cysteine availability, we monitored obesity-related variables in 4 dietary groups for 12 weeks: control-fed (CF), methionine-restricted (MR), MR supplemented with 0.5% L-cysteine (MR+Cys) and CF+Cys rats. MR lowered weight gain and FM/BW% despite higher food intake/weight than CF, and lowered serum cysteine. Hepatic Scd1 expression was decreased, with decreased serum SCD1 activity indices (calculated from serum fatty acid profile), decreased serum insulin, leptin and triglycerides, and higher adiponectin. Cysteine supplementation (MR+Cys) essentially reversed all these phenotypes and raised serum cysteine but not methionine to CF levels. Adding extra cysteine to control diet (CF+Cys) increased serum taurine but did not affect serum cysteine, lipids, proteins, or total weight gain. FM/BW% and serum leptin were modestly decreased. Our results indicate that anti-obesity effects of MR are caused by low cysteine and that dietary sulfur amino acid composition contributes to SCD1 regulation.—Elshorbagy, A. K., M. Valdivia-Garcia, D. A. L. Mattocks, J. D. Plummer, A. D. Smith, C. A. Drevon, H. Refsum, and C. E. Perrone. Cysteine supplementation reverses methionine restriction effects on rat adiposity: significance of stearoyl-coenzyme A desaturase. J. Lipid Res. 2011. 52: 104–112.

Supplementary key words

sulfur amino acids • diet • fat mass • food intake • weight gain • insulin • leptin • adiponectin • fatty acid profile • desaturase indices • taurine

Accumulating evidence suggests that the type and quantity of dietary proteins affects body weight. In humans, high-protein diets and leucine supplementation promote weight loss, improve body composition, and decrease insulin when used in weight loss programs (1). These effects may be related to enhanced satiety and increased energy expenditure (2). On the other hand, high habitual protein intake is epidemiologically associated with greater body mass index (BMI) (3) and body fat (3, 4). Rats fed high-protein diets develop insulin resistance (5), and type 1 diabetic patients on a high-protein diet have reduced insulin sensitivity compared with those with lower protein intake (6). Specifically, higher intake of animal-derived protein is associated in large cross-sectional studies with greater BMI and higher prevalence of obesity (7–9). Prospective studies show that subjects with higher animal protein consumption gain more weight over time (10) and are more likely to develop diabetes (11, 12). The possibility that specific amino acid constituents mediate metabolic effects of different protein types is likely but has not been clearly documented.

Methionine, an essential sulfur-containing amino acid mainly ingested in animal-derived foods (13), is a potential mediator of adverse effects of excess animal protein intake (14), and, at high intake levels, is associated with...
increased BMI and cardiovascular risk (15). Methionine is the precursor of homocysteine, a nonproteinogenic amino acid that can in turn be converted to cysteine via the intermediate, cystathionine (16). Although cysteine is the precursor of two sulfur compounds with antioxidant properties, taurine and glutathione (16), plasma total cysteine (tCys) levels are positively associated with body fat mass (17), obesity (18), hypercholesterolemia (19), and metabolic syndrome (20) in humans.

Methionine restriction (MR) is a recognized intervention that reduces fat mass and enhances insulin sensitivity in rats via a host of changes in enzymes, adipokines, and transcription factors involved in lipid and energy metabolism (21–23). In particular, adiponectin, which is a specific inverse predictor of diabetes incidence in humans (24), is markedly elevated by MR (23). Moreover, MR rodents exhibit a hypermetabolic phenotype (23, 25, 26), linked to increased BMI and cardiovascular risk (25). Methionine is an essential amino acid that can in turn be converted to cysteine via the interconversion of cystathionine (16). Although cysteine is the precursor of homocysteine, a nonproteinogenic amino acid that can in turn be converted to cysteine via the intermediate, cystathionine (16). Although cysteine is the precursor of two sulfur compounds with antioxidant properties, taurine and glutathione (16), plasma total cysteine (tCys) levels are positively associated with body fat mass (17), obesity (18), hypercholesterolemia (19), and metabolic syndrome (20) in humans.

Methods

Animal husbandry, diets, and sampling

The study was approved by Orentreich Foundation for Advancement of Science, Inc.’s. Institutional Animal Care and Use Committee and performed in accordance with the National Institutes of Health guidelines for use of animals in research laboratories.

Four-week-old male Fischer-344 rats from Taconic Farms (Germantown, NY) were maintained one rat per cage in a controlled 12 h light/dark cycle with free access to water and a standard diet (LabDiet 5001; PMI Nutrition International, LLC, Brentwood, MO) for 2 weeks. The rats were then randomly assigned to one of four dietary groups consuming chemically defined AIN-76-based diets with protein replaced by different amino acid mixtures (Dyets Inc., Bethlehem, PA) for 12 weeks: 1) Control-fed (CF, 0.86% methionine); 2) methionine-restricted (MR, 0.17% methionine); 3) CF supplemented with 0.5% l-cysteine (CF+Cys); and 4) MR supplemented with 0.5% l-cysteine (MR+Cys). Certificates of analysis indicated that the CF+Cys and MR+Cys diets contained 0.453% and 0.476% (by weight) cysteine, respectively. The un-supplemented control and MR diets were devoid of cysteine and other sulfur amino acids apart from methionine as described previously (29). To compensate for the reduced amino acid content in the MR diet, glutamic acid content was raised on an equal weight basis. Food and water were provided ad libitum.

