Maternal gut microbiota \textit{Bifidobacterium} promotes placental morphogenesis, nutrient transport and fetal growth in mice

Jorge Lopez-Tello\textsuperscript{1} · Zoe Schofield\textsuperscript{2} · Raymond Kiu\textsuperscript{2} · Matthew J. Dalby\textsuperscript{2} · Douwe van Sinderen\textsuperscript{3} · Gwénaëlle Le Gall\textsuperscript{4} · Amanda N. Sferruzzi-Perri\textsuperscript{1} · Lindsay J. Hall\textsuperscript{2,4,5}

Received: 10 March 2022 / Revised: 9 May 2022 / Accepted: 14 May 2022 / Published online: 28 June 2022
© The Author(s) 2022

Abstract
The gut microbiota plays a central role in regulating host metabolism. While substantial progress has been made in discerning how the microbiota influences host functions post birth and beyond, little is known about how key members of the maternal gut microbiota can influence feto-placental growth. Notably, in pregnant women, \textit{Bifidobacterium} represents a key beneficial microbiota genus, with levels observed to increase across pregnancy. Here, using germ-free and specific-pathogen-free mice, we demonstrate that the bacterium \textit{Bifidobacterium breve} UCC2003 modulates maternal body adaptations, placental structure and nutrient transporter capacity, with implications for fetal metabolism and growth. Maternal and placental metabolome were affected by maternal gut microbiota (\textit{i.e.} acetate, formate and carnitine). Histological analysis of the placenta confirmed that \textit{Bifidobacterium} modifies placental structure via changes in \textit{Igf2P0}, \textit{Dlk1}, \textit{Mapk1} and \textit{Mapk14} expression. Additionally, \textit{B. breve} UCC2003, acting through \textit{Slc2a1} and \textit{Fatp1-4} transporters, was shown to restore fetal glycaemia and fetal growth in association with changes in the fetal hepatic transcriptome. Our work emphasizes the importance of the maternal gut microbiota on feto-placental development and sets a foundation for future research towards the use of probiotics during pregnancy.

Keywords Pregnancy · Metabolism · Microbiota · Fetus · \textit{Bifidobacterium}

Introduction
All nutrients and metabolites required for feto-placental growth are provided by the mother, which in turn is thought to be influenced by the maternal gut microbiota through the breakdown of complex dietary components [1]. During gestation, liberated metabolites may be used by the placenta for morphogenesis, and transported across the placenta for use by the fetus for growth and development [2, 3]. This is highly important across gestation, particularly at later stages, when fetal growth is maximal. Notably, there are also alterations in the maternal microbiota throughout pregnancy with levels of the bacterial genus \textit{Bifidobacterium} rising from trimester 1 onwards [4–6]. Failure of the mother to provide nutrients and metabolites to the fetus can result in pregnancy
complications including small for gestational age, fetal loss and stillbirth. However, the contribution of the maternal gut microbiota in determining fetal outcomes is largely unexplored. Knowledge in this area would be highly valuable for developing treatments to improve fetal growth, with benefits for population health.

Studies performed with germ-free (GF) mice have identified that the microbiota is a key regulator for adequate development, early immune education and metabolism [7–11]. However, little is known about how maternal gut microbiota influences feto-placental growth and placental structure and function. Here, we hypothesized that the maternal gut microbiota, and specific microbiota members, regulate fetal growth by modulating placental development and nutrient supply. We tested this hypothesis by comparing conceptus growth across a range of microbiome complexity; using conventional specific-pathogen-free (SPF) mice as a model for standard microbial colonization, and as a baseline to define correct feto-placental growth [11]; GF mice which represent a completely clean and naïve microbiome system; and a mono-colonized maternal GF model—GF mice colonized with Bifidobacterium breve UCC2003 (group referred throughout the manuscript as BIF) [12]. Bifidobacterium, including B. breve UCC2003, is known to beneficially modulate the wider gut microbiota and host responses [13–15]. Certain species and strains are defined as probiotics “live microorganisms, which when ingested or locally applied in sufficient numbers confer one or more specified demonstrated health benefits for the host” (FAO/WHO; [16]). Therefore, B. breve may represent a suitable option for treating pregnancy complications by exerting metabolic effects on maternal physiology and associated fetoplacental growth. Indeed, B. breve induced changes in placental morphogenesis and the abundance of placental glucose and lipid transporters, which were associated with improvements in the growth and metabolism of the fetus.

Materials and methods

Bifidobacterium breve UCC2003/pCheMC

B. breve UCC2003/pCheMC was generated by introducing the plasmid pCheMC to electrocompetent B. breve UCC2003 as described previously to allow antibiotic tagging of B. breve for subsequent culture studies [17]. In brief, B. breve UCC2003 was grown until mid-log phase, chilled on ice and washed twice with ice-cold sucrose citrate buffer (1 mM citrate, 0.5 M sucrose, pH 5.8) and then electroporation of cells was carried out under the following conditions: 25MF, 200Ohms, 2 kV. Transformed cells were incubated for 2 h in reinforced clostridial medium (RCM) at 37 °C in a controlled anaerobic chamber then plated [18] on RCM agar plates with selective antibiotics. Colonies were sub-cultured 3 times on RCM agar plates with selective antibiotics. Antibiotics were used at the following final concentrations erythromycin 2 μg/mL.

Lyophilised B. breve

B. breve was grown in De Man, Rogosa and Sharpe agar (MRS) under anaerobic conditions overnight. The bacterial cell pellet was resuspended in 10% milk powder and lyophilised in 200 mL quantities. Lyophilised B. breve was reconstituted with 500 μL PBS. Concentration of B. breve was 10^{10} CFU/mL. All batches were tested for contamination upon the reconstitution of Luria–Bertani (LB) and brain–heart infusion (BHI) plates under anaerobic and aerobic conditions at 37 °C. No contamination of B. breve was detected.

Mice

All mouse experiments were performed under the UK Regulation of Animals (Scientific Procedures) Act of 1986. The project license PDADA180C under which these studies were carried out was approved by the UK Home Office and the UEA Ethical Review Committee. All mice were housed in the Disease Modelling Unit at the University of East Anglia, UK. Animals were housed in a 12:12 h light/dark, temperature-controlled room and allowed food and water ad libitum (food/water intake was not recorded). Female germ-free C57BL/6J (GF) and specific pathogen free (SPF) mice aged 6–8 weeks were used for the study. GF mice were bred in germ-free isolators (2 females to 1 male) and on gestational day (GD) GD9.5, pregnant mice (confirmed by weight gain) were removed from the GF isolator and transferred to individually ventilated cages. The sterility of these cages was previously tested and found to be suitable for housing GF mice for 1 week. Sterile water was changed every 2 days. We assessed responses at 2 gestational phases—the majority of studies were carried out at GD16.5, whilst the RNASeq studies utilized fetal livers harvested at GD18.5. A total of 6 SPF mice were used for GD16.5 assessments (no SPF mice were studied on GD18.5). For the GF group, a total of 5 (GD16.5) and 3 (GD18.5) dams were used. For the BIF mice, a total of 6 (GD16.5) and 4 (GD18.5) dams were used.

B. breve colonisation levels

Mice were given 100 μL of reconstituted lyophilised B. breve UCC2003 by oral gavage (containing 10^{10} CFU/mL) at GD10, GD12 and GD14 or 100 μL vehicle control (PBS, 4% skimmed milk powder), with this dosing regimen reflecting a more realistic time frame for women who are more likely to take probiotics once their pregnancy is confirmed.
At GD16.5 and GD18.5, mice were sacrificed by cervical dislocation and samples collected for molecular and histological analysis. The experimental design can be found in Fig. 1A.

