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Women with type 1 diabetes exhibit a progressive increase in gut *Saccharomyces cerevisiae* in pregnancy associated with evidence of gut inflammation

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Abbreviations: ASCA, Anti-*Saccharomyces cerevisiae* antibodies; ASV, Amplicon sequence variants; bp, Base pair; CPM, Counts per million; DQESv2, Dietary Questionnaire for Epidemiological Studies version 2; ENDIA, Environmental determinants of islet autoimmunity; FDR, False discovery rate; GEE, Generalized estimating equations; HbA1c, Haemoglobin A1c; HLA, Human leukocyte antigen; HREC, Human research ethics committee; I-FABP, Intestinal fatty-acid binding protein; IgA, Immunoglobulin A; IgG, Immunoglobulin G; ITS1, Internal transcribed spacer 1; LCBD, Local contribution to beta diversity; LogFC, Log2 fold-change; mg, milligram; OTU, Operational taxonomic unit; PCoA, Principal components analysis; RMA-PERMANOVARe, Repeated measure-aware permutation analysis of variance; SCFA, Short-chain fatty acid; SD, Standard deviation; T1D, Type 1 diabetes; WEHI, Walter and Eliza Hall Institute of Medical Research; WMS, Whole metagenomic sequencing; Zinb, Zero-inflated negative binomial regression; 1,5-AG, 1,5-Anhydroglucitol

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ABSTRACT

Aims: Studies of the gut microbiome have focused on its bacterial composition. We aimed to characterize the gut fungal microbiome (mycobiome) across pregnancy in women with and without type 1 diabetes.

Methods: Faecal samples (n = 162) were collected from 70 pregnant women (45 with and 25 without type 1 diabetes) across all trimesters. Fungi were analysed by internal transcribed spacer 1 amplicon sequencing. Markers of intestinal inflammation (faecal calprotectin) and intestinal epithelial integrity (serum intestinal fatty acid binding protein; I-FABP), and serum antibodies to *Saccharomyces cerevisiae* (ASCA) were measured.

Results: Women with type 1 diabetes had decreased fungal alpha diversity by the third trimester, associated with an increased abundance of *Saccharomyces cerevisiae* that was inversely related to the abundance of the anti-inflammatory butyrate-producing bacterium *Faecalibacterium prausnitzii*. Women with type 1 diabetes had higher concentrations of calprotectin, I-FABP and ASCA.

Conclusions: Women with type 1 diabetes exhibit a shift in the gut mycobiome across pregnancy associated with evidence of gut inflammation and impaired intestinal barrier function. The relevance of these findings to the higher rate of pregnancy complications in type 1 diabetes warrants further study.

1. Introduction

The autoimmune T-cell response that destroys insulin-producing beta cells in the pancreatic islets in type 1 diabetes (T1D) is thought to be promoted by environmental factors on a background of genetic susceptibility. A role for the gut bacterial microbiome, at the interface with the external environment, in the development of T1D is supported by animal models [1,2,3]. In humans, a decrease in compositional diversity and the relative abundance of potentially beneficial short-chain fatty acid-producing bacteria has been documented before and after the clinical presentation of T1D [4–11]. In addition, we recently reported that women with T1D exhibit a shift towards a more pro-inflammatory gut bacterial microbiome during pregnancy [12]. However, the gut microbiome is a complex ecosystem that comprises not only bacteria, but fungi, viruses, archaea and protozoa [13–16]. Fungi are ubiquitous in the environment and may interact with and modify other components of the microbiome, thus contributing directly and indirectly to the role of the microbiome in health and disease. Fungal species from the genera *Candida, Saccharomyces, Penicillium, Aspergillus, Cryptococcus, Malassezia, Cladosporium, Galactomyces, Debaryomyces* and *Trichosporon* have been identified in the healthy human gut, representing 0.1%-1.0% of the gut microbiota [13,15,17]. Based on culture methods, the outgrowth of *Candida* genera in the gastrointestinal tract has been linked to poor glycaemic control in T1D [18,19,20], but the gut mycobiome has not been characterized in this disease or in pregnancy. In the present study, we analysed the gut mycobiome in each trimester of pregnancy in a cohort of women with and without T1D in the Australia-wide Environmental Determinants of Islet Autoimmunity (ENDIA) pregnancy-birth cohort study [21].

2. Methods

2.1. Participants

The study comprised 70 pregnancies in 70 women, 45 women with established T1D on daily insulin treatment and 25 women with no history of T1D, being a representative sample of those in the ENDIA pregnancy-birth cohort study. One hundred and sixty-two faecal samples were collected across all trimesters (34 in trimester 1, 65 in trimester 2 and 63 in trimester 3; Supplementary Fig. 1). These samples were also included in a recent study of the bacterial microbiome in

pregnancy [12]. The main criterion for participation in ENDIA was a child with a first-degree relative with T1D. Table 1 summarizes and compares characteristics of the T1D and non-T1D participants.

Women who provided written informed consent were enrolled in the study from 2013 to 2016 at eight clinical sites. Each had up to three study visits during pregnancy, ideally one in each trimester. The study was approved by a Human Research Ethics Committee (HREC) at each clinical site, with the Women's and Children's Hospital HREC in Adelaide acting as the lead under the Australian National Mutual Acceptance Scheme (reference number HREC/16/WCHN/066). ENDIA is registered on the Australia New Zealand Clinical Trials Registry (ACTRN1261300794707).

2.2. Collection-processing of faecal samples

ENDIA participants self-collected faecal samples and placed them into a sterile 70 mL collection jar, which was stored in home refrigerator for 6–24 h before delivery to the laboratory in an insulated container. Sample aliquots were transferred into 6x sterile 5 mL screw cap tubes and stored at −80 °C until further processing as described [22].

2.3. DNA extraction

DNA was extracted from approximately 100 mg of faeces with the Zymobiomics kit (Zymo Research) as per manufacturer's instructions, quantified using a Qubit dsDNA BR (Broad Range) assay kit (Thermo Fisher Scientific) and stored at −80 °C until further processing.

2.4. Fungal ITS1 amplification and sequencing

For fungal analysis, the internal transcribed spacer 1 (ITS1) region was first amplified from faecal DNA. ITS1 primers were ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS1R (GCTGCGTTCTTCATCGATGC) [17]. Two PCRs were performed. Cycling conditions for the first PCR were: initial denaturation at 95 °C for 3 min, 20 amplification cycles at 94 °C for 30 s 56 °C for 90 s and 72 °C for 3 min, followed by a final extension step at 72 °C for 10 min. Conditions for the second PCR to add unique index barcodes to each sample were: initial denaturation at 95 °C for 3 min, 25 amplification cycles at 94 °C for 45 s, 57 °C for 60 s and 72 °C for 90 s, followed by a final extension step at 72 °C for 10 min. PCR products were visualized by agarose gel electrophoresis and quantified with Tape Station (Agilent 2100 Bioanalyzer). Equimolar amounts of purified amplicons were pooled and sequenced on the Illumina MiSeq platform using the Illumina MiSeq v3 600-cycle (2 × 300 bp) kit.