Body weight was registered at baseline and weekly throughout the experiment. Food intake was measured weekly by weighing residual food at the end of a 48 h period, and the average intake over 24 h was calculated. At baseline and after 6 weeks and 12 weeks on the dietary regimen, nonfasting blood samples were collected from the subclavian vein after anesthetizing the rats using an E-Z Systems apparatus (Palmar, PA).

After completing the dietary regimens for 12 weeks, the rats were euthanized by Euthanex E-Z Systems. White adipose tissue fat pads (inguinal, epididymal, peri-intestinal, retroperitoneal), interscapular brown fat (obtained by dissecting away the white superficial fat and harvesting the deeper brown fat between the shoulder blades), liver, and skeletal (quadriceps) muscle were immediately harvested, weighed, frozen in liquid nitrogen, and stored at −80°C.

Serum biochemistry

Liquid chromatography tandem mass spectrometry was used to analyze total homocysteine (tHcy), methionine, cystathionine, tCys, and total glutathione (tGSH) concentrations (30). Taurine and glutamate were measured by a modification of a previously described liquid chromatography tandem mass spectrometry method (28).

Serum concentrations of glucose, lipids, albumin, and protein were measured on a Beckman Coulter AU400 semi-automated clinical chemistry analyzer (31). Commercially available rat ELISA kits were used to measure leptin (Assay Design, Ann Arbor Michigan), IGF-1 (IDS, Fountain Hills, AZ), insulin (Alpco, Salem, NH), and adiponectin (Millipore, Billerica, MA).

Fatty acid profiles in serum total lipids were assayed by gas liquid chromatography with flame ionization detection at AS Vitas, Oslo Innovation Center, Oslo, Norway (www.vitas.no). The method has been validated for quantification of polynsaturated fatty acids according to US FDA Guidance Document on Bioanalytical Method Validation. In a separate prevalence study, efficacy of the direct transmethylation step has been found to be 96–104% for the lipid classes cholesterol esters, triglycerides, and phospholipids. Fatty acid content was calculated based on the area % of peaks and response factors relative to stearic acid (C18:0).

Estimated indices of desaturase and elongase enzyme activities

Desaturase and elongase activity indices were estimated as product/precursor ratios of fatty acids in serum (32). SCD1-16 activity was estimated as 16:1n-7/16:0, SCD1-18 activity as 18:1n-9/18:0, Δ5-desaturase (D5D) activity as 18:3n-6/18:2n-6; Δ6-desaturase (D6D) activity as 18:2n-6/18:1n-9, and elongase activity as 20:4n-6/20:3n-6 and 20:4n-6/20:3n-6; and elongase activity as 18:0/16:0 (32).

Gene expression profiling

Total RNA was isolated from inguinal white adipose tissue, liver, and quadriceps muscle tissue using Qiagen’s RNeasy kits. RNA concentrations were determined spectrophotometrically and their integrity was assessed by agarose gel electrophoresis. MRNA (mRNA) was transcribed into cDNA using a High-Capacity cDNA Reverse Transcription Kit in a Perkin-Elmer (Wellesley, MA) GeneAmp PCR System 9600 as previously described (21). Multiplexed PCR was conducted in a StepOnePlus Real-Time PCR System with the prepared cDNA and commercially available TaqMan primer-probe sets (Life Technologies, Carlsbad CA). Gene expression was assessed by the comparative CT (ΔΔCT)
In summary, supplementation of the MR diet with cysteine prevented most of the MR effects on serum lipids and protein markers, whereas addition of cysteine to the control diet had no effect on these parameters.

Serum fatty acid profile

The fatty acid profile in total serum lipids was determined after 12 weeks of dietary intervention. Marked differences were noted between the MR and CF groups, which were essentially blunted by cysteine (MR+Cys) (Table 2). MR lowered the levels of monounsaturated fatty acids palmitoleic (16:1,n-7) and oleic acids (18:1,n-9), and raised stearic acid (18:0), with consequent decreases in estimated indices of SCD1-16 and SCD1-18 desaturase activities (Fig. 2). These effects were reversed by cysteine supplementation of the MR diet (MR+Cys). Furthermore, cysteine supplementation of the MR diet markedly lowered estimated indices of D5D, D6D, and elongase activities (Fig. 2).
MR also raised the omega 6 (n-6) polyunsaturated fatty acids γ-linoleic acid (18:3, n-6) and docosadienoic acid (22:2, n-6), and reduced eicosadienoic acid (20:2, n-6). These effects were also reversed by cysteine. In contrast, cysteine supplementation of the control diet produced no effects. In summary, supplementing the MR diet with cysteine (MR+Cys) restored plasma tCys and cystathionine to control levels, but only partly raised taurine levels and partly lowered tHcy. After 12 weeks of intervention, serum tHcy in MR+Cys group (25.5 ± 2.2 μmol/L) was lower (P < 0.001) compared with MR (37.7 ± 3.4 μmol/L), but remained higher (P = 0.016) than CF (18.2 ± 0.6 μmol/L). Serum methionine was not raised by adding cysteine to the MR diet (Fig. 4).

No consistent difference in sulfur amino acid levels was observed between the CF+Cys and CF groups, except for an approximate 25% and 15% increase of serum taurine at 6 weeks and 12 weeks, respectively (P ≤ 0.005 at both time points; Fig. 4). Notably, serum tCys was not higher in CF+Cys versus CF after 6 or 12 weeks on the diets (Fig. 4).

