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Associations of disordered eating with the intestinal microbiota and short-chain fatty acids among young adults with type 1 diabetes

Daria Igudesman, PhD, RD[1,3], **Jamie Crandell, PhD**[2], **Karen D. Corbin, PhD RD**[3], **Dessi P. Zaharieva**[4], **Ananta Addala, DO, MPH**[4], **Joan M. Thomas**[1], **Cynthia M. Bulik, PhD**[1,5,6], **Ian M. Carroll, PhD**[1], **Brian W. Pence, PhD**[7], **Richard E. Pratley, MD**[3], **Michael R. Kosorok, PhD**[2], **David M. Maahs, MD PhD**[4], **Elizabeth J. Mayer-Davis, PhD RD**[1,8]

[1]Department of Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, USA 27599

[2]Department of Biostatistics, University of North Carolina at Chapel Hill, Chapel Hill, USA 27599

[3]AdventHealth Translational Research Institute, Orlando, USA 32804

[4]Department of Pediatrics, Division of Endocrinology, Stanford University, Stanford, USA 94304

[5]Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, USA

[6]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

[7]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, USA 27599

[8]Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, USA 27599

Abstract

Background and Aims—Disordered eating (DE) in type 1 diabetes (T1D) includes insulin restriction for weight loss with serious complications. Gut microbiota-derived short chain fatty acids (SCFA) may benefit host metabolism but are reduced in T1D. We evaluated the hypothesis that DE and insulin restriction were associated with reduced SCFA-producing gut microbes, SCFA, and intestinal microbial diversity in adults with T1D.

Methods and Results—We collected stool samples at four timepoints in a hypothesis-generating gut microbiome pilot study ancillary to a weight management pilot in young adults with T1D. 16S ribosomal RNA gene sequencing measured the normalized abundance of SCFA-producing intestinal microbes. Gas-chromatography mass-spectrometry measured SCFA (total,

Corresponding Author: Daria Igudesman, AdventHealth Translational Research Institute, 301 E Princeton St, Orlando, FL 32804, daria.igudesman@adventhealth.com.

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Competing Interests

All authors declare no competing interests.

acetate, butyrate, and propionate). The Diabetes Eating Problem Survey—Revised (DEPS-R) assessed DE and insulin restriction. Covariate-adjusted and Bonferroni-corrected generalized estimating equations modeled the associations. COVID-19 interrupted data collection, so models were repeated restricted to pre-COVID-19 data.

Data were available for 45 participants at 109 visits, which included 42 participants at 65 visits pre-COVID-19. Participants reported restricting insulin “At least sometimes” at 53.3% of visits. Pre-COVID-19, each 5-point DEPS-R increase was associated with a −0.34 (95% CI −0.56, −0.13, p=0.07) lower normalized abundance of genus *Anaerostipes*; and the normalized abundance of *Lachnospira* genus was −0.94 (95% CI −1.5, −0.42), p=0.02 lower when insulin restriction was reported “At least sometimes” compared to “Rarely or Never”.

Conclusion—DE and insulin restriction were associated with a reduced abundance of SCFA-producing gut microbes pre-COVID-19. Additional studies are needed to confirm these associations to inform microbiota-based therapies in T1D.

Keywords

type 1 diabetes; disordered eating; insulin restriction; gut microbiota; short-chain fatty acids; obesity

Introduction

Type 1 diabetes (T1D) is a risk factor for disordered eating (DE), potentially triggered by the intense focus on diet required for insulin dosing and weight management to mitigate cardiovascular risk (1), and enhanced susceptibility to both T1D and DE during adolescence (the peak age of T1D onset is 10-14 years) (2, 3). Weight stigma may also be a predisposing factor for DE (4), as the prevalence of overweight and obesity among people with T1D now mirrors that of the general population (5, 6). Substantial weight loss at T1D diagnosis is often followed by rapid weight regain upon initiation of insulin therapy, which may contribute to body dissatisfaction (7, 8). Binge eating, excessive exercise, and bulimia nervosa are twice as prevalent in individuals with T1D compared to controls without T1D (2, 3).

Higher scores on the validated Diabetes Eating Problem Survey, Revised (DEPS-R), which screens for mismanagement of insulin and blood glucose, food restriction, binge eating, purging, and over-evaluation of weight and shape, are associated with elevated hemoglobin A1c (HbA1c) and body mass index (BMI)—established cardiovascular disease risk factors (9, 10). Insulin mismanagement, which encompasses insulin restriction for weight loss or overdosing to enable binge eating, has a prevalence as high as 30% in T1D and can lead to uncontrolled blood glucose, life-threatening diabetic ketoacidosis, and microvascular complications such as retinopathy, nephropathy, and neuropathy (11-13). Mortality is synergistically increased when T1D and anorexia nervosa co-occur compared to either condition alone (11).

