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TITLE

Associations between diet, the gut microbiome and short chain fatty acids in youth with islet autoimmunity and type 1 diabetes

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Author contributions:

JEH: researched data, wrote manuscript, oversaw project and is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis. RTL: reviewed and analysed dietary data and contributed to discussion, reviewed manuscript. JMW: facilitated participant recruitment and study visits, reviewed/edited manuscript, contributed to discussion. JL: analysed data, wrote manuscript. AJRS: performed bioinformatic analysis of DNA, generated figures, wrote manuscript. RJB: collected ACAES data. KMN: performed DNA extraction and sequencing. MASP: developed sample collection methodology, reviewed/edited manuscript and contributed to discussion. PGC: supervised islet autoantibody assays, reviewed/edited manuscript. MEC: facilitated participant recruitment, oversaw NSW study site, reviewed/edited manuscript. SCB: analysed data, reviewed/edited manuscript. CDT: reviewed manuscript. MM: reviewed manuscript and contributed to discussion. LCH: supervised DNA sequencing and analysis, oversaw Victorian study site and wrote manuscript. JJC: oversaw project and wrote manuscript and is the guarantor of this work and, as such, had full access to all the

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ABSTRACT

Aim: We aimed to characterise associations between diet and the gut microbiome and short chain fatty acid products in youth with islet autoimmunity or type 1 diabetes (IA/T1D) in comparison with controls.

Research design and methods: 80 participants (25 diagnosed with T1D, 17 with confirmed IA, 38 sibling or unrelated controls) from the Australian Type 1 Diabetes Gut Study cohort were studied [median (IQR) age 11.7 (8.9,14.0) years, 43% female]. A Food Frequency Questionnaire characterised daily macronutrient intake over the preceding 6 months. Plasma and fecal short chain fatty acids were measured by gas chromatography; gut microbiome composition and diversity by 16S rRNA gene sequencing.

Results: A 10g increase in daily carbohydrate intake associated with higher plasma acetate in IA/T1D [adjusted estimate +5.2 (95% CI 1.1, 9.2) µmol/L p=0.01] and controls [adjusted estimate +4.1 (95% CI 1.7, 8.5) µmol/L p=0.04]. A 5g increase in total fat intake associated with lower plasma acetate in IA/T1D and controls. A five percent increase in non-core (junk) food intake associated with reduced richness [adjusted estimate -4.09 (95%CI -7.83, -0.35) p=0.03] and evenness [-1.25 (95% CI -2.00, -0.49) p<0.01] of the gut microbiome in IA/T1D. Fiber intake associated with community structure of the microbiome in IA/T1D.

Conclusions: Modest increments in carbohydrate and fat intake associated with plasma acetate in all youth. Increased junk food intake associated with reduced diversity of the gut microbiome in IA/T1D alone. These associations with the gut microbiome in IA/T1D support future efforts to promote short chain fatty acids by using dietary interventions.

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INTRODUCTION

Diet shapes the composition and function of the gut microbiota, with rapid adaptation to dietary change.[1–5] An individual's gut microbiome remains relatively stable over time after the rapid changes of the first few years of life.[6] Breast milk intake and weaning have major impacts on the gut microbiome during infancy[7] with rapid shifts in composition and diversity following weaning and the introduction of solid foods, leading to a relatively stable phase by 2-3 years of age.[6–8] However, long-term diet plays an important role in shaping the composition of the gut microbiome. Certain dietary patterns associate with certain microbiota. For example, high carbohydrate intake tends to be associated with Genus *Prevotella*,[9] and high protein and animal fat intake with *Bacteroides.*[9] Distinct microbiome profiles are described with dietary patterns such as Mediterranean, vegetarian, vegan and high meat diets.[2,4,5,10–13] Sustained dietary changes, including increased fiber and plant-based diets can shift gut microbiota composition and increase short chain fatty acids (SCFA)[10,14] raising the possibility of diet as a disease-modifying therapy; health benefits have been already demonstrated with diets such as the Mediterranean diet.[2,4,10]

