**Supplemental Digital Content 4: Sensitivity analysis of fecal bile acid profile of the HC and NAFLD groups using the analysis of covariance model with BMI and HOMA-IR as covariates.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | P-value |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | HC | HC | MF |
| Fecal bile Acid(μmol/g) | HC |  | NAFLD (MF) |  |  | NAFLD (AF) |  | vs | vs | vs |
| (n = 88) | 　 | 　 | (n = 104) | 　 | 　 | (n = 95) | 　 | AF | MF | AF |
| *Total BA* | 1096.3  | ( | 731.4  | - | 1461.3  | ) |  | 1394.6  | ( | 1128.9  | - | 1660.4  | ) |  | 1813.8  | ( | 1493.1  | - | 2134.5  | ) | 0.03 | 0.4 | 0.1 |
|  | Conj BA | 5.3  | ( | -0.7  | - | 11.3  | ) |  | 6.3  | ( | 2.0  | - | 10.7  | ) |  | 17.9  | ( | 12.6  | - | 23.2  | ) | 0.02 | 1.0 | 0.002 |
|  | Unconj BA | 1091.0  | ( | 728.1  | - | 1453.9  | ) |  | 1388.3  | ( | 1124.1  | - | 1652.5  | ) |  | 1795.9  | ( | 1477.0  | - | 2114.7  | ) | 0.04 | 0.4 | 0.1 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Primary BA | 6.9  | ( | -28.3  | - | 42.2  | ) |  | 30.8  | ( | 5.2  | - | 56.4  | ) |  | 114.9  | ( | 84.0  | - | 145.9  | ) | 0.0003 | 0.6 | <.0001 |
|  | Secondary BA | 1089.4  | ( | 729.3  | - | 1449.5  | ) |  | 1363.8  | ( | 1101.7  | - | 1626.0  | ) |  | 1698.9  | ( | 1382.5  | - | 2015.3  | ) | 0.08 | 0.5 | 0.2 |
|  | S/P ratio | 0.2  | ( | -0.5  | - | 0.9  | ) |  | 0.2  | ( | -0.3  | - | 0.7  | ) |  | 0.5  | ( | -0.1  | - | 1.2  | ) | 0.8 | 1.0 | 0.7 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| *Total CA* | 3.6  | ( | -1.3  | - | 8.6  | ) |  | 8.3  | ( | 4.7  | - | 11.8  | ) |  | 23.1  | ( | 18.8  | - | 27.5  | ) | <.0001 | 0.3 | <.0001 |
|  | Unconj CA | 3.4  | ( | -0.7  | - | 7.4  | ) |  | 6.7  | ( | 3.8  | - | 9.7  | ) |  | 17.8  | ( | 14.2  | - | 21.3  | ) | <.0001 | 0.4 | <.0001 |
|  | Conj CA | 0.3  | ( | -1.7  | - | 2.3  | ) |  | 1.5  | ( | 0.1  | - | 2.9  | ) |  | 5.4  | ( | 3.6  | - | 7.1  | ) | 0.003 | 0.6 | 0.002 |
| *Total CDCA* | 3.3  | ( | -30.2  | - | 36.8  | ) |  | 22.5  | ( | -1.8  | - | 46.9  | ) |  | 91.8  | ( | 62.4  | - | 121.3  | ) | 0.002 | 0.7 | 0.001 |
|  | Unconj CDCA | 3.2  | ( | -30.1  | - | 36.6  | ) |  | 22.1  | ( | -2.1  | - | 46.4  | ) |  | 90.1  | ( | 60.8  | - | 119.3  | ) | 0.003 | 0.7 | 0.001 |
|  | Conj CDCA | 0.1  | ( | -0.8  | - | 0.9  | ) |  | 0.4  | ( | -0.2  | - | 1.0  | ) |  | 1.7  | ( | 1.0  | - | 2.5  | ) | 0.04 | 0.8 | 0.02 |