2.5. Fungal sequence analysis

ITS1 amplicon sequence data were processed with QIIME2 (version 2017.12) [23]. Briefly, Q2-dada2 denoise-paired [24] was used to construct features (amplicon sequence variants; ASV). Due to the variable size of the ITS1 region (300–900 bp) to avoid loss of information paired-end reads were not merged and posterior analysis was performed only on single-end read mode using read 1. Representative sequences from read 1 were taxonomically classified using a naive Bayes classifier pre-trained with the UNITE (version 7-99 01.12.2017) eukaryotic nuclear ribosomal ITS region database [25], and a phylogenetic tree was built within qiime2 using first the mafft function to align the sequences and the fasttree function to infer the approximately-maximum-likelihood phylogenetic tree. Only sequences classified as fungi were analysed. The resulting taxonomic profile, i.e., features and their counts per sample, was imported into the phyloseq [26] package in R [27] and agglomerated into operational taxonomic units (OTUs) based on a phylogenetic tree (a cophenetic distance of 0.02 was used as proxy for the difference between fungal species) with the phyloseq function tip_glom. OTUs with a total abundance across samples < 0.01% of the total and unclassified OTUs at the Kingdom taxonomic level were removed. The resulting OTUs were further agglomerated based on species classification when available (i.e., where two or more OTUs were classified to the same species they were merged into one OTU). Due to the large difference in library sizes, samples were normalized, i.e., subsampled without replacement to the size of the smallest library (i. e. 1000 sequences) per sample using the phyloseq function rarefy even_depth. Normalized data was used for all statistical analyses.

2.6. Bacterial sequence analysis

Bacterial sequences for a subset of 84 samples were obtained from metagenomic shotgun sequencing that had been performed in a separate study [12].

2.7. Estimation of fibre intake

Maternal diet during pregnancy was measured at the third trimester visit using a validated 74 item food frequency questionnaire, Dietary Questionnaire for Epidemiological Studies version 2 (DQESv2) [28]. Even though this was administered only in the third trimester, evidence for stability of dietary intake over the course of the pregnancy was obtained from a separate, purpose-built ENDIA Pregnancy Lifestyle Questionnaire administered before each of the three study visits during pregnancy. This assessed consumption of milk (dairy and non-dairy), caffeinated and decaffeinated tea and coffee, caffeine-containing soft drinks, dairy products, soy, gluten containing cereals (wheat, barley, and rye) and non-gluten containing cereals (rice, corn, and oats). Analysis across the study visits revealed that on 86% of occasions respondents reported either the same unit or within one-unit difference between visits 1–2, visits 1–3, and visits 2–3. Magnitude changes of four or five units were reported on < 2% of occasions. This supports the DQESv2 as being reflective of the whole pregnancy period.

2.8. Measurement of serum 1,5-anhydroglucitol (1,5-AG)

Serum 1,5-anhydroglucitol (1,5-AG), an index of glucose control in pregnancy [29], was measured by GlycoMark (Nippon Kayaku Co. Ltd., New York, NY, US).

Table 1 – Summary of characteristics of non-T1D and T1D pregnancies.

General	Non-T1D	T1D	P-value **
Overall number of samples: n (%)	59 (36)	103 (64)	
Trimester 1	15 (18.8)	23 (18.5)	
Trimester 2	21 (43.5)	40 (47.1)	
Trimester 3	23 (37.7)	40 (34.5)	
All three trimesters (% pregnancies)	12 (46)	17 (38)	
All three trimesters (% samples)	12 (20)	17 (17)	
Gestational age in days at faecal sample: mean (SD)			
Trimester 1	85.4 (18.7)	80.2 (14.7)	0.34
Trimester 2	164 (20.1)	159 (19.5)	0.31
Trimester 3	246 (16.9)	232 (15.0)	**0.0011**
Maternal			
Overall number of pregnancies	26	45	
Age in years at conception: mean (SD)	32.6 (3.24)	31.5 (4.28)	0.29
Assisted conception: n (%)	2 (8)	4 (9)	0.86
Twin pregnancy: n (%)	0 (0.0)	0 (0.0)	
Nulliparous: n (%)	7 (26.9)	26 (57.8)	**0.014**
Pre-eclampsia: n (%)	0 (0)	8 (18)	**0.0050**
Group B Streptococcus positive: n (%)	6 (23)	5 (11)	0.19
Genito-urinary infections: n (%)	2 (8)	4 (9)	0.86
Pre-pregnancy BMI: mean (SD)	26.1 (5.59)	26.0 (5.00)	0.998
Underweight (<18.5): n (%)	0 (0)	1 (2)	
Normal weight (18.5–24.9): n (%)	14 (54)	18 (40)	
Overweight weight (25–29.9): n (%)	5 (19)	15 (33)	
Obese (>30): n (%)	7 (27)	11 (24)	
Gestational weight gain (kg): Mean (SD)	12.6 (3.99)	11.9 (5.02)	0.51
Paternal			
Age in years at conception: mean (SD)	34.9 (6.32)	32.9 (3.85)	0.090
Pre-pregnancy BMI: mean (SD)	27.7 (4.15)	27.6 (4.14)	0.89
Underweight (BMI < 18.5): n (%)	0 (0)	0 (0)	
Normal weight (BMI18.5–24.9): n (%)	4 (15)	8 (18)	
Overweight (BMI 25–29.9): n (%)	10 (38)	9 (20)	
Obese (>30): n (%)	4 (15)	11 (24)	
Missing: n (%)	8 (31)	17 (38)	
Maternal demographics			
Born in Australia: n (%)			
Yes	21 (81)	39 (87)	0.51
Education beyond High School: n (%)			
Yes	21 (81)	37 (82)	0.88
Lives in a metro area: n (%)	24 (92)	41 (91)	0.86

General	Non-T1D	T1D	P-value **
Socio-Economic Indexes for Areas (SEIFA) Index of Relative Socio-Economic Disadvantage (IRSD)			
Quintile 1n (%)	2 (8)	2 (4)	
Quintile 2n (%)	2 (8)	5 (11)	
Quintile 3n (%)	6 (23)	9 (20)	
Quintile 4n (%)	5 (19)	10 (22)	
Quintile 5n (%)	11 (42)	19 (42)	0.90
Smoking during pregnancy: n (%)	1 (4)	1 (2)	0.67
Household smoking during pregnancy: n (%)	3 (12)	5 (11)	0.91
Adults in house during pregnancy: n (%)			
One	0 (0)	3 (7)	
Two	24 (92)	39 (87)	
More than two	1 (4)	3 (7)	0.62
Children in house during pregnancy: n (%)			
None	7 (27)	24 (53)	
One	7 (27)	13 (29)	
Two	7 (27)	6 (13)	
More than two	4 (15)	1 (2)	**0.0068**
Furred pet ownership during pregnancy: n (%)	14 (54)	27 (60)	0.66
Diet and physical activity in pregnancy			
Diet: mean (SD)			
Energy/day (kJ)	7770 (1710)	6950 (2180)	0.14
Fat (g)	82.2 (20.3)	78.7 (27.7)	0.61
Protein (g)	88.5 (17.9)	86.5 (28.3)	0.77
Carbohydrate (g)	193 (47.6)	152 (52.4)	**0.0042**
Fiber (g)	20.6 (4.18)	19.9 (5.83)	0.61
Diet: Missing: n (%)	4 (15)	7 (16)	
Alcohol consumed: n (%)			
Yes	6 (23)	6 (13)	
Unknown	1 (4)	1 (2)	0.28
Total level of physical activity (MET) (h/week): mean (SD) [65]	258 (97.1)	278 (110)	0.57
Biological data			
HbA1c (%)			
Trimester 1: median (IQR)	–	6.5 (1.1)	
Trimester 2: median (IQR)	–	6.2 (0.95)	
Trimester 3: median (IQR)	–	6.4 (0.75)	
Trimester 1: missing	–	4 (17)	
Trimester 2: missing	–	5 (13)	
Trimester 3: missing	–	14 (35)	
Serum 1,5-anhydroglucitol (AG) (ug/mL)			
Trimester 1: median (IQR)	14.1 (11.1)	3.2 (1.8)	
Trimester 2: median (IQR)	10.8 (6.2)	2.4 (2.3)	
Trimester 3: median (IQR)	9.2 (5.4)	2.4 (2.1)	
Trimester 1: mean (SD)	14.3 (5.3)	3.92 (2.5)	**<0.001**
Trimester 2: mean (SD)	11.0 (3.9)	2.66 (1.9)	**<0.001**
Trimester 3: mean (SD)	9.34 (3.7)	2.79 (2.0)	**<0.001**