SCFA are produced in the gut lumen by the microbial fermentation of insoluble dietary fiber and resistant starch. SCFA act on the gut epithelium to promote integrity[15–17] and have favourable immune effects to induce expression of anti-inflammatory genes and the production of regulatory T cells.[17–21] Multiple beneficial metabolic benefits of SCFA including appetite suppression, improved insulin sensitivity and weight control are described.[22,23] The three main SCFA produced in the gut are acetate, propionate and butyrate with each having large biological gradients from gut to systemic circulation. Most of the SCFA produced are absorbed, leaving only 5% excreted in faeces.[24] Systemic concentrations are particularly low for propionate and butyrate due to hepatic clearance. The circulating blood levels of acetate, propionate and butyrate are estimated at 36%, 9% and 2% respectively of the gut lumen concentration.[25]

The gut microbiome is implicated in the pathophysiology of type 1 diabetes (T1D) both before the development of islet autoimmunity (IA) and in disease progression to hyperglycaemia and insulin dependence.[15,26,35–37,27–34] The disturbances described in the gut microbiome in the progression to IA and T1D are modest, but a reduced production of SCFA is a consistent finding.[27,36–42] In the Australian Type 1 Diabetes and the Gut (TIGs) cohort we recently described a gut microbiome dysbiosis in youth with IA and T1D characterised by a decreased abundance of SCFA producing bacteria in those who progressed to type 1 diabetes. Lower levels of circulating acetate associated with lower richness of the gut microbiome. Consistent with these changes progressors to T1D also had increased small intestinal permeability.[39] With this knowledge of the underlying dysbiosis in youth with IA/T1D, we hypothesised that associations between diet and the gut microbiome and its SCFA products in youth with IA/T1D would be less frequently detectable than in controls. We therefore aimed to characterise associations between diet and the gut microbiome and its SCFA products in youth with IA/T1D in comparison with controls within the TIGs cohort.

RESEARCH DESIGN AND METHODS

Study design and setting

This cross-sectional study included participants from the TIGs cohort. Briefly, the TIGs cohort followed 88 youth with IA or type 1 diabetes and sibling and age and gender matched unrelated controls longitudinally at 6 monthly intervals for up to 3 years as described.[39] TIGs inclusion criteria were newly diagnosed type 1 diabetes from 4 weeks to 2 years post diagnosis, youth with islet autoantibodies (IA) or a sibling or friend of the above, aged 5-30 years. Exclusion criteria were, known coeliac disease or coeliac autoimmunity, current gastrointestinal symptoms, infection or fever, or probiotic or antibiotic ingestion within 4 weeks of sample collection. From the TIGs cohort 81/88 participants completed a dietary questionnaire at the time of collection of paired fecal and

plasma samples; the gut microbiome was sequenced in 80/81, leaving 80 participants enrolled in this study. An in depth description of this cohort, including HLA-type has previously been described.[39] Overall, 42/80 participants had IA or type 1 diabetes (25 with recent-onset type 1 diabetes, 17 IA) and 38/80 were controls who were either IA-negative siblings of the participants with IA or type 1 diabetes (n=28: termed sibling controls), or friends (n=10: termed unrelated controls) of participants with IA or type 1 diabetes. Each participant completed a dietary questionnaire and blood collection at the clinic visit that was paired with a home stool sample collected within 24 hours of the visit. The protocol was approved by the Women's and Children's Health Network Human Research Ethics Committee # HREC/13/WCHN/29 and Royal Melbourne Health Human Research Ethics Committee #HREC/15/MH/11 under the Australian National Mutual Acceptance scheme. Informed written consent was obtained from parents or carers, and participants over 16 years.