In summary, supplementing the MR diet with cysteine restored serum tCys, cystathionine, tHcy, and taurine to or toward control levels, with no effect on methionine. Adding cysteine to a control diet failed to increase serum tCys but raised serum taurine.

**Gene expression profile**

We examined changes in expression of some genes known to be responsive to MR (Fig. 5) (21). Consistent with previous reports, MR enhanced hepatic expression of peroxisome proliferator activated receptor γ (Pparg), and markedly suppressed Scd1. Cysteine (MR+Cys) reversed or partly reversed these effects. However, cysteine supplementation of the control diet (CF+Cys) had no effect on either gene.

In contrast to hepatic changes, Scd1 expression was greatly induced in white adipose tissue from MR rats, as previously observed (21). Cysteine (MR+Cys) lowered adipose tissue Scd1 expression to control levels, and further suppressed adipose tissue Scd1 expression below control values when added to the control diet (CF+Cys). Supplementing the control diet with cysteine (CF+Cys) also decreased the expression of cytochrome c oxidase subunit IV.

### Table 1. Serum concentration of glucose, lipids and proteins in rats fed different diets

|                | CF          | CF+Cys      | MR          | MR+Cys      |
|----------------|-------------|-------------|-------------|-------------|
| Glucose, mM    | Baseline    | Final       | Baseline    | Final       |
|                | 10.11 ± 0.19| 10.30 ± 0.21| 9.67 ± 0.12 | 10.09 ± 1.28|
| Glycerol, μM   | Baseline    | Final       | Baseline    | Final       |
|                | 174 ± 10    | 173 ± 8     | 173 ± 12    | 149 ± 19    |
|                | 230 ± 14    | 222 ± 16    | 210 ± 21    |             |
| Triglycerides, mM | Baseline | Final | Baseline | Final |
|                | 1.12 ± 0.11 | 0.89 ± 0.06 | 0.84 ± 0.07 | 0.80 ± 0.11 |
| Free fatty acids, mM | Baseline | Final | Baseline | Final |
|                | 0.56 ± 0.03 | 0.56 ± 0.03 | 0.53 ± 0.03 | 0.52 ± 0.07 |
| Total-C, mM    | Baseline    | Final       | Baseline    | Final       |
|                | 2.22 ± 0.02 | 2.13 ± 0.03 | 2.08 ± 0.05 | 2.26 ± 0.28 |
| LDL-C, mM      | Baseline    | Final       | Baseline    | Final       |
|                | 0.29 ± 0.01 | 0.30 ± 0.01 | 0.35 ± 0.02 | 0.32 ± 0.04 |
| HDL-C, mM      | Baseline    | Final       | Baseline    | Final       |
|                | 1.36 ± 0.01 | 1.31 ± 0.02 | 1.40 ± 0.04 | 1.40 ± 0.18 |
| Protein, g/L   | Baseline    | Final       | Baseline    | Final       |
|                | 56.4 ± 0.4  | 56.7 ± 0.3  | 57.2 ± 0.4  | 56.8 ± 0.4  |
| Albumin, g/L   | Baseline    | Final       | Baseline    | Final       |
|                | 29.8 ± 0.2  | 29.6 ± 0.3  | 29.6 ± 0.3  | 29.2 ± 3.6  |

*P < 0.05 compared with CF.

1 P < 0.01 compared with CF.

MR+Cys significantly different from MR, P < 0.001.
tion of plasma tCys with obesity (17, 18), we investigated weight gain and fat mass% and higher energy expenditure MR rats Cysteine supplementation of MR diet: MR+Cys versus and in fact, tended to decrease adiposity. However, cysteine supplementation reversed by cysteine. However, cysteine supplementation anti-obesity effects of MR, as these effects were essentially reflecting secondary to MR is the major factor underlying the underlying diet. Our results indicate that reduced cysteine diets would increase fat accumulation in rats. Cysteine also modestly decreased expression of carnitine palmitoyl transferase 1b (Cpt1b) and fatty acid-binding protein 4 (Fabp4) in adipose tissue. In skeletal muscle, cysteine reversed a 2-fold upregulation of uncoupling protein 3 (Ucp3) induced by MR, and also modestly decreased expression of carnitine palmitoyl transferase 1b (Cpt1b) (CF+Cys vs. CF and MR+Cys vs. MR) and Cox4i1 (MR+Cys vs. MR).

**DISCUSSION**

To study the potential link between anti-obesity effects of MR in rats (21, 23, 28) and the epidemiologic association of plasma tCys with obesity (17, 18), we investigated whether cysteine supplementation of either MR or control diets would increase fat accumulation in rats. Cysteine supplementation had different effects depending on the underlying diet. Our results indicate that reduced cysteine supply secondary to MR is the major factor underlying the anti-obesity effects of MR, as these effects were essentially reversed by cysteine. However, cysteine supplementation of a control diet did not promote further fat mass gain, and in fact, tended to decrease adiposity.

### Cysteine supplementation of MR diet: MR+Cys versus MR rats

Low-methionine dietary rodent models feature lower weight gain and fat mass% and higher energy expenditure (Cox4i1), Pparg, and fatty acid-binding protein 4 (Fabp4) in adipose tissue.