Two metabolically relevant factors that are altered in individuals with T1D and in those with eating disorders compared to disease-free controls are the intestinal microbiota—the vast community of microbes residing in the intestinal tract—and SCFA, byproducts of microbial

fiber fermentation that may benefit metabolism (14, 15). According to prior studies, individuals with T1D and those with anorexia nervosa may have a reduced abundance of SCFA-producing gut microbes and SCFA, as well as reduced intestinal microbial diversity, compared to controls (15, 16).

It is plausible that disordered eating behaviors may further limit the already-reduced abundance of beneficial SCFA-producing gut microbes in T1D—inhibiting their potential to promote blood glucose and lipid homeostasis, resolve inflammation, mitigate adiposity, and reduce appetite (17). However, the intestinal microbiota and SCFA have not been studied specifically in the context of DE in T1D, which involves unique behaviors that can only be enabled in the setting of exogenous insulin therapy (e.g., insulin restriction to promote glucosuria and weight loss) and may worsen the already-dysregulated metabolic phenotype of T1D (18, 19). Furthermore, it is feasible to augment the abundance of SCFA and their fermentative gut microbes through dietary intervention (e.g., after inpatient treatment for an eating disorder or provision of dietary fiber) (14, 20). Therefore, in this hypothesis-generating study, we assessed whether increased DE, including insulin restriction, was associated with a reduced abundance of SCFA-producing gut microbes, fecal SCFA, and intestinal microbial diversity among young adults with T1D.

Methods

The design and main results of the Advancing Care for Type 1 Diabetes and Obesity Network (ACT1ON) study have been described elsewhere (21, 22). Briefly, ACT1ON was a 9-month feasibility pilot conducted at the University of North Carolina at Chapel Hill (UNC) and Stanford University. The 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 among young adults with T1D. Participants were assigned to an initial diet at study enrollment, after which the trial adapted dynamically to participant responses by re-randomizing those who met pre-specified rerandomization criteria (<2% weight reduction [unless weight loss resulted in a BMI <25 kg/m^2], HbA1c increase ≥0.5%, self-reported increased or problematic hypoglycemia, or self-reported diet unacceptability (23)) at 3- and 6-months. Diets were assigned using permuted block randomization stratified by site (24). The primary parent study outcomes were weight (kg), HbA1c (%, venous blood samples collected in clinic during study visits and analyzed in a central laboratory [Northwest Lipid Research Laboratory, WA] using high-performance liquid chromatography [HPLC]), and percent of time in clinical hypoglycemia (54-69 mg/dl) assessed by continuous glucose monitoring (CGM) at the end of each of the three diet periods. Secondary outcomes were percent body fat assessed by dual-energy x-ray absorptiometry (DXA) and percent of time in target glucose range (CGM, 70-180 mg/dl) (25). The parent study outcomes are reported in a separate manuscript (22). UNC coordinated the study. The experimental protocols and the process for obtaining informed consent were approved by the UNC and Stanford institutional review boards.

Participants completed four measurement visits at baseline and following each of three, 3-month, dietary periods. All study visits were completed between 11/12/2018 and 02/22/2021. As of 04/27/2020, the study moved to a virtual format via a HIPAA-secure

Zoom account in continued response to COVID-19; dietary counseling and data collection were done virtually. Due to COVID-19 related research clinic closures and the uncertainty of the timeline for resumption of normal research activities, the decision was made to cease recruitment (we enrolled 68 of 72 anticipated participants). Using standardized protocols with support from study staff, participants collected HbA1c samples (BIO-RAD Hemoglobin Capillary Collection System for HbA1c Testing, assayed using HPLC at the Diabetes Diagnostic Lab [University of Missouri, Columbia], concordance between venous and capillary HbA1c has an R^2 of 0.99 (26)) and inserted CGM sensors at home. DXA was discontinued.

Study Population

Participants were young adults with T1D aged 19-30 years (T1D duration ≥1 year), literate in English, HbA1c <13.0% (<119 mmol/mol) and body mass index (BMI) 27-39.9 kg/m^2 enrolled in the NIH-funded ACT1ON Sequential Multiple Assignment Randomized Trial (SMART) pilot for weight and glycemic management (1DP3DK113358-01, NCT03651622). Individuals were excluded if they had a history of a diagnosed eating disorder, gastrointestinal or bowel disorder, dietary restrictions that precluded following the study diets (including a vegan diet, although individuals following a vegetarian diet were eligible), were pregnant or lactating, had any episode of diabetic ketoacidosis or hypoglycemia requiring third-party assistance in the prior six months, or were weight unstable (change of ±10 lbs in the prior six months). ACT1ON participants included in the present analysis participated in the ancillary gut microbiome pilot study.