Main outcome measures

Food frequency questionnaire The Australian Child and Adolescent Eating Survey (ACAES version 1.2; University of Newcastle, Newcastle, Australia) was administered on one occasion by one registered dietitian (RB). The ACAES is a 120-item, semi-quantitative food frequency questionnaire (FFQ) that is validated in Australian children and adolescents.[43] Nutrient intakes were computed from current food composition databases of Australian foods, the Australian AusNut 1999 database Revision 17 for all foods and AusFoods Revision 5 for all brands (Australian Government Publishing Service, Canberra, Australia) to generate individual daily macro and micronutrient intakes. Dietary measures used for the analysis were each macronutrient (protein, carbohydrate and fat – total, unsaturated and saturated) by amount (kJ) and percentage of total energy, fiber (grams) and measures of diet quality, which included categorisation of core (nutrient dense) and non-core (discretionary or junk) foods, and the Australian recommended food score.[43,44] The percentage intake values were recalculated using the raw reported intakes as a percentage of total energy intake. The raw intakes were converted from grams to kilojoules using carbohydrate: 16kJ per gram, protein: 17kJ per gram, fats (total, saturated and unsaturated): 37kJ per gram. The Australian Recommended Food Score was derived from within the Australian Child and Adolescent Eating Survey using a subset of 70 items from the full FFQ. The Australian Recommended Food Score is a diet quality index that reliably estimates macronutrient and micronutrient intakes. It was developed to align with the Australian Dietary Guidelines and has been validated in adults and children.[45,46]

Plasma SCFA Plasma acetate, propionate and butyrate were measured by gas chromatography after liquid-liquid extraction. 50 µL of cold 200 mM heptanoic acid in 1 M phosphoric acid was added to 200 µL of plasma on ice. 4 mL of ether was added, and after repeated mixing and centrifuging the ether layer was removed and the infranatant dried under nitrogen at 40°C. The residue was re-dissolved in 30 µL of 1 M phosphoric acid and samples were analyzed on an Agilent 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA) as previously described.[39] Intra-assay CVs were 14.2%, 11.8% and 10.3% for acetate, propionate and butyrate, respectively.

Fecal sample collection

Fecal samples were collected at home using the OMNIgene GUT (OMR-200; DNA Genotek, Ontario, Canada) tube. [47,48] Samples were stored at room temperature and transported to the laboratory within 24 hours for storage at -80˚C.

Fecal SCFA (within Omnigene samples) were measured by gas chromatography with flame ionization detection (Omega Quant, South Dakota, USA). Cooled samples were transferred to a screw-cap vial which contained heptanoic acid as an internal standard (C7:0 FFA) (Nu-Chek Prep, USA). De-Ionized Water and 50% Sulfuric Acid Solution (Fisher Scientific, NJ, USA (3.5:1 v/v) was added to each vial. After homogenization, anhydrous ethyl ether (Fisher Scientific, NJ, USA) was added to each vial and vortexed for 1 minute. Samples were then centrifuged at 4°C for 10 minutes at 3800rpm. The ethyl

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ether layer was analyzed using a GC2010 Plus Gas Chromatograph equipped with an AOC-5000 autosampler with stack cooler (Shimadzu Corporation, Columbia, MD), and a HP-INNOWAX 30m column (0.25 mm internal diameter, 0.25 um film thickness; Agilent J&W, USA). Fatty acids were identified by comparison with a standard mixture of SCFA (Sigma, St. Louis, MO) which was also used to determine individual fatty acid calibration curves. The 7:0 FFA was used to calculate recovery efficiency of the assay. The following fatty acids were identified: acetic, propionic, iso-butyric, butyric, valeric, iso-caproic, and hexanoic. Fatty acid level was expressed as a percent of total identified fatty acids.

Microbiome analysis. Bacterial 16S rRNA genes were amplified from faecal samples as previously described[39,48] and the sequences clustered into Features using QIIME2 (version 2017.12). An abundance filter was applied to the Features table, leaving only those Features for which total relative abundance across all samples was higher than 0.01%. After the abundance filter the resulting table had 434 different Features across 80 samples. The mean (SD) library size by group was 17,083 (7,006) sequences for type 1 diabetes, 16,617 (4,845) for IA, 18,797 (5,311) for sibling control and 20,131 (5,993) for unrelated control. Alpha and beta diversity analyses were performed in R using Phyloseq[49] and Vegan.[50] For alpha diversity analysis (Observed Richness or Number of Features, and Inverse Simpson index or 'evenness'), the Features table was rarefied without replacement to the number of sequences (5,432) in the smallest sample. For beta diversity analysis, the Feature tables were normalized using cumulative sum scaling (CSS) and log-transformed. PCoA plots of the Bray-Curtis distances between pairs of samples were used for ordination analysis. DNA sequences from differentially abundant taxa were further searched against the non-redundant nucleotide database with the Basic Local Alignment Search Tool.[51] Relative abundance was calculated by dividing the counts of a specific taxon in a given sample by the total number of counts in that sample and multiplying by 100. Relative abundance was performed with limma as previously described.[39]

