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A diet of U.S. military food rations alters gut microbiota composition and does not increase intestinal permeability☆

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Abstract

Interactions between gut microbes and dietary components modulate intestinal permeability (IP) and inflammation. Recent studies have reported altered fecal microbiota composition together with increased IP and inflammation in individuals consuming military food rations in austere environments, but could not isolate effects of the diet from environmental factors. To determine how the U.S. Meal, Ready-to-Eat food ration affects fecal microbiota composition, IP and inflammation, 60 adults (95% male,18–61 years) were randomized to consume their usual *ad libitum* diet for 31 days (CON) or a strictly controlled Meal, Ready-to-Eat-only diet for 21 days followed by their usual diet for 10 days (MRE). In both groups, fecal microbiota composition was measured before, during (INT, days 1–21) and after the intervention period. IP and inflammation [high-sensitivity C-reactive protein (hsCRP)] were measured on days 0, 10, 21 and 31. Longitudinal changes in fecal microbiota composition differed between groups (P=.005), and fecal samples collected from MRE during INT were identified with 88% accuracy using random forest models. The genera making the strongest contribution to that prediction accuracy included multiple lactic acid bacteria (*Lactobacillus, Lactococcus, Leuconostoc*), which demonstrated lower relative abundance in MRE, and several genera known to dominate the ileal microbiota (*Streptococcus, Veillonella, Clostridium*), the latter two demonstrating higher relative abundance in MRE. IP and hsCRP were both lower (34% and 41%, respectively) in MRE relative to CON on day 21 (P<.05) but did not differ otherwise. Findings demonstrate that a Meal, Ready-to-Eat ration diet alters fecal microbiota composition and does not increase IP or inflammation.

1. Introduction

The gut microbiota and intestinal barrier coexist in a dynamic, bidirectional relationship that generally reflects a mutualistic symbiosis between host and microbiota. However, that relationship can be perturbed by various environmental and physiologic stressors, initiating a cycle in which translocation of antigens from the gut lumen (*e.g.*, bacterial lipopolysaccharide) activates immune and inflammatory responses that promote intestinal barrier dysfunction and increase intestinal permeability (IP) [1–3]. Sequelae of increased IP and associated inflammation can include gastrointestinal distress [1,4]; impaired nutrient absorption and metabolism [5]; decrements in cognition and physical performance [6]; and increased risk of illness, infection and chronic disease [2,7]. As such, there is increasing interest in identifying factors that influence gut microbiota–intestinal barrier interactions and the resulting impact on human health [2].

Interactions between gut microbes, and both nutritive and nonnutritive dietary components are now recognized as impacting intestinal barrier health and function. Macronutrients are thought to be especially critical. For example, low-fiber diets, often as part of "Western-style" diets containing high amounts of saturated fat and sugar, have been shown to induce intestinal barrier damage and dysfunction in animal models [8–11]. Underpinning mechanisms include macronutrient-mediated changes in ratios of beneficial and

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proinflammatory gut bacteria and bacterially derived metabolites, with reduced bacterial synthesis of the short-chain fatty acids (SCFA) acetate, propionate and butyrate thought to be particularly important [12,13]. These bacterial byproducts of fiber fermentation, and butyrate in particular, have several beneficial health effects which include enhancing intestinal barrier function and integrity [14]. Less attention has been given to the effects of other dietary factors on gut microbiota–intestinal barrier interactions. However, intakes of food-borne microbes [15,16], non-nutritive food additives (*e.g.*, emulsifiers and artificial sweeteners) [17–19], micronutrients [20,21] and various other plant- and animal-derived compounds [22–24], as well as food form and processing [25–27], have all been shown to alter the composition and/or metabolic activity of the gut microbiota and, in some cases, intestinal barrier health [18,19,23,28,29]. As such, a shift in diet macronutrient composition is not the only dietary factor expected to influence interactions between the gut microbiota and intestinal barrier.

Every year, hundreds of thousands of U.S. military personnel and civilians involved in natural disasters shift from consuming their habitual diets to subsisting on the U.S. Armed Services Meal, Ready-to-Eat food ration. The macronutrient proportions and fiber density of the ration are similar to an average American diet, and the micronutrient content is made to comply with U.S. military dietary reference intakes through fortification [30]. However, the ration differs from most diets in that it contains only commercially sterile, highly processed items and no fresh foods [31]. Recent studies have reported changes in gut microbiota composition and microbiota-related metabolites along with increased IP and inflammation in individuals consuming military food rations in austere environments [32–35]. However, those studies could not separate effects of the unique ration-based diets from confounding environmental factors known to impact the gut microbiota and IP [36]. As a result, the impact of military food rations, and the Meal, Ready-to-Eat ration in particular, on the gut microbiota and IP is unclear. This study aimed to address that gap by isolating the effects of a Meal, Ready-to-Eat diet on gut microbiota composition and IP. Secondary outcomes included fecal SCFA concentrations, gastrointestinal symptoms, and circulating markers of inflammation and intestinal barrier function.

2. Materials and methods

2.1. Participants

Sixty-one men and three women 18–61 years of age and recruited from the Natick, MA, area participated in this trial conducted between June 2015 and March 2017 at the U.S. Army Research Institute of Environmental Medicine. Both military personnel and civilians were enrolled. Study exclusion criteria included BMI >30 kg/m^2; any antibiotic use during the previous 3 months; history of any gastrointestinal disease; infrequent bowel movements (<4 weekly); regular use of medications impacting gastrointestinal function (*e.g.*, laxatives, stool-softeners or antidiarrheals); colonoscopy within the previous 3 months; inability to avoid NSAID use; following a vegetarian diet; pregnant or lactating at time of participation; and actively trying to lose or gain body weight. Participants were instructed to discontinue use of any probiotic, prebiotic or other dietary supplements beginning 2 weeks prior to study participation.

The study was reviewed and approved by the US Army Research Institute of Environmental Medicine Institutional Review Board. 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 prior to participation. The trial was registered on www.clinicaltrials.gov as NCT02423551.

2.2. Study design

This parallel-arm, randomized, controlled study consisted of a 9-day baseline period (days −8 to 0), a 21-day intervention period (INT; days 1–21) and a 10-day washout period (POST; days 22–31). Upon enrollment, participants were randomized using computer-generated randomization to one of two study groups. The control group (CON) did not receive any diet intervention but was instructed to continue following their habitual diet throughout the study. The intervention group (MRE) was instructed to follow their habitual diet during baseline and POST but was provided with and instructed to consume nothing but the Meal, Ready-to-Eat U.S. military ration during INT. The 21-day feeding period was selected in accordance with Army policy stipulating 21 days as the maximum time period in which the Meal, Ready-to-Eat ration can serve as the sole source of subsistence for soldiers.

All participants met with research dietitians at baseline to complete a 3-day food record (2 weekdays and 1 weekend day) to estimate usual dietary intake. Throughout the subsequent 31-day study period, participants met with study staff 3 d/wk for the study-related activities detailed below.

2.3. Study diets and diet assessment

Participants in MRE were provided with two to three Meal, Ready-to-Eat meals daily during INT. The Meal, Ready-to-Eat is a general purpose ration used by the U.S. Armed Services, and also by several disaster relief organizations, to provide nutrition when food availability is limited [37]. The ration is comprised of 24 menus containing shelf stable, precooked and ready-to-eat foods which include an entrée, a starch, a spread (cheese, peanut butter, jam/jelly), a dessert and/or snack, a beverage powder, instant coffee or tea and chewing gum. On average, an individual Meal, Ready-to-Eat menu provides 1340 kcal, 13% energy from protein, 50% energy from carbohydrate and 37% energy from fat. The average menu also provides 12 g fiber (9 g fiber/1000 kcal), but the physicochemical characteristics of that fiber (*e.g.*, soluble *vs.* insoluble, low *vs.* highly fermentable) have not been characterized. The ration is made nutritionally complete using micronutrient fortification, and the macro- and micronutrient compositions of the ration meet the Nutritional Standards for Operational Rations which are derived from the military dietary reference intakes [30].

