Concentration-Dependent Linkage of Dietary Methionine Restriction to the Components of its Metabolic Phenotype
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Objective: Restricting dietary methionine to 0.17% produces a series of physiological responses through coordinated transcriptional effects in liver and adipose tissue. The goal of the present work was to determine the threshold concentrations above and below 0.17% at which the beneficial responses to 0.17% dietary methionine are preserved.

Methods: Diets were formulated to restrict methionine to different degrees, followed by evaluation of the transcriptional and physiological responses to the different diets.

Results: Restriction of dietary methionine to 0.25%, but not 0.34%, was partially effective in reproducing the metabolic phenotype produced by restriction of methionine to 0.17%, while restriction of methionine to 0.12% reproduced the responses produced by restriction to 0.17% but failed to support growth and caused excessive weight loss. Restriction beyond 0.12% initiated responses characteristic of essential amino acid deprivation including food aversion and rapid weight loss.

Conclusions: Restriction of dietary methionine to levels above 0.25% was without effect, while restriction to levels below 0.12% produced responses characteristic of essential amino acid deprivation. In addition, although restriction of dietary methionine to 0.12% did not evoke essential amino acid deprivation responses, it provided insufficient methionine to support growth. The ideal range of dietary methionine restriction was from 0.17% to 0.25%.

Introduction
Dietary methionine (Met) restriction is accomplished using diets formulated from elemental amino acids that reduce Met content from control levels of 0.86% to restricted levels of 0.17%. The diets also lack cysteine; thus, cysteine and its downstream metabolites, glutathione and taurine, must be formed through the trans-sulfuration pathway (1,2). The initial description of Met restriction showed that diets containing 0.17% Met increased life-span by 30% in Fischer 344 rats (3). Although longevity was the primary end point of these studies (4-6), recent studies have expanded their focus to the short-term metabolic effects of Met restriction (7-9). With rare exception (10-12), most contemporary studies examining effects of Met restriction use a Met concentration of 0.17%. The assumption is that restriction of Met to 0.17% is optimal for increasing both longevity and short-term metabolic effects. Given that an important goal of our work is to develop therapeutic tools based on the biology of Met restriction, a key objective of the present work is to identify upper and lower thresholds of Met restriction within which metabolic responses are optimal.

Diet completely devoid of single essential amino acids (EAA) such as Met produce a well-documented series of responses including food aversion, increased energy expenditure (EE), rapid loss of body weight (BW) and adiposity, and death (13-18). In contrast, responses evoked by dietary Met restriction are fundamentally different, increasing both metabolic health and life-span (4-12,19,20). Two important unresolved questions include the following: (1) what is the upper threshold for dietary Met above 0.17% at which beneficial metabolic effects are initially detected, and (2) at what concentrations of Met below 0.17% are the counterproductive effects of EAA deprivation initiated? Using a combination of in vivo and ex vivo phenotyping approaches and carefully formulated diets, we report...
that a range of dietary Met concentration between 0.12% and 0.25% is the effective range for dietary Met restriction.

Methods
Animals and diets
Experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Pennington Biomedical Research Center based on guidelines established by the Animal Welfare Act, National Research Council, and Public Health Service Policy on humane use of laboratory animals.

Four experiments were conducted using male C57BL6/J mice obtained from Jackson Laboratory (Bar Harbor, Maine). Mice were 5 weeks of age in Experiments 1, 2, and 4 and 18 weeks of age in Experiment 3. Mice were single-housed in shoebox cages with corn-cob bedding and given a control (CON) diet containing 0.86% Met until randomized to either CON or Met-restricted diets. Diets containing 0.86%, 0.34%, 0.25%, 0.17%, 0.12%, 0.08%, 0.04%, or 0% Met were formulated as extruded pellets (Dyets Inc., Bethlehem, Pennsylvania) and provided ad libitum in each experiment. The energy content of all diets was 15.96 kJ/g, with 18.9% from fat (provided as corn oil), 64.9% from carbohydrates, and 14.8% from a custom mixture of L-amino acids. Met-restricted diets were supplemented with L-glutamic acid to compensate for the reduced Met content. Water was provided ad libitum in all experiments. Room temperature was 22°C to 23°C, and lights were on 12 h/d from 7 AM to 7 PM. Food consumption was assessed at various intervals in each experiment by weighing the unconsumed and wasted food.

