Hepatic FGF21 production is increased in late pregnancy in the mouse

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Cui Y, Giesy SL, Hassan M, Davis K, Zhao S, Boisclair YR. Hepatic FGF21 production is increased in late pregnancy in the mouse. Am J Physiol Regul Integr Comp Physiol, 2014, 307: R290–R298. First published June 4, 2014; doi:10.1152/ajpregu.00554.2013.—Female mammals call on hormonally driven metabolic adaptations to meet the energy demand of late pregnancy and lactation. These maternal adaptations preserve limiting nutrients and promote their transfer to the uterus during pregnancy or mammary gland during lactation. The novel metabolic hormone fibroblast growth factor-21 (FGF21) was recently shown to increase suddenly at the onset of lactation in dairy cows, but whether FGF21 is induced during the reproductive cycle of other mammals is unknown. To start addressing this question, we studied subsets of mice when virgin (V), on day 18 of pregnancy (P18) and on lactation day 1 (L1), L5 and L14. Plasma FGF21 increased from nearly undetectable levels to over 8 ng/ml between V and P18 and returned to V levels by L1. Gene expression studies showed that liver was the major source of plasma FGF21 at P18 with little or no contribution from other known expressing tissues or from the developing placenta and mammary epithelial cells. The increased FGF21 production at P18 was dissociated from plasma nonesterified fatty acids and liver lipids, unlike that seen in fasted V mice. Changes in FGF21 signaling components in target tissues were modest except for reduced β-Klotho and FGFR1c expression in P18 adipose tissue. The placenta expressed both β-Klotho and FGFR1c, raising the possibility that it responds to FGF21. In conclusion, maternal FGF21 is increased when products of conception account for ~40% of maternal weight, suggesting that FGF21 orchestrates some of the adaptations needed to meet the energy demand of late pregnancy.

MOUSE FIBROBLAST GROWTH FACTOR (FGF) 21 is a 210 amino acid protein originally cloned as an FGF-related protein expressed in mouse liver and thymus (34). FGF21 belongs to a branch of the FGF superfamily that includes FGF15 (mouse ortholog of human FGF19) and FGF23 (4, 31). Members of this branch are not trapped within the extracellular matrix of the producing tissue because they lack the high affinity heparin-binding region found in other FGFs (31, 52). As a consequence, they enter the circulation and have the ability to act in an endocrine manner (4, 31, 36, 52). FGF21 signals via a subset of ubiquitously expressed FGF receptors (FGFR) but only in the presence of the coreceptor β-Klotho (4, 36, 52). This requirement effectively restricts FGF21 signaling to the limited set of tissues expressing β-Klotho, which include liver, adipose tissue, and pancreas (14, 29, 52). Functional in vitro and in vivo data indicate that FGF21 prefers signaling complexes containing FGFR1c (3, 29) even though β-Klotho is also capable of interacting with FGFR2c, FGFR3c, and FGFR4 (25).

Interest in FGF21 as a metabolic regulator grew from the seminal observation that FGF21 promoted glucose uptake in the mouse adipocyte cell line 3T3 and improved insulin action in the obese, insulin-resistant ob/ob and db/db mice (26). These results were confirmed by a variety of approaches, including gain or loss of function mouse models (5, 26) and chronic administration of FGF21 and its mimetics to diabetic rodents, rhesus monkeys, and humans (2, 16, 49). Beneficial effects of FGF21 therapy stem from a variety of actions, including stimulation of oxidative lipid metabolism and ketogenesis in liver, increased energy expenditure, reduction of adipose tissue mass, and promotion of β-cell health (31, 36, 52). More recently, FGF21 was also shown to stimulate secretion of the insulin-sensitizing adipokine, adiponectin (17, 30), providing a mechanism for effects on tissues lacking β-Klotho, such as skeletal muscle.

