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Rapid Postnatal Upregulation of Intestinal FXR-FGF19 Signaling in Premature Pigs

Victoria Smith, M.S.[1], **Yanjun Jiang, Ph.D.**[2], **Thomas Thymann, Ph.D.**[3], **Per Sangild, Dsc**[3], **Magdalena Maj, Ph.D.**[4], **Rodrigo Manjarin, Ph.D.**[1], **Douglas Burrin, Ph.D.**[2,*]

[1]Animal Science Department, California Polytechnic State University, San Luis Obispo, CA

[2]U.S. Department of Agriculture/Agriculture Research Service, Childre's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030

[3]Comparative Pediatrics and Nutrition, University of Copenhagen, Copenhagen, Denmark

[4]Biological Sciences Department, California Polytechnic State University, San Luis Obispo, CA

Abstract

Objectives: Bile acid (BA) homeostasis is regulated by intestinal cellular signaling involving the farnesoid X receptor (FXR) and fibroblast growth factor 19 (FGF19) secretion. Using preterm and term pigs as a model, we examined postnatal changes in expression of the FXR-FGF19 axis that is poorly characterized in human infants.

Methods: Pigs delivered by cesarean-section at 10 d preterm and near full term (115 d gestation) were fitted with orogastric and umbilical arterial catheters. Pigs were fed combined parenteral nutrition and minimal enteral nutrition for 5 d, followed by milk formula until 26 d. Plasma and tissue samples were collected at d 0, 5, 11, and 26. Plasma FGF19 concentration and liver and distal intestinal gene expression of FGF19 and other FXR target genes were quantified.

Results: Plasma FGF19 levels were lower in preterm vs. term newborn pigs ($P < 0.05$), increased markedly by 5 d, especially in preterm pigs, and decreased in both groups until d 26. Likewise, intestinal FXR and FGF19 expression was lower ($P \leq 0.05$) in premature vs term newborn pigs and decreased ($P \leq 0.05$) between d 5 and 26. Hepatic expression of cholesterol 7α-hydroxylase (CYP7A1) was inversely correlated with plasma FGF19 in both groups.

Conclusions: We conclude that the activity of FXR-FGF19 axis is lower in preterm than in term newborn pigs but increases transiently and then declines by the first month of age. We also provide supportive evidence of negative feedback between plasma FGF19 and hepatic CYP7A1 expression.

Keywords

bile acids; premature pig; FXR-FGF19 axis

[*]Corresponding Author: Douglas Burrin, USDA Children's Nutrition Research Center, 1100 Bates St., Houston, TX, Tel: 713-798-7049, Fax: 713-798-7057, doug.burrin@usda.gov.

INTRODUCTION

Prematurity is a world-wide problem and leads to suboptimal postnatal growth, stunting and adverse neurodevelopmental outcomes (1, 2). Premature infants also have suboptimal digestive function and fat digestion that has been linked to poor development of digestive lipases and bile salt production (3, 4). Bile acids (BAs) are a key detergent like molecules synthesized by the liver and secreted into bile and function as essential cofactors in the digestion of lipids (5). The developmental regulation and expression of genes involved in BA homeostasis are poorly understood in premature infants.

A major nuclear receptor that regulates BA homeostasis is farnesoid X receptor (FXR) which is highly expressed in the liver and intestine (6, 7). Bile acids act as ligands that activate FXR and regulate the expression of target genes involved in synthesis and transport of BAs. Fibroblast growth factor 19 (FGF19) is a key FXR target gene whose secretion is triggered by luminal BAs and functions as an intestinal endocrine hormone (8). FGF19 secreted after a meal in response to gut BA flow functions mainly as a negative feedback signal to suppress hepatic BA production via inhibition of the rate limiting enzyme in bile synthesis, cholesterol 7α-hydroxylase (CYP7A1). However, FGF19 also has insulin-like functions on the liver and skeletal muscle metabolism and thus may function as a nutrient-responsive anabolic endocrine hormone (9, 10).

There are few reports describing the changes in FXR-FGF19 pathway in response to feeding and/or development during early development and the premature stage (11, 12). To our knowledge, there is only one report detailing FGF19 levels in preterm vs term neonates (13). Newborn preterm infants had high levels of FGF19 while their CYP7A1 activity, as measured by 7α-hydroxy-4-cholestene-3-one (C4) levels in plasma was undetectable in newborns less than 30 weeks' gestation. Further understanding of FXR-FGF19 signaling may be considered important for premature infants and have implications for feeding protocols.