In skeletal muscle, cysteine reversed a 2-fold upregulation of uncoupling protein 3 (Ucp3) induced by MR, and also modestly decreased expression of carnitine palmitoyl transferase 1b (Cpt1b) (CF+Cys vs. CF and MR+Cys vs. MR) and Cox4i1 (MR+Cys vs. MR).

#### TABLE 2. Serum fatty acid profile in total lipids in rats fed different diets

| Fatty Acid CF | CF + Cys | MR | MR + Cys |
|---------------|----------|----|---------|
| **Saturated** |          |    |         |
| C14:0 (myristic) | 0.50 ± 0.03 | 0.51 ± 0.07 | 0.30 ± 0.02<sup>a</sup> | 0.61 ± 0.07<sup>a</sup> |
| C15:0 (pentadecanoic) | 0.16 ± 0.01 | 0.16 ± 0.01 | 0.12 ± 0.02<sup>a</sup> | 0.22 ± 0.03<sup>a</sup> |
| C16:0 (palmitic) | 17.86 ± 0.40 | 18.01 ± 0.75 | 15.98 ± 0.59<sup>b</sup> | 19.76 ± 0.92<sup>b</sup> |
| C17:0 (heptadecanoic) | 0.17 ± 0.01 | 0.17 ± 0.01 | 0.15 ± 0.01<sup>c</sup> | 0.17 ± 0.02<sup>c</sup> |
| C18:0 (stearic) | 8.65 ± 0.64 | 8.45 ± 0.87 | 10.95 ± 0.52<sup>d</sup> | 6.25 ± 0.91<sup>d</sup> |
| **Monounsaturated** |          |    |         |
| C20:0 (arachidic) | 0.17 ± 0.01 | 0.16 ± 0.00 | 0.23 ± 0.02<sup>c</sup> | 0.18 ± 0.02<sup>c</sup> |
| C22:0 (behenic) | 0.17 ± 0.01 | 0.19 ± 0.02 | 0.36 ± 0.02<sup>c</sup> | 0.18 ± 0.02<sup>c</sup> |

**Polyunsaturated, n-3**

| C20:4, n-6 (arachidonic) | 21.23 ± 2.39 | 21.19 ± 2.81 | 23.70 ± 2.31 | 12.15 ± 2.33 |
| C22:4, n-6 (docosahexaenoic) | 0.14 ± 0.11 | 0.98 ± 0.13 | 1.12 ± 0.13 | 0.56 ± 0.10<sup>c</sup> |
| C22:5, n-3 (docosapentaenoic) | 0.08 ± 0.01 | 0.08 ± 0.01 | 0.11 ± 0.01 | 0.07 ± 0.01 |
| C22:6, n-3 (docosahexaenoic) | 0.14 ± 0.27 | 0.14 ± 0.15 | 0.13 ± 0.13 | 0.50 ± 0.10<sup>c</sup> |
| C18:2, n-6 (linoleic) | 0.35 ± 0.06 | 0.31 ± 0.03 | 0.28 ± 0.04<sup>c</sup> | 0.42 ± 0.05<sup>c</sup> |
| C18:3, n-6 (linolenic) | 0.40 ± 0.04 | 0.37 ± 0.03 | 0.50 ± 0.07<sup>c</sup> | 0.30 ± 0.03<sup>c</sup> |
| C18:3, n-3 (α-linolenic) | 0.28 ± 0.06 | 0.30 ± 0.03 | 0.26 ± 0.05 | 0.41 ± 0.08<sup>c</sup> |
| C20:4, n-6 (arachidonic) | 1.79 ± 0.24 | 15.29 ± 1.72 | 19.76 ± 0.92<sup>c</sup> |
| C20:5, n-3 (eicosapentaenoic) | 0.05 ± 0.01 | 0.06 ± 0.00<sup>c</sup> | 0.07 ± 0.01<sup>c</sup> |
| C20:3, n-6 (dihomo-γ-linolenic) | 0.05 ± 0.01 | 0.05 ± 0.01 | 0.06 ± 0.00<sup>c</sup> | 0.07 ± 0.01<sup>c</sup> |
| C18:1, n-9 (oleic) | 12.48 ± 0.97 | 12.41 ± 1.71 | 8.55 ± 1.14<sup>d</sup> | 15.29 ± 1.72<sup>d</sup> |
| C16:0 (palmitic) | 17.86 ± 0.40 | 18.01 ± 0.75 | 15.98 ± 0.59<sup>b</sup> | 19.76 ± 0.92<sup>b</sup> |
| C15:0 (pentadecanoid) | 0.16 ± 0.01 | 0.16 ± 0.01 | 0.12 ± 0.02<sup>a</sup> | 0.22 ± 0.03<sup>a</sup> |
| C18:1, n-11 | 0.17 ± 0.01 | 0.17 ± 0.01 | 0.15 ± 0.01<sup>c</sup> | 0.17 ± 0.02<sup>c</sup> |
| C16:1 (palmitoleic) | 1.80 ± 0.17 | 1.58 ± 0.33 | 0.82 ± 0.11<sup>c</sup> | 1.58 ± 0.63<sup>c</sup> |
| C17:0 (heptadecanoic) | 0.17 ± 0.01 | 0.17 ± 0.01 | 0.15 ± 0.01<sup>c</sup> | 0.17 ± 0.02<sup>c</sup> |
| C18:0 (stearic) | 8.65 ± 0.64 | 8.45 ± 0.87 | 10.95 ± 0.52<sup>d</sup> | 6.25 ± 0.91<sup>d</sup> |
| C19:0 (eicosanoic) | 0.19 ± 0.01 | 0.19 ± 0.01 | 0.19 ± 0.05 | 0.18 ± 0.01 |
| C20:0 (arachidic) | 0.17 ± 0.01 | 0.19 ± 0.02 | 0.36 ± 0.02<sup>c</sup> | 0.18 ± 0.02<sup>c</sup> |
| C22:0 (behenic) | 0.17 ± 0.01 | 0.19 ± 0.02 | 0.36 ± 0.02<sup>c</sup> | 0.18 ± 0.02<sup>c</sup> |