Ancillary Gut Microbiome Pilot Study

We established an ancillary gut microbiome pilot under the umbrella of the parent ACT1ON study, in which we invited ACT1ON study participants from the larger parent dietary intervention trial who had not taken antibiotics in the prior month to voluntarily provide stool samples via home collection during the two weeks in which all other measurement visit data were collected. We originally planned to collect samples only at baseline and Measurement Visit 2, which we did pre-COVID-19. During COVID-19, due to participant dropout and diminished sample size, we invited participants who provided stool samples at the baseline visit to provide additional samples at Measurement Visits 3 and 4 if they reported no antibiotic use in the month prior.

Sixty-eight parent ACT1ON participants completed 200 visits across the four study timepoints. Forty-five participants provided 112 stool samples across the four study timepoints for the ancillary gut microbiome pilot study (we excluded n=6 participants for antibiotic use, n=2 who did not return the stool sample before diet randomization due to shipment issues, n=2 who had difficulty with producing a sample, n=4 who declined participation, and n=9 who initially agreed to participate but did not return the sample prior to randomization). We excluded n=3 samples from SCFA analysis and analysis of gut microbial composition and diversity due to missing DE data from the DEPS-R. We excluded another n=6 samples from analysis of gut microbial composition and diversity as they did not pass quality controls in the Quantitative Insights Into Microbial Ecology (QIIME2) analysis pipeline. The final sample size for analysis of SCFA included 45 participants at

109 visits (including 42 participants at 65 visits pre-COVID-19), and 43 participants at 103 visits for analysis of the intestinal microbiota (including 42 participants at 62 visits pre-COVID-19). We show a CONSORT diagram with sample sizes for each outcome in Supplemental Figure 1.

Measures

Disordered eating—DE was measured by the validated 16-item DEPS-R (9). Items are scored on a Likert scale ranging from 0-5 and correspond to the semantic labels “Never,” “Rarely,” “Sometimes,” “Often,” “Usually,” or “Always”. The total score is the sum of individual response items (range 0-80). We dichotomized insulin restriction as “At least sometimes” if a response of “Sometimes” or greater was provided for at least one of two DEPS-R items: 4. “When I overeat, I don’t take enough insulin to cover the food.” or 13. “After I overeat, I skip my next insulin dose.” If the response for both items was “Rarely” or “Never,” insulin restriction was classified as such.

Stool collection and processing—Participants received thorough verbal and written instructions on home stool collection. Consistent with methods used in multiple clinical trials (27-29), stool samples were stored on ice in a 4°C fridge and then shipped priority overnight to UNC. Samples were held at 4°C during transportation. Transporting fecal samples for up to 48 h at 4°C has been reported to have a negligible impact on gut microbial composition (27-29). Given evidence that storage medium has a modest effect on the composition of the gut microbiota, samples were collected in a sterile collection cup and stored without any added reagents (30). Immediately upon receipt at UNC (or within one hour if first stored at 4°C), samples were homogenized with a spatula and aliquoted in a biological safety cabinet under aerobic conditions, and frozen at −80°C. Given rolling study recruitment, aliquots were stored between 1 year and 2.5 years prior to analysis. To prevent batch effects, samples were randomized prior to laboratory analysis.

Gut Microbiota Characterization—We isolated genomic microbial DNA from human fecal samples using a phenol-chloroform extraction combined with a bead beating step using 0.1 mm glass beads (Bio Spec products, Bartlesville, OK) to physically disrupt bacterial cells and a DNA clean-up kit (Qiagen DNeasy Blood and Tissue extraction kit, Valencia, CA), as previously described (31). We characterized fecal microbiotas using the variable 4 region of the 16S rRNA gene to create sequencing libraries via polymerase chain reaction (PCR) and sequencing on the Illumina MiSeq platform (Illumina, San Diego, CA) at the High-Throughput Sequencing Facility in the Carolina Center for Genome Sciences at the UNC School of Medicine (32).

We managed 16S rRNA gene sequences generated by the Illumina MiSeq platform via the QIIME2 pipeline, including demultiplexing and denoising reads via the Divisive Amplicon Denoising Algorithm (DADA2) (33). We generated sequence variants at 100% identity threshold using DADA2. There were 11,105,926 (98,558.5 [interquartile range, Q1, 78,072.0, Q3 129,325.8] per sample) sequence reads and 2,339 sequence variants. We normalized read counts according to a previously published method (34):

$$\log_{10}\left(\left[\frac{Raw\ count\ in\ sample\ (i)}{\#\ of\ sequences\ in\ sample\ (i)} \times Average\ \#\ of\ sequences\ per\ sample\right] + 1\right)$$