General	Non-T1D	T1D	P-value **
Trimester 1: missing n (%)	0 (0)	0 (0)	
Trimester 2: missing n (%)	0 (0)	3 (8)	
Trimester 3: missing n (%)	0 (0)	9 (23)	
Serum vitamin D (nmol/L): mean (SD)			
Trimester 1	83.5 (23.8)	81.0 (22.6)	0.74
Trimester 2	95.1 (24.0)	89.3 (26.7)	0.34
Trimester 3	88.8 (31.3)	94.5 (31.6)	0.52
Trimester 1: missing n (%)	2 (13)	1 (4)	
Trimester 2: missing n (%)	1 (5)	3 (8)	
Trimester 3: missing n (%)	2 (9)	6 (15)	
Maternal HLA: n (%)			
DR34	14 (54)	22 (49)	
DR3 or DR4	4 (15)	18 (40)	
DRXX	8 (31)	5 (11)	**0.031**
Known supplements pre-pregnancy and pregnancy			
Antibiotics: n (%)	NA	NA	NA
	7 (27)	14 (31)	0.71
Anticoagulants: n (%)	0 (0)	1 (2)	0.998
	3 (12)	16 (36)	**0.021**
Antihypertensive agents: n (%)	NA	NA	NA
	0 (0)	9 (20)	**0.0028**
Known other supplements pre-pregnancy and pregnancy			
Biotin: n (%)	10 (39)	9 (20)	0.095
	23 (89)	41 (91)	0.72
Calcium: n (%)	11 (42)	10 (22)	0.078
	23 (89)	43 (96)	0.28
Iron	12 (46)	9 (20)	**0.023**
	25 (96)	44 (98)	0.69
Magnesium: n (%)	12 (46)	9 (20)	**0.023**
	24 (92)	42 (93)	0.87
Selenium: n (%)	10 (39)	9 (20)	0.095
	23 (89)	41 (91)	0.72
Vitamin B1: n (%)	12 (46)	9 (20)	**0.023**
	24 (92)	42 (93)	0.87
Vitamin B2: n (%)	12 (46)	9 (20)	**0.023**
	24 (92)	42 (93)	0.87
Vitamin B3: n (%)	12 (46)	9 (20)	**0.023**
	24 (92)	42 (93)	0.87
Vitamin B5: n (%)	6 (23)	7 (16)	0.43
	12 (46)	29 (64)	0.14
Vitamin B6: n (%)	12 (46)	9 (20)	**0.023**
	24 (92)	42 (93)	0.87
Vitamin B9 (folate): n (%)	11 (42)	14 (31)	0.34
	25 (96)	45 (100)	0.997
Vitamin B12: n (%)	12 (46)	9 (20)	**0.023**
	24 (92)	41 (91)	0.86

General	Non-T1D	T1D	P-value **
Vitamin D: n (%)	12 (46)	10 (22)	**0.039**
	25 (96)	43 (96)	0.90
Vitamin E: n (%)	11 (42)	9 (20)	**0.048**
	21 (81)	38 (84)	0.69
OTHER			
Vaccine: n (%)			
Yes (Flu only)	1 (4)	4 (9)	
Yes (Pertussis only)	3 (12)	7 (16)	
Yes (Flu and Pertussis)	9 (35)	14 (31)	0.80
Mode of delivery: n (%)			
Vaginal	20 (77)	15 (33)	
Caesarean (with labour)	0 (0)	8 (18)	
Caesarean (without labour)	6 (23)	22 (49)	**<0.001**

Log transformation was used for Maternal BMI, Metabolic equivalent of task, 1,5-anhydroglucitol in trimesters 1 and 2 and for Vitamin D in trimesters 1 and 3.
Square root transformation was used for 1,5-anhydroglucitol in trimester 3 and Vitamin D in trimester 2.
Nine questionnaires had to be excluded due to unrealistic dietary intake (energy < 4500 kJ/day or >20,000 kJ/day), as previously reported in other pregnancy studies (http://dx.https://doi.org/10.1111/j.1740-8709.2007.00104.x).
Hb1Ac, serum 1,5-AG (μg/mL) and serum vitamin D are based on samples rather than pregnancies

2.9. Measurement of faecal calprotectin

Calprotectin, an index of intestinal inflammation [30], was measured in faecal samples from trimester three by enzyme-linked immunoassay (CALPRO$_{TM}$ Oslo, Norway) according to the manufacturer's instructions. The protein extracts were prepared using 50 mg of the same faecal sample used for the mycobiome analysis. The concentration of faecal calprotectin was expressed as milligrams per kg, the normal range being 5–50 mg/kg [31,32].

2.10. Measurement of serum intestinal fatty-acid binding protein (I-FABP)

Serum intestinal fatty acid-binding protein (I-FABP), a marker of intestinal epithelial damage [33], was measured across the three trimesters by enzyme-linked immunoassay (Hycult Biotech, The Netherlands) according to the manufacturer's instructions. The concentration of I-FABP was expressed as pg/mL, the normal range being 20–485 pg/mL [34].

2.11. Measurement of serum IgA/IgG antibodies to Saccharomyces cerevisiae

Serum IgA and IgG antibodies specific for *Saccharomyces* (ASCA) were measured across the three trimesters of pregnancy by enzyme-linked immunoassay (QUANTA Lite ASCA, Inova Diagnostics, San Diego, CA, US) according to the manufacturer's instructions. Results were expressed as arbitrary units based on the values of the positive and negative controls, with a cut-off for positivity of 10 U/mL.

2.12. Statistical analyses

Alpha diversity (diversity within microbial communities) was obtained from the number of observed OTUs and InvSimpson index using the function estimate_richness from the R package phyloseq. For testing differences in alpha diversity between women with and without T1D, Generalized Estimating Equations (GEEs) [35] were applied using the R function geeglm from package geepack v1.2-1 [36] to account for possible correlation of multiple measurements within a participant over time. Parameter family was set to default "Gaussian". The default empirical (robust or 'sandwich') estimator was used to ensure that estimates were robust to misspecification of the correlation structure. The model used for the regression included T1D status and trimester as well as their interaction term (T1D $\times$ trimester) to test if differences in alpha diversity between T1D and non-T1D women changed across trimesters and was adjusted for the human leukocyte antigen (HLA) category. Beta diversity (diversity between microbial communities) was determined with phyloseq (function distance, method= "bray") on proportional log transformed data. This function calculates Bray-Curtis coefficients, which measure the distance between communities based on the taxa that they contain and their abundances. Differences in beta diversity were evaluated with a modified version of the Adonis function from the vegan package [37], which performs a repeated measure aware (RMA) PERMANOVA test [38]. This statistical model included T1D status and time with their interaction adjusted for HLA category as in the alpha diversity model. In addition, interactions between time and other factors were also tested as shown in the Results. Due to the small number of taxa (at each taxonomic level from OTU to phylum) with a prevalence greater than 50% in women with and without T1D, differential abundance analysis was performed in a univariate manner, i.e., a test was applied to each individual taxon. Given that zero inflation and over dispersion were present in the data a zero inflated negative binomial generalized linear mixed model, using the NB1 parametrization was the preferred model after considering other models in this analysis. This was fit with the function 'glmmTMB' from the glmmTMB R package [39]. The model included the T1Dstatus * trimester interaction term and was adjusted for HLA category and a random factor for mothers that accounts for the correlation between counts from the same mother at different trimesters. Because none of the predictors influenced the excess zero logistic part of the model, the 'ziformula' parameter was set to 1. If the interaction term was statistically significant, the package 'emmeans' [40] from R was used for post-hoc comparisons between T1D and non-T1D women at each trimester and for comparisons between trimesters within T1D or non-T1D women and for estimating marginal means and their 95% confidence intervals (CI), and as this is a complex model calculated by simulation. The latter was performed averaging over the HLA, considering the differing effects of each category of HLA (Table 1). Predicted counts, i.e. estimated marginal or adjusted means and standard errors generated based on the zero inflated negative binomial linear model for T1D and non-T1D participants within each trimester averaged over HLA were plotted. A P-value ≤ 0.05 was considered significant. Zero inflated negative binomial linear (zinb) mixed models were fit with the function zeroinfl (R package pscl) [41] to determine associations of the relative abundance of *S. cerevisiae* with dietary intake (fibre, fat, carbohydrate, and specific carbohydrates [resistant starch, soluble and insoluble fibre]) in average grams consumed daily, and with the concentrations of serum 1,5-AG, faecal calprotectin, serum I-FABP and serum ASCA IgA and IgG as well as with the abundance of bacteria. Models were fitted with two terms, one with six levels for the combination of T1D status and trimester and the other for the covariate/variable of interest. For this, a hierarchical approach to testing significance (i.e., backward elimination) was used in which the interaction was tested before the main effects in both parts of the model (i.e., negative binomial and zero inflated) using the likelihood ratio test, function lrtest from R package lmtest [42]. When the interaction was not significant the model was further

