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Gut microbiota of type 1 diabetes patients with good glycaemic control and high physical-fitness is similar to matched non-diabetic controls: an observational study

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What's new?

- This study is the first to explore the gut microbiota in patients diagnosed with type 1 diabetes (T1D), but otherwise have excellent glycaemic control and high physical-fitness
- The gut microbiota from the T1D patients with good glycaemic control and high physical-fitness was comparable to matched non-diabetic healthy controls

Abstract

Aim: Type 1 diabetes (T1D) is the product of a complex interplay between genetic susceptibility and exposure to environmental factors. Existing bacterial profiling studies focus on patients who are most at risk at the time of diagnosis; there is limited data on the gut microbiota of patients with long standing T1D. This study compared gut microbiota of T1D patients with good glycaemic control and high levels of physical-fitness with matched non-diabetic controls.

Methods: Ten male type 1 diabetes patients (T1D**)** and ten matched controls (CON) were recruited; groups were matched for age, BMI, VO_{2max}, 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 functional content of the bacterial OTUs.

Results: Faecalibacterium sp., Roseburia sp., and Bacteroides sp. were typically the most abundant members of the community in both T1D and CON 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 T1D compared with CON. The functional profiles were more conserved and the T1D group were comparable to that of the CON group.

Conclusions: We show that both gut microbiota and resulting functional bacterial profiles from patients with longstanding T1D in good glycaemic control and high physical-fitness levels are comparable to matched non-T1D controls.

Introduction

Type 1 diabetes (T1D) 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 at 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 (GF) mice models of T1D may acquire the disease at higher rates, but this has been challenged with no significant differences between GF and colonized mice [6]. In the same study a Gram-positive organism was isolated which 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 T1D through modulation of the gut microbiota, where certain antibiotic combinations were recently 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 patients with T1D had greater Bacterodetes relative to Firmicutes and reduced overall diversity [9]. More recently in a Spanish cohort patients with T1D had increased abundance of Clostridium, Bacteroides and Veillonella and reduced abundance of Bifidobacterium and Lactobacillus compared to 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, T1D has a wide spectrum of severity and these studies tend to

71 focus on patients at who are most at risk at the time of diagnosis. Thus an important
72 knowledge gap remains in the literature regarding the status of patients in adulthood with
73 longstanding diabetes. Moreover, there is limited data examining such individuals who are
74 intensively managed, demonstrating good glycaemic control and high levels of physical
75 fitness.
76 This study seeks to explore gut microbiota in T1D patients with good glycaemic control and
77 high levels of physical-fitness and matched non-T1D controls. While the gut microbiota
78 potentially contributes to the T1D onset, we aimed to determine if long-term active suffers
79 are able to develop a gut microbiome comparable to healthy controls or if important
80 differences persist long after onset.

Materials and Methods

Participant recruitment and preliminary testing

Fully informed written consent was obtained from all patients 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 cardio-respiratory 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 ºC until processing. Tissue paper was sterilised under UV and a negative control sample of toilet paper was also carried out.

T1D patient eligibility criteria consisted of being aged between 18-35 years, a duration of diabetes > 5 years, and an HbA_{1c} < 8.0% (64 mmol/mol). In addition, patients 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 minutes at a time, at least three times per week). Ten male T1D patients were recruited (aged 27±2 years, BMI 23.5±0.7 $kg.m^2$, VO_2peak 51.3±2.2 ml/kg/min, duration of diabetes 12±2 years, HbA_{1c} 7.1±0.4% [54.5±2.1 mmol/mol]). Patients were treated with a basal-bolus regimen composed of long-acting insulins glargine (n = 8) or detemir (n = 2), and rapid-acting insulin aspart. Eligibility criteria for non-diabetic control participants consisted of being between 18-35 years, regularly and consistently undertaking exercise. Ten non-diabetic control participants (CON) were recruited (aged 27±2 years, BMI 22.4±0.8 kg/m^2, VO_2max 50.9±1.2 ml/kg/min). T1D and CON 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 hour recall. Patient demographics are summarised in Table 1.

