Intrauterine Growth Restriction Not Only Modifies the Cecocolonic Microbiota in Neonatal Rats But Also Affects Its Activity in Young Adult Rats

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ABSTRACT

Objective: Elucidating why intrauterine growth restriction (IUGR) predisposes to some intestinal pathologies would help in their prevention. Intestinal microbiota could be involved in this predisposition; its initial setup is likely to be altered by IUGR because IUGR delays perinatal intestinal development and strongly interacts with intestinal physiology. Furthermore, because initial colonization determines adult intestinal microbiota, an IUGR-induced defect in initial microbiota would have long-term consequences. Thus, to characterize the effect of IUGR on intestinal microbiota, we compared the composition and activity of cecocolonic microbiota from birth to adulthood in rats with and without IUGR.

Materials and Methods: IUGR was induced by gestational isocaloric protein restriction. Pups were fed by unrestricted lactating mothers. At different ages (days 5, 12, 16, 22, 40, and 100), cecocolonic contents from rats with IUGR and controls were analyzed for concentrations of bacterial end products and numbers of main bacterial groups, and submitted to in vitro fermentation tests.

Results: IUGR affected gut colonization: bacterial density was increased at day 5 and decreased at day 12. In adulthood, rats with IUGR still differed from controls, harboring fewer *Bifidobacterium* sp at day 40 and more bacteria related to *Roseburia intestinalis* at day 100. In vivo, propionate concentration was decreased by IUGR before weaning, whereas the concentrations of other short-chain fatty acids were decreased at day 40, although the in vitro metabolic capability was unaffected overall.

Conclusions: We showed that IUGR induced, per se, some neonatal and long-lasting alterations of the intestinal microbiota. The physiological consequences of these changes and their relation to the predisposing effect of IUGR to gut pathologies must now be explored.

Key Words: fetal programming, intestinal microbiota, intrauterine growth restriction, short-chain fatty acids

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Intrauterine growth restriction (IUGR) is defined as fetal growth less than normal considering the studied population and taking into account the theoretical growth potential of an infant. It often results in the ''small for gestational age'' neonates whose birth weight is below the 10th percentile of the birth weight values for neonates of the same gestational age (1). IUGR increases perinatal mortality (10% of which is a consequence of IUGR) and morbidity (2) and is considered a high risk for a variety of neonatal complications (3). IUGR is also a known risk factor in necrotizing enterocolitis (NEC) (2), which is the most common gastrointestinal emergency of the neonate (4). Understanding the mechanisms underlying this increased susceptibility would help in devising preventive strategies for this intestinal disease.

NEC etiology is complicated with risk factors including prematurity, enteral feeding, and mucosal injury, and NEC pathogenesis remains unclear (4,5). A few observations clearly indicate that intestinal microbiota is crucially involved in the development of the disease: NEC does not occur in germ-free animal models (6); although the occurrence of NEC has not been systematically related to 1 particular pathogen (7), both quantitative and qualitative changes in the fecal microbiota before the onset of NEC have been observed (8,9); NEC can be induced experimentally in animal models using some clostridial species (10); and mucosal injuries similar to those observed in NEC can be induced using colonic infusions of some of the main end products (ie, short-chain fatty acids [SCFA]) resulting from fermentation by the intestinal microbiota (11).

Gut colonization by bacteria and the fermentation activity of the resulting intestinal microbiota may be altered in infants who have experienced IUGR compared with healthy infants because of the effect of IUGR on the small intestine. Indeed, IUGR is known to reduce both the length and the weight of the intestine in humans and in several animal models (12–14) and to alter the development of the small intestinal mucosa (number and length of the villi, number of cells per villus and per crypt) and its proliferative capacity (15,16). IUGR is also reported to reduce the activities of several enzymes, such as disaccharidases and aminopeptidases (17–19), and the absorptive capacities in the duodenum and jejunum of pups during the neonatal period (20). All of these alterations could increase the amount of undigested compounds in the lumen of the small intestine and, consequently, the provision of fermentable substrates to the colonizing bacteria, which is known to modulate the composition and/or activity of the colonic microbiota (21). We therefore hypothesized that IUGR affects neonatal gut colonization by bacteria, which would explain why IUGR predisposes to NEC.

An altered initial colonization of the gut is likely to induce intestinal microbiota dysbiosis throughout adult life. The present assumption arises from the idea that an intestinal microbiota resembling that present in adults is definitively structured between birth and the first years of life, which is the time when the gut evolves from sterility to a highly populated bacterial ecosystem (22). To our knowledge, no appropriate follow-up study demonstrates this assumption in either animal models or humans; however,

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such a long-lasting effect of the initial gut colonization is exemplified by the fact that the initial differences in the fecal microbiota composition, which are induced by the baby delivery mode (ie, vaginal vs caesarean) (23), are still perceived in 7-year-old children (24).

Taking into account that the microbiota is considered a critical factor in colorectal cancer (CRC) development (25), if the scenario of a long-lasting effect of an initial defect in gut colonization is verified, the possible influence of IUGR on initial gut colonization could be responsible for the association between IUGR and a higher risk of developing CRC in later adult life that has recently been suggested by epidemiological studies (26,27). Therefore, we also considered, in this study, the long-lasting effect of IUGR on adult microbiota.

To investigate whether IUGR alters the intestinal microbiota, we characterized the composition and activity of cecocolonic microbiota from birth to adulthood in rats subjected to IUGR compared with rats that had experienced normal intrauterine growth.

MATERIALS AND METHODS

Animal Experiments

Experiments were carried out in accordance with the recommendations of the local Animal Care and Use Committee of Nantes (France). The animal facilities were approved by the government agency in charge of experimental facilities at the French Department of Agriculture, and the investigators were accredited by the National Veterinary Agency. Eight-week-old virgin female and male Sprague Dawley rats (Janvier, Le Genest Saint Isle, France) were caged under standard laboratory conditions with tap water and a maintenance diet (16% protein: A04, Safe, Augy, France) provided ad libitum, in a 12:12 hour light/dark cycle. After 10 days of habituation, female rats were mated overnight with male rats. During the whole gestation (21 days), pregnant dams were fed either a normal protein (NP) diet (20% protein) or an isocaloric low protein (LP) diet (8% protein), both purchased from Arie Block (Woerden, the Netherlands), as described previously (28). At day 19 of gestation (G19), feces from pregnant dams of the 2 groups (NP and LP) were aseptically collected for bacterial quantification. At birth, male pups born to both restricted (pups with IUGR) and normally fed mothers (control pups) were systematically adopted by normally fed mothers (NP) until the end of lactation (8 pups/litter) to limit the growth restriction to the gestational period only. At 21 days of life, pups were separated from the dams (full weaning) and were fed the NP diet until 40 days old, when they received a maintenance diet (A04, Safe) until 100 days old.

A minimum of 8 rats per group (IUGR and control) were sacrificed at days 5, 12, 16, 22, 40, and 100 of postnatal development.

Protocol at Slaughtering and Sampling

The jejunum was removed, cleaned of adhering tissue, and flushed with sterile 0.9% saline to remove luminal contents. Segments were then weighed, frozen in liquid nitrogen, and stored at −80°C until determination of lactase activity.

Pooled cecocolonic contents (days 5 and 12) or individual cecal contents (older ages) were aseptically collected, weighed, and immediately used for in vitro fermentation tests or extracted in Tris-EDTA (Tris 0.05 mol/L, EDTA 0.002 mol/L, pH 8) for 20 minutes at 65°C and then centrifuged (20 minutes, 10,000*g*) before storing the supernatants and pellets at −20°C with a view to end-product quantification and bacteria enumeration, respectively.

