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Gut microbiota of Type 1 diabetes patients with good glycaemic control and high physical fitness is similar to people without diabetes: an observational study

C. J. Stewart[1,2], A. Nelson[1], M. D. Campbell[1,3], M. Walker[4], E. J. Stevenson[1,4], J. A. Shaw[4], S. P. Cummings[1] and D. J. West[1,4]

[1]Faculty of Health and Life Sciences, Northumbria University, Newcastle-upon-Tyne, UK, [2]Alkek Center for Metagenomics and Microbiome Research, Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, USA, [3]Carnegie Research Institute, Leeds Beckett University, Leeds and [4]Institute of Cellular Medicine, Newcastle University, Newcastle-upon-Tyne, UK

Correspondence to: Christopher J. Stewart. E-mail: christopher.stewart@northumbria.ac.uk

What's new?

- This study is the first to explore the gut microbiota in patients with Type 1 diabetes, but who otherwise have good glycaemic control and high physical fitness.
- The gut microbiota from people with Type 1 diabetes and good glycaemic control and high physical fitness was comparable with that from matched healthy controls without diabetes.

Abstract

Aim Type 1 diabetes is the product of a complex interplay between genetic susceptibility and exposure to environmental factors. Existing bacterial profiling studies focus on people who are most at risk at the time of diagnosis; there are limited data on the gut microbiota of people with long-standing Type 1 diabetes. This study compared the gut microbiota of patients with Type 1 diabetes and good glycaemic control and high levels of physical-fitness with that of matched controls without diabetes.

Methods Ten males with Type 1 diabetes and ten matched controls without diabetes were recruited; groups were matched for gender, age, BMI, peak oxygen uptake (VO_{2max}), and exercise habits. Stool samples were analysed using next-generation sequencing of the 16S rRNA gene to obtain bacterial profiles from each individual. Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) was implemented to predict the functional content of the bacterial operational taxonomic units.

Results *Faecalibacterium* sp., *Roseburia* sp. and *Bacteroides* sp. were typically the most abundant members of the community in both patients with Type 1 diabetes and controls, and were present in every sample in the cohort. Each bacterial profile was relatively individual and no significant difference was reported between the bacterial profiles or the Shannon

diversity indices of Type 1 diabetes compared with controls. The functional profiles were more conserved and the Type 1 diabetes group were comparable with the control group.

Conclusions We show that both gut microbiota and resulting functional bacterial profiles from patients with long-standing Type 1 diabetes in good glycaemic control and high physical fitness levels are comparable with those of matched people without diabetes.

Introduction

Type 1 diabetes is the product of a complex interplay between genetic susceptibility and exposure to environmental factors [1]. Environmental exposure has long been implicated in the pathogenesis of the disease and now, with decades of evidence mapping an increased rate of incidence, it is clear that disease progression occurs at a rate for which genetic change alone cannot be solely accountable [2].

Previous research has shown that the gut microbiota, which is the collection of microorganisms colonizing the gut, has important roles in the disease [3–5]. Germ-free mouse models of Type 1 diabetes may acquire the disease at higher rates, but this has been challenged with no significant differences between germ-free and colonized mice [6]. In the same study, a Gram-positive organism was isolated that reduced the incidence of the disease. Administering 'probiotic' (live microorganisms which confer health benefits) to mouse models further demonstrated the potential of intervention targeting the gut microbiota to reduce disease incidence [6]. Antibiotic administration earlier in life may also predispose patients to Type 1 diabetes through modulation of the gut microbiota, and certain antibiotic combinations have recently been found to increase diabetes risk [7], although in mice the incidence was reduced with vancomycin from birth to weaning [8].