After 12 weeks on the experimental diets. Data represents mean ± SEM, g/100g fatty acid methyl ester. CF, control-fed; CF+Cys, control-fed supplemented with cysteine; MR, methionine-restricted; MR+Cys, methionine-restricted supplemented with cysteine. N = 6 per group. All fatty acids showed significant differences by ANOVA (P < 0.05), except C20:5, n-3. Pairwise comparisons are presented only for CF versus each of the other groups, and for MR versus MR+Cys.

* P < 0.05 compared with CF.
* P < 0.001 compared with CF.
* MR+Cys significantly different from MR, P < 0.05.
* MR+Cys significantly different from MR, P < 0.001.

**Fig. 2.** Estimated indices of desaturase and elongase activities in CF (control-fed), CF+Cys (control-fed supplemented with cysteine), MR (methionine-restricted), and MR+Cys rats after 12 weeks. Data represents mean for six rats per group. All indices show significant differences by ANOVA (P < 0.05). *P < 0.05 compared with CF; ** P < 0.001 compared with CF; MR+Cys significantly different from MR, P < 0.05; ***MR+Cys significantly different from MR, P < 0.001.
Cysteine supplementation, SCD1 and adiposity

kinase underlies the hypermetabolism induced by hepatic SCD1 inhibition (27, 39). Further, cysteine reduced skeletal muscle CPT1b mRNA, which is translated into the rate-limiting enzyme in transport of long-chain fatty acid into mitochondria for $\beta$-oxidation (40). Cysteine also reversed the MR-induced up-regulation of muscle Ucp3. Mice overexpressing Ucp3 are hypermetabolic and lean, at least partly due to uncoupling of substrate oxidation from ATP production (41), although whether UCP3 has a physiological function in regulating energy expenditure is uncertain (42). Overall, cysteine induced transcriptional changes that favored fatty acid partitioning away from $\beta$-oxidation.

The possible contribution of the integrated stress response to the SCD1 and fat mass changes of MR rats deserves investigation. Integrated stress response, triggered by numerous factors including imbalance or deprivation of essential amino acids, involves phosphorylation of the $\alpha$-subunit of the eukaryotic initiation factor 2 complex (eIF2$\alpha$). Phosphorylated eIF2$\alpha$ induces subsequent changes in transcription and translation of genes involved in adaptations to dietary stress (43). It was recently shown that MR rats feature enhanced eIF2$\alpha$ phosphorylation in conjunc-

![Fig. 3. Changes in serum hormones and adipokines over 12 weeks in CF (control-fed), CF+Cys (control-fed supplemented with cysteine), MR (methionine-restricted), and MR+Cys rats. Data represents mean ± SEM; N = 8 per group. All adipokines studied showed significant differences by ANOVA ($P < 0.05$). For relevant pairwise comparisons, see text. One outlier value was excluded from the CF+Cys 6-week insulin data.](image1)

![Fig. 4. Changes in serum sulfur amino acids and glutamate over 12 weeks in CF (control-fed), CF+Cys (control-fed supplemented with cysteine), MR (methionine-restricted), and MR+Cys rats. Data represents mean ± SEM; N = 8 per group. All amino acids illustrated show significant group differences by ANOVA ($P < 0.05$). For relevant pairwise comparisons, see text.](image2)
tion with upregulation of various components of the integrated stress response (44). The same pathway was found to mediate both SCD1 suppression and decreased abdominal fat mass in response to leucine deprivation (45). This raises the possibility that the changes in SCD1 and fat mass observed in MR rats, and their reversal by cysteine, may be mediated by elements of the integrated stress response.

One limitation of this study is that SCD1 indices were calculated from fatty acid profiles of total serum lipids, rather than of VLDL, which better reflects hepatic Scd1 expression (32). However, measuring fatty acid profile in specific lipid fractions is particularly indicated in human studies in which SCD1 indices are used to infer gene expression (32, 46). In the present study, Scd1 mRNA was measured in liver and white adipose tissue and the serum fatty acid profile clearly coincided with hepatic gene expression. In contrast to liver, adipose tissue showed enhanced Scd1 expression in MR rats, an effect that was reversed by cysteine supplementation. Cysteine also markedly decreased the activity indices of elongase, D5D, and D6D. Although these serum indices have been linked to the reduction of abdominal fat mass by cysteine supplementation. Cysteine also markedly decreased the activity indices of elongase, D5D, and D6D. Although these serum indices have been linked to the reduction of abdominal fat mass by cysteine supplementation. Cysteine also markedly decreased the activity indices of elongase, D5D, and D6D.