Approximately 20 mg of proximal colon wall was cleaned with sterile 0.9% saline, frozen in liquid nitrogen, and stored at −80°C for later mRNA analysis.

Analysis and Measurement

Determination of Lactase Activity

Jejunal segments were thawed and homogenized in distilled water with Polytron at 4°C. The lactase (EC 3.2.1.23) activity was assayed by the method of Dahlqvist (29) using lactose as a substrate. The glucose produced was determined using the glucose-6-phosphate dehydrogenase–hexokine kit (Boehringer, Mannheim, Germany). Lactase activity was then expressed as nanomoles of hydrolyzed substrate per minute per milligram of tissue.

In Vitro Fermentations

In vitro fermentations were miniaturized from Rycroft et al (30). In brief, basal nutrient medium was supplemented (5 mg/mL) with potato protein (KMC, Brande, Denmark) or Raftilose P95 (Beneo-Orafti, Paris, France) and then autoclaved. Inside an anaerobic cabinet ($N_2/CO_2/H_2$: 85/10/5), 0.9-mL aliquots of each medium were inoculated with 0.1 mL of slurries (15%, w/v) prepared by homogenizing the cecocolonic contents from rats in autoclaved sodium phosphate buffer (0.1 mol/L, pH 7). Incubations were carried out inside the anaerobic cabinet at 37°C. After 24 hours of incubation, pH was measured and then incubation media were frozen before analysis of bacterial end products.

Bacterial End-product Analysis

Thawed samples from the luminal contents or in vitro fermentation were centrifuged at 10,000 *g* for 20 minutes. SCFA (acetate, propionate, butyrate, and minors; ie, isobutyrate, valerate, and isovalerate) concentrations were determined in supernatants diluted with 0.5 mol/L oxalic acid and then analyzed by gas chromatography (31). D- and L-Lactate concentrations were determined using an assay kit according to the manufacturer's instructions and adapted to microplates (Biosentec, Toulouse, France).

The concentrations of amines were determined by high-performance liquid chromatography (HPLC). Samples were dansylated according to a protocol modified from Eerola et al (32). In brief, samples were centrifuged for 15 minutes at 10,000*g*. Twenty-five microliters of internal standard DAH (1,7-diaminoheptane), 50 μL of 2N NaOH, and 75 μL of saturated sodium bicarbonate were added to 150 μL of supernatant. Next, 500 μL of freshly prepared dansyl chloride solution (10 mg in 1 mL of acetone) was added. The reaction mixture was incubated at 40°C for 45 minutes in darkness. Then, residual dansyl chloride was removed by adding 25 μL of ammonia. After 30 minutes in darkness, 350 μL of acetonitrile was added and the reaction mixture was centrifuged for 10 minutes at 4000*g*. Dansylated amines were separated by reverse-phase liquid chromatography on a C18 LUNA column (4.6 mm × 250 mm, particle size 5 μm, Phenomenex, Le Pecq, France) with an HPLC apparatus (Waters 600S Multisolvent Delivery System and Waters 2487 Dual λ Absorbance Detector). The gradient elution was carried out with eluent A (ammonium acetate, 0.1 mol/L) and eluent B (acetonitrile). The gradient began with 45% solvent A/55% solvent B and ended with 10% solvent A/90% solvent B after 15 minutes (temperature 40°C, flow rate 1 mL/minute). Putrescine, cadaverine, tyramine, spermidine, and spermine were detected by ultraviolet absorption at 254 nm and quantified using the Galaxie software (Varian, France) and reference calibration curves. Results from the cecocolonic contents or in vitro fermentation were expressed as micromoles per gram of contents or millimoles per liter, respectively.

Enumeration of Bacteria

Bacteriological counts were carried out on DNA extracts from cecocolonic contents using real-time quantitative polymerase

chain reaction (qPCR). DNA was extracted using the QIAamp DNA Stool Mini kit (Qiagen, Courtaboeuf, France) after chemical and mechanical disruptions. Frozen contents (<220 mg) were added to 180 μL of a solution of lysozyme (20 mg/mL) and Triton (1.2%) in Tris-HCl/EDTA (20/2 mmol, pH 8) and then incubated at 37°C for 30 minutes. Approximately 300 mg of zirconium or silica beads (diameter 0.1 mm, BioSpec products, VWR International, Fontenay-sous-Bois, France) and 1.4 mL of ASL buffer from the QIAamp DNA Stool Mini kit were then added before the bacterial suspensions were disrupted in a mini-bead beater (MM301, Retsch GmbH & Co, Haan, Germany) at 30 Hz for 3 minutes. Subsequently, the bacterial DNA was isolated from these suspensions using the kit according to the manufacturer's instructions.

Real-time PCR was conducted using the primers (manufactured by MWG Biotech, Roissy, France) listed in Table 1 and amplification was performed with the iCycler iQ real-time PCR detection system instrument (Biorad Laboratories, Hercules, CA). Reaction mixtures consisted of a total volume of 15 μL containing 7.5 μL of 2X SYBR Green Master Mix (Qiagen), 3.5 nmol of each specific primer, 0.5 μL of a 3-mg/mL bovine serum albumin solution, and 5 μL of bacterial DNA. The conditions for amplification were 1 cycle at 95°C for 15 minutes, followed by 30 to 40 cycles of 15 seconds at 95°C, 60 seconds at annealing temperature (Table 1), and 30 seconds at 72°C.

For each quantified bacterial population, a standard curve was constructed and used to extrapolate the number of copies present in the analyzed samples. For this, pellets from 10-fold successive dilutions of bacterial cultures using reference strains (Table 1) were submitted to DNA extraction, as described above. Bacteria in these cultures were counted using epifluorescence microscopy (×100) after staining with 4′,6-diamidino-2-phenylindole (DAPI). Thus, the results are expressed as $\log_{10}$ of equivalent bacteria per gram of contents.

RNA Isolation and TaqMan Real-Time RT–PCR

RNA was isolated from snap-frozen proximal colon using the NucleoSpin RNA kit (Macherey-Nagel EURL, Hoerdt, France). Total RNA was submitted to DNase digestion following the manufacturer's instructions. Total RNA quality (RNA integrity number higher than 7) was verified using the Bioanalyser 2100 and RNA 6000 Nano Assay Kit and Chips (Agilent Technologies, Massy, France). cDNA was synthesized from 1 μg of total RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Courtaboeuf, France). TaqMan low-density arrays (TLDA, Applied Biosystems) were carried out according to the manufacturer's instructions using the 7900HT Fast Real-Time PCR System (Applied Biosystems). The expression of 6 selected genes involved in epithelial butyrate uptake or metabolism was analyzed (accession numbers and assay IDs are shown in Table 2). Expression levels of target genes were normalized to 3 housekeeping genes (β-actin, Rn00667869_m1; GAPDH, Rn99999916_s1; and β2-microglogulin, Rn00560865_m1) using the median of the 3 housekeeping genes threshold cycle (CT). Gene expression was then calculated using the ΔΔCT method (43) with data normalized to controls.

Statistical Analysis

Statistical analysis was performed using the Statview 5.0 package (SAS Institute, Cary, NC) for most of the statistical tests. For mothers' weight gain on the 19th day of gestation and for pups' birth weight ($n > 30$), normality was verified using a Shapiro-Wilk test (xlstat version 2009.5.01); therefore, data were expressed as means (SEM), and differences among groups were assessed by a parametric t test. For all of the other parameters, data were expressed as medians (first quartile Q1, third quartile Q3) and differences among groups were assessed by a nonparametric Mann-Whitney test. In all of the cases, the differences between means or medians were considered significant at $P < 0.05$ without applying any correction for the large number of tests performed. This choice was made because, in the case of intestinal microbiota, 1 sole parameter being affected is sufficient to demonstrate a general effect on colonic microbiota.

