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Research Article

Maternal metabolic, immune, and microbial systems in late pregnancy vary with malnutrition in mice†

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Abstract

Malnutrition is a global threat to pregnancy health and impacts offspring development. Establishing an optimal pregnancy environment requires the coordination of maternal metabolic and immune pathways, which converge at the gut. Diet, metabolic, and immune dysfunctions have been associated with gut dysbiosis in the nonpregnant individual. In pregnancy, these states are associated with poor pregnancy outcomes and offspring development. However, the impact of malnutrition on maternal gut microbes, and their relationships with maternal metabolic and immune status, has been largely underexplored. To determine the impact of undernutrition and overnutrition on maternal metabolic status, inflammation, and the microbiome, and whether relationships exist between these systems, pregnant mice were fed either a normal, calorically restricted (CR), or a high fat (HF) diet. In late pregnancy, maternal inflammatory and metabolic biomarkers were measured and the cecal microbiome was characterized. Microbial richness was reduced in HF mothers although they did not gain more weight than controls. First trimester weight gain was associated with differences in the microbiome. Microbial abundance was associated with altered plasma and gut inflammatory phenotypes and peripheral leptin levels. Taxa potentially protective against elevated maternal leptin, without the requirement of a CR diet, were identified. Suboptimal dietary conditions common during pregnancy adversely impact maternal metabolic and immune status and the microbiome. HF nutrition exerts the greatest pressures on maternal microbial dynamics and inflammation. Key gut bacteria may mediate local and peripheral inflammatory events in response to maternal nutrient and metabolic status, with implications for maternal and offspring health.

Summary Sentence

Malnutrition alters immune and metabolic systems in pregnancy, and gut microbes may mediate the maternal response to these suboptimal dietary exposures.

Key words: developmental origins of health and diseases, immunology, metabolism, nutrition, pregnancy.

Introduction

Health and nutrition are unquestionably linked. For much of the developed world, nutrient intake trends are increasing, in parallel with increased rates of obesity and diabetes [1] and excessive weight gain in pregnancy [2, 3]. Yet food insecurity continues to be a problem worldwide, notably affecting children and women of reproductive age [3, 4]. Poor maternal nutrition (both undernutrition and overnutrition) and metabolic status before and during pregnancy have profound effects on the health of the pregnancy and fetal development [5, 6], adversely impact maternal health postpartum, and program disease risk in the next generation [6, 7].

During normal pregnancy, maternal metabolic and immune status is profoundly altered. In early pregnancy, the mother increases her adipose tissue deposition [8] whilst late pregnancy is characterized by an insulin resistant state [9] to provide resources for the developing fetus and placenta. Inflammatory pathways are also activated in normal pregnancy [10, 11]. However, mothers entering pregnancy overweight/obese are already in a state of decreased insulin sensitivity and increased inflammation [12], which may have adverse consequences including earlier and greater substrate delivery to the fetus [13, 14].

Recently, gut microbes have been implicated in pregnancy-associated metabolic changes, promoting weight gain and insulin insensitivity in the third trimester [15]. Gut microbes from women in late pregnancy, naturally a time of increased inflammation [10, 11], have also been shown to induce a slight inflammatory response in germ-free mice [15]. Moreover, inflammatory conditions associated with altered gut microbial communities [16] result in adverse outcomes in pregnancy [17, 18]. Gut microbiota may modify permeability of key cellular barriers [19, 20] and it is estimated that up to one third of blood metabolites are of bacterial origin [21, 22], suggesting that bacteria are participants in the crosstalk between blood-borne factors and local tissues, and that metabolic and immune signals regulating host physiology may converge at the gut. If microbes also modify maternal blood metabolites, and these signals target not only maternal tissues but also those of the placenta and fetus, we must consider that the maternal gut microbiome represents another genome (in addition to the maternal and fetal genomes) orchestrating the immune and metabolic changes that occur in maternal and intrauterine tissues with normal pregnancy.

Diet, metabolic, and inflammatory status in the nonpregnant state play dominant roles in shaping the gut microbial community [16, 23, 24]. Inflammation at local sites such as the gut may influence peripheral immune and metabolic signals with effects on metabolic function and bodyweight, likely mediated through the gut bacteria [25]. Pregravid and/or antenatal changes in maternal nutrition or metabolic status may alter these host–microbe relationships, provoke undesirable pregnancy outcomes, and shift offspring developmental trajectories. Yet studies examining nutrition during pregnancy and its effects on the maternal gut microbiome are lacking, despite the prevalence of increased caloric intakes in reproductive aged and pregnant women, and even the persistent problem of undernourishment globally.

To address this complexity, we sought to determine the extent to which inadequate nutrition, including undernutrition and excessive caloric intake/high fat diet, would alter maternal metabolic status, inflammation, and the microbiome in late pregnancy, and may suggest a role for interactions between the nutritional environment, host, and microbes, in shaping maternal physiology. We hypothesized that mothers who were undernourished or overnourished during pregnancy would have a different composition of their gut microbiomes at the end of pregnancy compared to normally nourished controls, and that these differences would be associated with changes in levels of key metabolic and inflammatory biomarkers. Furthermore, we asked whether we could identify bacterial taxa that may have the potential to be protective against adverse effects of suboptimal maternal nutrition.

Materials and methods

Animal model

Ethical approval was obtained from the Animal Care Committee at Mount Sinai Hospital. In brief, male and female C57BL/6 mice (Jackson Laboratories) were housed in a single room under constant temperature of 25°C with a 12:12 light-dark cycle and free access to food and water. Females were randomized to one of three nutritional groups (n = 5/group): (1) mice fed a control chow diet (Dustless Precision Pellets S0173, BioServe, Frenchtown, NJ, USA; 23.4% saturated fat by weight) ad libitum before mating and throughout pregnancy (CON); (2) *or* mice fed a chow diet ad libitum before mating and until day 5.5 of gestation, and then calorically restricted by 30% of controls from gestational days 5.5–17.5, after which females were fed control chow ad libitum for the remainder of the study (CR; to induce weight faltering in mothers and growth restriction in fetuses [26]); (3) *or* mice fed a high fat diet (60% kcal as fat, 37.1% saturated fat by weight; D12492, Research Diets, New Brunswick, NJ, USA) ad libitum from 8 weeks before mating and throughout pregnancy (HF; to reflect increased maternal adiposity seen in maternal overweight/obesity). All breeding males were fed control chow diet ad libitum. Power calculations were performed based on experimental data from an independent animal study with diet interventions [27]. Calculations were conducted using the observed shifts in the microbial taxa to determine an expected range of effect sizes. A minimum of four animals per group were determined to be required to achieve the minimum effect sizes that were associated with statistically significant shifts in the complex microbiome community. Stage of estrous was assessed in females by probing and assessing the vaginal smear. Females with vaginal smears that predominantly contained cornified epithelial cells, typically indicating estrus, were housed with a male overnight only. Mating occurred at approximately 10 weeks of age and was confirmed by the presence of a vaginal sperm plug the following morning (E0.5; term = 19 days), at which time pregnant females were housed individually in cages with free access to water and their respective diets. Dams were weighed and food intakes recorded weekly before pregnancy and daily during pregnancy.

Biospecimen collection

At E18.5, dams were killed by cervical dislocation. Immediately following, tail glucose was measured using a commercial glucose meter (Roche Accucheck), dams were decapitated, and trunk blood was collected into heparin-coated tubes for plasma collection. Fetuses were removed from the uterus; fetal number, sex, resorptions and anthropometric measures were determined; and fetal and tissues were collected. Fetal sex was confirmed by PCR assay [28].

The maternal gastrointestinal tract (GIT) from stomach to rectum was dissected from the peritoneal cavity. The small intestine (SI) was isolated from the rest of the GIT by dissecting it from 5 mm from below the stomach to 5 mm above the cecum; cleaned

of fat, mesentery, and Peyer's patches; and flushed until devoid of all undigested matter. SI tissue was used for the isolation of SI intraepithelial lymphocytes (SI IEL) as described in the supplementary methods. The cecum was dissected from the remaining portion of the ileum at the ileocecal valve and proximal colon, and cecal contents were carefully extruded into a sterile tube and flash frozen in liquid nitrogen, then stored at –80°C until analyses.

Cytokine analysis from maternal plasma and SI IEL protein

Maternal plasma and SI IEL proteins were assayed for cytokine levels according to the manufacturer's instructions using the Bio-Plex Pro Mouse Cytokine 23-Plex Assay (Bio-Rad, Hercules, CA, USA) and the Luminex system (Bio-Rad; software v6.0). Cytokine concentrations are expressed as pg/ml for plasma and pg/mg protein for SI IEL total protein. Cytokines with values above or below the standard curve range were excluded from analyses leaving 19 and 15 cytokines for maternal plasma and SI IEL analyses, respectively.

Plasma biomarker analyses

Validated mouse-specific plate assays were used according to the manufacturer's instruction. Biomarkers measured in maternal plasma included insulin (Ultrasensitive Mouse ELISA, ALPCO, Salem, NH, USA), leptin (Mouse ELISA, Crystal Chem, Downers Grove, IL, USA), adiponectin (Mouse High Molecular Weight ELISA, ALPCO), and triglycerides (LabAssay Triglyceride Kit, Wako, Richmond, VA, USA). Leptin assays were further validated and data verified through percent recovery tests in consultation with the assay manufacturer. Coefficients of variation for all of the plate assays ranged from <5% to 15%.

Cecal DNA extraction, 16S rRNA sequencing, and analysis

DNA was extracted from frozen cecal contents by bead beating followed by full-length V1-V9 16S rRNA gene sequence amplification as detailed in the supplementary methods. Amplicons were hybridized to the G3 PhyloChip and scanned using Gene Array (Affymetrix). Images were transformed into abundance and incidence tables for each operational taxonomic unit empirically determined from the dataset (eOTUs) as previously described by Probst [29, 30] and in supplementary methods.