The number of meals and specific foods provided to MRE participants was individualized and intended to match the mean macronutrient distribution of the composite Meal, Ready-to-Eat ration while keeping macronutrient intake across MRE participants consistent and maintaining body weight. In so doing, the prescribed macronutrient distribution of the MRE diet was constant throughout INT within and between participants in MRE. Weight maintenance energy needs were calculated at baseline using a combination of energy intake measured from the 3-day food record, physical activity energy expenditure estimated using the International Physical Activity Questionnaire [38] and resting metabolic rate estimated using the Harris Benedict equation. MRE participants were instructed to consume all ration food items issued with no outside food or beverage other than water and 2–3 cup/d of black coffee if desired. Individual ration items were removed or added to match weight maintenance energy needs while maintaining the prescribed macronutrient distribution, and energy prescriptions were adjusted if a trend for weight gain or loss that met or exceeded 1 kg was measured over several study visits.

Dietary intake during INT was monitored in MRE participants using ration-specific food logs completed at the time of consumption and by collecting all empty food packaging and uneaten food items. Food logs and trash were reviewed by research dietitians, and any inconsistencies were adjudicated with participants. Nutrient intake was then analyzed using a database composed of chemically analyzed nutrient profiles for each ration item. Following INT, MRE participants returned to their usual eating habits. During this time, dietary intake was *ad libitum* and measured using three 24-h food records completed on nonconsecutive days.

Dietary intake in CON was measured using nine separate 24-h food records (six during INT and three during POST) which were completed on nonconsecutive weekend days and weekdays. All food records for MRE and CON were individually reviewed with a research dietitian and then entered in to Food Processor SQL (v11.0, ESHA, Salem, OR) for macro- and micronutrient analysis.

2.4. Anthropometrics

Height was measured at baseline using a portable stadiometer. Body weight was measured at baseline and 3 d/wk thereafter using a calibrated digital scale.

2.5. Questionnaires

All participants were asked to maintain their normal physical activity patterns throughout the study. Adherence to that instruction was measured using the International Physical Activity Questionnaire [38] which was administered at baseline and weekly thereafter to monitor physical activity energy expenditure over the previous week.

To assess gastrointestinal symptoms over the previous week, modified versions of the Irritable Bowel Syndrome-Symptom Severity Scale (IBS-SSS) [39] and the Gastrointestinal Quality of Life Index (GIQLI) Questionnaire [40] were administered weekly throughout the study. The IBS-SSS was scored out of 500 total points, with a higher score indicating greater severity. Participants were categorized as having no IBS symptoms (score <75) or mild IBS symptoms (score 75–175) [41]. Throughout the study, only two IBS-SSS scores exceeded the 175 point cutoff for mild IBS symptoms, and those two observations were categorized into the mild group for analysis. The GIQLI Questionnaire asked participants to subjectively rate the frequency of several GI-related symptoms (*e.g.*, flatulence, constipation, loose stool, cramping) which were then used to compute an overall GIQLI score in which lower scores indicate worse symptomology [40].

2.6. Fecal sample collection and analysis

A fecal sample was collected twice during baseline between study days −7 and −3 (BL-1 and BL-2), once weekly during INT [study days 8–10 (INT-1), days 15–17 (INT-2) and days 20–21 (INT-3)], and twice between study days 28 and 31 (POST-1 and POST-2) to measure microbiota community composition. Short-chain (SCFA) and branched-chain (BCFA) fatty acid concentrations were also measured in the BL-1 sample, all INT samples and the POST-2 sample. All samples were collected into plastic collection containers and processed within 12 h [median (interquartile range)= 52 min (95 min)] of sample collection. Aliquots were immediately frozen and stored at −80°C until processing.

2.6.1. Fecal metabolites

Fecal fatty acid concentrations were measured as previously described [32]. Fecal aliquots were thawed immediately prior to extraction, homogenized in distilled water (1:4 w/v) and centrifuged. Samples were then acidified using 50% H_2SO_4 (1:2 w/v), and fatty acids were extracted using diethyl ether (2:5 w/v). After incubating on ice for 2 min, samples were centrifuged, the organic layer was removed, and ethyl butyric acid was added as an internal standard. Samples were then stored at −80 °C until analysis. Acetic, propionic, butyric, isobutyric, isovaleric and valeric acids were quantified using an Agilent 7890A GC system with Flame Ionization Detection (60 m × 250 μm × 0.25 μm; DB-FFAP, Agilent J&W). Samples (1 μl) were injected by autosampler in triplicate using a split ratio of 10:1. The temperature program started with an initial temperature of 110°C for 2 min, increased 10°C/min up to 180°C and was then maintained at 180°C for 5 min. The carrier gas was nitrogen with a constant flow of 1 ml/min. Calibration standards were included for each fatty acid and used for peak identification and quantification.

2.6.2. Fecal microbiota composition

Fecal microbiota composition was assessed as previously described [32]. DNA was extracted from fecal samples using the MoBio PowerFecal DNA isolation kit (Qiagen, Germantown, MD, USA). Primers designed to amplify the V3–V4 region of the 16S rRNA gene were used for PCR amplification, and all samples were sequenced in triplicate on the Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA) over five separate sequencing runs. Sequencing data were processed using Quantitative Insights Into Microbial Ecology (QIIME) v.1.9.1 [42]. Read quality assessment, filtering, barcode trimming and chimera detection were performed on demultiplexed sequences using Trimmomatic [43]. Reads were joined in QIIME using a minimum overlap of 32 bp and a maximum percent difference within the overlap of 20%. Operational taxonomic units (OTU) were assigned by clustering sequence reads at 97% similarity and aligned against the Greengenes database core set v.13_8 [44] using PyNAST [45]. Taxonomic assignment was completed using the RDP classifier v.2.2 [46].

The median read count for each sample was 59,207 reads (range: 493–184,665 reads/sample). Reads were classified into 77,483 unique OTUs which could be assigned to 243 unique genera and 18 unique phyla. Prior to analysis, 23 samples which clustered with sequencing controls were removed from the data set, and the remaining 392 samples (n=5 missing samples) were used for differential abundance analyses. For diversity analyses, six samples with low read counts were also removed from the data set, and diversity metrics were then calculated for the remaining 386 samples after rarefaction at 22,526 reads/sample. Within-sample diversity (α-diversity) was calculated in QIIME using the Shannon and observed OTU diversity metrics. Between-sample diversity (β-diversity) was measured using Bray–Curtis dissimilarity, and both weighted and unweighted UniFrac distances were calculated using the R packages stats v.3.4.3 and phyloseq v.1.16.2. Ordinations of β-diversity metrics were completed using principal coordinates analyses within the R package ape.

2.7. Blood biochemistries

Blood samples were collected in the morning by venipuncture following a ≥12-h fast during baseline (study day 0), INT (study days 10 and 21) and POST (study day 31) to assess markers of intestinal barrier function and inflammation. All samples were separated into serum or plasma, frozen and stored at −80°C until analysis. Serum concentration of GLP-2, a pleiotropic enteroendocrine hormone shown to stimulate intestinal epithelial cell proliferation [47] and modulate effects of diet–gut microbe interactions on IP [48], was measured by enzyme-linked immunosorbent assay (ELISA) according to manufacturer instructions (EMD Millipore, St. Charles, MO, USA). Plasma concentration of intestinal fatty acid binding protein (I-FABP), a cytosolic protein found within mature enterocytes and a marker of enterocyte turnover and intestinal cell wall damage [2], was measured by ELISA according to manufacturer instructions (Hycult Biotech; Wayne, PA, USA). Plasma concentration of claudin-3, a tight junction protein and marker of paracellular barrier integrity loss [2], was measured by ELISA according to manufacturer instructions (MyBioSource; San Diego, CA, USA). Plasma concentration of lipopolysaccharide binding protein (LBP), an acute-phase protein secreted by the liver in response to bacterial lipopolysaccharide and an indirect measure of bacterial lipopolysaccharide translocation from the gut lumen into circulation [49], was measured by ELISA according to manufacturer instructions (Abonva, Taipei, Taiwan). Finally, serum high-sensitivity C-reactive protein (hsCRP) was measured by immuno-assay (Immulite 2000; Siemens, Malvern, PA) as a marker of inflammation.