RESULTS

Protein Restriction During Pregnancy-induced IUGR, Low-birth-weight Pups, and Altered Lactase Maturation

Mothers fed an LP diet gained less weight ($P = 0.001$) during gestation (until G19), had a tendency to eat more food ($P = 0.065$) but actually consumed less protein (−57%), and had the same number of pups per litter compared with mothers fed an NP diet (Table 3).

Pups born to mothers fed an LP diet (pups with IUGR) had a significantly lower birth weight (Table 3) compared with control pups ($P \leq 0.0001$). We verified that the median birth weight of pups with IUGR (6.1 g) was lower than the 10th percentile birth weight distribution of the control pups (6.4 g). In both groups, the pup survival rate was 100%.

From early weaning until puberty, rats with IUGR tended to be or were lighter than controls ($P = 0.059$ at day 16; $P = 0.002$ at day 22; and $P = 0.029$ at day 40; Fig. 1); however, whatever their age, the 2 groups of animals did not exhibit different food intakes: from 17.5 and 21.0 g/day for rats with or without IUGR, respectively, at day 35 ($P = 0.593$) to 26.7 and 26.6 g/day for rats with or without IUGR, respectively, at day 95 ($P = 0.521$).

At day 5, the lactase activity (Fig. 2) in the jejunum of rats with IUGR was lower than that in controls ($P = 0.029$). In controls, lactase activity significantly decreased between day 5 and day 16 ($P = 0.001$). In rats with IUGR, the age-induced reduction in lactase activity appeared later (between day 16 and day 22, $P = 0.011$).

Bacterial Quantification in Feces of Pregnant Dams

At G19, the number of total bacteria was higher in the feces of pregnant dams fed the NP diet than in those fed the LP diet (10.08 vs 9.69 $\log_{10}$ eq bact/g of feces, $P = 0.013$). No significant difference between the NP and LP groups was observed when considering the number of *Bifidobacterium* sp (5.66 vs 5.40, $P = 0.247$), *Lactobacillus* sp (6.76 vs 6.59, $P = 0.189$), *Escherichia coli* (7.34 vs 7.43, $P = 0.494$), *Bacteroides* sp (8.52 vs 8.61, $P = 0.674$), *Faecalibacterium prausnitzii* (8.06 vs 8.10, $P = 0.636$), or the number of bacteria from both the clostridial clusters IV (8.56 vs 8.60, $P = 0.713$) and XIVa (9.92 vs 9.63, $P = 0.270$) or from the *Roseburia intestinalis* cluster (6.76 vs 7.05, $P = 0.172$).

Bacterial Quantification in Cecocolonic Contents of Rats During Development

At day 5, the number of *Bacteroides* sp ($P = 0.009$) as well as the number of bacteria from the clostridial cluster IV ($P = 0.009$ and $P = 0.016$, for *Clostridium leptum* cluster and *F prausnitzii*, respectively) and from the *R intestinalis* cluster ($P = 0.009$) were higher in rats with IUGR than in controls (Table 4). The numbers of all other quantified bacteria (total bacteria, *Bifidobacterium* sp, *E coli*, *Lactobacillus* sp, and bacteria from the clostridial cluster XIVa) also tended to be higher in rats with IUGR than in controls at the

TABLE 1. Characteristics of the qPCR assays carried out in the present study

Target	Primers	Sequence (5′-3′)	References	Annealing temperature, °C	Bacterial strain used for calibration	Efficacy (medians ± interquartiles)	Detection limit (log_{10} eq bact/g)
All bacteria (16S)	Uni331modF	TCCTACGGGAGGCAGCAGTG	Modified from (33)	60	Pooled cultures of different bacteria*	101.4 ± 13.2	6.8
	E533modR	TTACCGCGGCTGCTGGCACG	Modified from (34)				
Bifidobacterium sp (16S)	BifRinF	TCGCGTC(C/T)GGTGTGAAAG	(35)	57	Pooled cultures of bifidobacteria**	100.1 ± 13.2	4.7
	BifRinR	CCACATCCAGC(A/G)TCCAC					
Clostridium coccoides/Eubacterium rectale group (16S)	Erec 482aF	CGGTACCTGACTAAGAAGC	(35)	57	*C coccoides* DSM935	93.1 ± 2.5	4.7
	Erec 688R	GTTCCTCCTAATATCTACGC	(36)				
C leptum group (16S)	Sg-Clept-F	GCACAAGCAGTGGAGT	(37)	51	*C leptum* DSM753	79.0 ± 6.1	4.8
	Sg-Clept-R3	CTTCCTCCGTTTTGTCAA					
Bacteroides sp/*Prevotella* sp (16S)	AllBac296F	GAGAGGAAGGTCCCCCAC	(38)	58	*B thetaiotaomicron* CIP 104206	96.8 ± 12.4	4.7
	AllBac412R	CGCTACTTGGCTGGTTCAG					
Escherichia coli (16S)	EcoliPenF	CATGCCGCGTGTATGAAGAA	(39)	60	*E coli* CIP106497	98.1 ± 16.7	4.9
	EcoliPenR	CGGGTAACGTCAATGAGCAAA					
Lactobacillus sp/*Leuconostoc* sp/*Pediococcus* sp (16S)	LAPB0159F	GGAAACAG(A/G)TGCTAATACCG	(40)	54	Pooled cultures of lactobacilli§	80.2 ± 9.8	5.1
	LAPB0677R	CACCGCTACACATGGAG					
Faecalibacterium prausnitzii (16S)	FPR-1F	AGATGGCCTCGCGTCCG	(41)	57	*F prausnitzii* ATCC27768	89.3 ± 0.7	4.0
	FPR2 (Fprau420R)	CCGAAGACCTTCTTCCTCC					
Roseburia intestinalis cluster (16S)	RrecF	GCGGTRCGGCAAGTCTGA	(42)	60	*R intestinalis* DSM14610	115.8 ±14.2	3.6
	Rrec630mR	CCTCCGACACTCTAGTMCGAC					

qPCR = quantitative polymerase chain reaction.

* The strains used were *Eubacterium eligens* DSM3376, *Eub hallii* DSM3353, *Roseburia intestinalis* DSM14610, *Clostridium polysaccharolyticum* DSM1801, *C coccoides* DSM935, *C leptum* DSM753, *Faecalibacterium prausnitzii* ATCC27768, *Ruminococcus albus* DSM20455, *Bacteroides capillosus* ATCC29799, *B thetaiotaomicron* CIP 104206, *Prevotella melaninogenica* CIP 105346, *Collinsella intestinalis* CIP106914, *Bifidobacterium longum* ATCC15707, and *Escherichia coli* CIP106497.

** The strains used were *B longum* ATCC15707, *B bifidum* CIP 56.7, *B catenulatum* CIP 104175, *B angulatum* CIP 104167, *B breve* CIP 64.69, *B infantis* CIP 64.67, *B pseudocatenulatum CIP* 104168, and *B pseudolongum* ATCC 25526.

§ The strains used were *Lactobacillus ruminis* CIP 106135, *L gasseri* CIP 103699, *L reuteri* CIP 101887, and *L intestinalis* CIP 10479.

