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Basic nutritional investigation

Sulfur amino acids in methionine-restricted rats: Hyperhomocysteinemia

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ABSTRACT

Objective: Dietary methionine restriction in Fischer-344 rats favorably influences visceral fat mass, insulin sensitivity, metabolic parameters, and longevity. However, little is known about the effects of methionine restriction on serum methionine and its downstream sulfur amino acids. We investigated the serum sulfur amino acid profile of male Fischer-344 rats fed a methionine-restricted diet for 3 mo.

Methods and results: Using tandem mass spectrometry, we observed marked reduction in serum concentrations of methionine, cystathionine, cysteine, and taurine in methionine-restricted rats compared with control ($P < 0.001$) and a 2.5-fold elevation of homocysteine ($P < 0.001$).

Conclusion: This suggests that homocysteine trans-sulfuration may be inhibited by methionine restriction, and that some of the effects of methionine restriction may be mediated by changes in sulfur amino acids downstream of methionine.

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Introduction

Methionine is the only essential sulfur-containing amino acid and the precursor of the sulfur amino acids homocysteine, cystathionine, cysteine, and taurine [1]. Upon activation to *S*-adenosylmethionine, methionine acts as a methyl donor producing *S*-adenosyl-homocysteine and subsequently homocysteine. Homocysteine can be re-methylated to synthesize methionine or irreversibly metabolized to cystathionine and, hence, cysteine by the consecutive actions of cystathionine β-synthase (CBS) and cystathionase enzymes [1]. Cysteine can be incorporated into proteins, used in the synthesis of glutathione, or oxidized to taurine [1].

Favorable effects of dietary methionine restriction in rodents have been observed on visceral fat, glucose tolerance, oxidative stress, and longevity [2–7], independently of caloric restriction [2,7], making this a potential intervention in humans [8]. Consistent with this, vegetarian diets, which are low in methionine, are associated with decreased risk of obesity [9] and diabetes [10] in humans.

Elevation of plasma total homocysteine (tHcy) with excessive methionine supplementation [11] raised the hypothesis that some of the beneficial effects of methionine restriction, especially on oxidative stress, may be mediated by controlling homocysteine levels [12,13]. However, apart from changes in cysteine and glutathione [14], little is known about the impact of methionine restriction on serum methionine and downstream sulfur amino acids. We therefore sought to investigate the serum sulfur amino acid profile of methionine-restricted (MR) rats.

Materials and methods

Methionine restriction

This study was approved by the Orentreich Foundation for Advancement of Science, Inc., institutional animal care and use committee and performed in accordance with National Institutes of Health guidelines for use of animals in research laboratories.

Four-week-old male Fischer-344 rats ($n = 22$) from Taconic Farms (Germantown, NY, USA) were maintained one rat per cage on a 12-h light/dark cycle and fed a standard diet (LabDiet 5001; PMI Nutrition International, LLC, Brentwood, MO, USA) for 2 wk. At 6 wk of age, the rats were randomly assigned to a control or an MR diet and maintained on these diets for 12 wk. Chemically defined AIN-76–based diets with protein replaced by amino acid mixtures containing 0.17% methionine (MR diet) or 0.86% methionine (control diet) were used (Dyets Inc., Bethlehem, PA, USA). Control and MR diets were devoid of cysteine and other sulfur-containing amino acids apart from methionine (diet composition detailed previously [7]). To compensate for reduced amino acid content of

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the MR diet, glutamic acid content was increased on an equal-weight basis. Food and water were provided ad libitum.

After completing the dietary regimen, the rats were weighed and then anesthetized using Euthenex Easy Anesthesia system (Palmar, PA, USA). Blood was collected from the subclavian vein before euthanasia. Visceral fat mass was determined by surgical excision and weighing of the epididymal and retroperitoneal fat pads.

Assay methods

Liquid chromatographic tandem mass spectrometry (LC-MS/MS) was used to analyze tHcy, methionine, cystathionine, total cysteine (tCys), and total glutathione (tGSH) concentrations using a modification of a previously described method [15].

Taurine measurement by LC-MS/MS

An internal standard ([$^{13}C_2$]taurine; Isotec, Sigma-Aldrich, Miamisburg, OH, USA) was prepared in 30 mmol/L of ammonium formate buffer (Riedstrasse, Steinheim, Germany) (pH 3.5) to a 50-μmol/L final concentration. Fifteen microliters of pooled plasma samples was used to prepare a control sample by adding 15 μL of labeled taurine solution and 75 μL of methanol. The concentration of taurine in this control was 28 μmol/L.

Sample preparation

Fifteen-microliter serum samples were mixed with 15 μL of [$^{13}C_2$]taurine internal standard, and then 75 μL of methanol was added for protein precipitation. The samples were mixed, centrifuged at 11 000 rpm for 10 min, and the supernatant transferred to new vials for analysis.

LC-MS/MS conditions

Samples were analyzed by LC-MS/MS using an API 365 PE SCIEX mass spectrophotometer upgraded to an API 3000 (Ionics MSV) system (Bolton, ON, Canada) equipped with atmospheric pressure ionization electrospray and Ionics HSID interface (Bolton, ON, Canada) and coupled to a Gilson 215 autosampler (Middleton, WI, USA) with two Perkin Elmer series 200 LC micropumps (Norwalk, CT, USA).