While the therapeutic potential of FGF21 is clear, the complete range of naturally occurring conditions where FGF21 is functionally important remains ill defined. In the mouse, plasma FGF21 is increased in conditions associated with increased lipid utilization such as fasting and consumption of lipid-rich diets (5, 19, 22), suggesting that it promotes the switch from glucose to lipid metabolism. In this context, we recently showed that plasma FGF21 rises suddenly in dairy cows at the onset of lactation when adipose-derived lipids covers ~30% of maternal energy requirements (40). These observations prompted us to ask whether increased plasma FGF21 is an obligatory adaptation of lactation. To do so, we chose the mouse, a species with a milk energy output similar to the modern dairy cow when expressed on a metabolic body weight basis (35, 37). We found that plasma FGF21 was also suddenly increased in the the mouse, but in contrast to the dairy cow, this induction occurred in late pregnancy. The increased plasma FGF21 of late pregnancy was accounted for by liver production with no contribution by tissues developing specifically during pregnancy [i.e., placenta and mammary epithelial cells (MEC)] and other known expressing tissues (i.e., pancreas, adipose tissue, and muscle). Finally, we found that the mouse placenta expressed significant levels of β-Klotho and FGFR1c, raising the possibility that it is an FGF21 target tissue.

MATERIALS AND METHODS

Animals. All procedures were approved by the Cornell Institutional Animal Care and Use Committee. FVB female mice were purchased when 5 to 6 wk old from The Jackson Laboratory (Bar Harbor, ME). They were housed at constant ambient temperature (22°C) and photoperiod (lights on between 0600 and 1800 h). A single rodent chow diet containing 5.8% fat and 19% protein was used throughout the experiment (Harlan Teklad 7912, Madison, WI).

Mice were divided into two experimental groups at 7 wk of age. The first group of mice was given an acclimatization period of 7 days and then placed with proven males (1 male/3 females) or kept in the virgin state. Mating females were checked every morning for the presence of a copulatory plug, with the day of detection designated as
Table 1. Primers used in real-time PCR analysis

<table>
<thead>
<tr>
<th>Gene*</th>
<th>Sequence†</th>
<th>Product Size (bp)</th>
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<tr>
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<td>171</td>
</tr>
<tr>
<td>R</td>
<td>GCCAGAGCTCCTGGTTCATTG</td>
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<tr>
<td>Fgf21 F</td>
<td>CCTCTAGAGTGTTTCGCAAACG</td>
<td>76</td>
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<tr>
<td>Fgfr1c F</td>
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<td>66</td>
</tr>
<tr>
<td>R</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>R</td>
<td>CTTGCTCGAGGAGCTCTCCATCAG</td>
<td></td>
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</table>

*Primers were designed to measure the abundance of 18S ribosomal RNA (18S), fibroblast growth factor-21 (Fgf21), fibroblast growth factor receptor (FGFR)-1c, FGFR2c, FGFR3c, FGFR4, β-Klotho (Klb), and perilipin (Plin1) transcripts. †Primer sequences are shown in a 5’ to 3’ orientation.

pregnancy day 1. Food was available in unlimited amount throughout the entire experiment. Subsets of mice were euthanized in the virgin state (V), on day 18 of pregnancy (P18), and on lactation day 1 (L1), L5, and L14 (n = 7 mice per group). The average number of fetuses ± SE at P18 was 9 ± 0.4, and all litters were normalized to 7–9 pups/dam upon lactation. The second experimental group consisted only of V mice (n = 5) and was studied in parallel and under the same conditions as the first V group, except that they were deprived of food for the 24-h period before euthanasia.

Both experimental groups were euthanized by CO2 asphyxiation between 1000 and 1200 h. Blood was collected via cardiac puncture into a heparinized syringe, and plasma was prepared by centrifugation. This was immediately followed by dissection of liver, adipose tissue (gonadal and retroperitoneal depots), pancreas, and gastrocnemius muscles. Tissues were weighed, snap frozen in liquid nitrogen, and stored at −80°C until further analysis.

A third group of pregnant mice was euthanized at P18 to obtain placenta and MEC (n = 5–7 for each tissue). After CO2 asphyxiation of the mice, the pregnant uterus was removed followed by dissection of an individual placenta and maternal tissues (pancreas, liver, gonadal adipose tissue, and the 2 abdominal mammary glands). All tissues were snap frozen in liquid nitrogen and stored at −80°C except the mammary glands, which were used to isolate MEC.