TABLE 2. mRNA expression of some genes involved in butyrate uptake or metabolism by TLDA in proximal colon tissue from animals with IUGR compared with controls

Gene	Gene description	Accession no.	Assays ID	mRNA expression							
				Day 12		Day 22		Day 40		Day 100	
				-Fold change	*P*	-Fold change	*P*	-Fold change	*P*	-Fold change	*P*
Slc16a1	Solute carrier family 16, member 1 (MCT1)	NM_012716.2	Rn00562332_m1	−1.10	NS	**−1.60**	**0.0374**	1.09	NS	−1.57	NS
Slc16a4	Solute carrier family 16, member 4 (MCT4)	NM_001013913.1	Rn01522015_m1	1.45	NS	1.82	NS	ND	ND	NR	
Slc5a8	Solute carrier family 5, member 8 (SMCT1)	XM_576209.2	Rn01503812_m1	2.80	NS	**−11.03**	**0.0176**	1.50	NS	−4.13	NS
Bsg	Basigin (CD147)	NM_012783.3	Rn00562874_m1	−1.15	NS	−1.36	0.0547	1.10	NS	−1.31	NS
Bucs1	Butyryl-CoA synthetase 1 (EC 6.2.1.2)	NM_001108502.1	Rn01506809_m1	NR		3.38	0.0782	ND	ND	NR	
Hmgcs2	3 Hydroxyl-3-methylglutaryl-CoA synthetase 2	NM_173094.1	Rn00597339_m1	1.08	NS	−1.15	NS	ND	ND	−1.02	NS

Results are shown in -fold change compared with control group at each day (n = 6 animals per group and per age). Significant differences between rats with IUGR and controls ($P < 0.05$, Mann-Whitney test) are shown in bold. IUGR = intrauterine growth restriction; ND = not determined; NR = not relevant, mRNA expression was not detected in any of the animal groups; NS = nonsignificant; TLDA = TaqMan low-density arrays.

same age. Despite these changes, neither the relative proportions of each bacterial group nor the Gram-positive to Gram-negative ratio (Table 4) nor the aerobes to anaerobes ratio (data not shown) were affected by IUGR.

From day 5 to day 12, the density of the microbiota strongly increased in both groups of rats, because we observed an increased number of total bacteria and of every bacterial group enumerated. In rats with IUGR, however, this increase was less pronounced than in controls, which ended up with a decrease in the numbers of total bacteria ($P = 0.001$), of *Bifidobacterium* sp ($P = 0.045$), of bacteria from the clostridial clusters IV and XIVa ($P = 0.022$ and $P = 0.001$), and of the relative proportion of the clostridial clusters XIVa (11.2 vs 4.4% of total bacteria, $P = 0.003$) in rats with IUGR at day 12. The ratio between facultative anaerobic and anaerobic bacteria was higher in rats with IUGR compared with controls (9.0 vs 28.5, $P = 0.011$) at this age.

At later stages of development, IUGR induced several modifications in cecal microbiota. A higher number of *Lactobacillus* sp ($P = 0.021$) and a lower number and proportion of *E coli* ($P = 0.037$ and 12.7 vs 43.9% of total bacteria, $P = 0.036$) were seen at day 16 compared with controls. Consequently, the Gram-positive to Gram-negative ratio increased in rats that experienced IUGR (32.3 vs 3.2, $P = 0.036$) at this age. At day 22, bacteria from the clostridial cluster XIVa ($P = 0.027$ for both *Clostridium coccoides* and *R intestinalis* clusters) increased, whereas both *Bifidobacterium* sp ($P = 0.093$) and the aerobes to anaerobes ratio (3.5 vs 1.1, $P = 0.054$) tended to be decreased by IUGR. At day 40, rats subjected to IUGR were still distinguishable from controls with respect to the number of *Bifidobacterium* sp, which was reduced ($P = 0.007$), the relative proportion of *Bacteroides* sp, which was increased (7.5 vs 5.1 %, $P = 0.018$), and the Gram-positive to Gram-negative ratio, which was decreased (10.9 vs 18.9, $P = 0.035$). Finally, 100 days after birth, an increased number ($P = 0.040$) of bacteria from the *R intestinalis* cluster was observed in rats that had experienced IUGR.

Quantification of Fermentation and Putrefaction End Products in Cecocolonic Contents

In rats with IUGR, the concentrations of total SCFA, acetate, propionate, and minor SCFA were reduced at day 12 ($P = 0.048$, $P = 0.097$, $P = 0.006$, and $P = 0.064$, respectively; Fig. 3). The concentration of propionate was decreased in cecocolonic contents of rats with IUGR compared with controls at day 16 ($P = 0.051$) and had a tendency to be lower at day 22 and day 40 ($P = 0.093$ and 0.072). At day 40, acetate and minor SCFA concentrations were diminished ($P = 0.015$ and $P = 0.007$, respectively), whereas butyrate concentration was halved ($P < 0.001$) in rats with IUGR compared with controls.

Whatever the rat group or age considered, spermine was not detected (<0.05 μmol/g of contents) and spermidine only occurred as traces (<0.1 μmol/g of contents) in cecocolonic contents. The concentrations of total amines (Fig. 3) putrescine, cadaverine, and tyramine decreased drastically between d16 and d22 ($P \leq 0.0001$) and remained under the threshold of quantification until d100. IUGR had no influence on the concentration of any of the quantified amines.

In Vitro Production of Fermentation and Putrefaction End Products

IUGR affected neither the pH acidification of the oligofructose and protein incubation media nor the production of acetate, minor

TABLE 3. Effect of dietary protein restriction during gestation on maternal weight gain and food intake, litter size, and pups' birth weight

	Mother fed	
	NP[†] diet (n = 34)	LP[†] diet (n = 33)
Weight gain on the 19th day of gestation, g[‡]	119.0 (3.5)	101.4 (3.3)[**]
Food intake through gestation, g[§]	371.6 (349.2–405.6)	397.6 (357.2–440.7)
No. pups per litter[§]	14 (12–14)	14 (12–15)
Birth weight of pups, g[‖]	6.86 (0.05)	6.19 (0.05)[***]

[†] NP = normal (20%) protein or LP = low (8%) protein diet.
[‡] Results are shown as mean (SEM).
[§] Results are shown as median (1st quartile; 3rd quartile).
[‖] Results are shown as mean (SEM) for n = 75 pups born to NP-diet mothers and n = 73 pups born to LP-diet mothers.
[***] $P < 0.001$ LP vs NP diet (*t* test).
[¶] $P < 0.0001$ LP vs NP diet (*t* test).

SCFA, and lactate from oligofructose or protein at day 12, day 40, or day 100 (Fig. 4). The production of butyrate from oligofructose or from protein in the presence of the microbiota from rats with IUGR was higher than that obtained in the presence of the control rats' microbiota at day 12 ($P = 0.004$ and $P = 0.055$, respectively). At day 100, propionate production from protein media was reduced by IUGR ($P = 0.054$). Whatever the rat group or age considered, spermine was not detected (<0.01 μmol/L) and spermidine occurred only as traces (<0.02 μmol/L of medium) in incubated media. IUGR did not alter putrescine or cadaverine production from protein incubation media but significantly reduced tyramine production at day 40 and day 100 ($P = 0.025$ and $P = 0.025$).

Expression of Genes Related to Butyrate Transport and Metabolism

At day 22, IUGR decreased or tended to decrease the expression of different genes related to butyrate uptake (Table 2). This was true for monocarboxylate transporter 1 (MCT1, $P = 0.037$), sodium monocarboxylate transporter 1 (SMCT1, $P = 0.018$), for basigin (*Bsg*, $P = 0.055$). Conversely, the metabolism of butyrate appeared to be stimulated in rats with IUGR compared with controls because the expression of butyryl-CoA synthetase 1 (*Bucs1*) tended to be higher ($P = 0.078$) in the former. At day 5, day 40, and day 100, IUGR did not induce any change in the expression of the genes studied.