Faecal samples were checked for contamination and *B. breve* colonization at GD12 and GD14 and GD16. Briefly, faecal samples from GF and BIF mice were diluted in 500 μL of PBS and agitated for 30 min at 4 °C on an Eppendorf MixMate 5353 Digital Mixer Plate Shaker. The faecal solution was passed through a 0.45 μm syringe filter. Faecal solution was diluted 1 in 100 and 20 μL was added to a De Man, Rogosa and Sharpe agar plate with erythromycin and incubated for 48 h in an anaerobic chamber at 37 °C. Colony-forming units were counted using a click counter. In SPF animals housed in the same animal facility we have previously shown that *Bifidobacterium* represents ~1% of the total gut microbiota [19].

**Blood hormones and circulating metabolites**

Maternal blood was obtained by cardiac exsanguination immediately after cervical dislocation. Blood was centrifuged and serum collected and stored at −80 °C until further analysis. Blood glucose and serum concentrations of leptin, insulin, triglycerides, cholesterol, and free fatty acids were determined as previously reported [20]. Fetal blood glucose levels were measured with a handheld glucometer (One Touch Ultra; LifeScan) immediately after decapitation of the fetus (fetuses were selected at random).

**Placental histology**

Placentas were cut in half and fixed in 4% paraformaldehyde overnight at 4 °C. Samples were washed 3 times with PBS for 15 min each and storage in 70% ethanol until embedding in wax. Embedded placentas were cut at 5 μm thickness and stained with haematoxylin and eosin for gross morphology. Placental layer volume densities (labyrinth zone, junctional zone and decidua) were calculated using point counting and the Computer Assisted Stereological Toolbox (CAST v2.0) and converted to estimated volumes by multiplying by the estimated volume of the placenta. For analysis of labyrinth components, sections were stained with lectin for the identification of fetal endothelial vessels and with cytokeratin for trophoblasts. Further details of the double-labelling immunohistochemistry can be found elsewhere [21]. Structural analysis of the labyrinth was performed as previously described [22–24]. Briefly, fetal capillaries, maternal blood spaces and trophoblast volume densities were calculated with a point counting system in 16 random fields and their densities were then multiplied by the estimated volume of the labyrinth zone to obtain the estimated component volume. To estimate the surface density of the maternal-facing and fetal-facing interhaemal membranes, we recorded the number of intersection points along cycloid arcs in a total 20 random fields of view. Both interhaemal membrane surfaces were converted to absolute surface areas and the total surface area for exchange calculated by averaging the two absolute surface areas. Fetal capillary length densities were obtained using counting frames with two contiguous forbidden lines [24] and then converted to absolute capillary length by multiplying the volume of the labyrinth zone. Fetal capillary diameter was estimated using the equation; 

\[
d = 2 \times \text{mean area}/\pi^{1/2}
\]

The interhaemal membrane barrier thickness was determined using orthogonal intercepts and measuring the shortest distance between fetal capillaries and the closest maternal blood spaces at random starting locations (at least 99) within the labyrinth zone [24].

For the analysis of placental glycogen, sections were stained with Periodic acid–Schiff (Sigma-Aldrich) previous incubation with 0.5% periodic acid (Thermo Fisher Scientific). Sections were counterstained with Fast-green (Sigma-Aldrich) and digitalized with the nanozoomer scanner (Hamamatsu). Analysis of placental glycogen accumulation was performed with Image J and conducted blinded to experimental groups. TUNEL staining for placental cell death was performed using the TUNEL Assay Kit—HRP-DAB (Abcam, ab206386) following manufacturer instructions except for the counterstaining which was substituted for Nuclear Fast Red (Vector). Sections were digitalized using a nanozoomer scanner (Hamamatsu) and the amount of apoptosis in the labyrinth zone was calculated in 5 random areas (×20 magnification) and analysed by Image J software.

**Western blotting**

Protein extraction was performed with RIPA buffer as described previously [25]. Lysates were separated by SDS-PAGE and incubated with antibodies against p-MAPK (Thr202/Tyr204) (Cell Signalling, 4370; 1/1000), t-MAPK 44/42 (Cell Signalling, 4695; 1/1000), DLK-1 antibody (Abcam, ab21682; 1/1000), p-P38MAPK (Cell Signalling, 4511; 1/1000) and t-P38MAPK (Cell Signalling, 8690; 1/1000). Reactive bands were detected by chemiluminescence (Thermo Scientific, Scientific SuperSignal West Femto) and quantified by Image J software. Proteins were normalized to Ponceau S staining [26].

**RNA extraction and qPCR**

Extraction of RNA from micro-dissected placental labyrinth zones was performed with RNeasy Plus Mini Kit (Qiagen) and reverse transcribed using the High Capacity cDNA RT Kit minus RT inhibitor (Applied Biosystems) according to manufacturer’s instructions. Samples were analysed using...
### A) GF mouse under isolator conditions vs. GF mice housed in individually ventilated cages (IVC) for treatment

| Time   | GD0.5 | GD9.5 | GD10 | GD12 | GD14 | GD16.5 | GD18.5 |
|--------|-------|-------|------|------|------|--------|--------|
| Matings |       |       |      |      |      |        |        |
| Plug detection |       |       |      |      |      |        |        |
| Mice not manipulated |   |       |      |      |      |        |        |
| Confirmation of pregnancy |       |       |      |      |      |        |        |
| Mice moved out from isolators |   |       |      |      |      |        |        |
| Treatment B. breve (BIF) or vehicle |       |       |      |      |      |        |        |
| Treatment B. breve (BIF) or vehicle |       |       |      |      |      |        |        |
| Maternal data |   |       |      |      |      |        |        |
| Fetal growth |   |       |      |      |      |        |        |
| Placental analysis |   |       |      |      |      |        |        |
| Metabolomics |   |       |      |      |      |        |        |
| Fetal liver transcriptomics (RNA-seq) |   |       |      |      |      |        |        |
| Terminal procedures | | | | | | | |

### B) Viable fetuses per litter (n)

| Viable fetuses (n) | 15 | 800 | 500 | 200 | 50 | 0 |

### C) Fetal weight (mg)

| Fetal weight (mg) | 800 | 800 | 400 | 200 | 100 | 0 |

### D) Glucose (mmol/L)

| Glucose (mmol/L) | 5 | 4 | 3 | 2 | 1 | 0 |

### E) Brain weight (mg)

| Brain weight (mg) | 80 | 80 | 60 | 40 | 20 | 0 |

### F) Liver weight (mg)

| Liver weight (mg) | 80 | 80 | 60 | 40 | 20 | 0 |

### G) GF vs BIF fetal liver GD18.5

- **Up-regulated**
- **Down-regulated**

### H) Gene expression analysis

#### GO: Molecular Function
- **(4)**
- Oxygen carrier activity
- Gas transport
- Oxygen binding
- Molecular carrier activity
- Hemoglobin alpha binding
- Unsaturated monocarboxylic acid metabolic process

#### GO: Biological Process
- **(4)**
- Organic acid metabolic process
- Response to organic substance
- Small molecule metabolic process
- Response to other organism
- Response to stimulus
- Lipid metabolic process
- Carbohydrate metabolic process
- Response to chemical stimulus

#### REACTOME
- **(10)**
- Phase I
- Biological oxidation
- Xenobiotics metabolism
- Metabolism
- Cytoskeleton
- Cell cycle
Maternal gut microbiota *Bifidobacterium* promotes placental morphogenesis, nutrient...
phosphate buffer solution at 600 μL. The mixture was then moved to an NMR tube (Merck) for subsequent NMR analysis. Metabolites from culture media Brain Heart Infusion (BHI; Oxoid) and spent media (BHI cultured with \textit{B. breve} UCC2003 for 48 h) were extracted as follows: 400 μL of the medium was transferred into a sterile microcentrifuge tube with the addition of 200 μL faecal phosphate buffer and mixed well. The mixture was then moved to an NMR tube (Merck) for further NMR analysis.

