Gastrointestinal Methionine Metabolism with Live Biotherapeutic SYNB1353 Results in Improved Plasma Methionine and Homocysteine Levels in Mice and Nonhuman Primates

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Introduction

- Methionine (Met) is an essential amino acid metabolized via the Met cycle and the transsulfuration pathway and is vital for protein synthesis required for normal growth and development.
- Like other amino acids, including phenylalanine and leucine, excess Met can lead to serious deleterious effects such as brain damage and death;
  - Met restriction extends lifespan and metabolic health in rodents and nonhuman primates.
  - Inborn errors of transsulfuration resulting from severe loss of function in cystathionine β-synthase (CBS) cause classic homocystinuria (HCU), which must be managed by a Met-restricted diet.

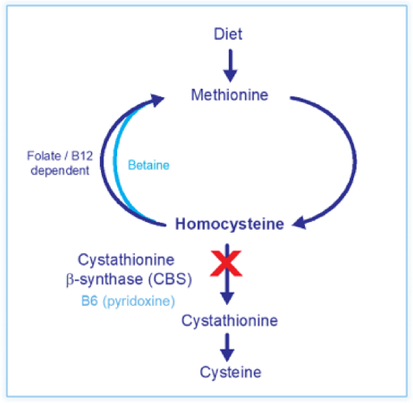


Figure 1. In HCU patients, mutations in the *CBS* gene result in accumulation of homocysteine. Pharmacotherapeutic options for the treatment of HCU consist of vitamin B6 (pyridoxine), which lowers homocysteine levels in B6-responsive patients, and betaine, which is involved in homocysteine remethylation to methionine.

- Synthetic Biotics are a new class of targeted live biotherapeutics that can be engineered to metabolize toxic dietary metabolites in the gastrointestinal tract to replicate the benefits of dietary restriction.
- Previous studies have shown that gastrointestinal levels of amino acids, including phenylalanine, come from two sources: dietary and enterorecirculation.

Study Design

Strain engineering

SYNB1353 was engineered from the probiotic *Escherichia coli* Nissle (EcN) to facilitate the entry of Met via MetP and metabolize Met via the methionine decarboxylase (MetDC) pathway, preventing its conversion to homocysteine (Hcy). To prevent the release of Met once it enters the cell, the *YjeH* gene, responsible for Met export, was deleted. SYNB1353 converts Met to 3-methylthiopropylamine (3-MTP).

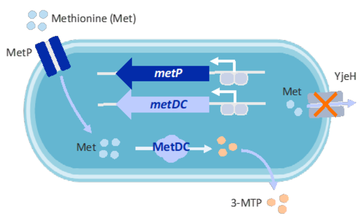


Figure 2. Schematic of SYNB1353. Met enters the cell via the importer MetP and is converted via MetDC to 3-MTP, preventing further conversion to Hcy.

Animal experiments

- Fasted mice and cynomolgus monkeys received an oral bolus of Met (monkeys: 100 or 300 mg/kg) or d_4-Met (mice: 50, 100, or 400 mg/kg), and blood and gut effluents were collected for Met and Hcy measurements while urine was collected for 3-MTP analysis.
- Hepatic CBS enzyme was downregulated using short hairpin RNA encapsulated in adeno associated viral (AAV) particles and administered to mice intravenously. AAV-CBS mice subsequently received an oral bolus of d_4-Met (50 mg/kg) with EcN (unengineered bacteria) or SYNB1353 at 5.4 x 10^{10} live cells and blood/urine were collected for Met, Hcy and 3-MTP measurements by LC-MS/MS.

