Donor human milk treated by high-pressure processing improves the body growth of growth-restricted mice pups

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ORIGINAL ARTICLE

Nutrition

Donor human milk treated by high-pressure processing improves the body growth of growth-restricted mice pups

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Abstract

Introduction: Pasteurized human donor milk (DM) is frequently used for feeding preterm newborns and extrauterine growth-restricted (EUGR) infants. Most human milk banks performed a pasteurization of DM using the standard method of Holder pasteurization (HoP) which consists of heating milk at 62.5°C for 30 min. High hydrostatic pressure (HHP) processing was proposed to be an innovative nonthermal method to pasteurize DM. However, the effect of different modes of DM pasteurization on body growth, intestinal maturation, and microbiota has never been investigated in vivo during the lactation.

Objectives: We aimed to study these effects in postnatally growth-restricted (PNGR) mice pups daily supplemented with HoP-DM or HHP-DM.

Methods: PNGR was induced by increasing the number of pups per litter (15 pups/mother) at postnatal Day 4 (PND4). From PND8 to PND20, mice pups were supplemented with HoP-DM or HHP-DM. At PND21, the intestinal permeability was measured in vivo, the intestinal mucosal histology, gut microbiota, and short-chain fatty acids (SCFAs) level were analyzed.

Results: HHP-DM pups displayed a significantly higher body weight gain than HoP-DM pups during lactation. At PND21, these two types of human milk supplementations did not differentially alter intestinal morphology and permeability, the gene-expression level of several mucosal intestinal markers, gut microbiota, and the caecal SCFAs level.

Conclusion: Our data suggest that HHP could be an attractive alternative to HoP and that HHP-DM may ensure a better body growth of preterm and/or EUGR infants.

KEYWORDS

DM, EUGR, HoP, HHP

Laure Dubernat and Augustin Lefevre are co-first authors. Delphine Ley and Jean Lesage are co-senior authors.

1 | INTRODUCTION

According to the World Health Organization, 15 million infants are delivered prematurely each year throughout the world.[1] After birth, these infants are frequently affected by extrauterine growth restriction (EUGR) which refers to insufficient growth during hospitalization and represents a significant clinical challenge. The incidence of EUGR is very high in preterm infants and it has been reported to affect 40% to 85% of them.[2] Human milk is the gold standard for the feeding of these vulnerable infants. In addition to its adapted nutritional content, human milk also contains many bioactive compounds that help to establish an optimal body growth and maturation of numerous biological functions of these fragile infants.[3] Human milk banks (HMBs) provide human donor milk (DM) as an alternative for the feeding of preterm infants when mother's own milk is not available or is in short supply to meet their nutritional requirements. To ensure the microbiological safety of DM, most HMBs performed a pasteurization of DM using the standard method of Holder pasteurization (HoP). HoP consists of heating milk at 62.5°C for 30 min in pasteurizers.[4] In the last decade, numerous studies have reported that HoP reduces numerous important heat-sensitive bioactive factors of DM.[5–8] Thus, for better preservation of DM properties, the European Association of Human Milk Banks (EMBA) has recommended to research and implement innovative processing methods for the sterilization of DM.[9]

Recent studies have demonstrated that high hydrostatic pressure (HHP) processing may be one of the best innovative nonthermal methods to pasteurize DM. For example, it was demonstrated that a moderate HHP protocol (four cycles of 5 min at pressure of 350 MPa, performed at 38°C) maintains the nutritional value of DM as well as various bioactive compounds such as immunoglobulins, lactoferrin, lysozyme, the bile salt-dependent lipase (BSSL), milk oligosaccharides and several hormones such as insulin, leptin, apelin, and glucagon-like peptide-1 (GLP-1) close to their initial levels in untreated DM.[5,6,8,10,11] These in vitro studies suggest that pasteurization through HHP might be more appropriate than HoP for the treatment of DM to ensure a better health and development of preterm infants. However, so far, no studies have evaluated in vivo the effects of different modes of DM pasteurization on perinatal development using experimental models or clinical studies.