2.8. Intestinal permeability

Regional and whole-gut intestinal permeability was assessed by quantifying the urinary excretion of orally ingested sugar and sugar substitutes [34,50] on study days 0, 10, 21 and 31. Measurements began immediately after the fasting blood draw by having participants consume 5 g sucrose, 5 g lactulose, 4 g mannitol and 2 g sucralose dissolved in 180 ml water. All urine produced over the subsequent 24 h was collected. Aliquots were taken after 1 h, 5 h and 24 h, immediately frozen and stored at −80°C until analysis. All participants remained under constant supervision for the first 2 h of collection, and many for the first 5 h. Participants remained fasted and sedentary during the first 5 h of collection but were allowed to consume water *ad libitum*. During the next 19 h, participants were free-living but instructed not to engage in strenuous exercise and not to consume any alcohol or foods/beverages containing sucralose, lactulose or mannitol.

Urine sucrose (0–1 h), lactulose (0–5 h), sucralose (0–5 h and 5–24 h) and mannitol (0–5 h) concentrations were measured by HPLC (Agilent 1100 HPLC, Santa Clara, CA, USA) as previously described [51,52]. Sucrose can be passively absorbed through the gastric mucosa and is used to assess gastric mucosal integrity [53]. Lactulose is excreted in proportion to paracellular permeability but is degraded by the colonic microbiota and therefore used to assess small intestinal permeability [2,53]. In contrast, sucralose is not degraded by the colonic microbiota, is excreted in proportion to paracellular permeability and is a marker of whole-gut and colonic permeability [50,53]. Finally, mannitol provides a control for differences in gastrointestinal surface area, hydration status and transit time [53]. Like lactulose, mannitol is degraded by the colonic microbiota and is therefore only useful for assessing small intestinal permeability.

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 fractional excretion of sucrose over 0–1 h was interpreted as a measure of gastric mucosal integrity. The lactulose concentration of the majority of samples was below the lower detectable limits of the assay and is not reported. Instead, the ratio of the fractional excretions of sucralose and mannitol from 0–5 h was used as a measure of small intestinal permeability. The fractional excretions of sucralose from 5–24 h and 0–24 h were interpreted as measures of colonic and whole-gut permeability, respectively.

Finally, 24-h urine creatinine concentrations were measured using the Jaffe enzymatic rate method (Dimension Xpand Plus; Siemens, Malvern, PA, USA) and used to calculate a creatinine ratio based on body mass [men: 24-h creatinine excretion/(body weight×24); women: 24-h creatinine excretion/[body weight×21)] [54]. A creatinine ratio of <0.07 was considered evidence of a potentially incomplete 24-h urine collection [55], and analyses were run both with and without participants who had ≥1 potentially incomplete 24-h urine collections.

2.9. Statistical analysis

Sample size estimates were calculated based on expected and clinically relevant mean differences and variance in IP. Specifically, Li et al. [34] reported a 0.03 greater lactulose:mannitol ratio during combat training in soldiers with elevated IBS-SSS scores relative to those with normal scores, suggesting a clinically relevant effect. The estimated number of subjects needed to detect an effect of that magnitude, using an expected S.D. of 0.026 [56] with power=0.80 and α=0.05, was determined to be 30 subjects/group. Unless otherwise noted, statistical analyses were completed using SPSS v.24 and data are presented as mean ± S.D. Statistical significance was set at $P \leq .05$, and P values between .05 and .10 were considered evidence of a trend for an effect.

Baseline participant characteristics were compared using t tests. Between-group differences in changes in body weight and dietary intake over time were examined using marginal models. All other outcomes, with the exception of data related to microbiota composition, were analyzed using marginal models which included time, diet and their interaction as fixed factors, and age, baseline BMI and baseline value of the dependent variable as covariates. Unstructured, compound symmetry and autoregressive covariance structures were examined, and Akaike's information criterion was used to select the best fit covariance structure for each model. Data were examined quantitatively and graphically for outliers, adherence to model assumptions was verified, and data transformations were used when necessary to meet model assumptions. Any significant main effects or interactions were further analyzed for within- and between-group differences using t tests with Bonferroni corrections.

For microbiota data, PERMANOVA implemented in the R (v 3.5) package vegan was used to determine main effects of diet, time and their interaction on community dissimilarities (Bray–Curtis, weighted UniFrac and unweighted UniFrac) while controlling for effects of age, BMI, sequencing run and correlations among repeated measurements on the same subject. The effects of diet, time and their interaction on Bray–Curtis dissimilarities from each time point relative to BL-1 were also examined using marginal models which included diet, time and their interaction as fixed factors, and age, baseline BMI and sequencing run as covariates. α-Diversity metrics were examined using the same model with the addition of baseline α-diversity as a covariate. Any significant main effects or interactions were further analyzed for within- and between-group differences using t tests with Bonferroni corrections.

There is currently a lack of consensus on the most appropriate approach for longitudinal analysis of individual taxa within microbial communities, with different

models each having strengths and weaknesses [57]. Therefore, multiple approaches were used to determine the effect of diet on the relative abundance of individual genera and phyla over time. First, random forest analysis was used to determine whether genus relative abundances could accurately discriminate samples collected from MRE and CON over time and to identify the genera making the largest contribution to the predictive accuracy of the analysis. Age, BMI and sequencing run were included in the random forest model which was grown using 10,001 trees. As discussed in Section 3.3, the random forest analysis accurately discriminated MRE samples collected during INT from other samples. The eight genera making the greatest contribution to that prediction accuracy, defined as genera with a mean decrease accuracy of ≥25%, were then extracted and subjected to two separate longitudinal analyses.

The primary longitudinal analysis used marginal models and arcsine-square root transformed taxa relative abundances [57]. Diet, time and their interaction were included as fixed factors and covariates included age, baseline BMI, sequencing run and baseline (BL-1) taxa relative abundance. One genus identified in the random forest analysis, *Leuconostoc*, was found to be present in only 28% of samples; therefore, its presence or absence over time was modeled using a binary logistic generalized linear model with the same factors and covariates noted above. Because marginal models do not explicitly handle sparse and overdispersed data, a second longitudinal analysis employing a *loess* spline model implemented in the R package SplinectomeR [58] was also used. The method uses *loess* splines to smooth longitudinal data before examining whether relative abundances of individual genera measured in two groups (*i.e.*, MRE and CON) follow more different trajectories over time than would be expected by random chance, and identifies time points at which relative abundances differ between groups. The model accounts for missing data, correlations among repeated measurements, and the sparse and compositional nature of microbiota community taxonomic profiles, but, in contrast to the marginal model, it does not currently allow for covariate adjustment. Using the spline model, each genus identified from the random forest analysis was analyzed individually over 999 permutations with a smoothing parameter of 0.5, and the number of intervals (*i.e.*, time points) set to 7. To adjust for multiple comparisons, $P<.01$ was used to identify between-group differences at individual time points.