| *Total DCA* | 765.6  | ( | 497.2  | - | 1034.0  | ) |  | 821.2  | ( | 625.8  | - | 1016.6  | ) |  | 1126.8  | ( | 890.9  | - | 1362.7  | ) | 0.2 | 0.9 | 0.11 |
|  | Unconj DCA | 761.2  | ( | 494.4  | - | 1028.0  | ) |  | 817.4  | ( | 623.2  | - | 1011.7  | ) |  | 1117.1  | ( | 882.7  | - | 1351.5  | ) | 0.2 | 0.9 | 0.11 |
|  | Conj DCA | 4.4  | ( | 0.9  | - | 7.9  | ) |  | 3.8  | ( | 1.2  | - | 6.3  | ) |  | 9.7  | ( | 6.6  | - | 12.8  | ) | 0.1 | 1.0 | 0.01 |
| *Total LCA* | 312.1  | ( | 159.6  | - | 464.7  | ) |  | 510.0  | ( | 398.9  | - | 621.0  | ) |  | 509.6  | ( | 375.5  | - | 643.6  | ) | 0.2 | 0.1 | 1 |
|  | Unconj LCA | 312.1  | ( | 159.6  | - | 464.7  | ) |  | 509.9  | ( | 398.9  | - | 621.0  | ) |  | 509.5  | ( | 375.5  | - | 643.6  | ) | 0.2 | 0.1 | 1 |
|  | Conj LCA | 0.0  | ( | 0.0  | - | 0.0  | ) |  | 0.0  | ( | 0.0  | - | 0.0  | ) |  | 0.0  | ( | 0.0  | - | 0.1  | ) | 0.3 | 0.9 | 0.18 |
| *Total UDCA* | 0.9  | ( | -3.3  | - | 5.1  | ) |  | 6.8  | ( | 3.7  | - | 9.9  | ) |  | 15.5  | ( | 11.8  | - | 19.2  | ) | <.0001 | 0.1 | 0.001 |
|  | Unconj UDCA | 0.9  | ( | -3.2  | - | 5.1  | ) |  | 6.7  | ( | 3.7  | - | 9.7  | ) |  | 14.9  | ( | 11.3  | - | 18.6  | ) | <.0001 | 0.1 | 0.001 |
|  | Conj UDCA | -0.1  | ( | -0.4  | - | 0.3  | ) |  | 0.1  | ( | -0.1  | - | 0.4  | ) |  | 0.6  | ( | 0.3  | - | 0.9  | ) | 0.04 | 0.7 | 0.05 |
| *Total HDCA* | 10.7  | ( | -3.4  | - | 24.9  | ) |  | 25.9  | ( | 15.6  | - | 36.2  | ) |  | 46.9  | ( | 34.5  | - | 59.4  | ) | 0.003 | 0.2 | 0.02 |
|  | Unconj HDCA | 10.2  | ( | -3.9  | - | 24.3  | ) |  | 25.4  | ( | 15.1  | - | 35.7  | ) |  | 46.4  | ( | 34.0  | - | 58.9  | ) | 0.003 | 0.2 | 0.02 |
| 　 | Conj HDCA | 0.6  | ( | 0.5  | - | 0.6  | ) | 　 | 0.5  | ( | 0.4  | - | 0.6  | ) | 　 | 0.5  | ( | 0.4  | - | 0.6  | ) | 0.7 | 0.6 | 1.0 |

Data are presented as mean ± 95% confidence interval.

AF, advanced fibrosis; BA, bile acid; BMI, body mass index; CA, cholic acid; CDCA, chenodeoxycholic acid; Conj, conjugated; DCA, deoxycholic acid; GCA, glycocholic acid; HDCA, hyodeoxycholic acid; HC, healthy control; HOMA-IR, homeostasis model assessment of insulin resistance; LCA, lithocholic acid; MF, mild fibrosis; NAFLD, nonalcoholic fatty liver disease; S/P, Secondary/primary; UDCA, ursodeoxycholic acid; Unconj, unconjugated