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Oral Recombinant Methioninase Inhibits Diabetes Onset in Mice on a High-fat Diet

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Abstract. *Background/Aim: We have recently shown that oral recombinant methionase (o-rMETase) prevents obesity in mice on a high-fat (HF) diet. The present study aimed to determine if o-rMETase can inhibit the onset of diabetes in mice on a HF diet. Materials and Methods: The mice on a HF diet were divided into two groups: 1) HF+phosphate buffered saline (PBS) group; 2) HF+o-rMETase group. Results: The blood glucose level in the HF+PBS group increased to average of 201 mg/dl during the experimental period of 8 weeks. In contrast, the blood glucose level in the HF+o-rMETase group maintained an average of 126 mg/dl (p<0.01, HF+PBS vs. HF+o-rMETase). The glucose tolerance test showed a significant increase in tolerance in the HF+o-rMETase group at 120 min after glucose injection compared to the HF+PBS group (p=0.04). Visceral adipose tissue was significantly less in the HF+o-rMETase group than the HF+PBS group (p=0.05). There was no difference in insulin levels, cholesterol or triglycerides between the HF+PBS and HF+o-rMETase groups. Conclusion: o-rMETase inhibited the onset of diabetes as well as prevented obesity on a high-fat diet, offering a possibility of a new and easy-to-use alternative to severe dieting or insulin injections.*

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Key Words: Methioninase, methionine restriction, MR, diabetes, high-fat diet, mice.

The population of diabetes patients is rapidly increasing and is currently estimated at more than 415 million adults worldwide (1). Diabetes can cause cardiovascular disease, kidney disease, cancer, and other health problems. Thus, decreasing the onset of diabetes is a primary health-care goal worldwide. Being overweight or obese increases the chances of developing diabetes; especially, accumulation of visceral fat is a high risk factor for diabetes (2). Methionine restricted (MR) diets in rodents and humans have been shown to improve glucose homeostasis and insulin sensitivity and prevent weight gain (3, 4). However, clinical trials based on dietary intervention often experience high drop-out rates (5, 6). MR diet is an onerous regimen with very little protein. While bariatric surgery is becoming wide-spread as the therapy for severe obesity and apparently for refractory diabetes, it can have complications, and obesity and diabetes recur at a constant rate after surgery (7, 8). We have previously shown that oral recombinant methionase (o-rMETase) prevents obesity in mice on a high-fat diet, a much more effective means of MR than an MR diet (9). In the present report, we show that o-rMETase inhibits diabetes onset in mice on a high-fat diet, thereby opening a new paradigm to prevent diabetes.

Materials and Methods

Animal studies. C57BL/6 mice, aged 8 weeks (AntiCancer Inc, San Diego, CA, USA), 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 α-deamino-γ-mercaptomethane 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,

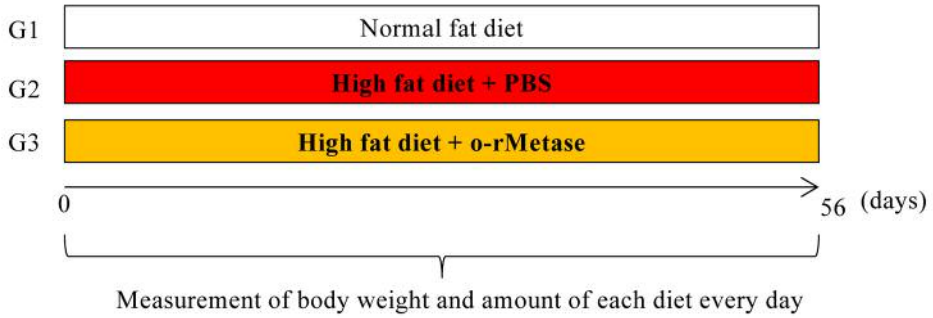

Figure 1. *Treatment schema. Treatment protocol. G1: Normal fat diet (untreated control) (n=5); G2: High-fat diet+PBS (50 μl/day, twice a day, oral gavage, daily); G3: High-fat diet+o-rMETase (50 units/dose, twice a day, oral gavage).*

