The Components of Age-Dependent Effects of Dietary Methionine Restriction on Energy Balance in Rats

Desiree Wanders¹, Laura A. Forney², Kirsten P. Stone², Barbara E. Hasek², William D. Johnson³, and Thomas W. Gettys²

Objective: Dietary methionine restriction (MR) improves biomarkers of metabolic health, in part through coordinated increases in energy intake and energy expenditure (EE). Some metabolic benefits of dietary MR are secondary to its effects on energy balance, so this study’s purpose was to examine how age at initiation of MR influences its effects on energy balance and body composition.

Methods: Energy balance was examined in rats provided control or MR diets for 9 months after weaning or in rats between 6 and 12 months of age.

Results: Rats provided the control diet for 9 months after weaning increased their body weight (BW) and fat mass by five- and eightfold, respectively, while BW and fat accumulation in the MR group were reduced to 50% of that of controls. In adult rats fed the respective diets between 6 and 12 months of age, dietary MR increased energy intake by ~23%, but the 15% increase in EE was sufficient to prevent increases in BW or fat mass.

Conclusions: Dietary MR produces comparable increases in EE in young, growing animals and in mature animals, but young animals continue to deposit new tissue because of the proportionately larger effect of MR on energy intake relative to maintenance requirements.

Introduction

Dietary methionine restriction (MR) produces an integrated series of metabolic and physiological responses that develop quickly after introducing the diet and improve many biomarkers of metabolic health (1-8). Two prominent physiological responses are increases in energy intake and expenditure (2), with the larger effect on energy expenditure (EE) slowing ongoing fat deposition by increasing the proportion of total energy intake required for maintenance of existing tissue. When the decrease in net energy available to support growth is integrated over time, it significantly limits normal age-associated growth and expansion of adipose tissue. The MR diet also increases in vivo insulin sensitivity through a combination of direct and indirect effects of the diet on the liver, adipose tissue, and muscle (6). In addition, improvements in overall insulin sensitivity accrue from diet-induced reductions in adiposity. However, the extent to which increased EE and reductions in adiposity are required for diet-induced improvements in insulin sensitivity has not been clearly established.

Most studies of MR assess the responses of young mice or rats during the postweaning phase of growth, but from a translational perspective, the more relevant strategy would be application of MR in adults with metabolic dysfunction. An additional concern with studying MR in a postweaning model is that metabolic changes could be secondary to developmental effects associated with slowed growth. Several studies of MR have examined the metabolic phenotypes of rats after long-term consumption of the MR diet (e.g., ~2 years) (1,2). Although improvements in biomarkers of metabolic health were documented in both studies, concerns remain that these benefits are secondary to developmental effects associated with postweaning introduction of the diet. Therefore, an important objective of the present work was to obtain a side-by-side comparison of the effects of dietary MR on energy balance in the standard postweaning MR model and also in an adult context in which dietary MR was initiated after attainment of ~80% of mature size. Using these two experimental models, we report here that dietary MR held body weight (BW) and body composition constant in adult rats between 6 and 12 months of age, despite increasing weight-adjusted food consumption by 20%. Dietary MR also increased energy intake and EE in young growing animals, but in this case, energy balance remained sufficiently positive to support a slower rate of growth and deposition of new tissue over the following 9 months.

Disclosure: The authors declared no conflicts of interest.