Table 1
Baseline participant characteristics, body weight change and dietary intake

	CON	MRE	*P* value [1] Diet	Time	Diet*time
n (M/F)	29/1	28/2	.55		
Age (year)	24 [18]	29 [27]	.26		
PAEE (kcal/d)	906 [1079]	812 [1114]	.31		
BMI (kg/m^2), day 0	25.6±2.9	26.0±3.1	.62		
Height (m)	1.73±0.09	1.76±0.07	.24		
Weight (kg)			.40	.01	<.001
BL, day 0	77.0±11.7^{a,b}	80.7±13.3^{a}			
INT, day 10	77.1±11.8^{a}	80.0±13.2^{b,c}			
INT, day 21	77.6±12.0^{b,c}	79.6±13.0^{b,c}			
POST, day 31	77.9±12.2^{c}	80.2±13.3^{a,c}			
Energy (kcal/d)			.01	.23	.03
BL	2651±667^{a}	2758±747			
INT, days 1–9	2360±574^{b}	2902±397 *			
INT, days 10–21	2464±730^{a,b}	3004±389 *			
POST, days 22–31	2626±673^{a,b}	2958±809			
Carbohydrate (%)			<.001	.31	.16
BL	45± 8	50±7 *			
INT, days 1–9	44± 6	50 ± 1			
INT, days 10–21	45± 7	50± 1			
POST, days 22–31	45±5	47±8			
Protein (%)			<.001	<.001	<.001
BL	18± 5^{a,b}	17 ± 3^{a}			
INT, days 1–9	19±4^{a}	13 ± 1^{b,*}			
INT, days 10–21	18±4^{b}	13±1^{b,*}			
POST, days 22–31	18± 3^{a,b}	17±3^{a}			
Fat (%)			.26	.11	.12
BL	37± 7	33±7			
INT, days 1–9	36 ± 6	1			
INT, days 10–21	37±6	36±1			
POST, days 22–31	37±5	36±7			
Saturated fat (%)			.78	.07	.47
BL	12± 4	11 ± 3			
INT, days 1–9	12±3	13 ± 1			
INT, days 10–21	12±3	13± 1			
POST, days 22–31	12±2	12±3			
Polyunsaturated fat (%)			.45	.001	<.001
BL	5 ± 2	4±2^{a,*}			
INT, days 1–9	5±2	6± 0.4^{b,*}			
INT, days 10–21	5±2	6±0.4^{b}			
POST, days 22–31	5±2	4±2^{a,*}			
Fiber (g/1000 kcal/d) [2]			.09	.02	.03
BL	7.7 [5.7]	8.5 [3.4]^{a,b}			
INT, days 1–9	7.0 [4.6]	9.1 [0.4]^{a,*}			
INT, days 10–21	6.0 [4.4]	9.0 [0.3]^{a,*}			
POST, days 22–31	6.7 [4.6]	7.4 [5.2]^{b}			

Values are mean ± S.D. or median [IQR]. PAEE, physical activity energy expenditure.
[1] Baseline participant characteristics and dietary intake compared between groups by χ^2 test, independent-samples *t* test, or Mann–Whitney *U* test. Main effects of diet and time, and their interaction on body weight and dietary intake were analyzed by marginal models with Bonferroni adjustments. Within a diet group, values not sharing a superscript letter are significantly different, $P<.05$.
[2] Log_{10}-transformed for analysis.
* Different from CON at same time point.

Table 2
Gastrointestinal symptom ratings

	CON (*n*=30)	MRE (*n*=30)	*P* value [1] Diet	Time	Diet*time
IBS-SSS score			.01	.004	.27
BL	32 [56]	45 [71]			
INT, week 1^{a}	35 [77]	50 [66]			
INT, week 2^{a,b}	32 [44]	62 [49]			
INT, week 3^{a,b}	22 [49]	60 [49]			
POST, week 4^{b}	12 [43]	34 [60]			
POST, week 5^{b}	22 [46]	35 [46]			
Mild IBS (%) [2]			.26	.01	.35
BL	21	30			
INT, week 1^{a}	37	35			
INT, week 2^{a,b}	22	32			
INT, week 3^{a,b}	18	25			
POST, week 4^{b}	7	26			
POST, week 5^{b}	12	19			
GIQLI score			.33	.002	.91
BL	84± 5	82± 8			
INT, week 1^{a}	85±5	83 ± 6			
INT, week 2^{a,b}	87± 4	85±5			
INT, week 3^{a,b}	87±5	85±5			
POST, week 4^{b}	87±5	86±4			
POST, week 5^{b}	88±5	87±4			

Values are mean ± S.D., median [IQR] or frequency. BL, baseline (study days −8 to 0); GIQLI, Gastrointestinal Quality of Life Index (higher scores indicate better quality of life); IBS-SSS; Irritable Bowel Syndrome Symptom Severity Scale; INT, intervention (study days 1–21); POST, postintervention (study days 22–31).
[1] IBS-SSS and GIQLI scores analyzed by marginal model with Bonferroni corrections controlling for age, baseline BMI and baseline value of the dependent variable. Within the full cohort, time points not sharing a superscript letter are significantly different, $P<.05$.
[2] Mild IBS defined as IBS-SSS score>75. Analyzed by generalized linear models with Bonferroni corrections controlling for age, baseline BMI and baseline value.

3. Results

Sixty participants completed the study and were included in the analysis (Table 1). Reasons for study withdrawal included gastrointestinal distress following the first IP measurement ($n=1$), personal reasons ($n=1$), nonadherence to study procedures ($n=1$) and time commitment ($n=1$).

Participants in MRE lost, on average, 1.1 kg [95% CI: 0.4–1.7 kg] body weight during INT and partially regained that weight (0.6 kg [95% CI: 0–1.3 kg]) during POST (Table 1). In contrast, participants in CON experienced a mean weight gain of 0.9 kg ([95% CI: 0.1–1.8 kg]) during the study (Table 1). Physical activity declined in both groups during the study (mean overall decrease in combined cohort=473 kcal/d [95% CI: 125–822 kcal/d]; main effect of time, $P=.005$) independent of diet group (diet-by-day interaction, $P=.58$).

3.1. Dietary intake

Diet adherence was high in MRE with 92%±6% of prescribed food and beverages reported as consumed. All but three participants

reported consuming >85% of the prescribed diet. As planned, actual macronutrient intakes in MRE were consistent with the mean macronutrient distribution in all 24 ration menus (Table 1). This resulted in fiber intake being higher in MRE relative to CON during INT, whereas the proportion of energy derived from protein was lower (Table 1). The proportion of energy derived from carbohydrate was higher in MRE relative to CON throughout the study (Table 1). Absolute macronutrient intakes, and both absolute and energy-adjusted intakes of micronutrients reported in the Meal, Ready-to-Eat chemical analysis database are reported in the Online Supplemental Material (Supplemental Tables 1 and 2). The Meal, Ready-to-Eat diet had higher micronutrient density than habitual diets, with energy-adjusted intakes of several vitamins (vitamin A, vitamin E, thiamin, riboflavin, vitamin B6, vitamin C) and minerals (magnesium, zinc) being higher in MRE relative to CON during INT.

3.2. Subjective and objective markers of gastrointestinal health and function

After adjusting for baseline scores, mean IBS-SSS scores during and after INT were higher in MRE relative to CON (Table 2). However, the clinical significance of that difference was likely minimal, as the incidence of "mild IBS" did not differ between groups at any time point. GIQLI scores increased (*i.e.*, improved) over time independent of diet group (Table 2). Bowel movement frequency demonstrated a tendency to be lower during the first 2 weeks of INT in MRE relative to CON (adjusted mean difference=1 bowel movement/wk at both time points, $P \leq .06$) but not thereafter (diet-by-time interaction, $P=.08$; data not shown).

Mean serum GLP-2 concentrations were 14%–25% lower in MRE relative to CON on study days 10 and 21 and did not differ on day 31 (Fig. 1A). Median plasma I-FABP concentrations were 15% lower in MRE relative to CON on day 10 but not did not differ thereafter (Fig. 1B). Plasma claudin-3 (data not shown; main effect of diet, $P=.57$; diet-by-time interaction, $P=.50$) and plasma LBP concentrations (Fig. 1C) did not differ between groups at any time. Finally, median hsCRP concentrations were 41% lower on study day 21 in MRE relative to CON but did not differ before or after (Fig. 1D).

Mean sucralose excretion during the 0–5-h urine collection period demonstrated a trend to be lower in MRE relative to CON on day 21 (P=.08), whereas mean mannitol excretion was 9% lower ($P=.01$) (Fig. 2A–B). As a result, the sucralose:mannitol ratio over the initial 5 h did not differ between groups on any day (Fig. 2C).