Isolation of MEC. Isolation of MEC were isolated according to a recently validated procedure (38). In brief, the lymph node was removed from both abdominal mammary glands and a small portion was snap frozen in liquid nitrogen. The remaining glands were chopped to a paste for 4 min followed by incubation in 5 ml of digestion buffer [50 mM sodium fluoride, 1 mM sodium orthovanadate in DMEM with 1 mg/ml trypsin (Life Technologies, Carlsbad, CA) and 2 mg/ml collagenase A (Sigma, St. Louis, MO)] at 37°C with shaking set at 200 rpm. After a 30-min period, the reaction was quenched with 1 ml of fetal bovine serum. The reaction volume was completed to 14 ml with wash buffer (50 mM sodium fluoride, 1 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride in phosphate-buffered saline), and MEC were isolated by centrifugation (9,300 g for 10 min at 4°C). The washing procedure was repeated two more times followed by total RNA isolation from the MEC pellet. The procedure reduced MEC expression of the adipose tissue marker perilipin to <13% of mammary gland expression.

Total RNA isolation and analysis of gene expression. Tissues were lysed in Qiagen and total RNA was isolated using RNeasy Mimi columns and on-column RNase-free DNase treatment (Qiagen, Valencia, CA). Quantity and integrity of RNA were determined using the RNA Nano Lab Chip Kit and BioAnalyzer (Agilent, Palo Alto, CA). Reverse transcription reactions were performed with 2 μg of RNA using the high-capacity cDNA reverse transcription kit with RNase inhibitor (Applied Biosystems, Foster City, CA). Real-time PCR assays were performed in duplicate in a 25-μl volume using Power SYBR Green Mix (Applied Biosystems). Reactions contained 500 nM each primer and 25 ng of reverse-transcribed RNA (except 1.25 ng was used for 18S). Each primer pair was shown to anneal in adjoining exons of the targeted gene and to yield a single product by melting curve analysis (see Table 1 for sequence of each primer pair). mRNA data for FGFR isoforms in the placenta were analyzed by the efficiency-corrected ΔCt method with all assays performed at the same detection threshold (14, 40). PCR efficiencies (E) were calculated from the slope of the standard curve using E = 10(−1/slope), and the quantity of each FGFR isoform and 18S RNA was calculated as quantity = E−ΔCt. An arbitrary expression level was obtained by normalizing the efficiency-corrected value for each FGFR isoform to 18S expression. All other mRNA data were analyzed using a relative standard curve based on serial twofold dilutions of pooled cDNA prepared from liver, adipose tissue, or pancreas as indicated in the figure legends. Unknown sample expression was determined from the standard curve, adjusted for 18S expression, and reported as a fold

| Table 2. Effects of pregnancy and lactation on whole animal and metabolic parameters |
|--------------------------------------|-------------------|-----------|
| Physiological State* | V | P18 | L1 | L5 | L14 | SE | P Value† |
| Body weight, g | 23.2±a | 38.4±b | 25.6±a | 29.2±a | 31.4±c | 0.8 | <0.001 |
| Liver, g | 1.05±a | 1.77±b | 1.65±b | 1.98±b | 2.34±d | 0.07 | <0.001 |
| Liver, % of live weight | 4.55±a | 4.59±b | 6.43±b | 6.78±b | 7.44±c | 0.13 | <0.001 |
| Gonadal fat, g | 0.68±a | 0.29±b | 0.23±b | 0.16±b | 0.08±b | 0.07 | <0.001 |
| Gonadal fat, % of live weight | 2.87±a | 0.76±b | 0.96±b | 0.56±b | 0.26±b | 0.22 | <0.001 |
| Plasma glucose, mg/dl | 193±a | 184±b | 145±b | 154±b | 163±b | 12± | 0.02 |
| Plasma NEFA, μM | 861±a | 924±b | 551±b | 509±b | 531±b | 64± | <0.001 |
| Plasma BHBA, mM | 0.86±a | 0.67±b | 0.45±b | 0.64±b | 0.55±b | 0.06 | 0.02 |
| Liver fat, % | 5.53 | 3.39 | 2.96 | 2.93 | 3.97 | 0.96 | NS |