Received: 22 December 2017; Accepted: 24 January 2018; Published online 4 March 2018. doi:10.1002/oby.22146
Methods

Animals and diets

All experiments were reviewed and approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee using guidelines established by the National Research Council, the Animal Welfare Act, and the Public Health Service Policy on the humane care and use of animals. Two experiments were conducted using male F344 rats obtained from Harlan (Indianapolis, Indiana) at 5 weeks of age (Experiment 1) or 5 months of age (Experiment 2). In Experiment 1, rats were fed Purina rodent diet (#5001) until 35 days of age, then singly housed in shoe box cages with corncob bedding and fed the control diet for 7 days. Then rats were randomly assigned to one of two dietary treatment groups. Using the experimental feeding paradigm described previously (2), control rats were provided with a purified diet containing 0.86% methionine and no cysteine, whereas rats in the MR group were provided with the same diet with methionine restricted to 0.17% and no cysteine. The diets were formulated as extruded pellets and provided ad libitum (2). The energy content of both diets (Dyets Inc., Bethlehem, Pennsylvania) was 15.96 kJ/g, with 18.9% of energy from fat (corn oil), 64.9% from carbohydrate, and 14.8% from a custom mixture of L-amino acids. The amino acid content of the diet on a weight basis was 14.1%. The details of diet composition are provided in Table 1. Temperature was maintained at 22°C to 23°C and lights were on 12 h/d from 7 AM to 7 PM. In Experiment 2, 5-month-old rats were fed the control diet for 1 month prior to assignment to the control or MR diet group.

Food consumption was measured by weighing food provided at the beginning of the feeding interval and by weighing unconsumed and wasted food for the previous 24 or 48 hours. The bedding was sifted through wire mesh to weigh unconsumed food. Body composition was determined by nuclear magnetic resonance (NMR) spectroscopy (Bruker Minispec, Billerica, Massachusetts).

Experiment 1: juvenile study

Two cohorts of 16 rats were provided the control or MR diet for 3 months or 9 months beginning at 6 weeks of age. After 3 and 9 months on the diet, eight rats from each dietary group were transferred to indirect calorimeters for measurement of EE (Oxymax System; Columbus Instruments, Columbus, Ohio). Rats were acclimated in the chambers for 24 hours prior to the measurement of oxygen consumption (VO₂) and carbon dioxide production (VCO₂) at 48-minute intervals for 72 hours. At the end of the 4-day period, the rats were removed from the calorimeter and euthanized. VO₂ is expressed as liters of oxygen consumed per hour, whereas the respiratory exchange ratio (RER) is the ratio of VCO₂ produced to VO₂ consumed. EE was calculated as (VO₂ × [3.815 + (1.232 × RER)]) × 4.019 kJ/h, and expressed as kilojoules per hour per rat as described by the manufacturer (Columbus Instruments). Group differences (e.g., diet, diet duration, time of day, diet × diet duration × time of day interaction) in EE (kilojoules per hour per rat) were compared using analysis of covariance (ANCOVA) (JMP Software, version 12; SAS Institute Inc., Cary, North Carolina), calculating least squares means that accounted for variation in EE attributable to differences in lean mass and fat mass among the rats. The significance of model effects and least squares means ± SEM for the three-way interaction were compared using residual variance as the error term (9).

Experiment 2: adult study

The effects of MR on energy balance in adult rats were examined in two cohorts of 16 rats beginning at 6 months of age. Rats were provided the control or MR diets for 3 months or 6 months thereafter. BW, body composition, and food consumption were determined, as before, and after 3 and 6 months EE was measured in cohorts of eight rats per group as before. The effects of diet, diet duration, and time of day on EE were assessed by ANCOVA as in Experiment 1.

Statistics

The energy balance response variables in Experiments 1 and 2 were analyzed using a two-way analysis of variance (ANOVA), with diet and diet duration as the main effects. The analysis tested for a diet × diet duration interaction using residual variance as the error term. Response variables at each diet duration were compared using least squares means from the ANOVA with the Bonferroni correction. Protection against type I errors was set at 5%.

Results

Experiment 1: juvenile study

In Experiment 1, the initial BW of all rats was ~84 g, and BW increased in controls by 3.9-fold at 3 months and 5.1-fold at...