16S rRNA gene bacterial profiling

Participants were provided 3 sections of toilet paper from the same roll that had all undergone UV sterilisation. 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 (MoBio, CA, USA) in accordance with the manufacturer's instructions. Bacterial profiling utilised the 16S rRNA gene targeting variable region 4 and was carried out by NU-OMICS (Northumbria University) based on the Schloss wet-lab MiSeq SOP and resulting. raw fastq data were processed using Mothur (version 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 was normalised by subsampling and rarefying all samples to 104,142 reads. The data was automatically transformed and analysed by principal coordinate analysis (PCA) using SIMCA 13.0 (Umetrics, Stockholm, Sweden) [15]. The community structure between the T1D and control groups were 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 T1D 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 T1D and CON and were present in every sample in the cohort (Figure 1). Levels of Bacteroides sp. tended to be higher in CON (P = 0.06) and Bifidobacterium sp. tended to be higher in T1D (P = 0.08), but neither was significant.

The bacterial profiles of T1D were comparable to the CON group with no distinct clusters based on the bacterial profiles (Figure 2A). To account for potential false negatives resulting from some T1D patients with HbA_{1c} outside the range for truly excellent control, further ordination analysis was conducted by stratifying T1D by HbA_{1c} by > or < 53 mmol/mol. PCA analysis with this classification showed no distinct clustering based on the overall bacterial community, with resulting PLS-DA predictive (Q) scores of -0.106 in >53 mmol/mol and 0.022 in <53, where scores of >0.5 represent significant differences and predictively between the groups (Supplementary Figure 1). Only 17 OTUs from a total of 3,062 were found to be significantly different between the groups (Table 2). Actinomyces sp. (OTU00428) was the most significant OTU (P = 0.008) in the T1D group and this was most associated with the T1D group in the PLS-DA loadings plot (Figure 2B). However, this OTU was detected in all but 2 patients (both from CON) and only compromised of 62 reads from a total of 2,082,840 (0.003%), where 49 reads were from T1D patients and 13 reads were from CON. No significant difference (P = 0.344) was found in the Shannon Diversity (H') between each group. The average T1D H' was 3.37 (range 2.16 – 3.92), whereas the CON H' was 3.13 (range 2.62 – 4.49).

157 PICRUSt was implemented to predict functional content of the bacterial OTUs. This showed
158 that despite the relatively large variation in of the bacterial community between individuals,
159 the functional profiles were much more comparable (Figure 3). Functional profiles from the
160 T1D group were comparable to that of the CON group.

Discussion

Alterations in the gut microbiota, whether causative or as a result of T1D, may have important implications for the health of patients. The aim of the present study was to explore gut microbiota in T1D patients with good glycaemic control and high levels of physical-fitness against matched non-T1D controls. We show for the first time that intensively managed T1D patients with optimal glycaemic control and good physical-fitness display comparable gut microbiota profiles to matched non-T1D individuals.

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 have been previously shown to be prevalent in a healthy adult gut microbiota [19]. The most significant OTUs driving the separation of the T1D 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 T1D group, only compromised of 62 reads (49 reads from T1D 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. While the cohort employed in this study is small, 10 T1D patients are comparable to that of previously published studies and should not influence the lack of clinically important OTUs discriminating T1D patients and controls [10]. Previous studies have also inferred associations at diagnosis of increasing *Bacteroides* and reduced *Bifidobacterium* in T1D [9,10]. While these organisms were relatively abundant overall we see opposing trends, with lower *Bacteroides* and increased *Bifidobacterium* in T1D; although

these differences are noteworthy they were not significant, but further work in a larger cohort is necessary to confirm these observations.

The Shannon diversity was comparable between T1D and controls with no significant difference found between the groups. Interestingly, previous studies suggest that children with T1D undergo dysbiosis of the gut microbiota, resulting in reduced diversity compared to controls [9,20]. The diversity reported in this study is comparable to that of a non-T1D adult population, but a lack of published aged-matched controls prevents any comparison with T1D adults. Nonetheless, the observation that active adults with T1D have a similar diversity to adults without T1D is important.