Samples in NMR tubes were subsequently subjected to NMR spectroscopy. The $^1$H NMR spectra were recorded at 600 MHz on a Bruker AVANCE spectrometer (Bruker BioSpin GmbH, Germany) running Topspin 2.0 software. The metabolites were then quantified using the software Chenomx® NMR Suite 7.0™.

### Statistical analysis

All statistical analysis and sample size are shown in each figure/table and in the corresponding figure/table legends. Only samples from viable fetuses were analysed. No statistical analysis was used to pre-determine sample size and samples were assigned code numbers and, were possible, analysis was performed in a blinded fashion. Statistical calculations were performed using the GraphPad Prism software (GraphPad v9, San Diego, CA), SAS/STAT 9.0 (Statistical System Institute Inc. Cary, NC, USA) and RStudio Version 1.4.1106 (RStudio Boston, MA) with R Version 4.0.3 (Vienna, Austria). Data reported as mean ± SEM. Morphometric parameters of mother, litters were significantly different after correction for multiple comparisons using the dunnTest function in the FSA package. The level of significance for all statistical tests used in this study was set at $P < 0.05$. All figures in the manuscript show individual values (raw data). However, P values and mean ± SEM within the graphs analysed by the general linear mixed model were corrected for repeated measures. Graphs containing the individual dots and graphs with corrected mean ± SEM were generated with Graphpad and merged with Adobe Illustrator.

### Results

**Germ-free mice treated with \textit{B. breve} have altered body composition and caecum metabolic profile**

To assess whether maternal microbiota can influence feto-placental growth, GF mice were treated orally with \textit{B. breve} UCC2003 from day 10 of gestation (treatment on days 10, 12 and 14; i.e. BIF group), and compared to GF and SPF dams (for an experimental overview see Fig. 1A). Timing and dosing were based on the fact that levels of \textit{Bifdobacterium} rise throughout pregnancy [5] (colonization levels during pregnancy can be found in Figure S1). Previous work has indicated three consecutive doses of \textit{B. breve} UCC2003 facilitates stable gut colonization, with the advantage of also avoiding repeated handling of the mice, which may induce spontaneous abortions [28, 39]. In addition, from a translational point of view, we also wanted to correlate our animal model with potential future supplementation studies in women at the point pregnancy is confirmed.

Maternal body composition differed between groups with GF and BIF mice showing increased digestive tract weight and lower pancreas mass compared to SPF mice. GF and BIF mice had similar circulating concentrations of glucose and insulin to SPF mice (Table 1). Compared to SPF mice, treatment with \textit{B. breve} reduced maternal gonadal fat depot, liver, and spleen weights in BIF mice. No differences were observed in the circulating concentrations of leptin, cholesterol, triglycerides, or free fatty acids in maternal serum (Table 1).

Metabolomics analysis in maternal caecum samples indicated that the concentration of 13 out of 81 metabolites were significantly altered (Table 1 and Table S2). Acetate was influenced by \textit{B. breve} (Table 1), with BIF dams having intermediate concentrations compared to SPF and GF mice (the low levels of acetate detectable in GF mice, most likely originated from the diet and/or are host-derived). These findings suggest that acetate producing \textit{B. breve} and the wider gut microbiota may exert selective effects on maternal metabolic (gonadal fat depot and liver) and immune organs (spleen).
Maternal gut microbiota and *B. breve* regulate fetal growth by controlling fetal glycaemia and hepatic transcriptome

The three experimental groups had similar numbers of viable fetuses per litter, although GF and BIF groups showed a higher variability compared to the SPF group (Fig. 1B). Compared to SPF and BIF mice, GF fetuses were growth restricted, hypoglycaemic and had reduced liver weight, but had preserved brain size (Fig. 1C–E). As the liver is a key organ for glucose storage and production and fetuses from BIF mice had heavier livers and improved glycaemia, we next determined if there were changes in the fetal hepatic transcriptome (livers were collected from a small cohort of mice on GD18.5, when fetal liver function is particularly active prior to term. Indeed, mouse fetal hepatocytes are mature from GD18.5, when they present a similar gene expression pattern to those in the postnatal liver [40]). A total of 602 genes were differentially expressed, with 94 significantly up-regulated and 508 down-regulated genes in BIF group, when compared to GF group (Fig. 1F–H). Functional enrichment analysis indicated that pathways involved in haemoglobin and oxygen transport-binding were significantly upregulated in the fetal livers of BIF mice (Fig. 1I and Table S3).

### Table 1

|                      | SPF (n=6) | GF (n=5) | BIF (n=6) | SPF vs GF | SPF vs BIF | GF vs BIF |
|----------------------|-----------|-----------|-----------|-----------|------------|-----------|
| Hysterectomy weight (g) | 26.01 ± 0.91 | 27.87 ± 0.78 | 27.17 ± 0.80 | NS        | NS         | NS        |
| Digestive tract (g)   | 2.76 ± 0.03 | 6.83 ± 0.32 | 7.25 ± 0.63 | < 0.0001  | < 0.0001   | NS        |
| Caeccum (g)           | 0.66 ± 0.03 | 3.47 ± 0.25 | 3.96 ± 0.41 | < 0.0001  | < 0.0001   | NS        |
| Small intestine (g)   | 1.66 ± 0.03 | 2.65 ± 0.11 | 2.59 ± 0.14 | < 0.0001  | < 0.0001   | NS        |
| Pancreas (mg)         | 315.40 ± 30.12 | 183.40 ± 24.74 | 190.60 ± 38.71 | 0.041 | 0.044 | NS        |
| Gonadal fat (mg)      | 433.10 ± 43.20 | 297.0 ± 37.02 | 272.0 ± 27.35 | NS      | 0.016      | NS        |
| Liver (g)             | 2.09 ± 0.10 | 1.79 ± 0.05 | 1.55 ± 0.08 | NS        | 0.001      | NS        |
| Spleen (mg)           | 117.90 ± 2.80 | 91.76 ± 10.60 | 83.03 ± 6.72 | NS      | 0.012      | NS        |
| Glucose (mmol/L)      | 8.08 ± 0.78 | 8.38 ± 1.18 | 8.88 ± 0.74 | NS        | NS         | NS        |
| Insulin (µg/L)        | 0.12 ± 0.004 | 0.19 ± 0.05 | 0.20 ± 0.06 | NS        | NS         | NS        |
| Leptin (pg/mL)        | 2465 ± 177.1 | 2739 ± 486 | 2425 ± 303 | NS        | NS         | NS        |
| Cholesterol (mmol/L)  | 1.33 ± 0.03 | 1.56 ± 0.08 | 1.41 ± 0.09 | NS        | NS         | NS        |
| Triglycerides (mmol/L)| 1.54 ± 0.08 | 1.79 ± 0.14 | 1.50 ± 0.11 | NS        | NS         | NS        |
| Free Fatty Acids (µmol/L) | 890.6 ± 101.3 | 1440 ± 362 | 1092 ± 114.5 | NS      | NS         | NS        |

