The Intestinal Microbiota and Short-Chain Fatty Acids in Association with Advanced Metrics of Glycemia and Adiposity Among Young Adults with Type 1 Diabetes and Overweight or Obesity

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ABSTRACT

Background: Comanagement of glycemia and adiposity is the cornerstone of cardiometabolic risk reduction in type 1 diabetes (T1D), but targets are often not met. The intestinal microbiota and microbiota-derived short-chain fatty acids (SCFAs) influence glycemia and adiposity but have not been sufficiently investigated in longstanding T1D.

Objectives: We evaluated the hypothesis that an increased abundance of SCFA-producing gut microbes, fecal SCFAs, and intestinal microbial diversity were associated with improved glycemia but increased adiposity in young adults with longstanding T1D.

Methods: Participants provided stool samples at ≤4 time points (NCT03651622: https://clinicaltrials.gov/ct2/show/NCT03651622). Sequencing of the 16S ribosomal RNA gene measured abundances of SCFAs-producing intestinal microbes. GC-MS measured total and specific SCFAs (acetate, butyrate, propionate). DXA (body fat percentage and percentage lean mass) and anthropometrics (BMI) measured adiposity. Continuous glucose monitoring [percentage of time in range (70–180 mg/dL), above range (>180 mg/dL), and below range (54–69 mg/dL)] and glycated hemoglobin (i.e., Hba1c) assessed glycemia. Adjusted and Bonferroni-corrected generalized estimating equations modeled the associations of SCFA-producing gut microbes, fecal SCFAs, and intestinal microbial diversity with glycemia and adiposity. COVID-19 interrupted data collection, so models were repeated restricted to pre-COVID-19 visits.

Results: Data were available for ≤45 participants at 101 visits (including 40 participants at 54 visits pre-COVID-19). Abundance of Eubacterium hallii was associated inversely with BMI (all data). Pre-COVID-19, increased fecal propionate was associated with increased percentage of time above range and reduced percentage of time in target and below range; and abundances of 3 SCFA-producing taxa (Ruminococcus gnavus, Eubacterium ventriosum, and Lachnospira) were associated inversely with body fat percentage, of which two microbes were positively associated with percentage lean mass. Abundance of Anaerostipes was associated with reduced percentage of time in range (all data) and with increased body fat percentage and reduced percentage lean mass (pre-COVID-19).

Conclusions: Unexpectedly, fecal propionate was associated with detriment to glycemia, whereas most SCFA-producing intestinal microbes were associated with benefit to adiposity. Future studies should confirm these associations and determine their potential causal linkages in T1D. This study is registered at clinical.trials.gov (NCT03651622; https://clinicaltrials.gov/ct2/show/NCT03651622).

Keywords: type 1 diabetes, gut microbiota, short-chain fatty acids, continuous glucose monitoring, dual-energy X-ray absorptiometry, hemoglobin A1c, glycemia, adiposity, body mass index

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Supplemental Figure 1 is available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.

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Abbreviations used: ACT1ON, Advancing Care for Type 1 Diabetes and Obesity Network; CGM, continuous glucose monitoring; DADA2, Divisive Amplicon Denoising Algorithm; FMT, fecal microbiota transplantation; GEE, generalized estimating equation; GLP-1, glucagon-like peptide 1; GPR, G-protein coupled receptor; Hba1c, glycated hemoglobin; IRB, Institutional Review Board; NDSP, Nutrition Data System for Research; NORC, Nutrition Obesity Research Center; QIIME2, Quantitative Insights Into Microbial Ecology 2; rRNA, ribosomal RNA; SMART, Sequential Multiple Assignment Randomized Trial; T1D, type 1 diabetes; UNC, University of North Carolina at Chapel Hill.
Introduction

Individuals with type 1 diabetes (T1D) are at a 3–7 times higher risk of micro- and macrovascular (cardiovascular) complications than those without diabetes (1). Comanagement of glycemia and adiposity is the cornerstone of cardiometabolic risk reduction for people with T1D. However, glycemic and weight targets are often not met, particularly in young adults, in whom glycated hemoglobin (HbA1c) peaks between the ages of 19 and 30 (2). Glycemia has not improved in the United States whereas the prevalence of obesity has increased in people with T1D in recent decades (3–5) despite numerous advances in and uptake of diabetes self-management technologies [e.g., continuous glucose monitoring (CGM) and insulin pump therapy] (6). There is a pressing need to identify novel factors that ease the burden of comanaging glycemia and adiposity in T1D.

One potential disruptor of comanagement of glycemia and adiposity is an altered composition of the intestinal microbiota—the vast community of microbes residing in the intestinal tract—and its efferent metabolites, short-chain fatty acids (SCFAs), produced through gut microbial fermentation of nondigestible carbohydrates such as fiber (7). Individuals with T1D may have a reduced abundance of SCFA-producing gut microbes and fecal SCFAs compared with controls without T1D (8–10). This altered microbial ecosystem could impact glycemia and adiposity via metabolic regulation. Specifically, SCFAs have been shown to bind to receptors in the liver and adipose tissue leading to improved hepatic and peripheral insulin sensitivity, enhanced neurologically mediated satiety, and glucagon-like peptide 1 (GLP-1) production by intestinal epithelial L-cells—which can enhance satiety through afferent signals to the brain's appetite regulatory centers (7, 11, 12). However, salient to weight management, specific gut microbes can increase energy harvest by fermenting fiber to SCFAs, which invites speculation that SCFA-producing intestinal microbes could contribute to positive energy balance and therefore weight gain (13–15). The notion that SCFAs can contribute to adiposity is supported by the finding that mice lacking the G-protein coupled SCFA receptor GPR41 are leaner than their wild-type littermates (16), although another preclinical study found a protective effect of SCFAs on adiposity through their signals to a different SCFA receptor, GPR43 (17).

Investigations of the role of the gut microbiota in T1D etiology have revealed differences in the abundances of major gut microbial taxa and their functional genomic potential (e.g., increases or decreases in facets of carbohydrate metabolism) in individuals with T1D compared with controls without T1D (8, 9, 18). However, prior associations of gut microbes and SCFAs with glucose metabolism and adiposity in animals, and in humans with metabolic syndrome (19, 20) have not been effectively translated to the metabolically unique setting of longstanding T1D. Therefore, in this hypothesis-generating study, we assessed whether an increased abundance of SCFA-producing gut microbes, fecal SCFAs, and intestinal microbial diversity were associated with improved glycemia, but also with increased adiposity, in young adults with longstanding T1D and overweight or obesity.

