

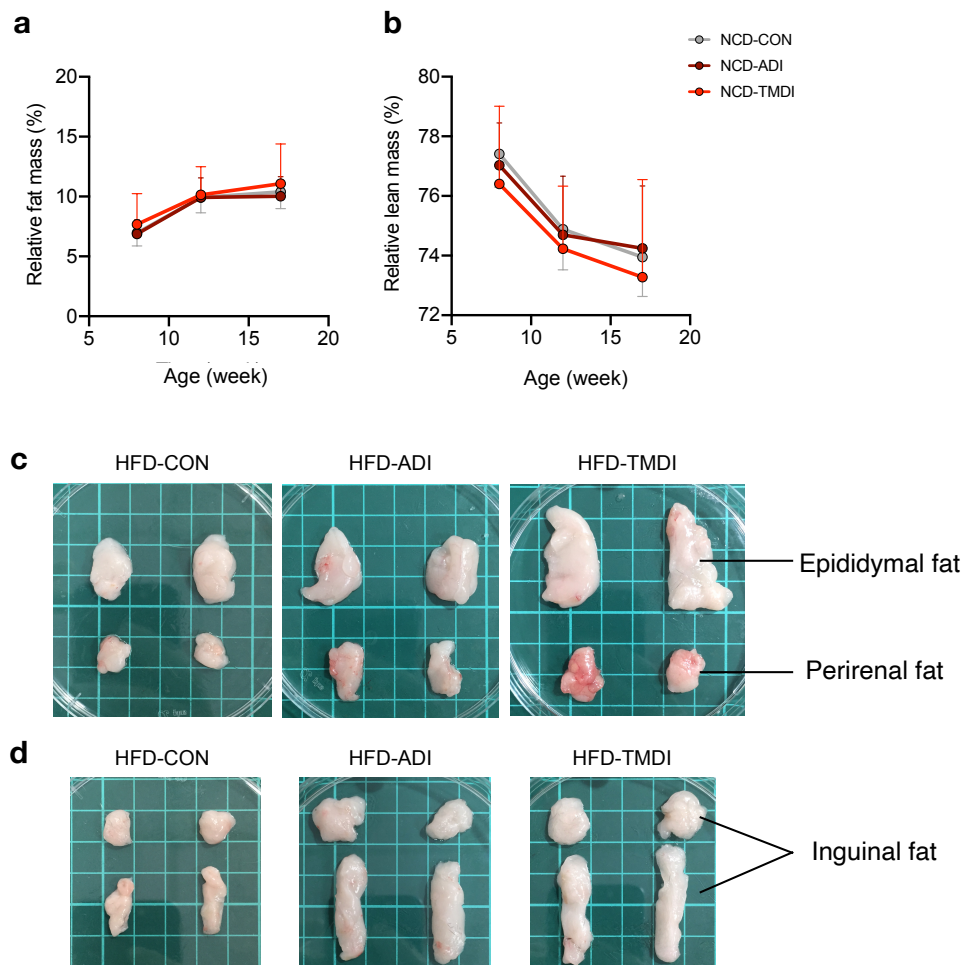
Dietary Exposure to Antibiotic Residues Facilitates Metabolic Disorder by Altering the Gut Microbiota and Bile Acid Composition

