

## Oral Recombinant Methioninase Prevents Obesity in Mice on a High-fat Diet

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**Abstract.** *Background/Aim:* obesity is a world-wide recalcitrant problem leading to many diseases. Dietary methionine restriction (MR) has been shown to prevent obesity, but it is an onerous regimen. The present study aimed to determine the effects of oral recombinant methioninase (*o-rMETase*), on preventing obesity in mice on a high-fat diet. *Materials and Methods:* Male C57BL/6J mice in the control group were fed a control diet (CD) (+6.5% fat) for 25 days, and others were fed a high-fat (HF) diet (+34.3% fat) for 25 days. Then, the mice were divided into three dietary groups: 1) HF + phosphate buffered saline (PBS) group; 2) HF + *o-rMETase* group; and 3) untreated non-HF group. *Results:* The mice on the CD increased in body weight by 14% during experimental period of 25 days; in contrast the mice in the HF+PBS group increased by 33%; however, the mice on the HF+*o-rMETase* group increased only by 14% ( $p=0.02$ , HF+PBS vs HF+*o-rMETase*). *Conclusion:* The HF+ *o-rMETase* group had the same weight increase as untreated mice on a normal fat diet, demonstrating the potential for *o-rMETase* to eliminate the need for dieting to maintain normal body weight.

The obesity population is rapidly increasing throughout the world (1). The World Health Organization (WHO) estimated

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in 2016, more than 1.9 billion adults aged 18 years and older were overweight (BMI >25), and of these over 650 million were obese (BMI >30). Approximately 13% of the world's adult population was obese in 2016; the world prevalence of obesity nearly tripled in about 40 years (2). Excess weight is often accompanied by high blood pressure, high blood cholesterol, type 2 diabetes, coronary heart disease, and other health problems. The total cost attributable to obesity-related diseases approaches \$100 billion annually in the US.

Dietary methionine restriction (MR) has prevented weight gain in mice and humans (3, 4). The potential health benefits of vegan and vegetarian diets may be linked to a lower methionine intake. Lowering methionine intake to levels characteristic of vegan diets, which have naturally low methionine levels (5, 6), may be associated with health benefits, including lower rates of obesity (7).

However, it is difficult to maintain dietary restriction in daily life. Dropout is a serious problem in weight-loss intervention programs for obese patients and up to 80%, depending on the type and setting of the treatment program (8-12), regained the lost weight or more. Bariatric surgery is wide-spread as a therapy for severe obesity and refractory diabetes. However, there are some complications, and obesity and diabetes recur at a constant rate after surgery (13, 14).

Dietary MR involves severe protein restriction and is unpalatable to most patients for cancer therapy. Enzymatic MR has also been described with methioninase (*L-methionine  $\alpha$ -deamino- $\gamma$ -mercaptomethane lyase*) (15-19). Our laboratory has developed recombinant methioninase (*rMETase*) with the *Pseudomonas putida* gene inserted in *Escherichia coli* and has targeted cancer in mouse models with high efficacy, especially in combination with chemotherapy (16-19). However enzymatic MR has limited potential as an injectable foreign protein.

Our laboratory has recently developed oral *rMETase* (*o-rMETase*) which has been shown to be highly effective in mouse models of cancer as injectable *o-rMETase* (20-27). In

the present report, we show that o-rMETase prevents obesity in mice on a high-fat diet, thereby opening a new paradigm for obesity prevention.

## Materials and Methods

**Animal studies.** C57BL/6 mice aged 8 weeks were used in this study. Mice were housed in a barrier facility on a high efficacy particulate air (HEPA)-filtered rack under standard conditions of 12-h light/dark cycles. Animal studies were performed with an AntiCancer Institutional Animal Care and Use Committee (IACUC)-protocol specially approved for this study and in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Animals under Assurance Number A3873-1.