Methods

Study sample

Participants were young adults with T1D aged 19–30 y (T1D duration ≥1 y), literate in English, HbA1c <13.0% (<119 mmol/mol), and BMI 27–39.9 kg/m² who enrolled in the NIH-funded Advancing Care for Type 1 Diabetes and Obesity Network (ACT1ON) Sequential Multi Assignment Randomized Controlled Trial (SMART) pilot for weight and glycemic management (1DP3DK113358–01, NCT03651622). Those included in the present analysis participated in an ancillary gut microbiome pilot study under the umbrella of ACT1ON. The study design of ACT1ON has been described elsewhere (21). Briefly, the ACT1ON SMART pilot was a 9-mo feasibility intervention conducted at the University of North Carolina at Chapel Hill (UNC) and Stanford University. Its objective was to identify acceptable dietary strategies (hypocaloric low carbohydrate, hypocaloric moderate low fat, or Mediterranean diet without calorie restriction) to co-optimize weight and glycemia in young adults with T1D. Per the SMART design, participants were assigned to an initial diet arm at study enrollment, after which the trial adapted dynamically to participant responses by rerandomizing those who rated the diet as being unacceptable or who did not achieve a minimum weight loss, or whose glycemic parameters deteriorated according to HbA1c or self-reported hypoglycemia. Diets were assigned using permuted block randomization stratified by site at 3 and 6 mo of the intervention (22–24). The primary parent study outcomes were weight, HbA1c, and percentage of time in clinical hypoglycemia (54–69 mg/dL) (25) assessed by CGM at the end of each of 3 diet periods. Secondary outcomes were percentage body fat assessed by DXA, and percentage of time in target glucose range (CGM, 70–180 mg/dL). (25) UNC coordinated the study.

We identified eligible young adults according to medical record data for participation in the parent ACT1ON SMART using a 2-step recruitment process (26). Participants completed 4 measurement visits. All study visits were completed between November 12, 2018 and February 2, 2021. As of April 27, 2020, the study moved to a virtual format via a Health Insurance Portability and Accountability Act–secure Zoom account in continued response to COVID-19. Dietary counseling and data collection were both done virtually, and recruitment ceased (we enrolled 68 participants whereas the target was 72). We conducted the first virtual visit during COVID-19 on June 17, 2020. Using standardized protocols with support from study staff, participants collected HbA1c samples and inserted CGM sensors at home. DXA was discontinued and measures of body composition were therefore only available pre-COVID-19.

Ancillary gut microbiome pilot study

We invited ACT1ON study participants who had not taken antibiotics in the prior month to provide stool samples for an ancillary hypothesis-generating gut microbiome study via a home collection during the 2 weeks in which all other measurement visit data were collected (i.e., before the beginning of each diet period). We originally planned to collect samples only at baseline and measurement visit 2, which we did pre-COVID-19. During COVID-19, we added voluntary stool collection at measurement visits 3 and 4 due to participant dropout and diminished...
sample size resulting in part from the COVID-19 pandemic. We invited participants who provided samples at the baseline visit to provide additional samples at follow-up visits if they reported no antibiotic use in the month prior to collection.

Sixty-eight parent ACT1ON participants completed 200 visits across the 4 study timepoints. Forty-five participants voluntarily provided stool for the ancillary gut microbiome pilot study, including 112 stool samples across the 4 study timepoints (we excluded 6 participants for antibiotic use, 2 who did not return the stool sample before diet randomization due to shipment issues, 2 who had difficulty with producing a sample, 4 who declined participation, and 9 who initially agreed to participate but did not return the sample prior to randomization). We restricted the analysis to visits with concurrently available 24-h dietary recall data, because it was necessary to adjust for fiber intake as an important potential confounder. Thus, we excluded 11 samples from study analysis due to missing diet data. SCFA and diet data were available for 101 samples, of which all had HbA1c and BMI data, 43 were missing DXA data (due to the post-COVID-19 virtual format), and 24 had missing (n = 9) or insufficient (n = 15) CGM data. An additional 4–5 samples (dependent on the outcome) did not pass quality controls [filtering and denoising in the Quantitative Insights Into Microbial Ecology 2 (QIIME2) analytic pipeline] and were therefore excluded from analysis of gut microbial taxonomy and diversity but still provided SCFA data. We showed a Consolidated Standards of Reporting Trials diagram with of gut microbial taxonomy and diversity but still provided SCFA data.

SCFA analysis. We analyzed total and specific fecal SCFAs using GC-MS (Agilent 7820), as previously described. Values were expressed in micromoles per gram (39).

CGM. Study participants wore a blinded CGM (Freestyle Libre Pro; Abbott Diabetes Care Inc) for 2 weeks following each measurement visit. We computed percentage of time in target glucose range (70–180 mg/dL), percentage of time above target range (i.e., hyperglycemia, >180 mg/dL), and percentage of time below range (i.e., clinical hypoglycemia, 54–69 mg/dL) for use as outcome variables in the present analysis. We did not use CGM values in the range of clinically serious hypoglycemia (<54 mg/dL) (25) because factors related to insulin dosing are likely to cause clinically serious hypoglycemic events, and because the amount of time in clinical hypoglycemia was limited (1.8%; IQR, 0.73%; 4.1%). We included observations with ≥1 wk of CGM data (i.e., ≥168 h regardless of gaps in readings) based on a recent consensus statement (25).

DXA. We quantified body fat percentage and percentage lean mass using a DXA scan (UNC: GE Lunar iDXA, GE Medical Systems Ultrasound & Primary Care Diagnostics; Stanford: Horizon Model A, Hologic).

HbA1c. We collected venous blood samples in person prior to COVID-19 and sent them to the Northwest Lipid Metabolism and Diabetes Research Laboratories at the University of Washington School of Medicine for determination of HbA1c. During COVID-19, participants obtained capillary blood samples at home using provided kits (BIO-RAD Hemoglobin Capillary Collection System for HbA1c Testing) with live instruction from study staff via Zoom. Participants mailed home kits to the Diabetes Diagnostic Lab at the University of Missouri, Columbia for deter-
ministration of HbA1c. In a prior study of 122 participants with T1D or type 2 diabetes at 22 clinical centers, venous HbA1c was highly correlated with capillary HbA1c ($R^2 = 0.993$), and 96.7% of measurements differed by $\leq 0.2\% (2.2 \text{ mmol/mol})$ (40).

**Anthropometrics.**
Weight (to the nearest 0.1 kilograms) was measured in person pre-COVID-19 at each measurement visit following standard procedures. During COVID-19, participants measured weights at home using Body-Trace Bluetooth scales, which were provided at study enrollment for voluntary weight tracking. We used baseline height measurements (to the nearest 0.1 cm) and the weight at each measurement visit to calculate BMI (kg/m$^2$).