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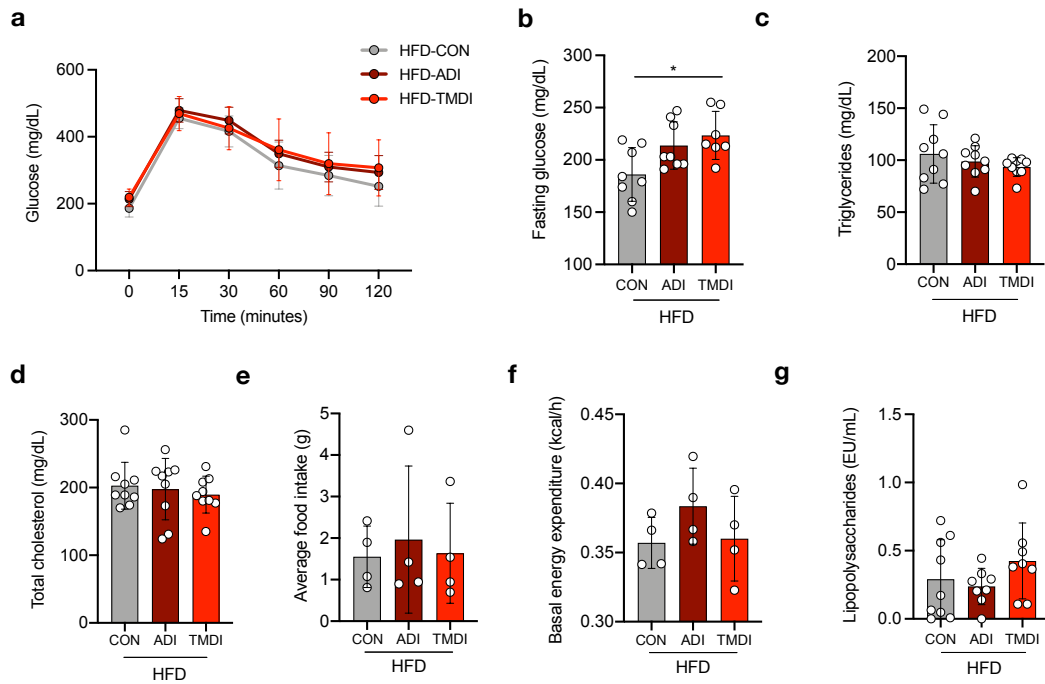
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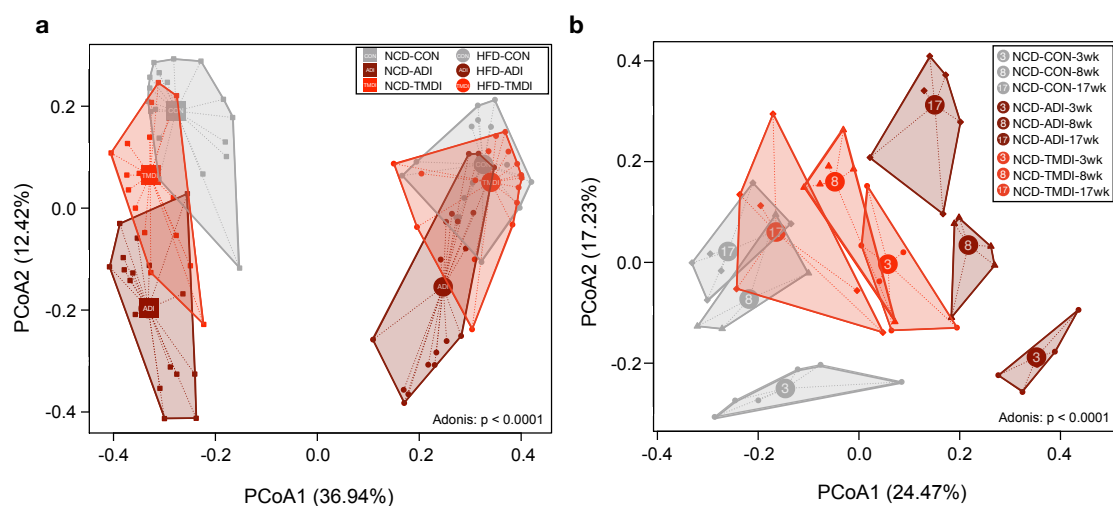
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Extended Data Fig.1 | Effects of tylosin on body composition and adipose tissue. a, Relative fat mass and **b,** relative lean mass change of NCD mice (n = 12/group). **c,** Size of epididymal and perirenal fat. **d,** Size of inguinal fat. Data are expressed as mean $\pm$ SD. Statistical analyses were performed by one-way ANOVA with Tukey's range test. Abbreviations: ADI, acceptable daily intake; CON, control; HFD, high-fat diet; NCD, normal chow diet; TMDI, theoretical maximum daily intake.



Extended Data Fig.2 | Effects of tylosin on metabolic parameters. **a**, Plasma glucose profile measured during the OGTT (n = 8–10/group). **b**, Plasma glucose **c**, triglycerides and **d**, total cholesterol level after overnight fasting (* $p < 0.05$). **e**, Average food intake (n = 4/group). **f**, Basal energy expenditure. **g**, Plasma lipopolysaccharides level (n = 8–10/group). Data are expressed as mean $\pm$ SD. Statistical analyses were performed by one-way ANOVA with Tukey's range test. Abbreviations: ADI, acceptable daily intake; CON, control; HFD, high-fat diet; TMDI, theoretical maximum daily intake.



