GPR120 controls neonatal brown adipose tissue thermogenic induction

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Abstract

Adaptive induction of thermogenesis in brown adipose tissue (BAT) is essential for the survival of mammals after birth. We herein show that G-coupled receptor protein-120 (GPR120) expression is dramatically induced after birth in mouse BAT. GPR120 expression in neonatal BAT is the highest among GPR120-expressing tissues in mouse at any developmental stage tested. The induction of GPR120 in neonatal BAT is caused by the postnatal thermal stress rather than by the initiation of suckling. GPR120-null neonates were found to be relatively intolerant to cold: close to one-third did not survive at 21ºC, but all such pups survived at 25ºC. Heat production in BAT was significantly impaired in GPR120-null pups. Deficiency in GPR120 did not modify brown adipocyte morphology or the anatomical architecture of BAT, as assessed by electron microscopy, but instead impaired the expression of UCP1 and the fatty acid oxidation capacity of neonatal BAT. Moreover, GPR120 deficiency impaired FGF21 gene expression in BAT and reduced plasma FGF21 levels. These results indicate that GPR120 is essential for neonatal adaptive thermogenesis.

INTRODUCTION

In mammals, the developing fetus does not perform adaptive heat production for the maintenance of body temperature; instead, the body temperature is maintained via maternal thermal homeostasis (10, 19). In most mammalian species, brown adipose tissue (BAT), which is the main site of non-shivering thermogenesis, begins to develop in the late fetal period due to ontogeny-programmed mechanisms, in the absence of environmental thermal stress stimuli (4,17,27). At birth, neonates must rapidly adapt from the warm intra-uterine environment (close to 37°C) to a colder extra-uterine environment. The achievement of this adaptation is crucial, since newborn hypothermia can be lethal (1, 4). Postnatal temperature maintenance is mainly achieved by BAT-mediated non-shivering thermogenesis (1, 4). The post-birth activation of BAT involves the up-regulation of uncoupling protein-1 (UCP1, which is key to providing the mitochondria of BAT with thermogenic properties) and induction of its proton conductance (21), the progressive biogenesis of mitochondria within BAT, and adaptations for active metabolic fuel oxidation to sustain thermogenesis (4,8). Sympathetic activation is believed to induce neonatal BAT thermogenesis in a manner similar to the cold-induced activation of BAT in adults. However, additional factors appear to be involved in the neonatal induction of BAT thermogenesis. For example, high levels of fibroblast growth factor 21 (FGF21) in neonatal plasma, originating in the liver in response to the initiation of milk intake, have been shown to activate neonatal BAT thermogenesis (12). FGF21 favors BAT activation and promotes the browning of white adipose tissue in adults (6, 12). FGF21 is expressed and secreted by the liver, and may also be produced by BAT under conditions that trigger thermogenic activation (13), but the role of locally synthetized FGF21 in neonatal BAT thermogenesis is unknown.

We recently identified an additional pathway through which BAT and WAT undergo thermogenic regulation via activation of G-protein coupled receptor 120 (GPR120, also called fatty acid receptor-4, FFAR4) (22), a finding further confirmed using pharmacological activation of GPR120 (25). The activation of the GPR120 receptor, which is mostly responsive to polyunsaturated fatty acids, has been reported to mediate potent anti-inflammatory and insulin sensitizing effects, and to protect against obesity and associated metabolic diseases (28). In our prior work (22), we also showed that activation of GPR120 in BAT and WAT promotes adaptive thermogenesis through the induction of FGF21 expression. Here, we show that, immediately after birth, an astonishingly high level of GPR120 expression is seen in BAT. Indeed, the expression levels of GPR120 in neonatal BAT are much higher than in any other mouse tissue at any other developmental stage. Based on these findings, we further analyzed the role of GPR120 in neonatal BAT adaptations.

MATERIALS AND METHODS

Animals. All animal experiments and selections of group sizes were performed in accordance with European Community Council Directive 86/609/EEC and approved by the Institutional Animal Care and Use Committee at the University of Barcelona. Swiss mice (Envigo) and C57/BL6 GPR120-heterozygous mice (Ffar4tm1(KOMP)Vlcg; MMRRC) were used. C57/BL6 GPR120-heterozygous mice were mated and experiments were performed on the obtained GPR120-/-, GPR120+/-, and GPR120+/+ littermates. For studies in fetuses, Cesarean sections were performed on day 19 of gestation (E19). Neonates were studied at birth (0 hour, when all pups had been born but had not yet started suckling), at 6 (P0.25), 12 (P0.5) and 24 (P1) hours after birth. For ontogeny expression studies, Swiss mice were sampled at 7 (P7), 14 (P14), 21 (P21), and 70 (adult) days after birth. For studies on the effects of postnatal starvation and environmental temperature, Swiss pups were separated from mothers prior to initiation of suckling and placed in a humidified thermostatically controlled chamber at 21°C or 37°C for 8 hours. BAT temperature was non-invasively estimated by measuring the iBAT skin-surface temperature using a high-sensitivity infrared thermography camera (FLIR T33) as previously reported (22). Mice were killed by decapitation and the liver, interscapular BAT, duodenum, jejunum, ileum, and colon were dissected. Blood was collected, glucose levels were determined from a portion of the blood sample using an Accutrend (Roche), and the remaining blood was centrifuged to obtain plasma.

Transmission electron microscopy. BAT samples were fixed with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) and post-fixed with 1% osmium tetroxide and 0.8% FeCNK in phosphate buffer. After dehydration in a graded acetone series, tissue samples were embedded in Spurr resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Jeol 1010 transmission electron microscope (Izasa Scientific, Barcelona, Spain), as described previously (3).