**Demographic and clinical covariates.**
Participants self-reported demographic data including age, gender, race and ethnicity, and insulin regimen (twice daily, 3 times daily, >3 times daily injections, or insulin pump) using standardized questionnaires. Self-reported race categories included African American, American Indian/Alaska Native, Asian, Native Hawaiian/Other Pacific Islander, Other race, or white. Ethnicity was classified as Spanish/Hispanic/Latino or not. Given sample size limitations, we collapsed race and ethnicity into a single indicator variable: Other race and ethnicity or non-Hispanic White. We provide information about raw race and ethnicity in all relevant table legends. We imputed 3 missing observations for insulin regimen forwards or backwards from the closest visit in time. Insulin regimen was dichotomized as insulin pump or injections due to sample size limitations.

**Dietary intake.**
Trained UNC NIH/National Institute of Diabetes and Digestive and Kidney Diseases Nutrition Obesity Research Center (NORC) staff administered 24-h dietary recalls via telephone at each measurement visit using a multipass method (41, 42). Staff collected recalls following a standard script on nonconsecutive days, ideally including 1 weekday and 1 weekend day. If 2 dietary recalls were available for a participant at a given measurement visit, the nutrient values were averaged across the 2 d. We removed unreliable dietary recalls according to the interviewer or that participants described as being “a lot more” or “a lot less” than they usually ate from analysis. NORC used the Nutrition Data System for Research (NDSR, version 2019; Nutrition Coordinating Center, University of Minnesota) (43) to derive nutrients associated with recalled foods and beverages.

**Design covariates.**
We constructed an indicator variable denoting whether each visit was completed during COVID-19; the duration (months) of each diet period given increased variability during COVID-19; the diet period (1, 2, or 3) to account for a likely greater impact of the intervention in the first diet period; diet assignment; and study site.

**Statistical analysis**
We compared the baseline demographic and clinical characteristics of ACT1ON study participants included and excluded from the analysis to assess representativeness. We conducted sensitivity analyses for outlier observations that were $\geq 2$ SDs from the mean by rerunning the models without the outlier. If the outlier had undue influence on the results, the outlying value was truncated (winsorized) to 10% above or below than the next highest or lowest absolute value (44). We chose this process because even when an outlier was determined not to be the result of measurement error, we did not want a single value to have undue influence on the results. We winsorized outlier values for fecal butyrate and the normalized abundance of *Anaerostipes*. An additional sensitivity analysis tested whether exclusion of 2 outlier observations for fecal acetate influenced results. They did not, so fecal acetate outliers were not winsorized.

**Effect size and power.**
After correction for multiple comparisons, we were powered to detect an $R^2$ of 0.07 with 80% power and an $R^2$ of 0.10 with 90% power given a sample size of $n = 101$ (the sample size for all available HbA1c or weight data when SCFA data were also available). Using the sample size of $n = 58$ for available DXA data (similar to the sample size for pre-COVID-19 data), we were powered to detect an $R^2$ of 0.13 with 80% power and an $R^2$ of 0.16 with 90% power. The magnitude of these effect sizes is smaller than those found in prior studies of the intestinal microbiota and adiposity (Spearman $\rho = 0.28–0.6$) with smaller sample sizes than ours ($n = 30–39$), suggesting that we were powered to detect observable effects (45, 46).

**Modeled analysis.**
We fit separate generalized estimating equation (GEE) models predicting outcomes (percentage of time in target glucose range, percentage of time above range, percentage of time below range, body fat percentage, percentage lean mass, BMI, and HbA1c) from each exposure variable [abundance of each SCFA-producing taxon, fecal SCFA (butyrate, propionate, acetate, and total) concentrations, and intestinal microbial diversity (number of unique taxa per sample)] using data from the 4 measurement timepoints (time 0 and roughly at 3, 6, and 9 mo).

We elected to use GEEs because they account for nonindependence of repeated measures. Although linear mixed models also have this capability, GEEs can better handle zero-inflated gut microbiome data (47). Because the adult fecal microbiome has high interindividual variability and temporal stability (48–50), substantial changes to diet are necessary to observe changes in the fecal microbiome; therefore, because ACT1ON was a free-living diet study, we designed this analysis as a repeated measures interindividual comparison rather than an intrindividual longitudinal analysis of how changes in the gut microbiome predict changes in glycemia and adiposity. We computed standardized $\beta$ coefficients by dividing each $\beta$ estimate from GEE models by its SE to allow for comparability across estimates and report these unitless standardized coefficients in the figures (51).

We repeated all modeled analyses restricted to pre-COVID-19 data, given changes in the mode of intervention delivery, in the assessment methods for the primary ACT1ON parent study outcomes of glycemia and weight, and reduced study retention and adherence to diet assignments during COVID-19. Analysis of the larger parent study outcomes revealed that the statistically significant $\sim 5$-lb ($\sim 2.27$-kg) mean weight loss at the end of the first diet period pre-COVID-19 was attenuated, although not to nonsignificance, when including participants who completed the first diet period during COVID-19 (D Igudesman, J Crandell, KD Corbin, DP Zaharieva, A Addala, JM Thomas, A Casu, MS Kirk-
TABLE 1 Baseline characteristics among ACT1ON study participants included or excluded from the analytic sample (total n = 68)\(^1\)

|                          | Included (n = 45) | Excluded (n = 23) | P value |
|--------------------------|------------------|-------------------|---------|
| Age, mean ± SD, y        | 25.4 ± 3.3       | 25.6 ± 2.8        | 0.73    |
| Female gender, n (%)     | 31 (68.9)        | 18 (78.3)         | 0.42    |
| Non-Hispanic White race and ethnicity, \(^2\) n (%) | 34 (75.6) | 11 (47.8) | 0.02    |
| UNC site, n (%)          | 27 (62.8)        | 12 (48.0)         | 0.23    |
| Diabetes duration, mean ± SD, y | 15.1 ± 6.4       | 11.8 ± 5.7        | 0.03    |
| Insulin pump use, n (%)  | 25 (58.1)        | 15 (60.0)         | 0.88    |
| BMI, median (Q1, Q3)     | 30.8 (28.2, 34.0)| 29.7 (27.1, 33.2)| 0.33    |
| HbA1c, mean ± SD, %      | 7.8 ± 1.4        | 8.0 ± 1.3         | 0.52    |
| Body fat percentage, median (Q1, Q3) | 41.6 (34.7, 45.1) | 39.2 (32.6, 43.8) | 0.46    |
| Percentage lean mass, median (Q1, Q3) | 55.2 (52.3, 62.1) | 57.6 (54.0, 64.2) | 0.40    |
| Time in range (>70–180 mg/dL), mean ± SD, % | 52.6 ± 22.0 | 44.1 ± 20.5 | 0.16    |
| Time above range (>180 mg/dL), mean ± SD, % | 40.2 ± 25.2 | 50.4 ± 22.8 | 0.14    |
| Time below range (54–70 mg/dL), median (Q1, Q3), % | 3.7 (1.7, 7.4) | 3.3 (1.0, 5.5) | 0.39    |

\(^1\) Group differences in continuous variables were tested using independent t-tests for normally distributed variables or Mann–Whitney U (Wilcoxon rank sum test) for non-normally distributed continuous variables. Group differences in categorical variables were tested using the \(\chi^2\) test for independence or the Fisher exact test if any cell sizes were less than \(n = 5\). Note: Body composition measures were only available pre-COVID-19 due to discontinuation of DXA after the transition to a virtual protocol during COVID-19. ACT1ON, Advancing Care for Type 1 Diabetes and Obesity Network; HbA1c, glycated hemoglobin; Q1, quartile 1; Q3, quartile 3; UNC, University of North Carolina at Chapel Hill.