Table 1: Composition of MR diet

| Ingredient | Concentration in diet (%) | Ingredient | Concentration in diet (%) |
|------------|---------------------------|------------|---------------------------|
| L-arginine | 1.12                      | L-phenylalanine | 1.16                          |
| L-lysine   | 1.80                      | Glycine     | 2.33                          |
| L-histidine| 0.33                      | Dextrose    | 20.00                         |
| L-leucine  | 1.11                      | Dyetrose    | 5.00                          |
| L-isoleucine| 0.82                   | cornstarch | 43.25                         |
| L-valine   | 0.82                      | Cellulose fiber | 5.00                         |
| L-threonine| 0.82                      | Choline bitartrate | 0.20                         |
| L-tryptophan| 0.18                  | Vitamin mix: AIN-76A | 1.00                          |
| DL-methionine | 0.17                | Mineral mix: AIN-76 | 3.50                          |
| Glutamic acid | 3.39                  | Corn oil | 8.00                          |

*Energy content of control and MR diets was 15.96 kJ/g.
*DL-methionine concentration of control diet was 0.86%.
*L-glutamic acid concentration of control diet was 2.70%.
9 months (Table 2). Mean fat mass in controls increased by 5.4-fold and 8.2-fold at 3 and 9 months, respectively, whereas mean fat-free mass increased by 3.5-fold at 3 months and 4.2-fold at 9 months (Table 2). The accumulation of fat-free mass paralleled the accumulation of BW, whereas the rate of fat accumulation over the same period exceeded the rate of accumulation of BW. MR slowed the increase in BW, fat mass, and fat free mass by ~50% at both time points, but the biggest impact was on fat mass at 9 months, at which point the MR group had deposited only 38% as much fat as the controls (Table 2). Despite the difference in BW of the two groups, rats in the MR group consumed 80% and 73% of the food consumed by controls at 3 and 9 months, respectively (Table 2). However, expressed per unit of BW, food consumption in the MR group was 48% higher than in controls during the day and 53% higher than in controls at 9 months (Table 2). These data show that rats in both groups were growing and depositing new tissue, although the MR group was growing at a significantly slower rate. Moreover, based on the high rate of energy intake per unit of BW in the MR group, the data suggest that the energy costs of maintaining existing tissue and/or adding new tissue in this group were significantly higher.

The compiled measurements of EE were analyzed by ANCOVA to assess the impact of diet, diet duration, time of day, lean mass, and fat mass on variation in EE between groups. The relative contributions of model components to variation in EE are shown as the t-ratio of each variable’s impact on total variation in EE (Figure 1). Although all model components had significant t-ratios, Figure 1 shows that time of day was the largest contributor to variation in EE among rats. The negative t-ratio for time of day indicates that EE was significantly lower during the day, irrespective of diet or diet duration. Diet duration had a significant independent effect on EE, as EE decreased with age in rats on both diets. Lean mass and fat mass had significant positive effects on variation in EE, and as predicted, the control diet had a negative effect on EE relative to the MR diet (Figure 1). Last, a significant time of day × diet interaction was detected based on the differential effect of the MR diet on EE measured during the day versus at night. This corresponds with increased nighttime activity and feeding and the larger effect of MR on nighttime EE versus its daytime effect. These effects are shown in Figure 2A, in which the impact of diet duration, irrespective of time of day and diet, is evident. Figure 2A also illustrates the independent effects of diet and time of day on EE at the two time points. The day-to-night increase in EE in controls at 3 months was 24%, whereas the corresponding increase in the MR group was 29%. At 9 months, EE in the MR group was 11% higher than that of controls during the day and 10% higher than that of controls during the night (Figure 2A).

The RER provides a real-time index of substrate utilization during the metabolic cycle and is based on the molar ratios of oxygen consumed and carbon dioxide produced during the oxidation of glucose.