Both serum methionine and tCys are decreased in MR rats (28). Cysteine blocked the effects of MR without increasing serum methionine. This suggests a relatively direct effect of cysteine rather than a methionine-sparing action of cysteine (49), and implicates lowered cysteine as the cause of reduced adiposity in MR. However, cysteine is the precursor of many biologically active compounds including taurine, GSH, and sulfate (16). A recent study showed decrease in weight gain and in hepatic lipogenesis in rats treated with an inhibitor of the rate-limiting enzyme in GSH synthesis, γ-glutamylcysteine synthetase (50). We observed no difference in plasma tGSH between the marginally decreased food intake in CF+Cys rats, and MR+Cys rats. Cysteine supplementation of Control diet: CF+Cys versus CF rats

Cysteine supplementation of control diet did not induce obesity. Compared with the CF group, CF+Cys rats showed no difference in serum tCys, or in hepatic SCD1 expression or activity indices, and had slightly lower fat mass%. We postulate that this response may have resulted from the marginally decreased food intake in CF+Cys rats, as observed by others (52, 53). Kawayami et al. (53) supplemented the same dose of t-cysteine used in the present study (0.5%) to four diets of different protein quality and quantity and found that cysteine reduced or tended to reduce food consumption. Further, cysteine enhanced weight gain only in groups where plasma tCys was increased above the unsupplemented group (53). Similarly, in the present study, supplementary cysteine enhanced SCD1 function and adiposity only in the model in which plasma tCys increased from subnormal (MR+Cys) levels. Cysteine supplementation in CF+Cys rats neither increased tCys nor adiposity above CF levels, and thus failed to replicate the elevated plasma tCys that is linked to human obesity (17, 18). This may result from the role of the liver, demonstrated in rodents (54), in disposing of excess dietary cysteine via conversion to taurine, as shown by raised serum taurine in CF+Cys rats.

Perspectives

It is tempting to speculate that the lean phenotype associated with low serum tCys in MR rats (28) and the human obesity associated with elevated plasma tCys (17, 18) are related processes. However, the evidence for this remains limited, and different mechanisms may be involved. Our data from rats indicates that MR increases energy expenditure by triggering a cellular response in which lipid synthesis and degradation pathways are simultaneously activated, initiating energy-consuming “futile cycles” (22). In contrast, mice fed a high cysteine diet feature decreased energy expenditure (Elshorbagy et al., unpublished observations), but whether the molecular pathways mirror those of MR remains to be determined. Further, in the present study the effect of dietary cysteine on SCD1 function and fat mass accretion clearly showed a ceiling effect that

![Fig. 5. Gene expression profile in CF (control-fed), CF+Cys (control-fed supplemented with cysteine), MR (methionine-restricted), and MR+Cys rats after 12 weeks of dietary intervention, in liver, inguinal white adipose tissue and quadriceps muscle. Data represents mean ± SEM; N = 8 per group. *P < 0.05 compared with CF; **P ≤ 0.001 compared with CF; #MR+Cys significantly different from MR, P < 0.05; $MR+Cys significantly different from MR, P ≤ 0.001.](https://www.jlr.org/content/52/5/110/f5)
stopped at control values for these parameters. This is at odds with human findings of a linear dose-response relation between tCys and body fat extending well into the obese range of BMI and fat mass (17, 18). The discrepancy could reflect a species difference in body weight response to excess dietary cysteine, or could alternatively indicate that high tCys in humans is not driven by excess cysteine intake.

Apart from the recognized induction of SCD1 in response to glucose oversupply to activate de novo lipogenesis (55), available data on nutritional regulation of SCD1 is limited. Our observation that SCD1 expression and activity indices are strongly responsive to dietary sulfur amino acid composition, with corresponding impact on adiposity and metabolic profile, is a key finding linking dietary protein quality with SCD1 regulation. Whether moderate inter-individual differences in sulfur amino acid intake, as observed between vegetarians and omnivores (35), might influence weight gain and metabolic risk (10, 12) through an effect on SCD1 function deserves investigation.

Author contributions

Amany K. Elshorbagy: Concept, study design, statistical analysis and interpretation, preparation of the manuscript. Maria Valdivia-Garcia: Development of liquid chromatography tandem mass spectrometry method for taurine and glutamate assay, conduction of all mass spectrometry says. Dwight A. Mattocks: Conduction of body and tissue data collection as well as gene expression analysis, review of the manuscript. Jason D. Plummer: Primary contact person regarding formulation of the cysteine supplemented diets as well as conduction of body and tissue data collection. A. David Smith: Concept, study design, critical revision of the manuscript. Christian A. Drevon: Study design related to fatty acid metabolism, critical revision of the manuscript. Heiga Refsum: Concept, study design, critical revision of the manuscript. Carmen E. Perrone: Study design, coordination of MR studies, conduction of MR experiment, tissue collections and serum hormone says and critical revision of the manuscript.

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REFERENCES

1. Devkota, S., and D. K. Layman. 2010. Protein metabolic roles in treatment of obesity. *Curr. Opin. Clin. Nutr. Metab. Care.* 13: 403–407.

2. Halton, T. L., and F. B. Hu. 2004. The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. *J. Am. Coll. Nutr.* 23: 373–385.

3. Silvestrein, K., A. L. Hasselbalch, T. Lallukka, L. Bogl, K. H. Pietiläinen, B. L. Heitmann, K. Schousboe, A. Rissanen, K. O. Kyyvik, T. I. Sorensen, et al. 2009. Modification effects of physical activity and protein intake on heritability of body size and composition. *Am. J. Clin. Nutr.* 90: 1096–1103.