*Age-matched mice were studied in the virgin state (V), on pregnancy day 18 (P18), or on lactation day 1, 5, or 14 (L1, L5, or L14). Values represent the means of 5–7 mice at each physiological state. NEFA, nonesterified fatty acids; BHBA, β-hydroxybutyrate; †Type I error probability for the overall ANOVA. NS, nonsignificant at P > 0.05. When P < 0.05 overall means were separated by Tukey’s test. a,b,c,dP < 0.05.
FGF21 is increased in late-pregnant mice

**Methods.** FGF21 mRNA abundance was measured relative to virgin liver expression. Within each tissue, each bar represents the mean ± SE of FGF21 mRNA abundance (n = 5–7 mice for each physiological state). A: hepatic mRNA abundance reported as a fold of virgin liver expression. Each bar represents the mean ± SE of FGF21 mRNA abundance (n = 5–7 mice for each physiological state). B: plasma FGF21 concentration. Each bar represents the mean ± SE of plasma FGF21 (ng/ml) relative to virgin liver expression. Within each physiological state, individual means were compared by pairwise comparisons with Tukey adjustment. Correlations were performed using the CORR procedure of SAS (SAS Institute). The level of statistical significance was set at P < 0.05.

**Results.**

Effects of pregnancy and lactation on whole animal and metabolic parameters. Mice were studied on day 18 of pregnancy and on day 1, 5, or 14 of lactation (L1, L5, or L14) and compared with a group of age-matched virgin (V) mice (Table 2). Expected changes were seen across physiological states for body weight, liver, and adipose tissue. Mice were 60% heavier at P18 than in V state (P < 0.05). This difference disappeared by L1 suggesting that it was accounted predominantly by the products of conception. Relative to V mice, liver mass increased by 60% in P18 mice and by 220% in L14 mice (P < 0.05). Once normalized to body weight, however, this effect persisted only during lactation (P < 0.05). In contrast, both absolute and relative gonadal fat mass were reduced by ~50% at P18 and by 90% at L14 (P < 0.05). Similar changes were seen for the retroperitoneal fat depot (results not shown).

Plasma NEFA concentrations did not differ between V and P18 mice but were uniformly reduced at all stages of lactation (P < 0.05). A similar pattern was seen for plasma glucose and BHBA, with lower glucose concentrations at L1 than V (P < 0.05) and lower BHBA concentrations at L1 and L14 than V if applicable as the fixed effect and animal as the random effect. Data from the fasting experiment were analyzed by a model accounting for feeding level (Fed vs. Fasted V) as the fixed effect and animal as the random effect. The relative expression of the various FGFR in the placenta was analyzed by a model accounting for receptor subtype (FGFR1c, FGFR2c, FGFR3c, or FGFR4) as the fixed effect and animal as the random effect. The statistical method for spatial expression of FGFR1c included tissue as the fixed effect and animal as the random effect. Whenever multiple comparisons were indicated, individual means were compared by pairwise comparisons with Tukey adjustment. Correlations were performed using the CORR procedure of SAS (SAS Institute). The level of statistical significance was set at P < 0.05.

**Fig. 2.** Effect of physiological state on FGF21 expression in nonhepatic tissues. Age-matched mice were studied in the virgin state (V), on pregnancy day 18 (P18), or on lactation day 1 (L1). A: FGF21 expression in tissues of fed virgin mice. FGF21 mRNA abundance was measured relative to virgin liver expression. Each bar represents the mean ± SE of FGF21 mRNA abundance (n = 5–7 mice for each tissue). B–D: FGF21 expression in pancreas, skeletal muscle, and adipose tissue across physiological state. FGF21 mRNA abundance was measured relative to virgin liver expression within each tissue. Each bar represents the mean ± SE of FGF21 mRNA abundance (n = 5–7 mice for each tissue). E: FGF21 expression in mammary epithelial cells (MEC) and placenta on pregnancy day 18 (P18). MEC were isolated from the mammary gland as described in MATERIALS AND METHODS. FGF21 mRNA abundance was measured relative to P18 liver expression. Each bar represents the mean ± SE of FGF21 mRNA (n = 5–7 mice for each tissue). **P < 0.001.