Glucose and palmitate oxidation. Samples of interscapular BAT from 1-day-old (P1) wild-type and GPR120-/- pups were dissected. Pieces of BAT (~ 3 mg) were placed in DMEM (Gibco) for 1 hour. Thereafter, the samples were incubated for 3 hours with ^{14}C-glucose (1 µCi/mL) (Perkin Elmer, NEC043X050UC) or ^{14}C-palmitate (0.4 µCi/mL) (NEC534050UC), the media were acidified, and the $^{14}CO_2$ released from incubated explants was trapped into Whatman paper for 45 min. The impregnated Whatman sheets were placed in Ecoscint H (National Diagnostics) and the dpm were quantified using a Liquid Scintillation Analyzer (TRI-CARB 2100 TR, Packard Bioscience Company).

RNA extraction and quantitative PCR with reverse transcription RNA was extracted from the tissue samples with a NucleoSpin® RNA kit (Macherey-Nagel, Düren, Germany). Reverse transcription was performed using random hexamer primers (Applied Biosystems, Foster City, CA, USA) and 0.5 µg RNA in a total reaction volume of 20 µl. For PCR, TaqMan Gene Expression Assay probes were used, with reaction mixtures containing 1 µl cDNA, 10 µl TaqMan Universal PCR Master Mix (Applied

Biosystems), 250 nM probes, and 900 nM of primers from the Assays-on-Demand Gene Expression Assay Mix (Applied Biosystems). The TaqMan probes used were : GPR120 (Ffar4), Mm00725193_m1; UCP1, Mm00494069_m1; Fgf21, Mm00840165_g1; Lpl, Mm00434764_m1; Plin1, Mm00558672_m1; PPARalpha, Mm00440939_m1; Acox1, Mm00443579_m1; Cpt1a, Mm01231183_m1; Ehhadh, Mm00619688_m1; 18S rRNA, Hs99999901_s1. The 18S rRNA was measured as a housekeeping reference gene. The mRNA level of each gene of interest in each sample was normalized to that of the reference control using the comparative ($2^{-\Delta CT}$) method. A transcript was considered to be non-detectable when CT≥40.

Western blot and ELISA. Western blot analysis of tissue extracts was performed following standard procedures, using primary anti-UCP1 (1:1000 Abcam, Cambridge, UK), and anti-GPR120 (1:150 sc-390752, Santa Cruz, USA). Loading controls were established using antibodies against α-tubulin (T9026, Sigma-Aldrich) or GAPDH (G9545, Sigma-Aldrich). Immunoreactive proteins were detected using an ECL (enhanced chemiluminescence) system (GE Healthcare). Signal intensities were quantified by scanning densitometry (Multi Gauge V3.0, Fujifilm). Plasma FGF21 levels were quantified with ELISA (RD291108200R, Biovendor).

Statistics. Results are expressed as mean ± SEM. Statistical analyses were performed using GraphPad Prism 6 (La Jolla, CA, USA). The statistical significances of differences were assessed using unpaired Student's t-tests, one-way ANOVA with Tukey's multiple comparison tests, or two-way ANOVA with Bonferroni post-testing, as appropriate.

RESULTS AND DISCUSSION

High levels of Gpr120 expression in neonatal BAT. The developmental regulation of GPR120 mRNA expression in mouse BAT is shown in Figure 1a, where it is compared with that in another tissue known to express the *Gpr120* gene (intestine sections) and a tissue known to minimally express *Gpr120* (liver) (11,15,20). In adults, the expression of the GPR120 transcript was significantly higher in BAT than in the other *Gpr120*-expressing tissue (intestine), which is consistent with the previous data (22, 23). GPR120 *mRNA* expression was low in fetal BAT as well as pups just born which did not initiated suckling yet (P0); by just 6 hours post-partum, however, the GPR120 mRNA level had increased so dramatically that it was much higher than those in adult BAT. This high-level expression of the GPR120 mRNA in BAT was maintained during the first days of life and declined progressively thereafter to reach adult levels. In the other GPR120-expressing sites GPR120 transcript expression was higher in adults than in neonates, and GPR120 mRNA levels tended to increase progressively throughout development. The colon was unique in showing an early postnatal induction of *Gpr120* gene expression, but the extent of this induction was much lower than that in BAT. Assessment of GPR120 protein levels confirmed these trends and indicated that the highest abundance of GPR120 was observed in neonatal BAT (Figure 1b).

Post-natal thermal stress induces Gpr120 expression in BAT. Given that GPR120 is a lipid sensor, we tested whether the early postnatal induction of its mRNA in BAT could be associated with the initiation of suckling or other events associated with birth, such as thermal stress. For this purpose, mouse pups were studied under four different conditions: just after birth but before sucking was initiated (0 hour) vs. 8 hours after birth, having been maintained with the mother and confirmed to have suckled; or having been separated from the mother before initiation of suckling and maintained under non-feeding conditions at an environmental temperature of 21°C or 37°C. Our results confirmed that GPR120 mRNA expression was induced in BAT at 8 hours after delivery under feeding conditions and that this behavior was shared by BAT thermogenesis-related genes, such as Ucp1 and Fgf21 (Figure 2). GPR120 transcript expression was also induced in non-fed pups that were maintained at 21°C, whereas it was totally blunted in non-fed pups maintained at 37°C (the same temperature as in the intrauterine environment). Again, the behavior of *Gpr120* gene expression under these conditions was similar to that of the thermogenesis-related genes, *Ucp1* and *Fgf21*. In contrast, another gene unrelated to specific thermogenic activation but to overall adipogenesis such as *PPARg* did not show a pattern of cold-induced expression in the early neonatal period. Together, our results revealed that, similar to *Ucp1* and *Fgf21*, the neonatal induction of *Gpr120* gene expression in BAT is mostly elicited by postnatal thermogenic stress rather than by the initiation of feeding.