reduced to investigate the significance of the main effects, which determined if the association with *S. cerevisiae* was significant. Since the "mother id" variance was essentially zero, this random factor was not included in the models.

3. Results

3.1. Study population and sequencing output

Forty-five pregnant women with T1D and 25 without T1D each provided up to three faecal samples across pregnancy (162 samples in total; Supplementary Fig. 1) for analysis by ITS-amplicon sequencing using the ITS1 sub-region. After quality filtering, 13,241 ± 8394 (mean ± SD) reads per sample were obtained with the Illumina MiSeq sequencing machine. Overall, a total of 234 fungal OTUs was identified with ranges per sample in women with T1D of 2–9 in trimester 1, 1–12 in trimester 2 and 1–14 in trimester 3 and in women without T1D of 2–8 in trimester 1, 1–9 in trimester 2 and 2–11 in trimester 3 (see Fig. 1 for more details).

3.2. Characterization of gut fungi in women with and without T1D in pregnancy

Out of 194 OTUs that were annotated up to the phylum level, 62 contained on average 63% of the total sequences per sample; the rest were classified only as Fungi (i.e., kingdom taxonomic level; Supplementary Fig. 2). As observed previously [17], most of fungal OTUs were specific to each individual. In general, samples were dominated by the phylum *Ascomycota* within which two OTUs were dominant, representing on average ~ 50% of the total OTUs. One was an unclassified fungus and the other was classified to the *S. cerevisiae* species (Supplementary Fig. 2). *S. cerevisiae* was present in 71.8% and 64.4% of women with and without T1D, at an average abundance of 42.3% and 34.9%, respectively. *Candida albicans* species, in the phylum *Ascomycota*, was present in 10.8% and 7.2% of women with and without T1D, at an average abundance of 56% and 30%, respectively.

In the alpha diversity analysis, a significant interaction (P = 0.023) was found between trimester and T1D status for fungal richness. This did not differ between women with and without T1D in trimester 1 or 2 (Fig. 1A, and 1B; Table 2) but decreased significantly in trimester 3 in women with T1D (Fig. 1C; Table 2). No interactions or significant differences in the InvSimpson index of alpha diversity were detected (Supplementary Fig. 3; Table 2). Beta diversity between women with and without T1D was not significantly different at any taxonomic level (Table 3), as expected by the low OTU frequency across samples in women in either group (Supplementary Fig. 4). However, there was a significant difference between trimesters at the class and phylum level (Table 3). Differential abundance analysis was performed in a univariate manner for taxa with a prevalence > 50% in

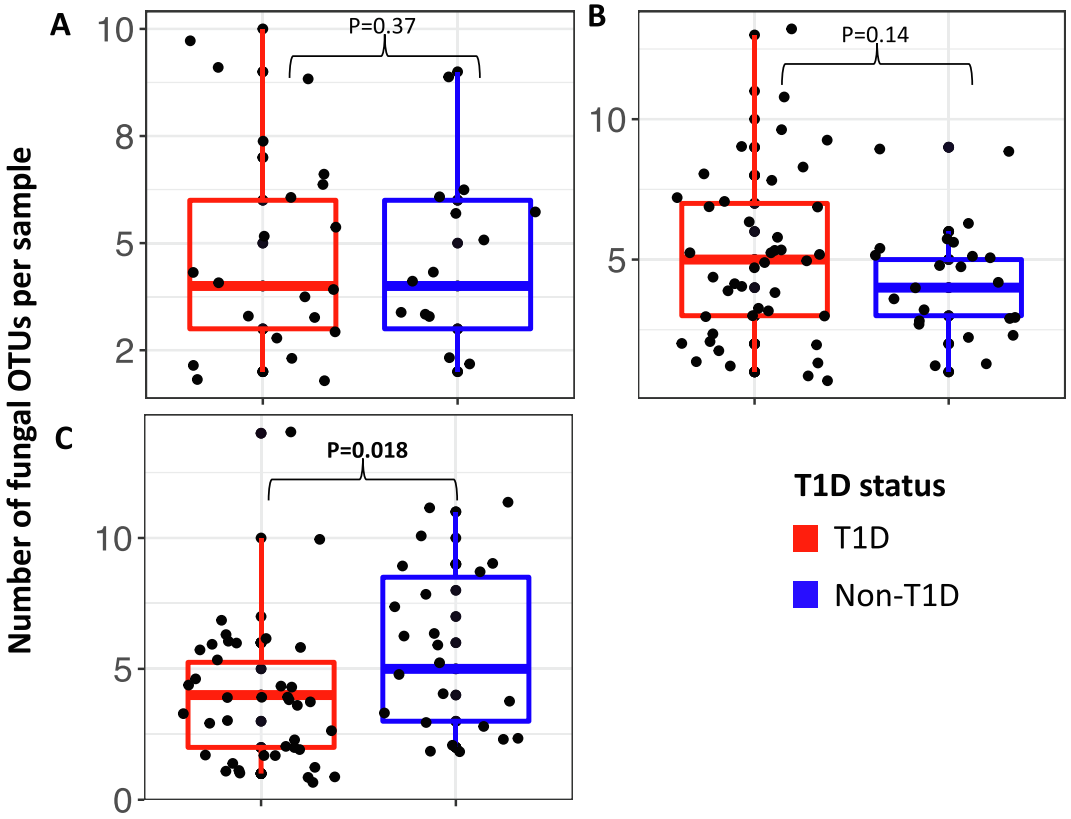

Fig. 1 – Alpha diversity (richness) by T1D status of women across trimesters. Number of fungal OTUs (richness) observed in samples from women with (T1D; red) and without T1D (non-T1D; blue) in A) the first (T1), B) second (T2), and C) third (T3) trimesters during pregnancy. Boxes show the inter-quartile range, and the line within the box indicates the median. The * denotes a significant difference (P-value < 0.05) between T1D and non-T1D.

Table 2 – Alpha diversity comparison (P-values).