Given the importance of the FXR-FGF19 signaling pathway in BA homeostasis and metabolism, we aimed to investigate the impact of premature birth on the FXR-FGF19 axis in the context of postnatal development. We quantified the concentration of circulating FGF19 and expression of key genes involved in the FXR-FGF19 pathway in preterm (90% gestation) and term pigs. The preterm pig has been extensively used as a model to investigate the effects of prematurity on the gastrointestinal (GI) system (14, 15). Additionally, preterm pigs have been shown to suffer from the same morbidities and GI and liver physiological dysfunctions as premature human infants (16, 17). Importantly, FGF19 is expressed in the intestine and liver of both humans and pigs, unlike rodents that express the human ortholog FGF15 only in the intestine (18), thus making pigs a relevant model of human FGF19 biology. We hypothesize that the transcription levels of key genes in the FXR-FGF19 pathway, and FGF19 protein secretion from the distal ileum (DI) are suppressed due to prematurity and upregulated by feeding and postnatal age.

METHODS

Study Design and Animal Protocols

All experimental procedures were approved by the National Ethics Committee on Animal Experimentation at University of Copenhagen. The plasma and tissue samples used in this study were collected during two animal studies in which the pigs were treated almost identically and are described in detail in recent reports (19–21). A total of 134 pigs (Danish Landrace × Large White × Duroc) from 12 sows were delivered by cesarean section at 90 % gestation age (Preterm; 106 d, n = 58) or 100 % gestation age (Term; 118 d, n = 59) as previously described (19). Umbilical catheters (infant feeding tube 4F; Portex, Kent, UK) and orogastric feeding tubes (infant feeding tube 6F; Portex) were placed in each piglet within 3 h of delivery as described previously (19, 20). All piglets received a subcutaneous injection of iron dextran post-catheterization (200 mg Uniferon; Pharmacosmos, Holbaek, Denmark), and 3 boluses of maternal sow plasma co-infused with parenteral nutrition after the cesarean section to provide passive immunity. Pigs were euthanized on d 1, d 5, and d 26 for Study 1 (19, 20); and d 1 and d 11 for Study 2 (21) using the same anesthesia protocol described for the sows followed by intracardiac injection of pentobarbital (see Figure, Supplemental Digital Content 1) . Animals were euthanized on d 5 because this was when parenteral nutrition ended and exclusive enteral feeding began (see below). Additionally, in Study 2, d 11 was chosen as an end-of-study time point because 11-d preterm pigs would have a postnatal age of 11d, and thus would be the same gestational age as the term pigs on d 1 of the study (term-corrected age for preterm pigs). Blood was drawn via cardiac puncture while animals were sedated prior to euthanasia, and the plasma fraction was snap-frozen. Tissue from DI and liver was immediately collected and snap-frozen in liquid nitrogen.

Nutritional Support

Pigs were weighed daily and the volumes of different forms of nutrition were adjusted per body weight in both studies (see Figure, Supplemental Digital Content 1). All pigs received minimal enteral nutrition (MEN) with enteral administration of bovine colostrum (Biofiber Damino, Vejen, Denmark) from 16 $ml \cdot kg^{-1} \cdot day^{-1}$ on d 1, increasing to 64 $ml \cdot kg^{-1} \cdot day^{-1}$ on d 5 (see Table, Supplemental Digital Content 2). Also during d 1–5, pigs received parenteral nutrition (PN; 96 $ml \cdot kg^{-1} \cdot day^{-1}$ on d 1) and on d 5, parenteral nutrition was discontinued (19). From d 5 until d 9 (Study 1) or d 11 (Study 2) all piglets were enterally fed increasing amounts of raw bovine milk via feeding troughs. On d 9 of Study 1 piglets were transferred to whole milk powder (Arla Foods Ingredients, Viby, Denmark) until d 26 as described previously (19). All pigs had ad libitum access to water from d 5.

Plasma FGF-19 Analysis

Concentration of FGF19 in plasma (Preterm n = 58, Term n = 55) was measured using a commercial porcine ELISA Kit (RayBiotech, Norcross, GA). Plasma was diluted 5-fold (preterm) or 10-fold (term) and 100 μL of diluted plasma was assayed.