**Fig. 1.** Effect of physiological state on hepatic fibroblast growth factor-21 (FGF21) mRNA abundance and plasma FGF21 concentration. Age-matched mice were studied in the virgin state (V), on pregnancy day 18 (P18), or on lactation day 1, 5, or 14 (L1, L5, or L14). A: hepatic mRNA abundance reported as a fold of virgin liver expression. Each bar represents the mean ± SE of FGF21 mRNA abundance (n = 5–7 mice for each physiological state). B: plasma FGF21 concentration. Each bar represents the mean ± SE of plasma FGF21 (ng/ml) relative to virgin liver expression. Within a tissue, each bar represents the mean ± SE of FGF21 mRNA abundance (n = 5–7 mice for each physiological state). **P < 0.001.
Liver and adipose tissue (Fig. 3B) abundantly expressed than any of the three other FGFRs FGFR2c, FGFR3c, or FGFR4) (25). FGFR1c was more capable of interacting with FGFR2c, FGFR3c, or FGFR4) (25). FGFR1c was more capable of interacting with β-Klotho (i.e., FGFR1c, FGFR2c, FGFR3c, or FGFR4) (25). FGFR1c was more abundantly expressed than any of the three other FGFRs (Fig. 3B, P < 0.001) and alone accounted for 93% of all relevant FGFR transcripts. We also compared FGFR1c expression across β-Klotho-expressing tissues. FGFR1c expression agreed with data previously reported in V mice (14), with adipose tissue expressing substantially higher levels than liver or pancreas (Fig. 3C, P < 0.01). Remarkably, FGFR1c expression in the placenta was 4.8-fold higher than that of adipose tissue (Fig. 3C, P < 0.001). The effects of pregnancy and lactation on FGF21 production. We started by measuring FGF21 mRNA in liver where production is subject to dynamic metabolic regulation (5, 19, 22). Relative to V liver expression, FGF21 mRNA abundance was increased over 60-fold at P18 (Fig. 1A, P < 0.001). FGF21 expression returned to V level by L1 and remained at this basal expression level at L5 and L14. Plasma FGF21 changed in parallel with hepatic expression, increasing from 0.14 ng/ml in V mice to over 8 ng/ml in P18 mice (Fig. 1B, P < 0.001), followed by a decline to V levels by L1. These data show that plasma FGF21 is increased in late pregnancy and identify liver as a contributor to this increase.

Next, we asked whether other tissues contributed to the rise of plasma FGF21 at P18. In mice, extra-hepatic FGF21 expression has been detected in the pancreas, adipose tissue, and skeletal muscle (1, 14, 23). In agreement with these data, FGF21 mRNA expression in V mice was 15-fold higher in the pancreas than liver (P < 0.001) and comparable in liver and adipose tissue (Fig. 2A). FGF21 expression was also detected in skeletal muscle, but expression was only 1% of V liver expression. When compared with V expression, FGF21 expression did not change significantly in the pancreas and muscle at P18 and L1 (Fig. 2, B and C) and was actually decreased at both of these stages in adipose tissue (Fig. 2D, P < 0.001). These data show that the pancreas and adipose tissue remain sites of FGF21 expression during pregnancy but do not contribute to the rise of plasma FGF21 occurring at P18.

Finally, we used a second cohort of pregnant mice to determine whether FGF21 could be produced at P18 by the rapidly developing mammary epithelial compartment or the placenta. For the mammary gland, we performed this analysis on isolated epithelial cells (MEC) to minimize contamination by the adipose tissue compartment (38). FGF21 transcripts were detectable in both MEC and placenta, but their abundance was only 0.12–0.17% of P18 liver expression (Fig. 2E). Overall, these studies show that the liver is the predominant source of plasma FGF21 at P18 and rule out meaningful contribution by other known FGF21 expressing tissues.

Effects of pregnancy and lactation on FGF21 signaling components. FGF21 signaling is absolutely dependent on the presence of the coreceptor β-Klotho (31, 52). Sites of significant β-Klotho expression in the mouse include liver, adipose tissue, and pancreas, but expression in the placenta and MEC has not been assessed (14). We first measured β-Klotho expression in the liver, adipose tissue, pancreas, and placenta of P18 mice. Similar to findings in V mice (14), β-Klotho expression in P18 mice was comparable in liver and adipose tissue and significantly lower in the pancreas (Fig. 3A, P < 0.05). β-Klotho expression in the placenta was lower than in liver and adipose tissue (Fig. 3A, P < 0.01) but similar to expression in the pancreas. In contrast, β-Klotho expression was undetectable in MEC isolated at P18.