\(^2\) Race and ethnicity were collapsed into non-Hispanic white and Other due to sample size limitations. To avoid the possibility of participant identification, we express frequencies with \(n\) values to be statistically significant at an \(\alpha\) level <0.1. We estimated power calculations with R software version 4.1.1. We conducted all other analyses using SAS version 9.4.

\(^3\) Six and 7 participants included in the analysis were missing data for diet and continuous glucose monitoring, respectively, at the baseline visit.

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| SCFA, μmol/g | Measurement visit 2 | Measurement visit 3 | Measurement visit 4 |
|-------------|---------------------|---------------------|---------------------|
|             | Baseline            | All data            | Pre-COVID-19        |                      |
| n           | 39                  | 25                  | 19                  | 15                  | 22                  |
| Acetate     | 52.9 ± 19.3         | 58.0 ± 24.1         | 56.4 ± 26.5         | 50.8 ± 16.9         | 56.9 ± 20.1         |
| Butyrate    | 7.0 ± 1.8           | 6.6 ± 1.9           | 6.5 ± 2.1           | 7.4 ± 2.2           | 6.6 ± 2.0           |
| Propionate  | 16.4 ± 6.1          | 16.6 ± 7.8          | 14.8 ± 7.5          | 17.7 ± 7.7          | 16.8 ± 5.6          |
| Total SCFA  | 76.3 ± 23.6         | 81.1 ± 29.7         | 77.6 ± 32.1         | 75.9 ± 16.0         | 80.3 ± 24.4         |

| CGM metrics | n       |               |                   |                   |
|-------------|---------|---------------|-------------------|-------------------|
|             | 26      | 19            | 14                | 13                | 19                |
| Time in range, mean ± SD, % | 52.4 ± 20.5 | 60.1 ± 19.2 | 61.1 ± 20.9 | 59.2 ± 17.9 | 53.6 ± 18.9 |
| Time above range (>180 mg/dL), mean ± SD, % | 40.8 ± 23.0 | 32.0 ± 21.8 | 32.1 ± 23.8 | 28.3 ± 23.0 | 36.9 ± 24.5 |
| Time below range (54–70 mg/dL), median (Q1, Q3), % | 3.7 (19, 7.2) | 3.6 (22, 10.7) | 3.6 (22, 6.2) | 7.0 (4.8, 10.7) | 4.0 (1.5, 9.1) |

| DXA metrics | Baseline | Measurement visit 2 | Measurement visit 3 | Measurement visit 4 |
|-------------|----------|---------------------|---------------------|---------------------|
| n           | 39       | 25                  | 19                  | 15                  | 22                  |
| Body fat percentage, median (Q1, Q3) | 42.8 (34.3, 45.2) | 36.6 (31.0, 43.9) | —                   | —                   |
| Percentage lean mass, median (Q1, Q3) | 54.7 (51.8, 62.8) | 60.5 (54.5, 65.5) | —                   | —                   |
| HbA1c, % (mmol/mol) | 8.0 ± 1.4 | 7.3 ± 1.2 | 7.2 ± 1.2 | 7.1 ± 1.2 | 7.4 ± 1.3 |
| Mean ± SD % (64 ± 15. mmol/mol) | (56 ± 13.1) | (55 ± 13.1) | (54 ± 13.1) | (57 ± 14.2) |

| BMI, kg/m² | n       |               |                   |                   |
|------------|---------|---------------|-------------------|-------------------|
| n          | 39      | 25            | 19                | 15                | 22                |
| Median (Q1, Q3) | 30.3 (28.0, 34.0) | 28.7 (27.3, 33.1) | 28.6 (27.0, 32.4) | 29.3 (27.6, 34.1) | 28.9 (26.8, 35.1) |

1 Data are from participants who had both SCFA and outcome data at the corresponding timepoint. Thirty-seven participants had CGM and SCFA data at 77 visits (32 participants with 40 visits pre-COVID-19); 45 participants had DXA and SCFA data at 58 visits pre-COVID-19; 45 participants had BMI and SCFA data at 101 visits (43 participants with 58 visits pre-COVID-19); 45 participants had HbA1c and SCFA data at 101 visits (40 participants with 54 visits pre-COVID-19). Stool collection was added to Measurement visits 3 and 4 only after COVID-19 began, and DXA was removed from the protocol when COVID-19 began; therefore, no participants have both DXA and gut microbiome data at Measurement visits 3 and 4. Note that baseline sample sizes may be smaller in Table 2 than in Table 1 because fecal samples were not available for all included participants at baseline. CGM, continuous glucose monitoring; HbA1c, glycated hemoglobin; Q1, quartile 1; Q3, quartile 3.
tion of 15.1 ± 6.4 y (Table 1). Over two-thirds of included participants identified with a female gender (68.9%), 62.8% were enrolled at UNC, and 58.1% used insulin pump therapy for their diabetes management. ACT1ON study participants who were included (n = 45) or excluded (n = 23) from analysis did not differ with respect to age, gender, enrollment across study sites, BMI, insulin pump use, dietary fiber intake, or any of the study outcomes related to glycemia or adiposity at the baseline visit (all P > 0.05). ACT1ON participants included in the analytic sample had a longer diabetes duration (15.1 ± 6.4 y) and were less racially and ethnically diverse (75.6% had a non-Hispanic white race and ethnicity) than those excluded (diabetes duration 11.8 ± 5.7 y, P = 0.03; 47.8% had a non-Hispanic white race and ethnicity, P = 0.02).

Mean or median values for SCFAs and the outcomes of glycemia and adiposity across all timepoints of the ACT1ON gut microbiome pilot study are displayed in Table 2. Collapsing across visits, the mean ± SD values for fecal SCFAs were 54.7 ± 20.3 μmol/g for acetate, 16.7 ± 6.6 μmol/g for propionate, and 6.9 ± 1.9 μmol/g for butyrate.