RT-qPCR Analysis

Total RNA was isolated from DI (Preterm n = 57, Term n = 52) and liver tissue (Preterm n = 28, Term n = 26) using TRIzol (Thermo Fisher Scientific, Waltham, MA) and expression of target genes was completed by reverse transcription polymerase chain reaction using standard protocols. Primer sequences for one reference gene, β-Actin, and 8 target genes: FXR, FGF19, small heterodimer partner (SHP), ileal lipid binding protein (ILBP), organic solute transporter (OST) α, apical sodium dependent BA transporter (ASBT), bile salt export pump (BSEP), and CYP7A1(see Table, Supplemental Digital Content 3); FXR, FGF19, SHP, ILBP, OSTα, and ASBT primers were previously designed (22). A relative standard curve was used to correct for differences on RT-qPCR reaction efficiency between plates (23). The $2^{-\Delta\Delta CT}$ method, as previously described (24) was used to compare gene expression levels between samples, which were analyzed to determine the fold change of mRNA expression.

Statistical Analysis

The results of Study 1 and 2 were combined, analyzed and presented as one study. We did this because the experimental protocols, gestational age at preterm and term birth, and postnatal nutrition support were the same in both studies. Plasma FGF19 concentrations and fold changes in gene expression were analyzed by a 2-way ANOVA using a linear mixed-model procedure (PROC MIXED) of SAS that included gestational age of birth (preterm vs term) and postnatal age (1, 5, 11, 26) as fixed effect. Normality of the residuals was tested using the Shapiro-Wilk test under the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Correlations between circulating FGF19 levels gene expression of FGF19 in DI and CYP7A1 in liver were determined using the PROC CORR in SAS. Pearson correlation coefficient (R) was considered significant at $P \leq 0.05$.

RESULTS

Plasma FGF19 concentrations.

Compared to d 1, plasma FGF19 concentrations increased on d 5 in both preterm and term pigs ($P \leq 0.05$ for preterm and term), and on d 11 in preterm group ($P \leq 0.001$), and were higher in term compared to preterm on d 1 ($P \leq 0.001$; Fig. 1.). There was no difference in plasma FGF19 concentration between preterm and term pigs on d 5, 11 and 26. In both preterm and term pigs, plasma FGF19 was lower ($P \leq 0.01$) at d 26 than at d 5.

Intestinal gene expression.

Gene expression of FXR in DI decreased in preterm and term animals on d 11 ($P \leq 0.001$ and ≤ 0.001 for preterm and term, respectively; Fig. 2A.) and d 26 ($P \leq 0.001$ and ≤ 0.001) compared to d 1, whereas DI expression of FGF19 increased on d 5 ($P \leq 0.001$; Fig. 2B.), 11 ($P \leq 0.001$), and 26 ($P \leq 0.001$) in preterm pigs, and was higher on d 5 ($P \leq 0.001$) and lower on d 11 ($P = 0.008$) in preterm compared to term. Compared to d 1, SHP levels in DI increased on d 11 in preterm pigs ($P = 0.005$; Fig. 2C.), and on d 26 in both preterm ($P \leq 0.001$) and term ($P \leq 0.001$) groups. Similarly, compared to d 1, both preterm and term pigs had increased DI gene expression of OSTα on d 5 ($P \leq 0.001$ and ≤ 0.001 in preterm and

term, respectively; Fig. 2D.), d 11 ($P \leq 0.001$ and ≤ 0.001), and d 26 ($P \leq 0.001$ and ≤ 0.001), increased ASBT on d 5 ($P = 0.006$ and 0.039; Fig. 2E.) and d 11 ($P = 0.007$ and ≤ 0.001), and increased ILBP on d 5 ($P = 0.026$ and 0.017; Fig. 2F.), d 11 ($P \leq 0.001$), and in term pigs, on d 26 ($P = 0.036$).

Hepatic gene expression.

Hepatic gene expression of FXR and SHP did not differ between term and preterm groups at any time point (Fig. 3A. and 3B.). Gene expression of BSEP in liver decreased on d 5 compared to d 1 in term animals ($P = 0.006$; Fig. 3C.) and was lower on d 5 ($P \leq 0.001$) and higher on d 26 ($P = 0.038$) in preterm compared to term. There was no significant difference in hepatic gene expression of BSEP in preterm animals (Fig. 3C.). Finally, compared to d 1, term pigs had decreased CYP7A1 expression in liver on d 5 ($P = 0.035$; Fig. 3D.).

There was a significant negative correlation between hepatic CYP7A1 expression and plasma FGF19 levels in both preterm ($R=0.685$, $P<0.001$) and term pigs ($R=0.677$, $P<0.001$) (Fig. 4, top panels). There was also a significant positive correlation between plasma FGF19 and intestinal FGF19 gene expression in preterm ($R=0.486$, $P<0.003$) and term ($R=0.336$, $P<0.028$) pigs (Fig. 4, bottom panels).