Other measures

Islet autoantibodies Autoantibodies to glutamic acid decarboxylase 65 (GADAb) and tyrosine phosphatase-like insulinoma antigen (IA2Ab) were measured by radioimmunoprecipitation assays and antibodies to insulin (IAA) were measured by fluid phase radiobinding assay. Antibodies to beta-cell specific zinc transporter 8 (ZnT8Ab) were measured by immunoprecipitation of ^{35}S-methionine-labelled recombinant human ZnT8 protein. The assays had 98%, 97%, 100% and 94%, specificity and 28%, 78%, 60% and 72% sensitivity for IAb, GADAb, IA-2Ab and ZnT8Ab respectively in the 2018 Islet Autoantibody Standardization Program (University of Florida). Positive cut-offs for autoantibodies were defined as GADAb >5 U, IA2Ab >3 U, IAA ≥0.7 mU/mL and ZnT8 ≥3.1 U. All positive results were confirmed by repeat testing in separate assays.

Physical examination Weight was measured in light clothing. Height was measured on a wall-mounted stadiometer (to 0.1cm). BMI z score was calculated using Centers for Disease Control and Prevention growth charts.[52]

Statistical analysis

Participants were analysed in two groups: first, IA or type 1 diabetes (IA/T1D) and second, controls (sibling or unrelated). Group differences between IA/T1D and controls were analysed using linear mixed models that were fitted for concentrations of plasma acetate and for percentage of total SCFA for fecal acetate, butyrate and propionate, and the alpha diversity measures of observed richness and Inverse Simpson. The potential for modification of the association by IA/T1D and control status was also explored by including an interaction term in the model and estimating the association separately for IA/T1D and controls. Sensitivity analyses excluded six participants with single IA from the data set.

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Potential correlation due to sibling associations was accounted for by including a random intercept (for each sibling cluster) in the models. Adjusted models included the covariates of participant age, gender, BMI and total energy intake was also adjusted for in models for the following diet variables: Carbohydrate (g), Total Fat (g), Saturated Fat (g), Unsaturated Fat (g), Fiber (g), Protein (g), Energy from Core Foods (kJ), Energy from Non-Core Foods (kJ).

Four participants were excluded from analyses of associations with energy intake (one type 1 diabetes, three sibling controls), one participant (type 1 diabetes) from analysis of percentage energy from total fat and unsaturated fat, and one (sibling control) from analysis of percentage energy from core foods and from analysis of the Australian Recommended Food Score, all due to implausible reported values. These participants were excluded from analyses for these variables but included in analyses for other dietary characteristics.

Dietary associations with relative abundance were limited to Features present in at least 50% of samples (n=45). Dietary associations with alpha diversity were performed in R using the *lme4* and *lmerTest* packages. Beta diversity was characterised by the Bray-Curtis dissimilarity and associations analysed by PERMANOVA using the *adonis function*[50] in the R *vegan* package. Stata v14.0 (StataCorp, College Station, TX) was used to fit the linear mixed models. A one-way ANOVA compared FFQ across groups. Results are expressed as mean differences between groups, with 95% confidence intervals (95% CIs). Statistical significance was set at a two-sided p-value of 0.05.

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RESULTS

Median (IQR) age for participants with IA/T1D was 11.5 (8.8, 13.1) years, 40% female, and in controls (unrelated and sibling) 11.7 (9.1,15.4) years, 44% female. Overall BMI z score was 0.2 (-0.4, 0.6). Overall, 78% were breast fed for a median (IQR) of 10.8 (4.5,12.8) months. In the IA/T1D group, 25 had type 1 diabetes with a median (IQR) duration of 6(3,14) months; 17 with IA included six participants with one IA, one participant with two IA, six with three IA and three with four IA to GAD, IA2, insulin or ZnT8. Age, gender distribution, BMI and breast-feeding history were similar between groups.

