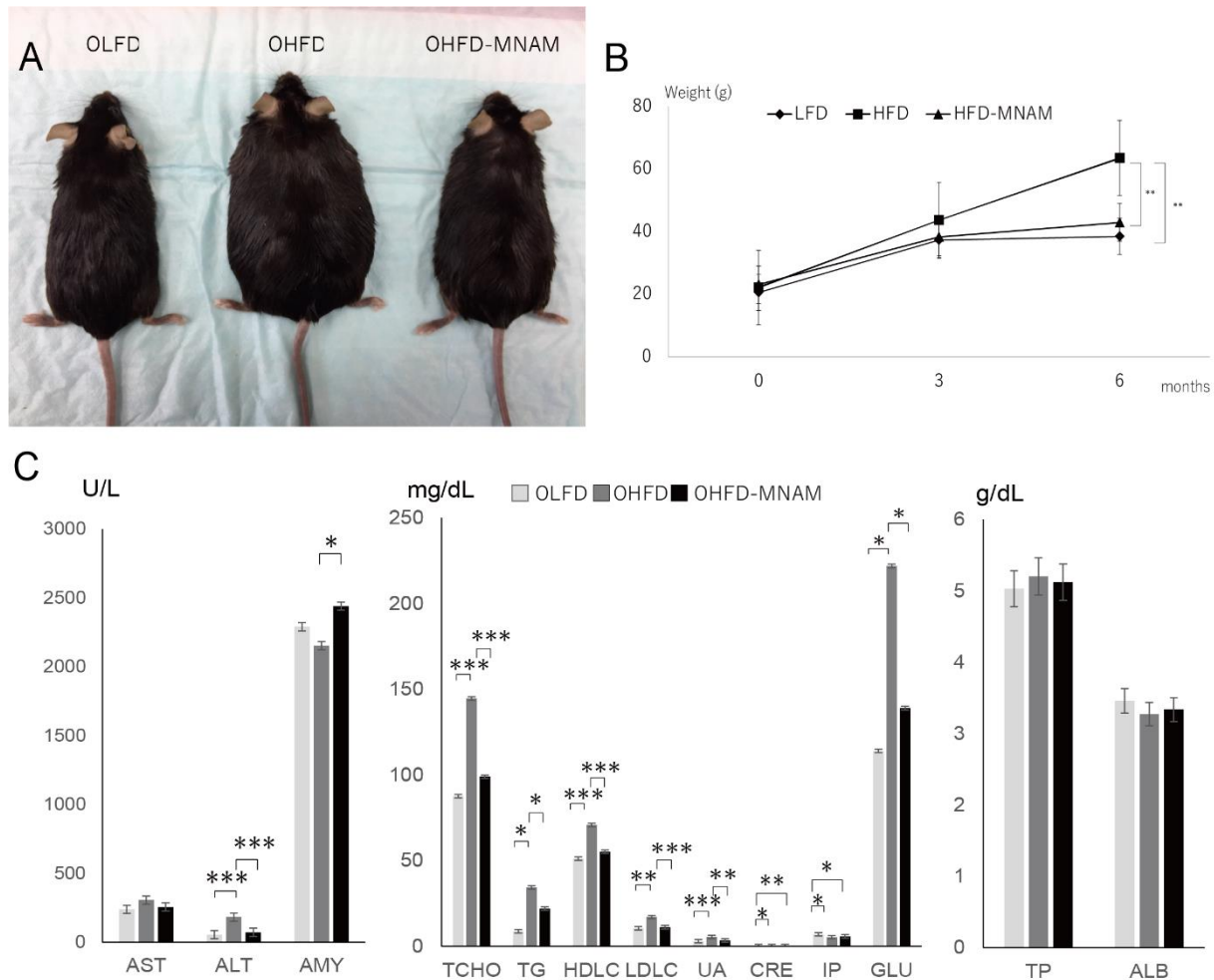


Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Figures



Supplementary Figure 1. Differences in weight