We performed taxonomic classification using the DADA2-formatted reference database Silva version 132 (Bremen, Germany). We used QIIME2 to derive intestinal microbial diversity—which we report as the number of unique sequence variants (i.e., unique taxa) per sample. To reduce potential bias stemming from imbalanced replication of sequence reads during PCR, we rarefied intestinal microbial diversity (i.e., normalized sequencing depth) to 3,000 sequence reads per sample (35). We retained "non-rare" genus and species-level taxa present in ≥25% of samples (36). Of the genus- and species-level SCFA producing taxa we identified through a rigorous literature review (37-41), we detected the following 24 SCFA-producing taxa in the stool of our study participants: *Akkermansia, Alistipes, Anaerostipes, Bacteroides, Bifidobacterium*, two members of the *Clostridium* genus (*Clostridium sensu stricto* cluster 1 and *Clostridium innocuum), Dialister*, three members of the *Eubacterium* genus (*E. eligens, E. hallii*, and *E. ventriosum), Faecalibacterium, Intestinimonas, Lachnospira*, two members of the *Prevotella* genus (*Prevotella* clusters 7 and 9), *Roseburia*, four members of the *Ruminococcus* genus (*Ruminococcus gnavus, Ruminococcus torques*, and *Ruminococcus* clusters 1 and 2), *Sutterella, Streptococcus*, and *Veillonella*. After removal of three taxa that were present in <25% of samples (*Sutterella* and *Prevotella* clusters 7 and 9), 21 taxa were used in analysis.

SCFA Analysis—We analyzed total and specific fecal SCFA using gas-chromatography/mass-spectrometry (Agilent 7820, Santa Clara, CA, USA), as previously described. Values were expressed in μmol/g (42).

Demographic and clinical covariates—Participants self-reported age, gender, race and ethnicity, and insulin regimen (twice daily, three times daily, more than three 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 n=3 missing observations for insulin regimen from the closest visit in time and dichotomized insulin regimen as insulin pump or injections due to sample size limitations.

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 (0 for baseline, 1, 2, or 3); 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.

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=109 (the number of visits with both DE and SCFA 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 given a sample size of n=65 (the number of visits with DE and SCFA data pre-COVID-19). These effects are smaller than or comparable to those in a prior study of the intestinal microbiota among participants with anorexia nervosa (R^2 = 0.15-0.27) with a smaller sample size (n=26), suggesting we were powered to detect observable effects (14).

Modeled analysis—We fit generalized estimating equations (GEE) predicting each outcome (normalized abundance of each genus- or species-level SCFA-producer, fecal SCFA [butyrate, propionate, acetate, and total] levels, and intestinal microbial diversity [number of unique taxa per sample]) from each DE exposure variable (total DEPS-R score or dichotomous insulin restriction). We conservatively estimated the change in each outcome that was associated with a five-point increase in the DEPS-R score given that a 3-month DE intervention for individuals with T1D and a clinically diagnosed eating disorder reduced DEPS-R score by ~15 points (43).

We elected to use GEEs as they account for nonindependence of repeated measures and are well-suited to handle zero-inflated gut microbiome data (44). Because the adult fecal microbiome has high inter-individual variability and temporal stability (45), 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 inter-individual comparison rather than an intra-individual 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 standard error to allow for comparability across estimates and report these unitless standardized coefficients in the figures (46).

We repeated all modeled analyses restricted to pre-COVID-19 data, given changes in the mode of intervention delivery, changes to the assessment methods for the primary ACT1ON parent study outcomes, reduced study retention and adherence to diet assignments during COVID-19, and attenuated weight loss for the parent study during COVID-19 (unpublished data, Igudesman *et al*).

Model 1 was unadjusted but accounted for within-subject correlations of repeated measures. To maximize utility of this pilot study sample, we used step-down approaches to evaluate which non-design potential confounders (age, gender, race and ethnicity, BMI, insulin regimen, and dietary fiber intake) to retain in Model 2 (47). We retained age and race and ethnicity as these were the informative variables ($p<0.1$). Model 2 also included design covariates (randomized diet assignment for each diet period, study site, the COVID-19 indicator, diet duration, and diet period). We did not adjust Model 2 for the COVID-19 indicator, diet duration, or diet period when restricting to pre-COVID-19 visits.

Given the hypothesis-generating nature of this study and our conservative method of correction for multiple comparisons, we considered Bonferroni-corrected (48) p-values to

be statistically significant at an alpha level of <0.1. We estimated power calculations with R software version 4.1.1 (Vienna, Austria). We conducted all other analyses using SAS version 9.4 (Cary, NC).

Results

At enrollment, participants included in the present analysis had an age of 25.4 ± 3.3 years, BMI of 30.8 kg/m^2 (Q1 28.2, Q3 34.0), and a diabetes duration of 15.1 ± 6.4 years (Table 1). Over two-thirds of included participants identified with a female gender (68.9%), 62.2% were enrolled at UNC, and 57.8% used insulin pump therapy for their diabetes management. ACT1ON study participants included in the present analysis were less racially and ethnically diverse than those who were excluded (75.6% of the n=45 included and 47.8% of the n=23 excluded had a Non-Hispanic White race and ethnicity, p=0.02) and had a longer diabetes duration (15.1 ± 6.4 years among those included and 11.8 ± 5.7 years among those excluded, p=0.03) but did not differ according to other baseline characteristics. Fourteen (31.1%) participants contributed data from one time point, 9 (20.0%) participants contributed data from two time points, 11 (24.4%) participants contributed data from three time points, and 11 (24.4%) participants contributed data from all four time points.

