Time of day and eating behaviors are associated with the composition and function of the human gastrointestinal microbiota

Jennifer L Kaczmarek,[1] Salma MA Musaad,[2] and Hannah D Holscher[1–3]

[1]Division of Nutritional Sciences, [2]Family Resiliency Center, and [3]Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL

ABSTRACT

Background: Preclinical research has shown that the gastrointestinal microbiota exhibits circadian rhythms and that the timing of food consumption can affect the composition and function of gut microbes. However, there is a dearth of knowledge on these relations in humans.

Objective: We aimed to determine whether human gastrointestinal microbes and bacterial metabolites were associated with time of day or behavioral factors, including eating frequency, percentage of energy consumed early in the day, and overnight-fast duration.

Design: We analyzed 77 fecal samples collected from 28 healthy men and women. Fecal DNA was extracted and sequenced to determine the relative abundances of bacterial operational taxonomic units (OTUs). Gas chromatography–mass spectroscopy was used to assess short-chain fatty acid concentrations. Eating frequency, percentage of energy consumed before 1400, and overnight-fast duration were determined from dietary records. Data were analyzed by linear mixed models or generalized linear mixed models, which controlled for fiber intake, sex, age, body mass index, and repeated sampling within each participant. Each OTU and metabolite were tested as the outcome in a separate model.

Results: Acetate, propionate, and butyrate concentrations decreased throughout the day (P = 0.006, 0.04, and 0.002, respectively). Thirty-five percent of bacterial OTUs were associated with time. In addition, relations were observed between gut microbes and eating behaviors, including eating frequency, early energy consumption, and overnight-fast duration.

Conclusions: These results indicate that the human gastrointestinal microbiota composition and function vary throughout the day, which may be related to the circadian biology of the human body, the microbial community itself, or human eating behaviors. Behavioral factors, including timing of eating and overnight-fast duration, were also predictive of bacterial abundances. Longitudinal intervention studies are needed to determine causality of these biological and behavioral relations. This trial was registered at clinicaltrials.gov as NCT01925560. *Am J Clin Nutr* 2017;106:1220–31.

Keywords: circadian rhythms, microbiome, eating patterns, eating frequency, early energy consumption, overnight-fast duration, timing of eating, humans, adults

INTRODUCTION

The composition and function of the human gastrointestinal microbiota are increasingly linked to metabolic health (1, 2), and there is keen interest in developing evidence-based strategies to modulate the gastrointestinal microbiota for health benefit. Clinical research findings indicate that diet and consumption of fiber and prebiotics affect the gut microbiota (3–5). Intriguingly, preclinical research suggests that gastrointestinal microbes are influenced by circadian rhythms (6–8). Circadian rhythms are cycles of gene expression, metabolism, and behaviors created by an internal clock to maximize an organism's metabolic efficiency (9). The underlying transcriptional and translational feedback loops will proceed without environmental input (e.g., pure biology), but they can also be affected by environmental factors such as light and food.

Cyclical variations in gastrointestinal bacteria are likely related to both biological circadian factors, as shown in the absence of enteral feeding (10), and to eating behaviors that result in cyclical abundance of food in the intestines (11, 12). Furthermore, there may be compounding effects of diet and circadian rhythms on the gastrointestinal microbiota, gut barrier function, and health (13, 14). However, at present, much of the literature is based on preclinical findings. Indeed, there is a dearth of knowledge on these relations in humans.

Robust circadian rhythms can be developed by aligning the phase and duration of feeding and fasting patterns with the environmental light-dark cycle. This entrainment means that peripheral clocks, which are affected by the presence of food, and the central clock, which is affected by the presence of light, are in sync. Importantly, synchronized circadian rhythms are associated with human health (15, 16). Behavioral patterns, such as time-restricted feeding and eating frequency, may also have health benefits in humans: time-restricted feeding positively affects body weight, blood lipids, and glucose homeostasis (16, 17) and greater eating frequency may be associated with improvements in metabolic

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Address correspondence to HDH (e-mail: hholsche@illinois.edu).

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health, although conflicting evidence exists (18–20). The consumption of a larger proportion of energy early in the day complements human circadian rhythms; for example, glucose tolerance and diet-induced thermogenesis are higher in the morning than in the evening (21, 22).

The aim of this study was to examine the relations between the human gastrointestinal microbiota and 2 closely intertwined elements—time, as it relates to biological circadian rhythms, and behavior, as it relates to time of eating. Given the rhythmic nature of the gastrointestinal microbiota in preclinical studies, we hypothesized that human gastrointestinal microbial abundances and metabolites vary throughout the day and are affected by behavioral patterns of eating timing.

METHODS

Participants

The study described herein was a secondary analysis of samples and data collected from the control period (0 g supplemental fiber) of a previously completed trial of agave inulin consumption in healthy adults (n = 28; 14 women) (23). The inclusion criteria for participants of the primary study were as follows: *1*) be between the ages of 20 and 40 y; *2*) have a BMI (in kg/m^2) >18.5 and <29.5; *3*) have no current or historical metabolic or gastrointestinal diseases; *4*) avoid medications known to affect gastrointestinal function; *5*) have no use of antibiotics for at least the past 8 wk; *6*) limit alcohol consumption to ≤2 servings/d (e.g., <28 g ethanol/d); *7*) avoid taking prebiotics or probiotics; *8*) consume a moderate-fiber diet, consistent with the US average of 12–19 g/d; *9*) maintain consistent vitamin and mineral supplementation as at baseline; *10*) maintain current level of physical activity; *11*) record detailed dietary and stool information daily; and *12*) meet with study personnel weekly. Female participants were excluded if they had menstrual cycles <27 or >29 d in length, or if they were pregnant or lactating. Before study initiation, all of the participants voluntarily signed a written informed consent as approved by the University of Illinois Institutional Review Board. This study was conducted from January 2013 to May 2013 and was registered with clinicaltrials.gov as NCT01925560.

Fecal samples

In the primary study protocol, participants consumed 0, 5.0, or 7.5 g agave inulin/d in a randomized order for 21 d with 7-d washouts between periods. They provided ≤3 fecal samples, each within 15 min of defecation, during days 16–20 of each of the 3 periods (maximum of 9 total samples/participant). Herein, the fecal samples from the 0-g supplemental fiber control period (maximum of 3 total fecal samples/participant) were used for this secondary analysis, which included 77 total fecal samples from 28 study participants. Additional analyses were conducted on the data from all 3 treatment periods (0, 5.0, or 7.5 g agave inulin/d) to determine whether there was an interaction of time and fiber treatment on the composition and function of the gut microbiota; those results are available as **Supplemental Tables 1–7**. Fecal samples were transported to the laboratory with Commode Specimen Collection Systems (Sage Products) on ice packs in coolers. On arrival, time of defecation was confirmed to be <15 min from delivery to the laboratory, and the time that the sample arrived for processing was recorded by a laboratory technician. Samples were manually homogenized, a pH measurement was taken (Denver Instrument), and then samples were placed into aliquots for individual assays.

Short-chain fatty acids

The fecal aliquot for short-chain fatty acids (SCFAs; acetate, propionate, butyrate) was immediately acidified with 2N-HCl (10% wt:vol) and frozen at −20°C until analysis. A separate aliquot was used for dry matter measurement according to the methods of the Association of Official Analytic Chemists (24). Fecal SCFA concentrations were analyzed by gas chromatography–mass spectroscopy as previously described and normalized on a dry matter basis (25).

Microbial analysis

The samples for microbial analysis were flash-frozen in liquid nitrogen and stored at −80°C until analysis. Fecal bacterial DNA was extracted according to the manufacturer's instructions by using the PowerLyzer PowerSoil DNA Isolation Kit (MO Bio Laboratories, Inc.). After extraction, the V4 region of the 16S bacterial ribosomal RNA gene was amplified by using a Fluidigm Access Array system (Fluidigm Corporation) before high-throughput sequencing on an Illumina HiSeq (Illumina Inc.). Sequencing was performed at the W. M. Keck Center for Biotechnology at the University of Illinois. High-quality (quality value >25) data derived from the sequencing process were analyzed with QIIME 1.8.0 and 1.9.1 (26). Briefly, sequences were clustered into operational taxonomic units (OTUs) by using closed-reference OTU picking against the Greengenes 13_8 reference OTU database (97% similarity threshold). After quality filtering, α and β diversity were calculated at an even sampling depth of 63,467 sequences/sample (27, 28).