Using all available data, the only associations among SCFAs-producing microbes, fecal SCFAs, and α-diversity with glycemia and adiposity that remained statistically significant after adjustment for potential confounders and Bonferroni correction were: 1) a negative association of the abundance of *Eubacterium hallii* with BMI (unstandardized β estimate −0.70 [95% CI −1.2, −0.24] P = 0.07); and 2) a negative association of the abundance of *Anaerostipes* with percentage of time in target glucose range (unstandardized β estimate −8.9% [95% CI −13.1, −4.0] P = 0.01).

Using pre-COVID-19 data, 4 covariate-adjusted and Bonferroni-corrected associations remained statistically significant for outcomes of glycemia, and 8 associations remained statistically significant for outcomes of adiposity. All statistically significant associations for glycemia used CGM data and had the following unstandardized β estimates: a 1 SD increase in fecal propionate or in total fecal SCFA was associated with a 11.7% [95% CI 5.6, 17.7] and an 11.7% [95% CI 4.9, 18.5] increase in the percentage of time spent in hyperglycemia (>180 mg/dL), respectively; and a 1 SD increase in fecal propionate was associated with a 1.6% (95% CI 2.6, 0.69) reduction in the percentage of time spent in clinical hypoglycemia (54–69 mg/dL) and a 9.3% [95% CI 14.8, 3.8] reduction in the percentage of time spent in target glucose range (70–180 mg/dL), respectively. The increased abundance of 3 SCFA-producing intestinal taxa (*Ruminococcus gnavus*, *Eubacterium ventriosum*, and *Lachnospira*) was associated with reduced body fat percentage; of these, increased *Ruminococcus gnavus* and *Eubacterium ventriosum* were also associated with increased percentage lean mass, and increased *Ruminococcus gnavus* was associated with reduced BMI. The normalized abundance of the SCFA producer *Anaerostipes* was associated with increased BFP, and with reduced percentage lean mass. The largest unstandardized β estimates corresponding to a 1 unit increase in the normalized abundance of the intestinal microbiota were a 1.7% [95% CI 0.92, 2.5%] percentage point increase in body fat percentage with increasing *Anaerostipes* and a −1.6% [95% CI −2.4, −0.78] percentage point reduction in body fat percentage with increasing *Ruminococcus gnavus*. Heatmaps with standardized β coefficients for covariate-adjusted and Bonferroni-corrected estimates are shown in Figure 1A (all data) and Figure 1B (pre-COVID-19 data). Crude unadjusted estimates are not presented due to major confounding by dietary fiber intake, whose inclusion in the adjusted models changed some point estimates by ∼5-fold or more.

Scatterplots of raw data for statistically significant associations of fecal SCFA with measures of glycemia are shown in Figure 2.

**Discussion**

To our knowledge, our hypothesis-generating study is the first to rigorously assess advanced metrics of glycemia and adiposity in associa-
FIGURE 2 Scatterplots of raw pre-COVID-19 data for associations of fecal SCFAs with measures of glycemia that were statistically significant after adjustment for potential confounders and correction for multiple hypothesis testing. (A, B) Increased total fecal SCFAs and fecal propionate were associated with reduced percentage of time in target glucose range. (C, D) Increased fecal propionate was associated with reduced percentage of time above target glucose range (C) and percentage of time in clinical hypoglycemia (D).

Our results suggesting that propionate may have a net negative impact on glycemia are unanticipated, given evidence from preclinical models and humans with metabolic syndrome that SCFAs, including propionate, can improve blood glucose homeostasis and help to resolve inflammation (54–59). Of note, although propionate was associated with a modest reduction in percentage of time in hypoglycemia, this was likely due to increased percentage of time in hyperglycemia. Therefore, we do not consider propionate to be protective from hypoglycemia. One preclinical study found that provision of propionate to healthy human-derived hepatocytes activated 5′-activated AMP kinase, which downregulated the expression of gluconeogenic enzymes and reduced gluconeogenesis, suggesting a potential benefit to glycemia (60). In a rat model, propionate decreased hepatic gluconeogenesis purportedly through stimulation of intestinal gluconeogenesis (61). However, several glucoregulatory mechanisms are disrupted in T1D, including deficient insulin, glucagon, and amylin production (62). This raises the possibility that propionate’s ability to regulate blood glucose is blunted in T1D.
The observed estimates for the association of propionate with hyperglycemia are clinically relevant if it is possible to manipulate the diet or the SCFA-producing capacity of the intestinal microbiota such that propionate is reduced by 1–2 SDs (i.e., a ~10–20% reduction in hyperglycemia). However, these associational data must be interpreted with caution and require confirmation in proof-of-mechanism studies to assess the direct biological effect of propionate administration on blood glucose excursions in individuals with T1D. Of note, calcium propionate is sometimes added to foods as a mold inhibitor (e.g., in certain bread products) (63) but is not calculated by the NDSR software, so we were unable to determine its dietary contribution. We assume that the major source of propionate is microbe-derived, although this requires further investigation (64).

Our results suggest that SCFAs may function differently in people with T1D than in those with metabolic syndrome. Prior use of fecal microbiota transplantation (FMT), or transfer of healthy donor stool to individuals with metabolic syndrome, suggests the potential to improve insulin sensitivity or energy expenditure through FMT, presumably through the action of the intestinal microbiota or its effector metabolites including SCFAs (20, 55, 57, 65). It is surprising that we did not find associations of butyrate (57, 66) with measures of glycemia or adiposity in our study, given its prior associations with metabolic benefits in preclinical models and humans with obesity or type 2 diabetes (67–69). Butyrate might indeed function uniquely in people with T1D: a recent randomized crossover study of 30 participants with longstanding T1D aged 18–65 y (BMI 18.5–25.0), did not find a benefit of daily oral supplementation with 4 g butyrate for 1 mo on immune or glycemic parameters (70). In people with non-alcoholic fatty liver disease, the abundance of butyrogenic Anaerostipes was associated inversely with fasting blood glucose (PMID: 31177662); however, in line with our results, Anaerostipes was associated positively with blood glucose among children with T1D from China (PMID: 34040330). Longer-term studies, including those that enroll adults with T1D and overweight or obesity, are required to fully understand the influence of SCFAs in the clinical management of T1D.