DISCUSSION

Bile acid homeostasis is critically important for lipid digestion as well as nutrient availability for rapidly growing preterm infants. Using preterm pigs as a model for preterm infants, we show that prematurity significantly reduces circulating FGF19 levels at birth. Additionally, the intestinal expression of FGF19 correlated with the level of FGF19 in circulation suggesting that FGF19 is partially transcriptionally regulated. The expression of FXR, the nuclear BA receptor that targets FGF19 was not different between preterm and term pigs but expression decreased in the first 26 d. The intestinal expression of several other FXR targets genes involved in BA homeostasis, namely OST, ASBT, and ILBP, were lower in both preterm and term newborns and increased with postnatal age. The developmental increase observed in plasma FGF19 and intestinal FXR target genes are similar to that reported previously in term infants and pigs (12). However, our results in preterm vs term pigs are in contrast to the recent report in preterm infants showing that plasma FGF19 is high at birth and declines with advancing gestational age (13). This difference between neonatal pigs and infant could be due to species differences in developmental ontogeny, but also potential confounding issues of heterogeneity of feeding practices in infants. The reduced secretion of FGF19 in preterm vs term pigs could be simply a function of lower intestinal mass relative to body weight observed previously in the pigs used from our study (19, 20). Further studies are warranted to test the FGF19 secretion patterns in response to feeding and different diets in preterm pigs.

After birth, the plasma FGF19 concentration surged in both preterm and term pigs likely triggered by the gradual increase in enteral feeding and resultant secretion of bile acids into the intestine. This finding demonstrates that despite having much lower plasma FGF19 at birth, the preterm pig has a remarkable adaptive capacity to upregulated FGF19 secretion within 5 d to levels comparable to term pigs. The intestinal FGF19 expression also increased

on d 5 in preterm and term pigs, after the onset of feeding, followed by a gradual decrease over time. The decline in plasma FGF19 between d 11 and 26 was consistent in both preterm and term pigs. The explanation for the decline in plasma FGF19 with advancing age is not clear, but may be related to changes in BA profiles marked by increases in hyocholic and hyodeoxycholic acids relative to chenodeoxycholic acid since the former BAs have been shown to be poor FXR agonists (12).

We also observed changes in hepatic FXR target genes involved in bile homeostasis, specifically CYP7A1 and BSEP. The expression of both genes tended to decrease between birth and 5 d of age and then rebounded by d- 26. CYP7A1 is the rate-limiting enzyme in BA synthesis, while BSEP is critical for hepatic BA efflux into bile canaliculi. Interestingly, hepatic CYP7A1 expression was negatively correlated with circulating levels of FGF19 in both preterm and term pigs between birth and d 26. This finding is consistent with the prevailing literature and suggests a functional negative feedback loop in neonatal pigs between circulating FGF19 and suppression of hepatic CYP7A1 and BA synthesis. We previously observed that plasma FGF19 concentrations are reduced during total parenteral nutrition, which may lead to unsuppressed CYP7A1 and hepatic synthesis and accumulation of BAs leading to parenteral nutrition-associated cholestasis (25).

In summary, the current study shows that plasma FGF19 concentrations are lower in preterm compared to term newborn pigs. We also show in both preterm and term pigs a surge in plasma FGF19 with the onset of enteral nutrition in the first two weeks followed by a decline after four weeks. The developmental patterns in expression of some FXR target genes correlate with plasma FGF19 and suggest a functional negative feedback with hepatic CYP7A1 expression. The metabolic significance of the postnatal surge in FGF19 in both pigs and human infants (11) and differences associated with prematurity and birth weight is unknown. Studies in mice show that FGF19 functions as an anabolic hormone with insulin-like actions on liver and skeletal muscle protein metabolism that are mediated via the mTOR pathway (9, 10). Very low birth weight, preterm infants suffer from growth failure in the early postnatal period and neurodevelopmental delays long-term. Importantly, our recent study in neonatal pigs shows that prematurity blunts the feeding-induced stimulation of translation initiation signaling and protein synthesis in muscle (26). Further studies are warranted to investigate the metabolic significance of FGF19 in infants and whether it functions as a nutrient-mediated growth factor.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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What is Known

- Premature birth is a worldwide problem and leads to growth failure and neurodevelopmental delay.
- Reduced BA production and circulation may limit lipid digestion in premature infants
- Fibroblast growth factor 19 (FGF19) is an important gut enterokine involved in BA homeostasis and metabolism.