Dietary data

Study participants were educated on dietary recording methods by a registered dietitian before study initiation. Dietary intake was recorded in a 7-d diet record during the study, and participants met with dietetic interns weekly to review their dietary records and to clarify ambiguities. Dietary record data were entered and analyzed by using Nutrition Data Systems for Research software, 2015 edition (University of Minnesota). The number of eating occasions on the day before fecal sample collection was calculated by counting every food or beverage event reported by the participants in the 24-h calendar day that contained ≥50 kcal (29). Overnight-fast duration was calculated as the time from the last kilocalorie consumption the previous night to the time of the first kilocalorie consumption on the day of sample collection. Water and other noncaloric beverages were not considered as breaking the fast, but occasions of low-energy (<50 kcal) consumption that would not be counted in the eating occasions calculation were considered here. Early energy consumption was calculated as the percentage of total kilocalories consumed at or before 1400 on the day before fecal sample collection. The time of 1400 was chosen because it encompassed what would be considered "lunch" by almost all of the participants.

Statistical analysis

Statistical analysis was performed by using SAS version 9.4 (SAS Institute Inc.). A probability of $P \leq 0.05$ was accepted as significant and was not adjusted for multiple testing because this is a preliminary study (30). To be as comprehensive as possible, genus-level OTUs that were present in ≥50% of the fecal samples were analyzed. Although the majority of preclinical literature in this field uses the JTK_cycle algorithm to detect cyclical features (31), design differences between this clinical study and the preclinical studies necessitated different statistical methods. First, these samples were only collected during the waking hours, and thus the use of an algorithm designed for 24-h data collection would not provide robust results. Second, the human gastrointestinal microbiota has been shown to be affected by age (32), sex (33), BMI (34, 35), and dietary fiber intake (5). This warranted statistical control of these variables within the models to allow for the detection of associations beyond these variables. Before using the statistical modeling described below, the methodology was confirmed to successfully replicate the majority of the findings from a preclinical circadian study of the murine cecal microbiota (11).

Mixed modeling was used to model normally (PROC MIXED) and non-normally (PROC GLIMMIX) distributed outcomes including person as a repeated effect. Each bacterial abundance and bacterial metabolite (SCFA) outcome were tested separately. SCFAs and bacterial genera distributions were examined to specify the best-fitting model. Model fit was assessed by using the ratio of the chi-square to its df. This ratio was used to assess residual variability that is not explained by the model (36). Values <2 were deemed to indicate appropriate model fit (37).

Three statistical models were used for data analysis. The first model was designed to examine the association of sample time (considered as a biological factor) with bacterial OTU relative abundances and SCFA concentrations. This model controlled for sex, age, BMI, and total dietary fiber per 1000 kcal and considered the within-subject correlation. The second model examined behavioral factors, including eating frequency, overnight-fast duration, and percentage of energy consumed before 1400, while controlling for the same covariates as model 1 and accounting for the within-subject correlation. The third, full, model included the biological variable (time) and the behavioral variables (eating frequency, overnight fast, and percentage of energy before 1400) together as well as the covariates (sex, age, BMI, and fiber) and the within-subject correlation to determine which factors contributed most strongly to existing relations and whether the inclusion of these factors in the same model would strengthen or weaken these relations.

For the results presented, the estimate (β coefficient) is the percentage change in the predicted value of the outcome variable for each 1-unit change in the predictor variable, if all the other predictors remain constant. It is important to note that when interpreting these estimates that "1 unit" is defined differently for each predictor. One unit of time or overnight-fast duration is 1 h. One unit of eating frequency is 1 eating occasion. For energy consumption before 1400, 1 unit is 1% of energy.

RESULTS

Fecal samples were collected between 0732 and 2200, with a mean clock time of 1136. Additional descriptive data on participant characteristics and the distributions of behavioral variables are presented in **Table 1**. In model 1, the biological model, there were significant relations between time of day and relative abundance of bacteria, as well as time of day and bacterial metabolites (**Table 2**). Acetate, propionate, and butyrate concentrations decreased with clock time (e.g., the concentrations of these bacterial fermentative end-products decreased throughout the day) (**Figure 1**). Among microbes, the relative abundances of *Roseburia*, *Veillonella*, *Haemophilus*, and an unspecified genus within the S24-7 family decreased with clock time. Alternatively, the relative abundances of *Adlercreutzia*, *Eggerthella*, *Anaerotruncus*, *Oscillospira*, *Ruminococcus*, *Holdemania*, *Desulfovibrio*, *Escherichia*, and an unspecified genus within the Enterobacteriaceae family increased with clock time. Significant results are also presented in **Figure 2**. Collectively, the genera that were associated with time represent 24% of the OTUs examined for a total of 7% of the bacterial community composition.

In the behavioral model (**Table 3**), the relative abundance of *Coprobacillus* increased with greater eating frequency. The relative abundances of *Actinomyces*, *Eggerthella*, *Anaerotruncus*, *Dialister*, *Veillonella*, and unspecified genera within the Barnesiellaceae and Ruminococcaceae families decreased with greater eating frequency. *Oscillospira*, *Megamonas*, *Coprobacillus*, *Holdemania*, and an unspecified genus within the Erysipelotrichaceae family had higher relative abundances when a greater percentage of daily energy was consumed before 1400. Alternatively, *Turicibacter*, *Coprococcus*, *Lachnospira*, *Roseburia*, *Veillonella*, and *Haemophilus* had lower relative abundances when a greater percentage of energy was consumed before 1400. *Turicibacter* decreased with longer overnight-fast duration. There were no significant behavioral relations observed with SCFAs in this model.

In the full model, which included both time and behavioral factors, most of the results from the preceding models remained, and new relations emerged (**Table 4**). Specifically, new associations included an increase in the relative abundances of *Bifidobacterium*, *Butyricimonas*, *Sutterella*, *Bilophila*, and an unspecified genus within the Rikenellaceae family with clock time and a reduction in the relative abundances of *Collinsella*, *Streptococcus*, and *Eubacterium* with clock time. *Roseburia* ($P = 0.17$), the unspecified genus within S24-7 ($P = 0.07$), and the unspecified Enterobacteriaceae genus ($P = 0.16$) were no longer significantly related to clock time when eating behaviors were included in the model. Overall, in this full model, 35% of OTUs, or 12% of the total bacterial community composition, were associated with time.

Behavioral relations were similar in the full model. All of the eating-frequency relations remained significant, except that the unspecified

TABLE 1
Descriptive characteristics of the sample[1]

Variable	Mean	Median	SD	Range
Age, y	28	27	4	21–36
BMI, kg/m^2	24.3	24.3	2.2	20.2–28.9
Sample clock time (24 h)	1136	1035	0307	0732–2200
Eating frequency, number of occasions	4.6	4.0	1.5	2–10
Early energy consumption (before 1400), %	47.4	46.7	14.7	9.6–84.0
Overnight-fast duration, h	11.8	11.5	2.4	7.0–21.0

[1] n = 28 participants (14 women) and 77 fecal samples.