Although our results should be interpreted with caution, they suggest that SCFA-producing gut microbes may uniquely protect against adiposity in people with T1D. However, these observational results should be interpreted with caution, especially given that the SCFA producer Anaerostipes was associated with increased adiposity in our study. Contrary to our results, a preclinical study in mice and a clinical study in people without T1D both found an increased abundance of Ruminococcus gnavus in a group with overweight or obesity compared with their lean counterparts, which the authors attributed to the microbe’s SCFA-producing (i.e., energy-harvesting) abilities (71, 72). In another preclinical mouse model of malnutrition, Ruminococcus gnavus ameliorated an impaired growth phenotype, potentially owing to its production of acylcarnitines—an energy source—from fermentation of branched-chain amino acids (73). Eubacterium ventriosum was similarly elevated in Japanese individuals with obesity compared with those who were lean (74), and Lachnospira decreased with decreasing body weight during a 3-mo weight loss intervention (75). Nonetheless, a well-established function of SCFAs is their ability to stimulate satiety hormone secretion (e.g., peptide YY and GLP-1) from intestinal epithelial L-cells, which signal to central homeostatic satiety regions (16, 76–78). It is possible that in people with T1D, the SCFA-stimulated increase in satiety hormones predominates over energy-accumulating pathways. Ultimately, novel strategies are needed to determine the net ecosystem-wide effects of the gut microbiota and its metabolites on host physiology and energy balance, particularly in people with T1D.

The results of our study should be interpreted in the context of its limitations. Given the hypothesis-generating nature of this study, its observational design, our conservative method of correction for multiple comparisons, modest sample size, and the small effect sizes that characterize associations with the intestinal microbiota, we cannot rule out the possibility of type I or type II error (79) or reverse causality. We focused on the SCFA-producing capacity of the intestinal microbiota, but other metabolic capabilities of the gut microbes we studied might have contributed to their inverse associations with adiposity—which can be investigated using whole-genome sequencing in future research.

We included individuals if they had not taken antibiotics in the prior month, which is long enough for many, but not all, intestinal microbes to reconstitute the intestinal tract (80). Thus, we cannot exclude the possibility that antibiotic use >1 mo prior to stool collection influenced the gut microbial composition such that it was not “usual.” Generalizability of our findings to individuals with T1D who do not have overweight or obesity, who are racially or ethnically more diverse than our study participants, who are middle-aged or older adults, or who were recently diagnosed with T1D could be limited. We lacked sufficient statistical power to adjust for race and ethnicity in their raw form and therefore used a combined binary specification of these variables.

The greater number of statistically significant findings when using pre-COVID-19 data compared with all available data might be due to changes in the composition of the intestinal microbiota and SCFAs during COVID-19 due to reduced exposure to the external environment, changes to diet, and other lifestyle factors such as physical activity, use of antibiotics, inflammatory responses to the COVID-19 virus or other infections, and changes to hygiene practices (81). It is also possible that participants’ management of glycemia and weight changed during COVID-19 (82), or that there was variability in study dropout according to variability in success with managing glycemia or adiposity. We used fecal SCFAs as a proxy for production, which is common in other studies but is not a direct measure (83, 84). SCFAs are absorbed across the intestinal epithelium with high efficiency and therefore represent ~5–10% of total SCFAs produced (85). Future in vivo tracer studies can directly measure SCFA production by the intestinal microbiota (86).

Our study also includes several strengths. We addressed a substantial gap in the literature by assessing the links among the intestinal microbiota, SCFAs, and clinical outcomes in people with longstanding T1D. We used novel and rigorous methods of analyzing the intestinal microbiota in association with advanced metrics of glycemia and adiposity, and we carefully adjusted models for potential confounding and design covariates. All statistically significant associations of the intestinal microbiota and SCFAs with glycemia were detected using CGM-based metrics, which highlights the utility of parsing hyper-, hypo-, and euhyperglycemia from HbA1c—a 3-mo average that is less informative for day-to-day management (87). The majority of statistically significant associations with adiposity used DXA-based...
metrics—which measure body composition directly and better predict metabolic risk than BMI (88)—so the contribution of intestinal microbes and SCFAs to adiposity may be better approximated using DXA.

Given that overweight status and glycemia have not improved or have worsened in people with T1D in the United States over the past several decades (3, 4, 89, 90), our hypothesis-generating study assessed whether the intestinal microbiota and SCFAs warrant further research in the clinical management of T1D. We identified 4 candidate microbes whose abundance was inversely associated with adiposity; and 1 SCFA (i.e., propionate) whose abundance was associated with a potential harm to glycemia. Additionally, the increased abundance of butyrogenic Anaerostipes was associated with more hyperglycemia and a higher body fat percentage, so additional studies should examine this gut microbe in people with T1D and overweight or obesity specifically.

These findings require confirmation in additional observational and mechanistic research, which could ultimately determine whether the intestinal microbiota or SCFAs are worthy of investigation in interventional trials to reduce the cardiometabolic risk factors of dysglycemia and adiposity in people with T1D.

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Data Availability
Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval by the ACT1ON Publications and Presentations Committee. If necessary, a data use or other data sharing agreement will be established.