	Interaction (T1D status:Time)	T1D STATUS (MAIN EFFECT)	TRIMESTERS (MAIN EFFECT)
Richness	**0.034**	–	–
InvSimpson	0.058	0.48	0.79
	INTERACTION TABLE: T1D vs. non-T1D within each trimester		
	T1	**T2**	**T3**
Richness	0.37	0.14	**0.018**
	INTERACTION TABLE: Time (trimester) within each of T1D and non-T1D		
	Time in T1D	**Time in non-T1D**	
Richness	0.15	0.19	

Note: In bold are significant P-values (<=0.05).
INTERACTION TABLE: T1D vs. non-T1D within each trimester: P-values obtained after testing T1D vs. non-T1D in each trimester, after the interaction between trimester and T1D status was significant.
INTERACTION TABLE: Time (trimester) within each of T1D and non-T1D: P-values obtained after testing differences between trimesters in women with or without T1D after the interaction between trimester and T1D status was significant.
Interaction (T1D status:Time): P-value from testing the interaction between terms T1D status and Time/Trimester. In the statistical model, the interaction between variables T1D status and Trimester were included to test if the possible differences in alpha diversity between T1D and non-T1D depend or are conditioned to trimester or T1D status (e.g. if there are differences between T1D and non-T1D only in one trimester or if there are differences between trimesters only in women with T1D).
T1D STATUS (MAIN EFFECT): P-value from testing differences between T1D and non-T1D women across the three trimesters, once the interaction between T1D status and trimester was tested and found not to be significant.
TRIMESTERS (MAIN EFFECT): P-value from testing differences between trimesters in women with and without T1D, once the interaction between T1D status and trimester was tested and was not significant.

Table 3 – Beta diversity comparison (P-values).

Beta diversity comparison between women with and without T1D - significant p-values (<0.05) in bold.

	Interaction (T1D status:Time)	T1D STATUS (MAIN EFFECT)	TRIMESTERS (MAIN EFFECT)
Species	0.058	0.92	0.43
Genus	0.17	0.76	0.07
Family	0.18	0.77	0.075
Order	0.088	0.45	0.051
Class	0.087	0.66	**0.024**
Phylum	0.15	0.37	**0.014**

Interaction (T1D status:Time): P-value from testing the interaction between terms T1D status and Time/Trimester. In the statistical model, the interaction between variables T1D status and Trimester were included to test if the possible differences in alpha diversity between T1D and non-T1D depend or are conditioned to trimester or T1D status (e.g. if there are differences between T1D and non-T1D only in one trimester or if there are differences between trimesters only in women with T1D).
T1D STATUS (MAIN EFFECT): P-value from testing differences between T1D and non-T1D women across the three trimesters, once the interaction between T1D status and trimester was tested and found not to be significant.
TRIMESTERS (MAIN EFFECT): P-value from testing differences between trimesters in women with and without T1D, once the interaction between T1D status and trimester was tested and was not significant.

women with or without T1D. The predicted counts, i.e. estimated marginal or adjusted means and standard errors generated based on the zero inflated negative binomial linear model of *S. cerevisiae* were significantly decreased in women with T1D in trimester 1 and then increased across pregnancy in women with T1D and decreased in women without T1D (Fig. 2; Table 4), being significantly higher in women with T1D in trimester 3. Similar patterns were observed for the genus *Saccharomyces*, the family *Saccharomycetaceae*, the order *Saccharomycetales*, the class *Saccharomycetes* and the phylum *Ascomycota*. This very similar pattern observed at different taxonomic levels is most likey explained by the percentage of Ascomycota reads classified as *S. cerevisiae* (Fig. 2; Table 4).

3.3. *Relationship between Saccharomyces and blood glucose control and diet in pregnancy*

To determine if the abundance of *Saccharomyces* was related to blood glucose control or diet, zero inflated negative binomial linear mixed models were fit with serum 1,5-AG, and with

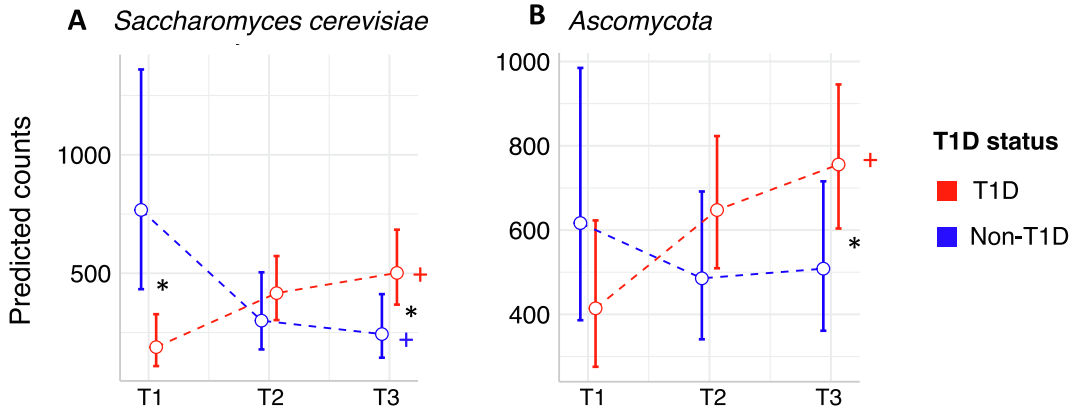

Fig. 2 – Differentially abundant taxa (mean ± SEM of predicted counts) detected by ITS1 amplicon sequencing between T1D (red) and non-T1D (blue) women and between trimesters. Predicted counts: estimated marginal or adjusted count means and standard errors generated based on the zero inflated negative binomial linear model. Predicted count means are shown as a point in each trimester for differentially abundant taxa in women with (red) and without (blue) T1D. An * between points denotes a significant difference (P-value < 0.05) between the groups in that trimester. A + sign denotes a significant difference in abundance between trimesters 1 and 3 and the colour denotes if the difference was observed in T1D (red) or non-T1D (blue) women. A) genus and B) Phylum.

dietary components (fat, carbohydrate, resistant starch, and total soluble and insoluble fibre). No significant associations were found (Table 5).

3.4. *Intestinal inflammation and anti-Saccharomyces cerevisiae antibodies in women with T1D*

Faecal calprotectin, released from neutrophils and monocytes, is a marker of intestinal inflammation that has been associated with impaired integrity and increased permeability of the epithelial barrier [43]. Serum intestinal fatty acid-binding protein (I-FABP) is a marker of intestinal epithelial damage [33]. We measured faecal calprotectin in trimester three and serum I-FABP in each trimester. Both markers were increased in women with T1D (Fig. 3; Supplementary Table 2 and Supplementary Table 3) and were correlated with each other (Pearson $R^2 = 0.58$; P = 0.001). Because gut inflammation with impaired epithelial barrier function could promote exposure and immunity to *Saccharomyces* we measured serum IgG and IgA ASCA. Both were higher in women with T1D across pregnancy (Fig. 4; Supplementary Table 1) but did not correlate with the abundance of *S. cerevisiae*.

3.5. *Relationship between Saccharomyces cerevisiae and gut bacteria*

We sought to address inter-microbial kingdom relationships and determine if the increase in *S. cerevisiae* in women with T1D was associated with a decrease in bacteria that produce anti-inflammatory short chain fatty acids (SCFAs), as we had recently observed [12]. A "zinb" linear mixed model approach was used to search for associations between *S. cerevisiae* and bacterial taxa identified as SCFA producers by metagenomic sequencing [12]. The species and genera of SCFA producers are listed in Table 6. The higher abundance of *S. cerevisiae* was associated with a significantly lower abundance of *Faecalibacterium prausnitzii* (Table 6; Fig. 5).

4. Discussion

As the fungal compartment is only 0.1–1.0% of the total microbiome [13,44,15,17] and is not well represented in whole genome metagenome sequencing [17], we employed fungal-specific ITS1amplicon sequencing of faecal DNA to analyse the gut mycobiota in pregnancy and T1D. Similar to what we observed, a high proportion of the Ascomycota phylum and, to a lesser extent, the Basidiomycota phylum, has been reported in the gut [17,45,46]. We found that Ascomycota was the dominant phylum in pregnant women and observed a decrease in fungal alpha diversity across pregnancy, mainly in trimester 3 in women with T1D. At this time, *Saccharomyces*, the most predominant genus within the Ascomycota phylum, increased in women with T1D. Changes in the gut mycobiome across pregnancy were distinct between women with and without T1D.