and blood analysis results

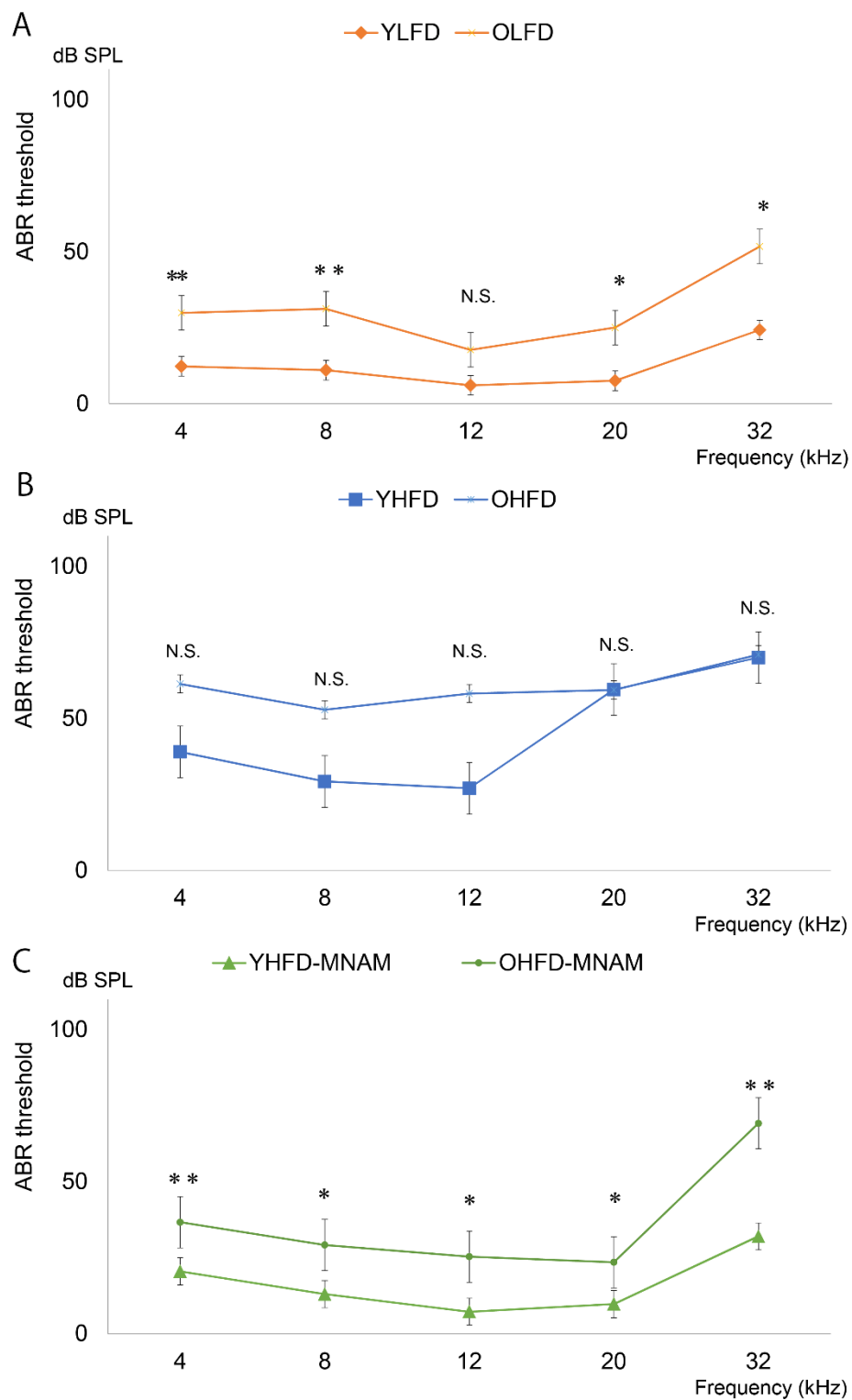
(A) Images of the animals in each group at 6 months after experiment initiation.