Research in children has shown that the gut microbiota in Finish people with Type 1 diabetes had greater Bacteroidetes relative to Firmicutes and reduced overall diversity [9]. More

recently in a Spanish cohort, people with Type 1 diabetes had an increased abundance of *Clostridium*, *Bacteroides* and *Veillonella*, and a reduced abundance of *Bifidobacterium* and *Lactobacillus* compared with controls [10]. Interestingly, the latter two organisms are regarded as beneficial and have been used extensively as probiotic candidates. Overall, these findings indicate that interactions between the intestinal microbiota and the innate immune system are critical for disease development [9,11]. However, Type 1 diabetes has a wide spectrum of severity and these studies tend to focus on people at who are most at risk at the time of diagnosis. Thus, an important knowledge gap remains in the literature regarding the status of people in adulthood with long-standing diabetes. Moreover, there are limited data examining such individuals who are intensively managed, demonstrating good glycaemic control and high levels of physical fitness.

This study seeks to explore the gut microbiota in patients with Type 1 diabetes and good glycaemic control and high levels of physical fitness, matched to people without diabetes. Although the gut microbiota potentially contributes to the onset of Type 1 diabetes, we aimed to determine whether active patients with long-term Type 1 diabetes are able to develop a gut microbiome comparable with that of healthy controls or if important differences persist long after diabetes onset.

Materials and Methods

Participant recruitment and preliminary testing

Fully informed written consent was obtained from all individuals following the study's approval from National Health Service NRES Committee – Tyne and Wear South. Participants attended the Newcastle National Institute for Health Research Clinical Research

Facility to establish peak cardiorespiratory parameters during the completion of an incremental–maximal treadmill running protocol, as previously described [12]. Participants provided stool material on tissue paper that was deposited in a sterile falcon tube and stored at –80°C until processing. Tissue paper was sterilized under UV and a negative control sample of toilet paper was also carried out.

Type 1 diabetes eligibility criteria consisted of being aged between 18 and 35 years, a duration of diabetes > 5 years, and an HbA_{1c} < 64 mmol/mol (8.0%). In addition, patients with Type 1 diabetes were required to be absent of diabetes-related complications, other than mild–background retinopathy, not receiving any medication other than insulin (assessed against recent medical notes), and regularly and consistently undertaking exercise (participating in aerobic-based exercise for a minimum of 30 min at a time, at least three times per week). Ten males with Type 1 diabetes were recruited [aged 27 ± 2 years, BMI 23.5 ± 0.7 kg/m^2, peak oxygen uptake (VO_{2max}) 51.3 ± 2.2 ml/kg/min, duration of diabetes 12 ± 2 years, HbA_{1c} 54.5 ± 2.1 mmol/mol (7.1 ± 0.4%)]. Patients were treated with a basal–bolus regimen composed of long-acting insulin glargine (n = 8) or detemir (n = 2), and rapid-acting insulin aspart. Eligibility criteria for controls without diabetes consisted of being between 18 and 35 years, and regularly and consistently undertaking exercise. Ten males without diabetes (controls) were recruited (aged 27 ± 2 years, BMI 22.4 ± 0.8 kg/m^2, VO_{2max} 50.9 ± 1.2 ml/kg/min). The Type 1 diabetes and control groups were matched for age, fitness and BMI (P > 0.05). Both groups were habitually consuming a predominantly carbohydrate-rich diet (> 60% carbohydrate) assessed via 24-h recall. Study demographics are summarized in Table 1.

16S rRNA gene bacterial profiling

Participants were provided three sections of toilet paper from the same roll that had all undergone UV sterilization. Following excrement, the participants used the toilet paper once, the soiled tissue was then collected in sterile universal tubes. Nucleic acid extraction of stool was carried out on a section of the soiled toilet paper using the PowerLyzer™ PowerSoil® DNA Isolation Kit (MO BIO Inc., Carlsbad, CA, USA) in accordance with the manufacturer's instructions. Bacterial profiling utilized the 16S rRNA gene targeting variable region 4 and was carried out by NU-OMICS (Northumbria University, Newcastle-upon-Tyne, UK) based on the Schloss wet-lab MiSeq SOP and resulting. Raw fastq data were processed using Mothur (v. 1.31.2) as described previously [13]. Briefly, combined reads were trimmed to 275 reads with 0 ambiguous bases. Chimeric sequences were detected by Chimera.uchime and removed from downstream analysis. Alignment was generated via the Silva v4 database [14] and Chloroplast, Mitochondria, unknown, Archaea and Eukaryota linages were removed from the analysis. In total, 5 165 964 reads were generated from the 20 samples. Sequences were deposited in MG-RAST under the accession numbers 4603090.3–4603109.3.