The species *S. cerevisiae* is recognised to colonize the human gut [47,48] and in some human populations is more abundant than *Candida* spp [49]. *S. cerevisiae* is present in most diets and its abundance in the gut may depend on nutrient availability. However, a study that reported associations between nutrients and fungal populations in faecal samples did not find a relationship between the nutrient composition of the diet and *Saccharomyces*, either recent or long-term [50]. We also found no association between diet and the abundance of *Saccharomyces*, but our dietary assessment tool did not specifically define yeast intake.

Poor glycaemic control in diabetes is associated with overgrowth of some fungi, especially *Candida albicans*, which is more prevalent in the faeces of individuals with T1D or T2D [51,52]. In our study, *C. albicans* was present in faecal samples from a minority of women with and without T1D and, in con-

Table 4 – Differential abundance analysis (P-values).

Differential abundant taxa between T1D and non-T1D women				Differential abundant taxa between trimesters 1 and 3			
Taxonomic level	Trimester	Classification	P.value	Taxonomic level	T1D status	Classification	P.value
OTU	T1	Saccharomyces_cerevisiae	**0.0006**	OTU	T1D	Saccharomyces_cerevisiae	**0.001**
OTU	T2	Saccharomyces_cerevisiae	0.28	OTU	non-T1D	Saccharomyces_cerevisiae	**0.004**
OTU	T3	Saccharomyces_cerevisiae	**0.015**	Genus	T1D	Saccharomyces	**0.001**
Genus	T1	Saccharomyces	**0.0006**	Genus	non-T1D	Saccharomyces	**0.004**
Genus	T2	Saccharomyces	0.28	Family	T1D	Saccharomycetaceae	**0.002**
Genus	T3	Saccharomyces	**0.015**	Family	non-T1D	Saccharomycetaceae	**0.005**
Family	T1	Saccharomycetaceae	**0.001**	Order	T1D	Saccharomycetales	**0.0003**
Family	T2	Saccharomycetaceae	0.23	Order	non-T1D	Saccharomycetales	**0.02**
Family	T3	Saccharomycetaceae	**0.017**	Class	T1D	Saccharomycetes	**0.0003**
Order	T1	Saccharomycetales	**0.005**	Class	non-T1D	Saccharomycetes	**0.02**
Order	T2	Saccharomycetales	0.43	Phylum	T1D	Ascomycota	**0.01**
Order	T3	Saccharomycetales	**0.004**	Phylum	non-T1D	Ascomycota	0.48
Class	T1	Saccharomycetes	**0.005**				
Class	T2	Saccharomycetes	0.43				
Class	T3	Saccharomycetes	**0.004**				
Phylum	T1	Ascomycota	0.22				
Phylum	T2	Ascomycota	0.17				
Phylum	T3	Ascomycota	**0.049**				

Note: In bold are significant P-values (i.e.<=0.05)

Table 5 – Results (p-values) from likelihood ratio test comparing zero inflated negative binomial models to test associations with the abundances of *S. cerevisiae*.

Counts (Negative binomial part of the model)				
	Interaction	**Variable**	**Trimester_T1D**	
Carbohydrates (g)	0.315	0.68	0.22	
Resistant starch (g)	0.243	0.422	0.21	
Fibre (g)	0.447	0.693	0.241	
Soluble fibre (g)	0.791	0.62	0.228	
Insoluble fibre (g)	0.3	0.96	0.225	
Fat (g)	0.376	0.932	0.234	
1,5-anhydroglucitol (AG) (ug/mL)	0.814	0.944	0.208	
HbA1c [only T1D women]	0.279	0.516	**0.048**	
I-FABP (pg/mL)	0.71	0.411	0.189	
Calprotectin (mg/kg) [only trimester 3]	0.603	0.76	0.65	
ASCA IgA (U/mL)	0.069	0.719	0.064	
ASCA IgG (U/mL)	**0.009****	0.45	**0.047**	For one unit increase in ASCA IgG the odds of having *S. cerevisiae* decrease by 1-exp(-9.034)) = 100% in trimester 1 in women with T1D
Binomial (zero in flation part of the model)				
	Interaction	**Variable**	**Trimester_T1D**	
Carbohydrates (g)	0.535	0.477	0.21	
Resistant starch (g)	0.36	0.766	0.193	
Fibre (g)	0.293	0.599	0.186	
Soluble fibre (g)	0.627	0.514	0.188	
Insoluble fibre (g)	**0.005*****	0.133	0.21	Interaction not significant in the full results below. So there is no association
Fat (g)	0.106	41	0.169	
1,5-anhydroglucitol (AG) (ug/mL)	0.622	0.82	0.613	
HbA1c [only T1D women]	0.866	0.426	0.29	
I-FABP (pg/mL)	0.462	0.705	0.527	
Calprotectin (mg/kg) [only trimester 3]	0.251	**0.01*****	0.079	For one unit increase in Calprotectin the odds of having *S. cerevisiae* decrease by 1-exp(-0.017) = 2%
ASCA IgA (U/mL)	0.561	0.056	0.484	
ASCA IgG (U/mL)	0.123	0.481	0.53	

Complete results from zinb test for ASCA IgG association with *S. cerevisiae

Call:

zeroinfl(formula = sac ~ ASCA IgG* t1dtri | 1, data = TTMetadataF, dist = "negbin")

Count model coefficients (negbin with log link):

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**	
(Intercept)	6.193	0.790	7.837	4.61E−15	
ASCA IgG	0.729	2.628	0.277	0.781	
t1dtrino_T2	−1.448	1.075	−1.346	0.178	
t1dtrino_T3	−1.484	0.992	−1.495	0.135	
t1dtriyes_T1	0.911	1.073	0.849	0.396	
t1dtriyes_T2	0.442	0.922	0.479	0.632	
t1dtriyes_T3	0.472	0.970	0.487	0.626	
FacI:t1dtrino_T2	4.146	4.236	0.979	0.328	
FacI:t1dtrino_T3	2.239	3.445	0.65	0.516	
FacI:t1dtriyes_T1	−9.034	3.298	−2.74	**0.00615**	For one unit increase in ASCA IgG the odds of having *S. cerevisiae* decrease by 1-exp(-9.034)) = 100% in trimester 1 in women with T1D

Table 5 – (*continued*)

FacI:t1dtriyes_T2	−1.672	2.758	−0.606	0.544	
FacI:t1dtriyes_T3	−1.693	3.050	−0.555	0.579	
Log(theta)	−0.134	0.156	−0.86	0.390	
Zero-inflation model coefficients (binomial with logit link):					
	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	−0.714	0.199	−3.58	0.000338	
***Complete results from zinb test for Calprotectin association with *S. cerevisiae*					
Call:					
zeroinfl(formula = sac ~ Calprotectin| t1dtri + Calprotectin, data = TTMetadataF, dist = "negbin")					
Count model coefficients (negbin with log link):					
	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	5.533	0.294	18.85	<2e-16	
Calprotectin	0.001	0.002	0.665	0.506	
Log(theta)	0.008	0.279	0.027	0.978	
Count model coefficients (negbin with log link):					
	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	−0.396	0.616	−0.642	0.521	
t1dtriyes_T3	1.398	0.828	1.687	0.092	
Calprotectin	−0.017	0.011	−1.564	0.118	For one unit increase in Calprotectin the odds of having *S. cerevisiae* decrease by 1-exp(-0.017) = 2%
***Complete results from zinb test for insoluble fiber association with *S. cerevisiae*					
Call:					
zeroinfl(formula = sac ~ Calprotectin| t1dtri + Insoluble fibre, data = TTMetadataF, dist = "negbin")					
Count model coefficients (negbin with log link):					
	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	5.982	0.313	19.099	<2e-16	
Insoluble fibre	0.003	0.033	0.095	0.924	
Log(theta)	−0.364	0.135	−2.698	0.007	
Count model coefficients (negbin with log link):					
	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	1.288	1.556	0.828	0.408	
Insoluble fibre	−0.095	0.134	−0.703	0.482	
t1dtrino_T2	−3.080	2.067	−1.49	0.136	
t1dtrino_T3	−6.964	3.257	−2.138	0.033	
t1dtriyes_T1	1.718	2.854	0.602	0.547	
t1dtriyes_T2	−2.280	1.832	−1.245	0.213	
t1dtriyes_T3	−3.794	1.875	−2.024	**0.043**	
FacI:t1dtrino_T2	0.178	0.182	0.975	0.330	
FacI:t1dtrino_T3	0.554	0.284	1.955	0.051	Interaction not significant