TABLE 2
Associations of metabolite concentrations and bacterial OTU relative abundances with time[1]

			Biological model, time (hours)		
	Participants, *n*	Fecal samples, *n*	Estimate ± SEE	95% CI	*P*
Short-chain fatty acids, μmol/g					
Acetate	23	63	−5.4 ± 1.88	−9.08, −1.72	<0.01*
Propionate	23	63	−4.61 ± 2.19	−8.9, −0.32	0.04*
Butyrate	23	63	−7.28 ± 2.27	−11.73, −2.83	<0.01*
Archaea, % of sequences					
Methanobrevibacter	23	64	9.96 ± 6.37	−2.53, 22.45	0.12
Actinobacteria, % of sequences					
Actinomyces	23	64	1.31 ± 4.98	−8.45, 11.07	0.79
Bifidobacterium	23	64	7.39 ± 4.65	−1.72, 16.5	0.12
Coriobacteriaceae unspecified genus	23	64	−9.01 ± 6.12	−21.01, 2.99	0.15
Adlercreutzia	23	64	12.01 ± 5	2.21, 21.81	0.02*
Collinsella	23	64	−6.06 ± 4.93	−15.72, 3.6	0.22
Eggerthella	23	64	27.95 ± 5.41	17.35, 38.55	<0.01*
Bacteroidetes, % of sequences					
Bacteroides	23	64	2.48 ± 1.27	−0.01, 4.97	0.06
Parabacteroides	23	64	0.27 ± 2.94	−5.49, 6.03	0.93
Rikenellaceae unspecified genus	23	64	2.68 ± 3.35	−3.89, 9.25	0.43
S24-7 unspecified genus	23	64	−19.29 ± 8.48	−35.91, −2.67	0.03*
Barnesiellaceae unspecified genus	23	64	6.13 ± 7.25	−8.08, 20.34	0.40
Butyricimonas	23	42	8.59 ± 4.62	−0.47, 17.65	0.07
Odoribacter	23	64	−4.84 ± 3.26	−11.23, 1.55	0.14
Firmicutes, % of sequences					
Granulicatella	23	64	0.32 ± 5.34	−10.15, 10.79	0.95
Lactococcus	23	64	−5.8 ± 11.35	−28.05, 16.45	0.61
Streptococcus	23	64	−4.08 ± 4.66	−13.21, 5.05	0.38
Turicibacter	23	64	0.3 ± 8.41	−16.18, 16.78	0.97
Clostridiales unspecified genus	23	64	0.26 ± 2.32	−4.29, 4.81	0.91
Christensenellaceae unspecified genus	23	64	−0.99 ± 6.33	−13.4, 11.42	0.88
Clostridiaceae unspecified genus	23	64	0.97 ± 4.15	−7.16, 9.1	0.82
Clostridium	23	63	−2.97 ± 3.12	−9.09, 3.15	0.34
Lachnospiraceae unspecified genus	23	64	0.34 ± 1.3	−2.21, 2.89	0.79
Anaerostipes	23	64	0.85 ± 3.69	−6.38, 8.08	0.82
Blautia	23	64	3.92 ± 2.33	−0.65, 8.49	0.10
Coprococcus	23	64	−2.01 ± 3.37	−8.62, 4.6	0.55
Dorea	23	64	6.53 ± 3.27	0.12, 12.94	0.05
Lachnobacterium	23	64	12.52 ± 10.46	−7.98, 33.02	0.24
Lachnospira	23	64	2.64 ± 4.11	−5.42, 10.7	0.52
Roseburia	23	64	−7.03 ± 3.39	−13.67, −0.39	0.04*
Ruminococcaceae unspecified genus	23	64	−2.05 ± 1.67	−5.32, 1.22	0.23
Anaerotruncus	23	64	23.52 ± 4.06	15.56, 31.48	<0.01*
Faecalibacterium	23	64	0.39 ± 2.17	−3.86, 4.64	0.86
Oscillospira	23	64	6.38 ± 2.07	2.32, 10.44	<0.01*
Ruminococcus	23	64	6.05 ± 2.83	0.5, 11.6	0.04*
Acidaminococcus	23	34	−18.75 ± 25.76	−69.24, 31.74	0.47
Dialister	23	64	−0.04 ± 6.35	−12.49, 12.41	1.00
Megamonas	23	41	0.68 ± 22.78	−43.97, 45.33	0.98
Megasphaera	23	42	12.64 ± 20.03	−26.62, 51.9	0.53
Phascolarctobacterium	23	64	−0.03 ± 4.48	−8.81, 8.75	0.99
Veillonella	23	64	−25.38 ± 9.84	−44.67, −6.09	0.01*
Mogibacteriaceae unspecified genus	23	64	1.54 ± 2.98	−4.3, 7.38	0.61
Erysipelotrichaceae unspecified genus	23	64	7.71 ± 4.42	−0.95, 16.37	0.09
Coprobacillus	23	64	2.45 ± 6.8	−10.88, 15.78	0.72
Holdemania	23	64	9.39 ± 3.21	3.1, 15.68	<0.01*
Eubacterium	23	61	−7.48 ± 5.25	−17.77, 2.81	0.16
Proteobacteria, % of sequences					
Sutterella	23	64	3.16 ± 1.76	−0.29, 6.61	0.08
Bilophila	23	64	3.26 ± 3.54	−3.68, 10.2	0.36
Desulfovibrio	23	64	20.33 ± 8.14	4.38, 36.28	0.02*
Enterobacteriaceae unspecified genus	23	64	15.16 ± 7.2	1.05, 29.27	0.04*

(Continued)

TABLE 2 (*Continued*)

	Participants, *n*	Fecal samples, *n*	Biological model, time (hours)		
			Estimate ± SEE	95% CI	*P*
Escherichia	23	64	17.74 ± 6.16	5.67, 29.81	<0.01*
Haemophilus	23	64	−19.76 ± 9.44	−38.26, −1.26	0.04*
Verrucomicrobia, % of sequences					
Akkermansia	23	64	12.8 ± 7.36	−1.63, 27.23	0.09

[1] Results of linear mixed-model analysis were adjusted for repeated sampling, age, BMI, sex, and normalized total fiber intake. Estimates represent the percentage change in the predicted value of the outcome variable for each 1-unit change in time (hours) if all of the other predictors remain constant. This analysis represents a linear relation between microbes or metabolites and time during the awake/feeding phase of the circadian cycle. A negative estimate indicates that the highest values were seen earlier and decreased throughout the day. A positive estimate indicates that the values increased throughout the day and were highest later. *Significant ($P < 0.05$). OTU, operational taxonomic unit.

Barnesiellaceae genus, which decreased with higher eating frequency in the behavioral model, only tended ($P = 0.05$) to be related to eating frequency when time was included in the model. All of the early energy consumption relations remained except that the positive relation between *Roseburia* and greater energy consumed before 1400 became a trend ($P = 0.06$) when time was included in the model. New relations emerged with overnight-fast duration when time was also included in the model, whereby the relative abundance of *Coprococcus* increased with longer overnight-fast duration, whereas *Holdemania* decreased with longer overnight-fast duration. Finally, propionate was present at higher concentrations with longer overnight-fast duration.

DISCUSSION

The relations between the human gastrointestinal microbiota and health and disease make it a promising target for lifestyle interventions. In parallel, research has shown the importance of circadian rhythms to normal metabolic homeostasis (38) and the presence of these rhythms in the bacterial community of the murine gastrointestinal tract (8, 11). Herein, we report for the first time, to our knowledge, a connection between time, eating behaviors, and human gastrointestinal microbiota composition and function.

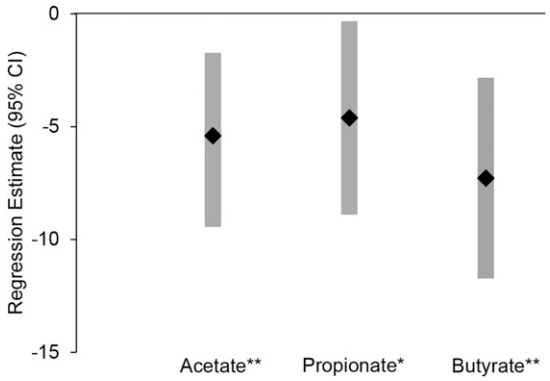

FIGURE 1 Associations of short-chain fatty acids with time in the biological model. Results of linear mixed-model analysis were adjusted for age, BMI, sex, normalized total fiber intake, and repeated sampling. Estimate represents the percentage of change in the predicted value of the outcome variable for each 1-unit change in time (hours) if all of the other predictors remain constant. The black diamonds indicate estimates; gray bars indicate 95% CIs. A negative estimate indicates that the highest concentrations were seen earlier and decreased throughout the day. *$P < 0.05$, **$P < 0.01$.

Our data show that the bacterial fermentative end-products, acetate, propionate, and butyrate decrease over the course of the day. Butyrate and propionate have previously been shown to behave rhythmically in murine models (10). In our study, butyrate concentrations decreased by 2–6 μmol · g^{-1} · h^{-1}, which represents a 5–12% decrease/h. Leone et al. (10) reported changes of 58 μmol · g^{-1} · h^{-1}, which represents a change of 22%/h in the ceca of mice. The larger effect in mice than in humans may be related to sample collection and analysis methods. For example, the murine study used cecal contents immediately harvested from killed mice, whereas our study used fecal samples collected from humans within 15 min of passing, and concentrations were reported on a dry matter basis.