What is New

- Plasma FGF19 concentrations are lower in preterm vs. term newborn pigs, but levels increase rapidly with enteral feeding after birth.
- We confirm evidence of a functional negative feedback relationship between plasma FGF19 and hepatic cholesterol 7α-hydroxylase (CYP7A1) in neonatal pigs.

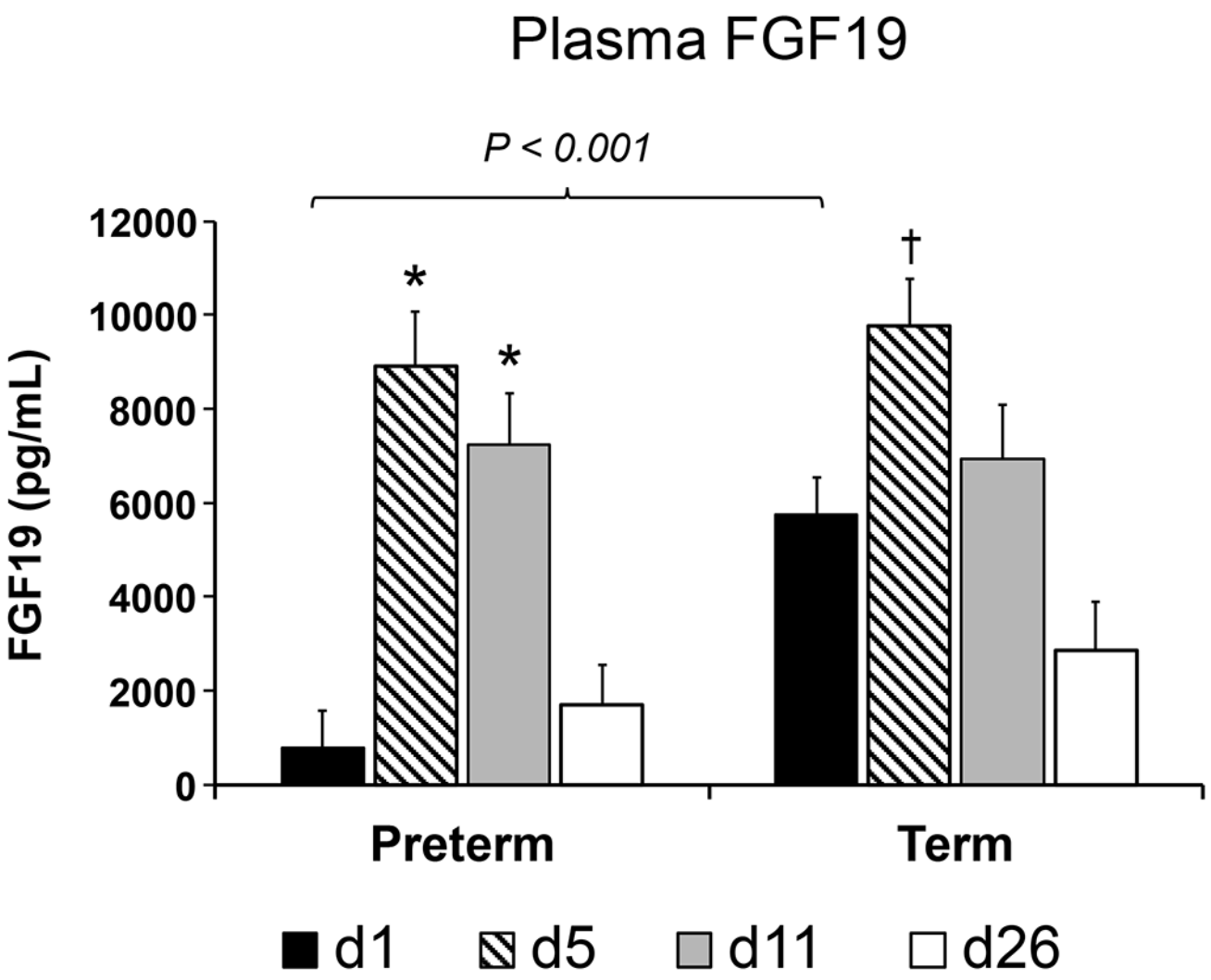

Fig. 1.

FGF19 concentration in pg/mL in peripheral plasma of preterm and term pigs on d-of-age 1, 5, 11, and 26. Values are least square means ± SE. Means differ from respective preterm or term D1 value *P ≤ 0.01, †P ≤ 0.05. Bracket denotes significant difference in values between preterm and term groups on the same day (d1 preterm n = 11, term n = 11; d5 preterm n = 8, term n = 10; d11 preterm n = 10, term n = 12; d26 preterm n = 10, term n = 10).