![Figure 1](image-url)
(1.00), lipid (0.70), and protein (0.80) (10-12). RERs typically range toward 1 during the switch to glucose utilization in the fed state and toward 0.7 during the switch to fat utilization during fasting. Figure 2B shows the expected diurnal fluctuations in the RER in each group, approaching 1 at night when rats consume 75% to 85% of their daily intake (13,14). The nighttime RER in the MR group slightly exceeded 1 after 3 months (Figure 2B), which occurs when glucose is used to support de novo lipogenesis (10,12). During the daytime postabsorptive state, RERs in the MR group dropped to 0.80 at both 3 and 9 months, indicating a greater shift to lipid as fuel than in controls. In addition, the range of day-to-night excursions in the RER are considered a measure of metabolic flexibility, and they were significantly larger in the MR group after both diet durations (Figure 2B). Considered together, the MR group oxidized more fat during the day than controls, and the nighttime shift from fat to carbohydrate was more complete.

Experiment 2: adult study
In Experiment 2, the starting weight of all rats was ~390 g and BW increased in controls by 13% at 3 months to 438 ± 7 g and by 22% at 6 months to 472 ± 12 g (Table 3). Fat mass increased by a modest 20% in controls at 3 months and by 38% at 9 months, whereas fat-free mass increased by 9% and 15% over this period (Table 3). Thus, fat...
mass accumulation outpaced the accumulation of fat-free mass and BW between 6 and 12 months in controls. In contrast, BW was essentially stable in the MR group during the study, with the MR group losing only 18 g or 5% of BW over 6 months (Table 3). Fat mass was also constant during this period, with final (84 ± 6 2.3 g) and starting fat mass (88 ± 6 1.7 g) not differing (Table 3). Fat-free mass at the end was 96% of its starting value (Table 3), indicating that, on average, BW and composition of the MR group remained almost constant during the study. Despite the difference in BW between control and MR rats at 3 and 6 months, food consumption did not differ between groups at either time point (Table 3). However, when food intake was expressed per unit of BW, energy intake in the MR group was 17% higher than in controls at 3 months and 23% higher at 6 months (Table 3). In contrast to the juvenile study, in which energy intake was used to support maintenance and growth in both groups, the MR group in the adult study used essentially 100% of their intake for maintenance. By definition, the MR group was relatively close to being in energy balance. Given that control rats continued to grow at the same level of energy intake, we conclude that energy costs of maintaining existing tissue were significantly higher in the MR group.

As in Experiment 1, the relative contributions of diet, diet duration, time of day, lean mass, and fat mass to variation in EE were assessed by ANCOVA and are shown as the t-ratio of each variable’s impact on total variation in EE. Figure 3 illustrates that time of day was the largest contributor to variation in EE, and as expected, EE was much lower in both groups during the day. Diet duration also had a significant effect on EE, but in contrast to the juvenile study, EE was significantly higher at 6 months than 3 months in both groups (Figure 3). Lean mass had a significant positive effect on variation in EE as predicted, but in this experiment, fat mass had no effect on variation in EE (Figure 3). As predicted from the data in Table 3, EE in the MR group, averaged across time of day and diet duration, was significantly higher than in the controls (Figure 3). Lastly, a significant time of day x diet interaction was detected based on the differential effect of the diets on nighttime EE averaged across diet duration. This is illustrated in Figure 4A, which shows a modest increase in EE between 3 and 6 months, irrespective of time of day and diet. The least squares means presented in Figure 4A also illustrate the independent effects of diet and time of day on EE at both times, with MR producing a 10% increase in EE over controls during the day at 3 months and a 15% increase over controls at night. After 6 months, EE in the MR group was 8% higher than in controls during the day and 21% higher than in controls at night (Figure 4A). These data show that dietary MR produced a consistent increase in EE over controls between 3 and 6 months and that the nighttime increase was approximately twice that of the daytime effect (Figure 4A).