Analysis of empirical operational taxonomic unit abundances and metadata covariates

The Welch test was performed on hierarchically aggregated eOTU HybScores to determine differences between dietary groups at every taxa level. Significant differences amongst metadata continuous variables (maternal weights, pregnancy outcomes, plasma biomarkers, local and peripheral inflammatory status) for weighted UniFrac distance were determined using the Adonis test. Spearman rank correlations test was used to determine relationships between each eOTU HybScore value and continuous variables. We corrected for multiple comparisons using the false discovery rate (FDR) Benjamini-Hochberg method and deemed corrected *P*-values (q-values) that were less than 0.05 to be significant. Heatmaps were generated to visualize associations between relative bacterial abundance and continuous variables. A hierarchical clustering technique was used to summarize these relationships in the form of a dendrogram. Biologically similar communities have a shorter branch length between them.

Phylogenetic relationships amongst differentially abundant taxa

A circular tree, rendered in iTOL [31], was used to display the phylogenetic relationships amongst differentially abundant eOTUs between maternal diets, as described in the supplementary methods.

Assessment of maternal metabolic and inflammatory states

To assess the extent to which maternal metabolic and inflammatory states were altered and how this may relate to metabolic syndrome and proinflammatory phenotypes, we derived four scores (see supplementary methods) summarizing data for (1) circulating biomarkers associated with metabolic dysfunction (leptin, glucose, insulin, adiponectin, leptin: adiponectin and triglycerides); (2) maternal weight characteristics (weight at conception and near term (E18.5), weight gain during pregnancy and weight gain in the first, second, and third trimesters), and key inflammatory markers associated with metabolic dysfunction (tumor necrosis factor alpha [TNF-α], interleukin 6 [IL-6], interleukin 1 alpha [IL-1α], interleukin 1 beta [IL-1β], and monocyte chemoattractant protein 1 [MCP-1]) in (3) plasma and (4) SI IEL.

Statistics

Differences between dietary groups for physiological outcome measures were determined by ANOVA with the Tukey post hoc or Kruskal-Wallis test with Steel-Dwass for nonparametric data ($P < 0.05$).

Results

The gut microbiome varies by maternal diet

We first aimed to determine whether a high calorie/high fat or undernourished diet would impact maternal weight gain during pregnancy and subsequently influence composition of the maternal gut microbiome. There was no difference in mean energy intake across pregnancy or within each trimester (early pregnancy, mid pregnancy, and late pregnancy) between mothers of the three dietary groups (Table 1). Despite consuming an HF diet prior to pregnancy, HF mothers were not significantly heavier than controls at conception, but were slightly heavier than mothers randomized to receive caloric restriction ($P < 0.05$, Table 1). By term, HF mothers remained similar in bodyweight to controls, whilst weight faltering occurred in CR mothers such that they were significantly lighter than both CON and HF mothers from the end of the first trimester until just before term ($P < 0.01$, Table 1). At the whole microbiome level (Adonis test), maternal weight at conception and at E18.5 were significantly associated with microbial abundance ($P < 0.05$), as was weight gain in trimester 1 ($P = 0.003$), but not trimester 2 or 3. These associations were dependent upon gestational age and were restricted to specific taxa (Figure 1A and B). Furthermore, fetal weight at E18.5 ($P < 0.05$), but not litter size, number of fetal resorptions, sex ratio, or placental weight, was significantly associated with maternal microbial abundance.

Bacterial richness was altered by maternal diet. The gut microbiome of HF mothers was less rich compared to both CR and CON, whilst levels of richness were similar between CR and CON

Table 1. Maternal characteristics during pregnancy and pregnancy outcomes.

	CON	CR	HF	*P*-value
Maternal weights				
Weight at conception (g)	22.4 ± 0.74[ab]	20.0 ± 0.40[b]	26.0 ± 2.1[a]	<0.05
Weight at E6.5 (end of T1) (g)	23.0 ± 0.38[a]	19.5 ± 0.38[b]	27.8 ± 1.6[a]	<0.01
Weight at E12.5 (end of T2) (g)	26.5 ± 0.60[a]	19.5 ± 0.37[b]	30.6 ± 1.5[a]	<0.01
Weight at E18.5 (end of T3) (g)	36.5 ± 0.69[a]	25.0 ± 0.56[b]	37.9 ± 0.64[a]	<0.01
Weight gain during pregnancy (g)	14.1 ± 0.60[a]	5.0 ± 0.43[b]	11.8 ± 1.53[a]	<0.01
Maternal energy intakes (kcal/g body weight/day)				
Mean energy intake during pregnancy	0.49 ± 0.03	0.48 ± 0.01	0.57 ± 0.1	NS
Mean energy intake trimester 1	0.53 ± 0.05	0.51 ± 0.02	0.68 ± 0.1	NS
Mean energy intake trimester 2	0.49 ± 0.02	0.46 ± 0.007	0.55 ± 0.1	NS
Mean energy intake trimester 3	0.46 ± 0.02	0.49 ± 0.01	0.45 ± 0.09	NS
Metabolic biomarkers at E18.5				
Glucose (mmol/l)	9.4 ± 0.56	7.6 ± 0.41	8.8 ± 0.65	NS
Insulin (ng/ml)	0.59 ± 0.11[a]	0.14 ± 0.04[b]	0.41 ± 0.07[ab]	<0.05
Leptin (ng/ml)	142 ± 12.5[a]	1.0 ± 0.22[b]	847 ± 300[a]	<0.001
Leptin:body weight	3.9 ± 0.35[a]	0.04 ± 0.01[b]	21.9 ± 7.6[a]	<0.001
Triglycerides (mg/dl)	136 ± 19.1[a]	59.6 ± 3.8[b]	72.2 ± 5.7[b]	<0.01
Inflammatory biomarkers at E18.5				
Plasma TNF-α (pg/ml)	489 ± 215[ab]	190 ± 85.4[b]	1008 ± 115[a]	<0.01
Plasma IL-6 (pg/ml)	4.6 ± 1.9	5.3 ± 1.4	8.1 ± 3.5	NS
Plasma IL-1α (pg/ml)	37.0 ± 9.5	38.9 ± 13.9	47.0 ± 11.8	NS
Plasma IL-1β (pg/ml)	115 ± 12.4[b]	130 ± 16.2[b]	257 ± 23.6[a]	<0.001
Plasma MCP-1 (pg/ml)	88.8 ± 32.2	90.6 ± 25.2	175 ± 30.1	NS
SI IEL TNF-α (pg/mg)	115 ± 64.8	97.5 ± 26.6	239 ± 157	NS
SI IEL IL-6 (pg/mg)	4.6 ± 1.9	5.3 ± 1.4	8.1 ± 3.5	NS
SI IEL IL-1α (pg/mg)	2.7 ± 0.47[b]	2.4 ± 0.17[ab]	17.7 ± 8.5[a]	<0.05
SI IEL IL-1β (pg/mg)	115 ± 12.4[b]	126 ± 16.2[b]	257 ± 23.6[a]	<0.001
SI IEL MCP-1 (pg/mg)	6.1 ± 0.64	6.9 ± 0.94	11.8 ± 1.3	<0.01
Fetal and placental weights at E18.5				
Fetal (male and female) weight (g)	1.08 ± 0.08[a]	0.76 ± 0.06[b]	1.12 ± 0.02[a]	0.002
Males (g)	1.07 ± 0.11	0.74 ± 0.08	1.19 ± 0.02	0.03
Females (g)	1.09 ± 0.12	0.78 ± 0.11	1.17 ± 0.04	NS (0.06)
Placental (male and female) weight (g)	0.13 ± 0.006[a]	0.096 ± 0.004[b]	0.12 ± 0.005[a]	0.002
Males (g)	0.14 ± 0.009[a]	0.097 ± 0.004[b]	0.12 ± 0.005[ab]	0.007
Females (g)	0.12 ± 0.004	0.095 ± 0.009	0.11 ± 0.007	NS
Pregnancy outcomes				
Fetal resorptions (n)	0.60 ± 0.55	0.80 ± 0.84	1.2 ± 0.84	NS
Litter size (n)	8.2 ± 0.49	7.8 ± 0.58	7.8 ± 0.58	NS

Data are mean ± SEM. Groups with different letters are significantly different (Tukey's or Steel-Dwass *post hoc*).
E = embryonic/gestational day (term = 19 days). T = trimester (referring to early, mid, and late pregnancy). SI IEL = small intestinal intraepithelial lymphocytes.

(Figure 2A), suggesting that diet, not obesity or weight faltering per se, influences the composition of the maternal gut microbiome. Interestingly, there were no archaea detected in samples from HF mothers, whilst the archaea *Methanogenium marinum* was detected in 4/5 CR and 2/5 CON mothers. Weight faltering associated with maternal CR did not impact species diversity, as evidenced by close clustering of CON and CR mothers by PCoA using the weighted UniFrac distance metric (Figure 2B and C). However, β-diversity of mothers fed an HF diet was distinct from both CON and CR ($P = 0.001$, Figure 2B; $P < 0.01$, Figure 2C). Whilst our research question was not to determine whether malnutrition had differential impacts on the gut microbiome in the pregnant versus nonpregnant state, we did evaluate whether nonpregnant females fed the control diet (NP) and housed in the same manner, at the same time, as control-fed pregnant dams, had different microbiomes. At the whole microbiome level, there were no differences in microbial abundance levels between NP and CON females (Adonis test). PCoA and hierarchical clustering using the weighted UniFrac distance metric showed that NP controls clustered with the CON and CR dams (Supplementary Figure S6).

