Gut microbiota-targeted dietary supplementation with fermentable fibers and polyphenols prevents hypobaric hypoxia-induced increases in intestinal permeability

Karl, J. P. ORCID: https://orcid.org/0000-0002-5871-2241, Fagnant, H. S., Radcliffe, P. N., Wilson, M., Karis, A. J., Sayers, B., Wijeyesekera, A. ORCID: https://orcid.org/0000-0001-6151-5065, Gibson, G. R. ORCID: https://orcid.org/0000-0002-0566-0476, Lieberman, H. R. ORCID: https://orcid.org/0000-0002-1519-0156, Giles, G. E. ORCID: https://orcid.org/0000-0003-1340-1703 and Soares, J. W. ORCID: https://orcid.org/0009-0006-1130-3420 (2025) Gut microbiota-targeted dietary supplementation with fermentable fibers and polyphenols prevents hypobaric hypoxia-induced increases in intestinal permeability. American Journal of Physiology: regulatory, integrative and comparative physiology, 329 (3). R378-R399. ISSN 1522-1490 doi: 10.1152/ajpregu.00109.2025 Available at https://centaur.reading.ac.uk/123823/

Am J Physiol Regul Integr Comp Physiol 329: R378–R399, 2025.
First published July 24, 2025; doi:10.1152/ajpregu.00109.2025

american physiological society

AMERICAN JOURNAL OF PHYSIOLOGY
REGULATORY, INTEGRATIVE AND COMPARATIVE PHYSIOLOGY

RESEARCH ARTICLE

Physiological Adaptations to Environmental Stressors and Challenging Conditions

Gut microbiota-targeted dietary supplementation with fermentable fibers and polyphenols prevents hypobaric hypoxia-induced increases in intestinal permeability

J. Philip Karl,[1] Heather S. Fagnant,[1] Patrick N. Radcliffe,[1,2] Marques Wilson,[1] Anthony J. Karis,[1] Briony Sayers,[3] Anisha Wijeyesekera,[3] Glenn R. Gibson,[3] Harris R. Lieberman,[1] Grace E. Giles,[4] and Jason W. Soares[5]

[1]*Military Nutrition Division, US Army Research Institute of Environmental Medicine, Natick, Massachusetts, United States;* [2]*Oak Ridge Institute for Science and Education, Oak Ridge, Tennessee, United States;* [3]*Food and Microbial Sciences Unit, Food and Nutritional Sciences, The University of Reading, Reading, United Kingdom;* [4]*Cognitive Science and Applications Branch, U.S. Army Combat Capabilities Development Command Soldier Center, Natick, Massachusetts, United States; and* [5]*Soldier Effectiveness Directorate, US Army Combat Capabilities Development Command Soldier Center, Natick, Massachusetts, United States*

Abstract

Interactions between the gut microbiota and intestinal barrier may contribute to the pathophysiology of high-altitude illnesses. This study aimed to determine the effects of targeting the gut microbiota using dietary supplementation with a blend of fermentable fibers and polyphenol sources on gut microbiota composition, fecal short-chain fatty acids (SCFAs), and intestinal function and permeability during hypobaric hypoxia exposure. Healthy adults participated in a randomized, placebo-controlled, crossover study. Food products containing oligofructose-enriched inulin, galacto-oligosaccharide, high-amylose corn starch, cocoa, green tea and cranberry extracts, and blueberry powder (FP) or maltodextrin (placebo; PL) were consumed daily during three 2-wk phases separated by a ≥1-wk washout. During the final 36 h of each phase, participants resided in a hypobaric chamber simulating low (LA; 500 m) or high (HA; 4,300 m) altitude creating three experimental conditions: PL + LA, PL + HA, and FP + HA. Twenty-six participants completed ≥1 study phase and 13 [12 male; 21 ± 3 yr; body mass index (BMI) 25.4 ± 2.4 kg/m^2] completed all three phases. Results demonstrated that FP mitigated hypoxia-induced increases in intestinal permeability within the small intestine and proximal colon while increasing *Bifidobacterium* relative abundance and decreasing gut microbiota α-diversity and colonic pH. Higher *Bifidobacterium* relative abundance and lower colonic pH were associated with greater reductions in intestinal permeability. However, FP did not alter fecal SCFA concentrations and increased gastrointestinal symptoms and altitude sickness during hypobaric hypoxia exposure. Findings suggest that targeting the gut microbiota with a combination of fermentable fibers and polyphenols can prevent hypobaric hypoxia-induced increases in intestinal permeability but that benefit does translate into a reduction in altitude illness symptoms.

NEW & NOTEWORTHY Dietary supplementation targeting the gut microbiota may provide novel approaches to improving physiologic responses to environmental stressors such as those experienced during sojourn at high terrestrial altitudes. This study demonstrated that gut microbiota-targeted dietary supplementation using a blend of fermentable fibers and polyphenol sources can prevent hypobaric hypoxia-induced decrements in intestinal permeability. Findings support the emerging concept that the gut microbiota may be a modifiable factor influencing physiologic responses in austere environments.

acute mountain sickness; gut health; inflammation; phenolic; prebiotic

INTRODUCTION

The gut microbiota is increasingly considered a potential mediator of host responses to environmental stress (1–3). One such stress is sojourn at high terrestrial altitudes (4). As altitude increases, the partial pressure of oxygen in the atmosphere decreases, resulting in a hypobaric hypoxia that reduces transport and delivery of oxygen to body tissues. In rodent models, exposure to real or simulated altitudes of 3,800–4,700 m can cause oxidative stress, inflammation,

Correspondence: J. P. Karl (James.p.karl.civ@health.mil).
Submitted 29 April 2025 / Revised 21 June 2025 / Accepted 17 July 2025

cellular atrophy, and dysfunction within the intestinal barrier (5–10). Similarly, human studies have reported endoscopic lesions and increases in markers of intestinal barrier damage, intestinal permeability, and systemic inflammation within hours-to-days of exposure to real or simulated altitudes of 4,000–4,600 m, particularly when coupled with exercise (11–15). Potential consequences of this hypoxia-induced intestinal barrier damage include dysbiosis within the gut microbiota (1), translocation of microbiota-derived antigens such as lipopolysaccharide (LPS) from the gut lumen into circulation, and subsequent systemic inflammatory responses (16). Though speculative, such responses may, in turn, contribute to the development of altitude illnesses (17, 18), including acute mountain sickness (AMS), which range in severity from nuisance to life-threatening and are commonly characterized by a constellation of symptoms that include several gastrointestinal issues (19, 20).

The gut microbiota modulates intestinal barrier function through various mechanisms including direct interactions with immune cells and production of barrier-protecting small molecules. Microbiota-derived short-chain fatty acids (SCFAs) may be particularly important due to their influence on immune responses, mucus secretion, inflammation, gene expression, cellular metabolism, and oxidative state within the gastrointestinal tract (21, 22). Notably, gut microbiota depletion prevented hypoxia-induced intestinal barrier dysfunction in mice exposed to 4,000 m simulated altitude (10), and fecal microbiota transplantation from mice exposed to simulated altitudes of ≥4,000 m caused intestinal damage and inflammation in recipient animals (8, 10). Furthermore, targeting the murine gut microbiota using probiotics and synbiotics has been shown to reduce damage and immune dysregulation within the intestinal barrier at simulated altitudes of 3,500–7,600 m (9, 23–25). Though human studies are scarce, some have reported increased abundances of LPS-producing gut bacteria and decreased abundances of beneficial, intestinal barrier protecting genera such as *Bifidobacterium* and *Lactobacillus*, concomitant with increases in inflammation and intestinal permeability in sojourners ascending to ≥4,300 m (14, 26). Furthermore, inverse associations between fecal SCFA concentrations and intestinal permeability have been reported during sojourn at 4,300 m (2), and SCFA treatment can temporarily enhance intestinal barrier function in in vitro enteroid models of hypoxia (27). Collectively, this evidence suggests that the gut microbiota may contribute to hypoxia-induced intestinal barrier damage and could be a novel intervention target for reducing intestinal permeability and related sequelae during high altitude sojourn.

Dietary supplementation with fermentable fibers and polyphenolic compounds provides one possible approach to favorably modulating host-gut microbiota interactions impacting intestinal barrier function. Fermentable fibers include certain nondigestible poly- and oligosaccharides such as high amylose maize starch, inulin, oligofructose, and galacto-oligosaccharides (28). Collectively, these fibers promote the growth of beneficial bacteria such as *Bifidobacterium* spp. and *Lactobacillus* spp. (29) and are metabolized by cross-feeding consortia of gut microbes into the SCFA acetate, propionate, and butyrate (30). Beneficial health effects of these SCFA, butyrate in particular, include reducing colonic pH and inflammation, providing an energy source for intestinal epithelial cells, and stimulating epithelial cell growth and intestinal barrier integrity (31, 32). Polyphenols are a diverse class of compounds ubiquitous in plant foods that have poor bioavailability in the small intestine (33). However, polyphenols are transformed into a variety of bioavailable compounds by the gut microbiota (34), and some may be considered candidate prebiotics (28). Those include polyphenols derived from cocoa, green tea, blueberry, and cranberry, which promote the growth of beneficial bacteria (35), deter the growth of proinflammatory microbes, and have anti-inflammatory, antioxidative, and antimicrobial properties (36) that benefit gut barrier function (37).

Consuming various fermentable fibers and polyphenolic compounds has been shown to promote intestinal barrier function in in vitro and animal models (37–39). Some, but not all, human studies have reported similar effects particularly when intestinal barrier dysfunction is induced experimentally or associated with a disease state (37, 38). The extent to which these gut microbiota-targeted dietary interventions attenuate intestinal barrier damage in healthy individuals exposed to hypoxic stress such as that experienced during high altitude sojourn is unknown. To address that gap, our team developed a dietary intervention containing a blend of three fermentable fiber and four polyphenol sources: oligofructose-enriched inulin, galacto-oligosaccharide, high-amylose corn starch, cocoa, green tea and cranberry extracts, and blueberry powder. Using an in vitro large intestine fermentation model, we demonstrated independent beneficial effects of the blends on the gut microbiota that were additive when combined and resulted in increased *Bifidobacterium* and *Lactobacillus* growth, SCFA production, and antioxidant potential (40). Herein, we report the results of a randomized, placebo-controlled crossover trial designed to determine the impact of the fermentable fiber and polyphenol blend on intestinal permeability during short-term exposure to simulated 4,300 m altitude. Secondary outcomes included gut microbiota composition, fecal SCFA, intestinal transit time and pH, circulating markers of intestinal barrier damage and inflammation, urinary metabolic profiles, and subjective measures of AMS and gastrointestinal symptoms. We hypothesized that acute exposure to simulated 4,300 m altitude would increase intestinal permeability and that fermentable fiber and polyphenol supplementation would mitigate that response while favorably modulating the gut microbiota and gastrointestinal environment as indicated by increases in *Bifidobacterium*, *Lactobacillus*, and fecal SCFA.

MATERIALS AND METHODS

Study Population

Generally healthy, physically active adults 18–39 yr of age were recruited from the U.S. Army Research Institute of Environmental Medicine, Natick, MA, and surrounding area between June 2019 and November 2022. Active-duty military personnel 17 yr of age were also allowed to participate in accordance with U.S. Department of Defense Instruction 3216.02. All participants met U.S. Army standards for physical fitness and body composition. Exclusion criteria included pregnancy, any use of oral antibiotics or colonoscopy within

3 mo of study participation, a history of gastrointestinal disease or gastrointestinal surgery, regular use of medications, metabolic or cardiovascular disease, swallowing disorder, sleeping disorder, musculoskeletal injury, anemia or sickle cell trait, and history of high altitude pulmonary or cerebral edema. Potential participants were also excluded if following a vegetarian or vegan diet, if born at an altitude of >2,100 m or if they had resided in or visited regions >1,200 m elevation for five or more days within the previous 2 mo. Participants were instructed to discontinue use of any probiotic, prebiotic, or other dietary supplement at least 2 wk before beginning study participation, to not consume probiotic-supplemented foods throughout study participation, and to avoid alcohol, nicotine-containing products and caffeine during controlled diet periods.

The study was reviewed and approved by the Headquarters US Army Medical Research and Development Command Institutional Review Board (approval number: M-10783). Investigators adhered to the policies regarding the protection of human subjects as prescribed in Army Regulation 70-25, and the research was conducted in adherence with the provisions of 32 CFR Part 219. All participants provided written informed consent before participation and were instructed that they could withdraw their consent at any time for any reason. The trial was registered on www.clinicaltrials.gov as NCT04111263.

Study Design

The study followed a 3-phase, randomized, placebo-controlled, crossover design (Fig. 1*A*). During baseline testing, participants completed a peak oxygen uptake ($\dot{V}O_{2peak}$) test, a 3-day food and activity record, and two steady-state exercise familiarization sessions. Thereafter, each study phase consisted of a 14-day diet supplementation period with snack bars that were custom-made for the study, and a ≥1 wk washout. During *days 1–7* of each phase, participants met with study staff each weekday to consume snack bars and complete questionnaires. During *days 8–12* of each phase, participants met with study staff daily to receive a provided diet and completed body weight measurements and questionnaires. During *days 13–14* of each phase, participants continued the provided diet while residing in a hypobaric chamber for 36 h under environmental conditions (18°C–22°C, 20%–50% relative humidity) mimicking either low altitude (500 m; barometric pressure = 720 mmHg; LA) or high altitude (4,300 m; 460 mmHg; HA). A 36-h chamber residence period was selected to provide time for a 24-h urine collection, to allow a brief adaptation phase before that urine collection and to capture the peak time course of AMS severity (commonly within 24–48 h of ascent) while also balancing logistical feasibility and volunteer burden. The altitude was selected to be consistent with our previous work demonstrating an increase in intestinal permeability 24 h after ascent to 4,300 m (14). During chamber residence, participants provided blood, urine, and stool samples; engaged in prescribed exercise; completed gastrointestinal transit and pH testing; and were administered several questionnaires (Fig. 1*B*). Following chamber residence, the provided diet was continued for up to 2 days until a stool sample was collected on the second day.

Three different treatments were tested during the study: dietary supplementation with fiber and polyphenols (FP) at HA (FP + HA) and placebo supplementation at LA (PL + LA) and HA (PL + HA). A fully balanced 2 × 2 design including a FP + LA condition was not used due to logistical constraints that limited duration of volunteer availability and the expectation that detecting changes in markers of intestinal barrier function in unstressed healthy adults with presumably normal barrier function would be unlikely. Participants were randomly assigned in groups of 2–4 participants to complete all three treatments in one of three sequence orders using a random number generator. Participants were blinded to the treatment. Attempts were made to blind participants to the altitude by not informing them of the environmental condition being tested, and having staff simulate high altitude testing (e.g., wearing nasal cannulas, keeping chamber doors locked) throughout all exposures. Study staff were aware of the altitude but were blinded to treatment during the HA conditions.

Diet and Intervention

Three separate snack bars, one intervention and two placebo products, were custom-made for the study by the US Army Combat Capabilities Command Soldier Center Combat Feeding Division, Natick, MA (see Supplemental Table S1 for ingredients list). The intervention snack bar contained a blend of fermentable fiber and polyphenol sources (per 50 g snack bar): 1.38 g Orafti Synergy1 (93.2% oligofructose enriched inulin by dry wt.; Beneo GmbH, Mannheim, Germany), 1.62 g Bimuno-galactooligosaccharides (85% B-GOS wt/wt; Clasado Biosciences, Reading, UK), 8.95 g Hi-Maize 260 (59% resistant starch wt/wt; Ingredion, Inc., Bridgewater, New Jersey), 300 mg cocoa seed extract (CocoActiv; 45.1% ± 2.6% total phenolics dry wt. catechin equivalents; Naturex; Avignon, France), 3.15 g wild blueberry powder (4.0% ± 0.1% total phenolics dry wt. gallic acid equivalents [GAE]; Naturex), 220 mg cranberry extract (Cystricran; 57.2% ± 1.3% total phenolics dry wt. GAE; Naturex), and 125 mg green tea leaf extract (100% total phenolics dry wt. GAE; Naturex). Placebo products substituted maltodextrin for the fiber and polyphenol sources. The PL + LA product differed slightly in color from the PL + HA and FP + HA products. However, taste, energy, and macronutrient content were similar between all products (Table 1).

