

SUPPLEMENTARY DATA

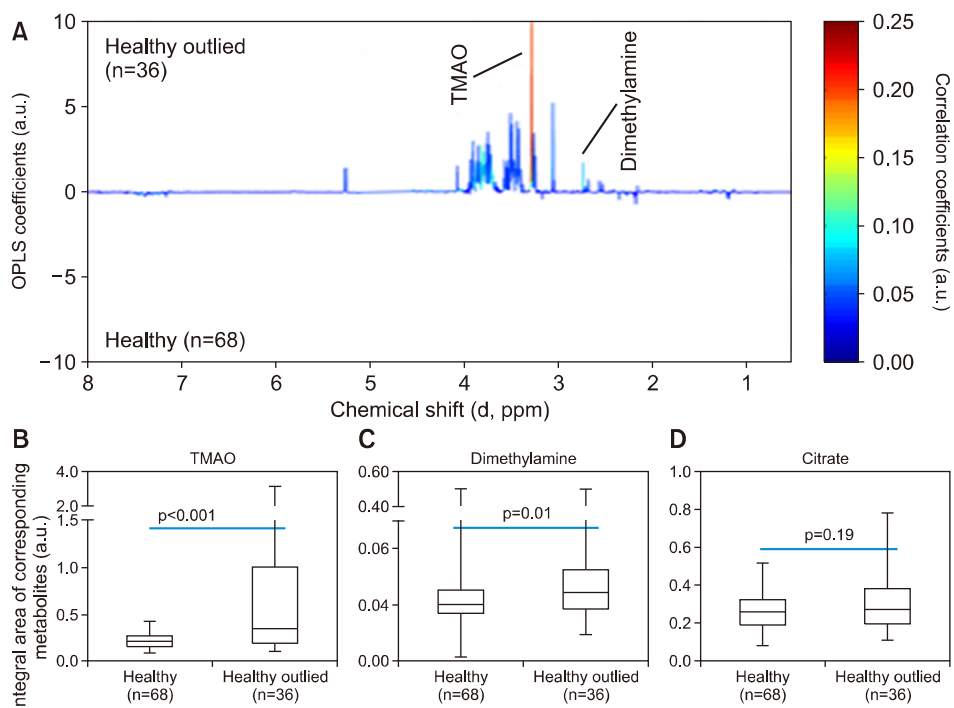
¹H NMR Spectroscopy of Urine Samples

Urine samples were prepared for NMR analysis by mixing 440 μ L of urine with 220 μ L of phosphate buffer (pH=7.0, which included 0.1% sodium azide to prevent bacterial contamination and 1 mM sodium 3-trimethylsilyl [2,2,3,3-²H₄]-1-propionate, TSP, in D₂O). The mixture was centrifuged to remove suspended particles. 550 μ L of the supernatant was transferred to an NMR tube. The ¹H NMR spectra of the urine samples were acquired using a Bruker 500 MHz spectrometer equipped with a cryogenic triple-resonance probe and an automatic sample changer (Bruker Biospin, Rheinstetten, Germany) operating at 500.13 MHz ¹H frequency and 300° K. The “noesyprsat” pulse sequence was used to suppress the water signal and to acquire the NMR spectrum. For each sample, 64 transients were collected into 32 K data points using a spectrum of 6,510.4 Hz with a relaxation delay of 2.0 s and a mixing time of 100 ms. A line-broadening function of 0.3 Hz was applied to all spectra prior to the Fourier transformation. The phase and base line of the resultant NMR spectra were manually corrected. The chemical shift was calibrated to δ 0.00 using the signal from the TSP with the phosphate buffer.

Supplementary Table 1. Sequences of the gene specific primers used for real-time PCR

Gene	Forward	Reverse
IL-6	TTCCATCCAGTTGCCTTCTTG	AGGTCTGTTGGGAGTGGTATC
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC
Nos2	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTAC
GAPDH	AACTTTGGCATTGTGGAAGG	GGATGCAGGGATGATGTTCT

PCR: polymerase chain reaction, IL: interleukin, Arg1: arginase 1, Nos2: nitric oxide synthase 2, GAPDH: glyceraldehyde 3-phosphate dehydrogenase.



Supplementary Figure 1. Variations in the levels of urinary TMAO and dimethylamine between healthy volunteers who were outliers and those who were not in the multivariate statistical analysis from Figure 1. (A), the OPLS loading plot between the two classes; (B), statistical analysis of urinary TMAO; (C), statistical analysis of urinary dimethylamine; (D), statistical analysis of urinary citrate. TMAO: trimethylamine-N-oxide, OPLS: orthogonal projections to the latent structures, a.u.: arbitrary unit.