Descriptive statistics for DE and fecal SCFA across all timepoints of the gut microbiome pilot study are displayed in Table 2. At baseline, DEPS-R score was 20.4 ± 10.1 and n=24 (53.5%) participants reported restricting insulin “At least sometimes.” Collapsing across visits, the mean ± SD values for fecal SCFA over time were 54.4 ± 19.9 μmol/g for acetate, 16.4 ± 6.5 μmol/g for propionate, and 6.8 ±1.9 μmol/g for butyrate.

Using all available data, no relationships between DE and measures of the intestinal microbiota or SCFA were statistically significant after Bonferroni correction, in either crude or covariate-adjusted models (Figure 1A). Using pre-COVID-19 data, two covariate-adjusted relationships remained statistically significant after correction for multiple comparisons. The unstandardized β estimates were: 1) a 5-point increase in DEPS-R score was associated with a −0.94 (95% CI −1.5, −0.42, p=0.02) decrease in the normalized abundance of SCFA-producing genus *Anaerostipes*; 2) among visits at which insulin restriction was reported “At least sometimes” compared to “Rarely or Never,” there was a −0.34 (95% CI −0.56, −0.13, p=0.07) reduction in the normalized abundance of the SCFA-producing genus *Lachnospira* (Figure 1B).

Discussion

In our sample of young adults with T1D and overweight or obesity at baseline who participated in a weight management trial, we found statistically significant inverse associations of DE (DEPS-R score and insulin restriction “At least sometimes” compared to “Rarely or Never”) with the normalized abundance of two SCFA-producing genera (*Anaerostipes* and *Lachnospira*) using pre-COVID-19 data—a characteristic of the gut microbiota that may be considered to be “dysbiotic” (i.e., reflective of a depletion of beneficial SCFA producing gut microbes) (49). However, no associations were statistically significant when using all available data.

Based on the results of previous studies, the genera *Anaerostipes* and *Lachnospira* may be reduced in various disease states but may be beneficial for metabolic health. In two independent studies, a reduced normalized abundance of the genus *Anaerostipes* was found in people with type 2 diabetes (50) or in those with anorexia nervosa (16) compared to control participants without diabetes or without an eating disorder. In a rodent model fed a Western diet, co-administration of *Anaerostipes rhamnosivorans* and myo-inositol for six weeks reduced fasting glucose, purportedly due to fermentation of myo-inositol—a substrate for propionate production (51). The genus *Lachnospira* was reduced in 30 children with T1D or mature onset diabetes of the young (MODY, a form of monogenic diabetes) and in a separate study of 15 children newly diagnosed with T1D in China compared to control children without diabetes (52, 53). The baseline depletion of *Lachnospira* in 40 older adults with type 2 diabetes compared to controls without diabetes was rectified through provision of a randomized fiber-rich diet for 21 days, which led to concurrent increases in the relative abundance of other SCFA-producing genera, including *Akkermansia, Roseburia*, and *Faecaelibacterium* (54). Collectively, these studies raise the possibility that the genera *Anaerostipes* and *Lachnospira* may serve as biomarkers of nutrient adequacy or maybe be needed in sufficient quantities to support metabolic health. Further inquiry into their mechanistic links with DE in T1D is needed to draw causal inferences.

One mechanism by which DE may influence the composition of the intestinal microbiota in T1D is by promoting pathways related to inflammation. As one example, insulin restriction for weight loss can elicit sustained hyperglycemia (12), which promotes systemic inflammation (55) and may incite gut permeability to allow an expansion of pathogenic intestinal microbes that outcompete beneficial microbes (e.g., those that produce SCFA) (56). The chronic low-grade inflammation that is characteristic of overweight and obesity (57) may further be at play in our sample of participants who had overweight or obesity at enrollment. Furthermore, increased self-reported DE scores among individuals with T1D are consistently associated with elevated HbA1C and BMI (9, 10), so it is possible that DE may further precipitate inflammation by contributing to increased hyperglycemia and body weight. These hypothesized mechanisms may compound the inflammatory nature of autoimmunity in T1D, which itself has been linked with an altered composition of the intestinal microbiota and gut permeability (58).