(B) Body weights of the mice at 3 and 6 months after experiment initiation. The body weights of the OHFD mice did not significantly increase compared with those of the LFD ($p = 0.10$) or the OHFD-MNAM mice ($p = 0.34$). The body weights of the OHFD mice significantly increased

compared with those of the OLF (p = 0.001) and the OHF-MNAM mice (p = 0.003; All groups, n = 5);

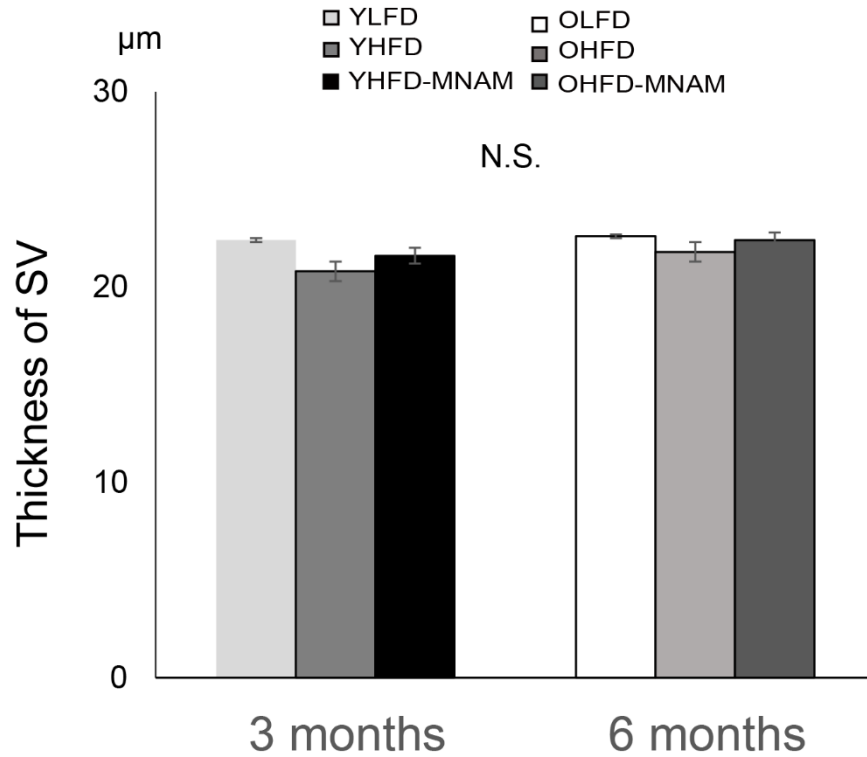
(C) Differences in the blood analysis results of all groups at 6 months after experiment initiation. The alanine aminotransferase (ALT), total cholesterol (TCHO), triglyceride (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), uric acid (UA), and glucose (GLU) levels in the OHF mice were significantly higher than those in the other two groups (vs OLF; p < 0.001, p = 0.001, p = 0.02, p = 0.003, p = 0.01, p < 0.001 and p = 0.01, vs OHF-MNAM; p < 0.001, p < 0.001, p = 0.02, p = 0.009, p = 0.007, p = 0.001 and p < 0.001). The creatinine (CRE) and inorganic phosphorus (IP) levels in the OLF mice were significantly higher than those in the other two groups (vs OHF; p = 0.03 and p = 0.003, vs OHF-MNAM; p = 0.008 and p = 0.01; All groups, n = 5; *: p < 0.05, **: p < 0.01, ***: p < 0.001)

[LFD, low-fat diet; HFD, high-fat diet; HFD-MNAM, high-fat diet plus 1% N¹-methylnicotinamide; OLF, low-fat diet-fed 6-month-old mice; OHF, high-fat diet-fed 6-month-old mice; OHF-MNAM, high-fat diet plus 1% N¹-methylnicotinamide-fed 6-month-old mice]



Supplementary Figure 2. Auditory brainstem responses (ABR)