RERs were comparable between groups during the day at 3 months, but MR produced a larger nighttime increase in the RER compared to controls.
with controls (Figure 4B). This is indicative of greater metabolic flexibility in the MR group. Diet duration was without effect in either group, but the daytime RER was slightly lower in the MR group compared with controls at 6 months (Figure 4B). The nighttime increase in the RER in the MR group was also significantly greater than that of controls after 6 months (Figure 4B). Therefore, although substrate selection between groups was comparable during the day, the nighttime shift to carbohydrate oxidation in the MR group was more complete at both time points.

Discussion

The most significant finding from the present work is that the impact of MR on energy balance depends on the age and/or size of the animal when the diet is introduced. In young, growing animals, the hyperphagic effect of MR provided an increase in energy intake that was sufficient to overcome the simultaneous increase in EE, leaving sufficient net energy to support continued, albeit slowed, deposition of new tissue. Energy intake in excess of expenditure is defined as net energy and is partitioned between fat and protein synthesis in a manner that determines the relative composition of tissue deposition during growth to maturity (15-17). However, net energy is always a remainder term determined by the difference between total energy intake and the energy required to meet daily maintenance requirements. For example, on any given day, the proportion of energy intake required for maintenance defines the energy available to support new growth. In practical terms, physiological changes that increase EE reduce energetic efficiency and effectively reduce the availability of net energy. It follows that energy intake and energetic efficiency are critical terms in the energy balance equation. The indirect calorimetry data in our juvenile study clearly establish that MR increases EE and therefore the energy required to support maintenance requirements. Maintenance energy is most simply defined as the energy intake required to maintain constant BW and composition (15-18) and is summative in the sense that it includes the energy costs of basal metabolism, thermoregulation, activity, and assimilation of food. Although activity was not measured here, previous studies have found no evidence that dietary MR affects EE by increasing activity (2,3,9,19). In fact, earlier work has shown that MR increases EE by increasing uncoupled respiration and enhancing futile substrate cycling (2,9). However, even with the increase in EE in the MR group, the simultaneous increase in energy intake compensated for increased maintenance costs and provided sufficient net energy to support a continued, yet slower, rate of growth. It is also possible that the reduced amount of methionine in the MR diet limited the rate of growth independently of the diet’s effect on EE. This seems unlikely, based on the ability of the MR diet to sustain a much higher BW and lean mass in the adult study.

Energy balance is defined as the point when energy intake and expenditure are equal, and it occurs in practice upon attainment of physical maturity when the rate of energy intake is sufficient to maintain a constant BW and composition. The data presented here make a compelling case that the MR diet, through a combination of increased energy intake (per unit of BW) and EE, brought the rats in this group into energy balance. Over the same 6-month period, the control group continued to grow and deposited an additional 40 g of protein, an additional 37 g of fat, and an additional 85 g of BW while consuming the same amount of food per rat as the MR group (Table 3). These findings illustrate the importance of the MR-dependent increase in weight-adjusted energy intake, for without it, the MR-dependent increase in EE would have put the rats in a state of significant negative energy balance. It is worth noting that rats in the adult study did not have obesity prior to the introduction of MR, so from a translational perspective, an important question is whether MR would induce weight loss if animals had obesity when MR was introduced. The answer is probably yes, based on recent work in which mice were initially fed high-fat diets to produce obesity, followed by 8 weeks of dietary MR (20). MR reduced BW from 44 g to 27 g and adiposity from 32% to 17% over the 8-week study (20). These findings argue that dietary MR would be effective in producing weight loss in the context of obesity and weight stability in individuals without obesity.