Statistical analysis

Data were normalized by subsampling and rarefying all samples to 104 142 reads. The data were automatically transformed and analysed by principal coordinate analysis (PCA) using SIMCA 13.0 (Umetrics, Stockholm, Sweden) [15]. The community structure between the Type 1 diabetes and control groups was analysed by Parsimony and weighted UniFrac analysis [16]. Significant operational taxonomic unit (OTUs) were classified by the metastats function in Mothur using 1000 permutations with multiple hypothesis testing correction [17].

Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) was implemented to predict functional content of the bacterial OTUs [18].

Results

The number of reads used in the subsampling (104 142) facilitated robust coverage of the gut microbiota of each individual in the cohort. No significant difference was found between the Type 1 diabetes and control groups using Parsimony ($P = 0.309$) and weighted UniFrac ($P = 0.107$). *Faecalibacterium* sp., *Roseburia* sp. and *Bacteroides* sp. were typically the most abundant members of the community in both the Type 1 diabetes and control groups, and were present in every sample in the cohort (Fig. 1). Levels of *Bacteroides* sp. tended to be higher in the control group ($P = 0.06$) and *Bifidobacterium* sp. tended to be higher in the Type 1 diabetes group ($P = 0.08$), but neither was significant.

The bacterial profiles of the patients with Type 1 diabetes were comparable with those of the control group with no distinct clusters based on the bacterial profiles (Fig. 2A). To account for potential false negatives resulting from some patients with Type 1 diabetes, where HbA_{1c} was outside the range for truly excellent control, further ordination analysis was conducted by stratifying Type 1 diabetes by HbA_{1c} by > 53 mmol/mol or < 53 mmol/mol. PCA with this classification showed no distinct clustering based on the overall bacterial community, with resulting partial least squares discriminant analysis (PLS-DA) predictive (Q) scores of –0.106 in > 53 mmol/mol and 0.022 in < 53 mmol/mol, where scores of > 0.5 represent significant differences and predictively between the groups (Fig. S1). Only 17 OTUs from a total of 3062 were found to be significantly different between the groups (Table 2). *Actinomyces* sp. (OTU00428) was the most significant OTU ($P = 0.008$) in the Type 1 diabetes group and this was most associated with the Type 1 diabetes group in the PLS-DA loadings plot (Fig. 2B).

However, this OTU was detected in all but two participants (both from the control group) and only compromised of 62 reads from a total of 2 082 840 (0.003%), where 49 reads were from patients with Type 1 diabetes and 13 reads were from controls. No significant difference ($P = 0.344$) was found in the Shannon diversity index (H') between each group. The average Type 1 diabetes H' was 3.37 (range 2.16–3.92), whereas the control H' was 3.13 (range 2.62–4.49).

PICRUSt was implemented to predict functional content of the bacterial OTUs. This showed that despite the relatively large variation in the bacterial community between individuals, the functional profiles were much more comparable (Fig. 3). Functional profiles from the Type 1 diabetes group were comparable to that of the control group.

Discussion

Alterations in the gut microbiota, whether causative or as a result of Type 1 diabetes, may have important implications for the health of people with Type 1 diabetes. The aim of this study was to explore gut microbiota in patients with Type 1 diabetes but good glycaemic control and high levels of physical fitness, matched to people without diabetes. We show for the first time that patients with intensively managed Type 1 diabetes with optimal glycaemic control and good physical fitness display gut microbiota profiles comparable with those of matched individuals without Type 1 diabetes.