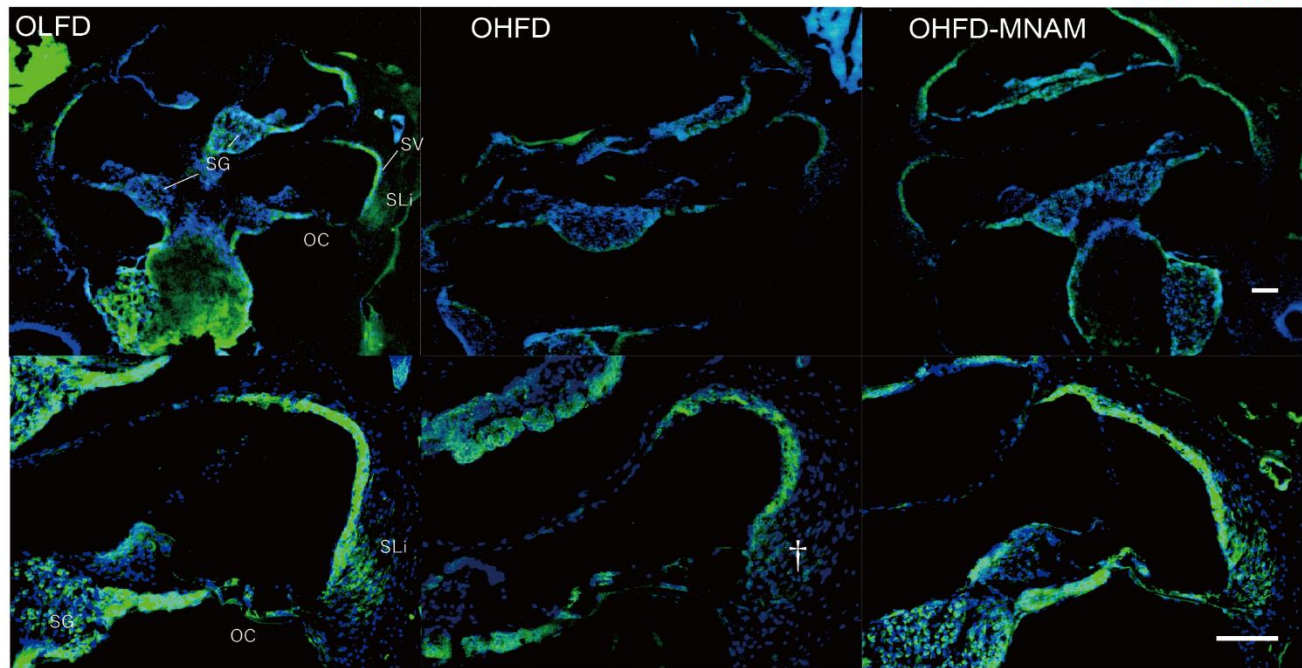
[YLFD, low-fat diet-fed 3-month-old mice; OLFD, low-fat diet-fed 6-month-old mice; YHFD, high-fat diet-fed 3-month-old mice; OHFD, high-fat diet-fed 6-month-old mice; YHFD-MNAM, high-fat diet plus 1% N¹-methylnicotinamide-fed 3-month-old mice; OHFD-MNAM, high-fat diet plus 1% N¹-methylnicotinamide-fed 6-month-old mice]



Supplementary Figure 3. Thickness of Stria vascularis (SV)

Analysis after immunostaining of SV revealed that there were no significant differences in thickness of SV among all groups.