**Maternal caecum metabolites**

|                      | SPF (n=3) | GF (n=4) | BIF (n=5) | SPF vs GF | SPF vs BIF | GF vs BIF |
|----------------------|-----------|-----------|-----------|-----------|------------|-----------|
| Butyrate (mmol/kg)   | 12.47 ± 7.97 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.006 | 0.008 | NS        |
| Citrulline (mmol/kg) | 0.30 ± 0.06 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.006 | 0.008 | NS        |
| Fucose (mmol/kg)     | 0.08 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.006 | 0.008 | NS        |
| Isobutyrate (mmol/kg)| 0.41 ± 0.24 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.006 | 0.008 | NS        |
| Isovalerate (mmol/kg)| 0.09 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.006 | 0.008 | NS        |
| Malonate (mmol/kg)   | 0.09 ± 0.02 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.006 | 0.008 | NS        |
| Methylvamine (mmol/kg)| 0.05 ± 0.02 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.006 | 0.008 | NS        |
| Propionate (mmol/kg) | 4.48 ± 1.99 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.006 | 0.008 | NS        |
| Trimethylamine (mmol/kg)| 0.06 ± 0.007 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.006 | 0.008 | NS        |
| Valerate (mmol/kg)   | 0.57 ± 0.27 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.006 | 0.008 | NS        |
| 2.methylbutyrate (mmol/kg)| 0.05 ± 0.01 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.006 | 0.008 | NS        |
| 5.Aminopentanoate (mmol/kg)| 0.33 ± 0.14 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.006 | 0.008 | NS        |
| Acetate (mmol/kg)    | 35.49 ± 20.66 | 0.55 ± 0.06 | 3.22 ± 1.39 | 0.006 | 0.129 | 0.094     |

Body composition and metabolites/hormones in serum were analyzed by one-way ANOVA followed by Tukey multiple comparisons test. Metabolites in maternal caecum were analysed by Kruskal–Wallis test followed by multiple comparisons using the Benjamini & Hochberg false discovery rate method and Dunn's test. ROUT test was used for the identification of outlier values. The level of significance was set at *P*<0.05. NS: not significant. Data presented as mean±SEM. The number of dams used for each group is annotated on the table and only data from dams at day 16.5 of gestation were used.
contrast, many metabolic pathways were downregulated in response to *B. breve* administration, including carboxylic acid and lipid metabolic processes, steroid hydroxylase activity, fatty acid metabolism and response to glucocorticoid (Fig. 1H; Table S3). Therefore, maternal *B. breve* appears to exert changes in fetal hepatic function with implications for fetal growth.

**Maternal gut microbiota and *B. breve* control placental morphogenesis**

To further understand the links between the maternal gut microbiota and the regulation of fetal growth, we assessed placental structure (performed on GD16.5, when placental growth in mice is maximal [24]). When compared to SPF mice, placentas were lighter in GF and BIF mice (Fig. 2A). Placental efficiency, defined as the grams of fetus produced per gram of placenta, was significantly improved in the BIF group compared to GF mice (Fig. 2B). Analysis of placental compartments showed that a lack of maternal gut microbiota significantly hampered the growth of the placental labyrinth transport zone (Lz), without compromising the endocrine junctional zone or decidua volumes (Fig. 2C). It also did not affect placental glycogen storage (Fig. 2D) or the volume of the trophoblast (Fig. 2E–F). Analysis of maternal blood spaces revealed that GF and BIF groups had reduced spaces compared to SPF mice, while the volume and the length of fetal capillaries were significantly reduced in the GF compared to SPF (Fig. 2F–G). Similarly, the surface area for exchange of the Lz was significantly decreased in GF compared to SPF mice (Fig. 2H). The barrier between maternal and fetal blood was also determined to be thinner in BIF vs GF mice (Fig. 2I). Lz apoptosis levels were similar between groups (Fig. 2J).

To define the molecular mechanisms behind the changes in the Lz, we quantified the expression of select genes in

**Fig. 2** Effects of maternal gut microbiome and *B. breve* supplementation during pregnancy on placental structure on day 16.5 of gestation. **A** Placenta weight. **B** Placental efficiency determined by dividing fetal by placental mass. **C** Placental regional analysis. Scale bar = 1 mm. **D** Representative staining of placental glycogen with PAS and glycogen abundance. Scale bar=2.5 mm and 250 μm. **E** Representative image of lectin and cytokeratin staining for labyrinth zone structural quantification. Scale bar=500 μm and 50 μm. **F–I** Stereological parameters determined in placental labyrinth zone. **J** Representative image of TUNEL staining for apoptosis quantification in labyrinth zone. Scale bar=2.5 mm and 100 μm. All data were analyzed by a general linear mixed model, taking into account litter size as a covariate and taking each fetus as a repeated measure followed by Tukey multiple comparisons test. ROUT test was used for the identification of outlier values. Dots represent raw data (individual values). However, the statistical analysis and the mean±SEM reported within the graphs were obtained with the general linear mixed model (further explanations can be found in the Materials and Methods statistical analysis section). Placental weight-efficiency was obtained from: SPF (49 fetuses/6 dams), GF (33 fetuses/5 dams), BIF (34 fetuses/6 dams). Laboratorial analysis was performed with: SPF (14–15 placentas from 6 dams), GF (10 placentas from 5 dams) and BIF (9–11 placentas from 6 dams). Only placentas collected on day 16.5 of gestation were analysed. One to three placentas per litter were randomly selected and used for assessment. Placentas were analysed blind to the experimental groups. (NS, not significant; *P*<0.05; ***P*<0.001). D decidua, Jz junctional zone, Lz labyrinth zone, TB trophoblasts, FC fetal capillaries, MBS maternal blood spaces.
Maternal gut microbiota *Bifidobacterium* promotes placental morphogenesis, nutrient…

Micro-dissected Lz. The angiogenic factor *Vegf* was similarly expressed between groups (Fig. 3A). However, the expression of signalling pathways involved in cell proliferation and growth, namely the MAPK pathway, was significantly altered by changes in maternal gut microbiota; *Mapk1* was shown to be increased in both GF and BIF, while *Mapk14* (also known as *p38Mapk*) was revealed to be specifically up-regulated in the Lz of BIF mice. In addition, *Dlk1* and *Igf2P0*, which are key genes implicated in metabolism and Lz formation, were significantly up-regulated in the BIF group compared to GF mice. The expression of *Akt* did not vary between groups (Fig. 3A). As informed by western blotting, activation of ERK was reduced in the placental Lz of GF compared to SPF mice, and this effect was reversed and the mean±SEM reported within the graphs (for qPCR data) were obtained with the general linear mixed model (further explanations can be found in the Materials and Methods, statistical analysis section). Gene expression analysis was performed with: SPF (13 placentas from 6 dams), GF (11 placentas from 5 dams) and BIF (14 placentas from 6 dams). Protein quantification was performed with: SPF (4 placentas from 4 dams), GF (5 placentas from 5 dams) and BIF (5 placentas from 5 dams). Only placentas collected on day 16.5 of gestation were analysed. For qPCR, one to three placentas per litter were assessed and selection of the samples was conducted at random. For protein expression analysis, 1 placenta per litter was selected (NS, not significant; *P* < 0.05; **P** < 0.01; ****P** < 0.0001).
by BIF (Fig. 3B). p38MAPK protein activity was similar between groups. DLK1 protein level was also lower in GF compared to SPF mice. However, BIF increased DLK1 protein levels when compared to both SPF and GF mice (Fig. 3B). Overall, these findings suggest that the maternal gut microbiota, and *B. breve*, regulate the development of the mouse placental Lz via the modulation of specific cell growth and metabolic genes/pathways.

Maternal gut microbiota and *B. breve* controls key placental nutrient transporters

To better understand the changes in fetal growth and glycemia between groups, we quantified the expression of selected amino acid, glucose and lipid transporters in the Lz. We found no difference in the expression of system A amino acid transporters (*Slc38a1, Slc38a2, Slc38a4*) between groups (Fig. 3C). However, the key glucose transporter *Slc2a1* was up-regulated in the Lz of BIF mice compared to GF mice (*Slc2a3* mRNA levels were similar between groups; Fig. 3D). Fatty acid transporters were also altered, with increased levels of *Fatp1* in the GF group compared to SPF and BIF, while *Fatp4* was increased in the BIF group compared to the GF (Fig. 3E; *Cd36* and *Fatp3,6* expression levels were unaltered). Collectively, these data suggest that maternal gut microbiota, and *B. breve*, may regulate fetal growth by inducing changes in the expression of key nutrient transporters within the placenta.