In Experiments 1, 2, and 4, body composition was assessed at baseline and weekly thereafter using nuclear magnetic resonance spectroscopy (Bruker Mini Spec, Billerica, Massachusetts). EE was measured using indirect calorimeters from either Columbus Instruments (Columbus, Ohio) or TSE Systems (Chesterfield, Missouri). Respiratory exchange ratio was calculated as the ratio between VCO2 produced and VO2 consumed. EE, expressed as kJ/h, was calculated as VO2 × [3.815 + (1.232 × RER)] × 4.1868. Body composition was measured immediately prior to and upon exit from the calorimeters and extrapolated over the period of EE measurement.

Mice were euthanized after a 4-hour fast using CO2-induced narcotics and decapitation. Inguinal white adipose tissue (IWAT), brown adipose tissue (BAT), and liver were harvested and snap frozen.

RNA isolation
Total RNA was isolated using RNeasy Mini Kits (QIAGEN, Valencia, California). cDNA obtained by reverse transcription was used for reverse transcription polymerase chain reaction (PCR) (Applied Biosystems, Foster City, California) of target genes and cyclophilin using primers listed in Table 1.

Insulin tolerance test
Insulin was administered via intraperitoneal injections after a 4-hour fast at a dose of 0.75 units/kg BW. Blood glucose was measured with a OneTouch Ultra™ blood glucometer at 15-minute intervals for 60 minutes, and the area under the glucose curve was determined.

Serum analyses
Serum insulin and FGF21 were analyzed via ELISA (Insulin, Millipore, Billerica, Massachusetts; FGF21, R&D Systems, Minneapolis, Minnesota).

Experiment 1
Diet containing 0.34% Met, 0.17% Met, or 0.86% Met (CON) were fed to eight mice per group for 7 weeks, followed by measurement of EE for 1 week by indirect calorimetry. Mice were returned to their home cage for 24 hours prior to tissue harvest. All mice received the diet they were initially assigned for the duration of the experiment.

Experiment 2
To further identify the upper threshold for Met restriction at which responses are first detected, diets containing 0.25% Met, 0.17% Met, or 0.86% Met were fed to eight mice per group for 8 weeks. Thereafter, EE was measured for 3 days. Mice were returned to their home cage for 1 week prior to insulin tolerance tests. Mice were allowed to recover for 1 week prior to tissue collection.

Experiment 3
To determine whether concentrations of dietary Met below 0.12% produced EAA deprivation responses, diets containing 0% Met, 0.04%, Met, 0.86% Met were formulated. Mice were randomized to the four diets (eight mice per group), and cumulative food consumption and BW change were measured at 2-day intervals. The study was terminated prematurely because BW loss in all but the CON 0.86% Met group exceeded 20% by day 8.

| Target gene | Forward (5’ to 3’) | Reverse (5’ to 3’) |
|-------------|--------------------|--------------------|
| Scd1        | CTGGCGTGCGGGCAAGGGCTTCT  | AGCCCAAGCTGCTACCTTCT |
| Fasn        | TCCTGGAGACGGAGACGGTCTTCT  | GAGAGTGTGCTCCTGAGCTTGT |
| Asns        | GGGGCACTGGAGACGGCTTCTTCT  | GGCGAAGCTTGCACCTGGAGCTTGT |
| Vldlr       | TGAGCGATGTTGGGGTTGAGTCG  | TGCGAAGGTCGACCTGGAGCTTGT |
| Fgf21       | TGGATCCATCGGAGGTGGATGAG  | GCAGCCAATGATGATGATGCTTAC |
| Leptin      | GTCCCTCCCAAAAAATATGGCTG  | TGATTTAATCGGAGCTGAGTGT |
| Ucp1        | GATCCAACTGGAGGTGGAAAGGCGAG  | GTTGACAACCTGCTGCTGCTGTTAGG |
| Bmp8b       | CGTGCATGGAGATGGAGGTGGAGG  | AGCAGACGATCGGAGGTGAGT |