We recently demonstrated that adult mice supplemented during 7 days with HHP-DM have a reinforcement of their intestinal barrier integrity and a better intestinal antioxidant defense as well as a reduced expression of inflammatory markers compared to adult mice supplemented with HoP-DM.[12,13] This suggests that DM pasteurization through HHP might be more beneficial for the intestinal health and maturation of preterm infants than the use of HoP to pasteurize DM. The aim of the present study was to test this hypothesis in a rodent model of EUGR during the early postnatal period of lactation. This model of EUGR was induced by increasing the number of pups per litter (15 pups/mother) during lactation. In previous studies, we demonstrated that these developing growth-restricted pups showed a drastic early postnatal growth restriction (PNGR), a delayed intestinal maturation and an increased intestinal permeability and pro-inflammatory state that augmented their susceptibility to DSS-induced chronic colitis in adulthood.[14,15] These physiopathological consequences observed in this experimental model are close to those observed in preterm/EUGR infants which are also affected by an early PNGR, an intestinal immaturity and an increased susceptibility to inflammatory bowel diseases.[16,17] In the present study, growth-restricted mice pups were supplemented by daily gavages from postnatal Day 8 (PND8) to PND20 with HoP-DM or HHP-DM. Their body weights were measured during lactation and, at weaning, segments of their intestine (jejunum, ileum, and colon) were analyzed (by means of histology and assessment of gene-expression levels of some cellular tight junctions, caecal short chain fatty acids (SCFAs) levels and microbiota composition). Finally, the intestinal paracellular permeability was measured in vivo after an oral administration of fluorescein isothiocyanate (FITC)-dextran.

What is Known

- Pasteurization of human donor milk (DM) with Holder pasteurization (HoP) was shown to reduce numerous bioactive factors.
- High hydrostatic pressure (HHP) processing is an innovative nonthermal method to pasteurize human DM.
- HHP processing maintains the nutritional value of human DM as well as numerous bioactive factors close to their levels in untreated DM.

What is New

- A daily supplementation with HoP-DM or HHP-DM to suckling mice pups is well tolerated.
- Growth-restricted mice pups display an improved body growth when supplemented with HHP-DM compared to HoP-DM supplementation.
- No differential intestinal effects including microbiota were observed between HHP-DM or HoP-DM mice pups.

2 | STUDY DESIGN/METHODS

2.1 | Milk collection and processing

Frozen DM samples from 10 donors were provided by the regional HMB (Lactarium Régional de Lille, Jeanne de Flandre Children's Hospital, CHU Lille). The study protocol was approved by the ethics committee of the Groupe Francophone d'Hépatologie, Gastroentérologie et Nutrition Pédiatrique (2023–51). Donors provided written and informed consent for the use of their milk (DM samples were collected between 02/2023 and 03/2023). Two batches of pooled DM were created by mixing DM samples. One batch was subjected to HoP according to the standard pasteurization protocol (62.5°C for 30 min) in the HMB of Lille's hospital and the second batch was subjected to HHP processing as previously described.[10] The set of HHP parameters was as follows: pressure = 350 MPa, temperature = 38°C, number of cycles = four cycles of 5 min. Aliquots of HoP-DM and HHP-DM were stored at –20°C until used for oral supplementations of mice pups.

2.2 | Animals and experimental design

All experimental protocols for animal study were approved by the regional Institutional Animal Care Ethics Committee (approval ID: #25481-2020050415255797). Eight-week-old females and males FVB/NRj mice (Janvier Labs) were maintained under standard conditions (12:12-h light-dark cycle) with ad libitum access to standard food (SAFE® diets R04-25) and water. The animal study was carried out in a specific pathogen free facility. After 7 days of acclimatation, female mice were mated with males during 7 days. Once pregnant, dams were housed individually and fed with a breeding diet (SAFE® diets R03-25, Augy, France) until the end of lactation. PNGR was induced by increasing litter size to 15 pups per litters as previously described at PND4.[14] From PND8 to PND20, all pups were daily supplemented (orogastric administration), with DM (5 mL/kg) processed by HoP or HHP ($n = 5$ litters/group; Figure 1A). DM was administered using a lubricated polyethylene tube (0.3 mm diameter) mounted on a 30G needle as previously detailed.[15] Mice pups were weighted every second day between PND4 and PND20 and at weaning (PND21). Regardless of its sex, the same supplementation was administered for each pup within a litter but only males were further analyzed to avoid sex-related outcome variance. At PND21, male pups were euthanized (HHP-DM group: $n = 33$; HoP-DM group: $n = 36$). Jejunum, ileum, and colon were excised and divided in two segments: one segment was fixed in 4% paraformaldehyde