DISCUSSION

We have hypothesized that IUGR modifies gut colonization by bacteria and has a long-lasting influence on the adult microbiota. First, the follow-up we have carried out to address these hypotheses provided complementary data about gut colonization in rat pups and illustrated that, although occurring with different kinetics, it reproduced the pattern that has been described for humans. With respect to our specific aim, our data support both of our hypotheses: between birth and early weaning, IUGR strongly affected the density and, to a lesser extent, the composition of the cecocolonic microbiota, and induced some changes in its metabolic activity as assessed both in vivo and in vitro. In later

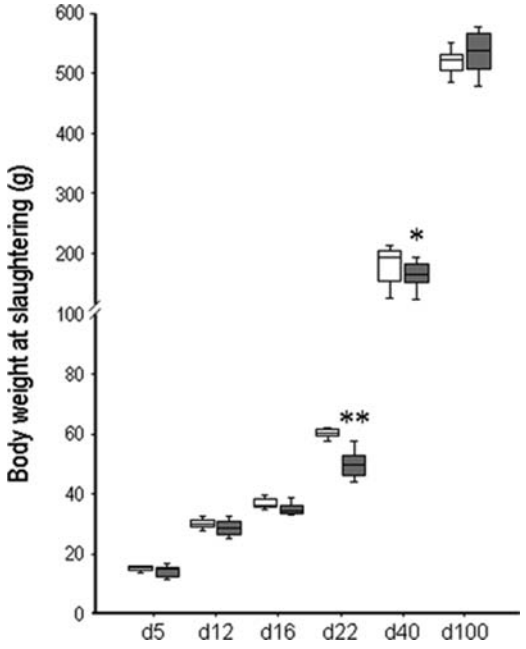

FIGURE 1. Body weight at slaughtering of rats with intrauterine growth restriction (gray boxes) and controls (white boxes). Boxes represent the 1st, 2nd, and 3rd quartiles, and whiskers show the 10th and 90th percentiles. $^{*}P < 0.05$, $^{**}P < 0.01$ rats with intrauterine growth restriction versus controls (Mann-Whitney test).

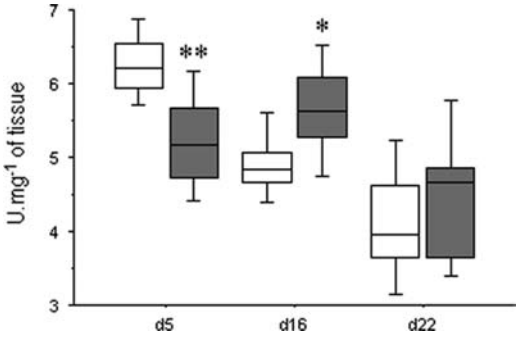

FIGURE 2. Lactase activity maturation in jejunum of rats with intrauterine growth restriction (gray boxes) and controls (white boxes). Data are shown in box plot and expressed in micromoles of substrate hydrolyzed per milligram of tissue. $^{*}P < 0.05$, $^{**}P < 0.01$ rats with intrauterine growth restriction versus controls at the same age (Mann-Whitney test).

TABLE 4. Bacterial quantification in cecocolonic content of rats that had experienced IUGR compared with CTL throughout development

	Age, days	5	12	16	22	40	100
All bacteria	CTL	8.6 (8.5–8.7)	11.5 (11.4–11.5)	11.2 (11.0–11.6)	11.4 (11.3–11.5)	11.6 (11.3–11.7)	11.3 (11.1–11.5)
	IUGR	8.9 (8.8–9.4)	11.2 (11.0–11.4)	11.3 (11.2–11.4)	11.5 (11.3–11.5)	11.6 (11.4–11.7)	11.4 (11.2–11.6)
	P	0.094	**<0.001**	0.674	0.371	0.767	0.753
Bifidobacterium sp	CTL	<4.7	5.5 (4.7–6.3)	6.1 (6.0–6.3)	6.5 (6.0–7.0)	6.6 (6.4–6.8)	8.0 (7.2–8.5)
	IUGR	4.7 (4.7–4.8)	4.7 (4.7–5.0)	6.1 (6.1–6.3)	5.7 (5.6–6.4)	5.4 (5.1–5.9)	7.9 (7.7–8.4)
	P	0.054	**0.045**	0.495	0.093	**0.007**	0.674
Lactobacillus sp/*Leuconostoc* sp/*Pediococcus* sp	CTL	8.3 (7.0–9.0)	10.8 (10.1–11.8)	11.3 (10.3–11.6)	11.1 (10.8–11.4)	7.7 (7.3–7.9)	9.3 (9.2–9.5)
	IUGR	9.2 (8.5–9.7)	10.1 (10.0–11.0)	11.7 (11.7–11.7)	10.9 (10.5–11.3)	7.8 (7.5–8.4)	9.4 (9.1–9.6)
	P	0.175	0.124	**0.021**	0.401	0.339	0.834
Escherichia coli	CTL	8.5 (8.4–8.7)	11.2 (10.7–11.4)	10.9 (10.7–11.0)	9.6 (9.4–10.0)	8.8 (8.3–9.1)	7.6 (7.4–7.9)
	IUGR	8.8 (8.7–9.4)	10.9 (10.4–11.2)	10.3 (9.8–10.7)	9.9 (9.7–10.0)	9.0 (8.8–9.2)	7.4 (7.2–7.8)
	P	0.076	0.291	**0.036**	0.401	0.410	0.494
Clostridium leptum group (clostridial cluster IV)	CTL	5.2 (5.0–5.3)	9.2 (8.1–9.7)	9.3 (8.9–9.5)	9.6 (9.4–9.7)	10.3 (10.1–10.7)	10.2 (10.0–10.4)
	IUGR	5.7 (5.6–5.9)	8.3 (7.9–8.5)	9.5 (9.0–9.7)	9.5 (9.0–9.9)	10.3 (9.9–10.6)	10.2 (10.0–10.3)
	P	**0.009**	**0.022**	0.294	0.834	0.742	0.834
C coccoides/Eubacterium rectale group (clostridial cluster XIVa)	CTL	6.9 (6.6–7.1)	10.5 (10.3–10.7)	10.4 (10.1–10.5)	10.4 (10.3–10.6)	11.6 (11.4–11.8)	11.3 (11.1–11.6)
	IUGR	7.2 (7.1–7.4)	9.7 (9.3–10.2)	10.5 (10.3–10.6)	10.7 (10.6–11.0)	11.5 (11.3–11.6)	11.4 (11.1–11.8)
	P	0.076	**<0.001**	0.345	**0.027**	0.448	0.674
Bacteroides sp/*Prevotella* sp	CTL	6.0 (5.8–6.1)	8.8 (6.9–9.2)	9.5 (9.4–9.9)	10.2 (9.9–10.4)	10.2 (10.0–10.5)	10.0 (9.8–10.2)
	IUGR	6.6 (6.4–6.6)	7.5 (6.2–9.0)	9.4 (9.2–9.4)	10.2 (10.0–10.5)	10.6 (10.2–10.6)	9.9 (9.6–10.1)
	P	**0.009**	0.228	0.128	0.430	0.129	0.462
Roseburia intestinalis cluster	CTL	4.5 (4.5–4.8)	8.8 (7.0–9.9)	9.6 (9.2–9.9)	6.1 (4.8–6.5)	9.4 (9.0–10.1)	8.0 (7.7–8.5)
	IUGR	5.3 (5.1–5.5)	7.7 (6.5–9.2)	9.4 (8.9–9.8)	7.3 (6.5–9.2)	8.9 (6.0–9.7)	8.8 (8.3–9.3)
	P	**0.009**	0.258	0.462	**0.027**	0.156	**0.040**
Faecalibacterium prausnitzii	CTL	5.2 (5.0–5.3)	6.1 (5.4–9.7)	7.9 (8.4–9.0)	8.8 (8.8–9.0)	8.8 (8.5–9.0)	8.1 (8.0–8.4)
	IUGR	5.8 (5.7–5.9)	5.8 (6.1–7.3)	7.2 (6.8–9.4)	8.6 (8.2–9.1)	9.0 (8.1–9.8)	8.1 (7.7–8.6)
	P	**0.016**	0.663	0.528	0.318	0.468	0.636
Gram-positive/Gram-negative ratio	CTL	0.9 (0.1–3.2)	2.7 (0.2–10.3)	3.2 (0.6–8.5)	7.8 (4.5–11.9)	18.9 (12.4–34.2)	22.8(21.0–25.9)
	IUGR	1.1 (0.8–2.8)	0.2 (0.1–2.6)	32.3 (7.3–58.0)	8.5 (2.9–14.3)	10.9 (7.4–21.0)	54.9 (19.4–67.9)
	P	0.602	0.083	**0.036**	0.674	**0.035**	0.142