During *days 1–7* of each phase, participants consumed two snack bars daily during breakfast. During *days 8–14*, participants consumed four snack bars daily split between breakfast and lunch (Fig. 1*B*). As such, participants were consuming 5.0 g/day oligofructose enriched inulin, 5.5 g/day Bimuno-GOS, 21.1 g/day resistant starch, and ∼2.1 g/day total phenolics from the intervention bars during the second week of FP + HA. The types and doses of fibers provided were intended to be consistent with those reported to favorably alter gut microbiota composition and increase SCFA production (29) while extending fermentation into more distal regions of the colon (41) and minimizing gastrointestinal side effects (42). The polyphenol sources used were selected to provide a broad representation of different polyphenol families found in plant-based foods and at an intake level

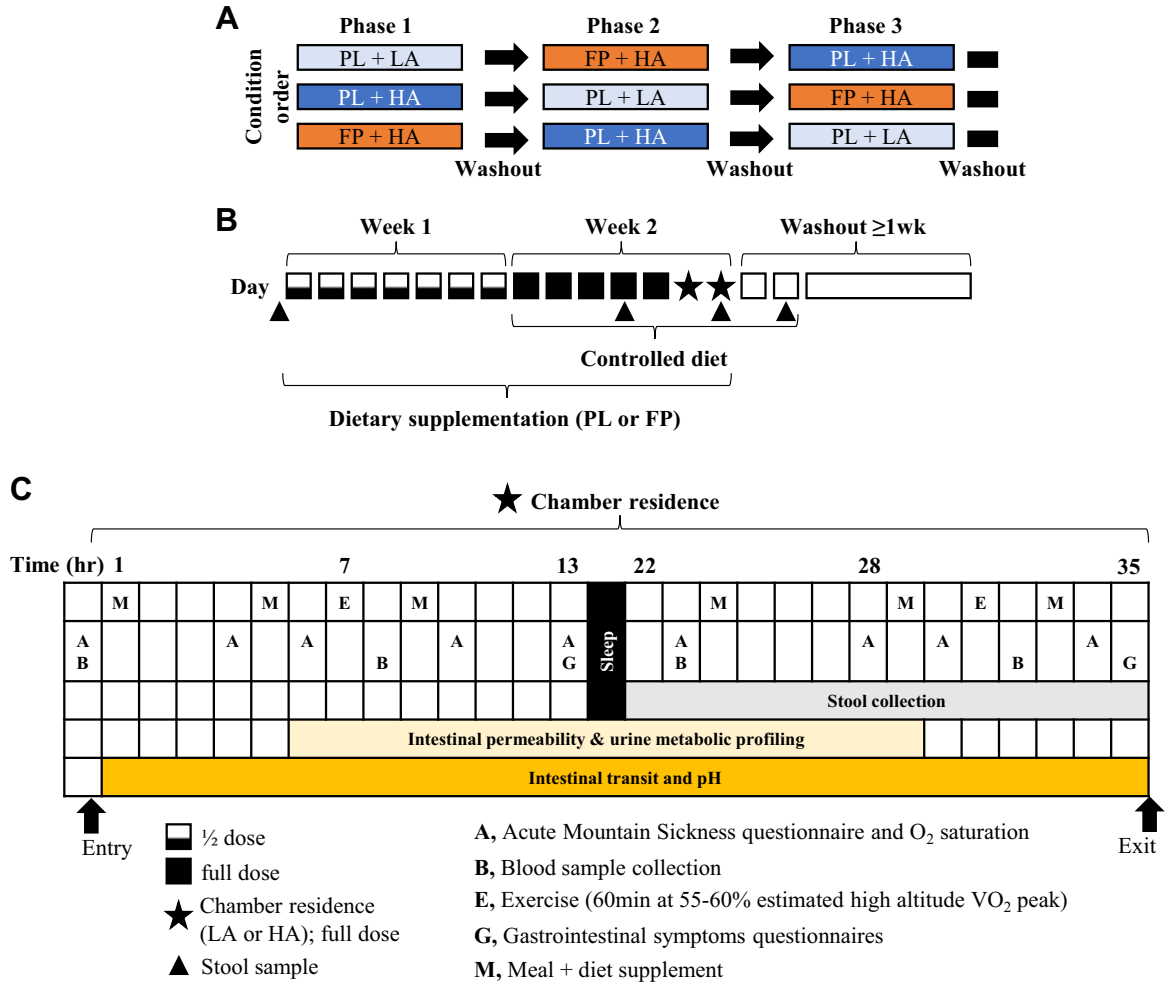

Figure 1. Study design. *A*: three-phase crossover trial wherein participants were randomly assigned to one of three condition orders. Each condition included dietary supplementation with a fermentable fiber and polyphenol-rich intervention (FP) or matched placebo (PL) before and during 36-h residence in a hypobaric chamber under low-altitude (LA, 500 m) or high-altitude (HA, 4,300 m) conditions. *B*: timeline within each study phase. During *week 1*, participant diets were supplemented with 2 snack bars daily. During *week 2*, participant diets were provided and included 4 supplemental snack bars daily. *C*: timeline for the 36-h chamber residence periods.

consistent with the upper end of habitual dietary intakes (43).

During *days 8–14*, participants consumed study-provided, body weight-maintaining diets (Fig. 1*B*). Weight maintenance energy needs were estimated using a combination of 3-day food and activity records administered during baseline testing and prediction equations [Harris-Benedict (44) and Mifflin-St. Jeor (45)]. All food record instruction and reviews were conducted by research dietitians who used Food Processor SQL v. 11.11 (ESHA Research; Salem, OR) to determine habitual intakes. Body weight was measured during each laboratory visit and energy intakes adjusted during *Phase 1* if consistent increases or decreases were observed. The diet provided during *Phases 2* and *3* was then matched to the final diet provided during *Phase 1*. Provided diets comprised primarily of products within the U.S. Armed Services Meals, Ready-to-Eat ration, and were designed to provide 55 ± 2%, 30 ± 2%, and 15 ± 2% total energy from carbohydrate, fat, and protein, respectively, and 9 ± 2 g total fiber/1,000 kcal. Consumption of breakfast, lunch, and the intervention snack bars was monitored by study staff. For snacks and dinners consumed off-site, participants returned food wrappers, containers, and any uneaten items. Foods and beverages were weighed before and after consumption. Actual energy and macronutrient intakes were then determined using ESHA Food Processor SQL (RRID:SCR_022528) and a database containing chemically analyzed energy and nutrient values for the ration products.

Exercise Testing

Peak oxygen uptake ($\dot{V}O_{2peak}$) was measured under normobaric normoxic conditions using a treadmill protocol in which participants ran at a fixed pace while the incline was progressively increased at 2-min intervals until volitional exhaustion. High-altitude $\dot{V}O_{2peak}$ was then estimated as 70% of measured normoxic $\dot{V}O_{2peak}$ (46). On both days of each chamber residence period participants completed 60 min of exercise on a treadmill at 55%–60% of estimated high altitude $\dot{V}O_{2peak}$ (~38%–42% of measured normoxic $\dot{V}O_{2peak}$; Fig. 1*C*). The walking pace and incline needed to elicit the prescribed intensity was determined during

Table 1. *Energy and macronutrient content of the experimental products (per 100 g)*

	FP	PL + HA	PL + LA
Serving size per bar, g	50	42	42
Energy, kcal[a]	401	397	407
Carbohydrate, g[a]	75	73	75
Fat, g[a]	10	10	10
Protein, g[a]	3	4	4
Total fiber, g[b,c]	19.6 ± 0.5	2.8	2.8
Insoluble fiber, g[b,c]	11.7 ± 0.2	1.1	0.9
Resistant starch, g[b,d]	7.3 ± 0.2	<2.0	<2.0
Soluble fiber, g[c]	7.9 ± 0.3	1.7	2.0
Fructan, g[e]	2.3 ± 0.2	<0.5	<0.5
Total phenolic content[f]			
CocoaActiv, mg CE	271		
Green tea extract, mg GAE	263		
Cystrican, mg GAE	250		
Wild blueberry powder, mg GAE	251		

Empty cells indiacte not measured. CE, catechin equivalents; FP, fiber and polyphenol intervention; GAE, gallic acid equivalents; HA, high altitude; LA, low altitude; PL, placebo. [a]Data are results of the chemical analysis of a composite of 3–4 bars for each product type; [b]Data are means ± SD from chemical analysis of 3 bars for the FP and the value from chemical analysis of a composite of 3–4 bars for PL products; [c]Determined by Association of Official Agricultural Chemists (AOAC) method 2011.25; [d]Determined by AOAC method 2002.02. Detection limit is 2.00 g; [e]Determined by AOAC method 999.03. Detection limit is 0.50 g; [f]Based on chemical analysis of raw ingredients measured in triplicate.

two familiarization sessions during baseline. That pace and incline was then maintained during all exercise sessions throughout the study. During all exercise testing, $\dot{V}O_2$ and $\dot{V}CO_2$ were measured by indirect calorimetry (TrueOne 2400; Parvo Medics, Inc., Sandy, UT), and peripheral oxygen saturation Sp_{O_2} was measured using finger pulse oximetry (PalmSat 2500; Nonin Medical, Plymouth, MN).

Gastrointestinal Transit Time and pH

Gastrointestinal transit time and pH were measured using the SmartPill wireless motility testing system (Covidien LLC; Indianapolis, IN). The system includes a SmartPill capsule that is ingested and transits the digestive tract transmitting pH (range 0.5–9.0, accuracy ± 0.5 units), temperature, and pressure readings every 20–40 s to a data receiver worn by each participant. During each study phase, a single SmartPill was ingested immediately following breakfast during the first day of chamber residence (Fig. 1*C*). After each capsule was passed, data from the receiver were downloaded and analyzed using MotiliGI software v. 3.1 (Given Imaging Buffalo, NY). Gastric emptying time was defined as the time from pill ingestion until an abrupt increase in pH of ≥3 units from a baseline gastric pH of <4 (47), corresponding with transit from the acidic environment of the stomach to the more alkaline environment of the duodenum. Transition of the pill from the small intestine to the colon through the ileocecal junction was determined by an abrupt drop in pH, generally between 30 min and 6 h after gastric emptying, reflecting the more acidic environment of the proximal ascending colon relative to the distal ileum (47). Elimination of the pill from the body was determined by a rapid drop in temperature.

Intestinal Permeability

Intestinal permeability was measured over 24 h during each chamber residence period by quantifying the urinary excretion of orally ingested sugar substitutes (Fig. 1*C*). Participants consumed 2 g sucralose and 2 g erythritol dissolved in 180 mL water 6 h after beginning chamber residence and then collected all urine produced over the subsequent 24 h. Aliquots of all urine collected from 0 to 5 h and 5 to 24 h were collected, frozen immediately, and stored at −80°C before being shipped on dry ice for analysis. Urine sucralose and erythritol concentrations were then measured using HPLC (Agilent 1100 HPLC, Santa Clara, CA) with a refractive index detector according to methods adapted from previous publications (48, 49). In brief, sucralose was measured using a Luna 5 µm C18(2) analytical column (250 mm × 4.6 mm; Phenomenex), a mobile phase consisting of 30% methanol, and phenyl β-d-glucopyranoside as an internal standard. Erythritol was measured using a Rezex RCM-Monosaccharide Ca^+ column (Phenomenex), a mobile phase consisting of HPLC-grade water and galactose as an internal standard. Calibration standards were used for both analytes. Fractional excretion of each probe was calculated by multiplying the measured concentration by the total volume of urine collected during the appropriate time period and dividing by the dose administered.

The ratio of fractional excretions of sucralose and erythritol from 0 to 5 h and 5 to 24 h were intended to be interpreted as measures of small intestinal permeability and colonic permeability, respectively. However, recent recommendations suggest restricting measurements of small intestinal and colonic permeability to 0–2 h and to 8–24 h after probe ingestion, respectively, as probes may be present in both the distal small intestine and proximal colon 2–8 h postingestion (50). Therefore, the 0–5 h collection period in this study likely reflected permeability within the small intestine and proximal colon, whereas the 5–24 h collection period likely reflected permeability in the distal small intestine and colon.

Urine Metabolic Profiling

Aliquots of urine collected from 0 to 24 h were collected, frozen immediately, and stored at −80°C before being shipped on dry ice to the University of Reading for an exploratory analysis using ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectroscopy-based untargeted metabolic profiling. Urine samples were prepared, and data were acquired using a ^{1}H-NMR spectrometer (Bruker Biospin, Rheinstetten, Germany) operating at 500 MHz, as previously described (51). Acquired spectroscopic data were processed and imported into the SIMCA software package (v. 13.0; Umetrics AB, Umeå, Sweden) for multivariate statistical analysis. Before analysis, metabolites were normalized to urinary creatinine levels measured using the NMR platform. Unsupervised modeling of the untargeted urinary metabolic profiles was then conducted using principal components analysis (PCA) to identify inherent patterns in the data. Next, supervised modeling was conducted using pairwise orthogonal projections to latent structures discriminant analysis (O-PLS-DA) (52) to maximize separation between the experimental conditions and identify metabolites characteristic of the different experimental

conditions. Models were calculated using one predictive component and two orthogonal components and were assessed based on variance explained (R^2Y) and predictive ability (Q^2Y) metrics. Metabolites were assigned by referencing against in-house chemical standard spectral data and published literature assignments. From this chemometric analysis, several metabolites were identified as being of interest and quantified by area under the curve from the ^{1}H-NMR spectroscopic data using the NMR Suite v10 Profiler (RRID:SCR_014682; Chenomx, Edmonton, Canada).

Blood Biochemistries

Blood samples were collected via antecubital venipuncture during each chamber residence period (Fig. 1*C*). Fasted morning samples were collected immediately before entering the chamber and after ~24 h of residence, and postexercise samples were collected immediately following steady-state exercise after 10 h and 34 h of chamber residence. Samples were separated into serum, frozen immediately, and stored at −80°C until analysis. Serum lipopolysaccharide-binding protein [LBP; Abonva, Taipei, Taiwan; intraassay coefficient of variation (CV) = 6.1%] and intestinal fatty acid-binding protein (I-FABP; Hycult Biotech; Wayne, PA; intraassay CV < 10%) and claudin-3 (MyBioSource; San Diego, CA; intraassay CV < 10%) concentrations were measured by ELISA according to the manufacturer's instructions. Serum interferon-γ, interleukin (IL)-1β, IL-1 receptor antagonist (1RA), IL-6, IL-8, IL-10, IL-17, and tumor necrosis factor-α concentrations were measured by multi-plex assay [Luminex 200 (RRID:SCR_018025); Luminex corporation, Austin TX; intraassay CV < 5%]. Serum cortisol concentrations were measured by immunoassay [intraassay CV = 2.8%; Beckman Coulter Access 2 (RRID:SCR_019607), Indianapolis, IN]. Finally, whole blood from a subset of participants (n = 11) was collected into EDTA tubes to measure hemoglobin and hematocrit concentrations using an automated hematology analyzer [CELL-DYN Emerald (RRID:SCR_026105); Abbott, Chicago, IL]. Hemoglobin and hematocrit were used to estimate percent change in plasma volume during chamber residence (53).

Stool Samples

Four stool samples were collected during each study phase to determine microbiota community composition and SCFA concentrations (Fig. 1*B*). Collection times were within 48 h of beginning each phase, within 48 h of beginning each chamber residence, after 24 h of chamber residence, and 24–48 h after chamber residence ended. All samples were collected into plastic collection containers with an anaerobic sachet (GasPak; BD, Franklin Lakes, NJ) added by the participant. Samples were transported at room temperature and then refrigerated until processed and placed in storage at −80°C within 4 h of collection. The median time between sample collection and processing was 28 min (IQR = 20 min; range = 0–240 min) across all conditions and time points and did not differ between conditions ($P_{interaction}$ = 0.50, $P_{condition}$ = 0.17, P_{day} = 0.61). Frozen aliquots for 16S amplicon sequencing were shipped overnight on dry ice to EzBiome (Gaithersburg, MD) and stored at −80°C until analysis. Frozen aliquots for fluorescence in situ hybridization (FISH) were shipped on dry ice to the University of Reading and stored at −80°C until analysis.

Short-chain fatty acids.

Fecal concentrations of acetate, butyrate, propionate, valerate, isovalerate, and isobutyrate were analyzed as described elsewhere (54). In brief, frozen aliquots were thawed, homogenized in distilled water, centrifuged (5 min at 8,000 *g* and 4°C), and acidified. SCFAs were then extracted using diethyl ether. The organic layer was removed after centrifugation (5 min at 12,000 *g* and 4°C) and ethyl butyric acid added as an internal standard before storing at −80°C until analysis. SCFAs were quantified using an Agilent 7890 A GC system with Flame Ionization Detection (60 m × 250 µm × 0.25 µm; DB-FFAP, Agilent J&W). Calibration standards were included for each fatty acid and used for peak identification and quantification. Fecal water content was measured by freeze-drying samples to a constant weight and used to calculate fecal SCFA concentrations on a dry weight basis.

DNA extraction and 16S rRNA amplicon sequencing.

Frozen samples were thawed at room temperature and then spiked with an internal spike-in control (ZymoBIOMICS Spike-in Control I; Zymo Research, Irvine, CA) at a final concentration of 0.1% to 10% (wt/vol). Samples were then thoroughly mixed by vortexing to ensure homogeneity in the distribution of the spike-in control within the fecal matrix. ZymoBIOMICS TruMatrix fecal reference was utilized as a positive control and nuclease-free water served as the negative control. Genomic DNA of all samples was extracted using a standard bead-based DNA extraction method (ZymoBIOMICS DNA Miniprep) with prelysis through overnight enzymatic digestions using the Metapolyzyme (Millipore Sigma, Burlington, MA) enzyme cocktail to ensure lysis of all microbial cells. DNA concentrations were quantified using the Qubit fluorometer (RRID:SCR_018095; Thermo Fisher, Waltham, MA) and Illumnina-based amplicon libraries of the 16S rRNA V3-V4 regions were then constructed with the primer pair Illumina-F: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and Illumina-R: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC using EzBiome's in-house validated Illumina-based amplicon protocols as described elsewhere (55). In brief, each 25 µL of PCR contained 12.5 ng of sample DNA as input, 12.5 µL 2X KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington, MA), and 5 µL of each primer (1 µM). PCR products were cleaned up from the reaction mix with Mag-Bind RxnPure Plus magnetic beads (Omega Bio-tek, Norcross, GA). A second index PCR amplification, used to incorporate barcodes and sequencing adapters into the final PCR product, was performed in 25 µL reactions, using the same master mix conditions as described above. The libraries were then normalized and pooled. The pooled library was checked using an Agilent 2200 TapeStation (RRID:SCR_014994) and sequenced on the MiSeq (RRID:SCR_016379; Illumina, San Diego, CA) on a 500 cycle (2 × 250 bp paired end) run.