References
1. De Ferrari SD, De Boer IH, Fonseca V, et al. Type 1 diabetes mellitus and cardiovascular disease: a scientific statement from the American Heart Association and American Diabetes Association. Circulation 2014;130(13):1110–30.
2. Miller KM, Foster NC, Beck RW, et al. Current state of type 1 diabetes treatment in the US: updated data from the T1D Exchange clinic registry. Diabetes Care 2015;38(6):971–8.
3. Liu LL, Lawrence JM, Davis C, et al. Prevalence of overweight and obesity in youth with diabetes in USA: the SEARCH for Diabetes in Youth study. Pediatr Diabetes 2010;11(1):4–11.
4. Malik FS, Sauter KA, Isom S, et al. Trends in glycemic control among young and young adults with diabetes: the SEARCH for Diabetes in Youth study. Diabetes Care 2022;45(2):285–94.
5. Wallace AS, Chang AR, Shin J-I, et al. Obesity and chronic kidney disease in US adults with type 1 and type 2 diabetes mellitus. J Clin Endocrinol Metab 2022;107(5):1247–56.
6. Tauschmann M, Hovorka R. Technology in the management of type 1 diabetes mellitus—current status and future prospects. Nat Rev Endocrinol 2018;14(8):464–75.
7. Chambers ES, Morrison DJ, Frost G. Control of appetite and energy intake by SCFA: what are the potential underlying mechanisms? Proc Nutr Soc 2015;74(3):328–36.
8. Murri M, Leiva I, Gomez-Zumaquero JM, et al. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. BMC Med 2013;11(1):46.
9. Brown CT, Davis-Richardson AG, Giongo A, et al. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. PLoS One 2011;6(10):e25792.
10. de Goffau MC, Luopajärvi K, Knip M, et al. Fecal microbiota composition differs between children with β-cell autoimmunity and those without. Diabetes 2013;62(4):1238–44.
11. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol 2015;11(10):577.
12. De Groot PF, Belzer C, Aydin Ö, et al. Distinct fecal and oral microbiota composition in human type 1 diabetes, an observational study. PLoS One 2017;12(12):e0188475.
13. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell 2016;165(6):1322–45.
14. David IA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505(7484):559–63.
15. Krajmalnik-Brown R, Ihlan ZE, Kang DW, DiBase JK. Effects of gut microbes on nutrient absorption and energy regulation. Nutr Clin Pract 2012;27(2):201–14.
16. Samuel BS, Shaito A, Motoike T, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, GPR41. Proc Natl Acad Sci U S A 2008;105(43):16767–72.
17. Kimura I, Ozawa K, Inoue D, et al. γ-Dependent switch from host physiology for type 1 diabetes. PLoS One 2011;6(10):e25792.
18. de Groot P, Nikolic T, Pellegrini S, et al. Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. Gut 2021;70(1):92–105.
19. den Besten G, Bleecker A, Gerding A, et al. Short-chain fatty acids protect against high-fat diet–induced obesity via a PPARγ-dependent switch from lipogenesis to fat oxidation. Diabetes 2015;64(7):2398–408.
20. Kootte RS, Levin E, Salojärvi J, et al. Improvement of insulin sensitivity after leandomorfeces in metabolicsyndromeisdrivenbybaselineintestinal lipogenesistofatoxidation. Diabetes 2015;64(7):2398–408.
21. Corbin K.D., Igudesman D., Addala A., Casu A., Crandell J., Kosorok M. R., et al. Design of the Advancing Care for Type 1 Diabetes and Obesity Network energy metabolism and sequential multiple assignment randomized trial nutrition pilot studies: An integrated approach to develop weight management solutions for individuals with type 1 diabetes. Contemp Clin Trials 2022;117:106765.
22. Kidwell KM. SMART designs in cancer research: past, present, and future. Clin Trials 2014;11(4):445–56.
23. Lavori PW, Dawson R. Dynamic treatment regimes: practical design considerations. Clin Trials 2004;1(1):9–20.
24. Murphy SA. An experimental design for the development of adaptive treatment strategies. Stat Med 2005;24(10):1455–81.

25. Battelino T, Danne T, Bergenstal RM, et al. Clinical targets for continuous glucose monitoring: data interpretation: recommendations from the International Consensus on Time in Range. Diabetes Care 2019;42(8):1593–603.

26. Standiford DA, Morwessel N, Bishop FK, et al. Two-step recruitment process optimizes retention in FLEX clinical trial. Contemp Clin Trials Commun 2018;12:68–75.

27. Fouladi F, Glenny EM, Bulik-Sullivan EC, et al. Sequence variant analysis reveals poor cor in microbial taxonomic abundance between humans and mice after gnotobiotic transfer. ISME J 2020;14(7):1809–20.

28. Kleiman SC, Glenny EM, Bulik-Sullivan EC, et al. Daily changes in composition and diversity of the intestinal microbiota in patients with anorexia nervosa: a series of three cases. Eur Eat Disord Rev 2017;25(5):423–7.

29. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13(7):581.

30. Lin H, Peddada SD. Analysis of microbial compositions: a review of normalization and differential abundance analysis. NPJ Biofilms Microbiomes 2020;6:60.

31. van den Berge K, Peraudeau F, Soneson C, et al. Observation weights unlock bulk RNA-seq tools for zero inflation and single-cell applications. Genome Biol 2018;19(1):24.

32. Noble EE, Hsu TM, Jones RB, Fodor AA, Goran MI, Kanoski SE. Early-life sugar consumption affects the rat microbiome independently of obesity. J Nutr 2017;147(1):20–8.

33. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 2012;41(D1):D590–6.

34. Willis AD. Rarefaction, alpha diversity, and statistics. Front Microbiol 2019;10:2407.

35. Noble EE, Hsu TM, Jones RB, Fodor AA, Goran MI, Kanoski SE. Early-life sugar consumption affects the rat microbiome independently of obesity. J Nutr 2017;147(1):20–8.

36. Wang Y, Wang H, Howard AG, et al. Circulating short-chain fatty acids are positively associated with adiposity measures in Chinese adults. Nutrients 2020;12(7):2127.

37. Remely M, Aumueller E, Merold C, et al. Effects of short chain fatty acids producing bacteria on epigenetic regulation of FFAR3 in type 2 diabetes and obesity. Gene 2014;537(1):85–92.

38. Louis P, Young P, Holtrop G, Flint HJ. Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. Environ Microbiol 2010;12(2):304–14.

39. van der Lelie D, Oka A, Taghavi S, et al. Rationally designed bacterial consortia to treat chronic immune-mediated colitis and restore intestinal homeostasis. Nat Commun 2021;12(1):1–13.

40. Nathan DM, Krause-Steinrauf H, Braffett BH, et al. Comparison of central laboratory HbA1c measurements obtained from a capillary collection versus a standard venous whole blood collection in the GRADE and EDIC studies. PLoS One 2021;16(11):e0257154.

41. Posner BM, Smigelaki C, Duggal A, Morgan J, Cobb J, Cupples L. Validation of two-dimensional models for estimation of portion size in nutrition research. J Am Diet Assoc 1992;92(6):738–41.

42. Beaton GH, Milner J, Corey P, et al. Sources of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. Am J Epidemiol 2003;157(4):364–75.

43. Mantel N. Why stepdown procedures in variable selection. Technometrics 1970;12(3):621–5.

44. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995;310(6973):170.

45. Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. Am J Clin Nutr 2005;82(3):559–67.

46. Weiss S, Xu ZZ, Peddada S, et al. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 2017;5(1):27.

47. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. Nature 2009;457(7228):480–4.

48. Faith JJ, Guruge JL, Charbonneau M, et al. The long-term stability of the human gut microbiota. Science 2013;341(6141):1237–43.

49. Costello EK, Lauber CL, Hamady M, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science 2009;326(5960):1694–7.

50. Hanley JA, Negassa A, Mølgaard N, Forrester JE. Statistical analysis of correlated data using generalized estimating equations: an orientation. Am J Epidemiol 2003;157(4):364–75.

51. Hanley JA, Negassa A, MD dE, Forrester JE. Statistical analysis of correlated data using generalized estimating equations: an orientation. Am J Epidemiol 2003;157(4):364–75.

52. Mantel N. Why stepdown procedures in variable selection. Technometrics 1970;12(3):621–5.

53. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. BMJ 1995;310(6973):170.

54. Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. Am J Clin Nutr 2005;82(3):559–67.