Results

Gut methionine levels are higher than plasma levels

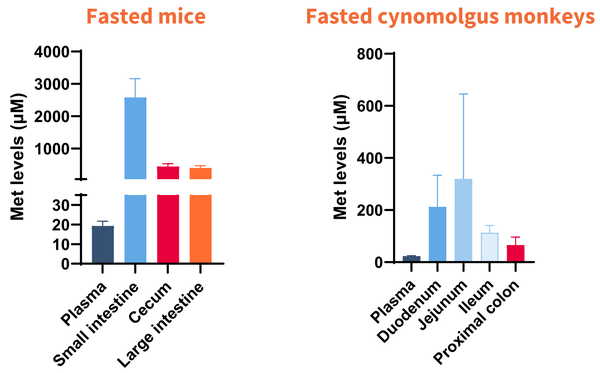


Figure 3. Met concentration in plasma and gut content from fasted mice and cynomolgus monkeys. Animals were fasted overnight, and plasma was collected after euthanasia. Intestines were harvested and flushed with PBS for methionine measurement by LC-MS/MS.

A fraction of the gastrointestinal pool of methionine comes from the systemic compartment

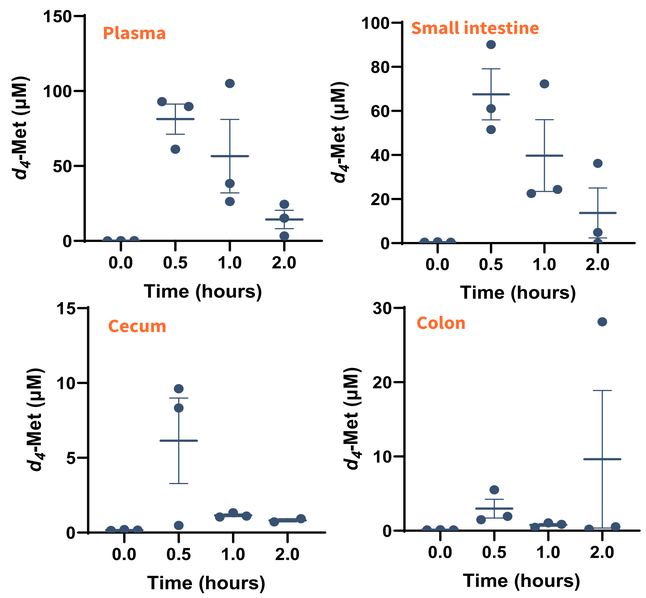


Figure 4. Plasma, small intestine, cecum, and colon d_4-Met levels (n = 3) following an intraperitoneal (IP) bolus of d_4-Met (100 mg/kg) in fasted mice. Blood (plasma) and intestinal contents were collected at the indicated timepoints.

Orally administered methionine results in rapid methionine and homocysteine exposures in mice and cynomolgus monkeys

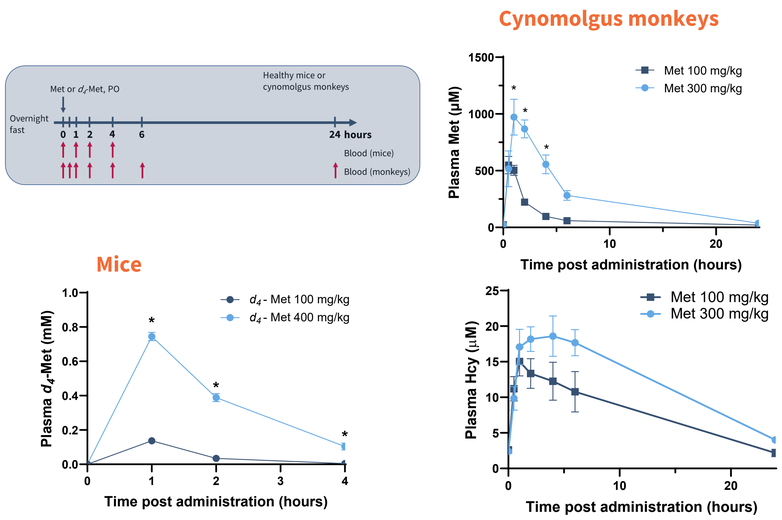


Figure 5. Effects of an oral Met/d_4-Met load in mice and monkeys. Labeled Met was administered at 100 or 400 mg/kg in mice. Met was administered at 100 or 300 mg/kg in monkeys. Plasma was collected throughout the study. *$p < 0.05$ versus 100 mg/kg Met/d_4-Met.