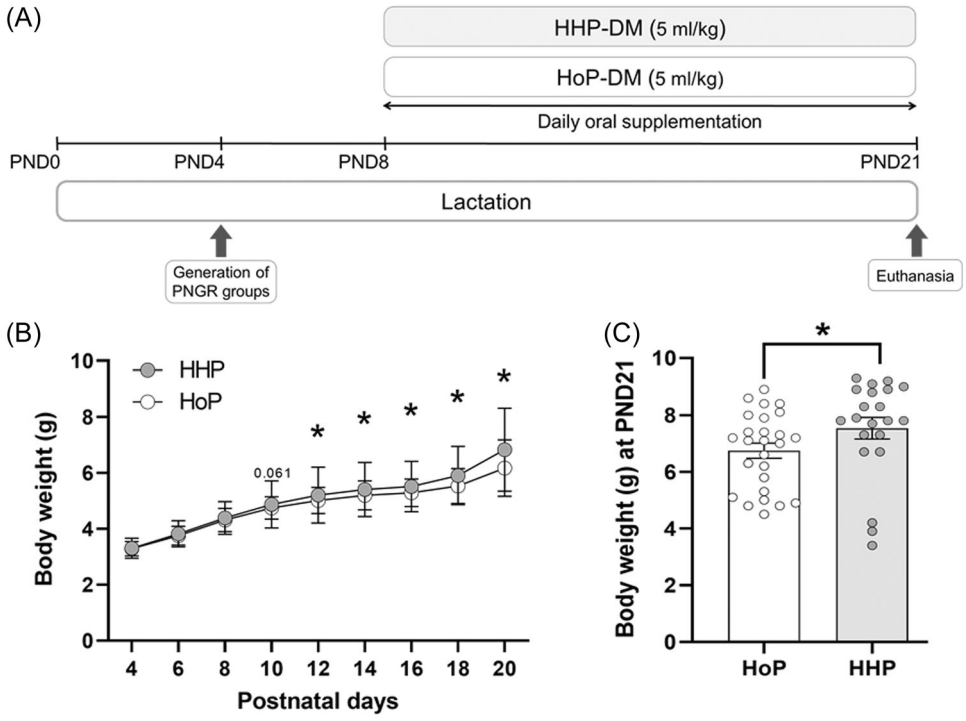

FIGURE 1 HHP-DM supplemented pups present a higher body weight gain during lactation compared to HoP-DM supplemented pups. (A) Experimental design of the study. (B) Body weight curves for HoP-DM and HHP-DM supplemented mice pups ($n = 33$–36 pups; *$p < 0.05$ HHP vs. HoP). (C) Body weight at PND21 ($n = 33$–36 pups; *$p < 0.05$ HHP vs. HoP). DM, donor milk; HHP, high hydrostatic pressure; HoP, Holder pasteurization; PND21, postnatal Day 21.

overnight and embedded in paraffin for histological analysis; the second segment was flash frozen and stored at −80°C for quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) analysis. Caecal and fecal contents were weighed, frozen, and stored at −80°C.

2.3 | Intestinal barrier permeability

At PND21, after an overnight fast, some pups ($n = 10$/group) were orally administered with a solution of 4 kDa FITC-dextran (440 mg/kg body weight in PBS, FD4, Sigma-Aldrich). Blood was collected by cardiac puncture after 4 h in anesthetized animals. Plasma FITC-dextran fluorescence was measured by fluorometry at 485 nm (FLUOstar Omega microplate reader, BMG Labtech) to measure intestinal paracellular permeability as previously described.[14]

2.4 | Intestinal histology

Hematoxylin and eosin (HE) staining was performed on 4-µm thick paraffin embedded intestinal sections. Using ImageJ Software (NIH), jejunal and ileal villus height and crypt depth as well as colonic mucosa thickness and colonic crypt depth were determined in a blinded manner by two independent observers. A total of eight pups per group were analyzed ($n = 1$–2 males/litter).

2.5 | Real-time quantitative polymerase chain reaction (RT-qPCR)

Total ribonucleic acid (RNA) was extracted from frozen jejunal, ileal, and colonic tissues ($n = 12$/group) using RNeasy Mini Kits (Qiagen). Reverse transcription was performed using iScript kit (Bio-Rad Laboratories). Using specific primers (Table S1), mRNA levels of several mucosal and cellular markers were quantified with SYBR Green (Thermo Fisher Scientific) in a StepOne system (Thermo Fisher Scientific). Intestinal transcripts expression was analyzed by the $2^{-\Delta Ct}$ method using the mean of beta-actin and GAPDH as reference genes as previously described.[15]