Next, we asked whether the placenta expressed any of the FGFR capable of interacting with β-Klotho (i.e., FGFR1c, FGFR2c, FGFR3c, or FGFR4) (25). FGFR1c was more abundantly expressed than any of the three other FGFRs (Fig. 3B, P < 0.001) and alone accounted for 93% of all relevant FGFR transcripts. We also compared FGFR1c expression across β-Klotho-expressing tissues. FGFR1c expression agreed with data previously reported in V mice (14), with adipose tissue expressing substantially higher levels than liver or pancreas (Fig. 3C, P < 0.01). Remarkably, FGFR1c expression in the placenta was 4.8-fold higher than that of adipose tissue (Fig. 3C, P < 0.001). The
significant expression of both β-Klotho and FGFR1c in the placenta raises the possibility that it is an FGF21 target tissue.

Finally, we asked whether the expression of β-Klotho and its subset of interacting FGFRs were altered during pregnancy and lactation (Fig. 4). We limited this analysis to the two most abundant FGFR expressed in liver (FGFR2c and FGFR4), adipose tissue (FGFR1c and FGFR2c), and pancreas (FGFR1c and FGFR2c) (14). The most prominent effects were seen at P18 in adipose tissue with reduction in the expression of both β-Klotho and FGFR1c ($P < 0.01$). In the liver, reductions in expression were seen for FGFR2c at L1 and FGFR4 at P18 ($P < 0.05$ or less), whereas β-Klotho expression remained unaffected. The only significant effect seen in the pancreas was a reduction in β-Klotho expression at L1 ($P < 0.05$).

Contrasting metabolic state associated with FGF21 induction in fasting and pregnancy. The mobilization of lipid reserves in late pregnancy in the mouse raises the possibility that plasma NEFA and hepatic lipid content contribute to FGF21 induction at P18, just as they do during fasting (22). To compare fasting and P18 within the same experimental setting, a separate cohort of age-matched V mice was fasted for 24 h and compared with the fed V mice. This period of fasting caused a 30% reduction in plasma glucose, a 40% increase in plasma NEFA, and a doubling in plasma BHBA and liver lipid content (Fig. 5A, $P < 0.01$). Fasting elevated plasma FGF21 from 0.14 to 5 ng/ml and caused a 35-fold induction in hepatic FGF21 expression (Fig. 5B, $P < 0.001$). Fasting tended to decrease FGF21 in the pancreas ($P < 0.06$) but had no statistically significant effect in adipose

![Fig. 4. Effect of physiological state on expression of β-Klotho and its subset of interacting FGF receptors.](http://ajpregu.physiology.org/)

A: β-Klotho, FGFR2c, and FGFR4 expression in liver. B: β-Klotho, FGFR1c, and FGFR2c expression in adipose tissue. C: β-Klotho, FGFR1c, and FGFR2c expression in the pancreas. For each tissue, expression of each gene is given relative to virgin expression, with each bar representing the mean ± SE of mRNA abundance ($n = 5–7$ mice for each physiological state). *$P < 0.01$; **$P < 0.05$. 


FGF21 IS INCREASED IN LATE-PREGNANT MICE

A

B

Fig. 5. Effect of fasting on plasma metabolic variables and FGF21 expression. Age-matched virgin mice were studied in the fed state (Fed) or after a 24-h period of fasting (Fasted). A: plasma concentration of glucose and nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHBA), and liver lipid content. Each bar represents the mean ± SE of the indicated variable (n = 5 mice for each treatment). B: plasma concentration and liver expression of FGF21. Each bar represents the mean ± SE of the indicated variable (n = 5 mice for each treatment). *P < 0.01; **P < 0.001.

tissue or muscle (data not shown). More importantly, both fasting and P18 led to a positive association between plasma FGF21 and hepatic FGF21 expression (Fig. 6A). Plasma FGF21 was positively related with plasma NEFA and liver lipid content but only in the fasting experiment (Fig. 6, B and C). These data suggest that plasma NEFA and liver lipid content play no role in the induction of hepatic FGF21 production in P18 mice.