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Figure 1 Physiological data for Experiment 1. (A) Body weight, (B) adiposity, and (C) food and (D) water intake were measured at weekly intervals over the course of the entire study. (E) Adipose tissue and (F) hepatic gene expression were measured via qPCR and expressed as the fold change between MR and CON. (G) Serum metabolites FGF21 and insulin were measured via ELISA, while (H) serum and hepatic triglyceride content was analyzed via colorimetric assays. All values are expressed as mean ± SEM. * indicates significant difference from CON at P < 0.05; groups not sharing a common letter are significantly different at P < 0.05. CON, control; MR, Met-restricted diets; IWAT, inguinal white adipose tissue; BAT, brown adipose tissue; Scd1, stearoyl CoA desaturase 1; Nrf2, nuclear factor erythroid 2-related factor 2; Gata2, glutathione S-transferase a2; Mgst3, microsomal glutathione S-transferase 3; Psat1, phosphoserine aminotransferase 1; ATF4, activating transcription factor 4; Asns, asparagine synthase; Vldlr, very low-density lipoprotein receptor; Fgf21, fibroblast growth factor 21; Ucp1, uncoupling protein 1; Bmp8b, bone morphogenetic protein 8b.
Experiment 4
To identify the lower threshold of dietary Met at which beneficial responses are retained but negative responses of EAA deprivation are avoided, diets containing 0.12% Met, 0.17% Met, or CON levels of 0.86% Met were provided after a 1-week adaptation to the CON diet. Thereafter, BW and composition were determined prior to randomization of eight mice per group to the three diets to compare the acute effects of 0.17% Met and 0.12% Met diets on EE over 14 days. Thereafter, mice were fed their respective diets for another 5 weeks prior to measurement of EE again for 1 week prior to tissue harvest.

Statistical analyses
BW, tissue composition, food intake, water intake, and gene expression data were analyzed using a one-way ANOVA and multiple t tests with the Holm-Sidak correction. For indirect calorimetry, group differences were compared by analysis of covariance using least-squares means that accounted for variation in EE attributable to differences in lean mass, fat mass, activity, and food intake between dietary groups as described previously (21). Insulin tolerance among groups was compared based on the area under the glucose clearance curves. Protection against type I errors was set at 5% (α = 0.05).

Results
Experiment 1
Restricting Met from control levels of 0.86% to 0.17% reduced BW and adiposity and increased food intake (Figure 1A-C) as before (9). Mice on the 0.17% Met diet increased water intake by twofold over the first 2 weeks of the study and maintained the increase for the duration (Figure 1D). In contrast, restricting Met to 0.34% produced no effects on BW, adiposity, or food intake relative to the CON diet (Figure 1A-C). The only indication of an effect of 0.34% Met was a small but significant 20% to 30% increase in water intake during weeks 2 to 4 but not for the remainder of the study (Figure 1D).

The transcriptional effects of the diets in adipose tissue and liver are illustrated in Figure 1E-F. Relative to CON, 0.17% Met reduced leptin mRNA and increased Ucp1 mRNA in IWAT and simultaneously increased thermogenic markers Bmp8b and Ucp1 mRNA in BAT (Figure 1E). The 0.34% Met diet was without effect in both tissues (Figure 1E). In the liver, 0.17% Met faithfully reproduced its previously reported reduction in hepatic Scd1 mRNA and activation of the NRF2 and ATF4 target genes (Figure 1F) (22,23). The 0.34% Met diet failed to decrease hepatic Scd1 mRNA or increase Gsta2, Mgst3, Psat1, Asns, Fgf21, or Vldlr mRNA (Figure 1F). A similar pattern emerged for circulating biomarkers, with 0.17% Met increasing serum FGF21 and reducing fasting insulin as before (Figure 1G) (20,23). In contrast, 0.34% Met failed to increase serum FGF21 or reduce fasting insulin (Figure 1G). These findings illustrate that 0.34% Met reproduces none of the biological responses produced by 0.17% Met.

Experiment 2
To further refine the threshold for biological efficacy, we tested the efficacy of restricting Met to 0.25%. Over 8 weeks, 0.25% Met produced a nonsignificant decrease in BW (Figure 2A). In contrast, both 0.25% Met and 0.17% Met decreased adiposity to similar extents relative to CON (Figure 2B). The 0.25% Met diet was also effective in increasing food and water intake above CON levels, although not to the extent of 0.17% Met (Figure 2C-D). For both variables, 0.25% Met showed intermediate efficacy between CON and 0.17% Met diets.