Results are expressed as $\log_{10}$ of equivalent bacteria per gram of cecocolonic content. Values are medians, and the 1st and 3rd quartiles are parenthesized. *P* values result from comparison between rats with IUGR versus controls using Mann-Whitney test, and significant differences ($P < 0.05$) are shown in bold. CTL = controls; IUGR = intrauterine growth restriction.

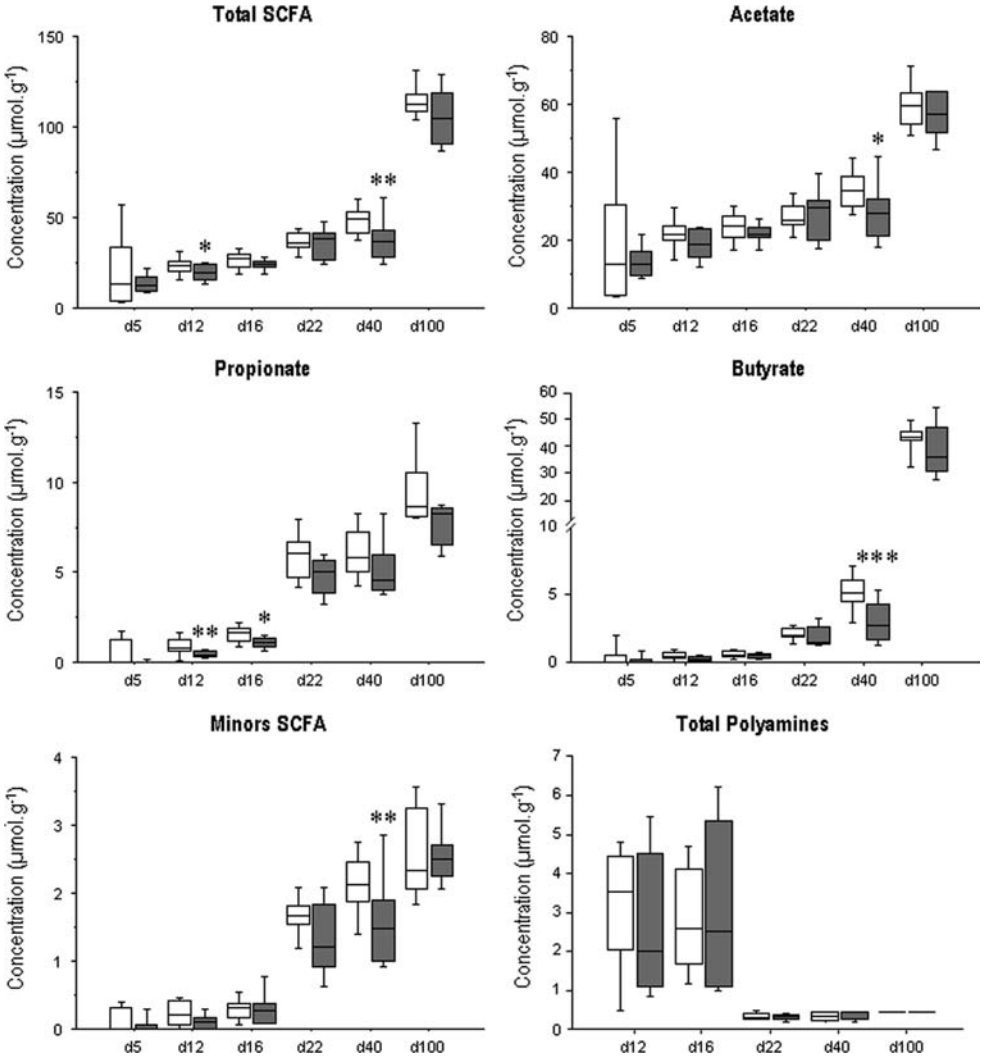

FIGURE 3. Concentrations of fermentation and putrefaction end products in cecocolonic contents of rats with intrauterine growth restriction (gray boxes) and controls (white boxes) during postnatal development. Data are shown in box plot and expressed in micromoles of end product per gram of cecocolonic content. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ rats with intrauterine growth restriction versus controls at the same age (Mann-Whitney test).

life (sexual maturity and adulthood), rats with IUGR were still distinguishable from controls with respect to intestinal microbiota characteristics. Such findings raise 2 main questions: what are the causes of these differences and what could be their physiological consequences for the host?

Intestinal Microbiota Maturation in Control Pups

In normal-growing rats, we observed that intestinal bacterial density markedly increased between days 5 and 12 and then stabilized, and that its composition, which was initially limited to enterobacteria (approximately 55% of total bacteria), lactobacilli (approximately 43%) together with a low level of bacteria from the clostridial cluster XIVa (approximately 2%), progressively evolved to become steadily dominated by bacteria from the clostridial clusters XIVa and IV (approximately 90% and 6%, respectively) and *Bacteroides* sp (approximately 4%) after day 22. These changes were paralleled by the progressive acquisition by the microbiota of the capability to produce propionate (from day 12) and then butyrate and minor SCFA (from day 16), which seems logical because these end products are not synthesized by enterobacteria or lactobacilli.