The most dominant phyla across all dietary groups were Firmicutes, Bacteroidetes, and Proteobacteria, and phylum-level abundance differences existed between dietary groups (Supplementary Table S1). It has been suggested that an increased Firmicutes: Bacteroidetes ratio is a marker for obesity/HF diet [32, 33]. Maternal HF diet did not alter the Firmicutes: Bacteroidetes abundance ratio in late gestation (CON, 3.9 ± 0.1; CR, 3.8 ± 0.1; HF, 4.1 ± 0.2). At the family level, bacterial abundance revealed further distinctions between dietary groups: of the top nine most abundant families Pseudomonadaceae were in greater abundance ($P = 0.0002$) and Catabacteriaceae were in lesser abundance ($P = 0.007$) in HF mothers compared to both CON and CR (Figure 3A and B). Since we observed that the microbiomes of HF-fed mothers were more dissimilar to CON and CR than CR microbiomes were to CON, we next

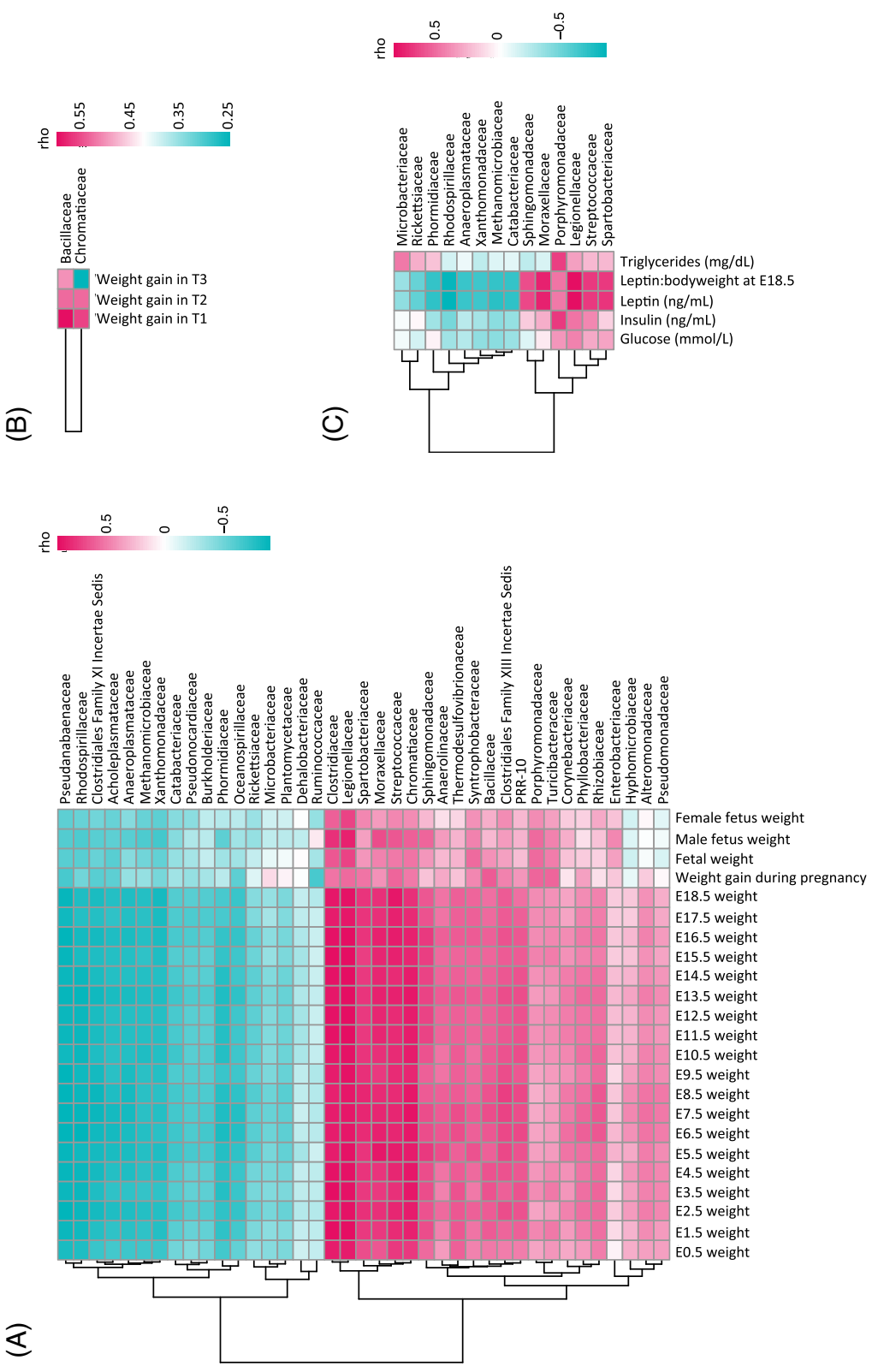

Figure 1. Associations between microbial taxa abundance (family-level), weight, and metabolic biomarkers during pregnancy. (A) Microbiome associations with maternal weight (grams of body weight) during pregnancy and fetal weight at E18.5. (B) Microbiome associations with maternal weight gain within each trimester. (C) Microbiome associations with maternal metabolic biomarkers at E18.5. Red indicates a positive association, blue indicates a negative association. Color saturation indicates the strength of relationship. Weight and weight gain in grams; E = embryonic day; fetal weight = all fetuses of litter; male/female fetus weight = only male or female fetuses of litter; T = trimester (referring to early, mid, and late pregnancy). See Supplementary Tables S3–S5 for corresponding correlation coefficients and q values of family-level heatmaps. See Supplementary Figure S4 for associations at the genus and species levels, and Supplementary Tables S9–S12 for corresponding correlation coefficients and q values of genus- and species-level heatmaps.

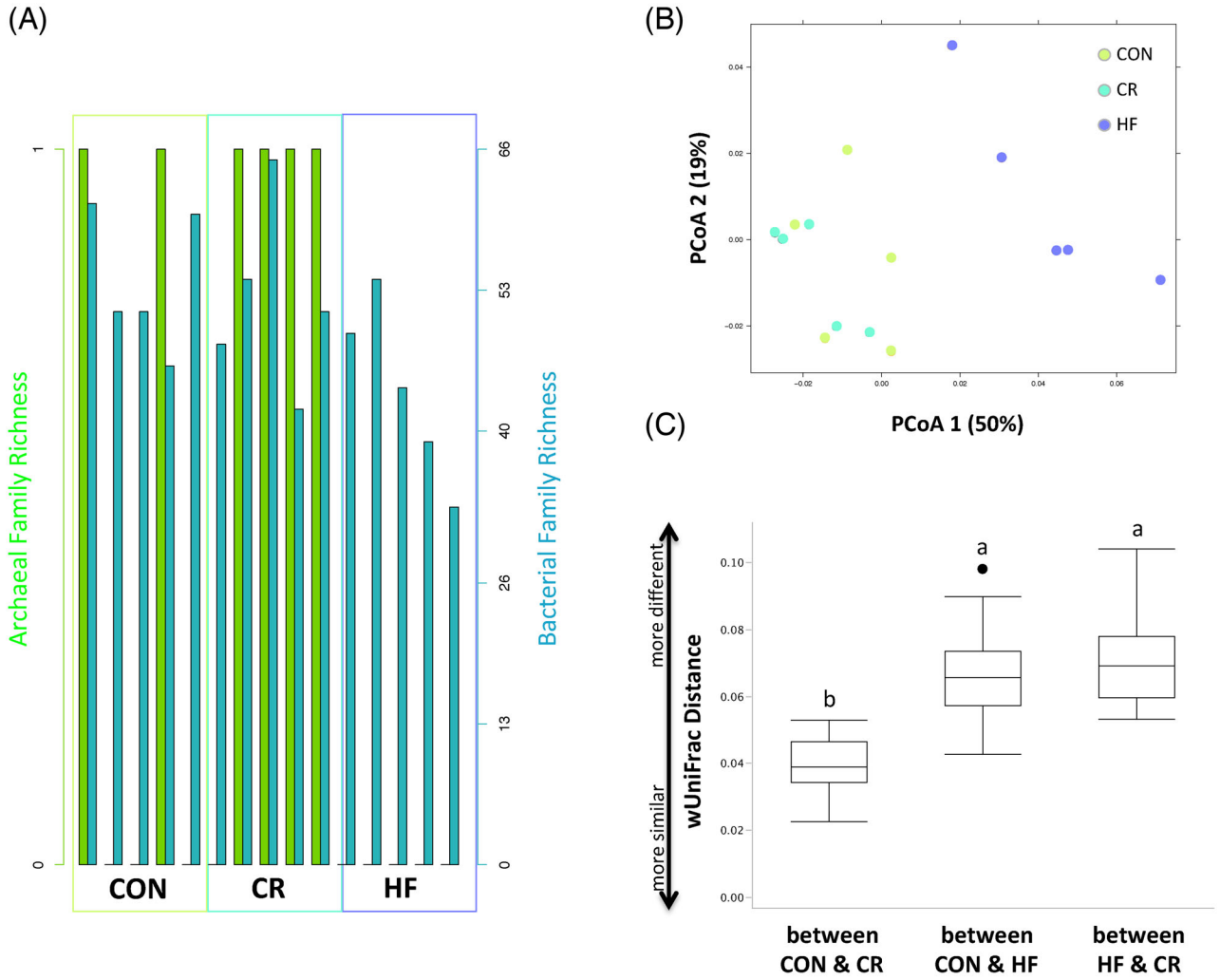

Figure 2. Characterization of maternal gut microbiome with altered nutrition. (A) Archaeal (green bars) and bacterial (blue bars) family-level richness. Bacterial richness was lower in HF mothers compared to CON and CR ($P = 0.05$). An archaeal species, *Methanogenium marinum*, was observed in CON and CR, but was not observed in HF mothers. (B) PCoA of weighted UniFrac distance between CON, CR, and HF mothers. Percent variation explained by the principal components is indicated in the parentheses. Each dot in the PCoA represents a mother and dot color indicates dietary group (yellow = CON, green = CR, blue = HF). (C) Weighted UniFrac distance between CON, CR, and HF mothers. Groups with different letters are significantly different ($P < 0.0001$).

determined which eOTUs were significantly altered by maternal HF diet by comparing HF mothers to all mothers fed the chow diet, irrespective of caloric intake. Bacterial abundance and prevalence were influenced by maternal nutrition (Supplementary Figure S1). Of the 12 taxa with the most significant abundance differences between diets, 7 were significantly underrepresented in HF-fed versus chow-fed mothers, whilst five were significantly overrepresented ($q < 1.0^{-7}$, Figure 3C and Supplementary Table S2). All eOTUs of the top 12 belonged to one of four phyla: Firmicutes (9), Proteobacteria (1), Bacteroidetes (1), and TM7–1 (1). To display taxonomic relationships amongst differentially abundant eOTUs, we constructed a circular tree using eOTUs selected by Welch test of bacterial abundance metrics between HF-fed and chow-fed groups (Figure 4). OTUs were both increasing and decreasing in abundance in HF samples compared to CON and CR, where no phylum contained only OTUs that were increasing, or only OTUs that were decreasing in abundance in one dietary group compared to another.