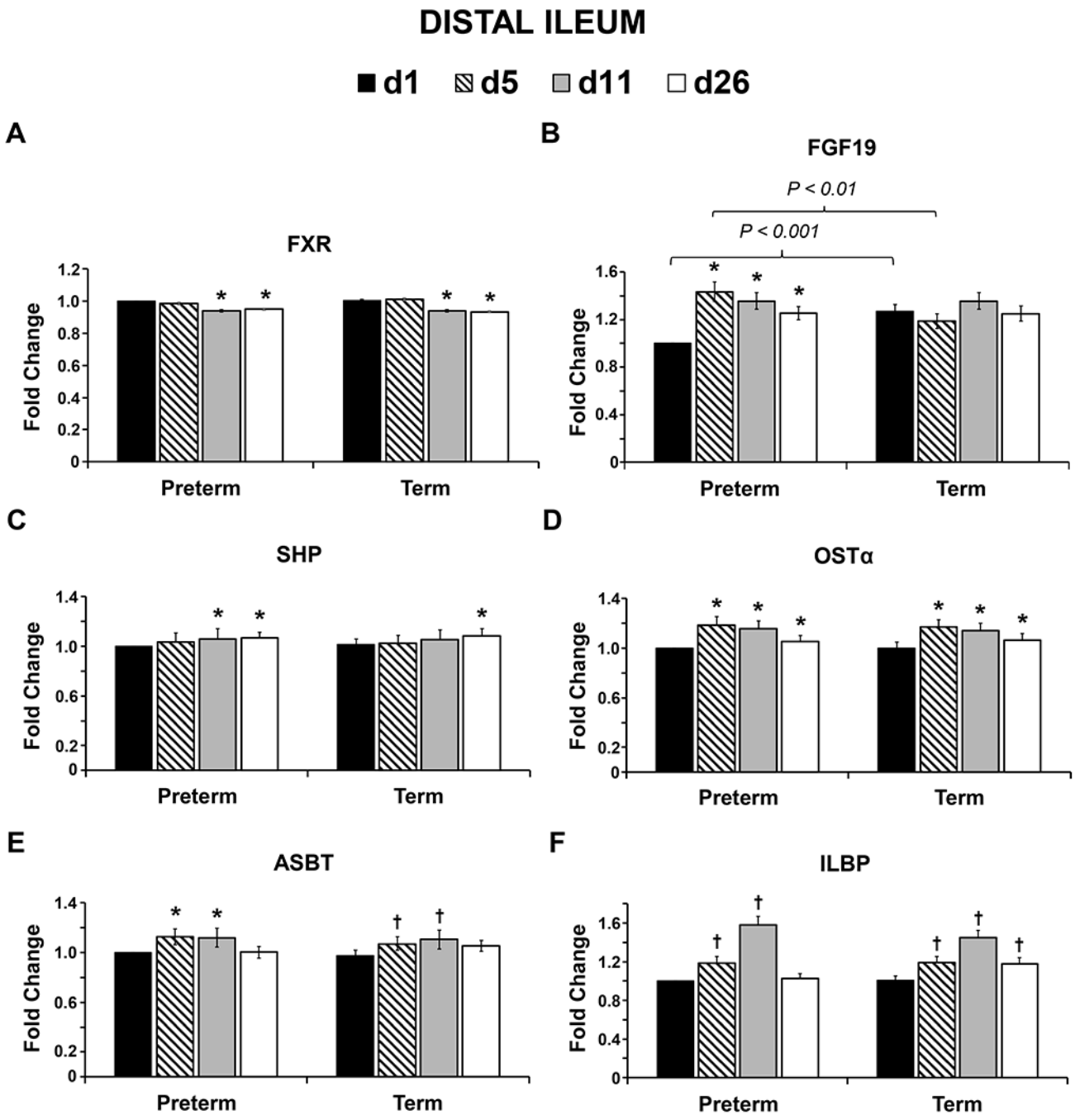

Fig. 2.

Relative mRNA abundance of farnesoid x receptor (FXR), fibroblast growth factor (FGF19), small heterodimer partner (SHP), organic solute transporter alpha (OSTα), apical sodium dependent bile acid transporter (ABST), and ileal lipid binding protein (ILBP) in the distal ileum of preterm and term pigs on d-of-age 1, 5, 11, and 26. Values are least square means ± SE. Means differ from respective preterm or term D1 value *P ≤ 0.01, †P ≤ 0.05. Brackets denote significant differences between preterm and term groups on the same day (d1 preterm

n = 7, term n = 7; d5 preterm n = 7, term n = 7; d11 preterm n = 7, term n = 7; d26 preterm n = 7, term n = 5).