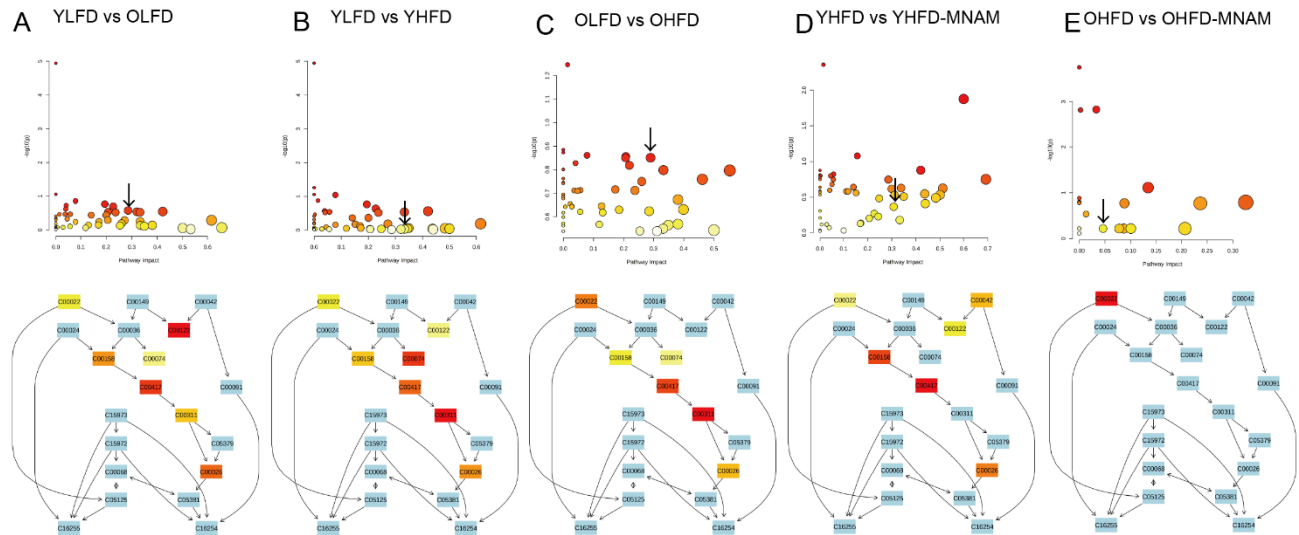
[SV, stria vascularis; YLFD, low-fat diet-fed 3-month-old mice; OLFD, low-fat diet-fed 6-month-old mice; YHFD, high-fat diet-fed 3-month-old mice; OHFD, high-fat diet-fed 6-month-old mice; YHFD-MNAM, high-fat diet plus 1% N¹-methylnicotinamide-fed 3-month-old mice; OHFD-MNAM, high-fat diet plus 1% N¹-methylnicotinamide-fed 6-month-old mice]



Supplementary Figure 4. Sirt 3 expression in the cochlea

SIRT3 staining (green) of the middle turn of the cochlea at 6 months after experiment initiation. The dagger symbol indicates a decrease in the SIRT3 protein expression in the SLi cells at 6 months after commencement of the experiment.

[SIRT3, Sirtuin 3; OCs, the organ of Corti; SGC, spiral ganglion cells; SLi, spiral ligament; SV, stria vascularis; OLFD, low-fat diet-fed 6-month-old mice; OHFD, high-fat diet-fed 6-month-old mice; OHFD-MNAM, high-fat diet plus 1% N¹-methylnicotinamide-fed 6-month-old mice]



Supplementary Figure 5. Metabolome pathway analysis and TCA cycle.

(A-E) Comparison between the cochlea of the YLFD and OLFD mice (A), the YLFD and YHFD mice (B), the OLFD and OHFD mice (C), the YHFD and YHFD-MNAM mice (D) and the OHFD and OHFD-MNAM mice (E). Arrow indicated TCA cycle.

C00022: Pyruvic acid, C00024: Acetyl-CoA, C00158: Citrate, C00149: Malate, C00036: Oxaloacetate, C00074: Phosphoenolpyruvate, C00417: Aconitate, C00042: Succinate, C00122: Fumarate, C00091: Succinyl-CoA, C00311: Isocitrate, C05379: Oxalosuccinate, C00026: 2-Oxoglutarate

[YLFD, low-fat diet-fed 3-month-old mice; OLFD, low-fat diet-fed 6-month-old mice; YHFD, high-fat diet-fed 3-month-old mice; OHFD, high-fat diet-fed 6-month-old mice; YHFD-MNAM, high-fat diet plus 1% N¹-methylnicotinamide-fed 3-month-old mice; OHFD-MNAM, high-fat diet plus 1% N¹-methylnicotinamide-fed 6-month-old mice]