Results are presented as a two-group comparison between islet autoimmunity or diabetes (IA/T1D), analysed as one group, and controls (unrelated and sibling) for all results except for the differential abundance analysis in which controls were separated into sibling and unrelated controls, as an anticipated difference was found, likely due to household effect.

Significant associations and statistically significant interaction terms, indicating for the latter a divergent association between IA/T1D and controls, are presented below.

Macronutrient intake

Macronutrient intake, proportion of energy from macronutrients, and proportion of fat intake across the groups is summarised in Table 1. There were no differences in micronutrient intake between IA/T1D and controls (data not shown). Macronutrient intake was similar between groups.

Table 1. Macronutrient intake and percentage of energy from macronutrients in IA/T1D and control groups

Dietary Variable	**IA/T1D (n=42)**	**Controls (n=38)**	**P value**
Energy (kJ) †	10,641 (2,900)	10,432 (3,033)	0.76
Carbohydrate (g)	314.9 (98.4)	338.3 (160.0)	0.43
Protein (g)	113.8 (36.5)	118.7 (44.0)	0.59
Fat (g)	95.6 (42.6)	101.9 (57.1)	0.58
Saturated fat (g)	44.0 (21.3)	46.3 (28.4)	0.69
Unsaturated fat (g)	43.4 (18.8)	47.0 (25.2)	0.47
Fiber (g)	30.9 (11.4)	34.4 (13.6)	0.22
Core foods (kJ)	6,831 (2,271)	7,201 (2,640)	0.50
Non-core foods (kJ)	4,158 (1,881)	4,533 (3,210)	0.52
Australian Recommended Food Score	31.1 (8.2)	34.4 (7.5)	0.06
Carbohydrate (% energy)	49.4 (6.3)	49.0 (4.9)	0.76
Protein (% energy)	17.7 (2.3)	17.7 (2.5)	0.96
Fat (% energy) †	32.8 (5.2)	33.5 (4.8)	0.51
Saturated fat (% energy)	15.2 (3.4)	15.0 (2.7)	0.84
Unsaturated fat (% energy) †	14.9 (2.2)	15.5 (2.3)	0.21
Core foods (% energy) †	62.4 (9.7)	63.8 (8.7)	0.52
Non-core foods (% energy)	37.6 (9.7)	37.3 (11.1)	0.92

Data are presented as mean (standard deviation); IA, islet autoantibody positive

† Note 4 outliers were excluded from Energy, 1 from Fat (% energy), 1 from unsaturated fat (% energy) and 1 from Australian Recommended Food Score, all due to implausible reported values.

Associations between diet and plasma SCFA

Associations between diet and plasma acetate

Carbohydrate intake was associated with higher plasma acetate in both IA/T1D and control groups. For each 10g increase in daily carbohydrate intake plasma acetate increased similarly in in IA/T1D

[adjusted estimate +5.2 (95% CI 1.1, 9.2) µmol/L p=0.01] and controls [adjusted estimate +4.1 (95% CI 1.7, 8.5) µmol/L p 0.04]. Total fat associated with lower plasma acetate in both groups. For each 5g increase in total fat plasma acetate decreased in IA/T1D [adjusted estimate -7.2 (95% -11.9, -2.1) µmol/L p <0.01] and in controls [adjusted estimate -9.6 (95% CI -15.8, -3.4) µmol/L p <0.01]. Both saturated and unsaturated fat intake also were associated with lower plasma acetate in IA/T1D and controls. (Table 2).

Table 2: Associations between dietary intake and plasma acetate in IA/T1D and controls

Dietary variable		Change in plasma acetate (µmol/L) corresponding to the increase in the dietary variable	
		Adjusted estimate (95% CI)	Adjusted p value
Carbohydrate	10g increase in IA/T1D	5.2 (1.1, 9.2)	0.01
	10g increase in controls	4.1 (1.7, 8.5)	0.04
Total Fat	5g increase in IA/T1D	-7.2. (-11.9, -2.5)	<0.01
	5g increase in controls	-9.6 (-15.8, -3.4)	<0.01
Saturated Fat	1g increase in IA/T1D	-2.5 (-4.1, -0.9)	<0.01
	1g increase in controls	-3.2 (-5.4, -1.0)	<0.01
Unsaturated Fat	1g increase in IA/T1D	-2.7 (-4.7, -0.7)	<0.01
	1g increase in control	-3.6 (-6.2, -1.1)	<0.01

Associations between diet and plasma butyrate and propionate

No dietary intake variable was associated with plasma butyrate or plasma propionate.