Although taking into account many more bacterial groups and species, particularly some poorly cultivable ones such as clostridial clusters XIVa and IV, the *R intestinalis* cluster, and *F prausnitzii*, our findings largely agree with the scarce published data related to gut colonization in newborn rats, which are based on quantitative bacterial culture (44–46) or on a qualitative culture-independent method (47). Indeed, these studies have shown that bacterial density increases from birth to early weaning (44), and that bacterial diversity evolves (45–47) with compositional changes being typified by a decrease in the fecal numbers of enterobacteria and—at least transitorily—of lactobacilli, and an increase in the *Bacteroides* and *Clostridium* sp detection frequencies occurring during breast-feeding and/or as weaning progresses (45,46). In contrast to the present work, none of these previous studies specified that, in newborn rats, these compositional changes result in a progressive complication of the metabolic activity of the intestinal microbiota. Postweaning

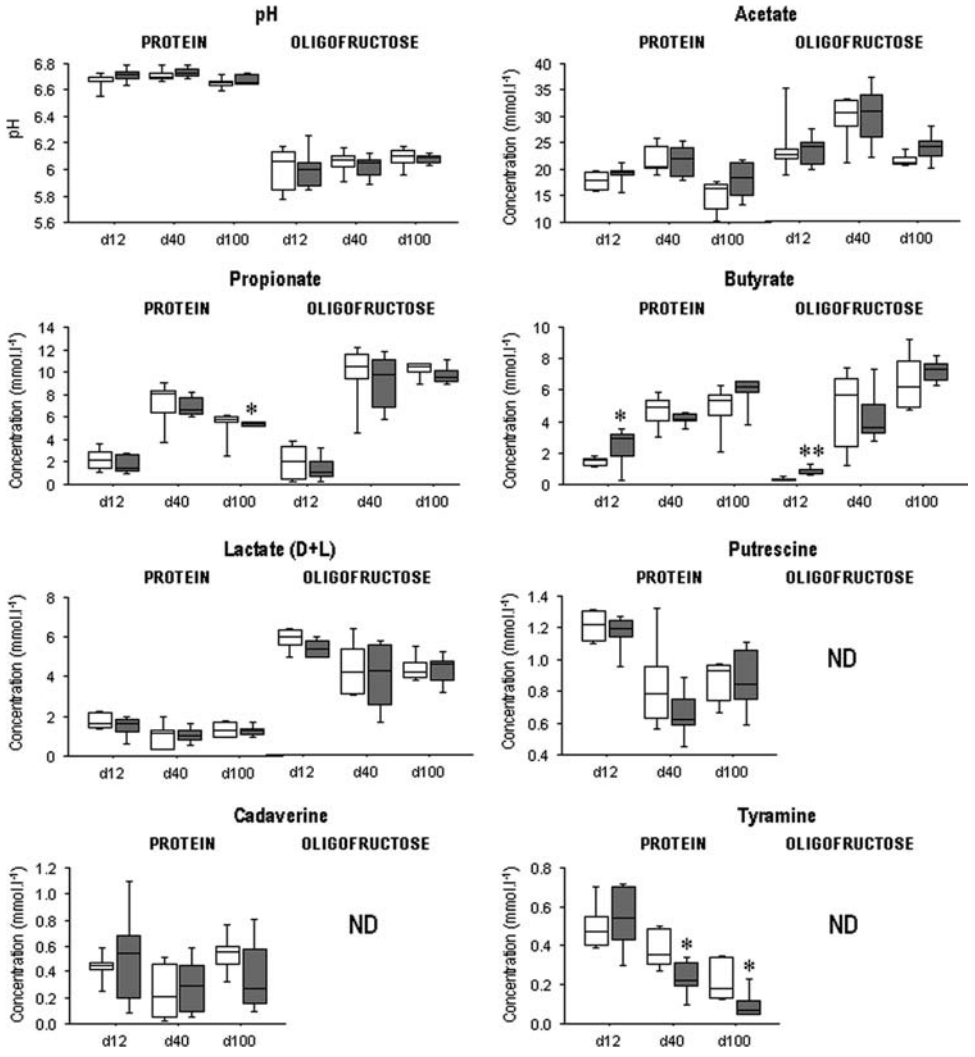

FIGURE 4. Concentrations of fermentation and putrefaction end products in oligofructose or protein media after an in vitro incubation of 24 hours in the presence of the microbiota from rats with intrauterine growth restriction (gray boxes) or from controls (white boxes). Data are shown in box plot and expressed in millimoles of end product per liter of incubation medium. $^{*}P < 0.05$, $^{**}P < 0.01$ rats with intrauterine growth restriction versus controls at the same age (Mann-Whitney test). ND = not determined.

follow-up carried out in rats suggested that intestinal microbiota with a composition resembling that of adult rats was acquired between the third and the fourth week of life (46,47).

When comparing the intestinal microbiota maturation observed in rats to that reported for humans, it appears that they are mostly similar, except that the total number of bacteria takes longer to reach its maximal level in rats than in neonates. Indeed, in full-term neonates, the total number of fecal bacteria is similar to that in adults by the end of the first week of life (48), whereas the maximal level is attained in more than 2 weeks in our rats. With this exception, the evolution of microbiota composition observed by us and previous authors before and during weaning is comparable in both rat and full-term neonates. The change from a microbiota dominated by aerobes toward one largely dominated by anaerobes, together with the diversification of its metabolic potential, reflect phenomena already described as occurring in the first 2 years of neonate life (22,48). Thus, despite the known differences between human and rat microbiotal composition (49), such a similarity confers to our findings relevance for human neonates.

Influence of IUGR on Intestinal Microbiota

More specifically, our study also describes for the first time the maturation of the intestinal microbiota in animals subjected to IUGR. Although this growth restriction probably affects bacterial gut colonization due to the changes it induces in the small intestine (see Introduction), such a topic has not yet been considered. The main reason for this is probably that most IUGR neonates also experience prematurity (1), which thus impedes the distinction of the specific IUGR effect on the microbiota from that of preterm birth and its subsequent medical care (50).

In the present study, we have considered both the immediate effects [soon after birth (day 5), before weaning (day 12), at early weaning (day 16), and once weaning is completed (day 22)], and the long-term influences [sexual maturation (day 40) and adult life (day 100)] because adult intestinal microbiota is thought to be definitively structured in the neonatal period (22,48). To assess changes in the intestinal microbiota, we have quantified the numbers of the main bacterial groups harbored in the colon and the luminal concentrations

of the main bacterial end products. We have also estimated the metabolic potential of cecocolonic microbiota by in vitro fermentation tests. Each of these parameters individually enables a change in cecocolonic microbiota to be demonstrated [eg, (51)].

The first effect we observed was that, for all of the enumerated bacterial groups except lactobacilli, the number of bacteria either tended to be or was higher in pups with IUGR compared with control pups at day 5. Because conversely, the bacterial density was lower in feces from LP dams compared with NP ones at the end of gestation, this increase is unlikely to result from higher bacterial transfers during delivery. Consequently, and considering the unspecific character of this increase, such an effect is likely to reflect the effect of IUGR on the overall accessibility to bacterial colonization, which may result from the modulation of factors favoring bacterial colonization. Among these factors are pH, intestinal motility, salt concentration, intestinal production of bactericidal molecules, type of epithelial glycoconjugates, redox potential of the intestinal lumen, and amount of available substrates (22). IUGR may affect most of these factors, considering its influence on the anatomy and activity of the small intestine already described (see Introduction). From our own data showing that lactase activity was reduced in day 5 pups with IUGR, it appears that IUGR could increase the overall bacterial density by increasing the amount of lactose because supplementing a continuous culture of intestinal microbiota with lactose has already been shown to stimulate bacterial proliferation (52).

Conversely, at day 12, the numbers of total and of some individual bacteria (*Bifidobacterium* sp, bacteria from clostridial clusters IV and XIVa) were lower in pups with IUGR than in control pups, indicating that the extent of bacterial colonization, which dramatically increased between day 5 and day 12 (ie, from 8.9 to 11.2 log on average), was reduced in pups that had been subjected to IUGR. Interestingly, this influence particularly affected strict anaerobes, which are supposed to require lowered oxygen tension and oxidation-reduction potential before being capable of colonizing the gut (22). This suggests that anaerobiosis would be reduced in IUGR, which could compare with the intestinal oxidative stress previously proposed in IUGR piglets (53). This could also be related to the IUGR-induced impairment of the colonic mucosa maturation because we have previously observed that the expression of mucin 2 (Muc2) and trefoil factor family 3 (Tff3) is reduced in rats with IUGR compared with control pups at d12 (28). Considering both the involvement of mucins in bacterial adhesion and proliferation (54) and the fact that mucolytic activity has been described mainly for anaerobes (55), a higher production of mucins in control pups compared with pups with IUGR could explain the observed higher numbers of bacteria in control pups.