Differences in placental labyrinth growth are linked to an altered placental metabolome

To gain a further mechanistic understanding of the changes observed in the placental Lz and fetal liver, we analysed > 80 metabolites at GD16.5 (Fig. 4 and Table S2). We found 5 metabolites significantly altered in the placental Lz (Fig. 4). 2-Aminoadipate was significantly increased in the Lz of both GF and BIF mice compared to SPF mice (Fig. 4A). Treatment with *B. breve* significantly reduced the concentrations of acetylcarnitine and carnitine in Lz tissue compared to SPF placenta, but not in fetal livers (Fig. 4B, C). Levels of formate in placental Lz were significantly elevated in both GF and BIF compared to SPF mice (Fig. 4D), with a similar trend (although not significant) in fetal liver samples. Acetate was also altered in the Lz (Fig. 4E), with concentrations significantly lower...
in the SPF compared to the GF group, whilst BIF samples showed intermediate levels (although these levels were much lower than observed in the maternal caecum). Similar to formate, concentrations of acetate in fetal liver followed similar directions to the Lz, yet were not statistically different between groups. These data suggest that maternal gut microbiota, and *B. breve*, regulate the fetal and placental growth via modulation of the placental Lz metabolome.

**Discussion**

In this study, we demonstrate that the maternal gut microbiota and the microbiota member *B. breve* regulate feto-placental growth. To the best of our knowledge, this is the first demonstration of a maternal gut bacterium remotely controlling placental structure and nutrient transporters, with important implications for fetal glycaemia and fetal growth. We observed that the effects of *Bifidobacterium* are partially mediated by altered metabolites in the maternal caecum and in placental Lz tissue, with alterations in the expression of key genes in the placental Lz and fetal liver.

*Bifidobacterium* is the dominant microbiota member in vaginally delivered, breast-fed infants, with certain species and strains known to stimulate and aid in maturation of the immune system [41]. *B. breve* UCC2003 also regulates responses at the gut barrier, inducing homeostatic epithelial cell programming, and protecting against inflammatory insults [28, 42]. Importantly, pregnancy is accompanied by increasing *Bifidobacterium* abundance in the gut of women and mice [5] and alterations in the abundance of *Bifidobacterium* are linked to the development of serious pregnancy complications like preeclampsia [43]. Recently, it has been demonstrated that the maternal gut microbiota regulate embryonic organ growth by promoting fetal neurodevelopment [44]. Our study shows that maternal gut microbiota induces changes in fetal organogenesis and that *B. breve* supplementation restored fetal glycaemia and liver weight. In this regard, fetal brain weight was unaltered in the three experimental groups, whilst liver mass was drastically reduced only in the GF group. Together, these results suggest that untreated GF fetuses prioritize the growth of the brain at expense of the liver. This fetal strategy, known as the ‘brain sparing effect’, is a protective mechanism to preserve oxygenation and nutrient delivery to the brain in situations of placental insufficiency [45–48]. Our RNAseq analysis shows an upregulation of genes involved in oxygen transport and haemoglobin binding, and downregulation of metabolic pathways such as steroid hydroxylase activity, carboxylic acid binding or fatty acid metabolism in the BIF group. These data, therefore, suggest that fetal defenses against growth retardation were better in the BIF group compared to the GF group and that the downregulation of the metabolic pathways could be due to the fact that BIF fetuses already achieved their hepatocyte maturation or their maximum liver growth potential earlier than the GF group. In fact, *B. breve* supplementation restored fetal glycaemia and weight, achieving similar values to that seen for SPF fetuses.

Previous in vivo studies show different strains of *Bifidobacterium* (including *B. breve*) modulate glucose handling [49], with this genus consistently associated with potential protection against human metabolic disorders e.g. type 2 diabetes [50, 51]. Our observations of reduced maternal gonadal fat mass and maternal liver weight in *B. breve*-treated dams compared to SPF dams, suggest that *Bifidobacterium*, or *B. breve* metabolites, could affect responses of key organs in the mother, and subsequently impact fetal resource allocation. *B. breve* UCC2003 appeared to induce changes in the metabolite milieu, including carnitine and acetate in the maternal caecum and/or placenta, which could be determinant of the effects observed on fetal growth. Carnitine is well known for mediating the transport of fatty acids into the mitochondrial matrix for fatty acid β-oxidation and BIF placentas had lower concentrations of acetylcarnitine and carnitine compared to SPF. These results suggest a potential greater reliance on these compounds for energy production or enhanced transfer of these fatty acids to the fetus [52]. On the other hand, acetate is a major bifidobacterial fermentation by-product, which directly mediates glucose homeostasis through the free fatty acid receptor 2 [53] and epithelial cell responses. Previous work in adult mice suggests that the elevation of gut acetate levels due to *Bifidobacterium* treatment plays a key role in regulating glucose handling systemically and reduces visceral fat accumulation [54]. Acetate also exerts systemic metabolic [55, 56] and immunological effects [57]. More generally, microbial-derived short-chain fatty acids (SCFAs) modulate multiple host physiological systems and during pregnancy are associated with maternal gestational weight, neonatal length and body weight, and protection against allergic airway disease in the developing fetus [58, 59]. Acetate crosses the placenta [59], so in our model, the elevated maternal *B. breve*-derived acetate may exert effects on feto-placental growth in three potential ways. First, higher maternal caecum acetate concentrations in SPF and *B. breve* supplemented dams vs GF dams could indicate maternal effects, through interactions within the maternal gut mucosa and subsequent impact on maternal organs (liver, adipose and spleen). Second, effects on the placenta, through the potential use of acetate for cellular metabolism, growth and function. Finally, effects on fetal metabolism following transport of acetate across the placenta to the fetus. Compared to the maternal caecum, levels of acetate were relatively low in the placental Lz and fetal liver (for all 3 groups). This suggests that *B. breve* (and SPF microbiota-derived acetate) may be used to support anabolic processes...
in utero (hence the very low levels detected). Moreover, the observed modulation of immune-associated pathways in the fetal liver, including those associated with G protein-coupled receptor signalling (e.g. *Dusp9*), also indicates a role in direct acetate-associated responses [60]. Further work is required to fully understand the mechanisms behind the differences observed in maternal organs between the SPF and BIF groups (liver, adipose and spleen) and how these changes impact materno-fetal resource allocation. Thus, future studies should assess the ontogeny of these changes and incorporate an additional pregnant SPF group treated with *B. breve* to fully understand the chemical, endocrine and metabolic interactions occurring between *B. breve*, maternal organs (gut, liver, adipose and spleen) and fetal metabolism.

