

**Supplementary:**

**S-methyl cysteine sulfoxide: Its effects on cardiometabolic outcomes in high-fat fed C57BL/6 mice and relevance to human health.**

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**Table S1.** Outcomes for body weight, energy intake, lipids and glucose in mice comparing those on a normal diet, high-fat diet, and a high-fat diet with 60, 170, or 350 mg kg<sup>-1</sup> BW day<sup>-1</sup> of S-methyl cysteine sulfoxide.

Variables	ND	HFD	HFD+60	HFD+170	HFD+350
Baseline weight (g) ^	24 ± 0.44 <sup>a</sup>	24 ± 0.49 <sup>a</sup>	23 ± 0.53 <sup>a</sup>	23 ± 0.59 <sup>a</sup>	24 ± 0.43 <sup>a</sup>
Total weight gain (g)	5.1 ± 0.29 <sup>****</sup>	12.8 ± 1.0	10.7 ± 0.77	11.3 ± 0.90	11.0 ± 0.96
Total weight gain (g), adjusted <sup>v</sup>	6.3 ± 1.6 <sup>****</sup>	14.7 ± 3.0	12.7 ± 1.2	13.1 ± 1.2	12.7 ± 1.2
Total energy intake (kJ)	3665 (3591–3960) <sup>***</sup>	5966 (5778–6246)	6284 (6057–6703)	6506 (4224–6777)	5775 (4817–6245)
Glucose tolerance test (AUC)	1445 ± 63 <sup>****</sup>	2416 ± 168	2446 ± 93	2259 ± 185	2343 ± 138
Insulin tolerance test (AUC)	635 ± 61	829 ± 85	749 ± 58	674 ± 65	658 ± 63
Fasting serum glucose (mmol/L)	11.0 ± 0.50	13.0 ± 0.79	13.0 ± 0.79	12.0 ± 1.3	12.0 ± 0.84
Total cholesterol (mmol/L)	3.0 ± 0.06 <sup>****</sup>	4.5 ± 0.15	4.1 ± 0.12	3.9 ± 0.24	4.1 ± 0.21
mLDL-C (mmol/L)	0.14 ± 0.01 <sup>***</sup>	0.23 ± 0.02	0.19 ± 0.01	0.21 ± 0.01	0.19 ± 0.01
HDL-C (mmol/L)	1.79 (1.75–1.86) <sup>***</sup>	2.15 (2.09–2.22)	2.15 (2.00–2.22)	1.89 (1.84–2.08) <sup>**</sup>	1.92 (1.81–2.10) <sup>*</sup>
Triglycerides (mmol/L)	0.59 (0.53–0.67)	0.64 (0.55–0.69)	0.63 (0.56–0.69)	0.61 (0.54–0.65)	0.54 (0.48–0.61) <sup>**</sup>
hs-CRP (mg/L)	0.07 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Hepatic lipid content (mg/g)	294 (250–334)	359 (295–385)	375 (356–426)	366 (259–411)	318 (269–456)

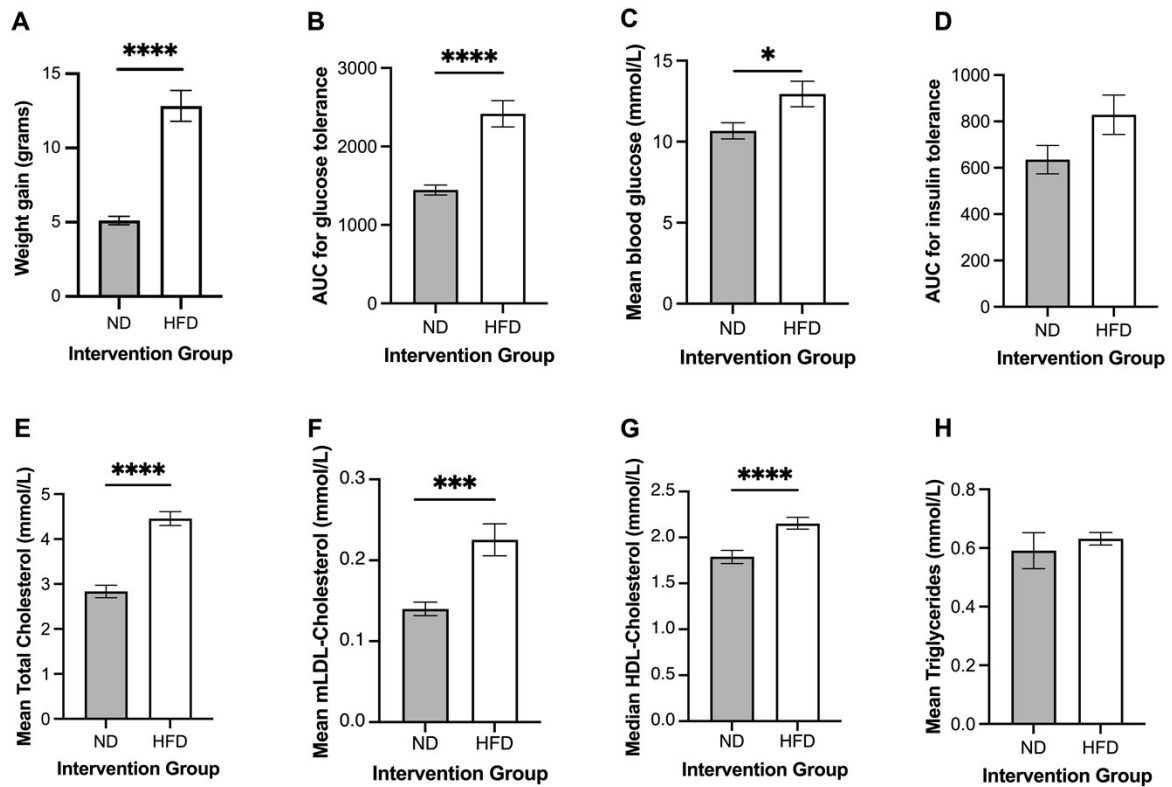
Data are presented as mean ± standard error of mean (SEM) differentiated by intervention feed group at the 12-week timepoint analysed using ANOVA, except for total energy intake, triglycerides, HDL-C, triglycerides, and hepatic lipid content which are reported as median and interquartile range (IQR) analysed by Kruskal-Wallis. The data from the ND, HFD+60, HFD+170, and HFD+350 groups were each statistically compared to those from the HFD. Significance is displayed as \**p* = 0.05, \*\**p* < 0.05,