2.6 | Fecal microbiota

Fecal microbiota composition was analyzed in PND21 animals ($n = 12$/group). Genomic DNA was extracted using a QIAamp DNA Stool Mini Kit (Qiagen), according to the manufacturer's instructions, including a bead-beating step. The V4 region of bacterial 16S rRNA gene was amplified using the primers 515F (GTGYCAGCGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT). Purified amplicons were sequenced using a MiSeq following the manufacturer's guidelines. Sequencing and demultiplexing was performed at MR DNA (www.mrdnalab.com; Shallowater). Sequences were processed using QIIME2 (version 2023.2).[18] The pipeline included Primer removal and Denoising using DADA2 to obtain the amplicon sequence variant (ASV) table.[19] Singletons (ASV present < 2 times) were discarded. Sequences were clustered based on a 0.99-percentage identity and chimeras were removed using the UCHIME algorithm (implemented in QIIME's vsearch plugin). Taxonomic classification was performed using a pre-trained naive Bayes classifier implemented in QIIME2 against the SILVA 138 reference database (silva138_AB_V4_classifier.qza).[20] Reads classified as mitochondria and chloroplast were filtered out while unassigned ASVs are retained. Taxa that could not be identified on genus-level are referred to the highest taxonomic rank identified. Alpha-diversity indexes (Shannon and Faith) and beta diversity index (Bray Curtis) were analyzed using Kruskal–Wallis and permutational multivariate analysis of variance statistical test, respectively.

2.7 | Caecal SCFAs content

Caecal contents ($n = 12$/group) were homogenized in 1.5 mL of NaOH at 0.005 M including internal standards (Acetate-D3, Propionate-D2, and Butyrate-13) using Precellys equipment. Total DNA was extracted following the next steps: 300 µL of supernatant were collected and 500 µL of propanol/pyridine mix (3:2 v/v) were added and then vortexed. Using propyl chloroformate, SCFAs were derivatized for chromatography mass spectrometry (GC/MS) and extracted using 0.5 mL of hexane. SCFAs were quantified by GC/MS using an ISQ LT™ equipped with a Triplus RSH (Thermo Fisher Scientific) and a fused-silica capillary column with a (5%-phenyl)-methylpolysiloxane phase (DB-5ms, J&W Scientific, Agilent Technologies Inc.) of 50 m × 0.25 mm i.d coated with 0.25-µm film thickness as previously described.[15]

2.8 | Statistical analysis

Statistical analysis was performed with GraphPad Prism 9.0. software. Grubb's test was used to detect any outliers. Normality of variables was evaluated by a D'Agostino-Pearson test. Statistical differences were

tested by Mann–Whitney or *t*-test according to sample normality assessment results. A p-value < 0.05 was considered significant.

3 | RESULTS

3.1 | PNGR pups displayed an improved body growth when supplemented with HHP-DM than pups supplemented with HoP-DM

From PND12 to PND21, mouse pups supplemented with HHP-DM displayed a significantly higher body weight gain than pups supplemented with HoP-DM (Figure 1B,C).

3.2 | Supplementation with HoP-DM or HHP-DM did not differentially affect intestinal morphology and permeability at PND21

No significant differences in the morphology (villus height, crypt depth, and/or mucosa thickness) in jejunal (Figure 2A), ileal (Figure 2B), and colonic (Figure 2C) segments were observed between experimental groups. Similarly, gene-expression levels of several tight junction proteins (Claudin-3, Claudin-4, Claudin-7, Occludin, and Zonula Occludens-1) were not different between groups in jejunum (Figure 2D), ileum (Figure 2E) and colon (Figure 2F). In addition, mRNA levels coding for mucin-2 (Figure 2G), lactase (Figure 2H), and sucrase-isomaltase (Figure 2I) were