Table 5 – (*continued*)				
FacI:t1dtriyes_T1	−0.641	0.438	−1.463	0.144
FacI:t1dtriyes_T2	0.110	0.171	0.644	0.520
FacI:t1dtriyes_T3	0.286	0.171	1.669	0.095

Note Trimester_T1D status was fitted as a single term with 6 levels corresponding to the combination of the 2 levels of T1D status and the 3 trimesters.
Sac: *Saccharomyces cerevisiae* abundance
Variable: This refers to the variable being tested, as named in each row (e.g. Carbohydrates [g], Resistant starch [g], etc)
Interaction: This refers to the p-value obtained from testing if there is as significant interaction between the "variable of interest" and the term "Trimester_T1D status". If the interaction is significant, it means that the possible differences in alpha or beta diversity between T1D and non-T1D depend or are conditioned to trimester and/or T1D status (e.g. if there are differences between T1D and non-T1D only in one trimester or if there are differences between trimesters only in women with T1D).

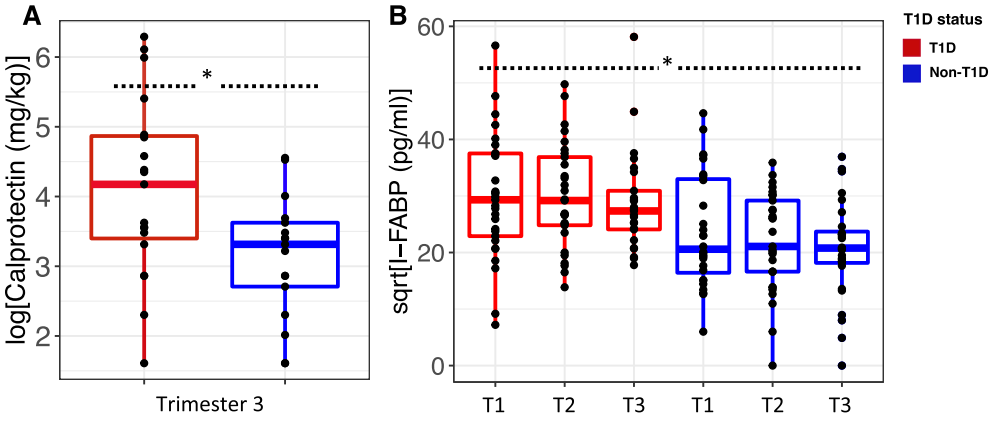

Fig. 3 – Faecal calprotectin and serum intestinal fatty-acid binding protein (iFABP) in pregnant women. Boxplots show the distribution of concentrations of faecal calprotectin in trimester 3 and serum I-FABP across trimesters in women with (red) and without (blue) type 1 diabetes. The * denotes a significant difference between T1D and non-T1D.

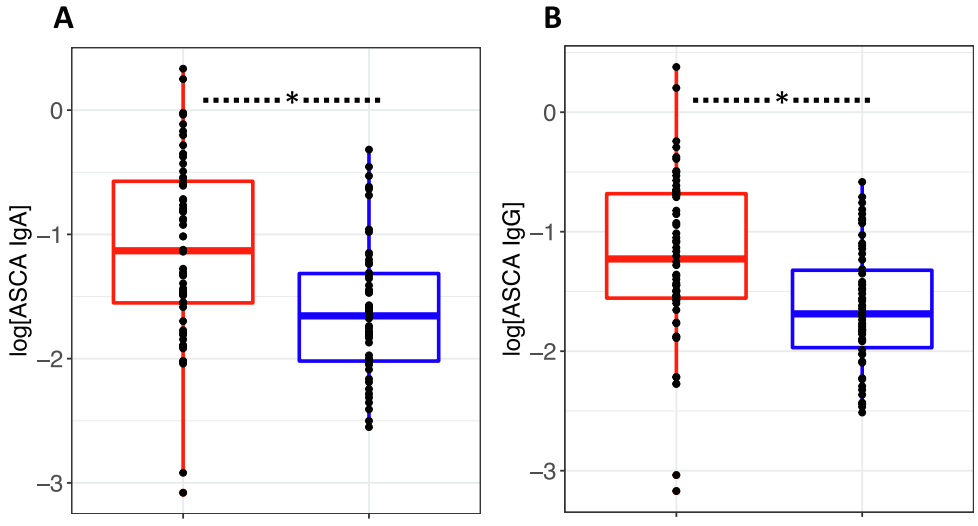

Fig. 4 – Anti-*Saccharomyces cerevisiae* antibodies in pregnant women. Serum from pregnant women with and without T1D across pregnancy was assayed for ASCA antibodies by ELISA. A) IgA ASCAs are higher in women with T1D compared to women without T1D across pregnancy. B) IgG ASCAs are higher in women with T1D compared to women without T1D across pregnancy. Results are expressed as absorbance (OD450). Boxplots showing the distribution for log transformed ASCA IgA and IgG measurements during pregnancy in women with (red) and without (blue) type 1 diabetes. The * denotes a significant difference between T1D and non-T1D women across pregnancy.

trast to *S. cerevisiae*, the difference in abundance between the groups was not significant. A possible explanation is that despite the difference in serum 1,5-AG between T1D and non-T1D women (Table 1), diabetes control in women with T1D was carefully monitored in pregnancy to minimise hyperglycaemia and prevent glycosuria. This was reflected by their median HbA1C across pregnancy of 6.33%, which is in the recommended range. In addition, we found no correlation between the concentration of serum 1,5-AG and the abundance of *S. cerevisiae*.

Sakly *et al* [53] reported higher concentrations of serum ASCA in individuals with T1D without celiac disease and suggested that this was due to increased intestinal permeability. Increased concentrations of serum ASCA have been noted in other autoimmune diseases, including antiphospholipid syndrome (APS) [54], systemic lupus erythematosus [55] and rheumatoid arthritis [56] in which impaired intestinal barrier function has been reported [57]. In women with T1D, intestinal inflammation was reflected by higher concentrations of faecal calprotectin and impaired epithelial integrity by higher concentrations of serum I-FABP. These are likely to be secondary to the lower abundance of *F. prausnitzii* and other producers of butyrate [58], as butyrate prevents gut inflammation and maintains the intestinal epithelial barrier [59,60]. We [11,12] and others [61] have previously reported impaired epithelial barrier function and gut permeability in T1D.

The ability of *S. cerevisiae* to produce a range of antimicrobial factors [62,63,64] may account for its reciprocal association with the abundance of *F. prausnitzii*, although why this bacterium is specifically affected is unclear and

Table 6 – Results (p-values) from likelihood ratio test comparing zero inflated negative binomial models.