Serum supernatants (50 μL) were injected into the chromatographic system using a 10-μL loop. Chromatographic separation was obtained using a HS F5 Discovery column (150 × 4.6 mm, 3 μm; Supelco, Sigma Aldrich, Bellefonte, PA, USA) with a 2-μm frit pre-column filter (Supelco, Sigma Aldrich) by isocratic separation (40% of 30 mmol/L of ammonium formate, pH 3.5, and 60% methanol) at a flow rate of 1000 μL/min. The liquid flow was passed through a pre-source splitter, and 33% of the eluant introduced into the mass spectrometer. Total running time was 4 min with taurine eluting at 1.88 min. The mass spectrometer was operated in positive mode. Nitrogen was used as the drying gas (at a flow rate of 7 L/min) and for collision-activated dissociation. The collision energy was 27 V, de-clustering potential was 31 V, and ion source temperature and voltage were 400 °C and 4500 V, respectively. Multiple-reaction monitoring mode produced the following ion transitions: taurine, m/z 126 → 108; [$^{13}C_2$] taurine, m/z 128 → 110.

Data acquisition and analysis was performed using Analyst 1.4.1. (MDS SCIEX, Applied Biosystems: Concord, ON, Canada) The interassay coefficient of variation for taurine was <2%.

Statistical analysis

Data are presented as median (25th–75th percentiles). Groups were compared by Mann-Whitney U test and $P < 0.05$ was considered statistically significant. Analyses were performed using SPSS 12.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results

Table 1 lists the distributions (median, 25th–75th percentiles) of serum concentrations of sulfur amino acids in MR and control-fed rats after 12 wk. Methionine and taurine were reduced by 62% and 64%, respectively, in MR rats compared with controls ($P < 0.001$ for both; Fig. 1). Cystathionine and tCys were decreased by approximately 44% ($P < 0.001$ for both), whereas serum tGSH was relatively maintained, showing a 17% reduction ($P = 0.004$). In contrast, tHcy was elevated by 2.5-fold in MR compared with control rats (46.1 versus 18.2 μmol/L, $P < 0.001$).

Table 1
Serum sulfur amino acids and body weight parameters after 3 mo in methionine-restricted and control-fed rats*

	Control-fed rats	Methionine-restricted rats
Sulfur amino acids and tGSH		
Methionine (μmol/L)	91 (81–124)	35 (34–38)†
tHcy (μmol/L)	18.2 (16.0–20.8)	46.1 (30.3–53.4)
Cystathionine (μmol/L)	2.40 (2.09–2.47)	1.38 (1.29–1.61)
tCys (μmol/L)	250 (238–268)	139 (136–155)
Taurine (μmol/L)	95 (91–134)	34 (23–40)
tGSH (μmol/L)	26.1 (24.1–28.9)	21.7 (19.4–24.3)‡
Body weight parameters		
Body weight (g)§	338 (315–385)	188 (188–184)
Visceral fat (g)	15 (11–17)	5 (5–6)
Visceral fat/body weight (%)	3.8 (3.4–4.6)	2.7 (2.6–3.0)

tCys, total cysteine; tGSH, total glutathione; tHcy, total homocysteine
* Data are presented as median (25th–75th percentiles); $n = 11$ rats per group.
† All parameters significantly different in methionine restriction versus control feeding by Mann-Whitney U test at $P < 0.001$, unless otherwise indicated.
‡ $P = 0.004$.
§ Previously reported at 3 mo as mean ± SEM/SD [2,3].

Similar to changes observed after long-term (80-wk) methionine restriction [2], median body weight and visceral fat mass were, respectively, 44% and 67% lower in MR rats after 12 wk compared with controls ($P < 0.001$). Visceral fat mass as a percentage of total body weight was 29% lower in MR rats (Table 1).

Discussion

A significant reduction in serum concentrations of methionine and downstream sulfur amino acids was observed in MR rats, apart from tHcy, which was markedly elevated.

Such paradoxical elevation of tHcy in MR rats, despite restriction of its precursor, methionine, could probably arise from reduced activity of CBS, the enzyme that catalyzes the first step of trans-sulfuration of homocysteine to form cysteine [1]. *S*-adenosylmethionine, synthesized from methionine, allosterically activates CBS [1,16], by relieving the inhibition exerted by the enzyme's C-terminal domain on the catalytic site [16]. This regulatory mechanism promotes disposal of excess methionine through irreversible trans-sulfuration to cysteine [16] and would

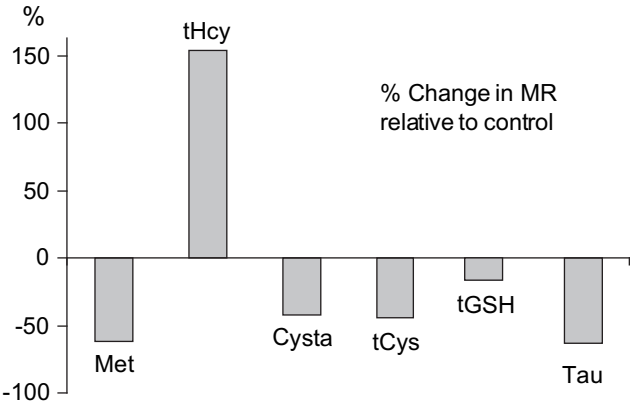

Fig. 1. Percent change of median serum sulfur amino acids in MR rats compared with controls. All differences are significant at $P \leq 0.004$ by Mann-Whitney U test. Cysta, cystathionine; Met, methionine; MR, methionine-restricted; Tau, taurine; tCys, total cysteine; tGSH, total glutathione; tHcy, total homocysteine.