Associations between diet and fecal SCFA

Fecal SCFA

There was no difference in the relative percentage of any of the fecal SCFAs between IA/T1D group and controls. However, a divergent association between fecal acetate concentration and energy intake from non-core (discretionary or junk) foods was observed when IA or T1D group was compared to controls. A 1000kJ increase in junk food intake was associated with a reduction of fecal acetate [adjusted estimate -3.8 (95% CI-6.5,-1.1) % of total fecal SCFA, p = 0.006] and an increase in fecal propionate [adjusted estimate 1.2 (95% CI 0.0, 2.5) % of total fecal SCFA, p=0.04] in controls, but not in IA/T1D (interaction term p=0.02). Fecal butyrate did not associate with any dietary variable and there was no evidence that the association differed between IA/T1D and controls.

Associations between diet and the gut microbiome

Alpha Diversity

Associations between diet and alpha diversity for both observed richness (number of features) and Inverse Simpson (evenness) are shown in Table 3.

In IA/T1D an increase in percentage energy from non-core (discretionary or junk) food was associated with a decrease in alpha diversity for both observed richness [adjusted estimate -4.09

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(95%CI -7.83, 0.35) p = 0.03] and Inverse Simpson [adjusted estimate -1.25) 95% CI -2.00, -0.49) p<0.01], but not in controls. A divergent association between alpha diversity and increased percentage energy intake from non-core (discretionary or junk) food, protein and saturated fat was seen in IA/T1D and control groups (Table 3). Notably, fiber intake and the Australian Recommended Food Score did not show an association with alpha diversity in either IA/T1D or controls (data not shown).

Table 3: Associations between diet and alpha diversity of the gut microbiome in IA/T1D and controls

	Observed Richness			**Inverse Simpson**		
Predictor	Adjusted Estimate (95% CI)	Adjusted p	Interaction term p value	Adjusted Estimate (95% CI)	Adjusted p	Interaction term p value
Percentage energy from non-core food			<0.01†			0.02†
Five percent increase in IA/T1D	-4.09 (-7.83, -0.35)	0.03		-1.25 (-2.00, -0.49)	<0.01	
Five percent increase in controls	4.10 (-0.51, 8.70)	0.08		0.12 (-0.76, 1.01)	0.78	
Percentage energy from protein			0.04†			0.04†
Five percent increase in IA/T1D	10.66 (-7.06, 28.39)	0.24		2.92 (-0.79, 6.63)	0.12	
Five percent increase in controls	-14.57 (-30.74, 1.60)	0.07		-2.49 (-5.80, 0.81)	0.14	
Percentage energy from saturated fat			0.05†			0.03†
Five percent increase in IA/T1D	-5.48 (-16.94, 5.99)	0.35		-1.50 (-3.85, 0.85)	0.21	
Five percent increase in controls	12.93 (-2.45, 28.31)	0.01		2.77 (-0.25, 5.79)	0.07	

†Interaction term p value. The interaction term determines whether the association with the dietary variable differed according to IA/T1D and control group status.

Beta diversity

Beta diversity was associated with (i) fiber intake [Sum of squares (SS)=0.39, p=0.05] and (ii) percentage energy from core foods (SS=0.53, p<0.01) in IA/T1D, and (iii) energy from non-core foods (SS= 0.42, p=0.04) in IA/T1D. The association between the Australian Recommended Food Score and beta diversity also differed in IA/T1D compared to unrelated controls (p<0.01). (Supplementary Table 1)

Relative abundance of taxa

The Australian Recommended Food Score was associated with the relativel abundance of five of the features measured, four identified from the *Bacteroides* genus and one from the Lachnospiraceae family. A higher (more favourable) Australian Recommended Food Score associated with increased relative abundance of these taxa in unrelated controls but reduced relative abundance in both the IA/T1D group and sibling controls (Supplementary Table 2).