Median sucralose excretion was 34% lower during the 5–24-h (colonic permeability) and 0–24-h (whole gut permeability) urine collection periods on study day 21 in MRE relative to CON but did not differ before or after (Fig. 2D–E). Those results were unchanged after adjusting for urine volume which did not demonstrate any between-group differences over either time period. Total 24-h urine creatinine excretion also did not differ between groups (data not shown; diet-by-day interaction, $P=.36$; main effect of diet, $P=.43$). However, 40 urine collections from 23 participants (CON: $n=11$, MRE: $n=12$, $P=.79$) were identified as being possibly incomplete. After removing these individuals from analyses of 5–24-h and 0–24-h urine collections, a main effect of diet (P-diet=.01), rather than a diet-by-time interaction, on 5–24-h sucralose excretion was observed with participants in MRE having lower excretion (data not shown). Results for 0–24-h sucralose excretion did not change.

Sucrose excretion in the 0–1-h urine collection (gastroduodenal permeability) did not differ by diet (Fig. 2F).

3.3. Fecal microbiota composition

Principal coordinates analysis of β-diversity measures did not show any distinct clustering of samples within MRE (Supplemental Fig. 1); however, PERMANOVA of Bray–Curtis dissimilarities did indicate a significant diet-by-time interaction ($P=.005$). To gain

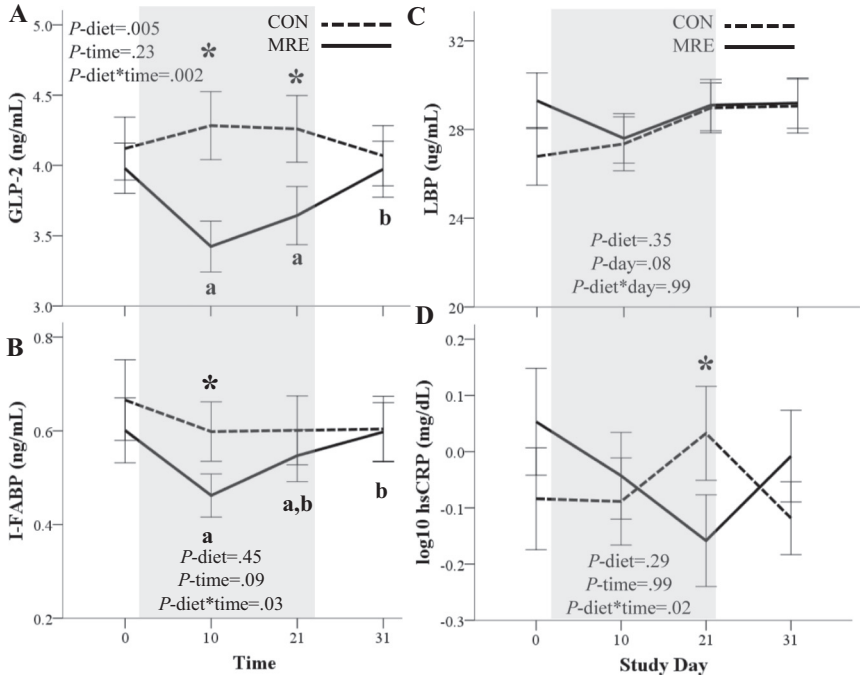

Fig. 1. Effects of a Meal, Ready-to Eat-only diet on markers of gastrointestinal barrier function and inflammation. Participants consumed their habitual diets for 31 days (CON, $n=30$) or a Meal, Ready-to-Eat ration diet (MRE, $n=30$) for 21 days (days 1–21) and then their habitual diet for 10 days (days 22–31). (A) Serum glucagon-like peptide (GLP)-2 concentrations (log_{10}-transformed for analysis); (B) plasma intestinal-fatty acid binding protein (I-FABP) concentrations (log_{10}-transformed for analysis); (C) plasma lipopolysaccharide binding protein (LBP) concentrations; (D) log_{10}-transformed high sensitivity serum C-reactive protein (hsCRP) concentrations. (A–D) Marginal model with Bonferroni corrections controlling for age, baseline BMI and baseline value of the dependent variable. Bars are mean ± S.E.M. *Different from CON on the same day, $P<.05$. Within MRE, values not sharing a superscript letter are significantly different, $P<.05$. Shaded area indicates time period during which MRE consumed the Meal, Ready-to-Eat diet.

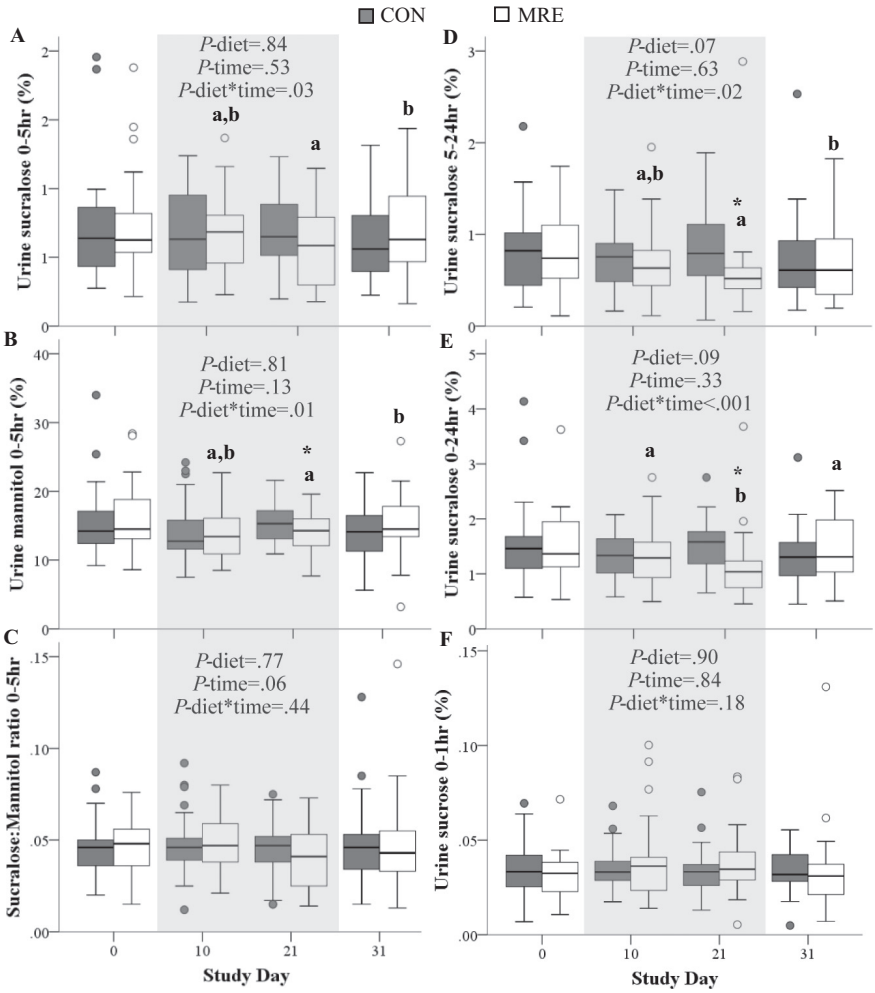

Fig. 2. Effects of a Meal, Ready-to Eat-only diet on gastrointestinal permeability. Participants consumed their habitual diets for 31 days (CON, $n=30$) or a Meal, Ready-to-Eat ration diet (MRE, $n=30$) for 21 days (days 1–21) and then their habitual diet for 10 days (days 22–31). Fractional urinary excretion of sucralose (A, D, E), mannitol (B) and sucrose (F), and the sucralose:mannitol ratio (C) analyzed by marginal models with Bonferroni corrections which included diet, time and their interaction as fixed factors, and controlled for age, baseline BMI and baseline value of the dependent variable. All measures over 5–24 h and 0–24 h were $\log_{10}$-transformed for analysis. Boxes show median and interquartile range; whiskers are 1.5 times the box height or min/max value if no values within that range. *Different from CON at the same time point ($P<.05$). Within MRE, values not sharing a superscript letter are significantly different ($P<.05$). Shaded area indicates time period during which MRE consumed the Meal, Ready-to-Eat diet.

further insight into that interaction, the similarity of each individual's fecal microbiota to their initial community composition (BL-1), as measured by Bray–Curtis distances (higher values indicate greater dissimilarity), was analyzed (Fig. 3A). This analysis revealed an initial increase in dissimilarity during INT within CON that was sustained thereafter. In contrast, within MRE, dissimilarity increased during INT but did not differ from BL during POST, suggesting an effect of diet on community composition. No significant between-group differences for any α-diversity metric was observed (Fig. 3B, C).