Taxonomic profiling was conducted by directly uploading the paired end reads to the EzBioCloud microbiome taxonomy profiling platform (www.ezbiocloud.net) as described elsewhere (56). In brief, low quality sequences with read

length <80 bp or >2,000 bp or average Q values < 25 were discarded. Denoising and extraction of nonredundant reads were conducted using DUDE-Seq (57). The UCHIME (RRID: SCR_008057) v. 4.1 algorithm (58) was applied against the EzBioCloud 16S chimera-free database to check and remove chimera. Dereplicated sequences were then subjected to taxonomic assignment using VSEARCH (RRID:SCR_024494) (59) to search and calculate sequence similarities of the query NGS reads against the EzBioCloud 16S database using 97% 16S similarity as the cutoff for species-level identification. All sequences that did not match any of reference sequences with at least 97% similarity were clustered using UPARSE (RRID:SCR_005020) (60) using 97% similarity as the cutoff. The raw sequencing reads were computed using the EzBioCloud 16S microbiome pipeline with default parameters and discriminating reads that were encountered in the reference database (PKSSU4.0).

Diversity metrics were calculated after rarefaction at 14,186 reads/sample. α-diversity was measured by the number of observed OTUs and the Shannon, Simpson, and Faith's Phyolgenetic Diversity (PD) indices. β-diversity was calculated using Bray–Curtis dissimilarities.

Fluorescence in situ hybridization.

Select bacterial populations were enumerated using FISH analyses using standard procedures (61). In brief, thawed samples were diluted to a 1:10 ratio and labeled oligonucleotide probes were used to hybridize genus-specific targets with fluorescent markers. Samples were screened using the Accuri C6 flow cytometer (RRID:SCR_019591; BD Biosciences, Franklin Lakes, NJ), measuring at 488 and 640 nm and analyzed using Accuri CFlow Sampler software. Probes used were (see Supplemental Table S2 for sequences) Bif164 for *Bifidobacterium* spp., Lab158 for *Lactobacillus/Enterococcus*, Bac303 for *Bacteroides–Prevotella* group, Erec482 for *Eubacterium rectale–Clostridium coccoides* group, Rrec584 for *Roseburia–E. rectale* group, Ato291 for *Atopobium* cluster, Prop853 for clostridial cluster IX, Fprau 645 for *Faecalibacterium prausnitzii* spp., Dsv687 for *Desulfovibrio* genus, and Chis 150 for most of the *Clostridium histolyticum* group (Clostridium clusters I and II). Total bacteria were enumerated using the Eub338 probe mix (Eub338, Eub338II, and Eub338III), and non-Eub was used as a negative control.

Gastrointestinal Symptoms

Gastrointestinal symptoms were assessed using modified versions of the Irritable Bowel Syndrome-Symptom Severity Scale (IBS-SSS) (62) and the Gastrointestinal Quality of Life Index (GIQLI) Questionnaire (63) that were administered five times weekly during the first week of each phase and daily during the second week (Fig. 1*C*). The modified IBS-SSS was scored out of 400 total points with a higher score indicating greater severity. The modified GIQLI Questionnaire asked participants to rate the frequency of several gastrointestinal-related symptoms such as abdominal pain, bloating, flatulence, constipation, nausea, loose stools, and uncontrolled stools. Ratings were then used to compute an overall score in which lower scores indicated worse symptomology (63). Volunteers were also asked to recall and report their total number of bowel movements each week.

Acute Mountain Sickness

The incidence and severity of acute mountain sickness (AMS) was determined using the shortened version of the Environmental Symptoms Questionnaire (64). The questionnaire was administered eight times daily during chamber residence: immediately before residence and then at 4, 6.5, 10.5, 13, 23, 28, 30.5, and 34.5 h of chamber residence (Fig. 1*C*). Cerebral factor scores (AMS-C) were calculated, and any participant with a score ≥0.70 at one or more timepoints was considered sick (65). After completing the questionnaire, peripheral oxygen saturation Sp_{O_2} was measured using finger pulse oximetry (PalmSat 2500; Nonin Medical, Plymouth, MN).

Statistical Analysis

Sample size calculations were based on an expected 60% increase in small intestinal permeability, which was previously reported within 36 h of ascent to 4,300 m in unacclimatized lowlanders (14). Using means and SD from that study, $n = 15$ was determined to be sufficient to detect a medium effect size for the main effect of the FP intervention on intestinal permeability at $\alpha = 0.05$ and power = 0.80.

All data were examined quantitively and graphically before analysis. Outcomes were analyzed using general linear models with correlated errors. Experimental condition (PL + LA, PL + HA, and FP + HA), study phase (first, second, and third), and condition order were included as fixed factors in all models. For outcomes measured at multiple time points during each phase, time and a condition-by-time interaction were included in the model as fixed factors. Participant was included as a random intercept in the models (linear mixed model). Marginal models were used instead of mixed models in cases where a mixed model could not be fit. Post-hoc testing was conducted when a statistically significant ($P < 0.05$) main effect or interaction was identified. For all models, the distribution and homoscedasticity of residuals were examined, and $\log_{10}$-transformations were applied if needed to meet model assumptions.

Analyses of 16S rRNA amplicon data focused on diversity and differential abundance. Between-condition differences in α-diversity were determined using linear mixed models as described above. Between-condition differences in β-diversity were determined using PERMANOVA using the adonis2 function in the vegan package (v. 2.6-4; R version 4.3.1). The model included the condition-by-day interaction, study phase, condition order, and subject as factors, and permutations were constrained within subject. Following a significant interaction, post hoc testing was conducted for each time point separately using models that substituted condition for the condition-by-day interaction. Differential abundances of genus-level relative abundances were tested using Linear model for Differential Abundance analysis (LinDA; v.0.2.0; RRID:SCR_025966) (66). Genera detected in ≤33% of samples were excluded. Models included condition, day and their interaction, study phase and condition order as fixed effects, and subject as a random intercept. Tests for main effects and interactions were conducted using Wald tests with P values adjusted by Benjamini–Hochberg correction using a cutoff of $q < 0.25$. Finally, correlations among variables were assessed using repeated measures correlation (67).

Restricted maximum likelihood estimation was used in all linear models to account for any missing data. When analyzing blood, urine, SmartPill, and questionnaire data for participants who completed only 1 day of a chamber residence period, all data collected through that day were included in the analysis. When analyzing stool sample data for participants who completed only 1 day of a chamber residence period, all available data from that phase were used if the participant continued following the study diet until the end of the phase. In cases where the participant did not continue following the study diet, only data collected before stopping the diet were included.

Analyses were conducted using SPSS v. 22.0 (RRID: SCR_016479) or R v.4.3.1 (RRID:SCR_001905). Graphs were created using GraphPad Prism v. 10.1.0 (RRID:SCR_002798). Primary analyses included the participants who completed all three study phases [completer cohort (CC)]. Secondary analyses including any participant who completed one or more study phases were also conducted for all outcomes [intention-to-treat cohort (ITT)]. Results of the CC analyses are presented in the main text and figures. Results for the ITT analyses are provided in the Supplemental Material, and any notable differences between the CC and ITT analyses are described in the text.

RESULTS

Thirty-one males and two females were enrolled into the study and randomized, and 26 completed at least one full study phase (ITT cohort; Table 2). Twelve males and one female completed all three study phases (completer cohort; Table 2). Three CC participants did not complete the second day of HA exposure during FP supplementation (FP + HA) due to AMS-related symptoms but continued to consume the provided diet throughout the intervention period and were included in the CC analysis. An additional participant completed the study but was unable to complete the second chamber residence period due to illness and was not included in the CC analysis. Of the 19 participants who attritted, 12 withdrew after not being available following institute-wide shutdowns related to the coronavirus-19 pandemic during their participation, two withdrew due to gastrointestinal symptoms experienced while consuming the FP intervention and study diet before the chamber residence period, one withdrew due to gastrointestinal symptoms during PL + HA, and four withdrew for personal reasons or due to relocation from the study area (Fig. 2).

Table 2. *Baseline volunteer characteristics*

	Intention-to-Treat Cohort	Completer Cohort
Male/female (*n*)	24/2	12/1
Race (*n*)		
White	19	9
Black	6	3
Native Hawaiian/Pacific Islander	1	1
Ethnicity (*n*)		
Non-Hispanic	19	9
Hispanic	7	4
Age, yr	22 ± 3	21 ± 3
Body weight, kg	78.3 ± 9.3	78.1 ± 8.7
BMI, kg/m^2	25.4 ± 2.5	25.4 ± 2.4
$\dot{V}O_{2peak}$, L/min	3.90 ± 0.51	3.87 ± 0.39

Values are *n* or means ± SD.

Dietary Intake and Body Weight

Adherence to the intervention was high, with a median consumption of 100% of all bars provided (Supplemental Table S3). As planned, total fiber intake was higher during FP supplementation relative to the PL conditions (Table 3 and Supplemental Table S3). No between-condition differences in energy, carbohydrate, fat, or protein intakes were observed before chamber residence (Table 3 and Supplemental Table S3). During chamber residence, energy, carbohydrate, fat, and protein intakes were all reduced during HA exposure independent of FP supplementation (Table 3 and Supplemental Table S3). The reduction in intake during HA exposure was attributed to AMS symptoms that prevented several participants from being willing to consume all provided food, including the intervention bars.

Body weight increased by 1.5 kg [95% confidence interval (CI): 1.0, 1.9; $P < 0.001$] during the prechamber residence period before decreasing by 1.4 kg (95% CI: 0.9, 1.9; $P < 0.001$) during chamber residence ($P_{day} < 0.001$) independent of HA exposure or FP supplementation ($P_{interaction} = 0.64$; Supplemental Table S4).

Exercise

Exercise intensity during chamber residence was not affected by HA exposure or FP supplementation (Supplemental Table S5). However, heart rate (mean difference [95% CI]: 28 beats/min [22, 34]) and perceived exertion (3 [2, 4]) were higher, and exercise Sp_{O_2} was lower (26% [24, 28]) while exercising at HA relative to LA independent of FP supplementation ($P_{condition} < 0.001$ for all; Supplemental Table S5).

Fecal Characteristics

Bristol Stool Scale scores were not affected by FP supplementation or HA exposure ($P_{interaction} = 0.70$; Supplemental Table S6). FP supplementation had no impact on fecal water content before chamber residence (Supplemental Table S6). In the ITT analysis ($P_{interaction} = 0.03$), but not the CC analysis ($P_{interaction} = 0.12$), a transient decrease in fecal water content was observed during LA but not HA exposure with fecal water content measured during chamber residence being lowest during PL + LA, highest during PL + HA, and intermediate during FP + HA (Supplemental Table S6).

Fecal Short-Chain Fatty Acids

In the CC analysis, fecal SCFA concentrations were not affected by FP supplementation or HA exposure ($P_{interaction} \geq 0.07$; Supplemental Table S6). In the ITT analysis, fecal butyrate concentrations measured immediately before chamber residence were lower during FP supplementation compared with PL (PL + LA, $P = 0.06$; PL + HA, $P = 0.03$). In addition, HA exposure, independent of FP supplementation, attenuated reductions in butyrate ($P_{interaction} = 0.05$) and valerate concentrations ($P_{interaction} = 0.02$) observed during LA exposure (Supplemental Table S6).

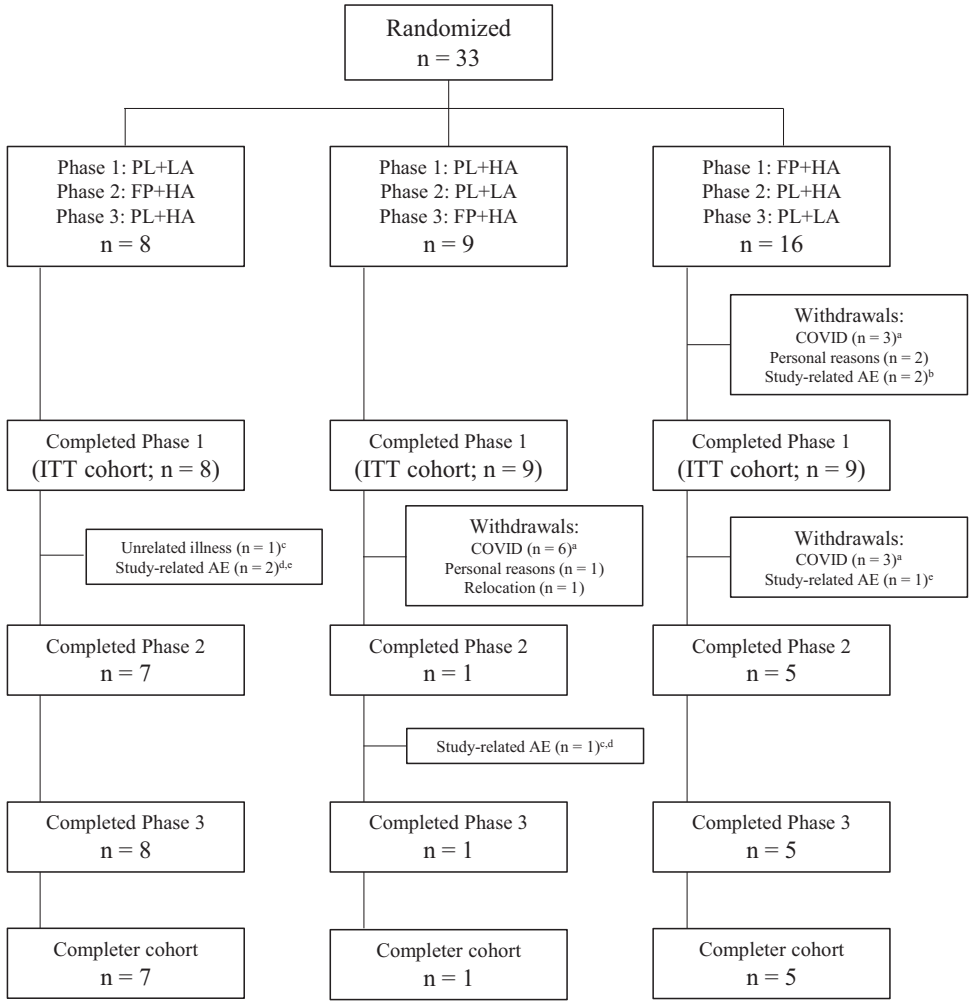

Figure 2. Participants were randomized to consume a fermentable fiber and polyphenol-rich intervention (FP) or matched placebo (PL) before and during 36-h residence in a hypobaric chamber simulating low-altitude (LA, 500 m) or high-altitude (HA, 4,300 m) conditions. AE, adverse event; ITT, intention-to-treat. [a]Unavailable after institute shutdown due to coronavirus (COVID)-19 pandemic; [b]gastrointestinal symptoms during consumption of study diet and intervention; [c]participant missed Phase 2 chamber residence due to illness but completed all of Phase 3; [d]participants completed one full day of chamber residence during the phase but remained on study diet through intervention period and were included in completer cohort; [e]symptoms consistent with acute mountain sickness.

Gut Microbiota Composition

PERMANOVA of Bray–Curtis dissimilarities revealed between-condition differences in gut microbiota composition at all time points except baseline ($P_{interaction} = 0.008$; Fig. 3*A* and Supplemental Fig. S1). FP supplementation reduced α-diversity throughout the supplementation period as measured by the Shannon, inverse Simpson, and Faith's PD metrics ($P_{interaction} \leq 0.03$; Fig. 3, *B* and C and Supplemental Table S6). Those reductions persisted to some extent at the

Table 3. *Energy and macronutrient intakes while consuming provided diets before and during chamber residence (completer cohort; n = 13)*

	PL + LA	PL + HA	FP + HA
Energy, kcal/day			
Week 2, days 1–5 (preresidence)	2,778 ± 373	2,782 ± 400	2,922 ± 454
Week 2, days 6–7 (chamber residence)	2,757 ± 434[a]	2,198 ± 503[b]*	2,341 ± 679[b]*
Carbohydrate, g/day			
Week 2, days 1–5 (preresidence)	393 ± 50	390 ± 57	415 ± 66
Week 2, days 6–7 (chamber residence)	423 ± 60[a]	344 ± 74[b]*	361 ± 104[b]*
Fat, g/day			
Week 2, days 1–5 (preresidence)	94 ± 13	95 ± 13	100 ± 14
Week 2, days 6–7 (chamber residence)	76 ± 16[a]*	61 ± 16[b]*	67 ± 21[b]*
Protein, g/day			
Week 2, days 1–5 (preresidence)	103 ± 18	104 ± 20	104 ± 21
Week 2, days 6–7 (chamber residence)	105 ± 21[a]	75 ± 23[b]*	81 ± 25[b]*
Total fiber, g/day			
Week 2, days 1–5 (preresidence)	23 ± 3[a]	24 ± 3[a]	58 ± 4[b]
Week 2, days 6–7 (chamber residence)	25 ± 3[a]	20 ± 5[b]*	52 ± 11[c]*

Data are means ± SD. Analyzed by linear mixed model with post-hoc testing following significant condition-by-time interactions ($P < 0.05$). Within a row, values not sharing superscript letters a and b are significantly different ($P < 0.05$). Between- and within-condition differences are the same for the intention-to-treat cohort ($n = 26$). FP, fiber and polyphenol intervention; HA, high altitude (4,300 m) condition; LA, low altitude (500 m) condition; PL, placebo. *Significantly different from *week 2, days 1–5* ($P < 0.05$).