**Recombinant methioninase.** Recombinant L-methionine  $\alpha$ -deaminoy-mercaptopmethane lyase [recombinant methioninase, (rMETase)] [EC 4.4.1.11] from *Pseudomonas putida* has been previously cloned and was produced in *Escherichia coli* (AntiCancer, Inc., San Diego, CA, USA). rMETase is a homotetrameric PLP enzyme of 172-kDa molecular mass (16).

**Study design.** Mice were randomized into three groups of 5 mice (Figure 1); standard diet (6.5% fat) without treatment (n=5); high-fat (HF) diet (34.3% fat), treated with phosphate-buffer saline (PBS) by oral gavage (n=5); HF diet with rMETase (100 units, per dose, twice a day, 25 consecutive days, oral gavage n=5). C57BL/6 mice were fed either a standard global rodent diet (Harlan Teklad 2020 $\times$ ) or HF diet chow containing 60% kcal from fat (Harlan Teklad TD.06414) for 25 days. Each mouse was given the experimental diet in accordance with a pair-feeding protocol. Therefore, daily food intake, and energy, protein and fat intake did not differ among the groups (Figure 2B).

**Body weight and calorie intake.** Body weight and dietary intake were recorded daily.

**Methionine calculation.** Methionine was determined with an HPLC procedure described previously (28, 29).

**Statistical analyses.** All data are presented as means $\pm$ standard error of the mean (SEM). Student's *t*-tests were performed.  $p < 0.05$  is considered significant.

## Results

**Efficacy of o-rMETase on the high-fat diet mice to prevent obesity in mice.** The mice on the control diet (CD) had a weight gain of 14% as they grew normally. The mice on the high-fat (HF) diet, in contrast, had a weight gain of 33%, approximately, 124% more than mice on the CD. However, the mice on the HF diet treated with o-rMETase gained only 14% body weight, the same as mice on the CD. o-rMETase could enable the mice to have normal weight despite being on the HF diet. ( $p=0.02$ ) (Figure 2A and Figure 3).

**o-rMETase regulates blood methionine levels.** o-rMETase reduces blood methionine levels to approximately 20% within 3 h compared to pre-o-rMETase level (Figure 4),

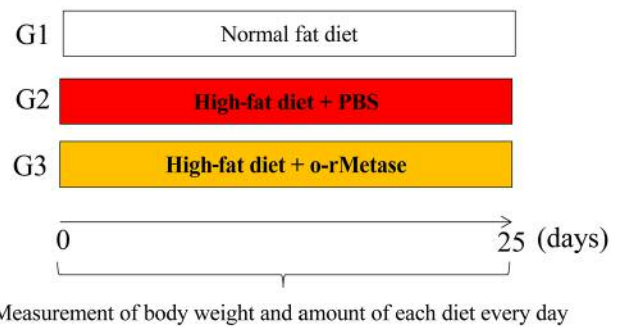


Figure 1. Treatment protocol. G1: normal fat diet + untreated control (n=5); G2: High fat diet + PBS (50  $\mu$ l/dose, twice a day, oral gavage, daily); G3: High fat diet + o-rMETase (50 units/ dose, twice a day, oral gavage).

## Discussion

Drug therapy, cognitive behavioral therapy and diet therapy are used for body weight loss for obesity patients. These therapies are temporarily efficient, with a high dropout rate (8-11, 12, 30). Body weight easily rebounds because it is difficult to maintain these regimens (31). Recently bariatric surgery has become wide-spread for obesity patients, but often involves complications.

Methionine is an essential amino acid that is absorbed in the small intestine. The absorbed methionine is used for protein synthesis and converted to S-adenosylmethionine, which plays an important role in DNA and other methylation and metabolic reactions. In general, a vegan diet has a low amount of methionine and several studies have suggested it has health benefits (6, 7). Dietary MR has many positive effects on metabolic health and has been shown to extend lifespan in rodents (32-34). However, it is difficult to maintain dietary MR in daily life, and we should consider the resulting high drop-out rate and weight rebound after MR.