Random forest analysis of all time points correctly classified 88% of samples collected from MRE during INT, but only 61% of samples from CON during INT and only 2%–20% of samples collected from either group at BL and POST (out-of-bag error rate for full model=63%). A 25% mean decrease accuracy cutoff was visually identified as the first obvious break point on a plot of the mean decrease accuracies calculated in the random forest analysis (Supplemental Fig. 2), and the genera with the strongest influence on the prediction accuracies of the classification (≥25% mean decrease accuracy) are shown in Fig. 4A. Several of these taxa demonstrated different between-group trajectories over time in one or both longitudinal analyses (Fig. 4 and Supplemental Fig. 3). *Ruminococcus, Veillonella, Clostridium* (all members of the Firmicutes phylum) and *Sutterella* (phylum: Proteobacteria) demonstrated a pattern of increasing in relative abundance during INT within MRE (diet-by-time interaction, $P<.05$ and/or effect of diet in spline model, $P<.05$; Fig. 4B–C,G–H and Supplemental Fig. 3), whereas *Leuconostoc*, *Lactococcus* and *Lactobacillus* (all Firmicutes) demonstrated decreases in relative abundance during INT within MRE (diet-by-time interaction, $P<.10$ and/or effect of diet in spline model, $P<.05$; Fig. 4D–F and Supplemental Fig. 3). Despite contributing to the prediction accuracy of the random forest analysis, *Streptococcus* (phylum: Firmicutes) did not differ within or between groups in longitudinal analyses (Fig. 4I).

In longitudinal analyses of phyla relative abundances, Firmicutes relative abundance was lower in MRE relative to CON (main effect of diet, $P=.05$; spline model $P=.02$; Supplemental Fig. 4), which resulted in a trend towards a lower Firmicutes:Bacteroidetes ratio in MRE (data not shown; main effect of diet, $P=.08$). Proteobacteria relative abundance was higher in MRE relative to CON at INT1 ($P=.002$) when analyzed using marginal models (diet-by-time interaction, $P=.07$; Supplemental Fig. 4) but did not differ between groups when analyzed using the spline model (spline model $P=.65$).

All of the genera that were decreased in MRE are lactic acid bacteria (LAB) and include species commonly used in food fermentations: *Lactobacillus* spp. in yogurt, cheeses and sausages; *Lactococcus* spp. in

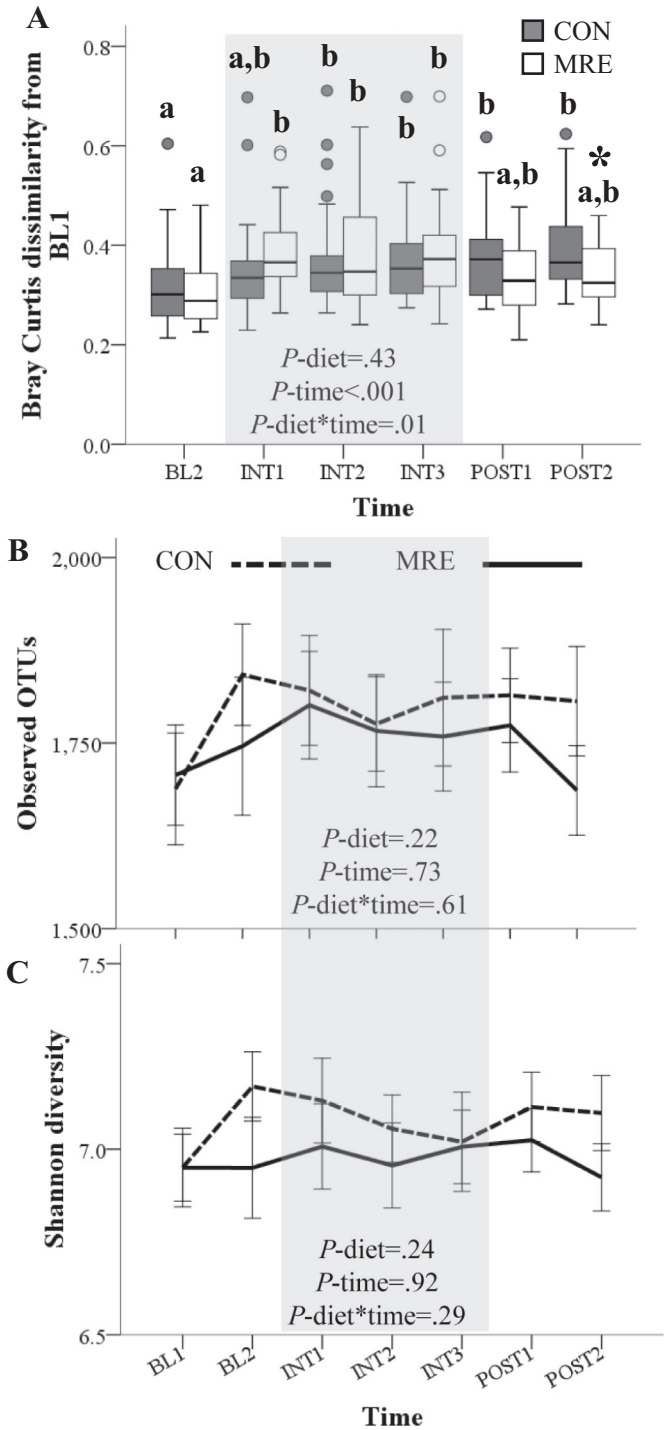

Fig. 3. Effects of a Meal, Ready-to Eat-only diet on fecal microbiota community diversity. After a baseline period (BL), participants consumed their habitual diets for 31 days (CON, n=30) or a Meal, Ready-to-Eat ration diet (MRE, n=30) for 21 days (INT-1 through INT-3) and then their habitual diet for 10 days (POST-1 and -2). (A) Bray–Curtis dissimilarity from first baseline (BL-1) sample (log_{10}-transformed for analysis). Analyzed by marginal models with Bonferroni corrections which included diet, time and their interaction as fixed factors, and controlled for age, baseline BMI and sequencing run. Boxes show median and interquartile range; whiskers are 1.5 times the box height or min/max value if no values within that range. *Different from CON on the same day, P=.05. Within a diet group, values not sharing a superscript letter are significantly different (P<.05). (B–C) Alpha diversity analyzed by marginal models with Bonferroni corrections which included diet, time and their interaction as fixed factors, and controlled for age, baseline BMI, sequencing plate and baseline diversity. Bars are mean ± S.E.M. Shaded area indicates time period during which MRE consumed the Meal, Ready-to-Eat diet.

cheeses; and *Leuconostoc* spp. in fermented dairy (*e.g.*, cheese, sour cream) and cabbage (*e.g.*, sauerkraut, kimchi) [59,60]. Therefore, intake of LAB-containing fermented foods was estimated using food records (Supplemental Table 3). A limitation of this estimation was that intakes for LAB-containing foods other than yogurt could only be approximated because LAB-containing foods that were an ingredient in another food (*e.g.*, cheese and pepperoni on pizza) could not be accurately estimated unless information on the quantities of individual ingredients was available (*e.g.*, slice of cheese on a sandwich). During BL, individuals in MRE were more likely to report consuming yogurt than individuals in CON (n=15 *vs* 7; Pearson χ^2=4.59, P=.03) and had higher overall yogurt intake (median [IQR]: MRE=20 g/d [123 g/d] *vs.* CON=0 g/d [9 g/d], P=.02). Cheese consumption was higher in CON (25 g/d [47 g/d]) relative to MRE during INT (0 g/d; cheese spreads in the Meal, Ready-to-Eat not included) but did not differ before or after. Intake of other LAB-containing fermented foods was infrequent (Supplemental Table 3).