Of 836, 553 eOTUs were significantly different between HF-fed mothers and chow-fed mothers ($q < 0.05$), with 11 of these annotated to the species level: 5 species had reduced abundance in HF-fed versus chow-fed mothers (*Trichodesmium erythraeum*, *Faecalibacterium prausnitzii*, *M. marinum*, and two species of *Allobaculum* sp. ID4), whilst 6 had increased abundance (*Clostridium cocleatum*, *C. septicum*, *Spirochaeta thermophilia*, *Agrococcus jenensis*, *Peptostreptococcus anaerobius*, *Jonesia dentrificans*). Further, six eOTU of the *Lactobacillus* genera were significantly increased in abundance in HF mothers ($q < 0.05$). One annotated species, *P. anaerobius*, was also significantly increased in abundance in CR vs CON ($P < 0.05$).

Maternal diet impacts maternal metabolic and immune function

Late pregnancy is a state associated with increased fat mass, metabolic changes, and altered immune function [8–11] to enable increased nutrient transfer to the growing fetus and allow the mother

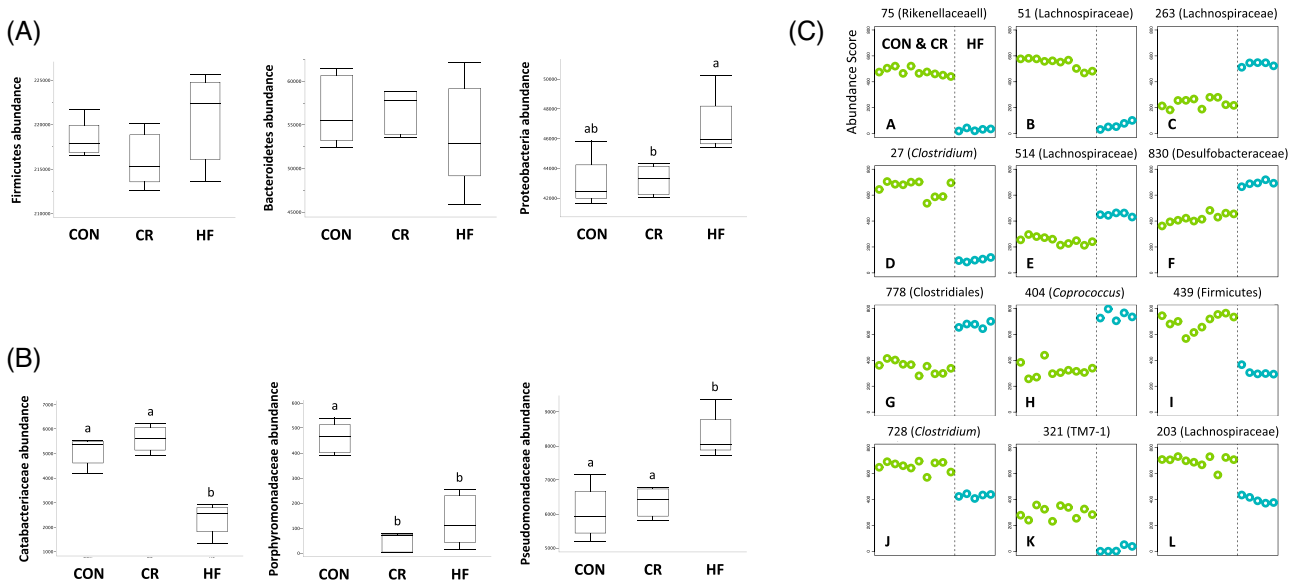

Figure 3. Maternal diet alters specific bacteria. Selected phyla- (upper panel, A) and family-level (lower panel, B) abundance differences with altered nutrition. Of the top three most abundant phyla, only Proteobacteria abundance was altered with maternal HF diet. However, within each phyla, taxon abundance differences emerged: of the top nine most abundant families Catabacteriaceae were in lesser abundance ($P = 0.007$) and Pseudomonadaceae were in greater abundance ($P = 0.0002$) in HF mothers compared to both CON and CR. Porphyromonadaceae were also in lesser abundance in HF and CR mothers compared to CON ($P = 0.005$). Groups with different letters are significantly different ($P < 0.05$). See Supplementary Table S1 for complete list of phylum-level abundance differences stratified by maternal diet, and Supplementary Figure S1 for proportional abundance differences at the family level stratified by diet. (C) Nearest shrunken centroid plots of 12 distinct taxa (eOTU) with the most significant abundance differences between dietary groups. Numbers indicate eOTU ID, names indicate the lowest level classification. Seven of 12 taxa were significantly decreased in HF vs. CON and CR mothers, whilst 5 of 12 taxa were significantly increased in HF vs. CON and CR mothers (q < 1.0–7). See Supplementary Table S2 for taxa annotations.

to meet the higher energetic demands of impending parturition and lactation. This phenotype is consistent with characteristics often seen with adverse cardiometabolic function. CR resulted in reduced insulin, leptin, and triglyceride levels in mothers at the end of pregnancy, and HF mothers also had reduced triglycerides compared to controls (Table 1). In contrast, HF nutrition, not CR, resulted in increased peripheral and SI IEL levels of some key inflammatory biomarkers known to increase with metabolic disease (Table 1). To determine whether maternal dietary or weight status impacted maternal metabolic and inflammatory states more generally, we analyzed summary scores of biomarker, weight, and systemic and local inflammatory outcomes (Supplementary Figure S2A–D). PCoA of summary scores revealed that CR mothers clustered distinctly from HF-fed and control mothers, whilst HF-fed mothers somewhat separated from controls (Figure 2E).

Variation in the gut microbiome is associated with metabolic and inflammatory state

Given the role of gut bacteria in regulating nutrient metabolism and immune function and the changes we observed in metabolic and immune biomarkers, we next asked whether bacterial abundance in late pregnancy is associated with maternal metabolic and inflammatory states and with changes in diet. Only leptin ($P = 0.002$) and leptin: body weight ratio ($P = 0.001$) were significantly associated with overall microbial abundance (Adonis test). Leptin concentrations and leptin: body weight ratio levels were also significantly associated with abundance of specific bacterial taxa ($q < 0.05$, Figure 1C). Triglyceride concentrations were only significantly associated with Porphyromonadaceae abundance. There were no significant associations between glucose or insulin concentrations with bacterial abundance at any taxonomic level.

Peripheral (TNF-α, IL-1α, interleukin 12 p40 [IL-12p40], interleukin 12 p70 [IL-12p70], interleukin 17 [IL-17], macrophage inflammatory protein 1 alpha [MIP-1α]; $P < 0.05$) and local (interferon gamma [IFN-γ], IL-1β, IL-12p40, IL-12p70, interleukin 2 [IL-2], MIP-1α; $P < 0.05$) levels of inflammation were associated with overall microbial abundance, and mothers with the highest levels of inflammation had microbiomes that were dissimilar to those from mothers with the lowest levels ($P = 0.02$, Supplementary Figure S3). Significant associations existed between abundance of specific taxa and levels of peripheral inflammatory factors (Figure 5A). These relationships persisted locally in the SI. Yet relationships between microbial taxa abundance and inflammation tended to be stronger in the SI than in the peripheral circulation, and we identified a greater number of classified taxa with abundance levels associated with inflammatory biomarkers in SI IELs than with levels in the circulation (Figure 5B).