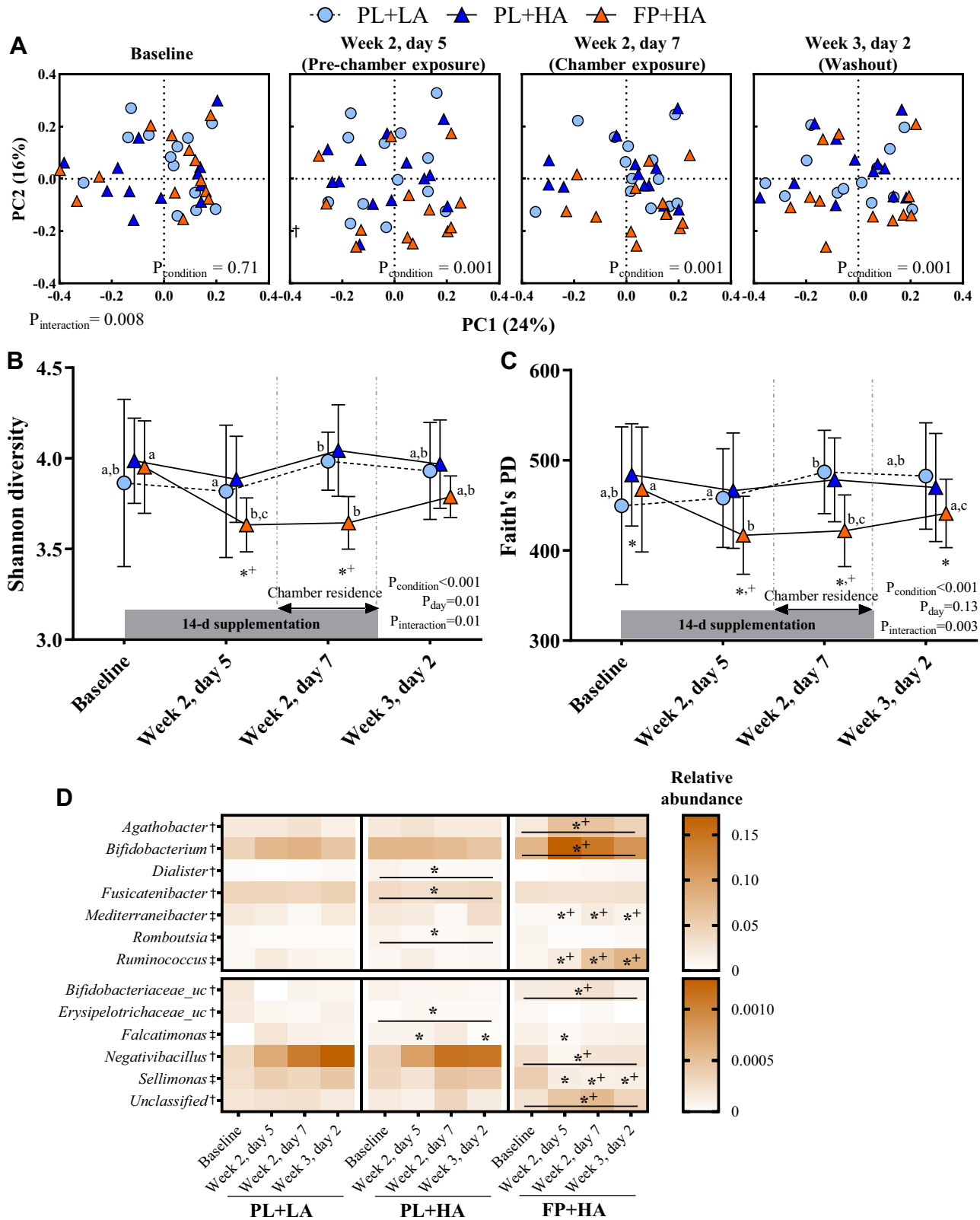

Figure 3. Consuming a fiber and polyphenol-rich intervention (FP) vs. matched placebo (PL) before and during 36-h residence in a hypobaric chamber simulating low altitude (LA, 500 m) or high altitude (HA, 4,300 m) alters gut microbiota composition (completer cohort, $n = 13$). *A*: principal coordinates analyses of Bray–Curtis dissimilarities analyzed using PERMANOVA. Changes in α-diversity measured by Shannon's index (*B*) and Faith's phylogenetic diversity (PD) (*C*). Analyzed by linear mixed model with post hoc testing following significant main effects and condition-by-day interactions ($P < 0.05$). Within a condition, timepoints not sharing superscript letters a, b, and c are significantly different ($P < 0.05$). Data are means ± SD. *D*: genera with relative abundances having †main effects of condition or ‡statistically significant interactions ($P < 0.05$, $q < 0.25$). Analyzed by Linear model for Differential Abundance analysis (LinDA). Heatmap shows median relative abundance. uc, unclassified. *Different from PL + LA; $P < 0.05$. $^{+}$Different from PL + HA; $P < 0.05$.

washout timepoint but were attenuated (Fig. 3, *B* and *C* and Supplemental Table S6). HA exposure alone (PL + HA vs. PL + LA) had little impact on α-diversity metrics though increases in the Shannon index (CC analysis only), and Faith's PD observed during LA exposure were not observed during HA exposure (Supplemental Table S6). Adjusting diversity analyses for Bristol Stool Scores had little impact on results for main effects or interactions (data not shown).

Differential abundance analyses identified several taxa impacted by FP supplementation (Fig. 3*D* and Supplemental Table S7). FP supplementation resulted in higher *Ruminococcus* relative abundance ($P_{interaction} = 0.002$, $q_{interaction} = 0.11$) and lower *Sellimonas* and *Mediterraneibacter* relative abundances ($P_{interaction} < 0.002$, $q_{interaction} \leq 0.11$) relative to both PL conditions. In addition, main effects of condition were observed for several taxa with mean relative abundances of *Bifidobacterium,* unclassified *Bifidobacteriaceae,* and *Agathobacter* all higher and mean relative abundance of *Negativibacillus* lower ($P_{condition} < 0.05$, $q_{condition} < 0.25$) throughout FP supplementation relative to both PL conditions. Results of the ITT analysis were largely consistent with those of the CC analysis though additional taxa were found to be affected by FP supplementation (Supplemental Fig. S2 and Supplemental Table S7). Most notably, FP supplementation increased relative abundances of *Bifidobacterium*, unclassified *Bifidobacteriaceae*, *Agathobacter*, and *Fecalibacterium* relative to both PL conditions ($P_{interaction} < 0.05$, $q_{interaction} < 0.25$). HA exposure alone had no effect on the relative abundance of any taxa during chamber residence (PL + LA vs. PL + HA) and the only taxa found to differ between PL conditions at the washout timepoint were *Falcatimonas* (CC analysis only; $P = 0.01$) and *Paraprevotella* (ITT analysis only; $P = 0.01$) (Fig. 3*D* and Supplemental Fig. S2).

Fluorescence in situ hybridization was used to complement the 16S rRNA amplicon sequencing by enumerating specific bacterial groups of interest in a subset of participants (completer cohort: $n = 9$; ITT cohort: $n = 22$; Supplemental Table S6). No clear effects of FP supplementation on any bacterial group were observed before chamber residence. However, abundance of *Clostridium* clusters I and II transiently decreased during chamber residence with FP supplementation but not PL supplementation at HA or LA (CC analysis only; $P_{interaction} \leq 0.03$; Supplemental Table S6). Across all timepoints, abundances of *Bifidobacterium* spp., *Clostridial* cluster IX, and *Atopium* cluster (CC analysis only) all demonstrated a similar pattern wherein mean cell counts were higher throughout FP supplementation versus PL + LA but not PL + HA ($P_{condition} \leq 0.03$; Supplemental Table S6). No effects of HA exposure alone were observed for any bacterial group (PL + HA vs. PL + LA).

Gastrointestinal pH, Transit Time, and Pressure

Gastrointestinal transit time was not affected by HA exposure or FP supplementation (Fig. 4, *A* and *B* and Supplemental Table S8). HA exposure alone also did not affect gastrointestinal pH (PL + HA vs. PL + LA). However, FP supplementation during HA exposure reduced pH near the ileocecal junction (FP + HA vs. PL + HA) and throughout the colon (FP + HA vs. PL + LA) (Fig. 4, *D* and *E* and Supplemental Table S8). In the ITT analysis (Supplemental Table S8), but not the CC analysis (Fig. 4*C*), pressure within the large intestine was reduced during HA exposure (PL + HA vs. PL + LA; $P = 0.001$) and the magnitude of that reduction was attenuated by FP supplementation ($P_{condition} = 0.01$).

Intestinal Permeability and Markers of Intestinal Barrier Damage

No between-condition differences in urine volume ($P_{condition} \geq 0.38$) or urine specific gravity ($P_{condition} \geq 0.25$) were observed during the 0–5 h or 5–24 h urine collection periods (Supplemental Table S9).

HA exposure increased sucralose excretion during the 0–5 h urine collection period (PL + HA vs. PL + LA) and that effect was fully mitigated by FP supplementation (Fig. 5*A* and Supplemental Table S9). Sucralose excretion during the 5–24 h urine collection period was not affected by HA exposure or FP supplementation (Fig. 5*B* and Supplemental Table S9).

Results for erythritol excretion and sucralose:erythritol ratios are shown in Supplemental Table S9 and Supplemental Fig. S3. Total urine erythritol excretion over the entire 24 h collection period was >100% of the administered dose (range: 30%–277%) for 30% of all collections suggesting contamination from the diet or endogenous contributions. These data were therefore considered unreliable and not considered when interpreting intestinal permeability, which is consistent with recent recommendations (50).

Circulating LBP and I-FABP concentrations were not affected by HA exposure or FP supplementation (Fig. 5, *C* and *D* and Supplemental Table S10). Mean serum claudin-3 concentrations were increased by HA exposure independent of FP supplementation (Fig. 5*E* and Supplemental Table S10). However, the effect of HA exposure on claudin-3 concentrations was not observed following adjustment for decreases in plasma volume experienced during HA exposure ($P_{condition} = 0.79$; Supplemental Table S10).

Repeated measures correlation revealed a positive association between 0 and 5 h urine sucralose excretion and pH near the ileocecal junction ($r = 0.44$, $P = 0.02$) and an inverse association between 0 and 5 h urine sucralose excretion and *Bifidobacterium* relative abundance measured during chamber residence ($r = -0.38$, $P = 0.05$) (Fig. 5*F*). Urine sucralose excretion (0–5 h) was also positively associated with the inverse Simpson index ($r = 0.40$, $P = 0.04$) and relative abundances of *Fusicatenibacter* ($r = 0.45$, $P = 0.02$), *Mediterraneibacter* ($r = 0.49$, $P = 0.009$), and *Sellimonas* ($r = 0.53$, $P = 0.0005$) measured during chamber residence (Fig. 5*F*).

Circulating Markers of Stress and Inflammation

Cortisol concentrations increased during HA exposure independent of FP supplementation (Fig. 6*A* and Supplemental Table S10). That effect was attenuated after adjusting for plasma volume changes during HA exposure, though a tendency toward a main effect of condition ($P_{condition} = 0.06$) was observed with mean cortisol concentrations measured during FP + HA ($P = 0.02$), but not PL + HA ($P = 0.08$), remaining higher than those measured during PL + LA (Supplemental Table S10).

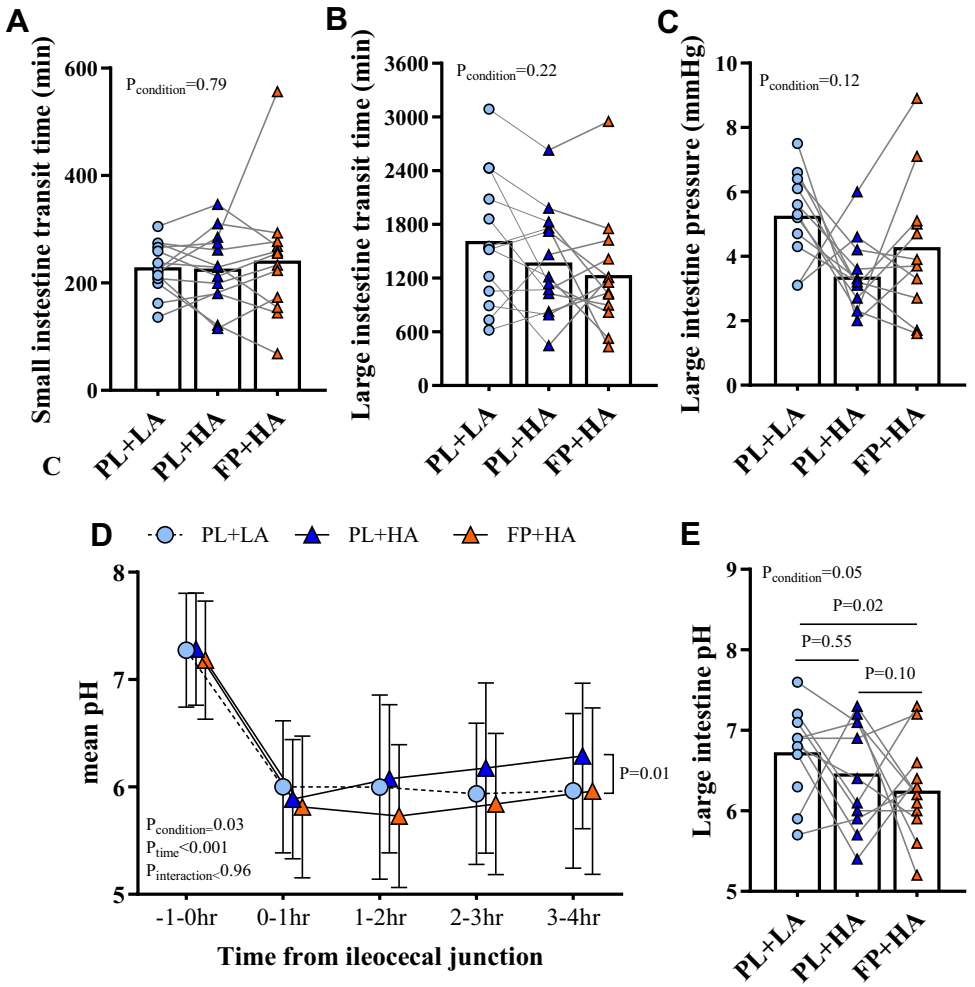

Figure 4. Effects of dietary supplementation with a blend of fermentable fibers and polyphenol sources on intestinal transit time, pressure, and pH during hypoxic stress. Transit time through the small (*A*) and large (*B*) intestines, mean pressure during large intestine transit (*C*), mean pH during transit through the ileocecal junction (*D*), and median pH during large intestine transit (*E*) measured by SmartPill during and after 36 h of residence in a hypobaric chamber under low-altitude (LA, 500 m) or high-altitude (HA, 4,300 m) conditions at the end of 2 wk dietary supplementation with a fermentable fiber and polyphenol-rich intervention (FP) or matched placebo (PL) (completer cohort; $n = 13$). Analyzed by linear mixed model with post hoc testing following significant main effects and condition-by-time interactions ($P < 0.05$). Bars are mean values. Line graph shows means ± SD.

Mean serum IL-6 concentrations were higher during HA exposure alone relative to LA (PL + HA vs. PL + LA) but that difference was fully mitigated by FP supplementation (Fig. 6*B*). Mean serum IL-1RA concentrations were also higher during HA exposure alone relative to LA (PL + HA vs. PL + LA). That difference was partially attenuated by FP supplementation in the ITT analyses, but not the CC analysis (Fig. 6*C*), and fully attenuated following plasma volume adjustment (Supplemental Table S10). Mean plasma volume adjusted TNFα concentrations, but not unadjusted concentrations, were lower during HA relative to LA exposure independent of FP supplementation ($P_{condition} = 0.003$; Supplemental Table S10). No effects of either HA exposure or FP supplementation were observed for any other cytokine (Supplemental Table S10).