Administration of *B. breve* significantly reduced the interhaemal membrane barrier thickness of the placenta (compared to GF group), which may facilitate the exchange of nutrients and gases. Previous work has shown that the barrier thickness is regulated by *Igf2P0*, as *Igf2P0* knockout mice have increased thickness of the exchange barrier and reduced passive permeability of the placentas [61]. In our case, *Igf2P0* was significantly elevated in the BIF group compared to the SPF and GF groups and although in vivo functional assays evaluating the passive and active transport of solutes are required to verify the implications of this effect on fetal nutrient allocation (e.g. performing unidirectional maternal–fetal transfer assays using $^{51}$Cr-EDTA or glucose and amino acid analogues, $^3$H-MeG or $^{14}$C-MeAIB [23, 61, 62]), this result explains, in part, the improvement in fetal weight observed in the BIF group. Moreover, IGFs have been implicated in the regulation of glucose transporters in a variety of organs by utilizing signalling pathways such as PI3K/AKT and MAPK [63], and among the different nutrient transporters evaluated, *Slc2a1* mRNA levels were significantly elevated in the BIF group compared to GF group. The other two transporters that were altered, *Fatp1* and *Fatp4*, changed in opposite directions suggesting that *B. breve* could modulate the expression of these two transporters in different ways depending on the direction and magnitude of fatty acid flux at the placental Lz [64, 65]. The divergent expression of *Fatp1* and *Fatp4* in the BIF compared to the GF group may also be linked to intracellular carnitine utilization, as *Fatp1* can interact with carnitine palmitoyltransferase 1 to promote fatty acid transport into mitochondria [66].

Maternal gut microbiota affected placental structure and its vascular bed, which is required for adequate fetal growth [23]. The mechanisms governing these structural changes could be partially mediated by changes in the expression of Igf2P0 and Dlk1 (two important imprinted genes in placental physiology [67]), and via MAPK upregulation. In addition to the changes above described for barrier thickness, deletion of *Igf2P0* results in fetus-placental growth restriction in association with reduced placental surface area for exchange and fetal capillary volume (reviewed by [68]); parameters that were significantly affected in the SPF vs GF groups, and partially restored by *B. breve* administration. Dlk1, a non-canonical ligand of the Notch signalling pathway localized to the endothelial cells of fetal capillaries in the placental Lz, regulates placental vascularisation and branching morphogenesis [69] and both, IGF2 and DLK1, can mediate cellular actions via the MAPK pathway [70–72]. We observed no differences in the mRNA levels of Dlk1 between SPF and GF mice. However, at the protein level, DLK1 in the Lz was controlled by the maternal gut microbiota and more specifically by *B. breve*. Similarly, *Mapk1* levels were increased in both GF and BIF groups compared to the SPF, but at the protein level, ERK activity was lower in the GF but not BIF group when compared to the SPF group, suggesting once again that *B. breve* is involved in the regulation of DLK-MAPK signaling. Another important signaling pathway for embryogenesis and placental Lz angiogenesis and vascular remodeling is p38MAPK (encoded by the gene *Mapk14*) [73]. This pathway has been linked to environmental stresses and inflammatory cytokines [74]. However, p38MAPK also regulates many normal cellular processes, including proliferation and cytoskeletal organisation. We observed that exposure to *B. breve* increases the mRNA abundance of *Mapk14* and carnitine, a metabolite that was found to be altered in the Lz of the BIF group compared to the SPF, group, can also promote p38MAPK signalling activation in cardiac tissue [75]. Taken together, our findings reveal that (1) maternal gut microbiota promotes fetal and placental growth in mice and (2) *B. breve* UCC2003 treatment may link to the altered metabolites/nutrient milieu in the mother, affecting placental nutrient transporter abundance and placental barrier thickness for exchange, with effects on fetal growth and development (when compared to GF).

**Limitations of the study**

While our study has clear strengths and strong translational implications for pregnancy complication treatments, it has limitations that are important to consider as they impact the conclusions drawn. First, our study only addresses the effects of *B. breve* UCC2003 in a completely clean and naive microbiome system (GF model). This is not representative of the human gut environment, and therefore future experiments could include the addition of a SPF group treated with *B. breve* UCC2003 and also a similar group treated with another probiotic species (e.g. *Lactobacillus acidophilus*), or a combination of species. This would help to define *Bifidobacterium*-specific effects (driven by key metabolites), including their efficacy, safety and potential use of...
probiotics during pregnancy. Moreover, it could be argued that the SPF group interferes in the interpretations of the \textit{B. breve} effects. However, there is a lack of fundamental knowledge on what is considered “normal or abnormal” in the GF system, as very little research has been done in understanding the role of the maternal gut microbiota on placental development (SPF vs GF). Therefore, the addition of the SPF group is required to define a baseline for adequate feto-placental growth, and it would also be important to understand how reconstitution of GF mice with SPF microbiota also modulates these responses. As previously mentioned, future work should evaluate the response of pregnant SPF mice to \textit{B. breve} UCC2003 supplementation (using microbial profiling [e.g. shotgun metagenomics] to follow microbiota changes), as well as the efficiency of \textit{B. breve} UCC2003 using other types of mouse models such as antibiotic-treated mice. These animal models may also reduce issues with the immune naïve physiology of the GF system [8]. Unsurprisingly, we did not see a full ‘rescue’ of placental phenotype in the monocolonised GF \textit{B. breve} (BIF) group, compared to the complex microbiota found in SPF dams. However, structural and functional adaptations of these placentas exposed to \textit{B. breve} were adequate enough to ‘rescue’ fetal weight and fetal glycaemia. An array of gut-associated signaling and a diverse metabolite pool are expected to provide more complete placental development. Indeed, other or additional \textit{Bifidobacterium} species and/or strains may be required for placental and fetal development, given strain-specific host physiology responses [42, 76]. Further studies should allow the relative contributions of other microbial- and \textit{Bifidobacterium}-derived factors to be elucidated. Moreover, ideally, future work should analyze fetal and placental growth each day of the supplementation period and use larger cohorts of pregnant mice. It would also be valuable to assess the impact of \textit{B. breve} supplementation from prior to, and/or during the whole pregnancy.

Exploring three different compartments (i.e. mother, placenta and fetus) with respect to metabolites and elucidating their role is a complex process, and makes interpretations and drawing definitive conclusions difficult. Further studies using e.g. 13C labeled \textit{Bifidobacterium} or specific metabolites for tracking experiments may allow more nuanced

### Fig. 5
Summary illustration showing the most relevant results on how the maternal gut microbiota and \textit{B. breve} affects mother, placenta and fetus during gestation. The effects of lacking maternal gut microbiota on maternal, placental and fetal phenotype are shown in red circles (SPF vs GF comparisons). Our results suggest that lacking maternal gut microbiota aside from inducing changes in the maternal digestive tract, pancreas and caecum metabolites, has important implications for the correct growth of the fetus and its placenta. The effects of \textit{B. breve} administration compared to the SPF and GF groups are shown in blue and red arrows, respectively. Overall, \textit{B. breve} induces changes in the maternal compartment that affect the structure, metabolome and function of the placenta in association with alterations in fetal metabolism, growth and hepatic transcriptome. SPF specific-pathogen-free mouse, GF germ-free mouse, BIF germ-free mouse treated with \textit{B. breve} UCC2003, Lz labyrinth zone, MBS maternal blood spaces, FC fetal capillaries, SA surface area for exchange, BT barrier thickness, DEG differentially expressed genes.
interactions to be uncovered in future work. Nonetheless, this study has revealed novel roles for the gut microbiota and specifically *Bifidobacterium* and provides the bases for future therapeutic strategies for treating pregnancy complications. These data suggest an opportunity for in utero programming through maternal *Bifidobacterium* and associated metabolites. Overall, although our study was performed in mice and is not representative of a clinical scenario, our study highlights the importance of the maternal gut microbiota during gestation and demonstrates that *B. breve* modulates maternal physiology, placental structure and nutrient transporter capacity with an impact on fetal glycaemia and fetal growth (Fig. 5). Our findings prompt an in-depth investigation into how additional members of the maternal gut microbiota impact on pregnancy outcomes. These future studies are important for the design of novel therapies to combat fetal growth restriction and other pregnancy complications.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00018-022-04379-y.

Acknowledgements Authors would like to thank Dr Ruben Bermejo-Poza (Complutense University of Madrid) for statistical advice and the Ferguson-Smith laboratory (University of Cambridge) for providing the DLK1 antibody.