We also report that certain members of the human gastrointestinal microbiota changed with time. *Eggerthella*, *Anaerotruncus*, and *Desulfovibrio* have the largest positive estimates, increasing by >20% of their relative abundances every hour throughout the day. However, these are low-abundance bacteria, together constituting <1% of the microbiota community. The 2 most abundant genera associated with time were *Roseburia* and *Ruminococcus*, which represent 2% and 3% of the bacterial community in our participants, respectively. Changes in microbial abundances and metabolites are likely related. We reported a relation between *Desulfovibrio* and time. Although *Desulfovibrio* has not previously been shown to cycle, hydrogen sulfide, a metabolite produced by *Desulfovibrio*, showed cyclical fluctuations in mice (10). In addition, we report that *Roseburia* and *Eubacterium* decreased throughout the day. Both genera produce butyrate (39), and our results likewise showed that butyrate concentrations decreased throughout the day.

In one murine study, 17% of OTUs were cyclical, with 20–83% of bacterial sequences at any one time belonging to OTUs that cycled (11). Genera found to cycle in murine models, which were also detectable in the human participants in our study, include *Bacteroides* (12, 40), *Lactococcus* (10, 11), *Lactobacillus* (11, 12), *Oscillospira* (10, 40), *Ruminococcus* (10), S24-7 (40), *Turicibacter* (40), *Sutterella* (40), *Akkermansia* (11), and *Bifidobacterium* (11). In addition, total bacterial load, number of mucosa-associated bacteria, and Firmicutes peak during feeding, whereas the other major phyla—Actinobacteria, Bacteroidetes, Proteobacteria, and Verrucomicrobia—peak during fasting in murine models (11, 40, 41). In the only human microbiota-focused circadian study to date, which included 2 individuals, oscillations were found in 10% of OTUs, including *Parabacteroides*, *Lachnospira*, and *Bulleida* (12). Herein, we reported that ≤12% of sequences in

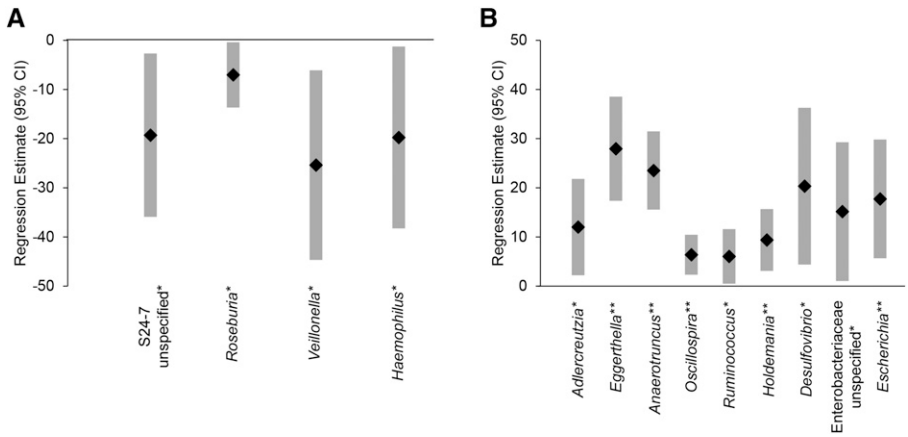

FIGURE 2 Associations of bacterial OTUs with time in the biological model. Results of linear-mixed model analysis were adjusted for age, BMI, sex, normalized total fiber intake, and repeated sampling. Estimates represent the percentage change in the predicted value of the outcome variable for each 1-unit change in time (hours) if all of the other predictors remain constant. The black diamonds indicate estimates; gray bars indicate 95% CIs. A negative estimate indicates that the highest relative abundances of the bacteria were seen earlier and decreased throughout the day. A positive estimate indicates that the relative abundances of the respective bacteria increased throughout the day and were highest later. (A) OTUs that significantly decreased throughout the day. (B) OTUs that significantly increased throughout the day. $*P < 0.05$, $**P < 0.01$. OTU, operational taxonomic unit.

the human gastrointestinal microbiota belong to an OTU that was associated with time. Of these, *Bifidobacterium*, S24-7, *Oscillospira*, *Ruminococcus*, and *Sutterella* replicated preclinical findings (10, 11, 40). Similar to previous clinical findings, we reported that *Lachnospira* tended ($P = 0.07$) to be associated with time and was related to early energy consumption in our full model, which suggests that both biological and behavioral factors influence the cyclical behaviors of this microbe in the human gastrointestinal tract. *Parabacteroides* and *Bulleida* were present in <50% of participants, and thus were not assessed in the current study. Thus, our results that human gastrointestinal bacteria fluctuate in abundance throughout the day are supported by changes in metabolite concentrations throughout the day, preclinical findings (10, 11, 40), and the results reported in a small ($n = 2$) human study (12).

Associations between time and bacterial abundances may be related to specific bacterial traits, such as bile resistance. For example, *Oscillospira* and *Bilophila*, which increased throughout the day in our cohort, are bile tolerant, and thus may have a competitive advantage during waking hours, when more bile is secreted due to food ingestion (42, 43). Alternatively, oscillations may be related to factors independent of the presence of food in the gastrointestinal tract: the murine microbiota has shown circadian variation even when parenteral nutrition is the only source of nutrition (10). Other factors independent of food intake could include hormonal signals from the host. For example, *Enterobacter aerogenes* is affected by melatonin, a circadian hormone (6).

Few studies exist on behavioral elements of eating timing and the gastrointestinal microbiota; one study in horses reported that increased meal frequency was associated with increased relative abundance of the genus *YRC22*, within the family Paraprevotellaceae, and decreased relative abundances of *Prevotella*, *Lactobacillus*, *Streptococcus*, *Coprococcus*, and *Phascolarctobacterium* (44). Although we also reported associations with eating frequency among phylogenetically diverse microbes, none of the microbes that were associated with feeding frequency in equine ceca were the same as those in the human gastrointestinal tract. With regard to overnight-fast duration, we reported that propionate concentrations and *Coprococcus*, a microbe that produces propionate (45), increased with increasing overnight-fast duration. Contrary to previous literature, *Akkermansia* and overnight-fast duration were not related (46, 47). Two possible explanations for this discrepancy include the following: *1*) as a mucosa-associated genus, *Akkermansia* measurements in stool (humans) may vary from that in the cecum (murine) (48, 49), and *2*) the type or amount of fiber in the diet of the study participants may have been adequate to keep the abundance of *Akkermansia* stable throughout the study (50).

This study was limited by the fact that it was a secondary data analysis and relied on self-report dietary records for behavioral factors. Intervention studies that modify biological and behavioral factors and assess the gastrointestinal microbiota as a primary outcome are necessary to determine causality. As with any observational study, we must also consider the possibility that the directionality of the associations is reversed. For example, microbes may signal via the gut-brain axis and influence appetite in a way that drives certain eating behaviors, rather than the behaviors themselves affecting the microbial composition. Indeed, microbial metabolites have been shown to affect hyperphagia in rodents (51, 52), and propionate reduced appetite in overweight adults (53). Furthermore, our data cover half of the circadian cycle, specifically the awake and feeding phase. Assessments over a 24-h period are needed to establish that human gastrointestinal microbes and metabolites that increase during the day show circadian rhythms (e.g., correspondingly decreasing at night).