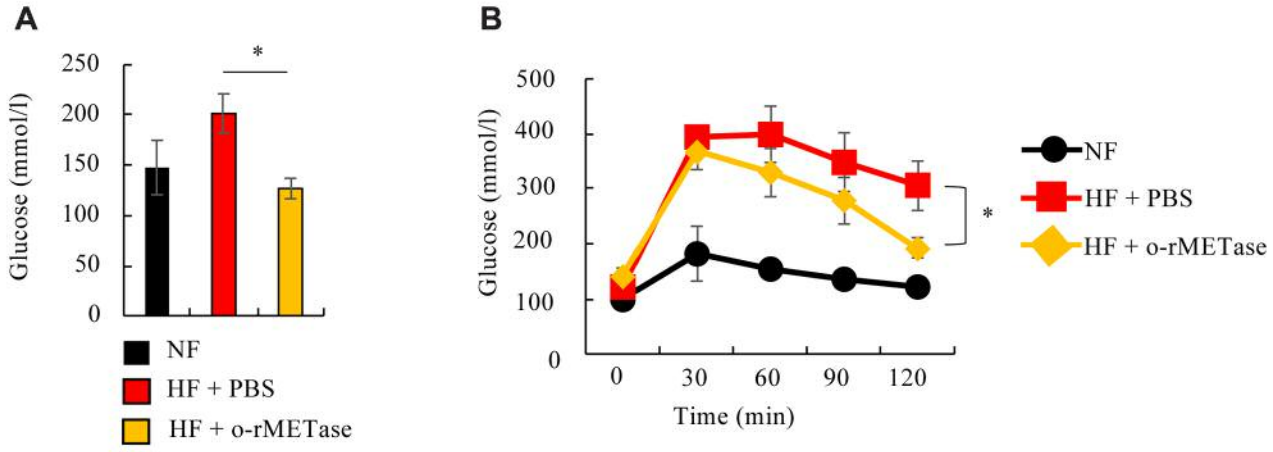

Figure 2. *o-rMETase maintains glucose homeostasis. (A) Average blood glucose level (±SEM, n=5 mice). (B) Glucose tolerance (±SEM, n=5 mice). *p<0.05; **p<0.01.*

CA, USA). rMETase is a homotetrameric PLP enzyme of 172-kDa molecular mass (10).

Study design. Mice were randomized into three groups of 5 mice; 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 o-rMETase (100 units per dose, twice a day, 56 consecutive days, oral gavage n=5) (Figure 1). C57BL/6 mice were fed either a standard global rodent diet (Harlan Teklad 2020×) or HF diet chow containing 60% kcal from fat (Harlan Teklad TD.06414) for 56 days. Each mouse was given the experimental diet in accordance with a pair-feeding protocol. Therefore, daily food intake, energy, and protein and fat intake did not differ among the groups (Figure 1).

Physiological and biochemical determinations. Body weight and dietary intake were recorded daily. A glucose tolerance test was performed on the mice after 16-h of fasting. Glucose (1 g/kg body weight) was injected intraperitoneally and blood glucose was measured with a GlucoGorx® (Innovus Pharmaceuticals Inc., CA, USA) before and 30, 60, 90, and 120 min after injection. Blood cholesterol and triglycerides were measured with a PRIMA® (PRIMA Lab SA., Balerna, Switzerland). Serum separated from orbital eye bleeding was measured for insulin levels with an insulin ELISA kit (Alpco, Salem, NH, USA) (11). A homeostasis model assessment of insulin resistance [HOMA-IR; (fasting immunoreactive insulin (μUml^{-1})×fasting glucose (mg per 100 ml))/405] was used as an index of insulin resistance. A homeostasis model assessment of β-cells [HOMA-β; (360×fasting immunoreactive insulin (μUml^{-1})/(fasting glucose (mg per 100 ml)-63)] was used as an index of insulin secretion (12). Visceral adipose tissue including perigonadal, retroperitoneal and mesenteric fat tissue was collected and measured after euthanasia (13).

Statistical analyses. All data are presented as means±standard error of the mean (SEM). The Student's *t*-test was performed. $p \leq 0.05$ was considered significant.

Results

Efficacy of o-rMETase to maintain normal blood glucose levels and tolerance in mice on a high-fat diet. After eight weeks on the high-fat diet, the HF+PBS group showed higher blood glucose levels than the HF+o-rMETase group ($p<0.01$) which was similar to the glucose levels of mice on a normal diet