Taxa potentially protective against diet-associated metabolic dysfunction

To evaluate whether specific taxa may be associated with conferring inflammatory and metabolic phenotypes in mothers, we sought to identify taxa that fit the following criteria: those that were significantly increased in HF mothers versus CON, but not in CR mothers (vs CON), and those that were increased in mothers with high biomarker concentrations versus those with low-to-moderate concentrations. Initially, we focused our comparisons on key inflammatory factors associated with obesity and metabolic dysfunction that also showed a heterogeneity in response within the dietary groups

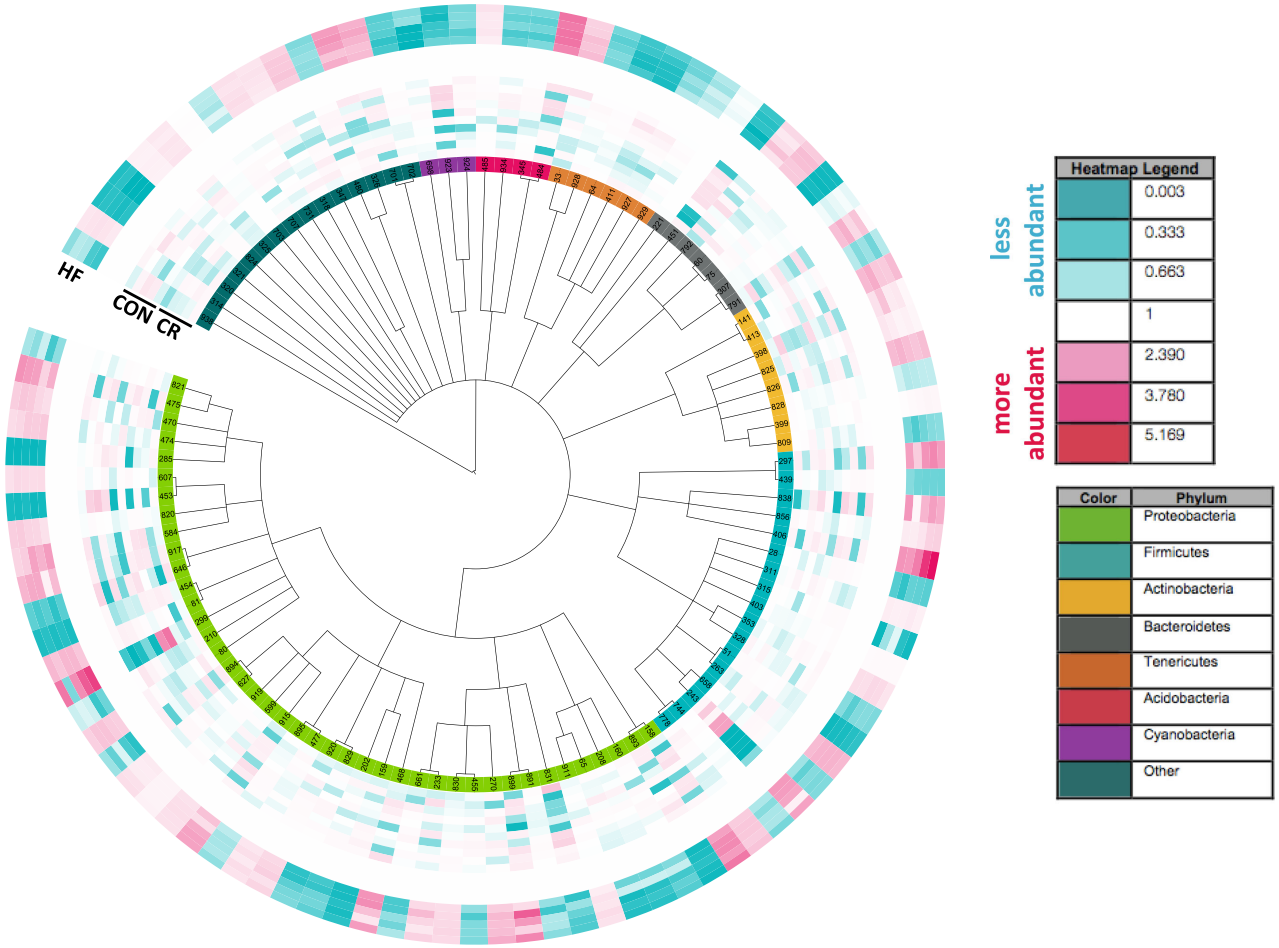

Figure 4. Circular tree comparing HF vs. CON and CR samples at the phylum level to display phylogenetic relationships amongst differentially abundant OTUs. Rings around the tree are a heatmap where HF samples comprise the outermost ring and CON and CR samples comprise the innermost ring. Red indicates an OTU was more abundant in HF than CON and CR; blue indicates an OTU was less abundant in HF than CON and CR. The color saturation indicates the degree of difference from the mean value of the CON and CR samples, where dark blue indicates a log ratio = 0.003, white = 1, dark red = 5.2, $P < 0.0001$. Phyla on the circular tree are indicated by different colors.

(leptin, IL-6, and granulocyte-macrophage colony-stimulating factor). With our stringent FDR, we did not find any taxa associated with inflammatory biomarker changes that fit these criteria. However, we did find 10 families and two species (*Allobaculum* sp. ID4 and *T. erythraeum*) that were enriched in mothers with low-to-moderate leptin levels, independent of a CR diet (Supplementary Table S8).

Discussion

It is now well accepted that diet plays a profound role in shaping the structure and function of the gut microbiome [24, 34, 35]. However, studies examining nutrition during pregnancy and its effects on the maternal gut microbiome are lacking. Such investigations are critical due to the growing problem of excessive nutrient intake in most populations worldwide [36], and that there still exists regions where undernourishment occurs, including for over 800 million people in developing countries and those facing food insecurity in North America [3, 4]. Both under- and overnutrition have adverse impacts on maternal adaptation to pregnancy and long-term health of the mother and importantly, situate children on developmental trajectories toward increased risk for metabolic diseases [6, 7, 37]. Our study concurrently examined these critical nutritional events in a well-controlled and phenotyped animal model using robust statistical analyses. Consistent with our hypothesis, we showed that maternal nutritional adversity was associated with an altered gut microbial community, and that gut microbes may influence pathways underlying metabolic and inflammatory status in mothers through their interactions with the maternal nutritional environment.

We found the most striking restructuring of the maternal gut microbiome occurred in mothers fed a high calorie/high fat diet, despite that these mothers were not heavier than control chow-fed mothers, and despite that mothers whose calorie intakes were restricted had significantly reduced weights by the end of pregnancy. The lack of significant change in the composition of gut microbiome with maternal undernutrition in our model is contrary to our hypothesis that both forms of malnutrition would result in gut microbial dysbiosis. Our study alone does not definitively determine that maternal diet plays a more dominant role than weight on the composition of the gut microbiome in pregnancy. However, our data are consistent with studies that suggest substrate type and availability, rather than weight or adiposity, exerts some of the greatest pressure on

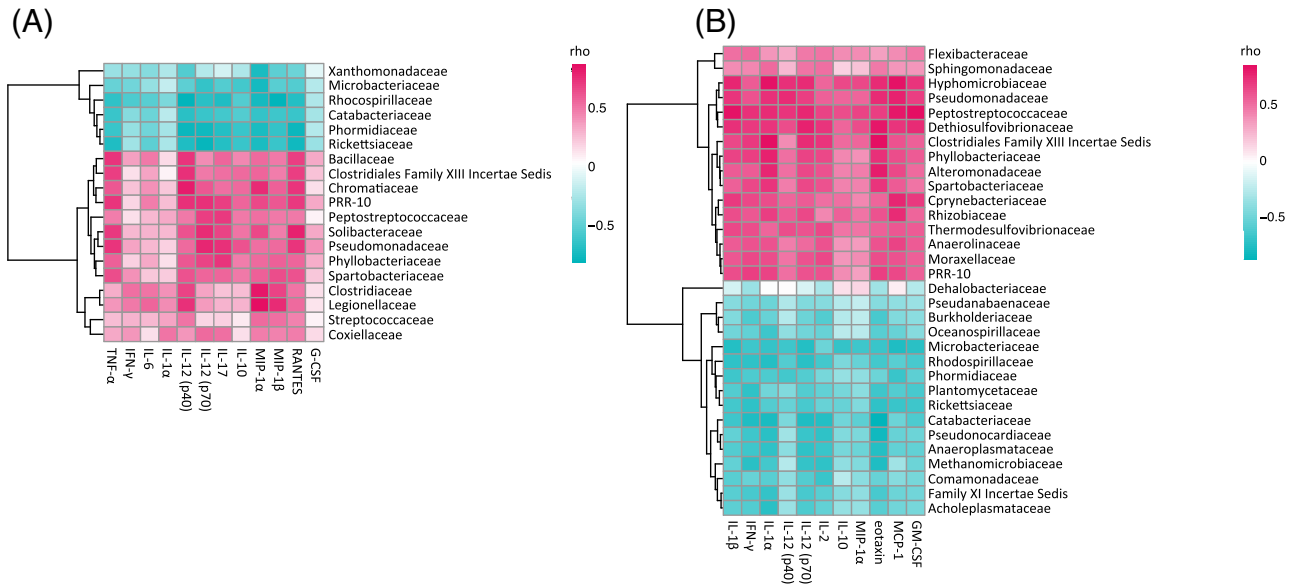

Figure 5. Associations between microbial taxa abundance (family-level) and inflammatory biomarkers in circulating plasma (A) or small intestinal intraepithelial lymphocytes (SI IEL) (B). Red indicates a positive association, blue indicates a negative association. Color saturation indicates the strength of relationship. Plasma inflammatory markers measured in pg/ml. SI IEL inflammatory markers measured in pg/mg. See Supplementary Tables S6–S7 for corresponding correlation coefficients and q values of family-level heatmaps. See Supplementary Figure S5 for associations at the genus and species levels, and Supplementary Tables S13–S16 for corresponding correlation coefficients and q values of genus- and species-level heatmaps.

microbial dynamics [38–40] and that although the gut microbiome quickly reacts to extreme changes in diet, long-term dietary changes may be required for stable reprogramming of community composition [24, 41]. Although one study did not find any impact of maternal diet on the gut microbiome, the Nordic population studied consumed a healthy and balanced diet [15] and the data may actually reflect the role of culture and nutritional history on gut microbiome composition [40]. The reduced microbial richness we observed in HF mothers is consistent with richness patterns seen in overweight/obesity and inflammatory phenotypes [42–45]. Interestingly, obese individuals with low bacterial richness have been shown to gain more weight over time, and short-term dietary restriction can partially restore richness and improve some of their anthropometric and metabolic biomarkers, with limited effects on inflammatory markers [43, 44]. Whether low bacterial richness could predispose to excessive weight gain during pregnancy remains to be determined, but it is intriguing to speculate given that many normal weight and overweight women gain more than the recommended amount of weight in pregnancy [2, 3].