Intolerance to post-natal cold in GPR120-null neonates. Our observation that the GPR120-coding gene is intensely regulated in neonatal BAT suggested that GPR120 could play a role in neonatal thermogenic adaptations. To analyze this possibility, we studied GPR120-/- (GPR120-KO) mouse neonates. Male and female C57/BL6 *Gpr120 (Ffar4*) +/- mice were mated, and females were maintained at a housing temperature of 21°C during pregnancy and after delivery. At this temperature, the mortality of GPR120-/- pups at 1 day after birth was high (27.3%), while that of GPR120-/+ pups was lower but still notable (16%) (Figure 3a). Increasing the environmental temperature at delivery by 4°C (to 25°C) suppressed the mortality of GPR120-/- pups. These findings indicate that GPR120 plays a key role in the thermogenic adaptations that occur during the neonatal period. For further studies, wild-type and GPR120-/- littermates were maintained at an environmental temperature of 25°C.

Impaired thermogenic activity of BAT in GPR120-null pups. Wild-type mice and GPR120-/- littermates were studied at E19, P0, P0.25, P0.5, P1 and P21. No significant difference was observed in total body weight, BAT weight, or liver weight in GPR120-/- pups compared with wild-type neonates. Blood glucose levels did not show any major between-genotype difference, although P1 GPR120-/- pups showed decreased blood glucose levels relative to wild-type littermates (Table 1). In wild-type littermates of GPR120-/- mice (C57/BL6 strain), we observed a strong postnatal induction of GPR120 mRNA similarly to what we observed in Swiss mice, although of lesser magnitude (Figure 3b). Heat production by BAT was determined using infrared thermography at the interscapular site of mouse pups, where the most prominent BAT depot is present. Our data indicated that significantly less heat was produced by BAT in P1 GPR120-/-

pups compared with wild-type neonates (Figure 3c). However, electron microscopy of the cellular morphology of BAT samples did not reveal any massive alteration and only a minor, non-significant, tendency to reduced lipid droplet size, due to *Gpr120* gene loss-of-function (Fig. 3d).

The postnatal induction of *Ucp1* gene expression was significantly reduced in BAT from GPR120-/- mice at P0.25 and P0.5 and UCP1 mRNA levels remained lower at postnatal day 21 (Figure 4a). UCP1 protein levels were also significantly lower in one day-old GPR120-/- pups relative to wild-type pups (Fig 4a). In fact, the GPR120-/- genotype was associated with a statistically significant reduction of UCP1 mRNA ($P \leq 0.05$) and UCP1 protein ($P \leq 0.05$) levels when all of the studied neonates were analyzed as a whole, according to two-way ANOVA factor analysis. UCP1 protein levels and unmasking of the UCP1-mediated protein conductance are considered to be essential for neonatal BAT thermogenesis (4, 21) and the altered levels of UCP1 found in GPR120-/- pups are consistent with reduced heat production in BAT.

Our analysis of the oxidative activity of BAT explants from P1 pups revealed that lack of GPR120 did not alter the glucose oxidation rate, but was associated with a dramatic reduction of fatty acid oxidation activity (Figure 4b). The expression of transcript encoded by genes involved in lipid catabolism indicated a trend to be reduced in BAT from GPR120-/- pups, which was statistically significant for *Lpl* and *Cpt1a* in neonates and for *Aox1* and *PPARα* in fetuses at term (Figure 4c). Moreover, the GPR120-/- genotype was associated with a statistically significant reduction of PLIN1 transcript ($P \leq 0.05$) when pups at the two distinct stages of development were analyzed as a whole, according to two-way ANOVA factor analysis. Such trend was maintained in mice studied at the age of weaning (P21) but only PLIN1 mRNA levels were significantly decreased in GPR120-/- mice relative to wild-type controls at that age (Fig 4c). Collectively, these data indicate the GPR120 is necessary for the adaptive thermogenic activation of neonatal BAT, but that it does not determine the cell differentiation or acquisition of gross BAT structure during neonatal development. The impairment in fatty acid oxidation pathways in BAT due to the lack of functional GPR120, occurring in concert with impaired BAT thermogenesis, is consistent with the preferential usage of fatty acids as metabolic sources for sustaining heat production in BAT (2). The impact of GPR120 loss-of-function in neonatal mice (compromised survival associated with impaired BAT function, and its rescue by high environment temperature) is reminiscent of similar observations in mice with targeted mutations of genes encoding key regulators of BAT development and function such as PACAP, a controller of norepinephrine release to BAT (9), PREF1 and DIO3 (5), and UCP1 itself (4). This highlights the importance of appropriate BAT function for postnatal thermoregulation and the key role of GPR120 in this process.

*GPR120 is required for the induction of FGF21 expression in BAT and FGF21 rise in blood from neonates***.** Previous reports showed that FGF21 plasma levels were increased during the first days of life in mice (12), and we previously found that GPR120-/- adult mice show reduced FGF21 circulating levels upon cold exposure (22).

The induction of FGF21 after birth is considered a neonatal-period adaptation that contributes to BAT thermogenesis (12). We herein found that pups deficient for GPR120 show impaired postnatal induction of plasma FGF21 levels at P0.5 and P1 and decreased levels of FGF21 were also present at P21 (Figure 5a). The FGF21 present in neonatal blood is considered to have a mainly hepatic origin (12), although adult BAT is known to express and release significant amounts of FGF21 when thermogenesis is activated (13). We found that FGF21 mRNA expression is markedly induced in BAT in the first hours of life, and that such induction is strongly impaired in GPR120-/- pups. Also, the postnatal increase in FGF21 mRNA expression in the liver was not affected by GPR120 gene loss-of-function (Figure 5b). Thus, we conclude that the effects of GPR120 on neonatal adaptation involve alterations of the FGF21 system in BAT, which may have consequences in systemic levels of FGF21. Considering previous data on the effects of FGF21 in BAT (12, 26), impaired signaling of FGF21 may be involved in the reduction in *Ucp1* gene expression and fatty acid oxidation in GPR120-null neonates.