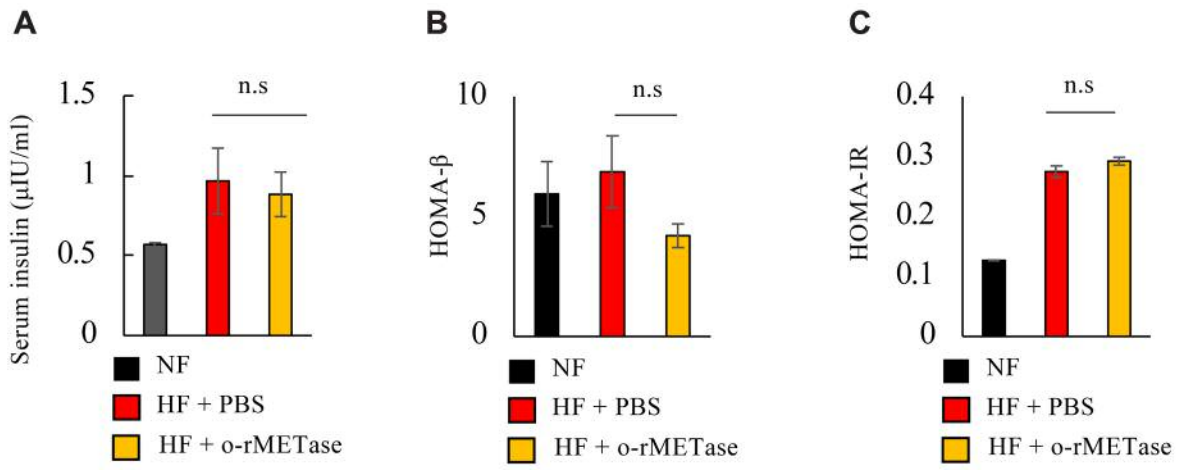

Figure 3. *Insulin secretion and resistance. (A) Average insulin secretion (±SEM, n=5 mice). (B) Average HOMA-β (±SEM, n=5mice). (C) Average HOMA-IR (±SEM, n=5 mice). HOMA-β: Homeostasis model assessment β-cells; HOMA-IR: homeostasis model assessment of insulin resistance.*

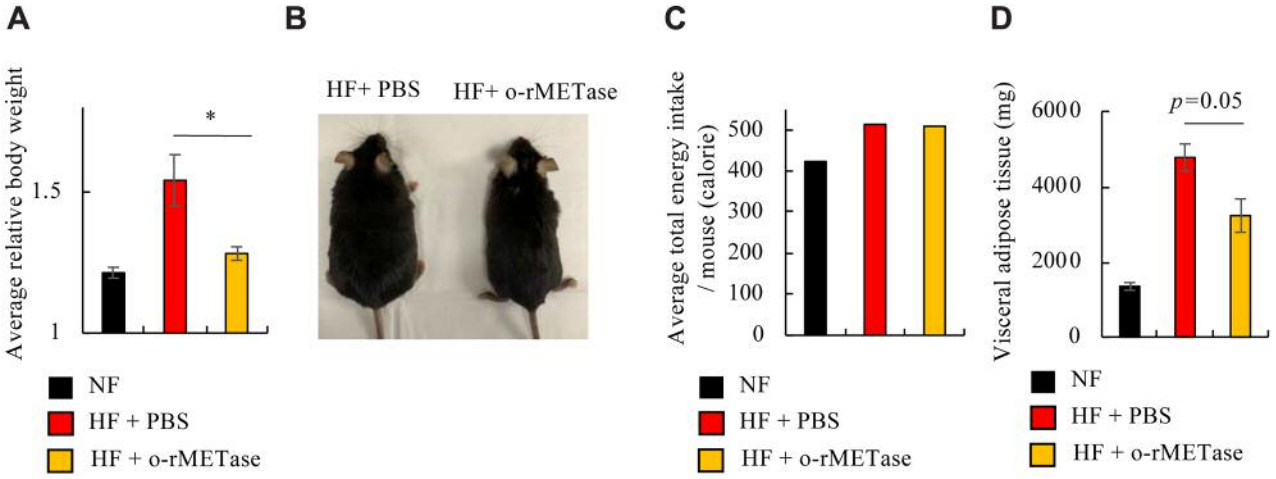

Figure 4. *o-rMETase prevents obesity. (A) Relative average body weight (±SEM, n=5mice). (B) Mice on a HF diet. Right: o-rMETase treated mouse. Left: PBS treated obesity mouse. (C) Average total energy intake per mouse. (D) Visceral fat tissue (±SEM, NF; n=3 mice, HF+PBS; n=3 mice, HF+o-rMETase; n=5 mice, p=0.05). *p<0.05.*

(Figure 2A). The glucose tolerance test showed an increase in tolerance in the HF+o-rMETase group after glucose injection compared to the HF+PBS group (p=0.04) (Figure 2B). Insulin secretion and resistance were not affected by o-rMETase in mice on the high-fat diet compared to the HF+PBS group (Figure 3A-C).

Efficacy of o-rMETase to reduce visceral adipose tissue increase on the high-fat diet. Visceral adipose tissue in the HF+o-rMETase group was reduced, along with reduced weight gain, compared to the HF+PBS group (p=0.05). While energy intake was similar among the groups (Figure 4).

Blood cholesterol and triglycerides in o-rMETase-treated and untreated mice on a high-fat diet. Blood cholesterol and triglycerides were not affected by o-rMETase in mice on the high-fat diet compared to the HF+PBS group (Figure 5A and B).