Despite some limitations, the study has several strengths. To our knowledge, it is the first of its kind to examine the biological and behavioral influences of time on the human gastrointestinal microbiota, and many of our findings replicate and extend those reported in preclinical studies. We used a robust statistical model that controlled for many of the factors known to be associated with the gastrointestinal microbiota [e.g., age (32), BMI (34), sex (33), and dietary fiber intake (5)]. Through the use of models that independently assessed biology and behavior, followed by a combination of both time and behavioral factors, we are able to discern how the variables may be interrelated. Interestingly, several bacteria associated with time were also affected by timing of eating, underscoring the potential relevance of how eating behavior

TABLE 3
Associations of metabolite concentrations and bacterial OTU relative abundances with behavioral patterns[1]

			Behavioral model								
			Eating frequency (occasions)			Early energy consumption (% of energy)			Overnight-fast duration (hours)		
	Participants, *n*	Samples, *n*	Estimate ± SEE	95% CI	*P*	Estimate ± SEE	95% CI	*P*	Estimate ± SEE	95% CI	*P*
Short-chain fatty acids, μmol/g											
Acetate	22	58	2.94 ± 4.92	−6.7, 12.58	0.55	−0.71 ± 0.46	−1.61, 0.19	0.13	2.09 ± 3.08	−3.95, 8.13	0.50
Propionate	22	58	4.37 ± 5.68	−6.76, 15.5	0.45	−0.1 ± 0.53	−1.14, 0.94	0.86	4.25 ± 3.56	−2.73, 11.23	0.24
Butyrate	22	58	1 ± 6.22	−11.19, 13.19	0.87	−0.78 ± 0.58	−1.92, 0.36	0.18	−0.28 ± 3.9	−7.92, 7.36	0.94
Archaea, % of sequences											
Methanobrevibacter	22	59	1.45 ± 15.69	−29.3, 32.2	0.93	−1.81 ± 1.48	−4.71, 1.09	0.23	11.39 ± 9.15	−6.54, 29.32	0.22
Actinobacteria, % of sequences											
Actinomyces	22	59	−32.17 ± 14.52	−60.63, −3.71	0.03*	0.62 ± 1.05	−1.44, 2.68	0.56	−1.94 ± 6.5	−14.68, 10.8	0.77
Bifidobacterium	22	59	−10.77 ± 11.96	−34.21, 12.67	0.37	2.05 ± 1.11	−0.13, 4.23	0.07	−0.18 ± 7.52	−14.92, 14.56	0.98
Coriobacteriaceae unspecified genus	22	59	15.32 ± 10.6	−5.46, 36.1	0.15	0.98 ± 1	−0.98, 2.94	0.33	−13.86 ± 8.82	−31.15, 3.43	0.12
Adlercreutzia	22	59	4.32 ± 14.9	−24.88, 33.52	0.77	0.87 ± 1.52	−2.11, 3.85	0.57	−8.89 ± 10.54	−29.55, 11.77	0.40
Collinsella	22	59	8.69 ± 13.87	−18.5, 35.88	0.53	−0.37 ± 0.98	−2.29, 1.55	0.71	5.68 ± 7.76	−9.53, 20.89	0.47
Eggerthella	22	59	−64.92 ± 21.53	−107.12, −22.72	<0.01*	0.42 ± 1.5	−2.52, 3.36	0.78	−0.89 ± 7.01	−14.63, 12.85	0.90
Bacteroidetes, % of sequences											
Bacteroides	22	59	−2.52 ± 3.42	−9.22, 4.18	0.46	−0.13 ± 0.22	−0.56, 0.3	0.55	1.84 ± 1.35	−0.81, 4.49	0.18
Parabacteroides	22	59	0.64 ± 7.99	−15.02, 16.3	0.94	−0.56 ± 0.52	−1.58, 0.46	0.29	2.69 ± 3.32	−3.82, 9.2	0.42
Rikenellaceae unspecified genus	22	59	−10.9 ± 7.26	−25.13, 3.33	0.14	−0.2 ± 0.68	−1.53, 1.13	0.77	1.02 ± 4.56	−7.92, 9.96	0.82
S24-7 unspecified genus	22	59	−0.74 ± 24.64	−49.03, 47.55	0.98	−2.55 ± 2.34	−7.14, 2.04	0.28	8.27 ± 14.04	−19.25, 35.79	0.56
Barnesiellaceae unspecified genus	22	59	−54.6 ± 26.15	−105.85, −3.35	0.04*	0.95 ± 1.6	−2.19, 4.09	0.56	−10.81 ± 10.83	−32.04, 10.42	0.32
Butyricimonas	22	38	−20.09 ± 11.4	−42.43, 2.25	0.09	0.68 ± 1.06	−1.4, 2.76	0.53	−3.06 ± 7.19	−17.15, 11.03	0.67
Odoribacter	22	59	0.33 ± 7.95	−15.25, 15.91	0.97	−0.17 ± 0.75	−1.64, 1.3	0.83	−3.51 ± 5.18	−13.66, 6.64	0.50
Firmicutes, % of sequences											
Granulicatella	22	59	9.39 ± 11.9	−13.93, 32.71	0.43	1.03 ± 1.22	−1.36, 3.42	0.41	2.65 ± 7.38	−11.81, 17.11	0.72
Lactococcus	22	59	−0.53 ± 22.97	−45.55, 44.49	0.98	−3.48 ± 2.32	−8.03, 1.07	0.14	8.5 ± 14.11	−19.16, 36.16	0.55
Streptococcus	22	59	−14.07 ± 11.4	−36.41, 8.27	0.22	0.09 ± 1.06	−1.99, 2.17	0.93	−0.64 ± 7.17	−14.69, 13.41	0.93
Turicibacter	22	59	−31.48 ± 20.02	−70.72, 7.76	0.12	−3.11 ± 1.31	−5.68, −0.54	0.02*	−29.28 ± 14.16	−57.03, −1.53	0.04*
Clostridiales unspecified genus	22	59	0.74 ± 5.92	−10.86, 12.34	0.90	0.12 ± 0.55	−0.96, 1.2	0.83	−0.07 ± 3.72	−7.36, 7.22	0.98
Christensenellaceae unspecified genus	22	59	0.15 ± 12.87	−25.08, 25.38	0.99	−0.51 ± 1.54	−3.53, 2.51	0.74	−9.54 ± 9.82	−28.79, 9.71	0.34
Clostridiaceae unspecified genus	22	59	4.35 ± 10.34	−15.92, 24.62	0.68	0.49 ± 0.96	−1.39, 2.37	0.61	−6.44 ± 6.5	−19.18, 6.3	0.33
Clostridium	22	58	12.43 ± 7.97	−3.19, 28.05	0.13	−1.38 ± 0.74	−2.83, 0.07	0.07	3.84 ± 5.03	−6.02, 13.7	0.45
Lachnospiraceae unspecified genus	22	59	0.56 ± 3.37	−6.05, 7.17	0.87	0.31 ± 0.22	−0.12, 0.74	0.16	−2.52 ± 1.39	−5.24, 0.2	0.08
Anaerostipes	22	59	−14.62 ± 9.13	−32.51, 3.27	0.12	0.47 ± 0.85	−1.2, 2.14	0.59	−4.45 ± 5.74	−15.7, 6.8	0.44
Blautia	22	59	−8.45 ± 5.5	−19.23, 2.33	0.13	0.2 ± 0.51	−0.8, 1.2	0.70	−0.05 ± 3.46	−6.83, 6.73	0.99
Coprococcus	22	59	0.02 ± 7.57	−14.82, 14.86	1.00	−1.69 ± 0.71	−3.08, −0.3	0.02*	8.99 ± 4.76	−0.34, 18.32	0.06
Dorea	22	59	2.82 ± 8.26	−13.37, 19.01	0.73	0.64 ± 0.77	−0.87, 2.15	0.41	1.38 ± 5.2	−8.81, 11.57	0.79
Lachnobacterium	22	59	−52.67 ± 30.19	−111.84, 6.5	0.09	−2.29 ± 2.2	−6.6, 2.02	0.30	−6.49 ± 13.74	−33.42, 20.44	0.64
Lachnospira	22	59	6.89 ± 9.88	−12.47, 26.25	0.49	−1.94 ± 0.92	−3.74, −0.14	0.04*	2.23 ± 6.22	−9.96, 14.42	0.72
Roseburia	22	59	12.09 ± 8.27	−4.12, 28.3	0.15	−1.55 ± 0.77	−3.06, −0.04	<0.05*	−3.7 ± 5.2	−13.89, 6.49	0.48
Ruminococcaceae unspecified genus	22	59	−8.42 ± 4	−16.26, −0.58	0.04*	−0.13 ± 0.37	−0.86, 0.6	0.73	−3.72 ± 2.52	−8.66, 1.22	0.15
Anaerotruncus	22	59	−44.25 ± 16.05	−75.71, −12.79	<0.01*	2.1 ± 1.07	0, 4.2	0.05	0.86 ± 5.96	−10.82, 12.54	0.89
Faecalibacterium	22	59	0.85 ± 4.81	−8.58, 10.28	0.86	−0.22 ± 0.45	−1.1, 0.66	0.63	−1.31 ± 3.02	−7.23, 4.61	0.67