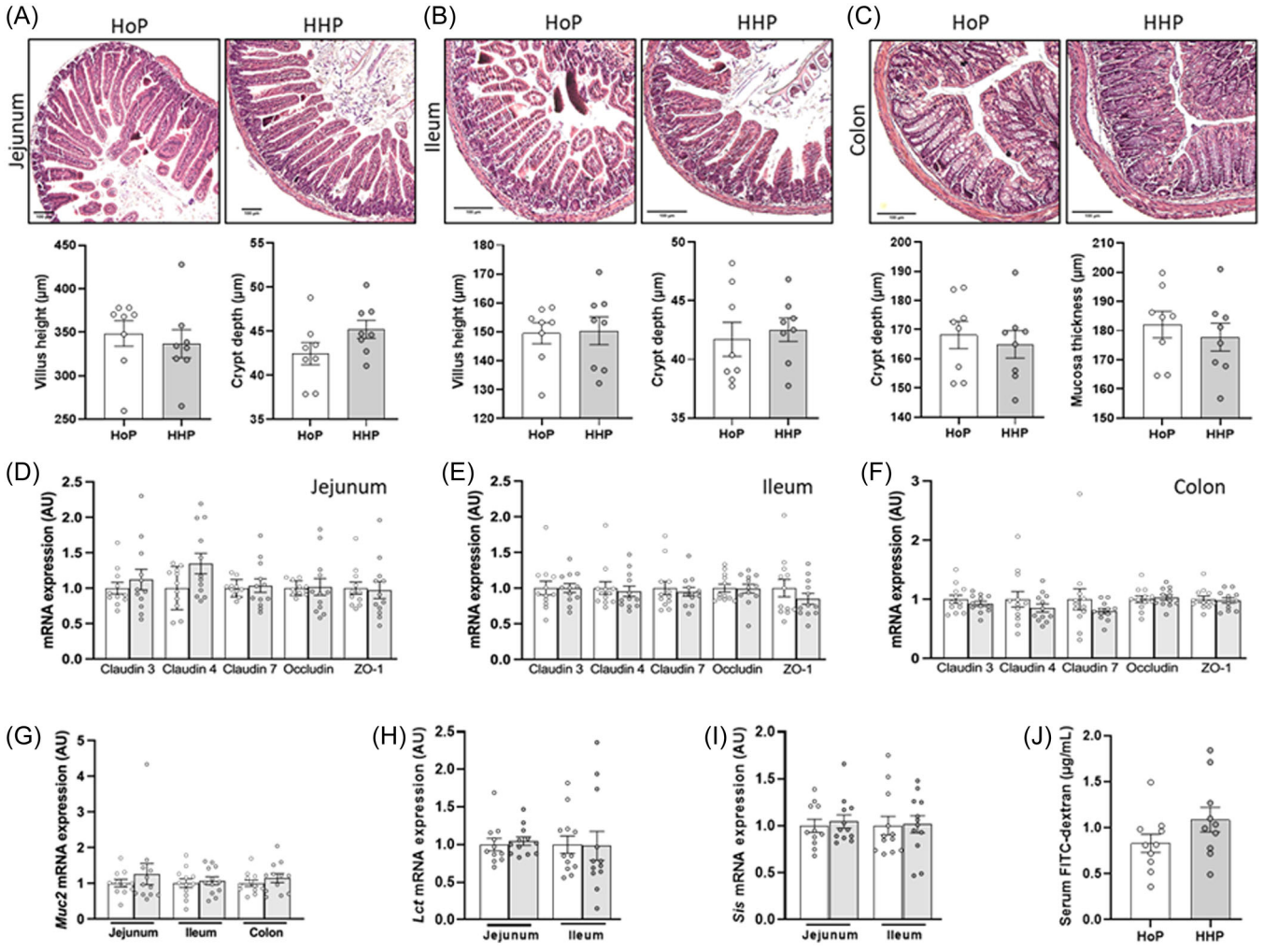

FIGURE 2 Intestinal morphology and markers in HoP-DM or HHP-DM pups at PND21. Representative hematoxylin and eosin staining of villus height crypt depth and/or mucosa thickness in jejunal (A), ileal (B), and colonic (C) segments from HoP-DM or HHP-DM supplemented pups ($n = 8$/group). (D) mRNA expression level of tight junctions in jejunal, ileal (E), and colonic (F) segments at PND21 ($n = 12$/group). (G) Mucin 2 (Muc2), (H) lactase (Lct), and (I) sucrase-isomaltase (Sis) mRNA expression level in intestinal segments ($n = 11$–12/group). (J) In vivo measurement of the paracellular permeability to FITC-dextran in both groups ($n = 10$/group). DM, donor milk; HHP, high hydrostatic pressure; HoP, Holder pasteurization; mRNA, messenger RNA; PND21, postnatal Day 21.

not different between groups. Finally, no differences between groups were observed for the intestinal paracellular permeability measured in vivo after FITC-dextran administration (Figure 2J).