Sensitivity analyses, excluding IA from the T1D group and excluding the six participants with single IA

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from the data set, were conducted for all outcomes (plasma and fecal SCFA, alpha and beta diversity and differential abundances) but these analyses did not alter any result.

DISCUSSION

We report for the first time, to our knowledge, the associations between diet and the gut microbiome and its SCFA products in youth with IA/T1D. Dietary associations with the gut microbiome's diversity and composition were seen no less frequently in IA/T1D, with some differences in comparison with controls. Key original findings were first, and most strikingly, diet associated with circulating SCFA similarly in youth with IA/T1D and controls. A small increase in carbohydrate intake associated with higher plasma acetate and a small increase in fat intake with lower plasma acetate. In controls a small increase in non-core (discretionary or junk) foods associated with a decreased ratio of fecal acetate to propionate as a percent of the total SCFA. Second, in IA/T1D, an increased intake of non-core associated with lower alpha diversity of the gut microbiome. Third, in IA/T1D an increase in intake of all food, both core (nutritionally dense) and non-core (discretionary or junk) foods, and an increase in fiber intake was associated with beta diversity (community structure) of the gut microbiome. A more favourable Australian Recommended Food Score, indicating a greater variety of core foods, differed in its association with the relative abundance of microbiota in diabetes and control groups.

Overall there were no major differences in macronutrient intake in IA/T1D and controls as reported in the full TIGS cohort,[39] and consistent with findings from an Australian study in similarly aged children with type 1 diabetes.[53] The majority of participants did not meet the Australian recommended daily intake of micronutrients and consumed excess saturated fat and sodium, also consistent with our previous research.[54,55] Fiber intake was relatively high in both groups (Table 1), and it is recognised that food frequency questionnaires may over report fiber intake.[56] The food frequency questionnaire was administered at least 3 months after clinical presentation of type 1 diabetes in the diabetes group so as to limit any influence of systematic nutrition advice that had been provided at the time of their clinical presentation.

To our knowledge this is the first time that the association between multiple dietary variables and plasma and fecal SCFA has been explored. It is noteworthy that very modest increases in daily intake of carbohydrate (10g) and fat (5g) associated with a measurable increase and decrease respectively in plasma acetate in the range of 4-10µmol/L in both IA/T1D and controls. Carbohydrate intake rather than fiber alone supported higher circulating levels of acetate, and both saturated and unsaturated fat intake associated with lower levels of plasma acetate. Our finding of an association between a higher intake of non-core (discretionary or junk) foods and a lower fecal acetate/proprionate ratio appear consistent with the association between lower carbohydrate and higher fat intake and lower acetate plasma levels. Interventions which increase circulating acetate either with prebiotics, by oral supplementation, or via direct infusion in vitro to the GI tract have induced beneficial metabolic benefits, such as improved insulin sensitivity and body weight control in individuals with metabolic abnormalities including obesity, impaired glucose tolerance and type 2 diabetes and also in healthy controls.[23] Further, in the nonobese diabetic mouse, dietary intervention that generates acetate and butyrate increases gut integrity and protects against the development of diabetes.[16] Therefore our and others' findings hold promise for the potential benefit of dietary interventions to increase circulating SCFA in IA/T1D.

Small differences in energy intake and percentage energy intake of non- core (discretionary or junk) foods reduced both measures of alpha diversity (richness and evenness) in participants with IA/T1D, in whom the gut microbiome is already characterized by reduced diversity,[39] but not in controls. Participants with IA/T1D had similar bacterial composition to their sibling controls, as expected with the known household effect on the gut microbiome.[57] The association of both core and non-core

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food energy intake and fiber intake with beta diversity (community structure) of the gut microbiome in IA/T1D was also as expected.[58]