DISCUSSION

We recently reported that plasma FGF21 is nearly undetectable in late-pregnant dairy cows, peaks at parturition, and then stabilizes at chronically elevated concentrations over the first few weeks of lactation (40). Early lactation in dairy cows is a nutritionally precarious period owing to a combination of near-maximal milk yield within days of parturition and inadequate voluntary feed intake (7, 12). Because early lactation is confounded with nutritional insufficiency in dairy cows, it is unclear whether increased plasma FGF21 is a lactation-specific adaptation or simply a response to the coincidental metabolic challenge.

To start answering this question, we studied FGF21 through the pregnancy-lactation cycle of the mouse. In contrast to the cow, late pregnancy, not early lactation, is the most nutritionally precarious phase of the mouse reproductive cycle. This is mainly because the products of conception in the late-pregnant mouse account for >40% of maternal weight versus a mere 5–6% in the cow (33, 41). This mass limits maternal gastrointestinal tract capacity and thus feed intake (42), necessitating a significant mobilization of maternal reserves to meet the metabolic demand of late pregnancy. After parturition, the nutritional status of the dam improves because milk output increases very gradually and feed intake augments in direct proportion to milk yield (33, 42). We found that plasma FGF21 was increased 60-fold at P18 but returned to virgin levels immediately upon lactation. These data indicate that increased FGF21 is not an obligatory or specific adaptation of lactation but more likely the consequence of maternal nutritional insufficiency. It would be useful to have FGF21 data in species that do not experience substantial energy deficits during either pregnancy or lactation. Such data are available in women (9), but only in the context of gestational diabetes and preeclampsia, with both conditions causing a two- to threefold elevation in plasma FGF21 near delivery relative to healthy controls (43, 44). Additional data collected across the entire reproductive cycle in women and other species are needed to evaluate effects of pregnancy and lactation.

Next, we surveyed known FGF21 expressing tissues to identify the source of increased plasma FGF21 at P18. First, we considered the liver because increased hepatic production often accounts for elevated plasma FGF21 (6, 19, 22). We observed that hepatic FGF21 expression tracked perfectly with changes in plasma FGF21, increasing 60-fold between the virgin state and P18, and plummeting when plasma FGF21 expression returned to basal level by L1. We also assessed the contribution of other known expressing tissues. In well-fed virgin mice, the highest site of expression is the pancreas, whereas expression in adipose tissue is nearly as high as in the liver and is also nutritionally regulated (13, 14). Our data provide no support for their contribution as FGF21 expression dropped in both tissues at P18. Moreover, we are not aware of any evidence that pancreas or adipose tissue-derived FGF21 enters the circulation. On the other hand, skeletal muscle experiencing mitochondrial dysfunction and activated brown adipose tissue (BAT) are capable of both high FGF21 expression and plasma contribution (18, 28). FGF21 expression, however, was barely detectable in muscle across all physiological states, including at P18. We did not assess FGF21 expression in BAT because pregnancy does not lead to its activation (46), a necessary condition for BAT con-
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Fig. 6. Relationships between plasma FGF21 and metabolic variables in fasted and late-pregnant mice. Data are shown for the fasted (Fasting) or pregnancy day 18 (P18) conditions. They were obtained from age-matched mice when either fed (Fed V, n = 4–5) or fasted for a 24-h period (Fasted V, n = 4–5), or from age-matched mice on day 18 of pregnancy (P18, n = 5–6). Fed virgin mice were used as the control for both conditions and are represented in both sets of graphs. A: relation between plasma FGF21 and hepatic FGF21 mRNA. B: relation between plasma FGF21 and plasma NEFA. C: relation between plasma FGF21 and liver lipid content. The regression equation, coefficient of determination ($R^2$), and level of significance ($P$) are reported for each graph.