3.3 | Supplementation with HoP-DM or HHP-DM did not differentially affect microbiota and SCFAs levels of PND21 pups

The analysis of the individual taxonomic relative abundance distribution of fecal microbial communities at the family level for each pup revealed no drastic differences in microbial profile between pups from the two experimental groups (Figure 3A). The beta diversity meaning the variation of gut bacteria between groups was analyzed by a principal coordinate analysis (PCoA). The PCoA plot based on the Bray–Curtis dissimilarity distances showed close similarities between the microbial composition from the two groups with an overlapping clustering (Figure 3B). Alpha-diversity indexes (Shannon and Faith's phylogenetic diversity [PD]) were analyzed to investigate the richness and evenness of gut bacteria between treatments. Shannon alpha diversity is sensitive to both the richness (total number of species in the community) and the evenness (relative abundance of different species). Faith's PD represents the number of phylogenetic tree-units within a sample. No significant differences of bacterial community diversity (Shannon, Figure 3C) and phylogenetic distance between operational taxonomic units (OTUs) in each group (Faith's PD index, Figure 3D) were found between groups. Finally, a comparable caecal level of the three major SCFAs (butyrate, acetate, and propionate) was found between HoP-DM and HHP-DM pups (Figure 3E–G).

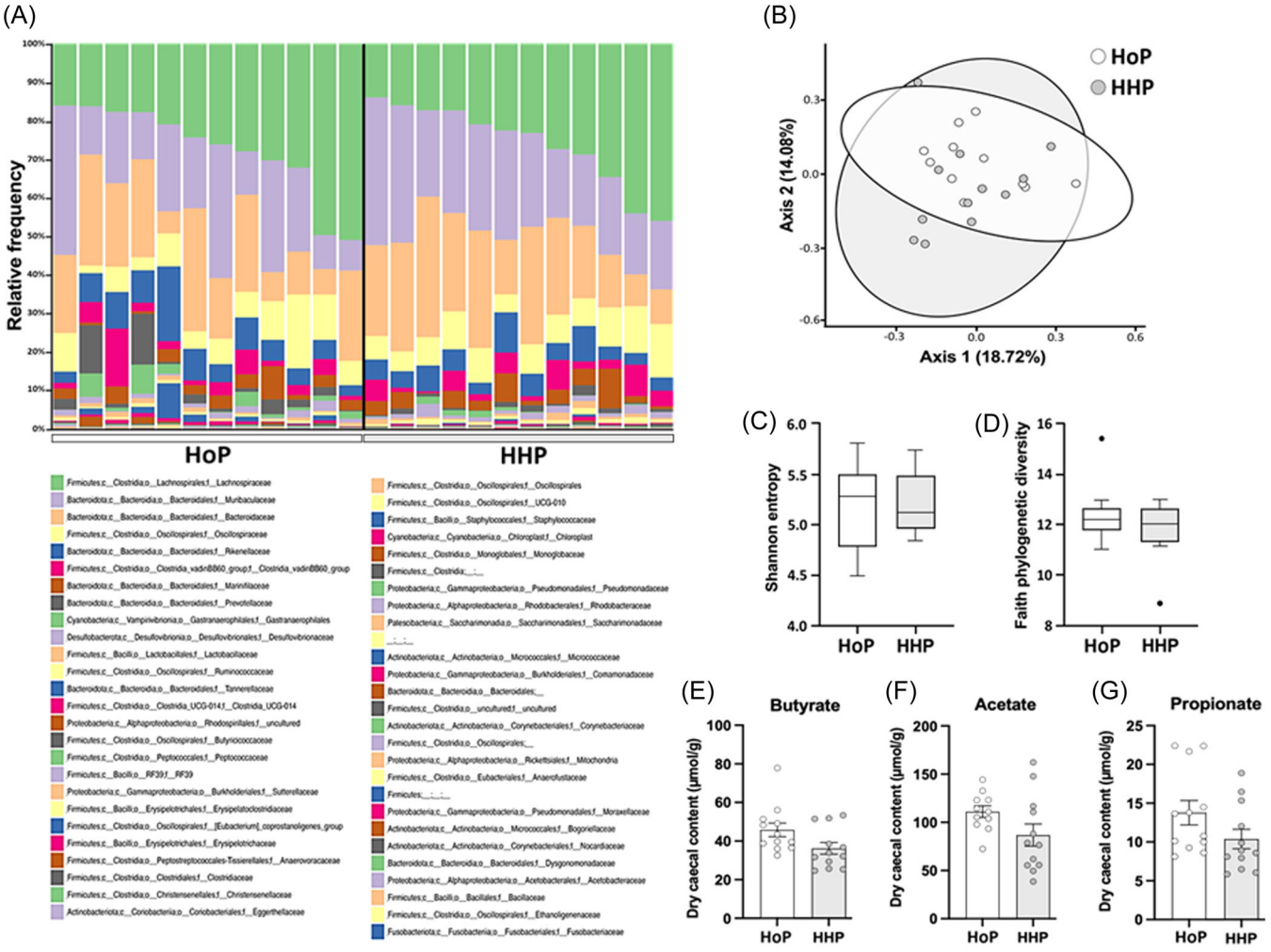

FIGURE 3 Differential DM processing method did not affect microbiota and SCFAs. (A) Individual taxonomic relative abundance distribution of fecal microbial communities at the family level for each pup ($n = 12$/group). (B) Principal coordinates analysis plot representing beta diversity as Bray–Curtis distances for the fecal microbiota of pups at PND21. (C) Distribution of Shannon entropy measures in fecal samples ($n = 12$/group). (D) Faith's phylogenetic diversity measures in fecal samples ($n = 12$/group). Butyrate (E), acetate (F), and propionate (G) concentrations in caecal samples of both groups ($n = 12$/group). DM, donor milk; HHP, high hydrostatic pressure; HoP, Holder pasteurization; PND21, postnatal Day 21; SCFAs, short-chain fatty acids.

4 | DISCUSSION

In this study, we explored for the first time in vivo the effect of HoP-DM or HHP-DM in growth restricted pups during lactation. Our study demonstrates that HHP-DM supplementation improves the postnatal growth of mice pups compared to HoP-DM supplementation. HHP-DM supplementation, compared with HoP-DM, did not alter the intestinal morphology and paracellular permeability, as well as the gene-expression level of several mucosal intestinal markers, gut microbiota, and caecal SCFAs levels including butyrate, propionate, and acetate.