4. Koppes, L. L., N. Boon, A. C. Nooyens, W. van Mechelen, and W. H. Saris. 2009. Macronutrient distribution over a period of 23 years in relation to energy intake and body fatness. *Br. J. Nutr.* 101: 108–115.

5. Rossetti, L., D. L. Rothman, R. A. DeFronzo, and G. I. Shulman. 1989. Effect of dietary protein on in vivo insulin action and liver glycogen repletion. *Am. J. Physiol.* 257: E212–E219.

6. Linn, T., R. Geyer, S. Prasse, and H. Laube. 1996. Effect of dietary protein intake on insulin secretion and glucose metabolism in insulin-dependent diabetes mellitus. *J. Clin. Endocrinol. Metab.* 81: 3938–3943.

7. Newby, P. K., K. L. Tucker, and A. Wolk. 2005. Risk of overweight and obesity among semivegetarian, lactovegetarian, and vegan women. *Am. J. Clin. Nutr.* 81: 1267–1274.

8. Murtaugh, M. A., J. S. Herrick, C. Sweeney, K. B. Baugnattner, A. R. Guiñano, T. Byers, and M. L. Slattery. 2007. Diet composition and risk of overweight and obesity in women living in the southwestern United States. *J. Am. Diet. Assoc.* 107: 1311–1321.

9. Lee, S. A., W. Wen, W. H. Xu, W. Zheng, H. Li, G. Yang, Y. B. Xiang, and X. O. Shu. 2008. Prevalence of obesity and correlations with lifestyle and dietary factors in Chinese men. *Obesity (Silver Spring).* 16: 1440–1447.

10. Rosell, M., P. Appleby, E. Spencer, and T. Key. 2006. Weight gain over 5 years in 21,966 meat-eating, fish-eating, vegetarian, and vegan men and women in EPIC-Oxford. *Int. J. Obs. (Lond).* 59: 1389–1396.

11. Vang, A. P. N. Singh, J. W. Lee, E. H. Haddad, and C. H. Brinigar. 2008. Meats, processed meats, obesity, weight gain and occurrence of diabetes among adults: findings from Adventist Health Studies. *Ann. Nutr. Metab.* 52: 96–104.

12. Stulpi, J. W., W. Beuleus, L. D. van der A, A. M. Spijkerman, D. E. Grobbbee, and Y. T. van der Schouw. 2010. Dietary intake of total, animal, and vegetable protein and risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL study. *Diabetes Care.* 33: 43–48.

13. Pennington, J. A., and Douglass, J. S. 2005. Bowes & Church’s Food Values of Portions Commonly Used. 18th edition. Lippincott Williams & Wilkins, Philadelphia.

14. McCarty, M. F., J. Barroso-Aranda, and F. Contreras. 2009. The low-methionine content of vegan diets may make methionine restriction feasible as a life extension strategy. *Med. Hypotheses.* 72: 125–128.

15. Virtanen, J. K., S. Voutilainen, T. H. Rissanen, P. Haapapalo, J. M. Mursu, J. A. Laakkanen, H. Poulsen, T. A. Kakka, and J. T. Salonen. 2006. High dietary methionine intake increases the risk of acute coronary events in middle-aged men. *Nutr. Metab. Cardiovasc. Dis.* 16: 113–120.

16. Stipanuk, M. H., J. E. Dominy, Jr., J. I. Lee, and R. M. Coloso. 2006. Mammalian cysteine metabolism: new insights into regulation of cysteine metabolism. *J. Nutr.* 136: 1652S–1659S.

17. Elshorbagy, A., G. Gjesdal, E. Nurm, C. G. Gjesdal, C. Tell, P. M. Ueland, O. Nygard, A. Tverdal, S. E. Vollset, and H. Refsum. 2008. Homocysteine, cysteine, and body composition in the Hordaland Homocysteine Study: does cysteine link amino acid and lipid metabolism? *Am. J. Clin. Nutr.* 88: 738–746.

18. Elshorbagy, A. K., H. Refsum, A. D. Smith, and I. M. Graham. 2009. The association of plasma cysteine and gamma-glutamyltransferase with BMI and obesity. *Obesity (Silver Spring).* 17: 1435–1440.

19. El-Hairy, L., P. M. Ueland, O. Nygard, H. Refsum, and S. E. Vollset. 1999. Lifestyle and cardiovascular disease risk factors as determinants of total cysteine in plasma: the Hordaland Homocysteine Study. *Am. J. Clin. Nutr.* 70: 1016–1024.

20. Giril, P., N. Jacob, C. Dourmap, B. Hansel, A. Carrie, E. Bruckert, X. Girerd, and M. J. Chapman. 2008. Elevated gamma-glutamyltransferase activity and perturbed thiol profile are associated with features of metabolic syndrome. *Arterioscler. Thromb. Vasc. Biol.* 28: 587–593.

21. Perrone, C. E., D. A. L. Mattocks, M. Jarvis-Morar, J. D. Plummer, and N. Orentreich. 2010. Methionine restriction effects on mitochondrial biogenesis and aerobic capacity in white adipose tissue, liver and skeletal muscle of F344 rats. *Metabolism.* 59: 1006–1011.