The analysis of EE by ANCOVA provided several additional insights into how diet, age, time of day, and body composition influenced variation in EE among the rats. In both studies, the most important effector of variation in EE was time of day, with daytime having a significant negative effect that was independent of diet and age. This is not surprising because rodents sleep during the day and are active at night. Therefore, the large impact of time of day on EE reflects the fact that it encompasses variation in EE associated with the nighttime increase in activity and food consumption of rodents in both diets at all ages. Second, diet duration had opposite effects on EE in the two studies, with increased age having a significant negative effect in the juvenile study and a modest positive effect in the adult study (Figures 1, 2A, 3, and 4A). No diet × diet duration interactions were detected, indicating that the observed effects of diet duration on EE were common to both diets in each study. It is interesting that lean mass and fat mass had significant positive effects on variation in EE in the juvenile study, indicating that deposition of each tissue type was positively related to and contributing to EE (Figure 1). However, the twofold-higher t-ratio for lean mass compared with fat mass was consistent with the expected greater impact of lean tissue on EE than fat tissue. In contrast, variation in fat mass had no significant effect on variation in EE in the adult study (Figure 3), whereas lean mass had the predicted positive effect. The reasons for the difference between studies are unclear but may relate to the fact that rats in the MR group, despite the significant increase in EE, were weight stable, with unchanging fat mass over the 6-month study. Thus, it is possible that the combination of stable fat mass in the MR group and the modest increase in adiposity of the controls provided insufficient variation to detect a covariation of fat mass with EE between groups. Considered as a whole, the most important finding from this analysis is that the MR diet had a significant positive effect on EE, regardless of the age when it was initiated, the diet duration, or the time of day when it was measured.

The ultimate goal of our work is to translate the documented preclinical efficacy of dietary MR into a therapeutic diet based on dietary MR. The most applicable context would be adults who are overweight and present with elements of metabolic disease. We have undertaken an initial proof of concept study in humans with metabolic syndrome (21). The approach involved the elimination of
meat, poultry, dairy, and grains from the diet and replacement of 100% of daily protein requirements with a commercial food (e.g., Hominex-2, Abbott Nutrition) containing a semisynthetic mixture of L-amino acids lacking methionine. In practice, we found that Hominex-2 meal replacements were supplying ~75% of daily energy requirements. Study participants were instructed to make up the calorie deficiency with unlimited fruit and vegetable intake and a limited intake of grains. In our study, the Hominex-2–based approach to dietary MR increased 24-hour fat oxidation and reduced hepatic lipid content as predicted (21). However, a retrospective analysis revealed a key limitation of the Hominex-2–based approach that limited its overall efficacy. Aside from poor palatability, Hominex-2 contains 0.9 g of cystine per 100 g, and based on the well-documented methionine-sparing effects of cystine (22), we believe that the cystine in Hominex-2 compromised the reduction in methionine produced by the diet. In subsequent preclinical work, we have established that MR is only effective within a defined concentration range (23) and that the addition of as little as 0.1% cystine to the MR diet reversed all but the direct transcriptional effects of MR on specific hepatic genes associated with de novo lipogenesis (e.g., stearoyl-CoA desaturase) (19,24). This could explain why the presence of cystine in Hominex-2 limited the diet’s effects to those on hepatic lipid metabolism (21). Viewed together, these findings argue that using MR to treat metabolic disease will involve developing palatable foods that eliminate cysteine and provide methionine within the defined range shown to be biologically effective.