Our finding of increased Proteobacteria abundance in HF mothers is consistent with the association of these bacteria with inflammatory conditions [15, 46]. At the same time, *F. prausnitzii* was significantly reduced in HF mothers. *F. prausnitzii* is a highly active member of the microbial community [47], is associated with anti-inflammatory effects [48], and its abundance is related to intestinal health [49]. Its role in metabolic health is less consistent, but it has been associated with reduced inflammation in obesity and diabetes [50]. Shifts in the proportion of Bacterioidetes to Firmicutes have been shown in some, but not all, cases of overweight [32, 33, 51, 52] and may be influenced by pregnancy [15, 38]. We did not observe a change in this ratio in HF mothers, and noted that taxa were both increasing and decreasing in abundance within all phyla across dietary groups. Yet, taken with our findings of distinct OTU abundance differences between dietary groups, our data support that a refined, rather than broad, assessment of the microbial community is required for identifying specific bacteria, and their combinations, that may influence host response to environmental challenges and subsequent phenotype.

Despite the expected weight gain in CON and HF mothers with advancing gestation, and the weight faltering in CR mothers from mid-late gestation, we found that the relationships between taxa abundance and gestational weight were consistent across pregnancy. This suggests, as in other studies [38], that factors beyond maternal weight (gain) during pregnancy are primary contributors to later microbial community structure. To our knowledge, there are no studies looking at the relationship between the maternal gut microbiome and fetal outcomes such as weight. We observed significant associations between abundance of taxa from the family to species levels and fetal weight, which corresponded to the associations between taxa abundance and maternal weight across gestation. Whether these taxa are involved in maternal weight regulation and fetal growth remains unknown, but their presence may more broadly reflect the altered metabolic environment of the mother during pregnancy and with nutritional adversity, which may place constraints on fetal development *in utero*. Growing evidence also points to a role for maternal diet during pregnancy in shaping the gut microbiome of offspring [53, 54]. This should not be surprising since the transfer of maternal microbes, which themselves can be altered by maternal diet and health states, occurs during delivery [55], through breastfeeding practices [56, 57], and perhaps even earlier *in utero* [58, 59], providing the first bacterial colonizers of the infant gut. It is now evident that the composition of this early life gut microbiome is critically important [60], as it can shape the development and health trajectories of the offspring [53, 57, 61].

We found significant associations between microbial taxa abundance at all levels and maternal leptin, but not glucose or insulin, concentrations. Changes in maternal leptin levels with advancing gestation have been seen in women with altered microbiomes in late

pregnancy [15], and obese mice that are leptin deficient also have altered gut microbiomes [33]. Leptin circulates in blood at levels proportional to body fat. Although HF mothers were not heavier than CON in late pregnancy, they likely had increased adiposity reflected by high leptin concentrations. We acknowledge that some variability seen in maternal biomarker variables may be due to the small n-number in each group, and this could conceal biological relationships between these biomarkers and the microbiome. We also observed high letpin: bodyweight ratios in HF dams, which may indicate that these mothers are becoming leptin resistant. Recent studies have examined the effects of dietary fats and sugars on the development of leptin resistance in rodents of normal bodyweight [62]. It is intriguing to speculate that HF diet and/or changes in the gut microbiome may represent additional mechanisms that mediate leptin resistance, even without elevations in body weight. Whether the differences we saw in leptin: bodyweight ratio and its relationships with microbial abundance levels in HF mothers reflect emerging leptin resistance due to HF diet and/or an altered gut microbiome remains to be determined.

We identified associations between taxa abundance and inflammatory biomarkers both locally at the level of the SI and in the periphery in late gestation. Stool from third trimester women has been shown to have increased inflammatory content compared to first trimester stool [15] and low grade inflammation, assessed by calprotectin level, has also been documented in late pregnancy stool [63]. The microbial abundance and inflammatory marker level associations in our study were consistent across the two sites, and suggest that peripheral measures of inflammation and their relationships with the gut microbiome may reflect immune–microbe relationships in the gut. Whether maternal diet remodels the gut microbial community, and these microbes influence locally resident immune cells, which in turn affect the peripheral immune system, remains to be clearly determined. Yet, our data are consistent with this model. HF mothers showed greatest changes in the microbiome, relationships between bacteria and inflammatory biomarkers were strongest in the SI, and when stratifying weighted UniFrac distance by plasma inflammation tertiles, we found that the greatest dissimilarity in microbiomes existed between mothers with the most divergent inflammatory states. Interestingly, the gut microbiomes of women in late pregnancy and 1 month postpartum appear to be similar [15, 63] and if our data followed the same pattern, it may suggest that adverse inflammatory changes associated with a dysfunctional microbiome may persist after pregnancy, exaggerating the postpartum inflammatory state [10].

Studies in obese and HF-fed hosts have shown that caloric restriction only partially improves inflammatory status [44, 64]. Our finding of two species enriched in mothers with low/moderate leptin levels independent of a calorie restricted diet, which were also in low abundance in HF mothers, may point to species that potentially protect against the known potent inflammatory and adipokine effects of leptin [65]. *Allobaculum* taxa include short-chain fatty acid producing bacteria [66]. These compounds have been shown to have anti-inflammatory effects in the gut and may play a role in ameliorating HF diet-induced adiposity, insulin resistance, and inflammation [66]. Less is known about *T. erythraeum* in mammalian physiology, but it thrives in nutrient poor ecological habitats and is critical in the storage of iron [67] due to its high requirements of iron for nitrogen fixation and for DNA protection against oxidative stress [68, 69]. Obesity is associated with iron deficiency [70] and oxidative stress [71], conditions that are linked to increased adipokine and inflammatory biomarker levels [72, 73]. Moreover, maternal iron deficiency, which often occurs in later pregnancy, impacts fetal growth and increases perinatal mortality [74]. Thus, findings from this study and others suggest that key bacterial species, including *Allobaculum* sp ID4 and *T. erythraeum*, may be important for adipokine, inflammatory, and micronutrient load in the host.

One must consider that the integrity of the intestinal barrier itself may well play a role in shaping the composition of the gut microbiome and host metabolic and inflammatory status. Many forms of malnutrition are associated with increased permeability of the gut epithelium or paracellular leakage [75]. This occurs through modulation of tight junctions by infectious microbes, reactive oxygen species, endotoxin lipopolysaccharide, and inflammatory cytokines [76–78]. Whether gut microbial dysfunction drives intestinal paracellular leakage, or a leaky gut drives microbial dysbiosis, or both, remains unclear. Regardless, the effects of the gut microbiome can extend beyond the local niche to impact peripheral inflammatory and metabolic signals [21, 25], which may occur through increased gut permeability. Fortunately, beneficial microbes have been found to diminish paracellular leakage [79] and hold therapeutic promise for restoring gut barrier integrity. Thus, both an altered maternal gut microbiome and a compromised gut barrier could contribute to the nutrient, metabolic, and inflammatory status of the mother during pregnancy.

Whilst our study was powered to detect differences in our primary outcomes (microbial abundance between groups), with FDR correction, and secondary outcomes (levels of metabolic and inflammatory biomarkers), we did not detect a significant difference in maternal weight before and during pregnancy between HF and CON dams. This may be due to the low n number in the experimental groups, or due to known differences in penetrance of obesity in individuals (across species) [38, 80–83]. Retrospective power analyses suggest that to detect a difference (at $\alpha = 0.05$) in weight between groups, we would need a sample size of 12 per dietary group. Observationally, we saw that HF dams were fatter at postmortem than both CON and CR, and HF dams did have high levels of leptin at E18.5, which circulates in the body at levels proportional to body fat, suggesting that HF mothers were likely fatter. Future experiments should include quantifying the mass of the maternal fat pads or performing a DEXA scan to determine fat and lean mass. As the rates of overweight and obesity in reproductive aged women are increasing worldwide [84], including in Canada where rates are over 30% [85], even overweight before and during pregnancy is a significant issue, as is poor nutrition in general [86]. Therefore, our findings may be relevant to consider with respect to common situations where there is no overt obesity, but where elevated weight and/or poor nutrition exist.

Our study provides insight on the role of maternal nutrition in shaping metabolic, immune, and microbial systems in late pregnancy, with potential implications for host–microbe interactions. An inappropriately balanced maternal gut microbiome leading into, or during pregnancy (a consequence of suboptimal nutrition, increased inflammatory load, or disease), could alter how the mother copes with the metabolic and inflammatory events that normally occur in pregnancy, and impact maternal nutrient metabolism. The consequences of this go beyond maternal health to impact fetal development, pregnancy outcomes, and likely health of the offspring long term.

Supplementary data

Supplementary data are available at *BIOLRE* online.

Supplementary Figure S1. Bubble plot of bacterial prevalence and relative abundance of nine distinct eOTU (classified to the family or genus level) observed either in HF-fed mothers only or chow-fed (CON and CR) mothers only. Seven eOTUs were observed in all of the HF mothers but in none of the CON or CR mothers, whilst two eOTUs were observed in all of the CON and CR mothers but in none of the HF mothers. Relative abundance of each eOTU is indicated by the size of the bubble.

Supplementary Figure S2. Characterization of maternal metabolic and immunologic function with altered nutrition. (A–D) Summary scores of gestational weight (a), key metabolic biomarkers (b), and key inflammatory biomarkers in the circulation (c) and in small intestinal intraepithelial lymphocytes (SI IEL) (d) in late gestation. Maternal CR was associated with altered weight gain ($P < 0.0001$) and metabolic biomarkers ($P = 0.0005$), whilst HF nutrition was associated with an elevated inflammatory status both in the periphery ($P = 0.05$) and locally at the SI ($P = 0.01$). Groups with different letters are significantly different. (E) PCoA of metabolic biomarker, inflammatory biomarker (peripheral and in SI IEL), and maternal weight summary scores. Percent variation explained by the principal components is indicated in the parentheses. Each dot represents a mother and dot color indicates dietary group (yellow = CON, green = CR, blue = HF).

Supplementary Figure S3. Maternal inflammatory status contributes to β-diversity. Weighted UniFrac distance within tertiles of plasma inflammation scores. Mothers with greater differences in levels of key plasma inflammatory biomarkers had different microbiomes ($P = 0.015$). Groups with different letters are significantly different.