METase was initially purified from *Clostridium sporogene* and catabolized methionine to  $\alpha$ -ketobutyrate, methanethiol and ammonia (15). rMETase was developed to lower the methionine levels *in vivo*. Our laboratory developed rMETase from *Pseudomonas putida*, cloned in *Escherichia coli*, as a very efficient means of MR (16). A big advance was our laboratory's development of o-rMETase which makes administration safe and easy without side-effects (20, 21-27).

The present study is the first demonstration of efficacy of o-rMETase to prevent obesity in mice on a high fat diet. In the present study, the body weight gain of o-rMETase-treated mice on a high-fat diet was significantly less than PBS-treated mice on a high fat diet (Figure 2A) and no more than untreated mice on a low-fat diet.

o-rMETase reduces 80% methionine levels within 3 h (Figure 4). Therefore o-rMET is a more powerful, means of

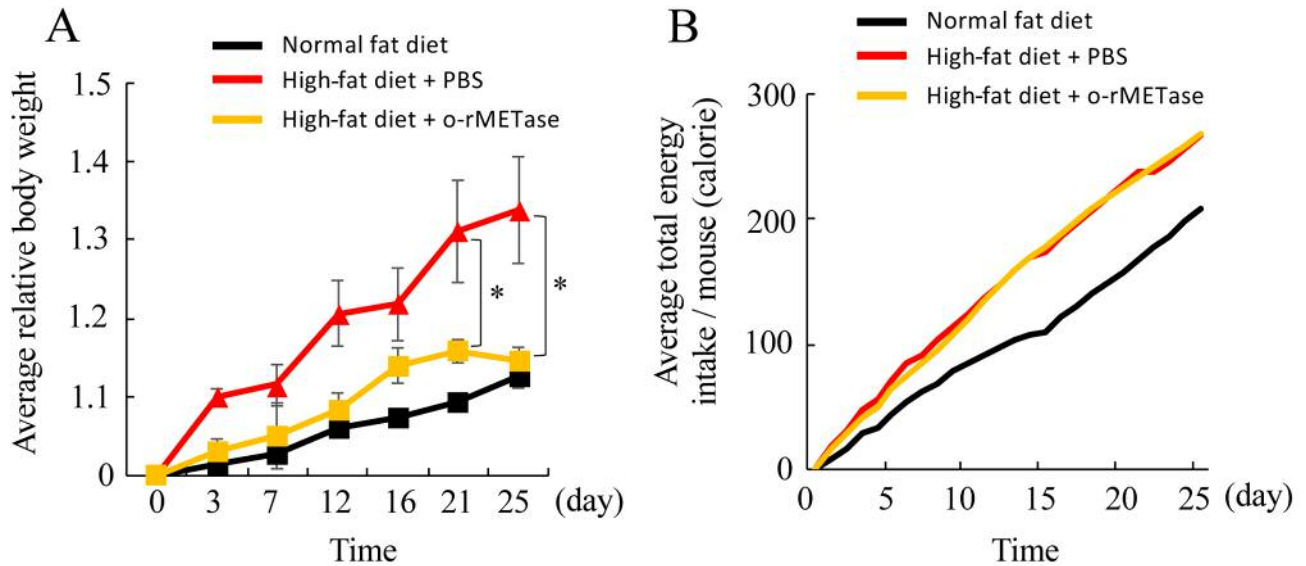


Figure 2. *o*-rMETase prevents obesity. (A) Relative average body weight (+SEM, n=5 mice). (B) Average total energy intake by mice. \* $p < 0.05$ .