3.4. Fecal short-chain fatty acid concentrations

Fecal acetate, propionate, butyrate and valerate concentrations did not differ between groups at any time point (Fig. 5). A trend for a diet-by-time interaction was observed for fecal isobutyrate and isovalerate concentrations (diet-by-time interaction, P≤.09), with *post hoc* testing indicating a trend (P≤.09) for lower concentrations of both fatty acids in MRE relative to CON during POST.

4. Discussion

This study is the first to examine changes in gut microbiota composition and IP in individuals subsisting on the U.S. Armed Services Meal, Ready-to-Eat food ration. In this study, consuming a diet comprised solely of the unique, commercially sterile and highly processed ration for 21-day altered gut microbiota composition, did not increase IP or inflammation, and did not result in clinically meaningful gastrointestinal symptoms when compared to typical American diets. These findings do not provide evidence to suggest that either the Meal, Ready-to-Eat ration itself or its effects on the gut microbiota promote decrements in gastrointestinal health and function in individuals consuming the ration for up to 21 days.

Changes in fecal microbiota community composition while consuming the Meal, Ready-to-Eat diet were subtle, and the changes detected varied depending on the method used to analyze the data (see Fig. 4 and Supplemental Figs. 1 and 3). However, analyses collectively suggested a transient shift in community composition during ration consumption (Fig. 3A) that could be characterized by reductions in the relative abundance of multiple LAB and increases in the relative abundance of two genera, *Veillonella* and *Clostridium* (Fig. 4), known to dominate the ileal microbiota. The reduction in fecal LAB proportions is likely attributable to changes in the dietary intake of those bacteria as the habitual diets of both MRE and CON participants contained LAB-containing foods (see Supplemental Table 3) which are largely absent from the ration. Further, foodborne microbes generally do not persist within the gut microbiota for more than a week after ingestion [61], and consuming LAB-containing foods increases LAB proportions within the fecal microbiota [15,16,62], while reductions in LAB such as *Lactobacillus* have been reported in individuals consuming sterilized diets [63]. These findings are also consistent with a recent observational study that reported reduced fecal LAB relative abundances in individuals consuming a Meal, Ready-to-Eat-based diet during a 21-day high-altitude sojourn [32], and with an *in vitro* colonic fermentation experiment conducted using fecal specimens collected from a subset of participants in this study which demonstrated that growth of *Lactobacillus* spp. was attenuated in fermentations following Meal, Ready-to-Eat consumption [64]. Therefore, consequences of

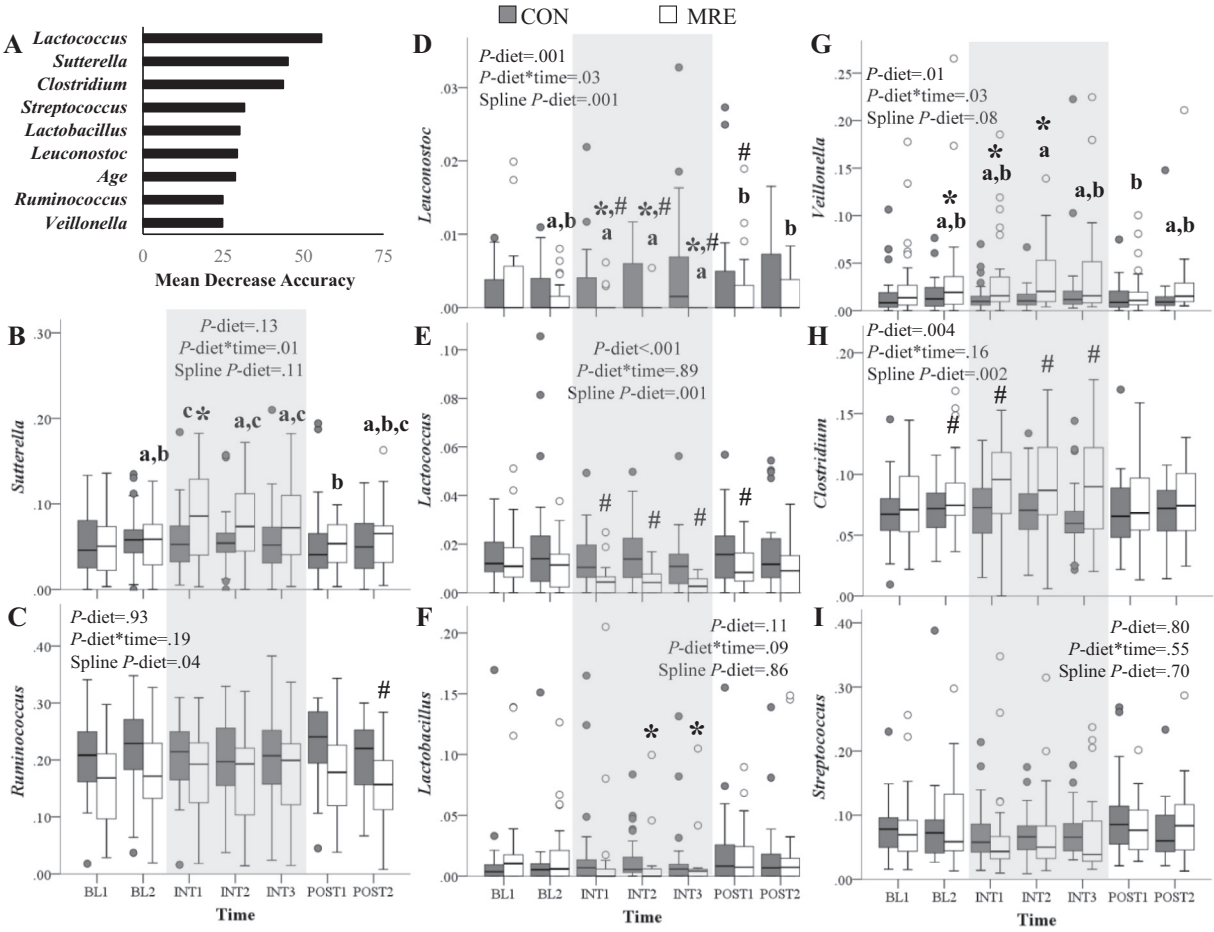

Fig. 4. Effects of a Meal, Ready-to Eat-only diet on genus relative abundances. After a baseline period (BL), participants consumed their habitual diets for 31 days (CON, $n=30$) or a Meal, Ready-to-Eat ration diet (MRE, $n=30$) for 21 days (INT-1 through INT-3) and then their habitual diet for 10 days (POST-1 and -2). Fecal samples were collected weekly during INT. (A) Results of random forest analysis. Genera and covariates with the strongest contribution to the prediction accuracy of the analysis (mean decrease accuracy ≥25%) are plotted. (B–I) Plotted values are arcsine-square root transformed relative abundances which were analyzed by marginal models or binary logistic generalized linear model (*Leuconostoc* only) with Bonferroni corrections. Models included diet (*P*-diet), time and their interaction (*P*-diet*time) as fixed factors, and controlled for age, baseline BMI, sequencing plate and baseline relative abundance. Also analyzed using permuted spline tests implemented in the R package Splinectome R (Spline P-diet). Boxes show median and interquartile range; whiskers are 1.5 times the box height or min/max value if no values within that range. *Different from CON at the same time point in marginal model ($P<.05$). #Different from CON at the same time point in spline model ($P<.01$). Within MRE, values not sharing a superscript letter are significantly different (marginal model, $P<.05$). Shaded area indicates time period during which MRE consumed the Meal, Ready-to-Eat diet.

consuming a commercially sterile Meal, Ready-to-Eat ration diet appear to include reduced LAB proportions within the gut microbiota resulting from decreased intake of LAB-containing foods.