Most studies evaluating differences in the composition of the gut microbiota between people with and without T1D have been limited to the time near the development of T1D autoimmunity (i.e., in childhood) (59-61), and have found a reduced gut microbial diversity and genus-level differences (e.g., a greater relative abundance of the genus *Bacteroides* (62) and a lower abundance of genera *Prevotella* and *Bifidobacterium* (63, 64)) among children with T1D or islet autoimmunity compared to controls without T1D. Further research is needed to evaluate the clinical relevance of both the compositional and functional differences in the gut microbiota in adults with and without T1D, especially in light of evidence that plasma SCFA—which can have direct metabolic consequences—are lower in adults with longstanding T1D than in those without T1D, despite a slightly higher fiber intake among the cases with T1D (15). Though the study authors found notable differences in gut microbial composition which led to clustering of samples by disease status, there was no difference in alpha diversity—which is concordant with our results (15) and the results

of another small case-control study of physically active adult males with longstanding T1D (65). Collectively, this suggests that disease status is only a driver of low alpha diversity near the time of T1D diagnosis, and that lifestyle behaviors such as diet and physical activity are more salient later in life. Our study participants were actively engaged in an intensive lifestyle intervention focused on a healthful diet and were free from an eating disorder at baseline, so the parent diet intervention was likely a stronger determinant of intestinal microbial diversity than disordered eating in our study sample.

DE behaviors are heterogeneous in T1D and can include those that exert opposing forces on weight management (e.g., food restriction, insulin restriction for weight loss, binge eating in response to hypoglycemia, and giving large insulin boluses to cover binge eating-induced hyperglycemia) (66). This is likely to induce adaptations by the intestinal microbiota in response to changing substrate availability. For example, the archaeon *Methanobrevibacter smithii* has the ability to enhance energy extraction in the setting of a very low calorie diet and is increased among individuals with anorexia nervosa compared to those who are lean or obese—a potential adaptation to the setting of extreme energy deficit (67). On the other hand, the relative abundance of the SCFA producer *Anaerostipes* was increased among 101 cases with binge-eating disorder compared to 39 controls without an eating disorder—which could conceivably be due, in part, to the surplus of calories ingested during binge-eating episodes (68). While numerous studies have determined that increased levels of circulating lipopolysaccharide—a pro-inflammatory bacterial toxin—can precipitate insulin resistance (69, 70), virtually no studies have examined the acute and long-term impact of variability in exogenously administered insulin—which is unique to the setting of insulin dependence such as in T1D—on the composition and functionality of the intestinal microbiota. This should be investigated in future studies, alongside the downstream metabolic consequences 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 nature, our conservative method of correction for multiple comparisons, modest sample size, and the small effect sizes that are characteristic of the intestinal microbiota in human studies, we cannot rule out the possibility of type I or type II error (71). Mechanistic studies and observational studies with larger sample sizes and longitudinal designs may be needed to derive unbiased estimates of effect. In future studies, whole genome sequencing could estimate the influence of disordered eating on functional gut microbial pathways.

Home stool collection poses challenges for measurement of SCFA, which are volatile (72). Studies that collect and snap-freeze feces on-site and process samples in an anaerobic environment provide a means to better preserve SCFA. The levels of fecal SCFA in our study participants are comparable to those of healthy participants whose data are stored in the Human Metabolome Database (measured by GC) (73), but lower than in a recent study of Danish adults with T1D (measured by GC-MS) (74, 75). Although fecal SCFA concentrations are partly dependent on participant age and sex (76), as well as weight (77), disease status (15, 16), diet (78), and geographic location (which impacts gut microbial composition and therefore SCFA production capacity (79)), it is possible that differences in stool collection and processing methods (i.e., lack of anaerobic stool processing) led to

lower fecal SCFA concentrations in our study. Nonetheless, we conclude that fecal SCFA levels were within the normal physiologic range in our study participants and that the high ratio of fecal acetate to propionate or butyrate reflects “normal” physiology (80).

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 may be limited.

Given the cross-sectional nature of our analytic design, we were not able to establish temporality in the association of DE with the intestinal microbiota; therefore, there is the potential for reverse causality, whereby the composition of the intestinal microbiota may influence DE behaviors in T1D through neuroendocrine pathways (81), or that the relationship is bidirectional, as has been suggested in anorexia nervosa (82). It is possible that DE may influence the intestinal microbiota through changes in dietary intake; although this was not our research question and dietary fiber was not an important explanatory variable in statistical models, it may be an important one to investigate in follow-up studies.

The lack of statistically significant findings using all available data may be explained by changing levels of DE during COVID-19 (83) or variability in study dropout during COVID-19 according to the degree of DE. The intestinal microbiota and SCFA may have changed during COVID-19 due to reduced exposure to the external environment, potential changes to diet and other lifestyle factors such as physical activity, use of antibiotics, inflammatory responses to COVID-19 or other infections, and changes to hygiene practices (84).

Individuals in our study sample were free from a medically diagnosed eating disorder at enrollment. Therefore, a greater severity of DE behaviors may be necessary to understand the true nature of the disordered eating-gut microbiome relationship in adults with T1D (85). Nonetheless, subthreshold DE in T1D is prevalent, can have serious health consequences, and is therefore important to monitor regularly in people with T1D (2, 3, 12). Development of objective assessments of disordered eating in T1D could avoid reporting bias associated with sensitive questions. Differential misreporting is unlikely here as participants were unaware of their gut microbiome outcome status.