(Continued)

TABLE 3 (*Continued*)

			Behavioral model								
			Eating frequency (occasions)			Early energy consumption (% of energy)			Overnight-fast duration (hours)		
	Participants, *n*	Samples, *n*	Estimate ± SEE	95% CI	*P*	Estimate ± SEE	95% CI	*P*	Estimate ± SEE	95% CI	*P*
Oscillospira	22	59	−3.04 ± 5.01	−12.86, 6.78	0.55	1.59 ± 0.47	0.67, 2.51	<0.01*	−3.4 ± 3.15	−9.57, 2.77	0.29
Ruminococcus	22	59	0.56 ± 7.89	−14.9, 16.02	0.94	−0.18 ± 0.74	−1.63, 1.27	0.81	1.86 ± 4.96	−7.86, 11.58	0.71
Acidaminococcus	22	31	27.44 ± 75.57	−120.68, 175.56	0.72	5.69 ± 6.28	−6.62, 18	0.37	−32.8 ± 34.34	−100.11, 34.51	0.35
Dialister	22	59	−34.97 ± 15.83	−66, −3.94	0.03*	−1.92 ± 1.16	−4.19, 0.35	0.10	−3.55 ± 8.33	−19.88, 12.78	0.67
Megamonas	22	37	78.85 ± 47.3	−13.86, 171.56	0.11	14.88 ± 4.47	6.12, 23.64	<0.01*	8.58 ± 31.45	−53.06, 70.22	0.79
Megasphaera	22	39	−3.8 ± 52.98	−107.64, 100.04	0.94	1.69 ± 4.8	−7.72, 11.1	0.73	−3.37 ± 27.7	−57.66, 50.92	0.90
Phascolarctobacterium	22	59	14.19 ± 10.29	−5.98, 34.36	0.17	0.62 ± 1.06	−1.46, 2.7	0.56	6.42 ± 6.59	−6.5, 19.34	0.33
Veillonella	22	59	−51.72 ± 20.89	−92.66, −10.78	0.02*	−6.61 ± 1.61	−9.77, −3.45	<0.01*	−3.19 ± 10.75	−24.26, 17.88	0.77
Mogibacteriaceae unspecified genus	22	59	−3.66 ± 7.57	−18.5, 11.18	0.63	−0.2 ± 0.66	−1.49, 1.09	0.77	4.61 ± 4.43	−4.07, 13.29	0.30
Erysipelotrichaceae unspecified genus	22	59	−8.46 ± 10.64	−29.31, 12.39	0.43	2.6 ± 0.99	0.66, 4.54	0.01*	−8.34 ± 6.69	−21.45, 4.77	0.22
Coprobacillus	22	59	24.69 ± 12.18	0.82, 48.56	<0.05*	3.15 ± 1.49	0.23, 6.07	0.04*	−7.91 ± 10.47	−28.43, 12.61	0.45
Holdemania	22	59	−11.69 ± 8.52	−28.39, 5.01	0.18	1.78 ± 0.76	0.29, 3.27	0.02*	−9.28 ± 5.26	−19.59, 1.03	0.08
Eubacterium	22	57	6.72 ± 13.45	−19.64, 33.08	0.62	0.06 ± 1.24	−2.37, 2.49	0.96	3.65 ± 8.41	−12.83, 20.13	0.67
Proteobacteria, % of sequences											
Sutterella	22	59	−5.23 ± 4.89	−14.81, 4.35	0.29	0.12 ± 0.32	−0.51, 0.75	0.72	−1.34 ± 2.06	−5.38, 2.7	0.52
Bilophila	22	59	12.55 ± 7.84	−2.82, 27.92	0.12	1.19 ± 0.81	−0.4, 2.78	0.15	−6.14 ± 5.89	−17.68, 5.4	0.30
Desulfovibrio	22	59	19.52 ± 20.3	−20.27, 59.31	0.34	3.35 ± 1.84	−0.26, 6.96	0.08	−14.17 ± 14.33	−42.26, 13.92	0.33
Enterobacteriaceae unspecified genus	22	59	12.38 ± 15.95	−18.88, 43.64	0.44	0.23 ± 1.74	−3.18, 3.64	0.90	17.46 ± 8.7	0.41, 34.51	0.05
Escherichia	22	59	15.24 ± 14.98	−14.12, 44.6	0.31	0.87 ± 1.56	−2.19, 3.93	0.58	13.78 ± 7.67	−1.25, 28.81	0.08
Haemophilus	22	59	−32.97 ± 20.98	−74.09, 8.15	0.12	−4.16 ± 1.41	−6.92, −1.4	<0.01*	−28.35 ± 14.55	−56.87, 0.17	0.06
Verrucomicrobia, % of sequences											
Akkermansia	22	59	2.4 ± 18.23	−33.33, 38.13	0.90	0.6 ± 1.85	−3.03, 4.23	0.75	0.42 ± 11.79	−22.69, 23.53	0.97

[1] Results of linear mixed-model analysis were adjusted for repeated sampling, age, BMI, sex, and normalized total fiber intake. Estimates represent the percentage of change in the predicted value of the outcome variable for each 1-unit change in the predictor variable if all of the other predictors remain constant. One unit of eating frequency is 1 eating occasion. One unit of energy consumption is 1% of daily energy intake. One unit of overnight-fast duration is 1 h. This analysis represents a linear relation between microbes or metabolites and behaviors during the awake/feeding phase of the circadian cycle. A negative estimate indicates an inverse relation between the outcome and the predictor (e.g., relative abundance of the bacterium was higher when eating frequency was lower). A positive estimate indicates a positive relation between the outcome and predictor (e.g., relative abundance of the bacterium was higher when eating frequency was higher). *Significant ($P < 0.05$). OTU, operational taxonomic unit.

TABLE 4
Associations of metabolite concentrations and bacterial OTU relative abundances with time and behavioral patterns in the full model[1]