In HMBs, the current recommended treatment for DM is HoP which requires heating milk at 62.5°C for 30 min followed by rapidly cooling to less than 10°C.[4,6] Although this time–temperature combination effectively inactivates most bacterial and some viral contaminants, HoP negatively affects the level of numerous bioactive milk compounds as well as some enzymatic activities including lipase and amylase enzymes.[21] For example, for these two types of enzymes, a complete degradation by HoP has been demonstrated for lipoprotein lipase and BSSL,[5–8,10,21,22] while amylase activity was partially retained in DM after this type of pasteurization.[22] It was thus postulated that a reduced absorption of nutrients through feeding with pasteurized DM rather than untreated DM, especially for lipids, may occur in infants fed with HoP-DM. To prevent these alterations, optimizing new thermal methodologies or applying non-thermal ones to DM are needed. HHP at mild temperature is used in the food industry to achieve the microbial decontamination of foods for up to 30 years.[23] We and others have demonstrated that this non-thermal method is able to pasteurize DM and to prevent the degradation of numerous important bioactive factors such as immunoglobulins, lactoferrin, BSSL, milk oligosaccharides, and several hormones.[5,6,8,10,11,24] In the present study, we observed that a daily supplementation with HHP-DM to growth-restricted mice pups improves their weight gain during lactation compared to HoP-DM supplementation without affecting the intestinal physiology nor fecal microbiota and cecal SCFAs levels. In accordance, in adult mice supplemented during 7 days with HHP-DM and compared to mice supplemented with HoP-DM, we recently demonstrated that these treatments did not affect caecal SCFAs levels and microbiota.[12] However, differential effects were found in these adult mice for some markers of the intestinal barrier integrity and on intestinal paracellular permeability.[12] Indeed, we observed that mice supplemented with HHP-DM had a reinforcement of their intestinal barrier integrity in the small intestine (measured in vivo after FITC-dextran administration) as well as an increased expression of some ileal tight junctions (Occludin, Cadherin-1) and of mucin 2. However, in their colons, opposite variations were found in these adult HHP-DM supplemented mice with a reduction of Cadherin-1 and tight junction protein-1 (Tjp1) expressions as well as for mucin 2 and mucin 4.[12] Thus, compared to present findings, we demonstrate that a supplementation with sterilized human DM has different intestinal effects when administered to adult mice or to developing mice pups during lactation which is a sensitive period for gut maturation processes.[14] Indeed, during the lactation period, the intestine of mice pups is very permeable and the intestinal barrier is not fully mature (low mucus, low tight-junctions' expressions, small intestinal villi in the small intestine). There is also a particular protein transport in enterocytes (vacuolated villus enterocytes) which was described in a previous study of our group in the PNGR model.[14] The microbiota is also not fully established as in adult. On the other hand, it is also known that several milk compounds have maturational effects on the intestine of pups (hormones, growth factors, oligosaccharides, milk metabolites). For all these reasons, it makes sense to observe large differential intestinal effects when mice are supplemented with human milk in adulthood or during the early postnatal period.

