Supplemental Methods

Sample preparation. Liquid chromatography (LC)-Mass spectroscopy (MS)/Mass spectroscopy (MS) analysis of short chain fatty acids (SCFAs) was performed according to a modified version of the protocol described by Han et al. (1). For water or other non-protein-containing aqueous solutions, 50 uL of aqueous sample for SCFA assay was combined with 50 uL of acetonitrile in a 2mL screw-top glass vial. For the plasma sample, 50 uL of plasma were vortexed with 100 uL of acetonitrile, then centrifuged at 15,000 xg for 5 minutes to precipitate proteins, and 100 uL of supernatant were transferred to a 2mL glass vial. To both types of sample, 15 µL of 200 mM 3-nitrophenylhydrazine (3-NPH, Sigma-Aldrich) in 50/50 acetonitrile/water (LC-MS grade from freshly opened bottles) and 15 µL of 120 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, Sigma-Aldrich) in 47/47/6 acetonitrile/water/pyridine were added. Sample vials were capped, vortexed, and allowed to react in a 40°C warming oven for 30 minutes. Samples were then diluted to 2mL with 50/50 acetonitrile/water (for aqueous test samples, water for this dilution was derived from same source as the sample water) and combined at a 1:1 ratio with a solution of ^{13}C-labeled SCFA standards prepared as described in Han et al.

LC-MS analysis. Samples were analyzed using an Agilent 6410 triple quadrupole LC-MS system. The chromatographic column was a Waters HSS T3, 2.1 mm x 100 mm, 1.7 µm particle size. Mobile phase A was 0.1% formic acid in water; mobile phase B was 0.1% formic acid in methanol. The gradient was as follows: linear ramp from 15% to 80% B from 0-12 min; step to 100%B from 12-12.1 min; hold 100%B from 12.1-16 min; step to 15%B from 16-16.1 min; hold 15%B from 16.1-20min. The injection volume was 5uL and the column temperature was 55 °C. MS parameters were as follows: gas temp 325 °C, gas flow 10 L/min, nebulizer 40 psi, capillary voltage 4000V, scan type MRM, negative ion mode, delta EMV 600. MRM parameters were as indicated in Table S1.

Quantitation was performed using MassHunter Quantitative Analysis software (Agilent Technologies, version 8.0) by measuring the ratio of peak area of an 3-NPH derivatized SCFA species to its ^{13}C-labeled internal standard. Linear standard curves were generated using dilutions of Sigma-Aldrich Volatile Fatty Acid Mix (1µM - 1000µM) to estimate SCFA concentrations in the samples.

Scan segments

Compound Group	Compound Name	ISTD?	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
acetate	C2-3NPH	☐	194.1	Unit	137.1	Unit	75	110	18	7	Negative
acetate IS	C2-3NPH_13C	☐	200.1	Unit	143.1	Unit	75	110	18	7	Negative
propionate	C3-3NPH	☐	208.1	Unit	137.1	Unit	75	110	18	7	Negative
propionate IS	C3-3NPH_13C	☐	214.1	Unit	143.1	Unit	75	110	18	7	Negative
butyrate	C4-3NPH	☐	222.1	Unit	137.1	Unit	75	110	18	7	Negative
butyrate IS	C4-3NPH_13C	☐	228.1	Unit	143.1	Unit	75	110	18	7	Negative
valerate	C5-3NPH	☐	236.1	Unit	137.1	Unit	75	130	18	7	Negative
valerate IS	C5-3NPH_13C	☐	242.1	Unit	143.1	Unit	75	130	18	7	Negative
hexanoate	C6-3NPH	☐	250.1	Unit	137.1	Unit	75	130	18	7	Negative
hexaoate IS	C6-3NPH_13C	☐	256.1	Unit	143.1	Unit	75	130	18	7	Negative
heptanoate	C7-3NPH	☐	264.1	Unit	137.1	Unit	75	130	18	7	Negative
heptanoate IS	C7-3NPH_13C	☐	270.1	Unit	143.1	Unit	75	130	18	7	Negative

Table S1. MRM scan parameters for LC-MS/MS SCFA assay on Agilent 6410 LC-MS

1. Han J, Lin K, Sequeira C, Borchers CH. 94 An isotope-labeled chemical derivatization method for the quantitation of short-chain fatty acids in human feces by liquid chromatography-tandem mass spectrometry. Anal Chim Acta. 2015 Jan 7;854:86-