Author contributions JL-T, ZS, ANS-P, LJH designed research; JL-T, ZS, RK, GLG conducted research, JL-T, ZS, RK, MJD contributed analytic tools and performed analysis; DvS contributed reagents; JL-T, ZS, ANS-P, LJH wrote the paper with feedback from all the authors.

Funding JL-T currently holds a Sir Henry Wellcome Postdoctoral Fellowship (220456/Z/20/Z) and previously a Newton International Fellowship from the Royal Society (NF170988 / RG90199). LJH is supported by Wellcome Trust Investigator Awards (100974/C/13/Z and 220876/Z/20/Z); the Biotechnology and Biological Sciences Research Council (BBRC), Institute Strategic Programme Gut Microbes and Health (BB/R012490/1), and its constituent projects BBS/E/F/000PR10353 and BBS/E/F/000PR10356. ANS-P is supported by a Lister Institute of Preventive Medicine Research Prize (RG93692).

Data availability The fetal liver RNA-Seq raw sequencing data are deposited at the National Center for Biotechnology Information (NCBI) under BioProject PRJNA748000. Relevant data are within the manuscript, individual figures and its Supporting Information files. Additionally, data are available from the corresponding authors on reasonable request. Scripts for differential gene expression analysis can be accessed at GitHub, https://github.com/raymondkiu/Maternal-foetal-microbiota-paper/.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate All mouse experiments were performed under the UK Regulation of Animals (Scientific Procedures) Act of 1986. The project license PDADA1B0C under which these studies were carried out was approved by the UK Home Office and the UEA Ethical Review Committee.