The gut microbiota profiles were highly individual across the whole cohort, but there is general conformity between the most dominant members of the community. *Faecalibacterium* sp., *Roseburia* sp. and *Bacteroides* sp. were found to be the most abundant in the cohort and generally represented a substantial proportion of the gut microbiota in each person. These species have previously been shown to be prevalent in a healthy adult gut

microbiota [19]. The most significant OTUs driving the separation of the Type 1 diabetes and control gut communities were generally low in abundance and reflected only a small proportion of the overall reads. For example, the *Actinomyces* sp. (OTU00428), which was the most significant OTU in the Type 1 diabetes group, compromised only 62 reads (49 reads from Type 1 diabetes group) from a total of 2 082 840 (0.003%). Thus, OTUs with such universally low relative abundance are unlikely to be contributing to disease pathophysiology and implying causality to disease should be avoided. Although the cohort employed in this study is small, with only 10 patients with Type 1 diabetes, it is comparable with that of previously published studies and should not influence the lack of clinically important OTUs discriminating people with Type 1 diabetes and controls [10]. Previous studies have also inferred associations at diagnosis of increasing *Bacteroides* and reduced *Bifidobacterium* in Type 1 diabetes [9,10]. Although these organisms were relatively abundant, overall we see opposing trends, with lower *Bacteroides* and increased *Bifidobacterium* in Type 1 diabetes; these differences are noteworthy but they were not significant, however, further work in a larger cohort is necessary to confirm these observations.

The Shannon diversity index was comparable between the Type 1 diabetes and control groups with no significant difference found between them. Interestingly, previous studies suggest that children with Type 1 diabetes undergo dysbiosis of the gut microbiota, resulting in reduced diversity compared with people without diabetes [9,20]. The diversity reported in this study is comparable with that of an adult population without Type 1 diabetes, but a lack of published aged-matched controls prevents any comparison with adults with Type 1 diabetes. Nonetheless, the observation that active adults with Type 1 diabetes have a similar diversity to adults without Type 1 diabetes is important.

Previous studies have suggested an increase in butyrate-producing and mucin-degrading bacteria in controls, whereas bacteria that produce short-chain fatty acids other than butyrate

were higher in disease cases [21]. Thus, synthetic pathways may represent a key aetiological trigger in the onset of Type 1 diabetes. Functional analysis of the bacterial community in this dataset demonstrated comparability between the bacterial pathways of the OTUs found in people with Type 1 diabetes and matched controls. Despite large variation at the OTU level, the function profiles showed much greater comparability, as reported previously [22]. It is noteworthy that these functional pathways represent only those of the bacterial community based on the classification OTUs and thus do not account for differential gene expression between the two groups.

Given the individual nature of the gut microbiota within each group of the cohort, it is perhaps not surprising that the ordination analysis of the bacterial profiles showed no distinct separation of people with Type 1 diabetes and matched controls. Thus, in adulthood, the gut microbiota is not significantly altered in active people as a result of being diagnosed with Type 1 diabetes. Notably this finding was not influenced when the Type 1 diabetes group was further stratified to account for ranging HbA_{1c}. Existing comparable data are limited, with studies to date focusing on differences in the gut microbiota in patients at the time of diagnosis (i.e. childhood) [9,10]. Although the gut microbiota may serve as an environmental trigger in the onset of Type 1 diabetes in patients in whom genetic elements alone cannot account for the pathogenesis, an important finding of this study is that active adults with Type 1 diabetes have a gut microbiota reflective of adults without Type 1 diabetes. Further work should sample greater numbers of people temporally and seek to include sedentary patients and those with poorer glycaemic control. Future work should also consider Type 1 diabetes patients with other pathologies, such as retinopathy or cardiovascular disease. Considering the lack of available data pertaining to the influence of exercise on gut microbiota, profiling patients across a range of glycaemic control and physical activity levels is warranted to ascertain whether alterations in gut microbiota are influenced by exercise,

glycaemic control or both, and whether intervention or therapeutic manipulation of the gut microbiota might confer improvements to well-being. The potential influence of differences in HLA genotype between those with and without Type 1 diabetes should also be considered in future studies.

In summary, this study confirmed existing data relating to the dominant bacterial organisms in the healthy active adult gut microbiota. Importantly, we show that both gut microbiota and resulting functional bacterial profiles from people with long-standing Type 1 diabetes in good glycaemic control and high physical fitness levels are comparable with matched people without diabetes.

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Competing interests

None declared.