In summary, we herein show that the GPR120-dependent pathway is essential for inducing adaptive neonatal BAT thermogenesis and the control of the FGF21 system. GPR120 (FFAR4) is considered a receptor for long chain fatty acids, preferentially of the n-3 PUFA type (18). N-3 PUFAs have been reported to induce brown fat activity in adult mice and in brown adipocytes in culture through interaction with GPR120 (16, 22). Initiation of lactation is associated with a massive and sudden intake of the lipids present in milk, including n-3 PUFAs (7), in the neonates, in contrast with the predominant glucose-based metabolic supply in the fetal period. Although we found that GPR120 expression itself is mostly regulated by cold stress rather than milk intake, our findings are consistent with the notion that fatty acids from milk may be essential regulators of neonatal thermogenic activation through the activation of the highly expressed GPR120 in BAT.

The current findings of a key role of GPR120 in energy metabolism in neonates may have relevant implications for neonatal and adult metabolic health. Although gene expression of GPR120 in human neonatal tissues is unknown, recent findings evidenced a postnatal surge in the levels of FGF21 (a main target of GPR120-dependent regulation according to our data above) in human neonates (24), analogously to rodents. However, the intensity of postnatal thermal stress in humans is likely to be lower than in rodents and, therefore, induction of GPR120 might be milder in human neonates. Moreover, a deleterious non-synonymous mutation that inhibits GPR120 signaling activity has been identified in human individuals, which increase the risk of obesity (14). However, in GPR120-deficient mice, obesity develops only after a high-fat diet (14). Thus, a similar quantitative role of GPR120 in energy balance among different species can not be assumed unequivocally. In any case, the strong impact of appropriate GPR120 signaling in the neonatal period metabolism found in mouse models may be of utmost importance not only in relation to perinatal health but also in relation to the adult consequences that metabolic alterations in early development may have.

AUTHOR CONTRIBUTIONS

TQL, SMR, and LC performed the animal experiments and monitoring of the pup deliveries. RI, AGN, and TQL performed the cesarean sections for fasting tests, differential temperature exposure experiments and substrates' oxidation assays. TQL, AGN and LC performed that quantitative analysis of transcripts and proteins. MG, FV, and TQL performed the data analysis and statistics; also cooperated in the writing of the article. All authors revised and accepted the manuscript details.

DISCLAIMERS

The authors declare that no conflicts of interest exist.

ACKNOWLEDGMENTS

We thank M. Morales and A. Peró for their help with the sample management.

GRANTS

This research was supported by grants from Ministerio de Economía y Competitividad (SAF2017-85722) and Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III (PI17/00420), co-financed by the European Regional Development Fund (ERDF). TQL thanks CONACyT (Consejo Nacional de Ciencia y Tecnología, México) for PhD scholarship.

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Figure legends

Figure 1. Developmental regulation of GPR120 gene expression. (a) Relative transcript levels of GPR120 (FFAR4) in tissues from Swiss mice obtained at the indicated developmental time points. E19, fetuses, embryonic day 19; A, adults, 70 days old. (b) Representative immunoblot of GPR120 (FFAR4) protein levels in BAT (left), ileum and colon (right). Images correspond to equally loaded lanes with identical incubation conditions and exposure time. Bars are means + sem of 4-10 pups from at least 3 independent litters. One-way ANOVA was performed and the statistically significant differences are shown as *P<0.05, **P<0.01, and ***P<0.001 compared to E19.

Figure 2. Effects of post-natal fasting and cold stress on GPR120 gene expression in BAT. Relative transcript levels of GPR120, Fgf21, UCP1 and PPARγ in neonates at birth (0h, before initiation of suckling) and 8-hour-old pups that had been allowed to suckle (Fed) or not allowed to suckle (Fasted) and maintained at environmental temperatures of 21ºC or 37ºC. Bars are means $\pm$ sem of 4-10 pups from at least 3 independent litters. One-way ANOVA was performed and the statistically significant differences are shown as *P<0.05, **P<0.01, and ***P<0.001 compared to E19.

Figure 3. Effects of GPR120 gene loss-of-function on BAT in the perinatal period. (a) Aggregate survival rates of GPR120-/-, GPR120+/- and wild-type neonates during the first hours of life. (b) Relative transcript levels of GPR120 in tissues from C57/BL6 GPR120+/+ neonatal mice at the indicated developmental time points. (c) Representative infrared thermography images (left) and surface temperature quantifications from interscapular BAT areas (right) of 1-day-old GPR120-/- pups and wild-type littermates. (d) Representative transmission electron microscopic images (scale bar: 10 µm) of interscapular BAT (up) and lipid droplet area in BAT (down). Data are presented as means $\pm$ sem from 5-7 individual samples from at least 4 different litters. Statistically significant differences are shown as *P<0.05 (one-way ANOVA) for comparison with E19 and +P<0.05 (two-tailed unpaired Student's t-test) for comparison between GPR120-/- and wild-type littermates.

