Dietary phytanic acid-induced changes in tissue fatty acid profiles in mice

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SUPPLEMENTARY FILE

Materials and Methods

Preparation of phytanic acid (PA)

Phytol obtained from Tama Biochemical CO., Ltd. (Tokyo, Japan) was converted into phytanol by hydrogenation using Adams' catalyst (PtO_2) as previously described (Patton and Benson, 1966). Ruthenium tetroxide-catalyzed oxidation with $NaIO_4$ (Prashad *et al.*, 1999) was used to convert phytanol into PA. The synthesized PA is a diastereomeric mixture of 3*R*, 7*R*, 11*R*- and 3*S*, 7*R*, 11*R*-isomers because chlorophyll-derived phytol (2*E*, 7*R*, 11*R*-isomer) was used as the raw material for synthesis.

- **Patton S and Benson AA** (1966) Synthesis of phytanic acid. *Journal of Lipid Research* **7**, 452-453.
- **Prashad M, Lu Y, Kim HY, Hu B, Repic O and Blacklock TJ** (1999) An improved and practical sharpless oxidation of primary alcohols to the carboxylic acids. *Synthetic Communications* **29**, 2937-2942.

Preparation of fatty acid methyl esters and gas chromatographic analysis

0.5 M KOH-methanol was added to the crude lipid extract, and the solution was heated at 100°C for 5 min. To the reaction solution was added 50% HCl-methanol (12 N HCl-methanol, 1:1), followed by incubation at 100°C for 5min. After the reaction was stopped by adding deionized water, n-hexane was added to the reaction solution. Following a vigorous shake, the reaction solution was centrifuged at 800 g for 5 min, and fatty acid methyl esters dissolved in the upper n-hexane phase were collected for gas chromatographic analysis. The fatty acid methyl esters were analyzed on a capillary column (BPX90, 100 m × 0.25 mm × 0.25 μm, SGE Analytical Science, Austin, Texas,

USA) in a GC-2010 gas chromatograph equipped with a flame-ionization detector and an automated injector (Shimadzu Corporation, Kyoto, Japan). The column temperature was programmed to hold at 140°C for 70 min, then increase by 2°C/min to 220°C and hold for 15 min, and to increase by 10°/min to a final temperature of 250°C and hold for 10 min. The temperature of injector and detector was set at 240°C and 250°C, respectively. Helium was used as the carrier gas and was maintained at a constant flow of 20 cm /sec. The outputs were calibrated against a 37-Component FAME Mix standard (Supelco, Bellefonte, PA, USA) supplemented with methyl esters of PA and pristanic acid (Sigma-Aldrich Co. LLC, St Louis, MO, USA). The fatty acid compositions were determined by calculating the peak areas for identified fatty acids and comparing them to each other.

Table S1. Composition of experimental diets (g/kg)

	Control	Phytanic acid
Casein	140	140
L-Cystine	1.8	1.8
Cornstarch	465.692	465.692
α-Cornstarch	155	155
Sucrose	100	100
Soybean oil	40	39.5
Phytanic acid	0	0.5
Cellulose	50	50
Mineral mix[a]	35	35
Vitamin mix[b]	10	10
Choline bitartrate	2.5	2.5
Tert-butylhydroquinone	0.008	0.008

[a] Mineral mixture, AIN-93M-MX (Nosan Corporation, Yokohama, Japan)

[b] Vitamin mixture, AIN-93-VX (Nosan Corporation, Yokohama, Japan)