Previous studies have suggested an increase of butyrate-producing and mucin-degrading bacteria in controls, whereas bacteria that produce short chain fatty acids (SCFAs) other than butyrate were higher in disease cases [21]. Thus synthetic pathways may represent a key etiological trigger in the onset of T1D. Functional analysis of the bacterial community in this dataset demonstrated comparability between the bacterial pathways of the OTUs found in patients with T1D and controls. Despite large variation at the OTU level, the function profiles showed much greater comparability, as has been previously reported [22]. Noteworthy is 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 patients with T1D and matched controls. Thus, in adulthood the gut microbiota is not significantly altered in active patients as a result of being diagnosed with T1D. Notably this finding was not influenced when T1D patients were further stratified to account for

ranging HbA_{1c}, with some patients in the T1D groups exhibiting HbA_{1c} outside the range considered excellent for glycaemic control. Existing comparable data is limited, with studies to date focusing on differences in the gut microbiota in patients at the time of diagnosis (i.e. childhood) [9,10]. While the gut microbiota may serve as an environmental trigger in the onset of T1D in patients where genetic elements alone cannot account for the pathogenesis, an important finding of this study is that active T1D adults have a gut microbiota reflective of non-T1D adults. Further work should sample greater numbers of patients temporally and seek to include sedentary sufferers and those with poorer glycaemic control. Future work should also consider T1D 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 if intervention or therapeutic manipulation of the gut microbiota could confer improvements to well-being. The potential influence of differences in HLA genotype between those with T1D and control participants 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 patients with longstanding T1D in good glycaemic control and high physical-fitness levels are comparable to matched non-T1D controls.

COMPETING INTERESTS

None to declare.

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Table 1 – Individual participant characteristics

Group	Patient 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		
T1D	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; BMI: Body mass index. Between group comparisons assessed with independent samples t-test.

Table 2 – OTUs which differ significantly between T1D and matched controls

Group	P value	OTU	Phylum	Class	Order	Family	Genus
CON	0.003	Otu00082	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified
CON	0.017	Otu01214	Firmicutes	Bacilli	Bacillales	Bacillaceae_1	Anoxybacillus
CON	0.019	Otu00865	Proteobacteria	Alphaproteobacteria	Rhizobiales	Aurantimonadaceae	Aurantimonas
CON	0.021	Otu00820	Deinococcus-Thermus	Deinococci	Deinococcales	Deinococcaceae	Deinococcus
CON	0.026	Otu00625	Firmicutes	Clostridia	Clostridiales	Clostridiaceae_1	Clostridium_sensu_stricto
CON	0.027	Otu00217	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus
CON	0.027	Otu00230	Proteobacteria	Betaproteobacteria	Burkholderiales	unclassified	unclassified
CON	0.032	Otu00807	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Schlegelella
CON	0.033	Otu01323	Proteobacteria	Betaproteobacteria	Burkholderiales	unclassified	unclassified
CON	0.036	Otu01060	Actinobacteria	Actinobacteria	Coriobacteriales	Coriobacteriaceae	unclassified
CON	0.039	Otu00363	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Zoogloea
CON	0.041	Otu00384	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	unclassified
T1D	0.008	Otu00428	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces
T1D	0.03	Otu00020	Actinobacteria	Actinobacteria	Coriobacteriales	Coriobacteriaceae	Collinsella
T1D	0.03	Otu00021	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified
T1D	0.047	Otu00023	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	unclassified
T1D	0.047	Otu00025	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Dialister

Figure Legends

Figure 1 – Bar Chart of OTUs from type 1 (T1) diabetes and matched controls. Each OTU represented as a % of the total community. Patients ordered by Faecalibacterium abundance.

Figure 2 – SIMCA analysis of type 1 (T1) diabetes samples and matched control. A) 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 OTU detected, where only the significantly different OTUs between cases and control 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 PICRUSt analysis from type 1 diabetes and matched controls. Each function represented as a % of the total community. Patients ordered in accordance with Figure 1.

Supplementary Figure Legends

Supplementary Figure 1 – PCA analysis of type 1 diabetes (T) samples and matched controls (C), with T1D patients split to account for differing glycaemic control. T1D samples split by HbA_{1c} >53 mmol/mol (orange) and HbA_{1c} <53 mmol/mol with PLS-DA scores of -0.106 and 0.022, respectively.