Supplementary Figure S4. Associations between microbial taxa abundance, maternal weight during pregnancy, and fetal weight at E18.5 at the genus (A) and species (B) levels and associations between microbial taxa abundance and maternal metabolic biomarkers at E18.5 at the genus (C) and species (D) levels. Red indicates a positive association, blue indicates a negative association. Color saturation indicates the strength of relationship. Weight in grams; E = embryonic day; fetal weight = all fetuses of litter; male/female fetus weight = only male or female fetuses of litter. See Supplemental Tables S9–S12 for corresponding correlation coefficients and q values of genus- and species-level heatmaps. For correlations with weight gain by trimester (data not shown), only the genera *Bacillus* (r = 0.525, q = 0.018) and the species *Clostridium septicum* (r = 0.629, q = 0.013) were significantly correlated with weight gain in the second trimester.

Supplementary Figure S5. Associations between microbial taxa abundance and circulating inflammatory biomarkers at the genus (A) and species (B) levels and associations between microbial taxa abundance and small intestinal intraepithelial lymphocyte inflammatory biomarkers at the genus (C) and species (D) levels. Red indicates a positive association, blue indicates a negative association. Color saturation indicates the strength of relationship. Plasma inflammatory markers measured in pg/ml. SI IEL inflammatory markers measured in pg/mg. See Supplemental Tables S13–S16 for corresponding correlation coefficients and q values of genus- and species-level heatmaps.

Supplementary Figure S6. Microbial abundance in pregnant and non-pregnant females. (A) PCoA of weighted UniFrac distance between nonpregnant (NP) and pregnant (P) females. Percent variation explained by the principal components is indicated in the parentheses. Each dot in the PCoA represents a female and dot color indicates pregnancy group (green = nonpregnant control-fed females, blue = pregnant females). NP females clustered with the pregnant CON and CR females, whilst the majority of the separation of the microbiome was between the HF pregnant females (far right dots) and all other females. (B) Hierarchical clustering based on weighted UniFrac distance between samples. NP control females cluster within the pregnant CON and CR females. The major separation was between pregnant females fed a high fat (HF) diet and females fed the control diet (NP, CON, and CR). Green = nonpregnant control-fed females, blue = pregnant females.

Supplementary Table S1. Phylum-level abundance differences between dietary groups.

Supplementary Table S2. Taxa annotations of the 12 most significantly different eOTUs between dietary groups based on bacterial abundance levels.

Supplementary Table S3. Family weight correlations.

Supplementary Table S4. Family weight gain correlations.

Supplementary Table S5. Family metabolic correlations.

Supplementary Table S6. Family plasma inflammatory biomarker correlations.

Supplementary Table S7. Family SI IEL inflammatory biomarker correlations.

Supplementary Table S8. (A) Family- and (B) species-level annotations of putatively protective taxa.

Supplementary Table S9. Genus weight correlations.

Supplementary Table S10. Species weight correlations.

Supplementary Table S11. Genus metabolic correlations,

Supplementary Table S12. Species metabolic correlations,

Supplementary Table S13. Genus plasma inflammatory biomarker correlations.

Supplementary Table S14. Species plasma inflammatory biomarker correlations.

Supplementary Table S15. Genus SI IEL inflammatory biomarker correlations.

Supplementary Table S16. Species SI IEL inflammatory biomarker correlations.

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Contributors

KLC, SJL: conceived and designed the experiments. KLC: performed the experiments:. KLC, CC, TZD, AA, LC: analyzed and interpreted the data. KLC, SJL, TZD, CC, AA, LC: wrote the paper and revised the manuscript.

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References

1. Seidell JC. Obesity, insulin resistance and diabetes–a worldwide epidemic. *Br J Nutr* 2000; **83**(Suppl 1):S5–S8.
2. Lowell H, Miller DC. Weight gain during pregnancy: adherence to Health Canada's guidelines. *Health Rep* 2010; **21**:31–36.
3. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, Ezzati M, Grantham-McGregor S, Katz J, Martorell R, Uauy R. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet North Am Ed* 2013; **382**(9890): 427–451.

4. McIntyre L, Glanville NT, Raine KD, Dayle JB, Anderson B, Battaglia N. Do low-income lone mothers compromise their nutrition to feed their children? *CMAJ* 2003; **168**:686–691.
5. Catalano PM, Thomas A, Huston-Presley L, Amini SB. Increased fetal adiposity: a very sensitive marker of abnormal in utero development. *Am J Obstet Gynecol* 2003; **189**(6):1698–1704.
6. Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ, Bleker OP. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. *Mol Cell Endocrinol* 2001; **185**(1-2):93–98.
7. Poston L, Harthoorn LF, Van Der Beek EM. Obesity in pregnancy: implications for the mother and lifelong health of the child. A consensus statement. *Pediatr Res* 2011; **69**(2):175–180.
8. Catalano PM, Roman-Drago NM, Amini SB, Sims EA. Longitudinal changes in body composition and energy balance in lean women with normal and abnormal glucose tolerance during pregnancy. *Am J Obstet Gynecol* 1998; **179**(1):156–165.
9. Catalano PM, Huston L, Amini SB, Kalhan SC. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. *Am J Obstet Gynecol* 1999; **180**(4):903–916.
10. Christian LM, Porter K. Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: effects of maternal body mass index. *Cytokine* 2014; **70**(2):134–140.
11. Mor G, Cardenas I, Abrahams V, Guller S. Inflammation and pregnancy: the role of the immune system at the implantation site. *Ann N Y Acad Sci* 2011; **1221**(1):80–87.
12. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. *J Clin Invest* 2011; **121**(6):2111–2117.
13. Catalano PM, Drago NM, Amini SB. Maternal carbohydrate metabolism and its relationship fetal growth and body composition. *Am J Obstet Gynecol* 1995; **172**(5):1464–1470.
14. Hirschmugl B, Desoye G, Catalano P, Klymiuk I, Scharnagl H, Payr S, Kitzinger E, Schliefsteiner C, Lang U, Wadsack C, Hauguel-de Mouzon S. Maternal obesity modulates intracellular lipid turnover in the human term placenta. *Int J Obes* 2017; **41**(2):317–323.
15. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Backhed HK, Gonzalez A, Werner JJ, Angenent LT, Knight R, Backhed F, Isolauri E et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012; **150**(3):470–480.
16. Balfour Sartor R, Mazmanian SK. Intestinal microbes in inflammatory bowel diseases. *Am J Gastroenterol Suppl* 2012; **1**(1):15–21.
17. Baiocco PJ, Korelitz BI. The influence of inflammatory bowel disease and its treatment on pregnancy and fetal outcome. *J Clin Gastroenterol* 1984; **6**:211–216.
18. Bush MC, Patel S, Lapinski RH, Stone JL. Perinatal outcomes in inflammatory bowel disease. *J Matern Fetal Neonatal Med* 2004; **15**(4):237–241.
19. Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Toth M, Korecka A, Bakocevic N, Guan NL, Kundu P, Gulyas B, Halldin C et al. The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* 2014; **6**(263):263ra158–263ra158.
20. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 2009; **58**(8):1091–1103.
21. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci USA* 2009; **106**(10):3698–3703.
22. Swann JR, Want EJ, Geier FM, Spagou K, Wilson ID, Sidaway JE, Nicholson JK, Holmes E. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. *Proc Natl Acad Sci USA* 2011; **108**(Supplement_1):4523–4530.
23. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012; **490**:55–60.
24. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014; **505**:559–563.
25. Verdam FJ, Fuentes S, de Jonge C, Zoetendal EG, Erbil R, Greve JW, Buurman WA, de Vos WM, Rensen SS. Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. *Obesity* 2013; **21**:E607–E615.
26. Knight BS, Pennell CE, Shah R, Lye SJ. Strain differences in the impact of dietary restriction on fetal growth and pregnancy in mice. *Reprod Sci* 2007; **14**:81–90.
27. Liu J, Kandasamy S, Zhang J, Kirby CW, Karakach T, Hafting J, Critchley AT, Evans F, Prithiviraj B. Prebiotic effects of diet supplemented with the cultivated red seaweed Chondrus crispus or with fructo-oligo-saccharide on host immunity, colonic microbiota and gut microbial metabolites. *BMC Complement Altern Med* 2015; **15**:279.
28. Clapcote SJ, Roder JC. Simplex PCR assay for sex determination in mice. *Biotechniques* 2005; **38**:702–706, 704, 706.
29. Probst AJ, Lum PY, John B, Dubinsky EA, Piceno YM, Tom LM, Andersen GL, He Z, De Santis TZ. Microarray of 16S rRNA gene probes for quantifying population differences across microbiome samples. In: He Z (ed.) *Microarrays. Current Technology, Innovations and Applications*. Norfolk: Caister Academic Press; 2014.
30. Probst AJ, Weinmaier T, DeSantis TZ, Santo Domingo JW, Ashbolt N. New perspectives on microbial community distortion after whole-genome amplification. *PLoS One* 2015; **10**:e0124158.
31. Letunic I, Bork P. Interactive Tree Of Life v2: online annotation and display of phylogenetic trees made easy. *Nucleic Acids Res* 2011; **39**:W475–W478.
32. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* 2005; **102**:11070–11075.
33. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**:1027–1131.
34. Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, Kau AL, Rich SS, Concannon P, Mychaleckyj JC, Liu J, Houpt E et al. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. *Science* 2013; **339**:548–554.
35. Subramanian S, Huq S, Yatsunenko T, Haque R, Mahfuz M, Alam MA, Benezra A, DeStefano J, Meier MF, Muegge BD, Barratt MJ, VanArendonk LG et al. Persistent gut microbiota immaturity in malnourished Bangladeshi children. *Nature* 2014; **510**:417–421.
36. Popkin BM, Adair LS, Ng SW. Global nutrition transition and the pandemic of obesity in developing countries. *Nutr Rev* 2012; **70**:3–21.
37. Galtier-Dereure F, Boegner C, Bringer J. Obesity and pregnancy: complications and cost. *Am J Clin Nutr* 2000; **71**:1242S–1248S.
38. Ma J, Prince AL, Bader D, Hu M, Ganu R, Baquero K, Blundell P, Alan Harris R, Frias AE, Grove KL, Aagaard KM. High-fat maternal diet during pregnancy persistently alters the offspring microbiome in a primate model. *Nat Commun* 2014; **5**:3889.
39. Muegge BD, Kuczynski J, Knights D, Clemente JC, Gonzalez A, Fontana L, Henrissat B, Knight R, Gordon JI. Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science* 2011; **332**:970–974.
40. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B et al. Human gut microbiome viewed across age and geography. *Nature* 2012; **486**:222–227.
41. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011; **334**:105–108.
42. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B et al. A core gut microbiome in obese and lean twins. *Nature* 2009; **457**:480–484.

43. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li J et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013; **500**:541–546.
44. Cotillard A, Kennedy SP, Kong LC, Prifti E, Pons N, Le Chatelier E, Almeida M, Quinquis B, Levenez F, Galleron N, Gougis S, Rizkalla S et al. Dietary intervention impact on gut microbial gene richness. *Nature* 2013; **500**:585–588.
45. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**:59–65.
46. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA* 2007; **104**:13780–13785.
47. Li M, Wang B, Zhang M, Rantalainen M, Wang S, Zhou H, Zhang Y, Shen J, Pang X, Wei H, Chen Y, Lu H et al. Symbiotic gut microbes modulate human metabolic phenotypes. *Proc Natl Acad Sci USA* 2008; **105**:2117–2122.
48. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G, Grangette C, Vasquez N et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA* 2008; **105**:16731–16736.
49. Miquel S, Martin R, Rossi O, Bermudez-Humaran LG, Chatel JM, Sokol H, Thomas M, Wells JM, Langella P. Faecalibacterium prausnitzii and human intestinal health. *Curr Opin Microbiol* 2013; **16**:255–261.
50. Furet JP, Kong LC, Tap J, Poitou C, Basdevant A, Bouillot JL, Mariat D, Corthier G, Dore J, Henegar C, Rizkalla S, Clement K. Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes* 2010; **59**:3049–3057.
51. Payne AN, Chassard C, Zimmermann M, Muller P, Stinca S, Lacroix C. The metabolic activity of gut microbiota in obese children is increased compared with normal-weight children and exhibits more exhaustive substrate utilization. *Nutr Diabetes* 2011; **1**:e12–e12.
52. Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, Flint HJ. Human colonic microbiota associated with diet, obesity and weight loss. *Int J Obes* 2008; **32**:1720–1724.
53. Chu DM, Antony KM, Ma J, Prince AL, Showalter L, Moller M, Aagaard KM. The early infant gut microbiome varies in association with a maternal high-fat diet. *Genome Med* 2016; **8**:77.
54. Myles IA, Fontecilla NM, Janelsins BM, Vithayathil PJ, Segre JA, Datta SK. Parental dietary fat intake alters offspring microbiome and immunity. *J Immunol* 2013; **191**:3200–3209.
55. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci USA* 2010; **107**:11971–11975.
56. Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. *Am J Clin Nutr* 2012; **96**:544–551.
57. Thompson AL. Developmental origins of obesity: early feeding environments, infant growth, and the intestinal microbiome. *Am J Hum Biol* 2012; **24**:350–360.
58. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. *Sci Transl Med* 2014; **6**:237ra265.
59. Prince AL, Ma J, Kannan PS, Alvarez M, Gisslen T, Harris RA, Sweeney EL, Knox CL, Lambers DS, Jobe AH, Chougnet CA, Kallapur SG et al. The placental membrane microbiome is altered among subjects with spontaneous preterm birth with and without chorioamnionitis. *Am J Obstet Gynecol* 2016; **214**:627.e1–627.e16.
60. Backhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, Khan MT, Zhang J et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe* 2015; **17**:690–703.
61. Paul HA, Bomhof MR, Vogel HJ, Reimer RA. Diet-induced changes in maternal gut microbiota and metabolomic profiles influence programming of offspring obesity risk in rats. *Sci Rep* 2016; **6**:20683.
62. Vasselli JR, Scarpace PJ, Harris RB, Banks WA. Dietary components in the development of leptin resistance. *Adv Nutr* 2013; **4**: 164–175.
63. Jost T, Lacroix C, Braegger C, Chassard C. Stability of the maternal gut microbiota during late pregnancy and early lactation. *Curr Microbiol* 2014; **68**:419–427.
64. Kalupahana NS, Voy BH, Saxton AM, Moustaid-Moussa N. Energy-restricted high-fat diets only partially improve markers of systemic and adipose tissue inflammation. *Obesity* 2011; **19**:245–254.
65. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998; **394**: 897–901.
66. Zhang X, Zhao Y, Zhang M, Pang X, Xu J, Kang C, Li M, Zhang C, Zhang Z, Zhang Y, Li X, Ning G et al. Structural changes of gut microbiota during berberine-mediated prevention of obesity and insulin resistance in high-fat diet-fed rats. *PLoS One* 2012; **7**:e42529.
67. Roe KL, Barbeau K, Mann EL, Haygood MG. Acquisition of iron by Trichodesmium and associated bacteria in culture. *Environ Microbiol* 2012; **14**:1681–1695.
68. Martinez A, Kolter R. Protection of DNA during oxidative stress by the nonspecific DNA-binding protein Dps. *J Bacteriol* 1997; **179**: 5188–5194.
69. Castruita M, Saito M, Schottel PC, Elmegreen LA, Myneni S, Stiefel EI, Morel FM. Overexpression and characterization of an iron storage and DNA-binding Dps protein from *Trichodesmium erythraeum*. *Appl Environ Microbiol* 2006; **72**:2918–2924.
70. Cepeda-Lopez AC, Aeberli I, Zimmermann MB. Does obesity increase risk for iron deficiency? a review of the literature and the potential mechanisms. *Int J Vitam Nutr Res* 2010; **80**:263–270.
71. Fernandez-Sanchez A, Madrigal-Santillan E, Bautista M, Esquivel-Soto J, Morales-Gonzalez A, Esquivel-Chirino C, Durante-Montiel I, Sanchez-Rivera G, Valadez-Vega C, Morales-Gonzalez JA. Inflammation, oxidative stress, and obesity. *Int J Mol Sci* 2011; **12**:3117–3132.
72. Chung B, Matak P, McKie AT, Sharp P. Leptin increases the expression of the iron regulatory hormone hepcidin in HuH7 human hepatoma cells. *J Nutr* 2007; **137**:2366–2370.
73. Chatterjee S, Ganini D, Tokar EJ, Kumar A, Das S, Corbett J, Kadiiska MB, Waalkes MP, Diehl AM, Mason RP. Leptin is key to peroxynitrite-mediated oxidative stress and Kupffer cell activation in experimental non-alcoholic steatohepatitis. *J Hepatol* 2013; **58**:778–784.
74. Christian P, Mullany LC, Hurley KM, Katz J, Black RE. Nutrition and maternal, neonatal, and child health. *Semin Perinatol* 2015; **39**: 361–372.
75. Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulzke JD, Serino M, Tilg H, Watson A, Wells JM. Intestinal permeability—a new target for disease prevention and therapy. *BMC Gastroenterol* 2014; **14**:189.
76. Menard S, Cerf-Bensussan N, Heyman M. Multiple facets of intestinal permeability and epithelial handling of dietary antigens. *Mucosal Immunol* 2010; **3**:247–259.
77. Leslie JL, Huang S, Opp JS, Nagy MS, Kobayashi M, Young VB, Spence JR. Persistence and toxin production by Clostridium difficile within human intestinal organoids result in disruption of epithelial paracellular barrier function. *Infect Immun* 2015; **83**:138–145.
78. Sheth P, Delos Santos N, Seth A, LaRusso NF, Rao RK. Lipopolysaccharide disrupts tight junctions in cholangiocyte monolayers by a c-Src-, TLR4-, and LBP-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**:G308–G318.
79. Ahrne S, Hagslatt ML. Effect of lactobacilli on paracellular permeability in the gut. *Nutrients* 2011; **3**:104–117.
80. Levin BE, Hogan S, Sullivan AC. Initiation and perpetuation of obesity and obesity resistance in rats. *Am J Physiol* 1989; **256**:R766–R771.
81. Harris RA, Alcott CE, Sullivan EL, Takahashi D, McCurdy CE, Comstock S, Baquero K, Blundell P, Frias AE, Kahr M, Suter M, Wesolowski S

et al. Genomic variants associated with resistance to high fat diet induced obesity in a primate model. *Sci Rep* 2016; **6**:36123.
82. Rabot S, Membrez M, Blancher F, Berger B, Moine D, Krause L, Bibiloni R, Bruneau A, Gerard P, Siddharth J, Lauber CL, Chou CJ. High fat diet drives obesity regardless the composition of gut microbiota in mice. *Sci Rep* 2016; **6**:32484.
83. Pigeyre M, Yazdi FT, Kaur Y, Meyre D. Recent progress in genetics, epigenetics and metagenomics unveils the pathophysiology of human obesity. *Clin Sci* 2016; **130**:943–986.
84. Institute for Health MetricsEvaluation (IHME). *GBD Cause Patterns*. Seattle: IHME, University of Washington; 2013.
85. Gotay CC, Katzmarzyk PT, Janssen I, Dawson MY, Aminoltejari A, Bartley NL. Updating the Canadian obesity maps: An epidemic in progress. *Can J Public Health* 2013; **104**:e64–e68.
86. International Food Policy Research Institute. *Global Nutrition Report 2016: From Promise to Impact: Ending Malnutrition by 2030*. Washington, DC.; 2016.