			Full model											
			Time (hours)			Eating frequency (occasions)			Early energy consumption (% of energy)			Overnight-fast duration (hours)		
	Participants, *n*	Samples, *n*	Estimate ± SEE	95% CI	*P*	Estimate ± SEE	95% CI	*P*	Estimate ± SEE	95% CI	*P*	Estimate ± SEE	95% CI	*P*
Short-chain fatty acids, μmol/g														
Acetate	22	58	−8.35 ± 2.02	−12.31, −4.39	<0.01*	−0.4 ± 4.35	−8.93, 8.13	0.93	−0.58 ± 0.4	−1.36, 0.2	0.15	5.4 ± 2.8	−0.09, 10.89	0.06
Propionate	22	58	−8.29 ± 2.43	−13.05, −3.53	<0.01*	1.06 ± 5.25	−9.23, 11.35	0.84	0.03 ± 0.48	−0.91, 0.97	0.95	7.53 ± 3.37	0.92, 14.14	0.03*
Butyrate	22	58	−9.76 ± 2.62	−14.9, −4.62	<0.01*	−2.9 ± 5.64	−13.95, 8.15	0.61	−0.63 ± 0.52	−1.65, 0.39	0.23	3.58 ± 3.62	−3.52, 10.68	0.33
Archaea, % of sequences														
Methanobrevibacter	22	59	7.84 ± 7.53	−6.92, 22.6	0.30	4.53 ± 16.09	−27.01, 36.07	0.78	−1.89 ± 1.47	−4.77, 0.99	0.20	8.21 ± 9.56	−10.53, 26.95	0.39
Actinobacteria, % of sequences														
Actinomyces	22	59	−2.82 ± 5.24	−13.09, 7.45	0.59	−33.21 ± 14.68	−61.98, −4.44	0.03*	0.67 ± 1.05	−1.39, 2.73	0.53	−0.65 ± 6.92	−14.21, 12.91	0.93
Bifidobacterium	22	59	11.62 ± 5.5	0.84, 22.4	0.04*	−4.73 ± 11.82	−27.9, 18.44	0.69	2.18 ± 1.09	0.04, 4.32	0.05	−4.71 ± 7.63	−19.66, 10.24	0.54
Coriobacteriaceae unspecified genus	22	59	−5.82 ± 6.31	−18.19, 6.55	0.36	13.13 ± 11.1	−8.63, 34.89	0.25	0.87 ± 1.03	−1.15, 2.89	0.41	−11.66 ± 9.35	−29.99, 6.67	0.22
Adlercreutzia	22	59	15.72 ± 5.78	4.39, 27.05	<0.01*	10.51 ± 13.36	−15.68, 36.7	0.44	0.47 ± 1.39	−2.25, 3.19	0.73	−13.04 ± 9.34	−31.35, 5.27	0.17
Collinsella	22	59	−10.06 ± 4.98	−19.82, −0.3	<0.05*	7.11 ± 13.26	−18.88, 33.1	0.59	−0.22 ± 0.94	−2.06, 1.62	0.82	8.84 ± 7.51	−5.88, 23.56	0.24
Eggerthella	22	59	24.81 ± 5.52	13.99, 35.63	<0.01*	−49.46 ± 16.03	−80.88, −18.04	<0.01*	−0.2 ± 1.25	−2.65, 2.25	0.87	−10.63 ± 5.94	−22.27, 1.01	0.08
Bacteroidetes, % of sequences														
Bacteroides	22	59	2.46 ± 1.67	−0.81, 5.73	0.15	−2.91 ± 3.4	−9.57, 3.75	0.40	−0.11 ± 0.21	−0.52, 0.3	0.62	0.92 ± 1.52	−2.06, 3.9	0.55
Parabacteroides	22	59	2.81 ± 3.93	−4.89, 10.51	0.48	0.08 ± 8.04	−15.68, 15.84	0.99	−0.55 ± 0.52	−1.57, 0.47	0.30	1.56 ± 3.72	−5.73, 8.85	0.68
Rikenellaceae unspecified genus	22	59	8.87 ± 3.74	1.54, 16.2	0.02*	−8.46 ± 8.04	−24.22, 7.3	0.30	0.01 ± 0.74	−1.44, 1.46	0.99	−3.26 ± 5.2	−13.45, 6.93	0.53
S24-7 unspecified genus	22	59	−21.27 ± 11.32	−43.46, 0.92	0.07	−10.49 ± 24.77	−59.04, 38.06	0.67	−1.99 ± 2.14	−6.18, 2.2	0.36	12.86 ± 14.96	−16.46, 42.18	0.39
Barnesiellaceae unspecified genus	22	59	6.5 ± 7.62	−8.44, 21.44	0.40	−50.84 ± 25.84	−101.49, −0.19	0.05	0.96 ± 1.61	−2.2, 4.12	0.55	−13.22 ± 11.17	−35.11, 8.67	0.24
Butyricimonas	22	38	15.25 ± 6.05	3.39, 27.11	0.02*	−12.65 ± 11.7	−35.58, 10.28	0.29	1.23 ± 1.06	−0.85, 3.31	0.26	−7.73 ± 7.32	−22.08, 6.62	0.30
Odoribacter	22	59	−4.4 ± 3.93	−12.1, 3.3	0.27	−1.27 ± 7.99	−16.93, 14.39	0.87	−0.13 ± 0.74	−1.58, 1.32	0.86	−1.73 ± 5.39	−12.29, 8.83	0.75
Firmicutes, % of sequences														
Granulicatella	22	59	−2.56 ± 5.77	−13.87, 8.75	0.66	8.53 ± 12.15	−15.28, 32.34	0.49	1.1 ± 1.24	−1.33, 3.53	0.38	3.63 ± 7.81	−11.68, 18.94	0.64
Lactococcus	22	59	−2.93 ± 11.69	−25.84, 19.98	0.80	−0.99 ± 23.39	−46.83, 44.85	0.97	−3.43 ± 2.38	−8.09, 1.23	0.16	9.95 ± 15.56	−20.55, 40.45	0.53
Streptococcus	22	59	−10.19 ± 4.76	−19.52, −0.86	0.04*	−16.81 ± 10.24	−36.88, 3.26	0.11	0.48 ± 0.94	−1.36, 2.32	0.61	4.86 ± 6.61	−8.1, 17.82	0.47
Turicibacter	22	59	6.75 ± 7.56	−8.07, 21.57	0.38	−28.69 ± 20.01	−67.91, 10.53	0.16	−3.15 ± 1.3	−5.7, −0.6	0.02*	−32.66 ± 14.9	−61.86, −3.46	0.03*
Clostridiales unspecified genus	22	59	−0.1 ± 2.83	−5.65, 5.45	0.97	0.7 ± 6.08	−11.22, 12.62	0.91	0.12 ± 0.56	−0.98, 1.22	0.83	−0.03 ± 3.92	−7.71, 7.65	0.99
Christensenellaceae unspecified genus	22	59	1.99 ± 7.37	−12.46, 16.44	0.79	0.81 ± 13.18	−25.02, 26.64	0.95	−0.55 ± 1.56	−3.61, 2.51	0.72	−10.41 ± 10.37	−30.74, 9.92	0.32
Clostridiaceae unspecified genus	22	59	1.57 ± 4.94	−8.11, 11.25	0.75	4.96 ± 10.6	−15.82, 25.74	0.64	0.47 ± 0.97	−1.43, 2.37	0.63	−7.06 ± 6.85	−20.49, 6.37	0.31
Clostridium	22	58	−3.88 ± 3.88	−11.48, 3.72	0.32	12.14 ± 8.16	−3.85, 28.13	0.14	−1.4 ± 0.75	−2.87, 0.07	0.07	5.5 ± 5.26	−4.81, 15.81	0.30
Lachnospiraceae unspecified genus	22	59	0.41 ± 1.75	−3.02, 3.84	0.82	0.35 ± 3.51	−6.53, 7.23	0.92	0.3 ± 0.22	−0.13, 0.73	0.18	−2.67 ± 1.59	−5.79, 0.45	0.10
Anaerostipes	22	59	−2.11 ± 4.35	−10.64, 6.42	0.63	−15.44 ± 9.36	−33.79, 2.91	0.11	0.49 ± 0.86	−1.2, 2.18	0.57	−3.61 ± 6.04	−15.45, 8.23	0.55
Blautia	22	59	1.75 ± 2.63	−3.4, 6.9	0.51	−7.53 ± 5.65	−18.6, 3.54	0.19	0.22 ± 0.52	−0.8, 1.24	0.68	−0.89 ± 3.65	−8.04, 6.26	0.81
Coprococcus	22	59	−6.33 ± 3.5	−13.19, 0.53	0.08	−2.43 ± 7.53	−17.19, 12.33	0.75	−1.6 ± 0.69	−2.95, −0.25	0.03*	11.51 ± 4.86	1.98, 21.04	0.02*
Dorea	22	59	5.22 ± 3.88	−2.38, 12.82	0.18	4.84 ± 8.34	−11.51, 21.19	0.56	0.57 ± 0.77	−0.94, 2.08	0.46	−0.7 ± 5.38	−11.24, 9.84	0.90
Lachnobacterium	22	59	9.51 ± 9.97	−10.03, 29.05	0.34	−47.04 ± 30.03	−105.9, 11.82	0.12	−2.46 ± 2.25	−6.87, 1.95	0.28	−10 ± 14.23	−37.89, 17.89	0.49
Lachnospira	22	59	8.27 ± 4.44	−0.43, 16.97	0.07	10.27 ± 9.53	−8.41, 28.95	0.29	−2.29 ± 0.88	−4.01, −0.57	0.01*	−1.52 ± 6.16	−13.59, 10.55	0.81
Roseburia	22	59	−5.41 ± 3.88	−13.01, 2.19	0.17	9.99 ± 8.33	−6.34, 26.32	0.24	−1.47 ± 0.77	−2.98, 0.04	0.06	−1.54 ± 5.38	−12.08, 9	0.78
Ruminococcaceae unspecified genus	22	59	0.56 ± 1.91	−3.18, 4.3	0.77	−8.28 ± 4.1	−16.32, −0.24	<0.05*	−0.14 ± 0.38	−0.88, 0.6	0.71	−3.95 ± 2.65	−9.14, 1.24	0.14

(Continued)

TABLE 4 (*Continued*)

