Supplemental Digital Content 2

Sample processing

Fecal samples were weighed, combined with 500 µL of ice-cold PBS and vortexed for 4 minutes at maximum setting. Samples were then centrifuged at 6k RCF, at 4 °C for 15 minutes and the supernatant transferred to a new 1.5 mL tube. The fecal pellet was freeze dried to obtain dry weight. The supernatant was spun down at 14k RCF, at 4°C for 10 minutes and transferred to a 3 kDa filter to remove microbes and proteins (14k RCF, 4°C for 45 minutes). Filtrate was spiked with internal standard containing 5 mM 3-(trimethylsilyl)-1-propanesulfonic acid-d6 (DSS-d_6), NaN_3, and D_2O at 10% final volume and adjusted to pH 6.8 ± 0.1 using HCl and/or NaOH. Milk samples were centrifuged at 11k RCF, at 4°C for 5 minutes to separate the aqueous layer from other milk components. An aliquot of aqueous milk was transferred to a 3 kDa filter and centrifuged at 14k RCF, at 4 °C for 45 minutes. The resulting filtrate was spiked with the DSS-d_6 internal standard at 10% final volume and adjusted to pH 6.8 ± 0.1 using HCl and/or NaOH. Plasma samples were thawed and transferred to a 3 kDa filter and centrifuged for 60 minutes at 14k RCF and 4 °C. 207 µL of filtrate was transferred to a clean 1.5 mL tube and combined with 23 µL of the DSS-d_6 internal standard. Sample pH was adjusted to 6.8 ± 0.1 using HCl and/or NaOH.

^{1}H NMR Spectroscopy

Metabolite concentrations from feces, plasma, and milk samples were determined using ^{1}H nuclear magnetic resonance (NMR) spectroscopy using a NOESY ^{1}H pre-saturation experiment on a Bruker Avance 600 MHz spectrometer (Bruker BioSpin, Germany) as previously described in He et al. (1). Chenomx NMR Suite v8.4 (Chenomx Inc, Canada) was used to manually phase and correct baseline spectra. Each metabolite was assigned manually and quantified using Chenomx Profiler.

16S rRNA gene sequencing

Fecal microbiota were characterized by amplification and sequencing of the V4 region of the 16S rRNA gene as described in Huda et al. (2). The resulting reads were processed using the DADA2 pipeline in QIIME2 as previously described (3, 4). Samples with less than 1500 reads were removed from the amplicon sequence variant (ASV) table before continuing with the analysis.

Fecal calprotectin

Quantitation of fecal calprotectin was accomplished using BÜHLMANN fCAL® ELISA (BÜHLMANN Laboratories AG, Switzerland), according to the manufacturer's instructions. Absorbance was measured at a wavelength of 450 nm and blanked at 620 nm using a Tecan Sunrise (Tecan Trading AG, Switzerland) plate reader. Samples were measured in duplicate and the average OD was used for analysis.

1. He X, Mishchuk DO, Shah J, et al. Cross-talk between E. coli strains and a human colorectal adenocarcinoma-derived cell line. *Sci Rep* 2013; 3:1–10. https://doi.org/10.1038/srep03416

2. Huda MN, Lewis Z, Kalanetra KM, et al. Stool microbiota and vaccine responses of infants. *Pediatrics* 2014; 134:e362. https://doi.org/10.1542/peds.2013-3937

3. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010; 7:335–336. https://doi.org/10.1038/nmeth.f.303

4. Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016; 13:581–583. https://doi.org/10.1038/nmeth.3869