When pups became older, some further IUGR-induced changes in the composition of the microbiota occurred, but, because these changes affected different bacterial populations at the different ages studied, no constant feature could be identified. Differences in colonic mucosa characteristics, which partly persist after weaning in rats that have experienced IUGR (28), may be responsible for such changes, but this can only be hypothetical due to the lack of knowledge about the specific factors controlling the colonization capability of each single bacterial group.

Together with these compositional changes, we observed some effects of IUGR on the cecocolonic concentrations of bacterial end products. Before weaning (day 12), they were typified by a decrease in propionate concentration and, long after weaning (day 40), by a decrease in acetate, butyrate, and minor SCFA concentrations. No further influence was noticed once the animals were moved to a maintenance diet (from day 41 to day 100).

Two phenomena can lead to in vivo changes in SCFA concentrations: either an increase in the use of SCFA by the cecocolonic mucosa or an alteration of the capability of the microbiota to produce SCFA.

Increases in SCFA use by the mucosa would involve an increase in either SCFA uptake or SCFA metabolism. Because butyrate is the main SCFA metabolized by the cecocolonic mucosa (56), we focused the testing of this possibility on butyrate. Thus, we measured the colonic gene expression of 2 enzymes involved in butyrate metabolism [butyryl-CoA synthetase 1 (EC 6.2.1.2) and 3 hydroxyl-3-methylglutaryl-CoA synthetase 2], of the 2 main colonic transporters for SCFA, MCT1, and SMCT1 (57,58), and of *Bsg* (CD147), a member of the immunoglobulin superfamily that facilitates proper localization of MCT1 at the cell surface and which is critical for butyrate transport activity (59). None of these gene expressions was stimulated in the colon of rats with IUGR at the time when the cecocolonic concentration of butyrate was decreased. This finding argues against the role of a favored absorption of SCFA in rats with IUGR compared with controls.

An alteration in the capability of the microbiota to produce SCFA may result from either the inherent characteristics of the microbiota or an insufficient or unbalanced provision of substrate. Differences in the inherent characteristics of the microbiota cannot explain all of the changes we observed. Indeed, whereas at day 12 the decrease in propionate concentration matched the lower numbers of bacteria from clostridial clusters IV and XIVa, which include numerous propionate-producing bacteria (60), this was no longer true at day 16. Similarly, the decrease in butyrate luminal concentration, which occurred at day 40, cannot be explained by the influence of IUGR on the microbiotal composition that we observed. Indeed, it was not paralleled by any decrease in the numbers of bacteria from the *R intestinalis* cluster or of *F prausnitzii*, which include most of the cecocolonic butyrate-producing bacteria (61). The sole compositional difference we detected was that *Bifidobacterium* sp was decreased by IUGR at this age. Although this genus is capable of producing acetate and lactate as major end products (60), 2 organic acids that have been identified as precursors for butyrate synthesis by other intestinal bacteria (61), the low number in which it occurs in rats (approximately 6.5 $\log_{10}$ eq bact/g) makes it unlikely to be responsible for the observed decrease in acetate and butyrate concentration. In theory, the decrease in luminal butyrate concentration could also result from a difference related to a bacterial group not considered (eg, bacteria from clostridial cluster I). This is possible, even though those we enumerated accounted for approximately 100% of the total bacteria detected at day 40. Such a difference would have induced differences in SCFA production observed after in vitro incubation in similar conditions. Yet, we did not demonstrate any influence of IUGR on the in vitro capability of the microbiota to produce SCFA.

Finally, besides the modulation of SCFA production by bacterial cross-feedings already mentioned (62), microbiotal capability to produce SCFA is also known to be affected both quantitatively and qualitatively by the amount, delivery rate, and N/C ratio of the medium or the ileal content that is provided (63,64). Because food consumption from day 22 to day 100 did not differ between rats that had experienced IUGR and controls, it has to be postulated that IUGR affected 1 of these parameters through the modulation of the orocecal transit time or of food digestibility in the small intestine, thus leading to differences in luminal SCFA concentrations.

Potential Physiological Repercussions of the IUGR-induced Microbiotal Changes

The intestinal microbiota is now recognized as playing a key role in numerous physiological processes, including growth, angiogenesis, optimization of nutrition, and stimulation of various arms of the innate and adaptive immune systems (65).

Therefore, any change in microbiota density, composition, and/or activity is likely to affect the health of the host. The present knowledge is far too insufficient to establish a straight link between microbiota composition and physiological effects. Thus, the health consequences of the changes we observed can only be speculative.

In our rat model, IUGR increased the bacterial density in the first days of life and the aerobes to anaerobes ratio just before weaning. Although bacterial overgrowth is frequently proposed as a risk factor for bacterial translocation (7), we did not observe any concomitant effect of IUGR on bacterial translocation to the spleen (data not shown). The delay of gut colonization by strict anaerobes could be perceived as beneficial because the opposite situation has been described in preterm neonates experiencing NEC (8,66). Nevertheless, because anaerobes include both opportunistic pathogens and the usual commensal intestinal bacteria, this change can hardly be interpreted with regard to gut physiology. In any case, considering the crucial role of intestinal bacteria in gut maturation (65), the changes we have observed are likely to affect the intestinal mucosa and intestinal immune system.

IUGR-induced compositional changes of microbiota occurring after weaning mainly concerned *Bifidobacterium* sp (decreased at day 40) and clostridial cluster XIVa or 1 of its components, the *R intestinalis* cluster (both increased at day 22 and at day 100 for the latter). Bifidobacteria are often said to be beneficial for health, mainly because of the extrapolation of biological or physiological effects induced by some bifidobacterial strains used as probiotics. The supposed health benefits of bifidobacteria mainly deal with immunomodulation (67). Therefore, the diminished number of bifidobacteria observed in young adults that have experienced IUGR could be interpreted as a weakening event. Similarly, the physiological consequences of increased numbers of bacteria from clostridial cluster XIVa may be discussed. On the one hand, this bacterial group has been associated with CRC or polyposis (25); on the other hand, the stimulation of these bacteria is now sometimes advised (21) because they include butyrate producers. Butyrate is indeed known to have numerous beneficial biological properties; thus, it is considered advantageous for the host (56), except for newborns (11). Butyrate is the major energy source for intestinal epithelial cells and stimulates the colonic defense barrier, thus enhancing protection against adverse luminal molecules (56). The expression of some genes involved in butyrate uptake was decreased at day 22 and luminal butyrate was decreased at day 40 in rats that had been subjected to IUGR. Such deficits in butyrate bioavailability could have a negative influence on the proliferation of colonocytes and the maintenance of colonic homeostasis by modulating the permeability (68), modulating the expression of some intestinal transcripts involved in barrier function (69), and reducing the secretion of mucins (70).

In conclusion, we have demonstrated that IUGR, per se, induced changes in intestinal microbiota composition and/or activity, and that these changes, although differing according to the age of the animal, persisted throughout its life. Considering the interplay between intestinal bacteria and health of the host and, more particularly, the possible physiological influences of the IUGR-induced modifications on butyrate production, the intestinal microbiota probably plays a role in the long-term health consequences of IUGR.

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