The only significant and differential effect of DM supplementation in the present study is an effect on body growth as HHP-DM pups displayed an improved body growth than HoP-DM pups. The question thus remains: what is the mechanism implicated in this positive effect of HHP-DM on body growth?

Several hypotheses can be postulated. At first, the nutritional value of HoP-DM and HHP-DM may be different. Piemontese et al.[25] demonstrated, using several hundred of DM samples, that HoP reduced macronutrient composition, especially in terms of lipids. This effect was proposed by Vincent et al.[26] to be attributed to the adherence of disrupted milk fat globules to container surfaces and to whether thermal treatment took place in pasteurizers. We recently confirmed these findings in a lipidomic and metabolomic study comparing HoP-DM and HHP-DM.[5] We found that multiple lipid classes were reduced by HoP, including fatty acids (FAs), some monoacylglycerols (MAGs), diacylglycerols (DAGs), medium-chain fatty acids, phosphatidyl-serine, and phosphatidyl-choline compounds.[5] However, in this previous study, we observed that HHP protocol also reduced multiple lipids such as DAGs, MAGs, phosphatidylcholine, and phosphatidylethanolamine compounds, as well as some medium chain FAs, lysophospholipids, and sphingomyelins whereas some ceramides were increased.[5] These changes in lipids may implicate the preservation of active endogenous lipases in HHP-DM that may play a role in these modifications. Finally, we also demonstrated that HoP and HHP treatments of human donor milk differentially affected several other classes of metabolites/nutrients including some amino acids, carbohydrates, and nucleotides that may have

nutritional and functional consequences in the control of the pup's body weight.[5]

A second hypothesis is that nutrients from HoP-DM or HHP-DM may be absorbed with different kinetics and/or capacity by mice pups. For example, as BSSL remains active in HHP-DM,[10] we can postulate that milk triacylglycerols and diacylglycerols can be hydrolyzed and absorbed faster and more efficiently metabolized in HHP-DM pups. For carbohydrates, we showed in a previous study that a chronic gavage with HoP-DM and HHP-DM differentially modulates the velocity of intestinal contractions and glucose metabolism in adult mice.[27] Indeed, we found that HHP-DM significantly reduced the amplitude of intestinal contractions in the duodenum and ileum which absorbed large amounts of glucose, but also that HHP-DM mice displayed an improve glucose tolerance through the modulation of the gut–brain axis activity.[27] Thus, a differential effect of HoP-DM and HHP-DM on the kinetic and capacity of intestinal nutrient absorptions and/or on the modulation of the gut-brain axis activity and carbohydrates metabolism may also be implicated in the differences observed here for pup's body weight.

Finally, a third hypothesis could involve milk hormones and the regulation of the energetic metabolism in mice pups. We previously showed that HoP-DM displayed reduced concentrations of numerous hormones including insulin, nesfatin-1, cortisol, leptin, apelin, and GLP-1 whereas in HHP-DM, all of these hormones were preserved from degradation.[8] These metabolic hormones may affect the regulation of the energetic metabolism in supplemented mice pups and lead to changes in body weight control of pups of our two experimental groups. For instance, some milk hormones such as insulin and apelin were shown to modulate intestinal transport of nutrients such as glucose and amino acids[28–30] which adds a further level of complexity to our hypotheses. Similarly, leptin, insulin, GLP-1, and apelin are also known to act on the hypothalamic neuronal network that controls the food intake in adults and in young mice.[31–35] Thus, a putative alteration of milk intake between pups of HoP-DM and HHP-DM groups cannot be ruled out to explain the observed differences of their body weights. Further studies are thus needed to investigate all of these hypotheses.

5 | CONCLUSION

We demonstrated that human donor milk sterilized by our HHP protocol (four cycles of a moderate pressure (350 MPa) for 5 min performed at 38°C) promotes an improved body growth of growth-restricted mice pups compared to human donor milk pasteurized by HoP. This reinforces the potential of HHP to treat human donor milk in HMBs to ensure a better growth and development of preterm and/or EUGR infants.

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CONFLICTS OF INTEREST STATEMENT

Patrice D. Cani is inventor on patent applications dealing with the use of specific bacteria and components in the treatment of different diseases. Patrice D. Cani was cofounder of The Akkermansia Company SA and Enterosys. The remaining authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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