55. De Groot P, Scheithauer T, Bakker GI, et al. Donor metabolic characteristics drive effects of faecal microbiota transplantation on recipient insulin sensitivity, energy expenditure and intestinal transit time. Gut 2020;69(3):502–12.

56. Canfora EE, van der Beek CM, Jocken JW, et al. Colonic infusions of short-chain fatty acid mixtures promote energy metabolism in overweight/obese men: a randomized crossover trial. Sci Rep 2017;7(1):1–12.

57. Vrieze A, van Nood E, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 2012;143(4):913–6.e7.

58. Singh N, Gurav A, Sivaprasakas S, et al. Activation of GPR109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. Immunity 2014;40(1):128–39.

59. Tedelind S, Westberg F, Kjerrulf M, Vidal A. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: a study with relevance to inflammatory bowel disease. World J Gastroenterol 2007;13(20):2826.

60. Yoshida H, Ishii M, Akagawa M. Propionibacterium propionicum suppresses hepatic gluconeogenesis via GPR43/AMPK signaling pathway. Arch Biochem Biophys 2019;672:108057.

61. De Vadder F, Kovatcheva-Datchary P, Goncalves D, et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell 2014;154(1):84–96.

62. Gerich JE, Langlois M, Noacco C, Karam JH, Forsham PH. Lack of glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha cell defect. Science 1973;182(4108):171–3.

63. Darzi J, Frost GS, Robertson MD. Effects of a novel propionate-rich sourdough bread on appetite and food intake. Eur J Clin Nutr 2012;66(7):789–94.

64. Hoyles L, Snelling T, Umlai T-U, et al. Microbiome–host systems interactions: protective effects of propionate upon the blood–brain barrier. Microbiome 2018;6(1):1–13.

65. Yu EW, Gao L, Stastka P, et al. Fecal microbiota transplantation for the improvement of metabolism in obesity: the FMT-TRIM double-blind placebo-controlled pilot trial. PLoS Med 2020;17(3):e1003051.

66. Engels C, Ruscheweyh H-J, Beerenwinkel N, Lacroix C, Schwab C. The common gut microbe Eubacterium hallii also contributes to intestinal propionate formation. Front Microbiol 2016;7:713.

67. Roshanravan N, Mahdavi R, Alizadeh E, et al. Effect of butyrate and inulin-type fructans in obese women. Gut 2013;62(8):1112–21.

68. Nadal I, Santacruz A, Marcos A, et al. Shifts in clostridia, bacteroides and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents. Int J Obes 2009;33(7):758–67.

69. Weiss S, Xu ZZ, Peddada S, et al. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 2017;5(1):27.

70. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. Nature 2009;457(7228):480–4.

71. Faith JJ, Guruge JL, Charbonneau M, et al. The long-term stability of the human gut microbiota. Science 2013;341(6141):1237–43.
69. Lin HV, Frassetto A, Kowalik EJ, Jr, et al. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. PLoS One 2012;7(4):e35240.

70. De Groot PF, Nikolic T, Imanaliyev S, et al. Oral butyrate does not affect innate immunity and ileal autoimmunity in individuals with longstanding type 1 diabetes: a randomised controlled trial. Diabetologia 2020;63(3):597–610.

71. Petriz BA, Castro AP, Almeida JA, et al. Exercise induction of gut microbiota modifications in obese, non-obese and hypertensive rats. BMC Genomics 2014;15(1):511.

72. Palmas V, Pisanu S, Madau V, et al. Gut microbiota markers associated with obesity and overweight in Italian adults. Sci Rep 2021;11(1):5532.

73. Blanton LV, Charbonneau MR, Salih T, et al. Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. Science 2016;351(6275):aad3311.

74. Kasai C, Sugimoto K, Moritani I, et al. Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. BMC Gastroenterol 2015;15(1):100.

75. Mayengbam S, Lambert JE, Parnell JA, et al. Impact of dietary fiber supplementation on modulating microbiota–host–metabolic axes in obesity. J Nutr Biochem 2019;64:228–36.

76. Tolhurst G, Heffron H, Lam YS, et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein–coupled receptor FFAR2. Diabetes 2012;61(2):364–71.

77. Ceriello A, Novials A, Ortega E, et al. Glucagon-like peptide 1 reduces endothelial dysfunction, inflammation, and oxidative stress induced by both hyperglycemia and hypoglycemia in type 1 diabetes. Diabetes Care 2013;36(8):2346–50.

78. Frost G, Sleeth ML, Sahuri-arisoylu M, et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. Nat Commun 2014;5:3611.

79. Ma Z, Li L, Gotelli NJ. Diversity–disease relationships and shared species analyses for human microbiome-associated diseases. ISME J 2019;13(8):1911–9.

80. Suez J, Zmora N, Zilberman-Schapira G, et al. Post-antibiotic gut mucosal microbiome reconstitution is impaired by probiotics and improved by autologous FMT. Cell 2018;174(6):1406–23.e16.

81. Finlay BB, Amato KR, Azad M, et al. The hygiene hypothesis, the COVID pandemic, and consequences for the human microbiome. Proc Natl Acad Sci U S A 2021;118(6):e2010217118.

82. Tornese G, Ceconi V, Monasta L, Carletti C, Faleschini E, Barb E. Glycemic control in type 1 diabetes mellitus during COVID-19 quarantine and the role of in-home physical activity. Diabetes Technol Ther 2020;22(6):462–7.

83. De la Cuesta-Zuluaga J, Mueller NT, Alvarez-Quintero R, et al. Higher fecal short-chain fatty acid levels are associated with gut microbiome dysbiosis, obesity, hypertension and cardiometabolic disease risk factors. Nutrients 2019;11(1):51.

84. Kim KN, Yao Y, Ju SY. Short chain fatty acids and fecal microbiota abundance in humans with obesity: a systematic review and meta-analysis. Nutrients 2019;11(10):2512.

85. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 1987;28(10):1221–7.

86. Boets E, Gomand SV, Deroover L, et al. Systemic availability and metabolism of colonic-derived short-chain fatty acids in healthy subjects: a stable isotope study. J Physiol 2017;595(2):541–55.

87. Beyond A1C Writing Group. Need for regulatory change to incorporate beyond A1C glycemic metrics. Diabetes Care 2018;41(6):e92–4.

88. Wang Y, Rimm EB, Stampfer MJ, Willett WC, Hu FB. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. Am J Clin Nutr 2005;81(3):555–63.

89. DuBose SN, Hermann JM, Tamborlane WV, et al. Obesity in youth with type 1 diabetes in Germany, Austria, and the United States. J Pediatr 2015;167(3):627–32.e1.

90. Conway B, Miller RG, Costacou T, et al. Temporal patterns in overweight and obesity in type 1 diabetes. Diabetes Med 2010;27(4):398–404.