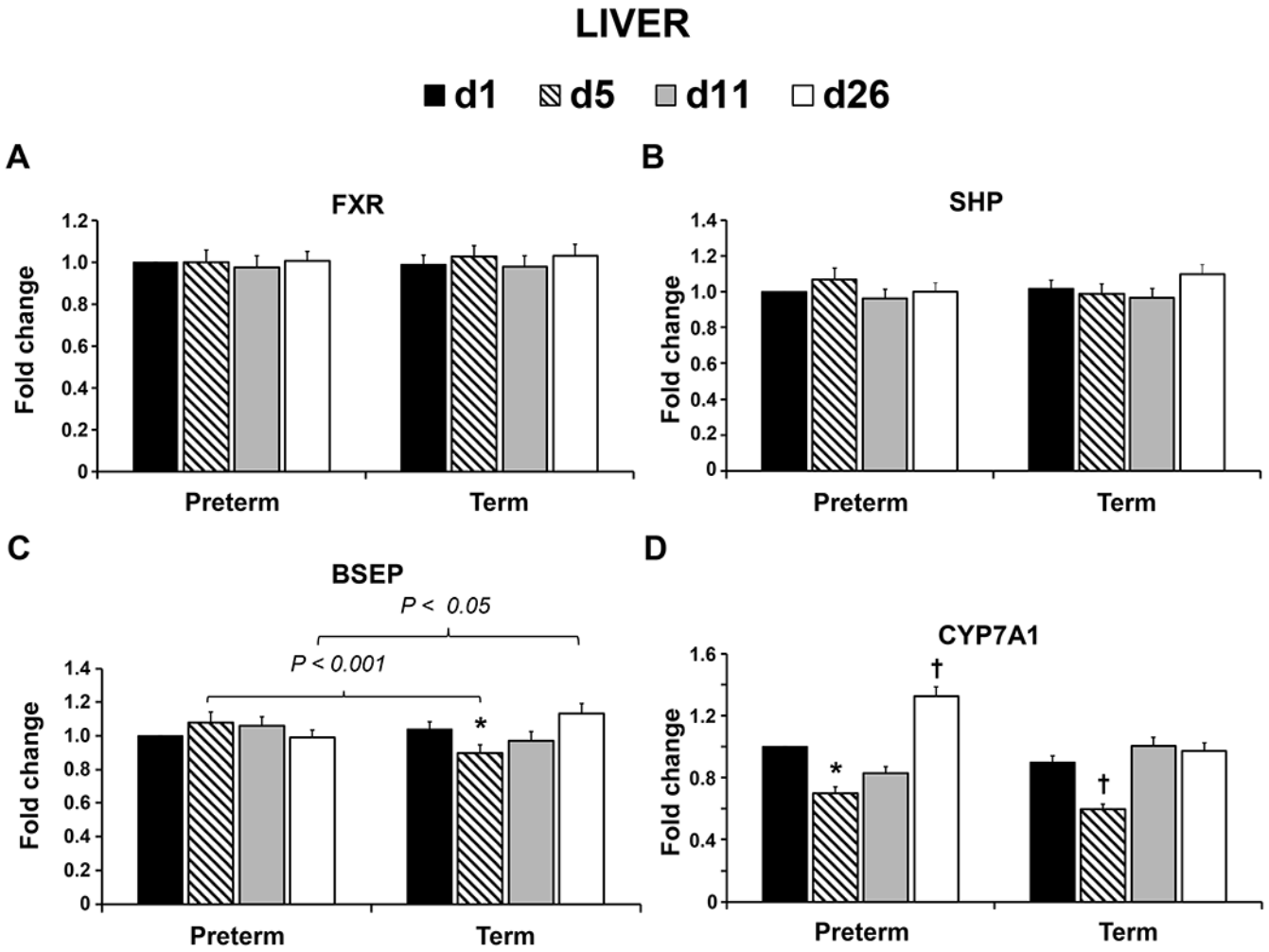

Fig. 3.

