

**Supplementary Fig. 1. Fecal lipid excretion and apparent fat absorption rate in rats fed a CA-supplemented diet.** (A) Fecal excretion of FFA and TG per day were calculated using fecal weight collected for 24 hours at the end of the experiment and the concentrations in the feces. Sum of the fecal excretion of FFA and TG was significantly reduced in the CA-fed rats (Control: 18.8 ± 4.8 mg/day vs CA: 11.0 ± 3.7 mg/day, *P* = 0.0028).  (B) Apparent fat absorption rate (Control: 98.3 ± 0.4% vs CA: 99.0 ± 0.28%, *P* = 0.0013) was calculated from fat intake (Control:1.13 ± 0.14 g/day vs CA: 1.13 ± 0.15 g/day) at the end of the experiment, fat content in diets (7% wt/wt), and the fecal lipid excretion shown in (A). There was no difference in body weight and food intake between control and the CA-fed rats (data not shown). Values are shown as the mean with the SEM (n = 8). Asterisks indicate a significant difference compared to control (*P* < 0.05).

***Supplemental methods***

*Animal experiments and sample collection*

The Institutional Animal Care and Use Committee of National Corporation Hokkaido University approved this study (approval number: 17-0119), and all animals were maintained following the guidelines of Hokkaido University Manual for Implementing Animal Experimentation. Male inbred WKAH/HkmSlc rats (3 weeks old; Japan SLC Inc., Shizuoka, Japan, NBRP Rat No: 0154) were housed individually in a controlled environment at 22 ± 2°C temperature and 55 ± 5% humidity. The light period was from 08:00 to 20:00. The rats had free access to food and water during the entire study period. The rats were acclimated for two weeks with the AIN-93G-based control diet [[1]](https://paperpile.com/c/ojny4w/48HXQ). Rats were divided into two groups (n = 8 per group) and fed a control diet or CA diet (0.5 g CA/kg diet) [[2]](https://paperpile.com/c/ojny4w/MRR9C) for 2 weeks. Body weight and food intake were measured every two days. Feces were collected for 24 hours at 2 weeks. The collected samples were stored at −80°C until analysis. Food deprivation was not conducted in the experiment.

***Fecal lipid analysis***

Fecal lipids were analyzed as described earlier [[3]](https://paperpile.com/c/ojny4w/Ub2M5). In brief, lipids were extracted from fresh liver and freeze-dried feces using chloroform: methanol = 2:1 (v/v) solution [[4]](https://paperpile.com/c/ojny4w/Vqz9O). The extracts were evaporated and dissolved in 2-propanol for measurement. Cholesterol (Chol), triglyceride (TG), and free fatty acid (FFA) levels in the extracts were determined using a cholesterol E-test, triglyceride E-test, and NEFA C-test Wako kits (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan), respectively.

[1] [Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.](http://paperpile.com/b/ojny4w/48HXQ)

[2] [Islam KBMS, Fukiya S, Hagio M, Fujii N, Ishizuka S, Ooka T, et al. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology 2011;141:1773–81.](http://paperpile.com/b/ojny4w/MRR9C)

[3] [Hori S, Hara H, Ishizuka S. Marginal iron deficiency enhances liver triglyceride accumulation in rats fed a high-sucrose diet. Biosci Biotechnol Biochem 2018;82:2140–8.](http://paperpile.com/b/ojny4w/Ub2M5)

[4] [Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497–509.](http://paperpile.com/b/ojny4w/Vqz9O)