Urine Metabolic Profiling

Data from a subset of participants (completer cohort: $n = 9$; ITT cohort: $n = 22$) were available for ^{1}H-NMR untargeted metabolic profiling. PCA did not identify any distinct clustering patterns across conditions (completer cohort: $R^2Y = 0.59$, $Q^2Y = 0.43$, ITT analysis $R^2 = 0.57$, $Q^2 = 0.47$; Supplemental Fig. S4). However, O-PLS-DA identified several metabolites associated with either HA exposure or FP supplementation: hippurate, propylene glycol, glycine, taurine, citrate, acetate, acetylcarnitine trimethylamine N-oxide (TMAO), phenylacetylglutamine, a metabolite tentatively identified as methylmalonate, and several metabolites that could not be identified from spectral databases and published literature assignments (Supplemental Fig. S5). Univariate analyses of those metabolites revealed that TMAO levels were significantly reduced during HA exposure alone (PL + HA vs. PL + LA) and that difference was fully mitigated by FP supplementation ($P_{condition} = 0.03$). In addition, methylmalonate (tentative identification) levels were increased during HA exposure independent of FP supplementation ($P_{condition} < 0.001$). Hippurate levels were not affected by HA exposure alone (PL + HA vs. PL + LA) but demonstrated a tendency to be higher during FP supplementation (FP + HA) relative to PL + LA ($P = 0.04$) in the CC analysis ($P_{condition} = 0.10$) and were increased by FP supplementation relative to both PL conditions in the ITT analysis ($P_{condition} = 0.04$). Hippurate was also the only identified metabolite found to discriminate the two HA conditions (FP + HA vs. PL + HA) based on Variable Importance in the Projection scores >2.0. That observation is consistent with the higher polyphenolic intake during FP supplementation given that hippurate is a glycine-conjugate of benzoic acid, a

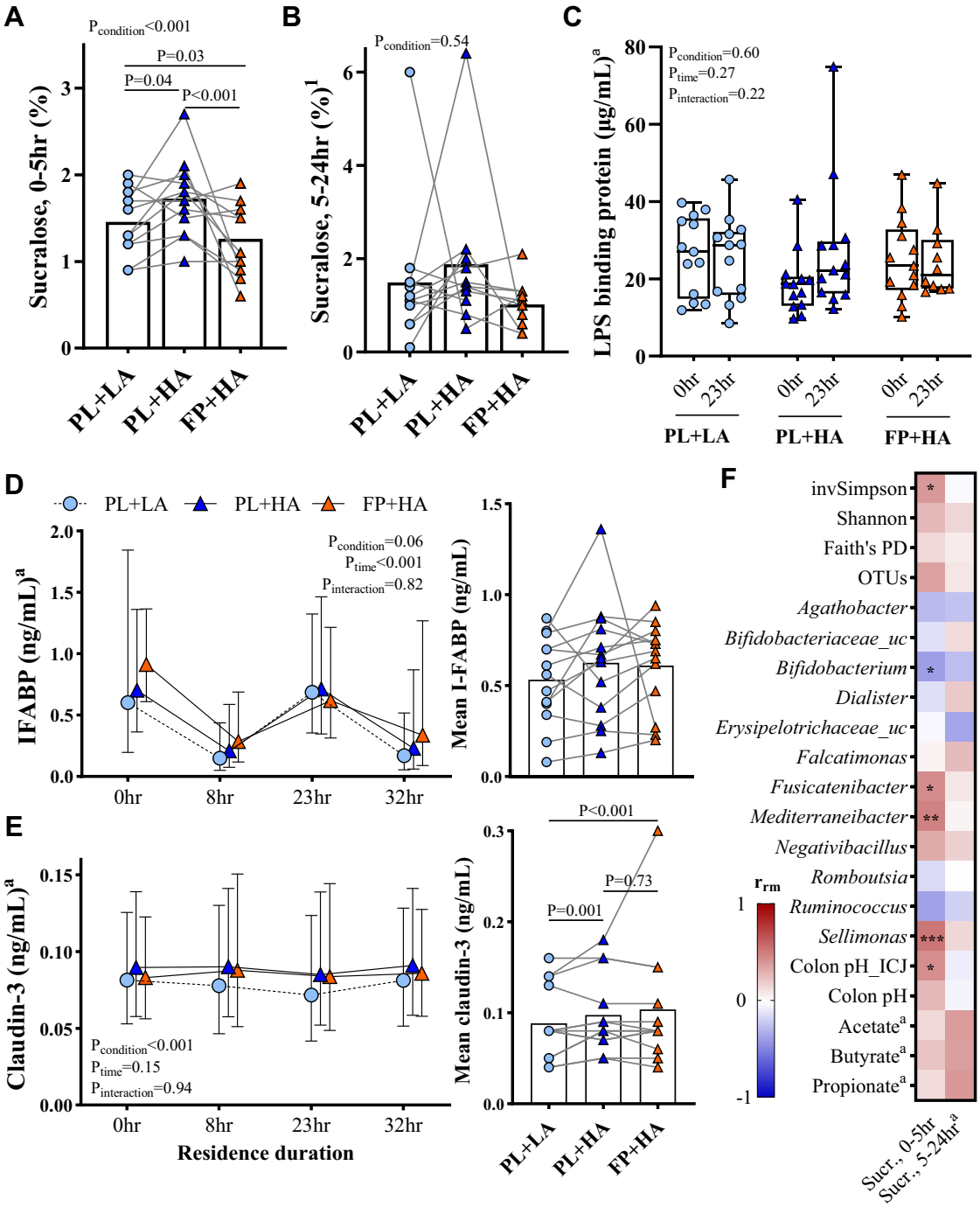

Figure 5. Effects of dietary supplementation with a blend of fermentable fibers and polyphenol sources on intestinal barrier integrity during hypoxic stress. Intestinal permeability markers measured during 36 h of residence in a hypobaric chamber under low-altitude (LA, 500 m) or high-altitude (HA, 4,300 m) conditions at the end of 2-wk dietary supplementation with a fermentable fiber and polyphenol-rich intervention (FP) or matched placebo (PL) (completer cohort; $n = 13$). *A*: urine sucralose excretion during initial 5 h postconsumption reflecting permeability of the small intestine and proximal colon. *B*: urine sucralose excretion from 5 to 24 h postconsumption reflecting permeability of the distal small intestine and colon. *C*: serum lipopolysaccharide (LPS) binding protein. *D*: serum intestinal fatty acid-binding protein (I-FABP); bars show mean of all four timepoints. *E*: serum claudin-3; bars show mean of all four timepoints. *A–E*: analyzed by linear mixed model with post-hoc comparisons conducted following statistically significant main effects and condition-by-time interactions ($P < 0.05$). Bars show mean values. Boxplots show median and IQR, with whiskers extending to minimum and maximum values. Line graphs show geometric means ± geometric SD. *F*: repeated measures correlations (r_{rm}) between intestinal permeability, gut microbiota features demonstrating between-condition differences and fecal short-chain fatty acid concentrations ($n = 26$). Gut microbiota features and short-chain fatty acid concentrations measured during chamber residence. All genus relative abundances were arcsine-square root transformed for analysis. ICJ, ileocecal junction; inv, inverse; OTUs, operational taxonomic units; PD, phylogenetic diversity; r, repeated measures correlation; Sucr., sucralose; uc, unclassified. ***$P < 0.001$; **$P < 0.01$; *$P \leq 0.05$. [a]Log_{10} transformed for analysis.

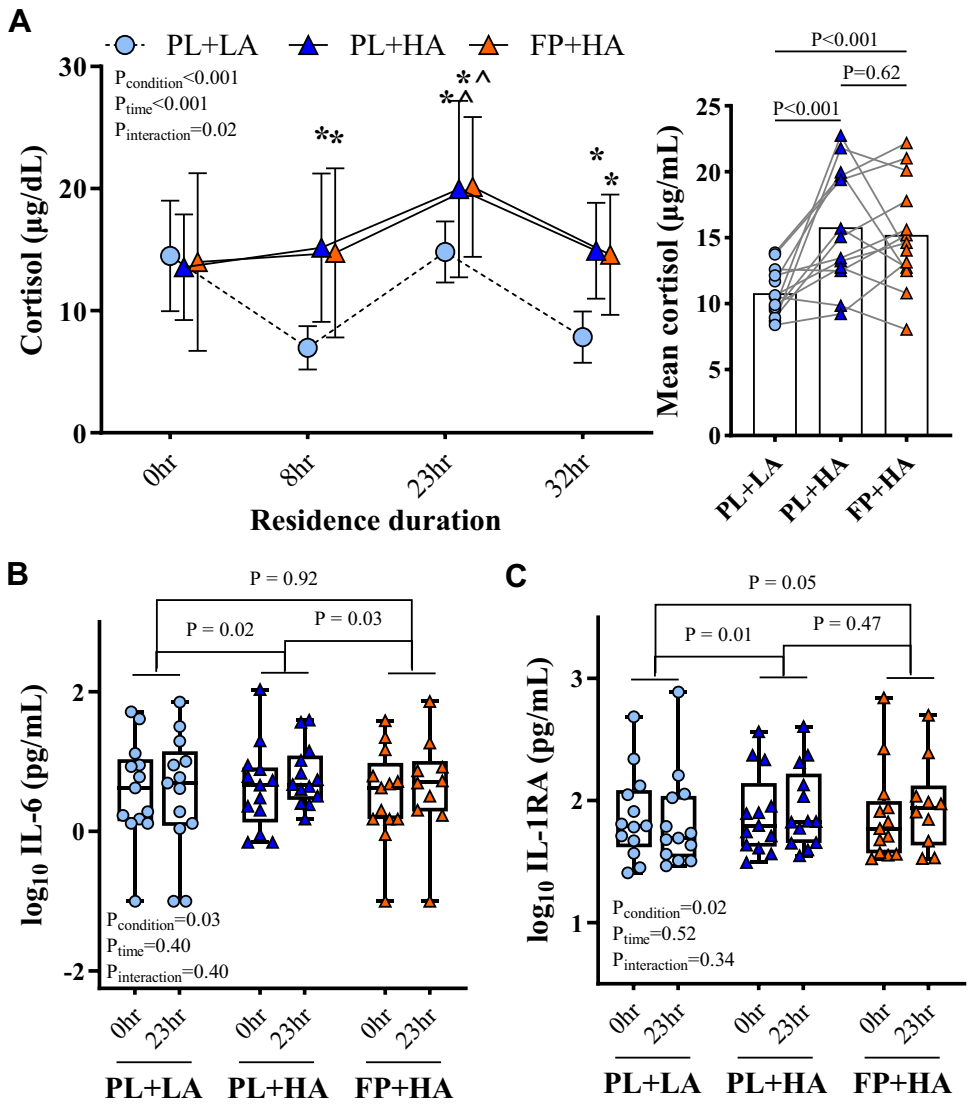

Figure 6. Effects of dietary supplementation with a blend of fermentable fibers and polyphenol sources on circulating markers of stress and inflammation during hypoxic stress. Circulating cortisol (*A*), interleukin (IL)-6 (*B*), and IL-1RA (*C*) concentrations measured over 36 h of residence in a hypobaric chamber under low-altitude (LA, 500 m) or high-altitude (HA, 4,300 m) conditions at the end of 2 wk of dietary supplementation with a fiber and polyphenol intervention (FP) or matched placebo (PL) (completer cohort, $n = 13$). Morning fasted concentrations measured at 0 h and 23 h, and postexercise concentrations measured at 8 h and 32 h of chamber residence. Analyzed by linear mixed model with post hoc comparisons conducted following statistically significant main effects or condition-by-time interactions ($P < 0.05$). Line graph shows means ± SD. Bar graph shows mean. Boxplots show median and IQR with whiskers extending to minimum and maximum values. *Different from PL + LA ($P < 0.05$); ^within-condition difference from the measurement 23–24 h prior ($P < 0.01$).

compound formed via gut microbiota-mediated metabolism of certain polyphenolic compounds (68).

Gastrointestinal Symptoms and Acute Mountain Sickness Severity

GIQLI and IBS-SSS scores were not affected by FP supplementation before HA exposure or by HA exposure alone (PL + HA vs. PL + LA) (Table 4). However, the combination of FP supplementation and HA exposure (FP + HA) resulted in a decrease in GIQLI scores, indicating an increase in gastrointestinal symptoms (Table 4 and Supplemental Table S11). A similar effect of FP supplementation during HA exposure on IBS-SSS scores was also observed though the interaction term was not statistically significant (Table 4 and Supplemental Table S11).

AMS-C scores were increased after 4 h of HA exposure and remained elevated throughout the exposure period (Fig. 7*A*). During HA exposure, FP supplementation resulted in higher mean AMS-C scores (FP + HA vs. PL + HA, $P = 0.03$) with peak scores being more than double those measured during PL supplementation (Fig. 7*B*). AMS incidence (FP + HA: 77% vs. PL + HA: 54%, $P = 0.02$) and frequency of lightheadedness, headache, dizziness, dim vision, nausea, anorexia, and feeling sick during HA exposure were all increased during FP supplementation relative to PL ($P_{condition} \leq 0.01$; Supplemental Table S11).

Oxygen saturation measured concomitant with AMS assessments was reduced throughout HA exposure (Fig. 7*C*). During HA exposure, FP supplementation improved oxygen saturation at 10.5 h and 28 h time points ($P < 0.05$) relative to PL and mean oxygen saturation during HA exposure was increased 1.8% [95% CI: 0.8, 2.8] during FP supplementation relative to PL (Fig. 7*D*).

DISCUSSION

In this randomized, placebo-controlled, crossover study, dietary supplementation with a blend of fermentable fibers and polyphenol sources targeting the gut microbiota mitigated hypobaric hypoxia-induced increases in intestinal permeability

Table 4. *Gastrointestinal symptoms measured before and during exposure to low altitude (LA, 500 m) and high altitude (HA, 4,300 m) conditions while consuming PL and FP products (completer cohort; n = 13)*

	PL + LA	PL + HA	FP + HA	P_C	P_D	$P_{C \times D}$
GIQLI Score				<0.001	0.01	0.02
Week 1, days 1–7	37 ± 3	38 ± 2	36 ± 3			
Week 2, days 1–5	36 ± 3	37 ± 2	36 ± 3			
Week 2, days 6–7 (chamber residence)	37 ± 3[a]	36 ± 2[a]	32 ± 7[b]*			
IBS-SSS	A	A, B	B	0.04	0.01	0.08
Week 1, days 1–7	36 [45]	39 [77]	43 [78]			
Week 2, days 1–5	36 [71]	54 [84]	56 [100]			
Week 2, days 6–7 (chamber residence)*	41 [79]	30 [74]	82 [149]			

Data are means ± SD or median [IQR]. Analyzed by linear mixed model with post hoc testing following significant main effects and condition-by-day interactions ($P < 0.05$). IBS-SSS was $\log_{10}$-transformed for analysis. Within a row, values not sharing superscript letters a and b are significantly different ($P < 0.05$). C, condition; D, day; FP, fiber and polyphenols; GIQLI, Gastrointestinal Quality of Life Index (range: 0–40; lower values indicate worse symptoms); HA, high altitude; IBS-SSS, Irritable Bowel Syndrome-Symptom Severity Score (range: 0–400; higher values indicate worse symptoms); LA, low altitude; PL, placebo. *Significantly different from *week 1* and *week 2, days 1–5* ($P < 0.05$).

within the small intestine and proximal colon while increasing *Bifidobacterium* relative abundance and decreasing gut microbiota α-diversity and colonic pH. However, supplementation did not alter fecal SCFA concentrations and increased gastrointestinal symptoms and AMS during hypobaric hypoxia exposure. Taken together, these findings demonstrate that targeting beneficial gut microbes with fermentable fibers and polyphenols can help preserve gut barrier function during

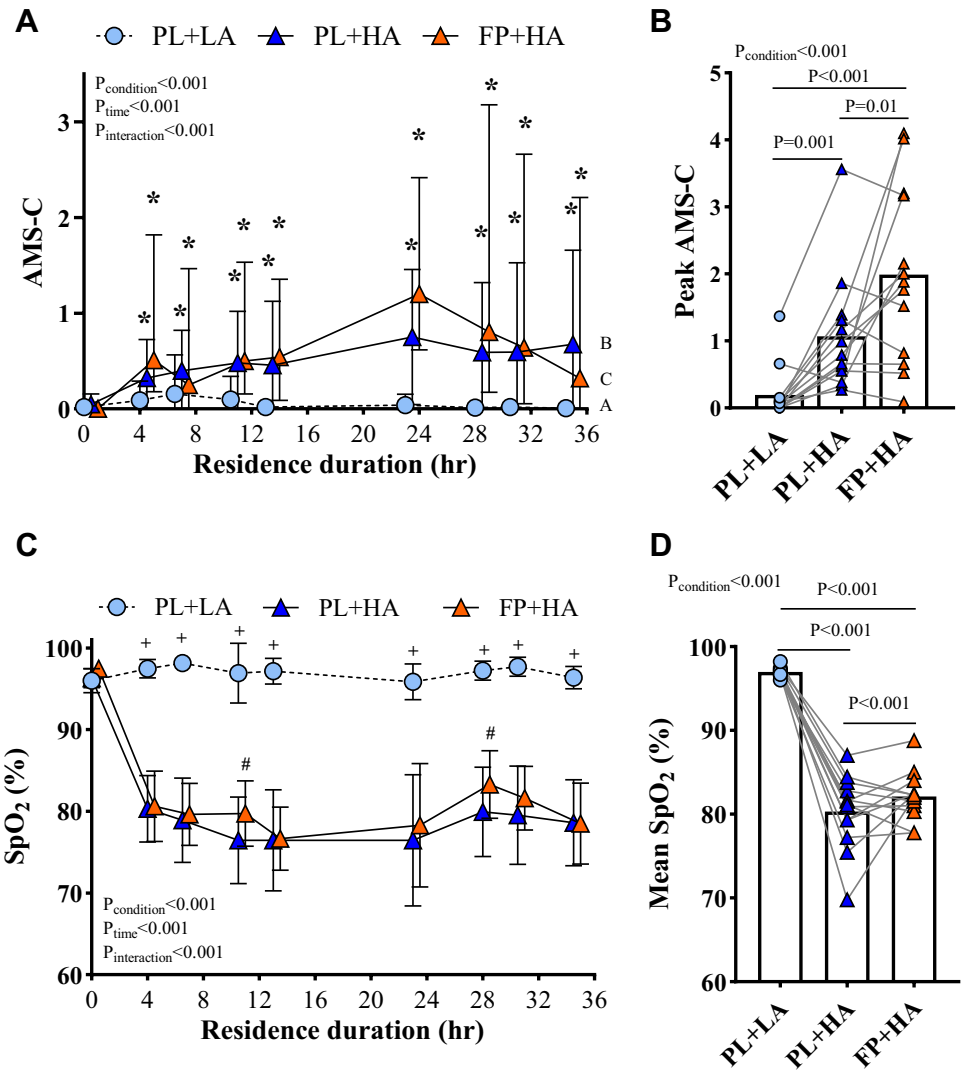

Figure 7. Effects of dietary supplementation with a blend of fermentable fibers and polyphenol sources on acute mountain sickness and peripheral oxygen saturation (Sp_{O_2}) during hypoxic stress. Acute mountain sickness-cerebral factors (AMS-C; *A* and *B*) scores and Sp_{O_2} (*C* and *D*) measured over 36 h of residence in a hypobaric chamber under low-altitude (LA, 500 m) or high-altitude (HA, 4,300 m) conditions at the end of 2-wk dietary supplementation with a fermentable fiber and polyphenol intervention (FP) or matched placebo (PL) (completer cohort, $n = 13$). AMS-C data were $\log_{10}$-transformed for analysis. Data analyzed by linear mixed model with post hoc comparisons conducted following significant main effects and condition-by-time interactions ($P < 0.05$). *Different from PL + LA ($P < 0.01$); + different from PL + HA and FP + HA ($P < 0.001$); #different from PL + HA ($P < 0.05$). Superscript capital letters indicate main effect of condition wherein mean values were highest during FP + HA, intermediate in PL + HA, and lowest in PL + LA. Line graph values are median [IQR] (*A*) or means ± SD (*C*). Bars show mean values.

hypobaric hypoxia-induced stress but that benefit does translate into a reduction in gastrointestinal or altitude illness symptoms.