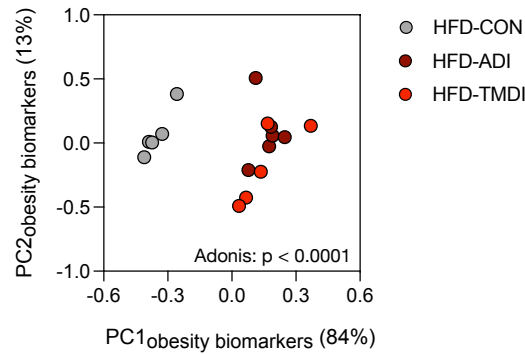
Extended Data Fig.3 | Effects of diet, tylosin, age on gut microbiota composition.

Bacterial community represented by principal coordinate analysis (PCoA) of Bray-Curtis distances of: **a**, Effects of diet and tylosin on gut microbiota composition ($n = 6/\text{group}$). **b**, Effects of tylosin in NCD-fed mice at 3-, 8-, and 17-week-old ($n = 6/\text{group}$). Abbreviations: ADI, acceptable daily intake; CON, control; HFD, high-fat diet; NCD, normal chow diet; TMDI, theoretical maximum daily intake.

a



Extended Data Fig.4 | Comparison fecal microbiota of HFD-CON and HFD-TMDI at amplicon sequence variant (ASV) level. Heatmap showing bacteria with significant differences ($p < 0.05$) based on $\log_2$ fold change >1 and significant difference ($p < 0.05$) using the Wilcoxon signed-rank test. Abbreviations: CON, control; HFD, high-fat diet; TMDI, theoretical maximum daily intake.

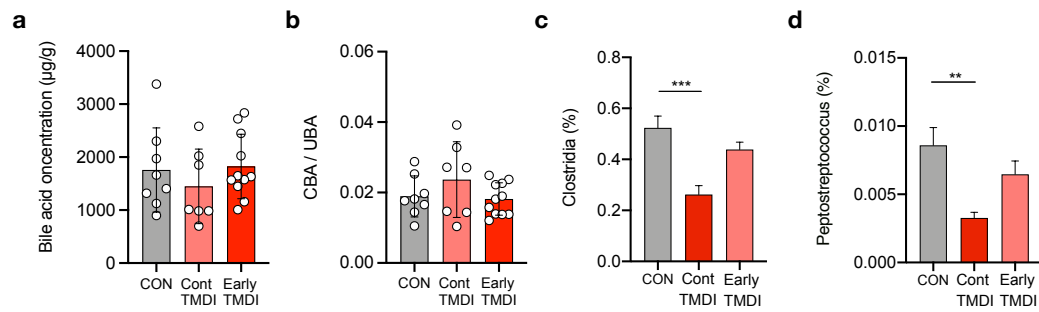


Extended Data Fig.5 | Principal component analysis of the obesity biomarkers of

mice. PCA of the obesity biomarkers of 17-week-old HFD-fed mice ($n = 5-6/\text{group}$).

The PCA plot was calculated from obesity biomarkers including body weight at 17 weeks of age, fat mass at 17 weeks of age, relative fat mass at 17 weeks of age, weight gain between 3 to 17 weeks of age, fat mass gain between 8 to 17 weeks of age, fasting glucose, OGTT_{AUC}, fasting insulin, HOMA-IR, weight of eWAT, and fatty liver score.

Adonis were performed to test the difference among groups. Abbreviations: ADI, acceptable daily intake; AUC, area under the curve; CON, control; HFD, high-fat diet; HOMA-IR, homeostasis model assessment of insulin resistance; OGTT, oral glucose tolerance test; TMDI, theoretical maximum daily intake.



Extended Data Fig.6 | Effects of tylosin TMDI on bile acid composition and bacteria involved in the conversion of primary bile acids to secondary bile acids. a, Total bile acid level in cecum (n = 7-11/group). **b,** Ratio of conjugated bile acids to unconjugated bile acids. **c-d,** Relative abundance of bacteria exhibiting bile acid hydroxylase, 7 α -dehydroxylase, and C-7 epimerase activity, including **(c)** *Clostridia* and **(d)** *Peptostreptococcus* (n = 8/group). Data are presented by mean $\pm$ SD (**a, b**) or mean $\pm$ SEM (**c, d**). Statistical analyses were performed by one-way ANOVA with Tukey's range test (* p < 0.05; ** p < 0.01; and *** p < 0.001). Abbreviations: CBA, conjugated bile acid; CON, control; TMDI, theoretical maximum daily intake; UBA, unconjugated bile acid.