Supplemental Results

An LC-MS/MS assay was used as a secondary confirmation of our GC-MS data that indicated substantial background levels of SCFA in water samples from various sources. As illustrated in Figure S1, an LC-MS total ion chromatogram (from summed MRM data for all SCFA species) shows clear, detectable peaks for SCFA species ranging from acetate to heptanoate in a solution of authentic standards, a representative water sample, and a porcine portal vein plasma sample (positive control). Figure S2 illustrates LC-MS estimated SCFA concentrations in water samples from a variety of sources as well as a control portal vein plasma sample. Although the profile of SCFAs differed somewhat from those detected by GC-MS, the data were consistent in that pervasive background peaks representing SCFA contamination were detected in all evaluated aqueous samples. The origin of this contamination remains unknown Taken together, these results suggest accurate quantitation of low-level SCFAs in respiratory samples such as exhaled breath condensate is challenging at best, should be carefully managed with appropriate negative and positive controls, and should be interpreted in the light of the possibility of interference from background contamination.

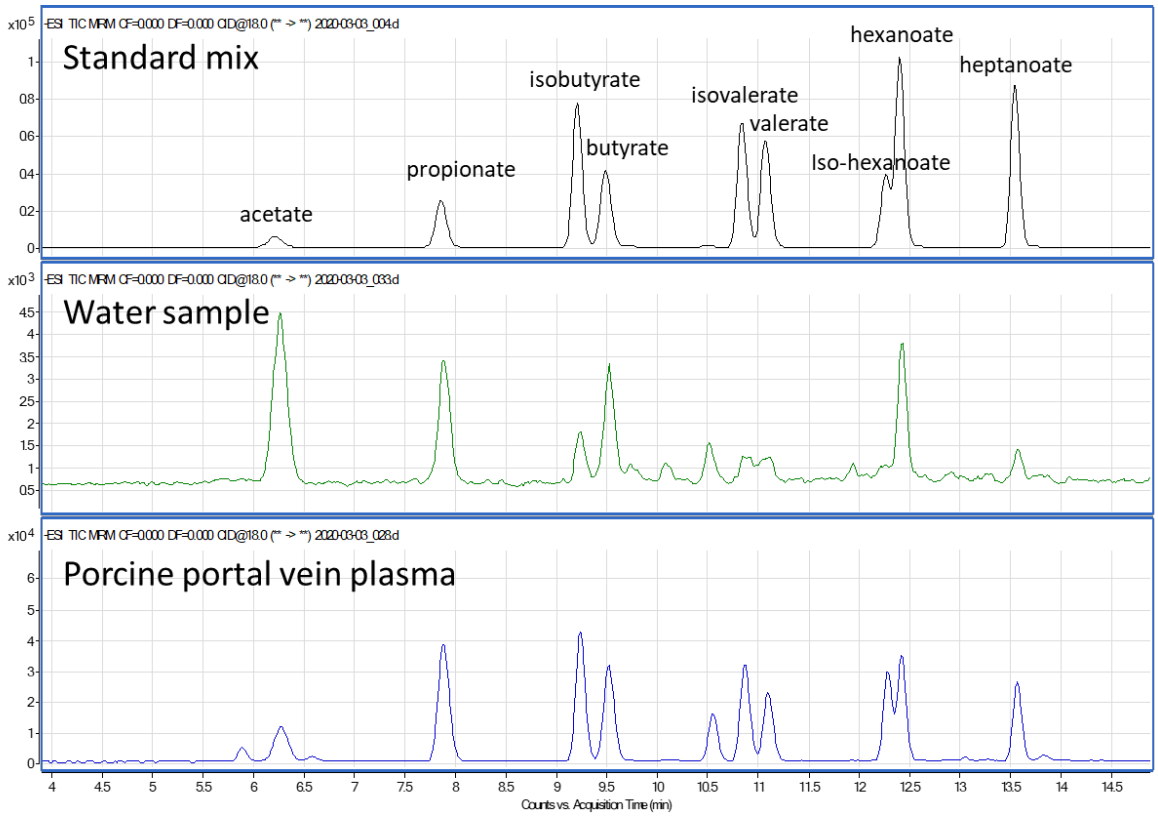

Figure S1. Total ion current chromatograms (summed from all MRM transitions) from LC-MS/MS analysis of a representative 3-NPH derivatized short chain fatty acid (SCFA) standard mix, a water sample, and a porcine portal vein plasma sample. The water sample is shown without signal from internal standard (C13) peaks to more clearly illustrate true background SCFA levels.