Hepatic CBS knockdown results in stably elevated homocysteine levels in mice

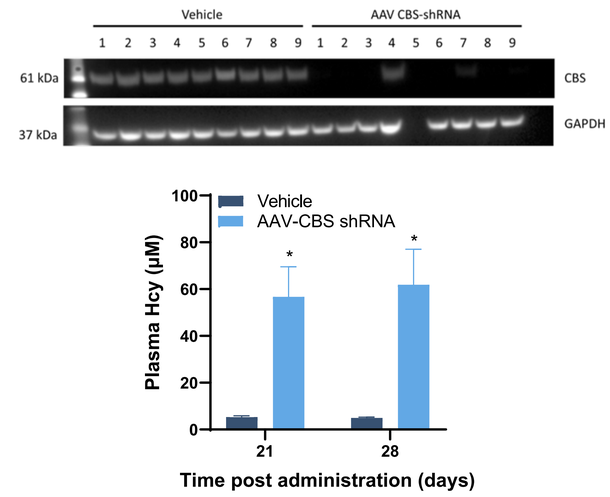
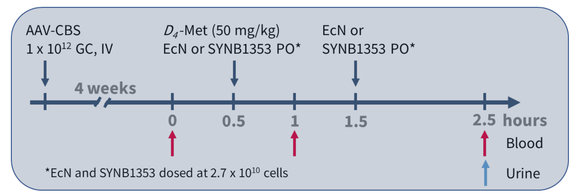


Figure 6. CBS knockdown results in significant elevations in plasma Hcy levels in mice. Mice received 1 x 10^{12} genomic copies of an AAV containing shRNAs targeting CBS or vehicle intravenously and blood was collected on days 21 and 28 and liver on day 35. Data presented as mean plasma Hcy ± SEM (n = 9). *$p < 0.05$.

Methionine is degraded by SYNB1353 in AAV-CBS mice



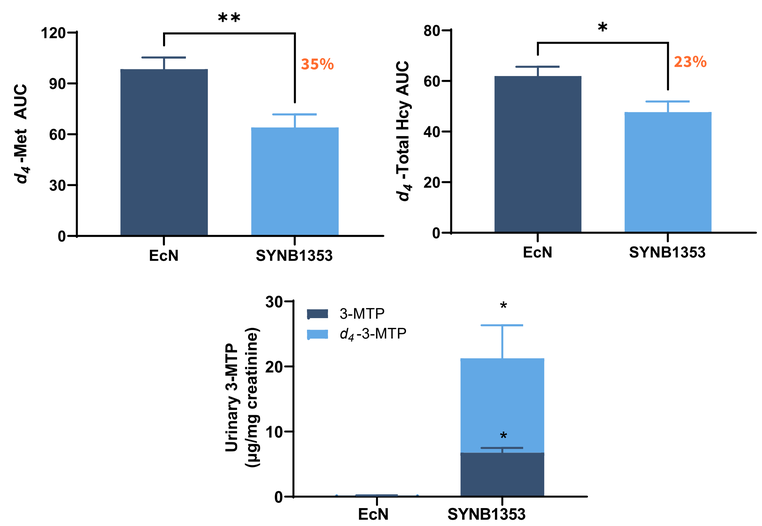


Figure 7. Effect of SYNB1353 (2 doses for a total of 5.4 x 10^{10} live cells) on plasma d_4-Met, plasma d_4-Hcy, and urinary 3-MTP/d_4-3-MTP recovery after an oral (PO) bolus of d_4-Met (50 mg/kg) in AAV CBS knockdown animals (n = 8/group). Blood and urine were collected at 0, 0.5, and 2 hours post first dose of bacteria. *$p < 0.05$, **$p < 0.01$.

Conclusions

- Met is readily available in the gut of mice and cynomolgus monkeys from dietary and systemic pools.
- Downregulation of hepatic CBS using shRNAs encapsulated in AAV particles results in stably elevated Hcy levels in mice.
- SYNB1353 can efficiently metabolize gastrointestinal Met to lower plasma Met and plasma Hcy in AAV mice.

Disclosures: This study was funded by Synlogic, Inc.

Presented at SIMD, Salt Lake City, UT, March 19, 2023