The 0.25% Met diet was effective in recapitulating many but not all of the transcriptional effects of 0.17% Met. For example, 0.25% and 0.17% Met produced comparable reductions in leptin mRNA and increases in Ucp1 mRNA in IWAT (Figure 2E). The 0.25% and 0.17% Met diets also increased BAT Ucp1 mRNA comparably. However, the BAT Bmp8b mRNA increase in the 0.25% Met group was significantly less than the increase produced by 0.17% Met (Figure 2E). In the liver, 0.25% Met and 0.17% Met produced concentration-dependent increases in the NRF2 and ATF4 target genes (Figure 2F). In contrast, both diets produced nearly identical reductions in hepatic Scd1 mRNA (Figure 1E). With insulin sensitivity, both the 0.25% Met and 0.17% Met diets enhanced insulin-dependent glucose clearance (Figure 2G). Glucose clearance curves showed that the response to the 0.25% Met diet was intermediate between the CON and 0.17% Met diets (Figure 2G inset). Lastly, the Met-restricted diets produced comparable increases in serum FGF21 and decreases in fasting insulin (Figure 2H). Collectively, this experiment shows that the upper threshold for biological efficacy of Met restriction was 0.25% Met.

Experiments 3 and 4
A significant remaining objective was to identify the lower threshold of Met restriction at which food-averse/weight-loss responses produced by EAA deprivation would be engaged. Based on life-span extension in mice after restricting Met between 0.12% and 0.15% (10,11), we ultimately compared the metabolic responses of 0.17% Met and 0.12% Met after initially examining responses to levels below 0.12% in Experiment 3. Experiment 3 used Met concentrations from 0% to 0.08% and was terminated prematurely at day 8 because of rapid weight loss exceeding 20% of BW resulting from reduced consumption of these diets (data not shown). Therefore, to determine whether restricting Met beyond 0.17% produced more robust biological responses than those produced by 0.17% Met while avoiding responses characteristic of EAA deprivation, a lower threshold of 0.12% Met was tested in Experiment 4. Mice consuming the 0.12% Met diet for 8 weeks weighed significantly less than mice on either the CON or 0.17% Met diets, and the BW of mice consuming the 0.17% Met diet was intermediate between CON and 0.12% Met (Figure 3A). The key difference was that the 0.12% Met group lost ~4 g of BW over the study while mice in the 0.17% Met group gained ~3 g (Figure 3A). Adiposity followed a similar pattern in that the percentage of body fat in mice on the 0.17% Met diet did not change over the study, while adiposity of mice in the 0.12% Met group decreased from 20% to ~16% (Figure 3B). Adiposity in the CON group increased from 20% to 28% over the same period (Figure 3B). Interestingly, both food and water intake increased to the same extent in mice on the 0.17% and 0.12% Met diets (Figure 3D-E). Together, these findings show that the 0.12% Met diet was no more effective than the 0.17% Met diet in increasing energy intake, but it was significantly more effective in producing loss of BW and adiposity. Comparison of lean mass among the three groups reveals an important distinction between the 0.12% Met and 0.17% Met diets in how they affected BW. Figure 3C shows that lean mass was essentially constant over the 8-week study in the CON and 0.17% Met diets.
Met groups, whereas mice in the 0.12% Met group lost a significant amount of lean (Figure 3C) and fat (Figure 3B) mass during the study. Thus, although the 0.12% diet did not evoke food-aversive responses (Figure 3D), the mice’s loss of lean mass over time, despite their hyperphagia, suggests that 0.12% Met provides insufficient Met to support growth or even maintenance of BW. The addition of small amounts of cysteine to the diet may spare sufficient Met to prevent this response (see below).
Figure 3 Physiological data for Experiment 3. (A) Body weight, (B) adiposity, (C) lean mass, (D) food intake, and (E) water intake were measured at weekly intervals over the course of the study. Expression of selected genes in (F) adipose tissue and (G) liver were measured via qPCR and expressed as the fold change of the MR group over the CON group. (H) Serum metabolites FGF21 and insulin were measured via ELISA. All values are expressed as mean ± SEM. Means annotated with an asterisk differ from CON at P < 0.05. In panels F and G, means not sharing a common letter differ at P < 0.05. CON, control; MR, Met-restricted diets; IDC, indirect calorimetry; IWAT, inguinal white adipose tissue; BAT, brown adipose tissue; Sod1, stearoyl CoA desaturase 1; Nrf2, nuclear factor erythroid 2-related factor 2; Gsta2, glutathione S-transferase a2; Mgat3, microsomal glutathione S-transferase 3; Psat1, phosphoserine aminotransferase 1; ATF4, activating transcription factor 4; Asns, asparagine synthase; Vldlr, very low-density lipoprotein receptor; Fgf21, fibroblast growth factor 21; Ucp1, uncoupling protein 1; Bmp8b, bone morphogenetic protein 8b.
The 0.17% Met and 0.12% Met diets produced comparable decreases in leptin mRNA in IWAT and increases in thermogenic genes in BAT (Figure 3F). The exception was IWAT *Ucp1* mRNA, of which the 0.12% Met diet produced a more substantial increase than the 0.17% Met diet (Figure 3F). In the liver, the two Met-restricted diets produced comparable decreases in *Scd1* mRNA and increases in two of the three NRF2 target genes and ATF4 target genes (Figure 3G). The lone exception was *Psat1*, of which the 0.12% Met diet produced a slightly larger induction than the 0.17% Met diet (Figure 3G). Finally, the 0.12% Met and 0.17% Met diets produced nearly identical increases in serum FGF21 and decreases in fasting insulin (Figure 3H). Taken together, these data indicate that while 0.12% Met effectively replicates many of the effects of 0.17% Met, it does not further amplify any of the effects except loss of BW, lean mass, and adiposity.