Our study also includes several strengths. To our knowledge, this is the first study to evaluate the associations of DE and insulin restriction with the intestinal microbiota and SCFA in T1D, as prior studies have focused on changes in the composition of the intestinal microbiota near the time of the development of autoimmunity in T1D (62, 64). Although the ACT1ON study experienced substantial changes in study conduct during COVID-19, all statistically significant associations were detected using pre-COVID-19 data—a time period during which the ACT1ON intervention was delivered as originally intended. We believe that the consistent directionality of our findings gives credence to our results. We utilized rigorous methods of statistical analysis and adjusted models for potential confounding variables.

In our sample of young adults with T1D and overweight or obesity, we found that increased subthreshold DE and insulin restriction were associated with a reduced normalized

abundance of SCFA-producing commensal microorganisms that may also be reduced in the nutrient-poor setting of anorexia nervosa and in the inflammatory state of type 2 diabetes but that may have metabolic benefits to glycemia. If reducing insulin restriction and other DE behaviors can promote replenishment of SCFA producers, which may in turn benefit metabolism, this is another reason to prioritize DE screening and intervention in T1D. Mechanistic studies and randomized trials designed to infer causality in the relationships of DE with the intestinal microbiota and SCFA in T1D can inform the development of novel therapeutic avenues (e.g., tailored diet recommendations or pre- or probiotic administration) for mitigating depletion of beneficial SCFA-producing gut microbes in the setting of DE in T1D.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflicts of Interest and Source of Funding:

DMM has had research support from the NIH, JDRF, NSF, and the Helmsley Charitable Trust and his institution has had research support from Medtronic, Dexcom, Insulet, Bigfoot Biomedical, Tandem, and Roche; and has consulted for Abbott, Aditxt, the Helmsley Charitable Trust, Lifescan, Mannkind, Sanofi, Novo Nordisk, Eli Lilly, Medtronic, Insulet, Dompe, and Biospex. EJM-D has consulted for Helmsley Charitable Trust. CMB reports: Shire (grant recipient, Scientific Advisory Board member); Idorsia (consultant); Lundbeckfonden (grant recipient); Pearson (author, royalty recipient); Equip Health Inc. (Clinical Advisory Board). IMC is a consultant for Vivilex; former consultant for Salix Pharmaceuticals and receives funding from NIH (R21-AI125800–01-02) and NIMH (R01-MN105684–03). DI, JC, KDC, DPZ, AA, JMT, BWP, RP, and MRK declare no conflict of interest. DI was supported by the Global Cardiometabolic Disease training grant (National Heart, Lung, and Blood Institute of the National Institutes of Health) awarded to the Department of Nutrition at the University of North Carolina at Chapel Hill under Award Number HL129969. DPZ is supported by ISPAD-JDRF Research Fellowship and Leona M. and Harry B. Charitable Trust. This study was supported by NIH/NIDDK (1DP3DK113358-01, PNC DK056350, and P30 DK034987).

Data Sharing Plan

Data will be shared as appropriate following formal submission of a paper proposal, to be approved by the ACT1ON Publications and Presentations Committee.

Abbreviations:

T1D	type 1 diabetes
BMI	body mass index
SCFA	short-chain fatty acids
ACT1ON	Advancing Care for Type 1 Diabetes and Obesity Network
SD	standard deviation
Q1	quartile 1

Q3	quartile 3
HbA1c	hemoglobin A1c
DEPS-R	Diabetes Eating Problem Survey, Revised

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- Disordered eating, including insulin restriction, is prevalent in type 1 diabetes
- Disordered eating can deplete beneficial gut microbes and their metabolites
- Disordered eating was associated with reduced *Anaerostipes*
- Insulin restriction was associated with reduced *Lachnospira*
- Future longitudinal studies should confirm these associations in type 1 diabetes

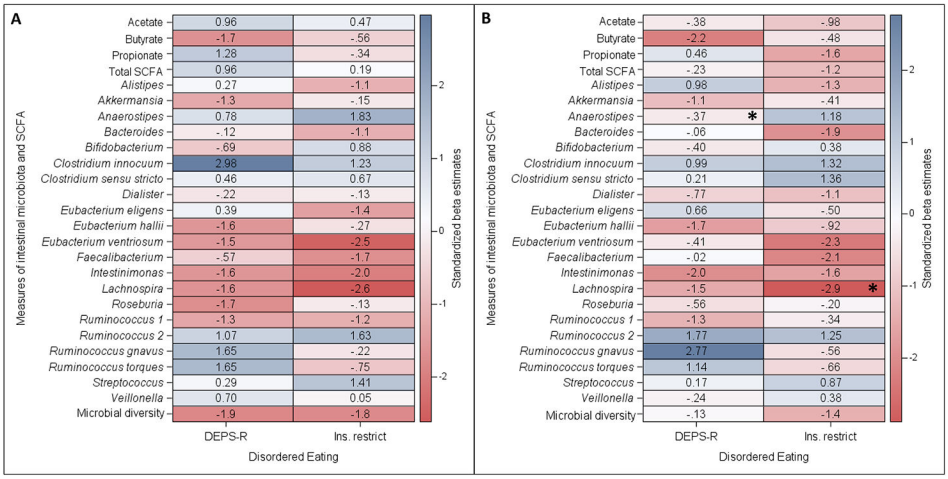

Figure 1.