\*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .  $n = 9-11$  per group. AUC, area under the curve; BW, body weight; kJ, kilojoule; HDL-C, high-density lipoprotein cholesterol; HFD, high fat diet; HFD+60, high-fat diet + 60 mg kg<sup>-1</sup> BW day<sup>-1</sup> of SMCSO; HFD+170, high-fat diet + 170 mg kg<sup>-1</sup> BW day<sup>-1</sup> of SMCSO; HFD+350, high-fat diet + 350 mg kg<sup>-1</sup> BW day<sup>-1</sup> of SMCSO; hs-CRP, high sensitivity C-reactive protein; mLDL-C, measured low-density lipoprotein cholesterol; ND, normal diet; SMCSO, S-methyl cysteine sulfoxide. ^ provides the mean  $\pm$  SEM of baseline weights for each group reported at baseline prior to randomisation, with each group compared against every other group, and significance displayed by different superscript letters. <sup>∇</sup> is presented as mean  $\pm$  SEM, with total weight gain adjusted for total energy intake, using linear regression.

**Table S2.** Urinary S-methyl cysteine sulfoxide concentration ( $\mu\text{g/ml}$ ) measured at 0, 6, and 12 weeks, according to allocated feed groups.

	<b>ND</b>	<b>HFD</b>	<b>HFD+60</b>	<b>HFD+170</b>	<b>HFD+350</b>
0 weeks ( $\mu\text{g/ml}$ )	$0.36 \pm 0.05$	$0.55 \pm 0.19$	$0.49 \pm 0.14$	$0.28 \pm 0.04$	$0.36 \pm 0.13$
6 weeks ( $\mu\text{g/ml}$ )	$0.33 \pm 0.04$	$0.37 \pm 0.13$	$0.98 \pm 0.2$	$2.4 \pm 1.10$	$5.3 \pm 0.58$
12 weeks ( $\mu\text{g/ml}$ )	$0.31 \pm 0.05$	$0.26 \pm 0.06$	$0.64 \pm 0.11$	$2.7 \pm 1.10$	$6.6 \pm 1.4$

Presented as mean  $\pm$  standard error of mean for each feed group at 0 weeks (baseline), 6-weeks and 12-week timepoints. The intervention feed groups were ND, HFD, HFD+60, HFD+170, and HFD+350. All intervention feed groups contained n = 11 except for ND which contained n = 10. BW, body weight; HFD high fat diet; HFD+60, high-fat diet + 60 mg kg<sup>-1</sup> BW day<sup>-1</sup> of SMCSO; HFD+170, high-fat diet + 170 mg kg<sup>-1</sup> BW day<sup>-1</sup> of SMCSO; HFD+350, high-fat diet + 350 mg kg<sup>-1</sup> BW day<sup>-1</sup> of SMCSO; ND, normal diet; SMCSO, S-methyl cysteine sulfoxide.



**Figure S1.** Data analyzed using Student *t*-tests showing differences in total weight gain (A), area under the curve (AUC) for glucose tolerance (B), fasting blood glucose (C), AUC for insulin tolerance (D), total cholesterol (E), measured LDL-C, (F), HDL-C (G), and triglycerides (H) comparing the mice group consuming a normal diet (ND) versus the mice group consuming a high-fat diet (HFD), after 12 weeks. Data are presented as mean ± standard error of the mean, except for HDL-C which is presented as median and interquartile range. ND contained n = 9; HFD contained n = 11. Statistical significance as \**p* = 0.330, \*\*\* *p* = 0.0017, \*\*\*\**p* < 0.0001. HFD, high-fat diet; HDL-C, High-density lipoprotein cholesterol; mLDL-C, Measured low-density lipoprotein cholesterol; SMCSO S-methyl cysteine sulfoxide