**Experiments 1, 2, and 4**

EE was measured by indirect calorimetry at the end of Experiments 1 and 2 and at the beginning and end of Experiment 4. Figure 4A-B illustrates that daytime and nighttime EE did not differ between mice on the 0.34% Met and CON diets but was significantly increased in the 0.17% Met group. In Experiment 2, EE in the 0.25% Met group was significantly higher than the CON group and...
TABLE 2 Methionine (Met) intake by diet

| Experiment | Met intake (mg/mouse) | Met intake (mg Met/g BW) |
|------------|-----------------------|-------------------------|
| **Experiment 1** |                       |                         |
| CON        | 33.3 ± 1.82a          | 1.38 ± 0.070a           |
| 0.34% Met  | 12.9 ± 0.48b          | 0.55 ± 0.021b           |
| 0.17% Met  | 8.3 ± 0.59c           | 0.39 ± 0.025c           |
| **Experiment 2** |                       |                         |
| CON        | 22.5 ± 0.51a          | 1.14 ± 0.023a           |
| 0.25% Met  | 7.4 ± 0.13b           | 0.38 ± 0.003b           |
| 0.17% Met  | 5.2 ± 0.14c           | 0.28 ± 0.005c           |
| **Experiment 4** |                       |                         |
| CON        | 29.5 ± 0.61a          | 0.95 ± 0.28a            |
| 0.17% Met  | 6.94 ± 0.17b          | 0.25 ± 0.004b           |
| 0.12% Met  | 4.44 ± 0.19c          | 0.18 ± 0.005c           |

Average Met intake per mouse for Experiments 1, 2, and 4, expressed as absolute Met intake and corrected for body weight. Values are expressed as mean ± SEM. Groups not sharing a common letter within experiments are significantly different at P < 0.05.

Met intake by dietary group

Absolute and relative Met intakes were calculated for Experiments 1, 2, and 4 to assess the impact of the compensatory hyperphagia in some groups. Met intake was reduced in all Met-restricted groups compared to CON (Table 2). The reduction in Met intake produced by the 0.34% Met diet was without notable effects, while the Met reductions observed in the 0.25% Met group (Experiment 2), the 0.17% Met group (Experiments 1, 2, and 4), and the 0.12% Met group (Experiment 4) were either partially or fully effective in producing the phenotypic profile (Table 2). Our findings indicate that there is a very precise threshold of reduced Met intake that must be reached for induction of these responses. A range of Met intake of 5 to 7 mg per mouse appears to be the ideal range, with intake of Met at 4 mg per mouse being insufficient to maintain lean body mass and BW.

Discussion

Dietary Met restriction produces a coordinated series of biochemical and physiological responses that improve biomarkers of metabolic health, limit fat accumulation, reduce tissue and circulating lipid levels, remodel WAT, and enhance overall insulin sensitivity in rats and mice (4,7,9,19,20). Individual components of the phenotype become evident soon after initiation of Met restriction. As part of an overarching goal to develop therapeutic diets to treat metabolic disease in domestic animals and humans, the goal of the present work is to identify the range of dietary Met concentrations on either side of 0.17% that reproduce these beneficial effects.