A number of factors are known to stimulate hepatic FGF21 production. In the mouse, FGF21 production is triggered by an increased flux of free fatty acids reaching the liver, arising either from mobilization of lipid stores or from ingestion of diets enriched in lipids (i.e., consumption of ketogenic diets in adults or milk by neonates) (5, 19, 22). Moreover, hepatic expression and/or plasma FGF21 are positively associated with liver fat content in humans and other species (47, 50). Mechanistically, these effects reflect fatty acid-mediated, peroxisome proliferator-activated receptor-α (PPAR-α)-dependent activation of FGF21 gene transcription (22, 32). This mechanism likely contributed to the increased hepatic FGF21 production seen in fasted mice in this experiment and in our previous work in early lactating dairy cows (40) because both groups of animals experience substantial increases in plasma free fatty acids and liver lipids. However, this mechanism is unlikely in the late-pregnant mouse because increased FGF21 production occurred without an increase in/or association with plasma NEFA and liver lipids. Another mechanism could be STAT5-dependent transcriptional activation of the FGF21 gene as recently proposed in cattle (51). In pregnant mice, hepatic STAT5 activation would follow engagement of the prolactin receptor by the high prevailing plasma levels of prolactin-related proteins (20). This mechanism is also unlikely because the mouse FGF21 promoter lacks the two STAT5 binding sites identified in the bovine gene (Y. R. Boisclair, unpublished observation). Finally, hepatic FGF21 production is also induced by a variety of stresses, including amino acid...
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deficiency, mitochondrial dysfunction, oxidative insults, and endoplasmic reticulum (ER) stress (10, 27, 28, 39). Each insult activates one of three stress-regulated kinases (PERK, GCN2, or HR1), leading to phosphorylation-dependent inhibition of the translational regulator eIF2α and increased synthesis of the transcription factor ATF4. ATF4 then drives increased FGF21 transcription via a pair of amino acid response elements (AARE) located in the proximal promoter (10, 39). The possibility that late pregnancy activates one or more stress kinases followed by ATF4-dependent FGF21 production warrants further investigation.

Glucose is the predominant oxidative fuel of the placenta and growing fetuses (8). Accordingly, maternal metabolism in late pregnancy is dominated by adaptations aimed at preserving the glucose supply and facilitating its placental uptake (8). Increased plasma FGF21 at this time could promote glucose sparing by increasing maternal reliance on lipid oxidation and ketogenesis. In support of this idea, exogenous FGF21 administration alleviates the defective lipid oxidation seen in fasted PPAR-α knockout mice, whereas FGF21 null mice have reduced hepatic lipid utilization when fed a ketogenic diet (5, 22). In this context, it is intriguing that the placenta expresses the obligatory FGF21 coreceptor β-Klotho at a level comparable to that of the pancreas, a recognized FGF21 target tissue (48). Moreover, placental FGF expression is dominated by FGFR1c, the receptor mediating FGF21 actions on glucose metabolism, including increased GLUT1 expression in adipose tissue (3, 26). GLUT1 is not only an FGF21 target gene, it is also a major glucose transporter in the mouse placenta (11). These observations suggest a model whereby increased maternal FGF21 at P18 not only safeguards the maternal glucose supply but also facilitates placental glucose uptake. A second role is suggested by the development of maternal insulin resistance during mouse pregnancy (21, 24). This natural adaptation promotes placental glucose transport (8) but also necessitates a substantial expansion of β-cell mass to preserve maternal normoglycemia (21, 24). Plasma FGF21 is often increased in individuals suffering from insulin resistance (50, 53), and FGF21 therapy reverses this condition (2, 16, 49). Accordingly, increased FGF21 in the late-pregnant mouse could serve to limit maternal insulin resistance and its negative consequences, such as β-cell malfunction and death (48).

Perspectives and Significance

We found that plasma FGF21 increases in the mouse in late pregnancy but becomes nearly undetectable in lactation. This is the exact opposite of the pattern we recently observed in the modern dairy cow, i.e., undetectable levels in late pregnancy followed by chronically elevated levels in early lactation (40). In both species, however, increased FGF21 overlaps with the period when maternal nutrition is the most precarious (i.e., late pregnancy in the mouse versus early lactation in the cow). This suggests that increased plasma FGF21 may be a signal coordinating maternal metabolism whenever nutritional insufficiency occurs during the reproductive cycle. It will be important in future studies to identify these FGF21-dependent adaptations and their roles in maintaining the metabolic well being of the dam and their ability to support placental and/or mammary functions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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