Figure 4. **Effects of GPR120 gene loss-of-function on UCP1 expression, glucose oxidation and fatty acid oxidation in BAT.** (a) Relative transcript levels of UCP1 (left) and UCP1 protein levels (right) in BAT from GPR120-/- pups and wild-type littermates at the indicated postnatal times. Representative immunoblot of UCP1 protein is shown (bottom of right panel). α-tubulin (ATUB), loading control. (b) Relative levels of $^{14}CO_2$ production from ^{14}C-Glucose and ^{14}C-Palmitate in BAT explants from 1-day-old pups incubated for 3 hours. (c) Relative transcript levels of the indicated gene in BAT from GPR120-/- fetuses, 6h-old neonates, and 21 day-old (weaning) mice, and wild-type littermates. Data are presented as means $\pm$ sem of 5-9 individuals from at least 5 different litters. Two-way ANOVA was performed and statistically significant differences are shown as *P<0.05, **P<0.01, and ***P<0.001 compared with E19, and

+ P<0.05 for comparison between GPR120-/- pups and wild-type littermates at each age.

Figure 5. FGF21 gene expression in neonatal GPR120-null mice. GPR120-/- pups and wild-type littermates were studied at the indicated ages. (a) Plasma levels of FGF21. (b) Relative transcript levels of Fgf21 in BAT. (c) Relative transcript levels of Fgf21 in liver. Data are presented as means ± sem of 5-9 individuals belonging to at least 5 different litters. Two-way ANOVA was performed and statistically significant differences are shown as *P<0.05, **P<0.01, and ***P<0.001 compared with the corresponding controls at E19; and as +P<0.05 and ++P<0.01 for comparison of GPR120-/- relative to wild-type pups at each age.