Counts (Negative binomial part of the model)					
		Interaction	Variable	Trimester_T1D	Interpretation
Species	Ruminococcus_torques	0.817	0.761	0.878	
	Eubacterium_rectale	0.083	0.604	0.882	
	Roseburia_intestinalis	0.369	0.756	0.913	
	Anaerostipes_hadrus	0.27	0.397	0.918	
	Lachnospiraceae_bacterium_5_1_63FAA	0.851	0.795	0.869	
	Roseburia_inulinivorans	0.988	0.963	0.877	
	Faecalibacterium_prausnitzii	0.625	**0.005****	0.972	For one unit increase in *Faecalibacterium_prausnitzii* the odds of having *S. cerevisiae* decrease by 1-exp(-0.085)) = 8%
Genus	Ruminococcus	0.588	0.17	0.697	
	Eubacterium	0.152	0.453	0.88	
	Roseburia	0.876	0.399	0.911	
	Anaerostipes	0.245	0.301	0.928	
	Faecalibacterium	0.634	**0.005****	0.972	For one unit increase in Faecalibacterium *prausnitzii* the odds of having *Saccharomyces* genus decrease by 1-exp(-0.085)) = 8%
Binomial (zeroin flation part of the model)					
		Interaction	Variable	Trimester_T1D	
Species	Ruminococcus_torques	0.419	0.121	0.064	
	Eubacterium_rectale	0.554	0.323	**0.036**	
	Roseburia_intestinalis	0.321	0.377	**0.037**	
	Anaerostipes_hadrus	0.35	0.088	**0.036**	
	Lachnospiraceae_bacterium_5_1_63FAA	0.937	0.746	**0.042**	
	Roseburia_inulinivorans	0.696	0.599	**0.049**	
	Faecalibacterium_prausnitzii	0.457	0.894	**0.043**	
Genus	Ruminococcus	0.961	0.427	**0.036**	
	Eubacterium	0.433	0.279	0.062	
	Roseburia	0.353	0.087	**0.033**	
	Anaerostipes	0.287	0.114	**0.037**	
	Faecalibacterium	0.444	0.899	**0.043**	
	***Complete results from zinb test for significant bacterial taxa associations with *S. cerevisiae*				
	Call:				
	zeroinfl(formula = sac ~ Faecalibacterium_prausnitzii | t1dtri, data = TTMetadata, dist = "negbin")				
	Count model coefficients (negbin with log link):				
		Estimate	Std. Error	z value	Pr(>|z|)
	(Intercept	6.320	0.203	31.081	**<2e-16**
	Faecalibacterium_prausnitzii	−0.085	0.024	−3.616	**0.0003**
	Log(theta)	−0.116	0.178	−0.655	0.512
	Zero-inflation model coefficients (binomial with logit link):				
		Estimate	Std. Error	z value	Pr(>|z|)
	(Intercept)	3.35E-01	7.96E-01	0.421	0.6739
Genus	t1dtrino_T2	−1.711	0.928	−1.843	0.065

For one unit increase in *Faecalibacterium_prausnitzii* the odds of having Saccharomyces decrease by 1-exp(-0.085)) = 8%

Table 6 – (*continued*)

t1dtrino_T3	−1.094	0.825	−1.325	0.1852	
t1dtriyes_T1	−17.970	2316.000	−0.008	0.994	
t1dtriyes_T2	−1.708	0.937	−1.823	0.068	
t1dtriyes_T3	−0.798	0.807	−0.989	0.323	
	Call:				
	zeroinfl(formula = sac ~ Faecalibacterium| t1dtri, data = TTMetadata, dist = "negbin")				
	Count model coefficients (negbin with log link):				
		Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
	(Intercept)	6.319	0.203	31.066	<2e-16
	Faecalibacterium	−0.085	0.024	−3.603	**0.0003**
	Log(theta)	−0.117	0.178	−0.66	0.509
	Zero-inflation model coefficients (binomial with logit link):				
		Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
	(Intercept)	0.338	0.796	0.424	0.671
	t1dtrino_T2	−1.711	0.928	−1.844	0.065
	t1dtrino_T3	−1.094	0.825	−1.326	0.185
	t1dtriyes_T1	−17.969	2315.989	−0.008	0.994
	t1dtriyes_T2	−1.709	0.937	−1.824	0.068
	t1dtriyes_T3	−0.799	0.807	−0.99	0.322

For one unit increase in Faecalibacterium *prausnitzii* the odds of having *Saccharomyces* genus decrease by 1-exp(-0.085)) = 8%

Note Trimester_T1D status was fitted as a single term with 6 levels corresponding to the combination of the 2 levels of T1D status and the 3 trimesters.

Sac: *Saccharomyces cerevisiae* abundance

t1dtri: Refers to composed factor of T1D status and trimester

Variable: This refers to the variable being tested, as named in each row (e.g. Carbohydrates [g], Resistant starch [g], etc)

Interaction: This refers to the p-value obtained from testing if there is as significant interaction between the "variable of interest" and the term "Trimester_T1D status". If the interaction is significant, it means that the possible differences in alpha or beta diversity between T1D and non-T1D depend or are conditioned to trimester and/or T1D status (e.g. if there are differences between T1D and non-T1D only in one trimester or if there are differences between trimesters only in women with T1D).

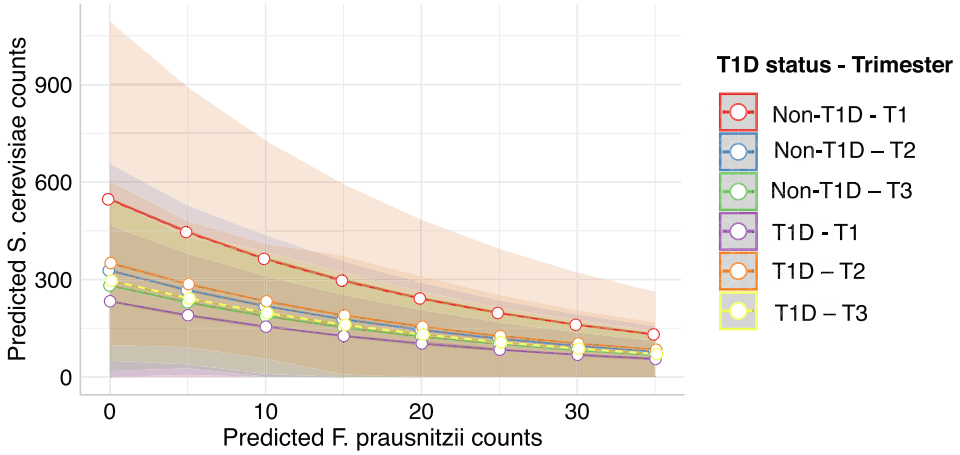

Fig. 5 – Predicted counts of the bacterium *Faecalibacterium prausnitzii* in relation to the predicted counts of *Saccharomyces cerevisiae* in relation to non-T1D and T1D status, and trimester variables. Predicted counts: estimated marginal or adjusted means and standard errors generated based on the zero inflated negative binomial linear model used to fit the data.

highlights the need to understand the complex community interactions within the gut microbiome. The increased abundance of *Saccharomyces* in later pregnancy in women with T1D, associated with a decrease in beneficial bacteria such as *F. prausnitzii*, could be detrimental to the health of the mother and child.

In summary, the gut mycobiome changes across pregnancy in both women with and without T1D. Women with T1D exhibit a progressive increase in the abundance of *S. cerevisiae*, associated with a decrease in the abundance of the bacterium *F. prausnitzii*, together with evidence of gut inflammation and impaired epithelial integrity. The relevance of these findings to the higher rate of pregnancy complications in women with T1D and the potential to ameliorate these complications by pro-biotic or anti-fungal interventions may be worthy of further study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Submission of sequencing data.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2022.109189.

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