The observed between-condition differences in several, but not all, measures of intestinal barrier integrity during the placebo treatments are consistent with localized hypoxia-induced decrements in intestinal permeability but fail to provide clear insight into the underlying mechanisms. Orally administered saccharide probes classically used to measure intestinal permeability are detected within the colon within 2 h of administration and almost exclusively in the colon within 8 h (69, 70). The method provides a functional measure of regional paracellular permeability impacted by both regulation of tight junctions and damage to structural features including tight junction complexes and epithelial cells (38, 71). Claudin-3 is a tight junction protein though to reflect paracellular barrier integrity loss throughout the gastrointestinal tract, whereas I-FABP is a cytosolic protein measured to assess epithelial cell damage, primarily within the jejunum (71). Therefore, the increase in 0–5 h but not 5–24 h sucralose excretion observed during hypobaric hypoxia exposure (i.e., PL + HA vs. PL + LA) likely reflects an increase in intestinal permeability localized to the small intestine and proximal colon. The parallel increase in claudin-3 but not I-FABP could implicate paracellular permeability resulting from tight junction loss rather than epithelial cell damage as a potential pathway. However, the claudin-3 results require cautious interpretation given that the hypoxia-associated increase in circulating concentrations appeared to be largely driven by shifts in plasma volume. The absence of a concomitant increase in LBP concentrations may further suggest relatively modest and localized barrier integrity loss, given that LPS, the antigen stimulating hepatic release of LBP, is a large compound unlikely to pass through paracellular routes absent of apoptosis or more severe epithelial cell damage (38). When considered together, these results appear most consistent with a modest hypobaric hypoxia-induced decrement in intestinal barrier function without strong evidence of major structural damage within the intestinal epithelium.

Previous studies of healthy adults have reported increased permeability in the small intestine and proximal colon within 24 h of sojourn at 4,300 m (14) and increases in circulating I-FABP (11, 12, 72–74), claudin-3 (11), endotoxin (15), and LBP (11) concentrations during 40–60 min of exercise at 4,000–4,600 m simulated altitude. Though blood biomarker results of the present study differ from those studies, differences in study design may explain the inconsistencies. Studies reporting hypoxia-induced increases in circulating biomarkers of intestinal barrier damage during exercise all used higher exercise intensities (50%–65% normoxic $\dot{V}O_{2peak}$ or 65% hypoxic $\dot{V}O_{2peak}$) and shorter hypoxic exposures (<3 h) than this study and, unlike this study, conducted measurements immediately before starting exercise (11, 12, 15, 73). Most of those studies also conducted exercise testing in the morning under fasted conditions rather than in the afternoon under fed conditions as in this study. Higher exercise intensities elicit more severe intestinal damage (75), and the effects of exercise on I-FABP concentrations may be blunted by feeding (76), likely due to greater splanchnic perfusion under fed conditions. Though speculative, diurnal fluctuations could also impact blood biomarker concentrations (77). The underpinning mechanisms and severity of hypoxia-induced intestinal barrier damage may also vary during the initial hours and days of hypoxia exposure. Our data add some insight into that time course and the effects of hypobaric hypoxia on intestinal barrier function absent the higher-intensity exercise investigated in previous trials. When considered together, these studies collectively suggest that acute (i.e., <36 h) exposure to hypobaric hypoxia modestly increases intestinal permeability within the small intestine and proximal colon and that effect may be exacerbated by moderate intensity exercise causing structural damage within the intestinal epithelium, especially when conducted under fasted conditions.

A novel finding of this study was that the hypobaric hypoxia-induced increase in intestinal permeability appeared to be preventable using dietary supplementation with fermentable fibers and polyphenols. Consuming polyphenol-rich foods (78, 79) and supplements (80) and fermentable fibers including inulin (81) and GOS (82) has been shown to reduce markers of intestinal permeability in generally healthy populations in whom intestinal permeability is elevated or experimentally increased. To our knowledge, similar supplementation strategies have not previously been tested in humans exposed to hypobaric hypoxia. However, rodent studies have shown that certain gut microbiota-targeted interventions containing various bacteria strains (9, 24) or bacteria strains combined with fermentable oligosaccharides such as fructo-oligosaccharide (23) and stachyose (25) can mitigate intestinal barrier damage in animals exposed to environmental hypoxia simulating 3,500–7,600 m. Findings of this study extend that concept to a human model by demonstrating that dietary interventions targeting the gut microbiota can maintain elements of gut barrier function in healthy adults exposed to hypobaric hypoxic stress.

Whether the beneficial effects of dietary supplementation on intestinal permeability were attributable to changes in gut microbiota composition and metabolic activity is unclear. Notably, fermentable fiber and polyphenol supplementation transiently reduced gut microbiota community richness and evenness. That response could be considered undesirable given that lower α-diversity is associated with community instability and certain disease states (83) and has been associated with greater stress-induced increases in intestinal permeability (84). However, multiple studies using the same or similar fiber sources as used herein have reported reductions in diversity (29, 85) despite recognized health benefits of the fibers (28). Indeed, no changes or reductions in community evenness are expected in fiber supplementation studies when the fibers used target a limited subset of higher abundant taxa (86) as is the case for inulin-type fructans, GOS, and resistant starch (28). Our use of multiple polyphenol sources may have also contributed to decreased α-diversity via anti-microbial effects of the compounds (87). For example, high dose cocoa-derived flavanol supplementation has been shown to reduce gut microbiota α-diversity by depleting rare taxa (88), whereas lower doses of polyphenols obtained from increasing intake of polyphenol-rich foods (78) and cranberry powder supplementation (89) did not effect α-diversity. Thus, the supplementation-induced decrease in gut microbiota community diversity likely reflects both a fermentable fiber-driven reduction in

community evenness and a polyphenol-mediated depletion of rare taxa.

Differential abundance analyses suggested that the fermentable fibers and polyphenols also targeted beneficial taxa that may promote gut barrier function. The supplementation-induced increase in *Bifidobacterium* is consistent with the well-established bifidogenic effects of various inulin-type fructans and GOS (29), with bifidogenic effects reported for various polyphenols such as cocoa (90) and with results of in vitro experiments using the same fiber and polyphenol blend (40). *Bifidobacterium* spp. are widely regarded as health promoting with some species having been shown to improve gut barrier function in various model systems (91). Those effects align with the inverse association between *Bifidobacterium* relative abundance and intestinal permeability observed in this study. The increase in *Ruminococcus* is consistent with our in vitro experiments (40) and with the known role for some *Ruminococcus* spp. in degradation of resistant starches, particularly maize-derived starches (92, 93). That role facilitates cross feeding by other gut commensals and production of butyrate, a preferred energy source for intestinal epithelial cells thought to promote gut barrier function (94). Inulin, GOS (95), and resistant starch (96) have also been shown to increase relative abundances of *Agathobacter*, itself a butyrate-producing genus.

Despite increases in SCFA-producing taxa, between-condition differences in fecal SCFA concentrations were not observed. However, that incongruency is consistent with other studies of fermentable fiber supplementation (85). Potential explanations include parallel decreases in relative abundance of SCFA-producing taxa such as *Mediterraneibacter* and *Sellimonas* and the rapid absorption and metabolism of microbiota-derived SCFA in situ which complicates interpretation of fecal concentrations (97). Of note, the O-PLS-DA analysis did identify urine acetate as being higher during fiber and polyphenol supplementation relative to both placebo conditions though the effect was weak and not statistically significant in univariate analysis.

A novel aspect of this study was the inclusion of in situ pH measurements throughout the gastrointestinal tract. Intestinal pH may provide an indirect measure of saccharolytic fermentation and SCFA production given that, as acidic compounds, SCFAs along with other fermentation byproducts reduce intestinal pH. Saccharolytic fermentation predominates in the proximal colon and decreases thereafter as carbohydrate sources are metabolized by the gut microbiota (98). The lower pH measured near the ileocecal junction during fermentable fiber and polyphenol supplementation relative to placebo is therefore consistent with a higher production of SCFA as would be expected following provision of rapidly fermentable oligofructose and GOS and based on in vitro observations showing increased SCFA production using the same compounds (40). Importantly, the supplementation-induced reduction in pH was correlated with lower intestinal permeability. Therefore, although underpinning mechanisms are likely multifactorial (39), increased fermentation and resulting SCFA production may have contributed to the prevention of hypoxia-induced intestinal permeability.

Increased fermentation and SCFA production could also help explain the higher oxygen saturation observed during fiber and polyphenol supplementation. In support, consuming fermentable substrate has been linked to improved hypoxic ventilatory responses in both humans (99) and rats (100). Most recently, a probiotic cocktail administered to adults sojourning at 3,800 m for 4 days increased mean daytime Sp_{O_2} by 3.6% and substantially reduced AMS symptoms (101). The probiotic-mediated increase in Sp_{O_2} in that study was correlated with the hypoxic ventilatory response and speculated to be related to an increase in SCFA production, though SCFA were not measured. Thus, while preliminary and requiring replication in large cohorts, these studies support the intriguing concept that gut microbiota modulation targeting beneficial microbes, and perhaps SCFA production, may provide novel strategies for improving host physiologic responses to hypobaric hypoxia in organ systems beyond the gut.

Our study, however, highlights that improving physiologic responses to hypobaric hypoxia by targeting the gut microbiota, and preventing increased intestinal permeability in particular, does not necessarily translate into clinically obvious benefits for subjective gastrointestinal symptoms or AMS incidence and severity. Notably, McKenna et al. (11) reported that increases in I-FABP, claudin-3, and LBP concentrations were positively associated with gastrointestinal symptom severity during moderate-intensity exercise (65% normoxic $\dot{V}O_{2peak}$) conducted under hypobaric hypoxia simulating 4,300 m. However, in contrast to our results, the hypoxia-induced increases in circulating concentrations of all three biomarkers in that study demonstrated large effect sizes. Considered together, these studies may suggest that more severe intestinal barrier damage than is induced by exposure to hypobaric hypoxia alone (up to 4300 m) may be required to induce gastrointestinal symptoms. The apparent disconnect between intestinal permeability and AMS symptoms in the present study is consistent with two studies that have likewise failed to observe associations between AMS and measures of intestinal barrier function during exposure hypobaric hypoxia (4,300–4,600 m) (14, 74). In addition, although upregulation of various cytokine pathways within the gut including IL-6 have been implicated as mechanisms by which the gut microbiota mediates hypoxia-induced intestinal barrier damage and dysfunction (8) and higher IL-6 concentrations have been associated with more severe AMS symptomology (102, 103), the hypobaric hypoxia-induced increases in circulating IL-6 and intestinal permeability in this study were small. When interpreted within the context of the multifactorial etiology of AMS (20), these results collectively suggest that intestinal barrier dysfunction may not play a prominent role in AMS development at altitudes up to 4,300 m.

That conclusion is somewhat complicated by the possibility that the increase in gastrointestinal symptoms due to fermentable fiber and polyphenol supplementation observed in this study masked any clinical benefit of maintaining intestinal permeability and even contributed to the intervention-mediated increase in AMS symptoms. Notably, gastrointestinal symptoms associated with fiber and polyphenol supplementation were only observed during chamber residence, which may suggest that hypobaric hypoxia increases sensitivity to byproducts of fermentation such as various gases. Studies investigating the effects of gut-targeted and

gut microbiota-targeted interventions expected to have minimal side effects therefore remain warranted, particularly in populations likely to engage in moderate-intensity or more vigorous physical activity at high altitudes such as military personnel and adventure travelers. The design and testing of those gut microbiota-targeted interventions should consider the potential for increased gastrointestinal sensitivity to certain interventions at high altitudes and measure additional outcomes that may be impaired by hypobaric hypoxia and improved by modulation of the gut microbiota and intestinal barrier such as immune or cognitive function.

Study strengths include the randomized, placebo-controlled design that included blinding to both the intervention and environmental conditions, tightly controlled diet, inclusion of complementary measures of intestinal barrier function, and integration of gut microbiota composition and metabolic activity with physiologic and subjective measures of responses to hypobaric hypoxia. The study also has several limitations. Foremost is high participant attrition that was not equally balanced across the condition orders. The unbalanced attrition resulted in a completer cohort that was underpowered for some outcomes and in whom the fermentable fiber and polyphenol intervention preceded the placebo-altitude condition for all but one volunteer. Both factors could result in an underestimation of the effects of hypobaric hypoxia and dietary supplementation on study outcomes. However, that concern is somewhat reduced by the general consistency of the ITT and CC analyses and because significant differences in intestinal permeability, microbiota composition and other measures suggest sufficient power were achieved for many outcomes. Effects of dietary supplementation could also be underestimated due to the short washout period and resulting potential for carryover effects. Of note, baseline, presupplementation differences in gut microbiota composition were generally not detected for any diversity metric, relative or absolute abundance of any taxa or fecal concentrations of any SCFA with the exceptions of fecal butyrate concentrations, Faith's PD, and *Bifidobacterium* absolute abundance, all of which differed between the PL + HA and PL + LA conditions. Between-condition comparisons for outcomes measured during chamber residence could also be impacted by differences in dietary intake during the placebo low-altitude condition relative to the two high-altitude conditions. However, comparisons between the two high-altitude conditions were not affected as dietary intake did not differ. An additional limitation is that plasma volume changes, urine metabolic profiles, and absolute bacterial abundances were only measured in a subset of participants. Furthermore, bacterial abundances were consistently lowest in the PL + LA condition independent of timepoint, which complicated determining whether supplementation-induced differences in gut microbiota reflected changes in absolute bacterial load. Finally, the study cohort was predominantly healthy men which may limit generalizability.

The gut microbiota is increasingly implicated in host responses to environmental stress. However, few studies have investigated whether dietary interventions targeting the human gut microbiota can improve how one responds to environmental stress. Study results help address that gap by demonstrating a dietary intervention containing a blend of fermentable fiber and polyphenol sources designed to target the gut microbiota can prevent increases in intestinal permeability during exposure to hypobaric hypoxia. Though the practical benefits of this supplementation regimen for high-altitude sojourners are unclear and perhaps limited, results do support the continued exploration of novel intervention strategies aiming to promote performance in austere environments, including high terrestrial altitudes, by targeting the gut microbiota.

DATA AVAILABILITY

Data will be made available upon reasonable request.

SUPPLEMENTAL MATERIAL

Supplemental Figs. S1–S5 and Supplemental Tables S1–S11: https://doi.org/10.6084/m9.figshare.26506825.

ACKNOWLEDGMENTS

We thank the study volunteers for their efforts and dedication to the study. We also thank Dr. Jennifer Rood and the team at the Pennington Biomedical Research Center for performing urine and blood biochemistries, Laura Lutz and the team at the US Army Research Institute of Environmental Medicine for performing blood biochemistries, and the team at EzBiome for conducting the amplicon sequencing and associated bioinformatics. We thank Kenneth Racicot for contributions to the study design; Danielle Anderson for designing, sourcing, and manufacturing the intervention products; and Dr. Andrew Young for editorial support. We thank Michael Aponte, Christopher Carrigan, Rebecca Cherian, Gino Estrada, Laurel Doherty, Kyle Driver, Michele Mahoney, Susan McGraw, Julie McNiff, Adam Nixon, Dylan Nugent, Frank Petrassi, Cornal Pounds, Jasmine Robinson-Espinosa, Marcus Sanchez, Claudia Toussaint, Claire Whitney, and all the USARIEM hypobaric chamber operators for their technical support and contributions to the study. Finally, we thank Clasado Bioscienecs, Ingredion Inc., Beneo GmbH, and Naturex for supplying ingredients for the study. Graphical Abstract created with a licensed version of BioRender.com.

GRANTS

This work was supported by the US Defense Health Program Joint Program Committee-5/Military Operational Medicine Research Program under Grant No. OM205010 and the US Army Research Laboratory—Army Research Office under grant number W911NF-19-0095.