LAB ingested in the diet can remain viable and metabolically active during intestinal transit [15,16], and likely compete with commensal gut bacteria for mono- and disaccharides within the gastrointestinal tract [61,65]. This competition has greater potential to influence community dynamics within the small intestine than in the colon due to the less dense commensal bacterial population in the small intestine [61]. Further, microbiota community dynamics within the small intestine are thought to be strongly influenced by the availability of simple carbohydrates which are metabolized by a cross-feeding network of bacteria wherein *Clostridium* and *Streptococcus* readily ferment carbohydrate while *Veillonella* and other lactic acid-utilizing bacteria rely on lactate produced by LAB such as *Streptococcus* during that fermentation [66–68]. Thus, changes in the competitive dynamics for carbohydrate due to a reduction in LAB in combination with the higher carbohydrate intake MRE could, in part, explain the observed increases in *Clostridium* and *Veillonella* relative abundances, although additional factors such as an undetected reduction in the proportions of other lactic-acid utilizers could also contribute.

Importantly, no decrements in gastrointestinal health or function could be attributed to either the Meal, Ready-to-Eat ration or its effects on the gut microbiota. In support, despite a reduction in *Lactobacillus* and an increase in *Sutterella* relative abundances, colonic permeability and systemic inflammation decreased on the ration diet, while small intestinal permeability and LBP concentrations were not affected. *Lactobacillus* is widely considered a beneficial LAB due to anti-inflammatory, antioxidative and antimicrobial activities, capability for SCFA production, and evidence supporting benefits on intestinal health and barrier function [59,69,70]. In contrast, *Sutterella* is a lipopolysaccharide-producing genus previously associated with increases in IP during military training [33] and with suppressed concentrations of secretory IgA, a critical modulator of the intestinal immune barrier [71]. These findings contrast with previous studies using similar methods which have reported 60% to threefold increases in IP in individuals consuming military food rations while training and living in austere environments [32–34]. However, the austere environments in which those studies were conducted, and in which military rations are commonly consumed, are characterized by environmental and physiologic stressors that have independently been shown to modulate gastrointestinal function and IP [36]. In this study, wherein ration consumption was isolated from those stressors,

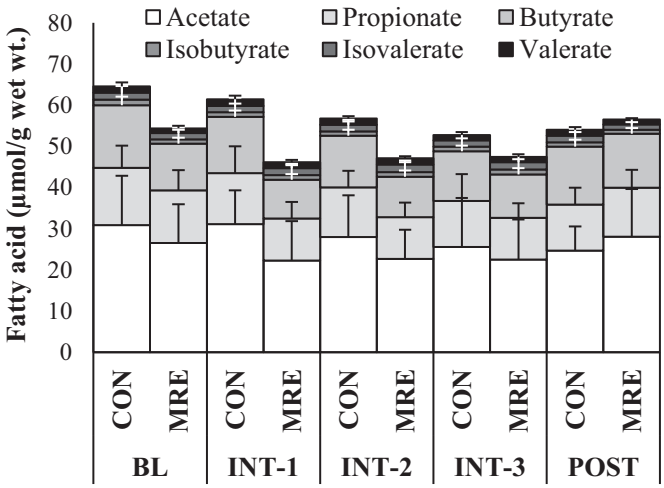

Fig. 5. Effects of a Meal, Ready-to Eat-only diet on fecal fatty acid concentrations. After a baseline period (BL), participants consumed their habitual diets for 31 days (CON, $n=30$) or a Meal, Ready-to-Eat ration diet (MRE, $n=30$) for 21 days (INT-1 through INT-3) and then their habitual diet for 10 days (POST). Fecal samples were collected weekly during INT. Analyzed by marginal models with Bonferroni corrections which included diet, time and their interaction as fixed factors, and controlled for age, baseline BMI and baseline fatty acid concentration. Acetate, propionate and butyrate were $\log_{10}$-transformed for analysis. Isobutyrate, isovalerate and valerate were square root-transformed for analysis. Bars are median + IQR/2. No statistically significant main effects or interactions were observed.

increased IP was not observed despite changes in gut microbiota composition that might be expected to increase IP. Any decrements in IP experienced within the austere environments in which the Meal, Ready-to-Eat is often consumed are therefore more likely attributable to stressors common to those environments than the ration itself or the ration's impact on the gut microbiota.

Fecal SCFA concentrations were measured to gain insight into one possible pathway through which interactions between Meal, Ready-to-Eat consumption and the gut microbiota may influence intestinal barrier function. These byproducts of bacterial fermentation have trophic effects on the intestine and enhance intestinal barrier function, in part by stimulating GLP-2 secretion from enteroendocrine cells [72]. In this study, GLP-2 concentrations decreased; however, this response did not result in increased IP or appear to be mediated by SCFA which were largely unaffected by the ration diet. Of note, changes in SCFA production may have gone undetected as fecal SCFA concentrations poorly represent *in situ* SCFA production due to rapid intestinal absorption [73]. Alternately, the reductions in GLP-2, I-FABP and mannitol excretion suggest intestinal epithelial cell proliferation, enterocyte turnover and intestinal surface area, respectively, decreased during ration consumption. Collectively, these observations suggest that the GLP-2 response could reflect a decrease in small intestinal mass, possibly resulting from the mild caloric restriction experienced in the MRE group [74] rather than an effect of any ration-mediated changes in SCFA production.

Study results should be interpreted within the context of several strengths and limitations. Strengths include providing controlled and measured diets to MRE, and assessing study outcomes before, during and after the intervention period. An important limitation was the observed body weight loss in MRE. Although diligent measures were taken to maintain energy balance and body weight loss was attenuated once detected, this weight loss does complicate interpretation of some outcomes as caloric restriction has been associated with reductions in inflammation and IP [75–77]. Additionally, the colonic and whole-gut permeability measurements used herein and in previous studies [33,34] did not include a control probe to account for any changes in intestinal surface area, hydration status and intestinal transit time. Hydration status, estimated by 24-h urine volume, did not differ between groups, and transit time, estimated by bowel movement frequency, was longer in MRE relative to CON, which would be expected to increase sucralose absorption. However, the decrease in mannitol excretion is consistent with reduced intestinal surface area, which could be a result of the slight energy deficit in MRE, and precludes concluding that the Meal, Ready-to-Eat ration reduced colonic permeability. Lastly, definitive conclusions as to which components or characteristics of the Meal, Ready-to-Eat were responsible for observed effects cannot be made because controlled diets were not provided to CON. However, the study was not designed to identify those dietary components *per se* but rather to approximate the real-world situation wherein individuals must quickly transition between eating *ad libitum* diets and military rations, and to isolate the effects of this dietary shift from stressors characteristic of environments in which military rations are generally consumed.

U.S. military personnel and civilians involved in natural disasters shift from consuming habitual diets to relying on the commercially sterile, highly processed Meal, Ready-to-Eat food ration for sustenance. The new findings from this study indicate that this shift has marginal impact on gut microbiota composition but may reduce proportions of LAB due to reduced intake of LAB-containing foods. Importantly, those reductions were reversed once usual *ad libitum* diets were resumed, and neither the ration itself nor its effects on the gut microbiota and SCFA concentrations resulted in increased IP or inflammation, or caused clinically meaningful gastrointestinal symptoms over 21 days. However, observed reductions in LAB abundance could have implications for military-relevant health outcomes not measured in this study given the multiple health benefits attributed to LAB such as various *Lactobacillus* species [78], especially in situations wherein reliance on the Meal, Ready-to-Eat ration must extend beyond 21 days. Research aimed at developing interventions to prevent *Lactobacillus* depletion during sustained periods of Meal, Ready-to-Eat consumption may therefore warrant consideration.

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Competing interests

There are no competing interests to report.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnutbio.2019.108217.

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