22. Perrone, C. E., D. A. Mattocks, G. Hristopoulos, J. D. Plummer, R. A. Krajcik, and N. Orentreich. 2008. Methionine restriction effects on 11-HSD1 activity and lipogenic/lipolytic balance in F344 rat adipose tissue. *J. Lipid Res.* 49: 12–23.

23. Malloy, V. L., R. A. Krajcik, S. J. Bailey, G. Hristopoulos, J. D. Plummer, and N. Orentreich. 2006. Methionine restriction decreases visceral fat mass and preserves insulin action in aging male Fischer 344 rats independent of energy restriction. *Aging Cell.* 5: 305–314.
37. Gall, W. E., K. Beeke, K. A. Lawton, K. P. Adam, M. W. Mitchell, P. J. Nakhle, J. A. Ryals, M. V. Milburn, M. Nannipieri, S. Camatra, et al. 2010. Alpha-hydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population. PLoS ONE. 5: e10883.

38. Jeyakumar, S. M., P. Lopamudra, S. Padmini, N. Balakrishna, N. V. Giridharan, and A. Vajreswari. 2009. Fatty acid desaturation index correlates with body mass and adiposity indices of obesity in Wistar NIN obese mutant rat strains WNN1/Ob and WNN1/GR-Ob. Nutr. Metab. (Lond). 6: 27.

39. Dobrzný, P., A. Dobrzný, M. Miyazaki, P. Cohen, E. Asilmaz, D. G. Hardie, J. M. Friedman, and J. M. Nambi. 2004. Stearoyl-CoA desaturase 1 deficiency increases fatty acid oxidation by activating AMP-activated protein kinase in liver. Proc. Natl. Acad. Sci. USA. 101: 6409–6414.

40. Ramsay, R. R., R. D. Gourdour, and F. R. van der Leij. 2001. Molecular enzymology of carnitine transfer and transport. Biochim. Biophys. Acta. 1546: 21–13.

41. Clapham, J. C., J. R. Arch, H. Chapman, A. Haynes, C. Lister, G. B. Moore, V. Piercy, S. A. Carter, I. Lehner, S. A. Smith, et al. 2000. Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. Nature. 406: 415–418.

42. Nabben, M., and J. Hoeks. 2008. Mitochondrial uncoupling protein 3 and its role in cardiac- and skeletal muscle metabolism. Physiol. Behav. 94: 250–269.

43. Kilberg, M. S., J. Shan, and N. Su. 2009. ATF4-dependent transcription mediates signaling of amino acid limitation. Trends Endocrinol. Metab. 20: 436–443.

44. Sikalidis, A. K., and M. H. Stipanuk. 2010. Growing rats respond to a sulfur amino acid-deficient diet by phosphorylation of the alpha subunit of eukaryotic initiation factor 2 heterotrimeric complex and induction of adaptive components of the integrated stress response. J. Nutr. 140: 1080–1085.

45. Guo, F., and D. R. Cavenzer. 2007. The GCN2 eIF2alpha kinase regulates fatty-acid homeostasis in the liver during deprivation of an essential amino acid. Cell Metab. 5: 103–114.

46. Flowers, M. T. 2009. The delta9 fatty acid desaturation index as a predictor of metabolic disease. Clin. Chem. 55: 2071–2073.

47. Murakami, K., S. Sasaki, Y. Takahashi, K. Uenishi, T. Watanabe, T. Kohri, M. Yamasaki, R. Watanabe, K. Baba, K. Shibata, et al. 2008. Lower estimates of delta-5 desaturase and elongase activity are related to adverse profiles for several metabolic risk factors in young Japanese women. Nutr. Res. 28: 816–824.

48. Warenjo, E., U. Riserus, and B. Vessby. 2005. Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. Diabetologia. 48: 1995–2005.

49. Finkelstein, J. D. 1988. Methionine metabolism in mammals. The methionine-sparing effect of cystine. J. Biol. Chem. 263: 11750–11754.

50. Brandtch, C., T. Schmidt, D. Behn, K. Weisse, A. S. Mueller, and G. I. Stangl. 2010. Glutathione deficiency down-regulates hepatic lipogenesis in rats. Lipids Health Dis. 9: 50.

51. Richie, J. P., Jr., D. Komminou, Y. Leutzinger, W. Kleinman, N. Orentreich, V. Malloy, and J. A. Zimmerman. 2004. Tissue glutathione and cysteine levels in methionine-restricted rats. Nutrition. 20: 800–805.

52. Okawa, H., T. Morita, and K. Sugiyama. 2007. Cysteine supplementation decreases plasma homocysteine concentration in rats fed on a low-casein diet in rats. Biosci. Biotechnol. Biochem. 71: 91–97.

53. Kawakami, Y., S. Ohuchi, T. Morita, and K. Sugiyama. 2009. Hypohomocysteinemic effect of cysteine is associated with increased plasma cysteine concentration in rats fed diets low in protein and methionine levels. J. Nutr. Sci. Vitaminol. (Tokyo). 55: 66–74.

54. Stipanuk, M. H. 2004. Role of the liver in regulation of body cysteine and taurine levels: a brief review. Nutr. Res. 29: 105–110.

55. Houdali, B., H. G. Wahl, M. Kresi, V. Nguyen, M. Haap, F. Machicoa, H. P. Ammon, W. Renn, E. D. Schleicher, and H. U. Haring. 2003. Glucose oversupply increases Delta9-desaturation expression and its metabolites in rat skeletal muscle. Diabetologia. 46: 203–212.