DISCLAIMERS

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Army or the Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations. Approved for public release; distribution is unlimited. PNR was supported by an appointment to the US Army Research Institute of Environmental Medicine administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the US Department of Energy and the US Army Medical Research and Development Command.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.P.K., A.W., G.R.G., H.R.L., G.E.G., and J.W.S. conceived and designed research; J.P.K., H.S.F., P.N.R., M.W., A.J.K., B.S., A.W., and J.W.S. performed experiments; J.P.K., H.S.F., and A.W. analyzed data; J.P.K. and A.W. interpreted results of experiments; J.P.K. and A.W. prepared figures; J.P.K. drafted manuscript; J.P.K., H.S.F., P.N.R., M.W., A.J.K., B.S., A.W., G.R.G., H.R.L., G.E.G., and J.W.S. edited and revised manuscript; J.P.K., H.S.F., P.N.R., M.W., A.J.K., B.S., A.W., G.R.G., H.R.L., G.E.G., and J.W.S. approved final version of manuscript.

REFERENCES

1. **Karl JP**, **Hatch AM**, **Arcidiacono SM**, **Pearce SC**, **Pantoja-Feliciano IG**, **Doherty LA**, **Soares JW.** Effects of psychological, environmental and physical stressors on the gut microbiota. *Front Microbiol* 9: 2013, 2018. doi:10.3389/fmicb.2018.02013.
2. **Pasiakos SM**, **Karl JP**, **Margolis LM.** Challenging traditional carbohydrate intake recommendations for optimizing performance at high altitude. *Curr Opin Clin Nutr Metab Care* 24: 483–489, 2021. doi:10.1097/MCO.0000000000000782.
3. **Rosenberg E**, **Zilber-Rosenberg I.** Do microbiotas warm their hosts? *Gut Microbes* 7: 283–285, 2016. doi:10.1080/19490976.2016.1182294.
4. **Netzer N**, **Strohl K**, **Faulhaber M**, **Gatterer H**, **Burtscher M.** Hypoxia-related altitude illnesses. *J Travel Med* 20: 247–255, 2013. doi:10.1111/jtm.12017.
5. **Li M**, **Han T**, **Zhang W**, **Li W**, **Hu Y**, **Lee SK.** Simulated altitude exercise training damages small intestinal mucosa barrier in the rats. *J Exerc Rehabil* 14: 341–348, 2018. doi:10.12965/jer.1835128.064.
6. **Luo H**, **Zhou DJ**, **Chen Z**, **Zhou QQ**, **Wu K**, **Tian K**, **Li ZW**, **Xiao ZL.** Establishment and evaluation of an experimental rat model for high-altitude intestinal barrier injury. *Exp Ther Med* 13: 475–482, 2017. doi:10.3892/etm.2016.4012.
7. **Zhang F**, **Wu W**, **Deng Z**, **Zheng X**, **Zhang J**, **Deng S**, **Chen J**, **Ma Q**, **Wang Y**, **Yu X**, **Kang S**, **Wang X.** High altitude increases the expression of hypoxia-inducible factor 1α and inducible nitric oxide synthase with intest-inal mucosal barrier failure in rats. *Int J Clin Exp Pathol* 8: 5189–5195, 2015.
8. **Li Y**, **Wang Y**, **Shi F**, **Zhang X**, **Zhang Y**, **Bi K**, **Chen X**, **Li L**, **Diao H.** Phospholipid metabolites of the gut microbiota promote hypoxia-induced intestinal injury via CD1d-dependent γδ T cells. *Gut Microbes* 14: 2096994, 2022. doi:10.1080/19490976.2022.2096994.
9. **Wan Z**, **Zhang X**, **Jia X**, **Qin Y**, **Sun N**, **Xin J**, **Zeng Y**, **Jing B**, **Fang J**, **Pan K**, **Zeng D**, **Bai Y**, **Wang H**, **Ma H**, **Ni X.** Lactobacillus johnsonii YH1136 plays a protective role against endogenous pathogenic bacteria induced intestinal dysfunction by reconstructing gut microbiota in mice exposed at high altitude. *Front Immunol* 13: 1007737, 2022. doi:10.3389/fimmu.2022.1007737.
10. **Wang Y**, **Shi Y**, **Li W**, **Wang S**, **Zheng J**, **Xu G**, **Li G**, **Shen X**, **Yang J.** Gut microbiota imbalance mediates intestinal barrier damage in high-altitude exposed mice. *FEBS J* 289: 4850–4868, 2022. doi:10.1111/febs.16409.
11. **McKenna ZJ**, **Fennel ZJ**, **Berkemeier QN**, **Nava RC**, **Amorim FT**, **Deyhle MR**, **Mermier CM.** Exercise in hypobaric hypoxia increases markers of intestinal injury and symptoms of gastrointestinal distress. *Exp Physiol* 107: 326–336, 2022. doi:10.1113/EP090266.
12. **Hill GW**, **Gillum TL**, **Lee BJ**, **Romano PA**, **Schall ZJ**, **Hamilton AM**, **Kuennen MR.** Prolonged treadmill running in normobaric hypoxia causes gastrointestinal barrier permeability and elevates circulating levels of pro- and anti-inflammatory cytokines. *Appl Physiol Nutr Metab* 45: 376–386, 2020. doi:10.1139/apnm-2019-0378.
13. **Fruehauf H**, **Vavricka SR**, **Lutz TA**, **Gassmann M**, **Wojtal KA**, **Erb A**, **Maggiorini M**, **Schwizer W**, **Fried M**, **Fox M**, **Goetze O**, **Greuter T.** Evaluation of acute mountain sickness by unsedated transnasal esophagogastroduodenoscopy at high altitude. *Clin Gastroenterol Hepatol* 18: 2218–2225.e2, 2020. doi:10.1016/j.cgh.2019.11.036.
14. **Karl JP**, **Berryman CE**, **Young AJ**, **Radcliffe PN**, **Branck TA**, **Pantoja-Feliciano IG**, **Rood JC**, **Pasiakos SM.** Associations between the gut microbiota and host responses to high altitude. *Am J Physiol Gastrointest Liver Physiol* 315: G1003–G1015, 2018. doi:10.1152/ajpgi.00253.2018.
15. **Machado P**, **Caris A**, **Santos S**, **Silva E**, **Oyama L**, **Tufik S**, **Santos R.** Moderate exercise increases endotoxin concentration in hypoxia but not in normoxia: a controlled clinical trial. *Medicine (Baltimore)* 96: e5504, 2017. doi:10.1097/MD.0000000000005504.
16. **McKenna ZJ**, **Gorini Pereira F**, **Gillum TL**, **Amorim FT**, **Deyhle MR**, **Mermier CM.** High-altitude exposures and intestinal barrier dysfunction. *Am J Physiol Regul Integr Comp Physiol* 322: R192–R203, 2022. doi:10.1152/ajpregu.00270.2021.
17. **Song TT**, **Bi YH**, **Gao YQ**, **Huang R**, **Hao K**, **Xu G**, **Tang JW**, **Ma ZQ**, **Kong FP**, **Coote JH**, **Chen XQ**, **Du JZ.** Systemic pro-inflammatory response facilitates the development of cerebral edema during short hypoxia. *J Neuroinflammation* 13: 63, 2016. doi:10.1186/s12974-016-0528-4.
18. **Zhou Y**, **Huang X**, **Zhao T**, **Qiao M**, **Zhao X**, **Zhao M**, **Xu L**, **Zhao Y**, **Wu L**, **Wu K**, **Chen R**, **Fan M**, **Zhu L.** Hypoxia augments LPS-induced inflammation and triggers high altitude cerebral edema in mice. *Brain Behav Immun* 64: 266–275, 2017. doi:10.1016/j.bbi.2017.04.013.
19. **Anand AC**, **Sashindran VK**, **Mohan L.** Gastrointestinal problems at high altitude. *Trop Gastroenterol* 27: 147–153, 2006.
20. **Luks AM**, **Swenson ER**, **Bärtsch P.** Acute high-altitude sickness. *Eur Respir Rev* 26: 160096, 2017. doi:10.1183/16000617.0096-2016.
21. **Ghosh S**, **Whitley CS**, **Haribabu B**, **Jala VR.** Regulation of intestinal barrier function by microbial metabolites. *Cell Mol Gastroenterol Hepatol* 11: 1463–1482, 2021. doi:10.1016/j.jcmgh.2021.02.007.
22. **de Vos WM**, **Tilg H**, **Van Hul M**, **Cani PD.** Gut microbiome and health: mechanistic insights. *Gut* 71: 1020–1032, 2022. doi:10.1136/gutjnl-2021-326789.
23. **Khanna K**, **Mishra KP**, **Chanda S**, **Ganju L**, **Singh SB**, **Kumar B.** Effect of synbiotics on amelioration of intestinal inflammation under hypobaric hypoxia. *High Alt Med Biol* 22: 32–44, 2021. doi:10.1089/ham.2020.0062.
24. **Song K**, **Ling H**, **Wang L**, **Tian P**, **Jin X**, **Zhao J**, **Chen W**, **Wang G**, **Bi Y.** Lactobacillus delbrueckii subsp. bulgaricus alleviates acute injury in hypoxic mice. *Nutrients* 16: 1465, 2024. doi:10.3390/nu16101465.
25. **Ren D**, **Ding M**, **Su J**, **Ye J**, **He X**, **Zhang Y**, **Shang X.** Stachyose in combination with L. rhamnosus GG ameliorates acute hypobaric hypoxia-induced intestinal barrier dysfunction through alleviating inflammatory response and oxidative stress. *Free Radic Biol Med* 212: 505–519, 2024. doi:10.1016/j.freeradbiomed.2024.01.009.
26. **Kleessen B**, **Schroedl W**, **Stueck M**, **Richter A**, **Rieck O**, **Krueger M.** Microbial and immunological responses relative to high-altitude exposure in mountaineers. *Med Sci Sports Exerc* 37: 1313–1318, 2005. doi:10.1249/01.mss.0000174888.22930.e0.
27. **Pearce SC**, **Karl JP**, **Weber GJ.** Effects of short-chain fatty acids on intestinal function in an enteroid model of hypoxia. *Front Physiol* 13: 1056233, 2022. doi:10.3389/fphys.2022.1056233.
28. **Gibson GR**, **Hutkins R**, **Sanders ME**, **Prescott SL**, **Reimer RA**, **Salminen SJ**, **Scott K**, **Stanton C**, **Swanson KS**, **Cani PD**, **Verbeke K**, **Reid G.** Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 14: 491–502, 2017. doi:10.1038/nrgastro.2017.75.
29. **So D**, **Whelan K**, **Rossi M**, **Morrison M**, **Holtmann G**, **Kelly JT**, **Shanahan ER**, **Staudacher HM**, **Campbell KL.** Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. *Am J Clin Nutr* 107: 965–983, 2018. doi:10.1093/ajcn/nqy041.
30. **Flint HJ.** The impact of nutrition on the human microbiome. *Nutr Rev* 70, *Suppl* 1: S10–S13, 2012. doi:10.1111/j.1753-4887.2012.00499.x.
31. **Macfarlane GT**, **Macfarlane S.** Bacteria, colonic fermentation, and gastrointestinal health. *J AOAC Int* 95: 50–60, 2012. doi:10.5740/jaoacint.sge_macfarlane.
32. **Blaak EE**, **Canfora EE**, **Theis S**, **Frost G**, **Groen AK**, **Mithieux G**, **Nauta A**, **Scott K**, **Stahl B**, **van Harsselaar J**, **van Tol R**, **Vaughan EE**, **Verbeke K.** Short chain fatty acids in human gut and metabolic

health. *Benef Microbes* 11: 411–455, 2020. doi:10.3920/BM2020.0057.
33. **Scalbert A**, **Williamson G.** Dietary intake and bioavailability of polyphenols. *J Nutr* 130: 2073S–2085S, 2000. doi:10.1093/jn/130.8.2073S.
34. **Duda-Chodak A**, **Tarko T**, **Satora P**, **Sroka P.** Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review. *Eur J Nutr* 54: 325–341, 2015. doi:10.1007/s00394-015-0852-y.
35. **Duenas M**, **Munoz-Gonzalez I**, **Cueva C**, **Jimenez-Giron A**, **Sanchez-Patan F**, **Santos-Buelga C**, **Moreno-Arribas MV**, **Bartolome B.** A survey of modulation of gut microbiota by dietary polyphenols. *Biomed Res Int* 2015: 850902, 2015. doi:10.1155/2015/850902.
36. **Tuohy KM**, **Conterno L**, **Gasperotti M**, **Viola R.** Up-regulating the human intestinal microbiome using whole plant foods, polyphenols, and/or fiber. *J Agric Food Chem* 60: 8776–8782, 2012. doi:10.1021/jf2053959.
37. **Bernardi S**, **Del Bo C**, **Marino M**, **Gargari G**, **Cherubini A**, **Andrés-Lacueva C**, **Hidalgo-Liberona N**, **Peron G**, **González-Dominguez R**, **Kroon P**, **Kirkup B**, **Porrini M**, **Guglielmetti S**, **Riso P.** Polyphenols and intestinal permeability: rationale and future perspectives. *J Agric Food Chem* 68: 1816–1829, 2020. doi:10.1021/acs.jafc.9b02283.
38. **Camilleri M**, **Lyle BJ**, **Madsen KL**, **Sonnenburg J**, **Verbeke K**, **Wu GD.** Role for diet in normal gut barrier function: developing guidance within the framework of food-labeling regulations. *Am J Physiol Gastrointest Liver Physiol* 317: G17–G39, 2019. doi:10.1152/ajpgi.00063.2019.
39. **Mavrogeni ME**, **Asadpoor M**, **Henricks PAJ**, **Keshavarzian A**, **Folkerts G**, **Braber S.** Direct action of non-digestible oligosaccharides against a leaky gut. *Nutrients* 14: 4699, 2022. doi:10.3390/nu14214699.
40. **Whitman JA**, **Doherty LA**, **Pantoja-Feliciano de Goodfellow IG**, **Racicot K**, **Anderson DJ**, **Kensil K**, **Karl JP**, **Gibson GR**, **Soares JW.** In vitro fermentation shows polyphenol and fiber blends have an additive beneficial effect on gut microbiota states. *Nutrients* 16: 1159, 2024. doi:10.3390/nu16081159.
41. **So D**, **Gibson PR**, **Muir JG**, **Yao CK.** Dietary fibres and IBS: translating functional characteristics to clinical value in the era of personalised medicine. *Gut* 70: 2383–2394, 2021. doi:10.1136/gutjnl-2021-324891.
42. **Grabitske HA**, **Slavin JL.** Gastrointestinal effects of low-digestible carbohydrates. *Crit Rev Food Sci Nutr* 49: 327–360, 2009. doi:10.1080/10408390802067126.
43. **Zamora-Ros R**, **Knaze V**, **Rothwell JA**, **Hémon B**, **Moskal A**, **Overvad K et al.** Dietary polyphenol intake in Europe: the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Eur J Nutr* 55: 1359–1375, 2016. doi:10.1007/s00394-015-0950-x.
44. **Harris JA**, **Benedict FG.** A biometric study of human basal metabolism. *Proc Natl Acad Sci USA* 4: 370–373, 1918. doi:10.1073/pnas.4.12.370.
45. **Mifflin MD**, **St Jeor ST**, **Hill LA**, **Scott BJ**, **Daugherty SA**, **Koh YO.** A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr* 51: 241–247, 1990. doi:10.1093/ajcn/51.2.241.
46. **Young AJ**, **Cymerman A**, **Burse RL.** The influence of cardiorespiratory fitness on the decrement in maximal aerobic power at high altitude. *Eur J Appl Physiol Occup Physiol* 54: 12–15, 1985. doi:10.1007/BF00426291.
47. **Tran K**, **Brun R**, **Kuo B.** Evaluation of regional and whole gut motility using the wireless motility capsule: relevance in clinical practice. *Therap Adv Gastroenterol* 5: 249–260, 2012. doi:10.1177/1756283x12437874.
48. **Anderson AD**, **Poon P**, **Greenway GM**, **MacFie J.** A simple method for the analysis of urinary sucralose for use in tests of intestinal permeability. *Ann Clin Biochem* 42: 224–226, 2005. doi:10.1258/0004563053857923.
49. **Marsilio R**, **D'Antiga L**, **Zancan L**, **Dussini N**, **Zacchello F.** Simultaneous HPLC determination with light-scattering detection of lactulose and mannitol in studies of intestinal permeability in pediatrics. *Clin Chem* 44: 1685–1691, 1998. doi:10.1093/clinchem/44.8.1685.
50. **Khoshbin K**, **Khanna L**, **Maselli D**, **Atieh J**, **Breen-Lyles M**, **Arndt K**, **Rhoten D**, **Dyer RB**, **Singh RJ**, **Nayar S**, **Bjerkness S**, **Harmsen WS**, **Busciglio I**, **Camilleri M.** Development and validation of test for "leaky gut" small intestinal and colonic permeability using sugars in healthy adults. *Gastroenterology* 161: 463–475.e13, 2021. doi:10.1053/j.gastro.2021.04.020.
51. **Wijeyesekera A**, **Wagner J**, **De Goffau M**, **Thurston S**, **Rodrigues Sabino A**, **Zaher S**, **White D**, **Ridout J**, **Peters MJ**, **Ramnarayan P**, **Branco RG**, **Torok ME**, **Valla F**, **Meyer R**, **Klein N**, **Frost G**, **Parkhill J**, **Holmes E**, **Pathan N.** Multi-compartment profiling of bacterial and host metabolites identifies intestinal dysbiosis and its functional consequences in the critically ill child. *Crit Care Med* 47: e727–e734, 2019. doi:10.1097/CCM.0000000000003841.
52. **Trygg J**, **Wold S.** Orthogonal projections to latent structures (O-PLS). *J Chemom* 16: 119–128, 2002. doi:10.1002/cem.695.
53. **Dill DB**, **Costill DL.** Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 37: 247–248, 1974. doi:10.1152/jappl.1974.37.2.247.
54. **Karl JP**, **Armstrong NJ**, **McClung HL**, **Player RA**, **Rood JC**, **Racicot K**, **Soares JW**, **Montain SJ.** A diet of U.S. military food rations alters gut microbiota composition and does not increase intestinal permeability. *J Nutr Biochem* 72: 108217, 2019. doi:10.1016/j.jnutbio.2019.108217.
55. **Brumfield KD**, **Raupp MJ**, **Haji D**, **Simon C**, **Graf J**, **Cooley JR**, **Janton ST**, **Meister RC**, **Huq A**, **Colwell RR**, **Hasan NA.** Gut microbiome insights from 16S rRNA analysis of 17-year periodical cicadas (Hemiptera: Magicicada spp.) Broods II, VI, and X. *Sci Rep* 12: 16967, 2022. doi:10.1038/s41598-022-20527-7.
56. **Yoon SH**, **Ha SM**, **Kwon S**, **Lim J**, **Kim Y**, **Seo H**, **Chun J.** Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 67: 1613–1617, 2017. doi:10.1099/ijsem.0.001755.
57. **Lee B**, **Moon T**, **Yoon S**, **Weissman T.** DUDE-Seq: fast, flexible, and robust denoising for targeted amplicon sequencing. *PLoS One* 12: e0181463, 2017. doi:10.1371/journal.pone.0181463.
58. **Edgar RC**, **Haas BJ**, **Clemente JC**, **Quince C**, **Knight R.** UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 27: 2194–2200, 2011. doi:10.1093/bioinformatics/btr381.
59. **Rognes T**, **Flouri T**, **Nichols B**, **Quince C**, **Mahé F.** VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4: e2584, 2016. doi:10.7717/peerj.2584.
60. **Edgar RC.** UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 10: 996–998, 2013. doi:10.1038/nmeth.2604.
61. **Kennedy JM**, **De Silva A**, **Walton GE**, **Poveda C**, **Gibson GR.** Comparison of prebiotic candidates in ulcerative colitis using an in vitro fermentation model. *J Appl Microbiol* 135: lxae034, 2024. doi:10.1093/jambio/lxae034.
62. **Francis CY**, **Morris J**, **Whorwell PJ.** The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther* 11: 395–402, 1997. doi:10.1046/j.1365-2036.1997.142318000.x.
63. **Eypasch E**, **Williams JI**, **Wood-Dauphinee S**, **Ure BM**, **Schmülling C**, **Neugebauer E**, **Troidl H.** Gastrointestinal Quality of Life Index: development, validation and application of a new instrument. *Br J Surg* 82: 216–222, 1995. doi:10.1002/bjs.1800820229.
64. **Beidleman BA**, **Muza SR**, **Fulco CS**, **Rock PB**, **Cymerman A.** Validation of a shortened electronic version of the environmental symptoms questionnaire. *High Alt Med Biol* 8: 192–199, 2007. doi:10.1089/ham.2007.1016.
65. **Sampson JB**, **Cymerman A**, **Burse RL**, **Maher JT**, **Rock PB.** Procedures for the measurement of acute mountain sickness. *Aviat Space Environ Med* 54: 1063–1073, 1983.
66. **Zhou H**, **He K**, **Chen J**, **Zhang X.** LinDA: linear models for differential abundance analysis of microbiome compositional data. *Genome Biol* 23: 95, 2022. doi:10.1186/s13059-022-02655-5.
67. **Bakdash JZ**, **Marusich LR.** Repeated measures correlation. *Front Psychol* 8: 456, 2017 [Erratum in *Front Psychol* 10: 1201, 2019]. doi:10.3389/fpsyg.2017.00456.
68. **Ticinesi A**, **Guerra A**, **Nouvenne A**, **Meschi T**, **Maggi S.** Disentangling the complexity of nutrition, frailty and gut microbial pathways during aging: a focus on hippuric acid. *Nutrients* 15: 1138, 2023. doi:10.3390/nu15051138.
69. **Camilleri M**, **Nadeau A**, **Lamsam J**, **Nord SL**, **Ryks M**, **Burton D**, **Sweetser S**, **Zinsmeister AR**, **Singh R.** Understanding measurements of intestinal permeability in healthy humans with urine