Relative mRNA abundance of farnesoid X receptor (FXR), small heterodimer partner (SHP), bile salt export pump (BSEP), and cytochrome P450 family 7 subfamily A member 1 (CYP7A1) in the liver of preterm and term pigs on d-of-age 1, 5, 11, and 26. Values are least square means ± SE. Means differ from respective preterm or term D1 value *P ≤ 0.01, †P ≤ 0.05. Brackets denote significant differences between preterm and term groups on the same day (d1 preterm n = 7, term n = 7; d5 preterm n = 7, term n = 7; d11 preterm n = 7, term n = 7; d26 preterm n = 7, term n = 5).

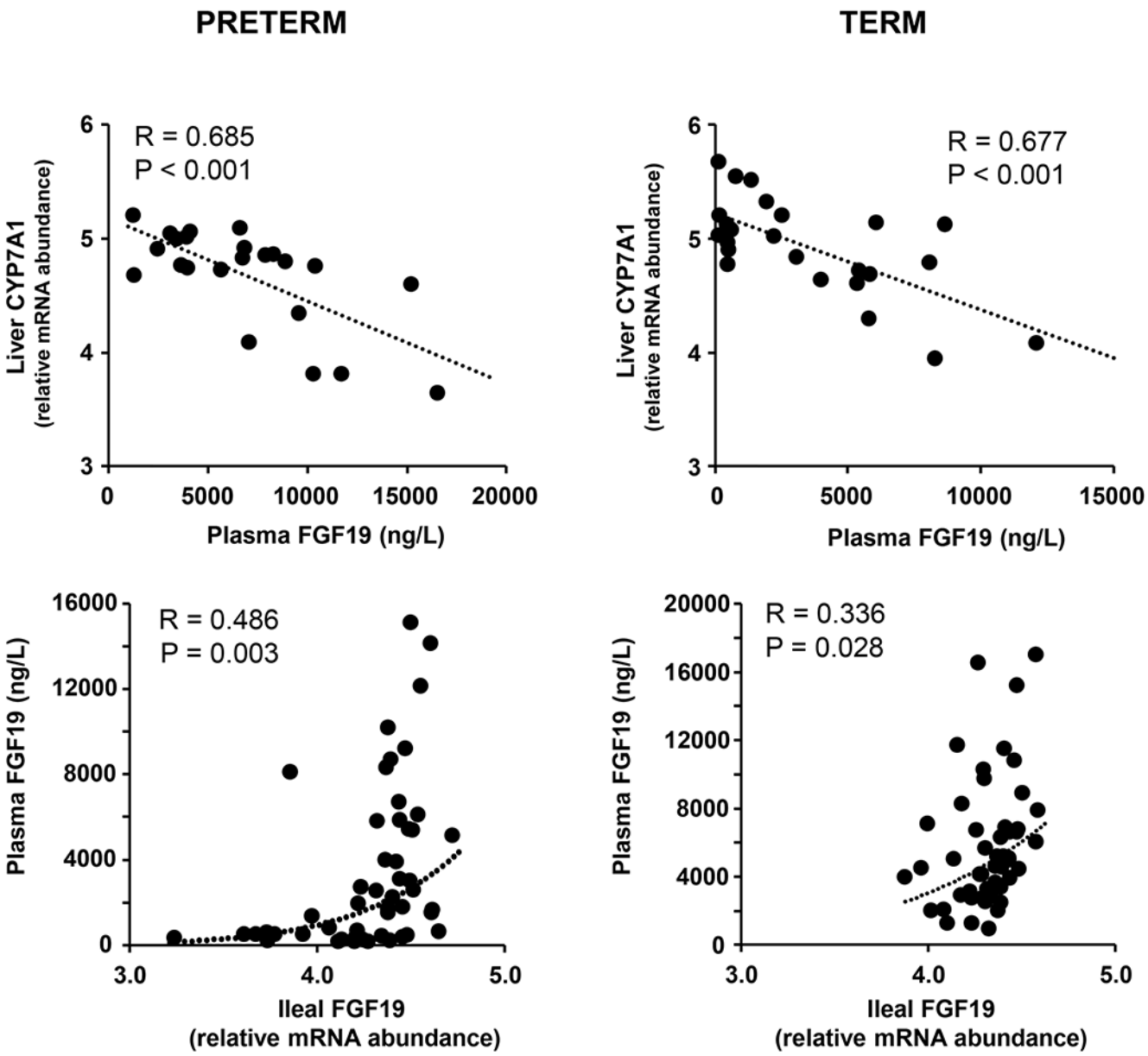

Fig. 4.

Correlations between circulating FGF19 (ng/L) and relative mRNA abundance of liver CYP7A1 (top panels) or relative mRNA abundance of ileal FGF19 (bottom panels) in preterm and term pigs.