			Full model											
			Time (hours)			Eating frequency (occasions)			Early energy consumption (% of energy)			Overnight-fast duration (hours)		
	Participants, *n*	Samples, *n*	Estimate ± SEE	95% CI	*P*	Estimate ± SEE	95% CI	*P*	Estimate ± SEE	95% CI	*P*	Estimate ± SEE	95% CI	*P*
Anaerotruncus	22	59	17.95 ± 3.88	10.35, 25.55	<0.01*	−34.93 ± 13.57	−61.53, −8.33	0.01*	1.23 ± 0.99	−0.71, 3.17	0.22	−4.91 ± 5.49	−15.67, 5.85	0.38
Faecalibacterium	22	59	−0.79 ± 2.29	−5.28, 3.7	0.73	0.53 ± 4.93	−9.13, 10.19	0.92	−0.21 ± 0.45	−1.09, 0.67	0.64	−0.96 ± 3.18	−7.19, 5.27	0.76
Oscillospira	22	59	7.1 ± 2.2	2.79, 11.41	<0.01*	−0.55 ± 4.72	−9.8, 8.7	0.91	1.48 ± 0.43	0.64, 2.32	<0.01*	−5.75 ± 3.05	−11.73, 0.23	0.07
Ruminococcus	22	59	7.99 ± 3.52	1.09, 14.89	0.03*	2.4 ± 7.56	−12.42, 17.22	0.75	−0.35 ± 0.69	−1.7, 1	0.61	−0.93 ± 4.88	−10.49, 8.63	0.85
Acidaminococcus	22	31	−8.77 ± 32.96	−73.37, 55.83	0.79	23.16 ± 78.81	−131.31, 177.63	0.08	5.68 ± 6.41	−6.88, 18.24	0.39	−28.98 ± 37.87	−103.21, 45.25	0.45
Dialister	22	59	−2.04 ± 5.98	−13.76, 9.68	0.73	−35.52 ± 16.15	−67.17, −3.87	0.03*	−1.8 ± 1.22	−4.19, 0.59	0.15	−2.79 ± 8.75	−19.94, 14.36	0.75
Megamonas	22	37	−6.06 ± 26.36	−57.73, 45.61	0.82	75.44 ± 50.34	−23.23, 174.11	0.15	15.13 ± 4.67	5.98, 24.28	<0.01*	10.58 ± 33.14	−54.37, 75.53	0.75
Megasphaera	22	39	16.68 ± 24.94	−32.2, 65.56	0.51	1.95 ± 54.15	−104.18, 108.08	0.97	2.04 ± 4.87	−7.51, 11.59	0.68	−9.49 ± 29.41	−67.13, 48.15	0.75
Phascolarctobacterium	22	59	−0.18 ± 5.43	−10.82, 10.46	0.97	14.11 ± 10.65	−6.76, 34.98	0.19	0.62 ± 1.07	−1.48, 2.72	0.56	6.49 ± 6.95	−7.13, 20.11	0.36
Veillonella	22	59	−29.46 ± 8.3	−45.73, −13.19	<0.01*	−60 ± 21.49	−102.12, −17.88	<0.01*	−5.64 ± 1.53	−8.64, −2.64	<0.01*	7.22 ± 11.61	−15.54, 29.98	0.54
Mogibacteriaceae unspecified genus	22	59	6.14 ± 3.3	−0.33, 12.61	0.07	0.37 ± 7.8	−14.92, 15.66	0.96	−0.27 ± 0.65	−1.54, 1	0.68	2.72 ± 4.49	−6.08, 11.52	0.55
Erysipelotrichaceae unspecified genus	22	59	7.33 ± 4.98	−2.43, 17.09	0.15	−5.62 ± 10.7	−26.59, 15.35	0.60	2.5 ± 0.98	0.58, 4.42	0.01*	−11.26 ± 6.91	−24.8, 2.28	0.11
Coprobacillus	22	59	6.7 ± 7.15	−7.31, 20.71	0.35	27.32 ± 12.64	2.55, 52.09	0.04	3.11 ± 1.51	0.15, 6.07	<0.05*	−10.83 ± 11.01	−32.41, 10.75	0.33
Holdemania	22	59	11.61 ± 3.1	5.53, 17.69	<0.01*	−7.64 ± 7.52	−22.38, 7.1	0.31	1.52 ± 0.68	0.19, 2.85	0.03*	−14.38 ± 4.89	−23.96, −4.8	<0.01*
Eubacterium	22	57	−12.4 ± 6.04	−24.24, −0.56	<0.05*	2.32 ± 13.21	−23.57, 28.21	0.86	0.25 ± 1.2	−2.1, 2.6	0.84	8.77 ± 8.52	−7.93, 25.47	0.31
Proteobacteria, % of sequences														
Sutterella	22	59	5.45 ± 2.25	1.04, 9.86	0.02*	−6.35 ± 4.57	−15.31, 2.61	0.17	0.13 ± 0.3	−0.46, 0.72	0.66	−3.51 ± 2.13	−7.68, 0.66	0.11
Bilophila	22	59	8.71 ± 4.2	0.48, 16.94	0.04*	15.43 ± 7.89	−0.03, 30.89	0.06	1.19 ± 0.8	−0.38, 2.76	0.14	−10.65 ± 6.28	−22.96, 1.66	0.10
Desulfovibrio	22	59	24.44 ± 8.86	7.07, 41.81	<0.01*	29.37 ± 18.51	−6.91, 65.65	0.12	3.2 ± 1.68	−0.09, 6.49	0.06	−18.01 ± 13.07	−43.63, 7.61	0.17
Enterobacteriaceae unspecified genus	22	59	11.58 ± 8.15	−4.39, 27.55	0.16	17.11 ± 16.37	−14.98, 49.2	0.30	−0.05 ± 1.73	−3.44, 3.34	0.98	12.85 ± 9.06	−4.91, 30.61	0.16
Escherichia	22	59	15.87 ± 6.7	2.74, 29	0.02*	20.07 ± 15.39	−10.09, 50.23	0.20	0.67 ± 1.59	−2.45, 3.79	0.67	7.28 ± 7.96	−8.32, 22.88	0.36
Haemophilus	22	59	−20.11 ± 8.56	−36.89, −3.33	0.02*	−42.83 ± 22.01	−85.97, 0.31	0.06	−3.96 ± 1.39	−6.68, −1.24	<0.01*	−22.93 ± 14.47	−51.29, 5.43	0.12
Verrucomicrobia, % of sequences														
Akkermansia	22	59	12.68 ± 8.6	−4.18, 29.54	0.15	8.67 ± 19.14	−28.84, 46.18	0.65	0.3 ± 1.9	−3.42, 4.02	0.88	−3.66 ± 12.26	−27.69, 20.37	0.77

[1] Results of linear mixed-model analysis were adjusted for repeated sampling, age, BMI, sex, and normalized total fiber intake. Estimates represent the percentage change in the predicted value of the outcome variable for each 1-unit change in the predictor variable if all the other predictors remain constant. One unit of eating frequency is 1 eating occasion. One unit of energy consumption is 1% of daily energy intake. One unit of time or overnight-fast duration is 1 h. This analysis represents a linear relation between microbes or metabolites and time or behaviors during the awake/feeding phase of the circadian cycle. A negative estimate for time indicates that the highest values were seen earlier and decreased throughout the day. A positive estimate for time indicates that the values increased throughout the day and were highest later. A negative estimate for eating behaviors indicates an inverse relation between the outcome and the predictor (e.g., relative abundance of the bacterium was higher when eating frequency was lower). A positive estimate for eating behaviors indicates a positive relation between the outcome and predictor (e.g., relative abundance of the bacterium was higher when eating frequency was higher). *Significant ($P < 0.05$). OTU, operational taxonomic unit.

may modulate circadian variation in the human gastrointestinal microbiome.

The relations reported between the human gut microbiota and time of day highlight several important points in the expanding area of microbiome research. First, associations between time and human gastrointestinal microbiota are modest compared with preclinical studies. However, because these relations may be of relevance, time of defecation should be recorded and considered as a potential covariate in analyses. Although this study did not examine health outcomes, the connections between the gastrointestinal microbiota and host health are too well documented to ignore. Thus, circadian variation within the microbiome, and the potential for eating behaviors to modify this variation, should be further studied as an avenue for health interventions. Future directions include the need for adequately powered, randomized controlled trials of timing interventions with the gastrointestinal microbiota as a primary outcome. These trials should use interventions of various eating window lengths, and with varying eating frequencies, preferably with participants serving as their own controls to minimize interindividual variation.

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