© 2018 The Obesity Society

References

1. Malloy VL, Krajcik RA, Bailey SJ, Hristopoulos G, Plummer JD, Orentreich N. Methionine restriction decreases visceral fat mass and preserves insulin action in aging male Fischer 344 rats independent of energy restriction. Aging Cell 2006;5:303-314.
2. Hasek BE, Stewart LK, Henagan TM, et al. Dietary methionine restriction enhances metabolic flexibility and increases uncoupled respiration in both fed and fasted states. Am J Physiol Regul Integr Comp Physiol 2010;299:R728-R739.
3. Plaisance EP, Henagan TM, Echlin H, et al. Role of β-adrenergic receptors in the hyperphagic and hypermetabolic responses to dietary methionine restriction. Am J Physiol Regul Integr Comp Physiol 2010;299:R740-R750.
4. Wanders D, Ghosh S, Stone K, Van NT, Gettys TW. Transcriptional impact of dietary methionine restriction on systemic inflammation: relevance to biomarkers of metabolic disease during aging. Biofactors 2013;40:113-26.
5. Hasek BE, Boudreau A, Shin J, et al. Remodeling the integration of lipid metabolism between liver and adipose tissue by dietary methionine restriction in rats. Diabetes 2013;62:3362-3372.
6. Stone KP, Wanders D, Ogeron M, Cortez CC, Gettys TW. Mechanisms of increased in vivo insulin sensitivity by dietary methionine restriction in mice. Am J Physiol Regul Integr Comp Physiol 2013;305:E372-E379.
7. Ables GP, Ouattara A, Hampton TG, et al. Dietary methionine restriction in mice elicits an adaptive cardiovascular response to hyperhomocysteinemia. Sci Rep 2015;5:8886. doi:10.1038/srep08886.
8. Perrone CE, Malloy VL, Orentreich DS, Orentreich N. Metabolic adaptations to methionine restriction that benefit health and lifespan in rodents. Exp Gerontol 2012;48:654-660.
9. Wanders D, Burkh D, Cortez CC, et al. UCPI is an essential mediator of the effects of methionine restriction on energy balance but not insulin sensitivity. FASEB J 2015;29:2603-2615.
10. Elia M, Livesey G. Theory and validity of indirect calorimetry during net lipid synthesis. Am J Clin Nutr 1988;47:591-607.
11. Ferrannini E. The theoretical bases of indirect calorimetry: a review. Metabolism 1988;37:287-301.
12. Simonsen DC, DeFronzo RA. Indirect calorimetry: methodological and interpretative problems. Am J Physiol 1990;258:E399-E412.
13. Farley C, Cook JA, Spar BD, Austin TM, Kowalski TJ. Meal pattern analysis of diet-induced obesity in susceptible and resistant rats. Obes Res 2003;11:845-851.
14. Liu M, Shen L, Liu Y, et al. Diurnal rhythm of apolipoprotein A-IV in rat hypothalamus and its relation to food intake and corticosterone. Endocrinology 2004;145:3232-3238.
15. Brody S. Bioenergetics and Growth. New York, NY: Reinhold; 1945.
16. Brody S. Nutrition. Annu Rev Biochem 1935;4:383-401.
17. Kleiber M. The Fire of Life: An Introduction to Animal Energetics. New York, NY: John Wiley & Sons; 1961.
18. Blaxter KL, Wainman FW. The fasting metabolism of cattle. Br J Nutr 1966;20:103-111.
19. Wanders D, Stone KP, Forney LA, et al. Role of GCN2-independent signaling through a non-canonical PERK/NRF2 pathway in the physiological responses to dietary methionine restriction. Diabetes 2016;65:1499-1510.
20. Wanders D, Forney LA, Stone KP, Burkh DH, Piersce A, Gettys TW. FGF21 mediates the thermogenic and insulin-sensitizing effects of dietary methionine restriction but not its effects on hepatic lipid metabolism. Diabetes 2017;66:858-867.
21. Plaisance EP, Greenway FL, Boudreau A, et al. Dietary methionine restriction increases fat oxidation in obese adults with metabolic syndrome. J Clin Endocrinol Metab 2011;96:E836-E840.
22. Di Buono M, Wykes LJ, Ball RO. Pencharz PB. Dietary cysteine reduces the methionine requirement in men. Am J Clin Nutr 2001;74:761-766.
23. Forney LA, Wanders D, Stone KP, Piersce A, Gettys TW. Concentration-dependent linkage of dietary methionine restriction to the components of its metabolic phenotype. Obesity (Silver Spring) 2017;25:730-738.
24. Elshorbagy AK, Valdivia-Garcia M, Mattocks DA, et al. Cysteine supplementation reverses methionine restriction effects on rat adiposity: significance of stearoyl-coenzyme A desaturase. J Lipid Res 2011;52:104-112.