Figure 1

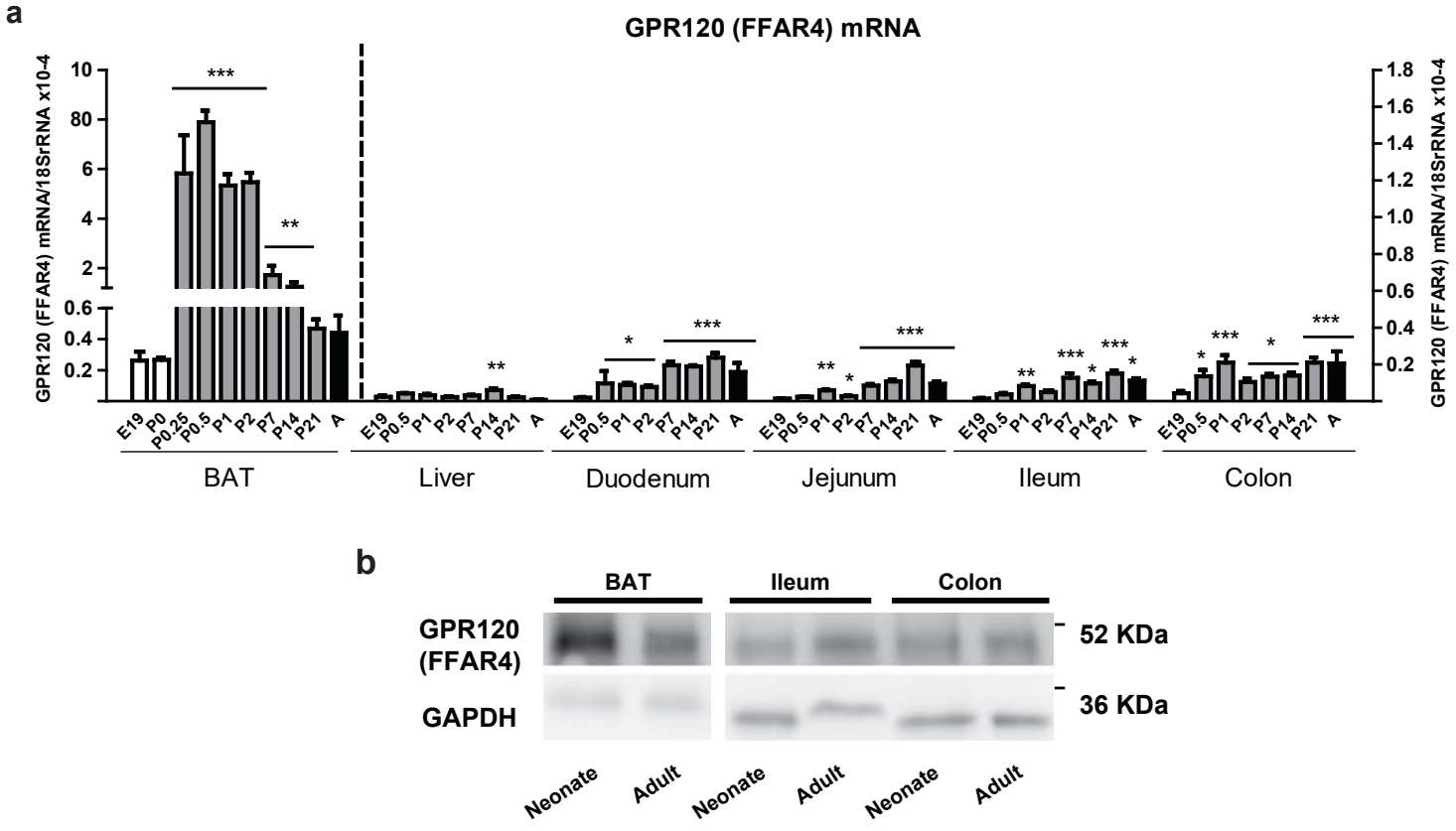

Figure 2

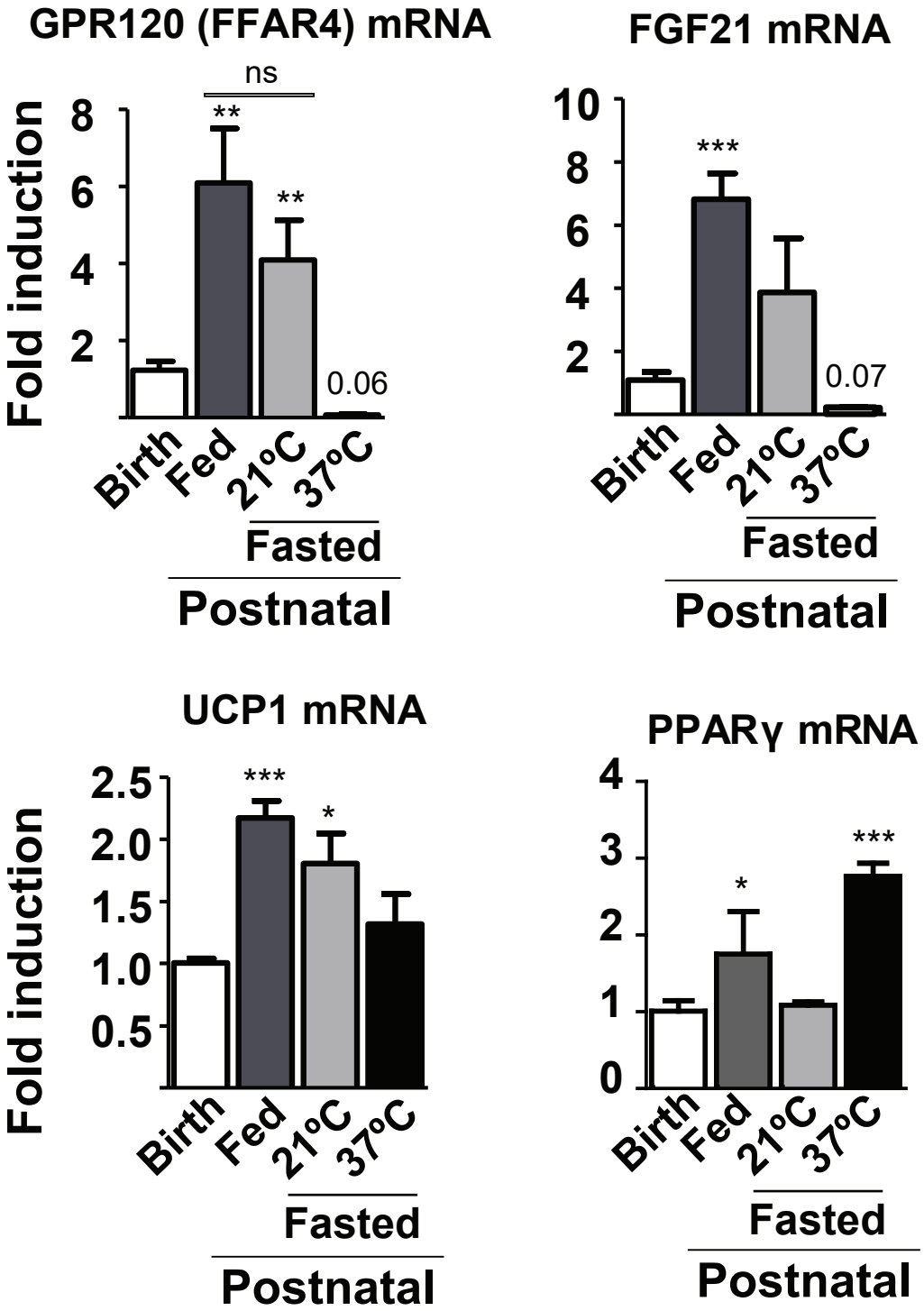

Figure 3

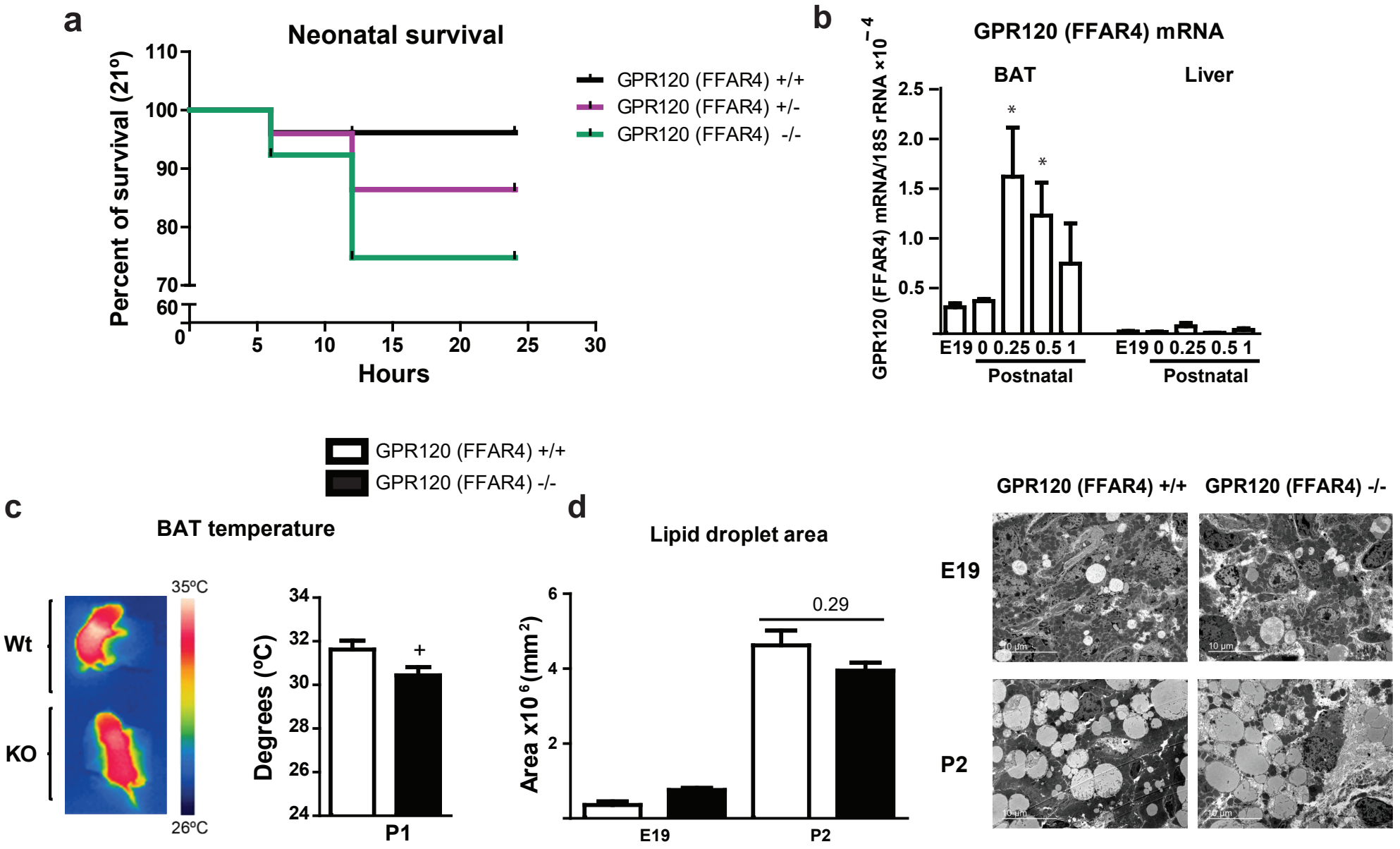

Figure 4

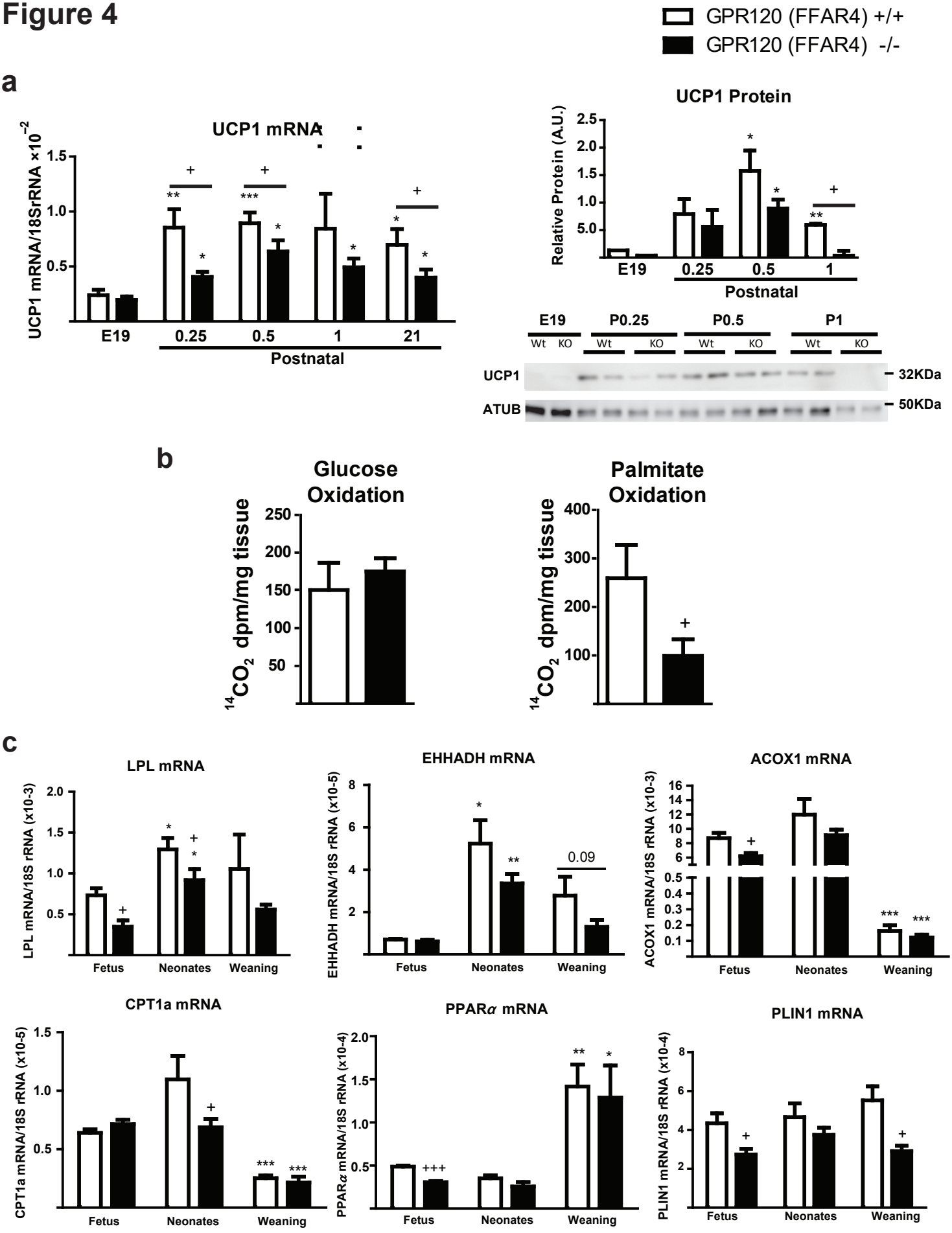

Figure 5

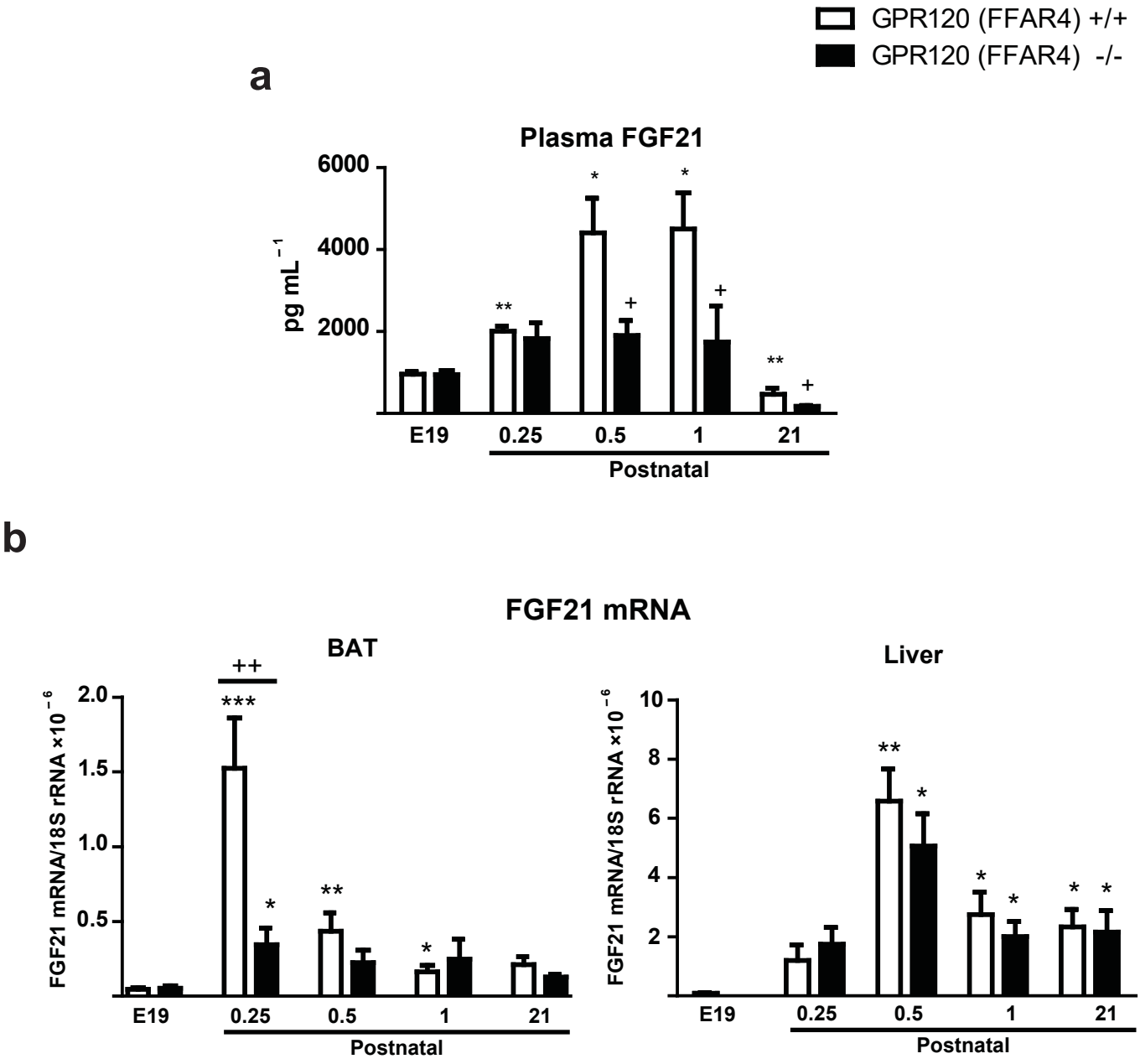

Table 1. Body weight, BAT and liver weights, and blood glucose											
	Fetus (E19)		6 h (P 0.25)		12 h (P 0.5)		24h (P 1)		P21		P value