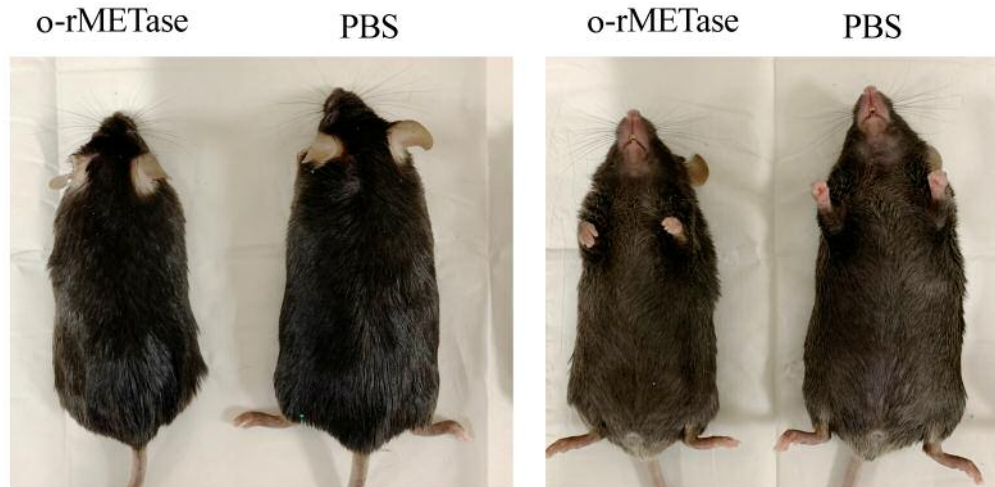


Figure 3. Mice on a high-fat diet.

MR than a low methionine diet (35). *o*-rMETase administration eliminates the need for dietary MR.

Thus, *o*-rMETase may be a new beneficial strategy for obesity patients and possibly other metabolic diseases associated with obesity. *o*-rMETase has the potential to eliminate the need for dieting to maintain body weight.

## Conclusion

In summary, we demonstrated for the first time the efficacy of *o*-rMETase to prevent obesity in mice on a high fat diet.

## Authors' Contributions

Y.T., S.R.S and R.M.H designed and performed experiments, analyzed data and wrote the paper; Q.H., M.Z. and Y.T. provided reagents; N.S., J.Y., H.N., A.T., M.M., M.B. and H.N. gave technical support and conceptual advice.

## Conflicts of Interest

The Authors declare no competing financial interests.

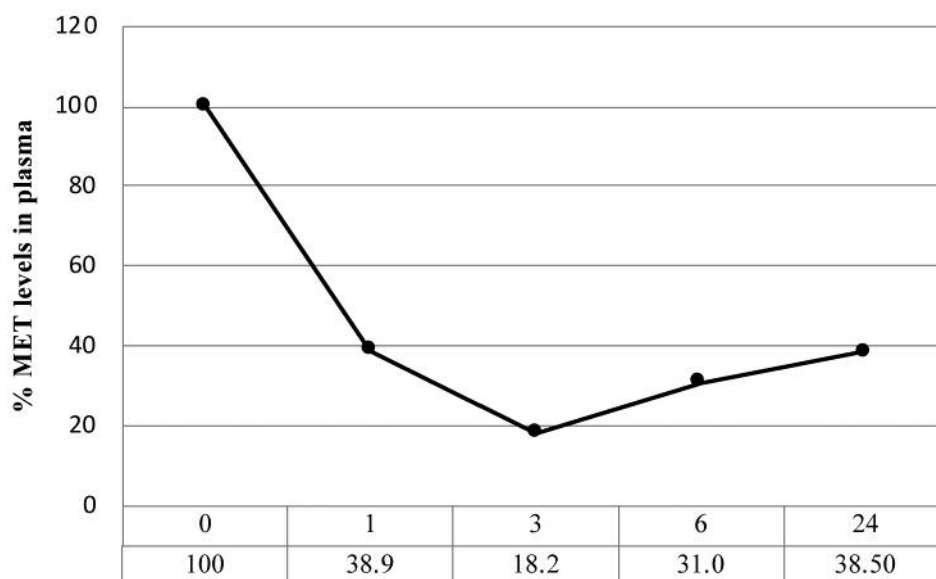


Figure 4. Time course of methionine depletion in mouse plasma after o-rMETase. 50 units of o-rMETase, twice a day and overnight fasting.

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