Our study has several limitations. The study design allowed us to explore associations between dietary intake and the gut microbiome cross-sectionally only. We would expect that both diet and the microbiome and its products would be relatively stable in this age group, but no causality could be implied. The numbers of participants within a group were sometimes too small to confidently interpret; as for the association between diet quality, as measured by the Australian Recommended Food Score, and the differential abundance of the Bacteroides genus and other genera in unrelated controls. Six of the participants in the IA and type 1 diabetes group had a single IA and their future risk of type 1 diabetes would be anticipated to be substantially less than those with multiple IA who have a lifetime risk of type 1 diabetes approaching 100%.[59] However, sensitivity analyses excluding these six IA participants from the dataset did not alter any results. In consideration of the fecal SCFA analyses, the low excretion of fecal SCFA limits the interpretation of their dietary associations. Measurement of fecal SCFA was compositional as the OMR-200 kit, chosen for its high reproducibility and stability for the gut microbiome,[48] precluded calculation of the absolute concentration of fecal SCFA in each sample.[23] Finally, our microbiome analysis used 16S rRNA sequencing and metagenomic sequencing would be required to examine bacterial species differences and allow functional profiling.

In conclusion, a poorer quality diet, in terms of increased junk food, was associated with lower diversity of the gut microbiome and, in terms of lower carbohydrate and higher fat intake, was associated with lower circulating SCFA in youth with IA/T1D. Dietary associations with the gut microbiome in IA/T1D were detected with similar frequency as those in controls. Importantly, the underlying microbiome dysbiosis in IA or type 1 diabetes did not mask associations between diet and the gut microbiome. Our findings should inform future efforts to promote SCFA and their beneficial effects by dietary interventions.

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Data availability

All microbiome 16S rRNA gene sequencing is available in the NCBI BioProject database. Bioproject ID PRJNA601279. Available at:https://www.ncbi.nlm.nih.gov/bioproject/601279.

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Supplementary Table 1: Associations between diet and beta diversity of the gut microbiome in IA/T1D and sibling and unrelated controls

Dietary Characteristic	**Adjusted SS**	**Adjusted p**
Fiber (g) in IA/T1D	0.39	0.05
Interaction sibling control	0.24	0.35
Interaction unrelated control	0.24	0.34
Percentage energy from core food in IA/T1D	0.53	<0.01
Interaction sibling control	0.27	0.25
Interaction unrelated control	0.31	0.17
Energy from non-core foods (kJ) in IA/T1D	0.42	0.04
Interaction sibling control	0.40	0.04†
Interaction unrelated control	0.18	0.62
Australian Recommended Food Score in IA/T1D	0.22	0.41
Interaction sibling control	0.20	0.54
Interaction unrelated control	0.71	<0.01†

†Interaction term's p value indicating that the association with the dietary variable differed by IA and diabetes or control group status

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Supplementary Table 2: Associations between the Australian Recommended Food Score and differential abundance of taxa in the gut microbiome in IA/T1D and sibling and unrelated controls

FEATURE Identification to family/genus	Adjusted Estimate (SE)	Adjusted p
efc2ec8d3da2ae7ecec5ee7cbad376d6† [Bacteroidaceae (f) *Bacteroides* (g)]		
IA/T1D	-1.75 (0.37)	0.02
Sibling control	-1.72 (0.37)	0.02
Unrelated control	1.79 (0.36)	0.02
f81e433e6d0de01f9694b6cd73dc9572 [Bacteroidaceae (f)*Bacteroides* (g)]		
IA/T1D	-1.43 (0.35)	0.02
Sibling control	-1.29 (0.36)	0.02
Unrelated control	1.45 (0.35)	0.02
4260624e4fe8b85d58e194d60df84bb4 [Lachnospiraceae (f)]		
IA/T1D	-1.4 (0.35)	<0.01
Sibling control	-1.39 (0.36)	<0.01
Unrelated control	1.39 (0.35)	<0.01
51c93f3c95bc4baae2b080f28e526603 [Bacteroidaceae (f) *Bacteroides* (g)]		
IA/T1D	-1.3 (0.35)	0.02
Sibling control	-1.31 (0.36)	0.02
Unrelated control	1.35 (0.35)	0.01
9abaf1ba8c18794a2020ed0047c329c5 [Bacteroidaceae (f) *Bacteroides* (g)]		
IA/T1D	-1.59 (0.36)	<0.01
Sibling control	-1.47 (0.36)	<0.01
Unrelated control	1.57 (0.35)	<0.01

f= identified to family; g= identified to genus
† Sequences were clustered into Features using QIIME2

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Key Words: islet autoimmunity, type 1 diabetes, diet, short chain fatty acids, gut microbiome

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