Short-Chain Fatty Acid (SCFA) Data Processing and Quality Control

Platform and Instrumentation
Serum short-chain fatty acids (SCFAs; C2–C6) were quantified using a targeted LC-MS/MS assay (Metabolon Method TAM148) on an Agilent 1290 / SCIEX QTRAP 5500 system equipped with a C18 reverse-phase UHPLC column operated in negative electrospray ionization (ESI) mode.

Sample Preparation
Samples were spiked with stable isotope-labeled internal standards and subjected to protein precipitation using an organic solvent. After centrifugation, an aliquot of the supernatant was derivatized prior to injection. Internal standards were used to correct for extraction efficiency and instrument variability.

Quantification
Quantitation was performed using weighted linear least-squares regression based on fortified calibration standards prepared concurrently with study samples. Multiple reaction monitoring (MRM) transitions were used with analyte product ions measured against corresponding internal standards. Concentrations are reported in ng/mL. Raw data were collected using SCIEX Analyst 1.7.3 and processed using SCIEX OS-MQ v3.1.6.

Outlier Detection
Multivariate outlier detection was performed using principal component analysis (PCA). Features with more than 20% missing values were temporarily excluded; remaining missing values were imputed using a random forest–based method solely for this quality assessment step. Abundance values were log2-transformed, mean-centered, and scaled to unit variance prior to PCA. Mahalanobis distance was calculated in principal component space, and samples exceeding the 99th percentile of the chi-square distribution were classified as outliers and removed. A