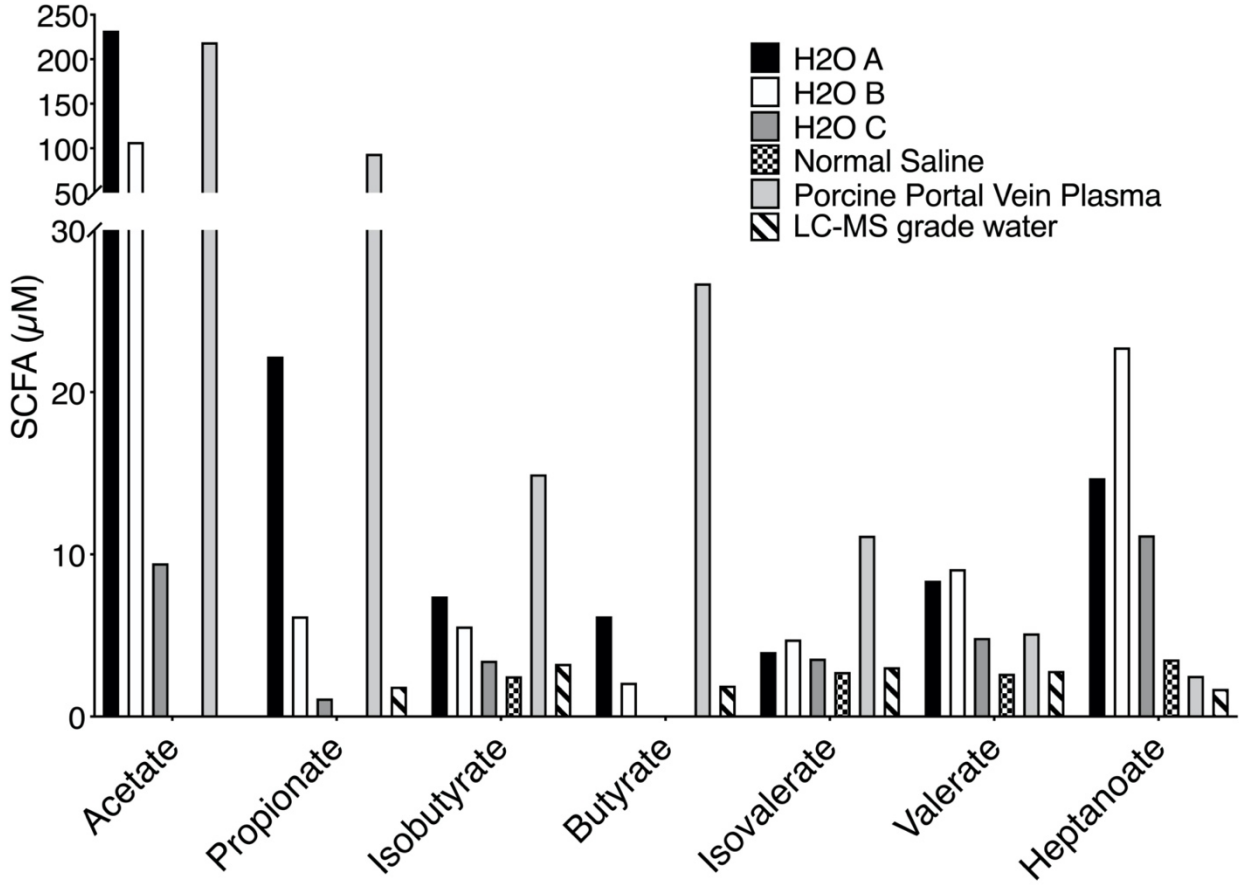

Figure S2. Short chain fatty acid (SCFA) concentrations in representative water samples and a porcine portal vein plasma sample measured by an LC-MS/MS-based assay as described in the supplemental methods. **H_2O A**: Millipore Milli-Q Academic deionizer, with a nominal resistance reading of 18.2 megaohms at 25°C that uses a Q gard 2 purification pack and a Quantum EX ultrapure cartridge with a 0.22 µm membrane filter; **H_2O B**: a newly opened bottle of HPLC-grade water (Fisher W5-4 HPLC grade, submicron 0.5um filtered); **H_2O C**: lab #2 Milli-Q Biocel A10 deionizer with a nominal resistance reading of 18.0 M-ohms.cm at 25°C that uses a Q gard 2 purification pack and a Quantum EZ ultrapure cartridge with a 0.22 µm membrane filter; **normal saline**: a sample from a newly opened bottle of normal saline from the bronchoscopy suite. A portal vein plasma sample acquired from a post-mortem swine at the termination of an unrelated experiment was used as a positive control. The LC-MS grade water is a representative sample from that which was used in the sample preparation process described in the supplemental methods.