lactulose and mannitol excretion. *Neurogastroenterol Motil* 22: e15–e26, 2010. doi:10.1111/j.1365-2982.2009.01361.x.

70. **Rao AS**, **Camilleri M**, **Eckert DJ**, **Busciglio I**, **Burton DD**, **Ryks M**, **Wong BS**, **Lamsam J**, **Singh R**, **Zinsmeister AR.** Urine sugars for in vivo gut permeability: validation and comparisons in irritable bowel syndrome-diarrhea and controls. *Am J Physiol Gastrointest Liver Physiol* 301: G919–G928, 2011. doi:10.1152/ajpgi.00168.2011.
71. **Bischoff SC**, **Barbara G**, **Buurman W**, **Ockhuizen T**, **Schulzke JD**, **Serino M**, **Tilg H**, **Watson A**, **Wells JM.** Intestinal permeability–a new target for disease prevention and therapy. *BMC Gastroenterol* 14: 189, 2014. doi:10.1186/s12876-014-0189-7.
72. **McKenna ZJ**, **Ducharme JB**, **Berkemeier QN**, **Specht JW**, **Fennel ZJ**, **Gillum TL**, **Deyhle MR**, **Amorim FT**, **Mermier CM.** Ibuprofen increases markers of intestinal barrier injury but suppresses inflammation at rest and after exercise in hypoxia. *Med Sci Sports Exerc* 55: 141–150, 2023. doi:10.1249/MSS.0000000000003032.
73. **Lee BJ**, **Thake CD.** Heat and hypoxic acclimation increase monocyte heat shock protein 72 but do not attenuate inflammation following hypoxic exercise. *Front Physiol* 8: 811, 2017. doi:10.3389/fphys.2017.00811.
74. **McKenna ZJ**, **Bellovary BN**, **Ducharme JB**, **Deyhle MR**, **Wells AD**, **Fennel ZJ**, **Specht JW**, **Houck JM**, **Mayschak TJ**, **Mermier CM.** Circulating markers of intestinal barrier injury and inflammation following exertion in hypobaric hypoxia. *Eur J Sport Sci* 23: 2002–2010, 2023. doi:10.1080/17461391.2023.2203107.
75. **Costa RJS**, **Snipe RMJ**, **Kitic CM**, **Gibson PR.** Systematic review: exercise-induced gastrointestinal syndrome-implications for health and intestinal disease. *Aliment Pharmacol Ther* 46: 246–265, 2017. doi:10.1111/apt.14157.
76. **Edinburgh RM**, **Hengist A**, **Smith HA**, **Travers RL**, **Koumanov F**, **Betts JA**, **Thompson D**, **Walhin JP**, **Wallis GA**, **Hamilton DL**, **Stevenson EJ**, **Tipton KD**, **Gonzalez JT.** Preexercise breakfast ingestion versus extended overnight fasting increases postprandial glucose flux after exercise in healthy men. *Am J Physiol Endocrinol Physiol* 315: E1062–E1074, 2018. doi:10.1152/ajpendo.00163.2018.
77. **Martchenko A**, **Martchenko SE**, **Biancolin AD**, **Brubaker PL.** Circadian rhythms and the gastrointestinal tract: relationship to metabolism and gut hormones. *Endocrinology* 161: bqaa167, 2020. doi:10.1210/endocr/bqaa167.
78. **Del Bo C**, **Bernardi S**, **Cherubini A**, **Porrini M**, **Gargari G**, **Hidalgo-Liberona N**, **González-Domínguez R**, **Zamora-Ros R**, **Peron G**, **Marino M**, **Gigliotti L**, **Winterbone MS**, **Kirkup B**, **Kroon PA**, **Andres-Lacueva C**, **Guglielmetti S**, **Riso P.** A polyphenol-rich dietary pattern improves intestinal permeability, evaluated as serum zonulin levels, in older subjects: the MaPLE randomised controlled trial. *Clin Nutr* 40: 3006–3018, 2021. doi:10.1016/j.clnu.2020.12.014.
79. **Nocella C**, **Cavarretta E**, **Fossati C**, **Pigozzi F**, **Quaranta F**, **Peruzzi M**, **De Grandis F**, **Costa V**, **Sharp C**, **Manara M**, **Nigro A**, **Cammisotto V**, **Castellani V**, **Picchio V**, **Sciarretta S**, **Frati G**, **Bartimoccia S**, **D'Amico A**, **Carnevale R.** Dark chocolate intake positively modulates gut permeability in elite football athletes: a randomized controlled study. *Nutrients* 15: 4203, 2023. doi:10.3390/nu15194203.
80. **Nieman DC**, **Kay CD**, **Rathore AS**, **Grace MH**, **Strauch RC**, **Stephan EH**, **Sakaguchi CA**, **Lila MA.** Increased plasma levels of gut-derived phenolics linked to walking and running following two weeks of flavonoid supplementation. *Nutrients* 10: 1718, 2018. doi:10.3390/nu10111718.
81. **Russo F**, **Linsalata M**, **Clemente C**, **Chiloiro M**, **Orlando A**, **Marconi E**, **Chimienti G**, **Riezzo G.** Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers. *Nutr Res* 32: 940–946, 2012. doi:10.1016/j.nutres.2012.09.010.
82. **Krumbeck JA**, **Rasmussen HE**, **Hutkins RW**, **Clarke J**, **Shawron K**, **Keshavarzian A**, **Walter J.** Probiotic Bifidobacterium strains and galactooligosaccharides improve intestinal barrier function in obese adults but show no synergism when used together as synbiotics. *Microbiome* 6: 121, 2018. doi:10.1186/s40168-018-0494-4.
83. **McBurney MI**, **Davis C**, **Fraser CM**, **Schneeman BO**, **Huttenhower C**, **Verbeke K**, **Walter J**, **Latulippe ME.** Establishing what constitutes a healthy human gut microbiome: state of the science, regulatory considerations, and future directions. *J Nutr* 149: 1882–1895, 2019. doi:10.1093/jn/nxz154.
84. **Karl JP**, **Margolis LM**, **Madslien EH**, **Murphy NE**, **Castellani JW**, **Gundersen Y**, **Hoke AV**, **Levangie MW**, **Kumar R**, **Chakraborty N**, **Gautam A**, **Hammamieh R**, **Martini S**, **Montain SJ**, **Pasiakos SM.** Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress. *Am J Physiol Gastrointest Liver Physiol* 312: G559–G571, 2017. doi:10.1152/ajpgi.00066.2017.
85. **Vinelli V**, **Biscotti P**, **Martini D**, **Del Bo C**, **Marino M**, **Meroño T**, **Nikoloudaki O**, **Calabrese FM**, **Turroni S**, **Taverniti V**, **Union Caballero A**, **Andres-Lacueva C**, **Porrini M**, **Gobbetti M**, **De Angelis M**, **Brigidi P**, **Pinart M**, **Nimptsch K**, **Guglielmetti S**, **Riso P.** Effects of dietary fibers on short-chain fatty acids and gut microbiota composition in healthy adults: a systematic review. *Nutrients* 14: 2559, 2022. doi:10.3390/nu14132559.
86. **Cantu-Jungles TM**, **Hamaker BR.** Tuning expectations to reality: don't expect increased gut microbiota diversity with dietary fiber. *J Nutr* 153: 3156–3163, 2023. doi:10.1016/j.tjnut.2023.09.001.
87. **Wan MLY**, **Co VA**, **El-Nezami H.** Dietary polyphenol impact on gut health and microbiota. *Crit Rev Food Sci Nutr* 61: 690–711, 2021. doi:10.1080/10408398.2020.1744512.
88. **Suther C**, **Alba B**, **Yurkevicius BR**, **Radcliffe PN**, **Fagnant HS**, **Castellani JW**, **Karl JP.** Effects of short-term, high-dose cocoa-derived flavanol supplementation on gut microbiota composition: secondary findings from a randomized, double-blind, placebo-controlled crossover study. *J Nutr Sci* 13: e22, 2024. doi:10.1017/jns.2024.17.
89. **Rodríguez-Morató J**, **Matthan NR**, **Liu J**, **de la Torre R**, **Chen CO.** Cranberries attenuate animal-based diet-induced changes in microbiota composition and functionality: a randomized crossover controlled feeding trial. *J Nutr Biochem* 62: 76–86, 2018. doi:10.1016/j.jnutbio.2018.08.019.
90. **Tzounis X**, **Rodriguez-Mateos A**, **Vulevic J**, **Gibson GR**, **Kwik-Uribe C**, **Spencer JP.** Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. *Am J Clin Nutr* 93: 62–72, 2011. doi:10.3945/ajcn.110.000075.
91. **Abdulqadir R**, **Engers J**, **Al-Sadi R.** Role of bifidobacterium in modulating the intestinal epithelial tight junction barrier: current knowledge and perspectives. *Curr Dev Nutr* 7: 102026, 2023. doi:10.1016/j.cdnut.2023.102026.
92. **Ze X**, **Duncan SH**, **Louis P**, **Flint HJ.** Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon. *ISME J* 6: 1535–1543, 2012. doi:10.1038/ismej.2012.4.
93. **Martínez I**, **Kim J**, **Duffy PR**, **Schlegel VL**, **Walter J.** Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS One* 5: e15046, 2010. doi:10.1371/journal.pone.0015046.
94. **Kelly CJ**, **Zheng L**, **Campbell EL**, **Saeedi B**, **Scholz CC**, **Bayless AJ**, **Wilson KE**, **Glover LE**, **Kominsky DJ**, **Magnuson A**, **Weir TL**, **Ehrentraut SF**, **Pickel C**, **Kuhn KA**, **Lanis JM**, **Nguyen V**, **Taylor CT**, **Colgan SP.** Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe* 17: 662–671, 2015. doi:10.1016/j.chom.2015.03.005.
95. **Song H**, **Jeon D**, **Unno T.** Evaluation of prebiotics through an in vitro gastrointestinal digestion and fecal fermentation experiment: further idea on the implementation of machine learning technique. *Foods* 11: 2490, 2022. doi:10.3390/foods11162490.
96. **Chen R**, **Zhang C**, **Xu F**, **Yu L**, **Tian F**, **Chen W**, **Zhai Q.** Meta-analysis reveals gut microbiome and functional pathway alterations in response to resistant starch. *Food Funct* 14: 5251–5263, 2023. doi:10.1039/d3fo00845b.
97. **Sakata T.** Pitfalls in short-chain fatty acid research: a methodological review. *Anim Sci J* 90: 3–13, 2019. doi:10.1111/asj.13118.
98. **Procházkova N**, **Falony G**, **Dragsted LO**, **Licht TR**, **Raes J**, **Roager HM.** Advancing human gut microbiota research by considering gut transit time. *Gut* 72: 180–191, 2023. doi:10.1136/gutjnl-2022-328166.
99. **Seredyński R**, **Pawłowska-Seredyńska K**, **Ponikowska B**, **Paleczny B.** Acute effects of increased gut microbial fermentation on the hypoxic ventilatory response in humans. *Exp Physiol* 106: 748–758, 2021. doi:10.1113/EP089113.
100. **O'Connor KM**, **Lucking EF**, **Bastiaanssen TFS**, **Peterson VL**, **Crispie F**, **Cotter PD**, **Clarke G**, **Cryan JF**, **O'Halloran KD.** Prebiotic administration modulates gut microbiota and faecal short-chain fatty acid concentrations but does not prevent chronic intermittent hypoxia-

induced apnoea and hypertension in adult rats. *EBioMedicine* 59: 102968, 2020. doi:10.1016/j.ebiom.2020.102968.

101. **Yu JJ**, **Moya EA**, **Cheng H**, **Kaya K**, **Ochoa T**, **Fassardi S**, **Gruenberg E**, **Spenceley A**, **DeYoung P**, **Young EV**, **Barnes LA**, **Lugo A**, **Sanchez-Azofra A**, **Orr JE**, **Heinrich EC**, **Malhorta A**, **Simonson TS.** Improved oxygen saturation and acclimatization with bacteriotherapy at high altitude. *iScience* 28: 112053, 2025. doi:10.1016/j.isci.2025.112053.

102. **Boos CJ**, **Woods DR**, **Varias A**, **Biscocho S**, **Heseltine P**, **Mellor AJ.** High altitude and acute mountain sickness and changes in circulating endothelin-1, interleukin-6, and interleukin-17a. *High Alt Med Biol* 17: 25–31, 2016. doi:10.1089/ham.2015.0098.

103. **Wang C**, **Jiang H**, **Duan J**, **Chen J**, **Wang Q**, **Liu X**, **Wang C.** Exploration of acute phase proteins and inflammatory cytokines in early stage diagnosis of acute mountain sickness. *High Alt Med Biol* 19: 170–177, 2018. doi:10.1089/ham.2017.0126.