The goal of our initial experiments was to identify the upper threshold of Met restriction that reproduced some or all of the effects of 0.17% Met. Preliminary studies showed that restriction of Met by up to 50% from CON Met levels had no discernible effect on any component of the metabolic phenotype. These findings suggested the threshold was much closer to 0.17% Met; thus, our initial experiment tested the efficacy of 0.34% Met, a doubling of the amount of Met in the 0.17% diet. The findings of Experiment 1 showed that the responses to 0.34% Met did not differ from the CON group (Figure 1). In Experiment 2, we reduced the amount of Met to 0.25% and found that this concentration, depending on end point, was either partially or fully effective in reproducing each component of the phenotype observed with 0.17% Met (Figure 2). Although the reduction in BW produced by 0.25% Met compared to the CON group was not significant, the reduction in adiposity produced by this diet was comparable to that produced by 0.17% Met (Figure 2B). The apparent dichotomy of this finding is explained by the intermediate effect of 0.25% Met on energy intake and EE relative to 0.17% Met. Transcriptional responses to 0.25% Met in liver and adipose tissue were indicative of intermediate efficacy in that this degree of Met restriction either partially or fully reproduced the changes in gene expression produced by 0.17% Met. Comparable decreases in fasting insulin and improvements in glucose tolerance support the view that these improvements in metabolic status are not secondary to effects of 0.25% Met’s effect on growth. Viewed together, the findings from Experiment 2 make a compelling case that diets formulated to provide a range of Met between 0.17% and 0.25% would be effective in producing the desired metabolic effects without untoward effects on BW.

Our second objective was to identify the lower threshold of Met restriction that retained the metabolic effects of 0.17% while avoiding the detrimental effects of EAA deprivation on BW and lean mass. The findings from Experiment 3 with Met concentrations below 0.1% were not reported because all concentrations caused food aversion and rapid weight loss and had to be stopped by day 8 because of excessive weight loss. In Experiment 4, we chose to evaluate the efficacy of 0.12% Met compared to 0.17% Met to test whether 0.12% Met would produce more robust metabolic responses. The 0.12% Met produced no greater increase in food intake and EE than 0.17% Met but a far greater loss of BW and lean mass and a slightly greater loss of adiposity. The biochemical and molecular responses between these two diets were comparable, supporting the interpretation that 0.12% Met did not produce more robust transcriptional responses than 0.17% Met. In contrast, we interpret the greater loss of BW and leanness produced by 0.12% Met as a failure to provide sufficient Met to support protein synthesis and growth. The 0.17% Met diet may also limit growth to some extent in young growing animals, whereas the 0.25% Met diet produced minimal limitation of growth.

A previously unexplored area of dietary Met restriction is the degree to which hyperphagia in Met-restricted groups compensates for...
reduced dietary Met content. Both absolute Met intake per mouse and BW-adjusted Met intake are presented in Table 2. The mice on all Met-restricted diets consumed significantly less Met when compared to the CON group. However, as the phenotype produced by the 0.34% Met diet did not differ from CON, the present work suggests that the effective range is 4 to 7 mg Met/mouse/day.

Considered together, the present findings make a compelling case that limiting Met to a range of 0.17% to 0.25% is most effective in increasing EE, limiting fat deposition, reducing de novo synthesis of hepatic triglyceride, and improving insulin sensitivity, while diets providing only 0.12% Met would be no more effective than 0.17% in improving these metabolic biomarkers but would include negative effects on BW and lean mass. Achieving the desirable range of 0.17% to 0.25% Met with natural sources of protein would also require consideration of their cysteine content, because, as shown previously (22), the addition of small amounts of cysteine (e.g., 0.2%) to the Met-restricted diet fully reversed all metabolic effects of Met restriction. Given the Met-sparing effect of dietary cysteine (1,2) and the narrow range of concentrations in which Met restriction is effective, it seems likely that even small amounts of dietary cysteine would counteract the effects of 0.25% Met. Perhaps with 0.17% Met, a small amount of cysteine (e.g., 0.05%) would not be counterproductive. It also seems that the negative effects of the 0.12% Met diet on BW and lean body mass could be counteracted by small amounts of cysteine without negating the positive metabolic effects. It will be important in future studies to carefully examine how much cysteine each degree of Met restriction will tolerate while still retaining positive metabolic effects. This information will be critical to developing effective therapeutic diets based on Met restriction.

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