Consent for publication For the purpose of open access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, and indicate if changes were made otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Krajmalnik-Brown R, Ilhan Z-E, Kang D-W, DiBaise JK (2012) Effects of gut microbes on nutrient absorption and energy regulation. Nutr Clin Pract 27:201–214. https://doi.org/10.1177/088433611436116
2. McDonald B, McCoy KD (2019) Maternal microbiota in pregnancy and early life. Science 365:984–985. https://doi.org/10.1126/science.aay0618
3. de Agüiero MG, Ganal-Vonarburg SC, Fuhrer T et al (2016) The maternal microbiota drives early postnatal innate immune development. Science 351:1296–1302. https://doi.org/10.1126/science.aad2571
4. Koren O, Goodrich JK, Cullender TC et al (2012) Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 150:470–480. https://doi.org/10.1016/j.cell.2012.07.008
5. Nuriel-Ohayon M, Neuman H, Ziv O et al (2019) Progesterone increases bifidobacterium relative abundance during late pregnancy. Cell Rep 27:730-736.e3. https://doi.org/10.1016/j.celrep.2019.03.075
6. Naso T, Yong H, Lopez-Tello J, Sferruzzi-Perri AN (2018) The role of placental hormones in mediating maternal adaptations to support pregnancy and lactation. Front Physiol. https://doi. org/10.3389/fphys.2018.01091
7. Lamoussé-Smith ES, Tseng A, Starnbach MN (2011) The intestinal flora is required to support antibody responses to systemic immunization in infant and germ free mice. PLoS ONE 6:11116. https://doi.org/10.3389/fphys.2018.01534
8. Kennedy EA, King KY, Baldridge MT (2018) Mouse microbiota models: comparing germ-free mice and antibiotics treatment as tools for modulating gut bacteria. Front Physiol 9:1534. https://doi. org/10.3389/fphys.2018.01534
9. Lu J, Synowiec S, Lu L et al (2018) Microbiota influence the development of the brain and behaviors in C57BL/6d mice. PLoS ONE 13:e0201829. https://doi.org/10.1371/journal.pone.0201829
10. Martin AM, Yabut JM, Choo JM et al (2019) The gut microbiome regulates host glucose homeostasis via peripheral serotonin. PNAS 116:19802–19804. https://doi.org/10.1073/pnas.1909311116
11. Faas MM, Liu Y, Borghuis T et al (2019) Microbiota induced changes in the immune response in pregnant mice. Front Immunol 10:2976. https://doi.org/10.3389/fimmu.2019.02976
12. Pokusaeva K, Fitzgerald GF, van Sinderen D (2011) Carbohydrate metabolism in bifidobacteria. Genes Nutr 6:285–306. https://doi.org/10.1007/s12263-010-0206-6
13. Turroni F, Ventura M, Buttò LF et al (2014) Molecular dialogue between the human gut microbiota and the host: a Lactobacillus and Bifidobacterium perspective. Cell Mol Life Sci 71:183–203. https://doi.org/10.1007/s00018-013-1318-0
14. Turroni F, Milani C, Duranti S et al (2018) Bifidobacteria and the infant gut: an example of co-evolution and natural selection. Cell Mol Life Sci 75:103–118. https://doi.org/10.1007/s00018-017-2672-0
15. James K, O’Connell Motherway M, Penno C et al (2018) *Bifidobacterium breve* UCC2003 employs multiple transcriptional regulators to control metabolism of particular human milk oligosaccharides. Appl Environ Microbiol 84:e02774-e2817. https://doi.org/10.1128/AEM.02774-17
16. Food and Agriculture Organization of the United Nations, World Health Organization (2006) Probiotics in food: health and nutritional properties and guidelines for evaluation. Food and Agriculture Organization of the United Nations: World Health Organization, Rome.
17. Mázé A, O’Connell-Motherway M, Fitzgerald GF et al (2007) Identification and characterization of a fructose phosphotransferase system in *Bifidobacterium breve* UCC2003. Appl Environ Microbiol 73:545. https://doi.org/10.1128/AEM.01496-06
18. Cronin M, Akin AR, Collins SA et al (2012) High Resolution in vivo bioluminescent imaging for the study of bacterial tumour targeting. PLoS ONE 7:e30940. https://doi.org/10.1371/journal.pone.0030940
19. Hughes KR, Schofield Z, Dalby MJ et al (2020) The early life microbiota protects neonatal mice from pathological small intestinal epithelial cell shedding. FASEB J 34:7075–7088. https://doi.org/10.1096/fj.20200042R
20. Musial B, Fernandez-Twinn DS, Vaughan OR et al (2016) Proximity to delivery alters insulin sensitivity and glucose metabolism in pregnant mice. Diabetes 65:851–860. https://doi.org/10.2337/db15-1531
21. De Clercq K, Lopez-Tello J, Vriens J, Sferruzzi-Perri AN (2020) Double-label immunohistochemistry to assess labyrinth structure of the mouse placenta with stereology. Placenta 94:44–47. https://doi.org/10.1016/j.placenta.2020.03.014
22. Sferruzzi-Perri AN, López-Tello J, Fowden AL, Constancia M (2016) Maternal and fetal genomes interplay through phosphoinositol 3-kinase (PI3K)-p110α signaling to modify placental resource allocation. Proc Natl Acad Sci 113:11255–11260. https://doi.org/10.1073/pnas.1602012113
23. López-Tello J, Pérez-García V, Khaira J et al (2019) Fetal and trophoblast PI3K p110α have distinct roles in regulating resource supply to the growing fetus in mice. Elife. https://doi.org/10.7554/eLife.45282
24. Coan PM, Ferguson-Smith AC, Burton GJ (2004) Developmental dynamics of the definitive mouse placenta assessed by stereology. Biol Reprod 70:1806–1813. https://doi.org/10.1095/biolreprod.103.024166
25. Salazar-Petres E, Carvalho DP, Lopez-Tello J, Sferruzzi-Perri AN (2021) Placental mitochondrial function, nutrient transporters, metabolic signalling and steroid metabolism relate to fetal size and sex in mice
26. Romero-Calvo I, Ocón B, Martínez-Moya P et al (2010) Reversible Poncette staining as a loading control alternative to actin in Western blots. Anal Biochem 401:318–320. https://doi.org/10.1016/j.ab.2010.02.036
27. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression using real-time quantitative PCR and the 2(-ΔΔC(T)) method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262
28. Kiu R, Treveil A, Harnisch LC et al (2020) *Bifidobacterium breve* UCC2003 induces a distinct global transcriptomic program in neonatal murine intestinal epithelial cells. Science 23:101336. https://doi.org/10.1126/sci.2020.101336
29. Chen S, Zhou Y, Chen Y, Gu J (2018) fastq: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:884–889. https://doi.org/10.1093/bioinformatics/bty560
30. Kopylova E, Noël L, Touzet H (2012) SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. Bioinformatics 28:3211–3217. https://doi.org/10.1093/bioinformatics/bts611
31. Bray NL, Pimentel H, Melsted P, Pachter L (2016) Near-optimal probabilistic RNA-seq quantification. Nat Biotechnol 34:525–527. https://doi.org/10.1038/nbt.3519
32. Zerbino DR, Achuthan P, Akann W et al (2018) Ensembl 2018. Nucleic Acids Res 46:D754–D761. https://doi.org/10.1093/nar/gkx1098
33. Pimentel H, Bray NL, Puente S et al (2017) Differential analysis of RNA-seq incorporating quantification uncertainty. Nat Methods 14:687–690. https://doi.org/10.1038/nmeth.4324
34. Kinsella RJ, Kähäri A, Haider S et al (2011) Ensembl BioMarts: a hub for data retrieval across taxonomic space. Database (Oxford) 2011:bar030. https://doi.org/10.1093/database/bar030
35. Raudvere U, Kolberg L, Kuzmin I et al (2019) g:Profiler: a web server for functional enrichment analysis and conversions of gene lists (2019 update). Nucleic Acids Res 47:W191–W198. https://doi.org/10.1093/nar/gkx369
36. Le Gall G (2015) Sample collection and preparation of biofluids and extracts for NMR spectroscopy. Methods Mol Biol 1277:15–28. https://doi.org/10.1007/978-1-4939-2377-9_2
37. Wu J, An Y, Yao J et al (2010) An optimised sample preparation method for NMR-based faecal metabolic analysis. Analyst 135:1023–1030. https://doi.org/10.1039/b927543f
38. Lazic SE, Essioux L (2013) Improving basic and translational science by accounting for litter-to-litter variation in animal models. BMC Neurosci 14:37. https://doi.org/10.1186/1471-2202-14-37
39. Fanning S, Hall LJ, Cronin M et al (2012) Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. PNAS 109:2108–2113. https://doi.org/10.1073/pnas.1115621109
40. Single-cell RNA-Seq analysis reveals dynamic trajectories during mouse liver development—PubMed. https://pubmed.ncbi.nlm.nih.gov/29202695/. Accessed 27 Jan 2022
41. Dalby MJ, Hall LJ (2020) Recent advances in understanding the neonatal microbiome. F1000Res 9:F1000 Faculty Rev-422. https://doi.org/10.12688/f1000research.22355.1
42. Hughes KR, Harnisch LC, Alcorn-Giner C et al (2017) *Bifidobacterium breve* reduces apoptotic epithelial cell shedding in an exopolysaccharide and MyD88-dependent manner. Open Biol 7:160155. https://doi.org/10.1098/rsob.160155
43. Miao T, Yu Y, Sun J et al (2021) Decrease in abundance of bacteria of the genus Bifidobacterium in gut microbiota may be related to pre-eclampsia progression in women from East China. Food Nutr Res. https://doi.org/10.29219/fnr.v65.i7
44. Vuong HE, Pronovost GN, Williams DW et al (2020) The maternal microbiome modulates fetal neurodevelopment in mice. Nature 586:281–286. https://doi.org/10.1038/s41586-020-2745-3
45. Godfrey KM, Haugen G, Kiserud T et al (2012) Fetal liver blood flow distribution: role in human developmental strategy to prioritize fat deposition versus brain development. PLoS ONE 7:e41759. https://doi.org/10.1371/journal.pone.0041759
46. López-Tello J, Arias-Álvarez M, Jiménez-Martínez M-Á et al (2021) The effects of sildenafil citrate on feto-placental development and haemodynamics in a rabbit model of intrauterine growth restriction. Reprod Fertil Dev 29:1239–1248. https://doi.org/10.1071/RF13530
47. López-Tello J, Arias-Alvarez M, Jimenez-Martinez MA et al (2017) Competition for materno-fetal resource partitioning in a rabbit model of undernourished pregnancy. PLoS ONE 12:e0169194. https://doi.org/10.1371/journal.pone.0169194
48. Giussani DA (2016) The fetal brain sparing response to hypoxia: physiological mechanisms. J Physiol (Lond) 594:1215–1230. https://doi.org/10.1113/jp271099
49. Kikuchi K, Ben Othman M, Sakamoto K (2018) Sterilized bifidobacteria suppressed fat accumulation and blood glucose level. Biochem Biophys Res Commun 501:1041–1047. https://doi.org/10.1016/j.bbrc.2018.05.105
50. Wu H, Esteve E, Tremaroli V et al (2017) Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nat Med 23:850–858. https://doi.org/10.1038/nm.4345
51. Solito A, Bozzi Cionci N, Calgaro M et al (2021) Supplementation with Bifidobacterium breve BR03 and B632 strains improved insulin sensitivity in children and adolescents with obesity in a cross-over, randomized double-blind placebo-controlled trial. Clin Nutr 40:4585–4594. https://doi.org/10.1016/j.clnu.2021.06.002
52. Sferruzzi-Perri AN, Higgins JS, Vaughan OR et al (2019) Placental mitochondria adapt developmentally and in response to hypoxia to support fetal growth. Proc Natl Acad Sci USA 116:1621–1626. https://doi.org/10.1073/pnas.1816056116
53. Fuller M, Priyadarshini M, Gibbons SM et al (2015) The short-chain fatty acid receptor, FFA2, contributes to gestational glucose homeostasis. Am J Physiol Endocrinol Metab 309:E840–E851. https://doi.org/10.1152/ajpendo.00171.2015
54. Aoki R, Kamikado K, Suda W et al (2017) A proliferative probiotic Bifidobacterium strain in the gut ameliorates progression of metabolic disorders via microbiota modulation and acetate elevation. Sci Rep 7:43522. https://doi.org/10.1038/srep43522
55. Fukuda T, Toh H, Hase K et al (2011) Bifidobacteria can protect against enteropathogenic infection through production of acetate. Nature 469:543–547. https://doi.org/10.1038/nature09646
56. González Hernández MA, Canfora EE, Jocken JWE, Blaak EE (2017) Bifidobacterium breve suppressed fat accumulation and blood glucose level. Biochem Biophys Res Commun 501:1041–1047. https://doi.org/10.1016/j.bbrc.2018.05.105
57. López-Tello J, Arias-Alvarez M, Jimenez-Martinez MA et al (2017) Competition for materno-fetal resource partitioning in a rabbit model of undernourished pregnancy. PLoS ONE 12:e0169194. https://doi.org/10.1371/journal.pone.0169194
58. Priyadarshini M, Thomas A, Reisetter AC et al (2014) Maternal irradiation. Eur J Pharmacol 804:7–12. https://doi.org/10.1016/j.ejphar.2017.04.003
59. Ruiz L, Delgado S, Ruas-Madiedo P et al (2017) Bifidobacteria and their molecular communication with the immune system. Front Microbiol 8:2345. https://doi.org/10.3389/fmicb.2017.02345

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.