	Wild-type	GPR120 (FFAR4) -/-	Wild-type	GPR120 (FFAR4) -/-	Wild-type	GPR120 (FFAR4) -/-	Wild-type	GPR120 (FFAR4) -/-	Wild-type	GPR120 (FFAR4) -/-	
Body Weight (BW) (g)	1.1 ± 0.05	1.0 ± 0.05	1.3 ± 0.03	1.3 ± 0.06	1.4 ± 0.05	1.3 ± 0.07	1.4 ± 0.07	1.4 ± 0.04	9.9 ± 0.2	9.8 ± 0.3	0,12
BAT Weight (mg/g BW)	9.0 ± 0.5	9.3 ± 1.2	7.2 ± 0.5	6.6 ± 0.6	7.2 ± 0.6	7.6 ± 0.7	6.3 ± 0.4	6.2 ± 0.3	4.9 ± 0.6	5.3 ± 0.3	0,70
Liver Weight (mg/g BW)	55.1 ± 1.9	46.0 ± 4.1	37.8 ± 1.3*	42.9 ± 1.5 +	37.0 ± 1.1	37.6 ± 1.6	33.8 ± 1.3	31.7 ± 2.4	43.7 ± 4.6	45.0 ± 2.4	0,41
Glucose (mg/dL)	52.8 ± 10.6	48.0 ± 5	60.0 ± 2.6	63.6 ± 10.5	63.7 ± 5.2	62.9 ± 4.9	81.0 ± 4.1	63.8 ± 4.9 +	154.8 ± 10.1	172.0 ± 15.5	0,93
Data are means ± SEM of 5 to 11 pups per group form at least 4 independent litters. Two-way ANOVA analysis was employed for determining genotype differences in expression (*P<0.05 for comparison between each age and its corresponding control; +P<0.05 for comparison between WT and GPR120-/- at a given age).											