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FIGURE 1 Bar chart of operational taxonomic units (OTUs) from Type 1 (T1) diabetes and matched controls. Each OTU is represented as a percentage of the total community. Samples are ordered by *Faecalibacterium* abundance.

FIGURE 2 SIMCA analysis of Type 1 (T1) diabetes samples and matched controls (CON). (A) Principal components analysis (PCA) score scatter plot. R2X[1] = 0.124, R2X[2] = 0.0998. (B) Loadings plot showing taxa associated with each group. Green (Y) represents each operational taxonomic unit (OUT) detected, where only the significantly different OTUs between cases and controls are labelled. Blue (X) shows different classification of the model, where OTUs associated with control samples are shown on the upper right and OTUs associated with cases are shown on the lower left.

FIGURE 3 Bar chart of phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) analysis from Type 1 diabetes and matched controls. Each function is represented as a percentage of the total community. Samples are ordered in accordance with Fig. 1.

Supporting Information

Additional Supporting Information is available in the online version of this article:

Figure S1. PCA analysis of Type 1 diabetes (T) samples and matched controls (C), with the Type 1 diabetes group split to account for differing glycaemic control.

Table 1 Individual participant characteristics

Group	Subject ID	Age (years)	BMI	VO_{2peak} (ml/kg/min)	Fasting blood glucose (mmol/l)	Diabetes duration (years)	HbA_{1c} (mmol/mol)
Control	C1	25	22.1	50	4.20		
	C2	23	21.4	51	4.32		
	C3	31	21.7	56	4.33		
	C4	30	20.1	52	3.87		
	C5	28	26.9	48	3.46		
	C6	26	21.4	55	4.02		
	C7	26	23.7	50	3.29		
	C8	30	25.4	51	4.22		
	C9	25	21.8	45	4.28		
	C10	26	20.4	49	4.22		
Type 1 diabetes	T1	29	22.8	57	5.44	5	54
	T2	24	25.9	48	5.75	11	42
	T3	19	22.5	64	5.01	12	49
	T4	34	22.4	50	3.90	5	60
	T5	21	22.5	56	8.43	12	55
	T6	33	27.1	52	7.32	19	58
	T7	29	26.9	41	6.45	5	58
	T8	25	22.8	51	6.31	24	43
	T9	24	22.4	45	3.45	13	50
	T10	31	22.5	46	3.22	19	61

VO_{2peak}, peak oxygen uptake. Between-group comparisons assessed with independent samples *t*-test.

Table 2 Operational taxonomic units (OTUs) that differ significantly between patients with Type 1 diabetes and matched controls

Group	*P*-value	OTU	Phylum	Class	Order	Family	Genus
Control	0.003	OTU00082	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
Control	0.017	OTU01214	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	*Anoxybacillus*
Control	0.019	OTU00865	Proteobacteria	Alphaproteobacteria	Rhizobiales	Aurantimonadaceae	*Aurantimonas*
Control	0.021	OTU00820	Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae	*Deinococcus*
Control	0.026	OTU00625	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	*Clostridium_sensu_stricto*
Control	0.027	OTU00217	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	*Coprococcus*
Control	0.027	OTU00230	Proteobacteria	Betaproteobacteria	Burkholderiales	Unclassified	Unclassified
Control	0.032	OTU00807	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Schlegelella*
Control	0.033	OTU01323	Proteobacteria	Betaproteobacteria	Burkholderiales	Unclassified	Unclassified
Control	0.036	OTU01060	Actinobacteria	Actinobacteria	Coriobacteriales	Coriobacteriaceae	Unclassified
Control	0.039	OTU00363	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	*Zoogloea*
Control	0.041	OTU00384	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Unclassified
Type 1diabetes	0.008	OTU00428	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	*Actinomyces*
Type 1diabetes	0.03	OTU00020	Actinobacteria	Actinobacteria	Coriobacteriales	Coriobacteriaceae	*Collinsella*
Type 1diabetes	0.03	OTU00021	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
Type 1diabetes	0.047	OTU00023	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unclassified
Type 1diabetes	0.047	OTU00025	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	*Dialister*