Discussion

The pandemic of diabetes is an urgent problem. Diabetes and obesity are closely related. Obesity is the greatest risk factor for diabetes progression and weight loss can prevent diabetes (14). Drug therapy, cognitive behavioral therapy and diet therapy are used for body-weight loss in obesity patients.

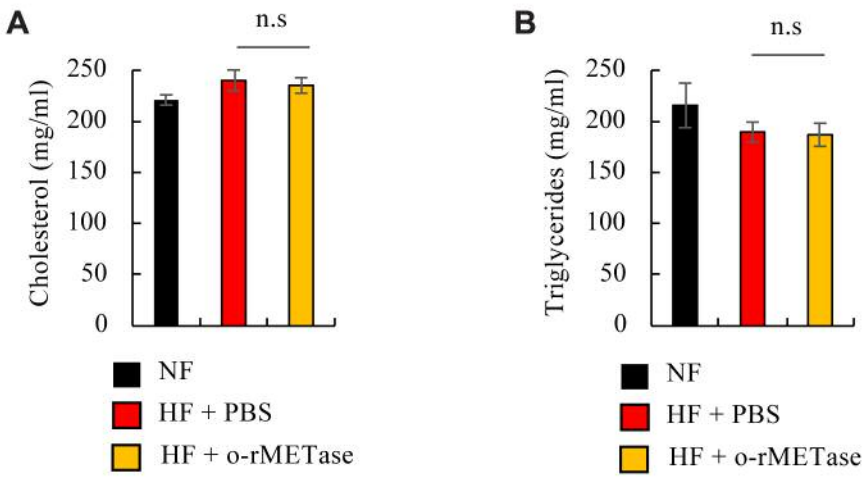

Figure 5. *Blood cholesterol and triglycerides. (A) Cholesterol (±SEM, n=5 mice). (B) Triglycerides (±SEM, n=5 mice).*

These therapies are temporarily efficient, with a high dropout rate (15-20). Body weight easily rebounds because it is difficult to maintain these regimens (21). While recently bariatric surgery has become wide-spread in obesity patients, it often involves complications, insufficient efficacy and high rates of recurrence (22-26).

Methionine is an essential amino acid, which is absorbed in the small intestine. The absorbed methionine is used for protein synthesis and is converted to S-adenosylmethionine, which plays an important role in DNA-methylation and other methylation reactions. In general, a vegan diet has low amounts of methionine and several studies have suggested that it has health benefits (16, 27). Dietary MR has been demonstrated to inhibit insulin resistance in diet-induced-obesity rodent models (28) and suggests a potential nutritional strategy to prevent diabetes (4, 29, 30). However, it is difficult to maintain dietary MR in daily life, due the resulting high drop-out rate and weight rebound after MR, as with other dietary interventions.

rMETase was initially purified from *Clostridium sporogene* and catabolized methionine to α-ketobutyrate, methanethiol and ammonia (31). rMETase was developed to lower the methionine level *in vivo*. Our laboratory developed rMETase from *Pseudomonas putida,* and cloned it in *Escherichia coli*, as a very efficient means of MR (10). We have shown that o-rMETase prevents obesity in mice on a high-fat diet (9). The present study is the first demonstration of the efficacy of o-rMETase to inhibit obesity-induced diabetes onset in mice on a high-fat diet. In the present study, blood glucose levels were significantly suppressed as a result of o-rMETase treatment and glucose tolerance of o-rMETase-treated mice on a high-fat diet was significantly better than that in PBS-treated mice on a high-fat diet (Figure 2A and B). Body weight gain, and visceral adipose tissue accumulation of o-rMETase-treated mice on a high-fat diet were significantly less than those in PBS-treated mice on a high-fat diet (Figure 4A, B and D).

Thus, o-rMETase may be a new beneficial strategy to prevent diabetes onset as well as to prevent obesity.

Metformin can regulate body weight and energy balance during treatment for diabetes and is the most prescribed drug for diabetes. However, metformin causes lactic acidosis and gastrointestinal side effects of nausea, abdominal pain, and bloating or diarrhea, thereby up to 20% of metformin-treated patients are intolerant (32). Insulin requires injection, unlike o-rMETase, and can cause hypoglycaemia, body weight gain and other problems (33).

o-rMETase has the potential to possibly eliminate the need for dieting and the administration of other drugs such as insulin and metformin to inhibit diabetes onset.

Conclusion

In summary, we demonstrate for the first time the efficacy of o-rMETase to inhibit diabetes onset in mice on a high-fat diet, suggesting a possible new paradigm for prevention of obesity and diabetes.

Conflicts of Interest

The Authors declare no competing financial interests regarding this study.

Authors' Contributions

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

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