Heatmaps with standardized beta estimates from covariate-adjusted generalized estimating equations. All data (**A**): 45 participants with 109 visits for SCFA, and 43 participants at 103 visits for the intestinal microbiota. Pre-COVID-19 data (**B**): 42 participants with 65 visits for SCFA, and 42 participants with 62 visits for the intestinal microbiota. P-values were Bonferroni corrected and statistically significant at $p<0.1$ (denoted by asterisks). Units are μmol/g for SCFA, normalized abundance for intestinal microbes, and the number of unique taxa for intestinal microbial diversity. Insulin restriction was dichotomized as “At least sometimes” (“Sometimes,” “Often,” “Usually,” or “Always”) or as “Rarely or Never.” Abbreviations: DEPS-R—Diabetes Eating Problem Survey, Revised; ins. restrict—insulin restriction; SCFA—short-chain fatty acids

Table 1:

Baseline characteristics of ACT1ON participants included and excluded from analysis (n=68)

	Included (n=45)	Excluded (n=23)	p-value
Age: Mean ± SD	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: N (%)	34 (75.6)	11 (47.8)	**0.02**
UNC site: N (%)	28 (62.2)	11 (47.8)	0.26
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
DEPS-R: Mean ± SD	20.4 ± 10.1	22.2 ± 9.2	0.48
Insulin restriction “At least sometimes”: N (%)	24 (53.3)	14 (60.9)	0.55
Diabetes duration (years): Mean ± SD	15.1 ± 6.4	11.8 ± 5.7	**0.03**
Insulin pump use: N (%)	26 (57.8)	14 (60.9)	0.81

Race and ethnicity were dichotomized as non-Hispanic White and Other due to sample size limitations. To avoid participant identification, we express frequencies with <3 individuals as “n<3”: n=4 (8.9%) included participants identified with an African American race, n<3 with an Asian race, n<3 with more than one race, and n=36 (86.0%) participants with a White race; n=6 (13.3%) identified with a Hispanic ethnicity. N<3 excluded participants identified with an African American race, n<3 with a Native Hawaiian/Other Pacific Islander race, n<3 participants with an Asian race, n<3 with Other race, n=5 (20.0%) participants with more than one race, and n=13 (56.5%) with a White race; n=5 (21.7%) identified with a Hispanic ethnicity.

Insulin restriction was dichotomized as “At least sometimes” (“Sometimes,” “Often,” “Usually,” or “Always”) or as “Rarely or Never.”

Unpaired t-tests or the Kruskal Wallis test compared continuous variables. The chi-square or fisher exact test compared categorical variables.

Abbreviations: BMI—body mass index; DEPS-R—Diabetes Eating Problem Survey, Revised; HbA1c—hemoglobin A1c; Q1—quartile 1; Q3—quartile 3; SD—standard deviation

Table 2:

Disordered eating and short-chain fatty acids among included ACT1ON participants

	Baseline	Measurement Visit 2		Measurement Visit 3	Measurement Visit 4
	All data (n=45)	All data (n=25)	Pre-COVID-19 (n=20)	All data (n=16)	All data (n=23)
Disordered eating					
DEPS-R score: mean ± SD	20.4 ± 10.1	16.1 ± 7.6	15.5 ± 6.6	14.9 ± 6.4	16.6 ± 7.9
DEPS-R insulin restriction “At least sometimes”: N (%)	24 (53.3)	12 (48.0)	9 (45.0)	8 (50.0)	13 (56.2)
Fecal SCFA (μmol/g): mean ± SD					
Acetate	54.7 ± 19.1	56.3 ± 24.8	54.9 ± 26.6	50.6 ± 15.0	54.2 ± 19.5
Butyrate	6.9 ± 1.9	6.4 ± 1.9	6.3 ± 2.1	7.0 ± 1.6	6.9 ± 2.1
Propionate	16.6 ± 6.7	16.4 ± 7.9	14.5 ± 7.4	15.1 ± 5.0	16.8 ± 5.5
Total SCFA	78.2 ± 23.8	79.1 ± 30.7	75.8 ± 32.3	72.7 ± 18.6	78.0 ± 23.7

Insulin restriction was dichotomized “At least sometimes” (“Sometimes,” “Often,” “Usually,” or “Always”) or as “Rarely or Never.” Gut microbiome data at Measurement Visits 3 and 4 were only available during COVID-19.

Abbreviations: DEPS-R—Diabetes Eating Problem Survey, Revised; SCFA—short-chain fatty acids; SD—standard deviation