

Apelin controls fetal and neonatal glucose homeostasis and is altered by maternal undernutrition

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ABSTRACT

The adequate control of glucose homeostasis during both gestation and early postnatal life is crucial for the development of the fetoplacental unit and adaptive physiological responses at birth. Growing evidences indicate that apelin and its receptor APJ, which are expressed across a wide range of tissues, exert important roles in glucose homeostasis in adult. However, little is known about the function of the apelinergic system during gestation. In this study, we evaluated in rats the activity of this system, the role of apelin in fetal and neonatal glucose homeostasis and its modulation by maternal food-restriction (FR). We found that: 1/ the apelinergic system is expressed at the fetoplacental interface and in numerous fetal tissues, 2/ ex-vivo, the placenta releases high amount of apelin in late gestation, 3/ i.v. apelin injection in mothers increases the transplacental transport of glucose, 4/ i.p. apelin administration in neonates increases glucose uptake in lung and muscle. Maternal FR drastically reduced apelinemia in both mother and growth-restricted fetuses and altered the expression of the apelinergic system at the fetoplacental interface. Together, our data demonstrate that apelin controls fetal and neonatal glucose homeostasis and is altered by fetal growth restriction induced by maternal undernutrition.

Key-Words: apelin, gestation, fetus, glucose homeostasis, neonates, IUGR

Introduction

Apelin is a regulatory peptide, identified as an endogenous ligand of the apelin receptor named APJ (1). Apelin gene encodes a 77-amino-acid preproprotein that generates during posttranslational processing several molecular isoforms such as apelin-36, apelin-17 and apelin-13 (2; 3). Moreover, the N-terminal glutamate of apelin-13 can be post-translationally modified thus creating the pyroglutamate apelin-13 ([pyr-1]-apelin-13) which is more protected from exopeptidase degradation (4). Apelin and APJ have a widespread distribution in the body and are involved in various physiological functions. Apelin and APJ mRNAs are expressed in heart, lung, placenta, mammary gland, several regions of the central nervous system, adipocytes and the gastrointestinal tract (5; 6). Depending on the cell type studied, APJ activation results in the activation of several intracellular effectors such as Extracellular signal-Regulated Kinases (ERKs), protein kinase B (PKB or Akt) and p70S6 kinase; but also in the inhibition of cAMP production (7; 8).

Recently, apelin has been extensively described as a beneficial factor regarding to glucose metabolism and is endowed with anti-diabetic properties (9; 10). Studies demonstrated that both short- and long-term apelin treatments improve insulin sensitivity in obese and insulin-resistant mice mainly by increasing glucose uptake in skeletal muscle (9-11). Apelin also modulates insulin secretion and increases pancreatic islet cell mass and β -cell insulin content in mice (12). During prenatal development, previous studies have revealed that APJ deficiency in mice causes early embryonic defects and leads to embryonic lethality due to growth retardation and cardiac malformations (13). However, the effect of apelin on glucose homeostasis *in utero* remains unknown. To gain further insight into apelin function during gestation and to study the effects of apelin on fetal and neonatal glucose homeostasis, the aims of the present study were to investigate in rats: 1/ the kinetics of apelin plasma levels in mother/fetus pairs, 2/ the ex-vivo placental apelin release, 3/ the gene expression levels of apelin and APJ in the fetoplacental unit, 4/ the effect of maternal apelin administration on transplacental glucose transfer and 5/ the effect of apelin administration to neonates on glucose uptake in several tissues. We subsequently studied the apelinergic (apelin and APJ) system in intrauterine growth-restricted (IUGR) fetuses from rat mothers that received only 30% of the food-intake of control mothers (FR30 model).

Research design and methods

Animals

Experiments were conducted in accordance with the institutional guidelines for the use of laboratory animals and were approved by the animal ethics committee (from University of Lille). Adult Wistar rats (Janvier) were housed at 22°C with a 12-h light/dark cycle with free access to a chow diet (16% protein, 3% fat, 60% carbohydrates). Females were mated with a male. Embryonic day 0 (E0) was defined the following day if spermatozoa were found in vaginal smears. Pregnant females were divided in two groups: 1/ a control group (n=50) in which dams were fed *ad libitum* and 2/ a food-restricted group (n=27) in which females received 30% of the food-intake of control mothers from E1 to E21. Five virgin adult females were used for apelinemia determination.

Plasma and tissue collections

Tail blood samples were collected at 9 AM in virgin and E7 females to measure apelinemia. At E13, E17 and E21, pregnant rats were killed by decapitation. Placentas, fetuses and mesometrial triangles were collected by caesarean section. Maternal and fetal plasma aliquots were stored at -20°C. Placentas and mesometrial triangles were frozen in liquid N₂ and stored at -80°C. Some E21 fetuses (n=5) were used to study the tissue distribution of apelin and APJ mRNAs using reverse transcriptase quantitative real time PCR (RT-qPCR) analysis. In fetuses, 17 selected tissues were dissected, frozen and stored at -80°C.

Endocrine and circulating parameters

Commercially available ELISA/EIA kits were used to measure plasma insulin (DRG International, catalog number: EIA-2943) and apelin (Phoenix Pharmaceuticals, catalog number: EK-057-23 that assayed all isoforms of apelin from apelin-12 to apelin-36) levels. Blood glucose was measured using a glucometer (Roche Diagnostics).

RT-qPCR analysis

Methods for RT-qPCR analysis have been previously described (14). RT-qPCR was performed with a Light Cycler 480 SYBR Green I master and a LightCycler480 (Roche). Primers for apelin, APJ, glucose transporter type 1 (GLUT1) and GLUT3 genes reported in supplementary table1 were designed using the Primer Premier software (Premier Biosoft International). Several housekeeping genes (*Gapdh*, *Ppib* and *Hprt1*) were used for the normalization and have been previously described (14).

Placental apelin secretion.

After cesarean section, E17 and E21 placentas (n=24) were collected rinsed in saline and incubated for 24 hours in dish plates containing 2 ml of DMEM (Gibco). Dish plates were placed at 37°C with 95% O₂/5% CO₂ and 95% humidity. Samples of medium were collected after 2, 6 and 24h of incubation for determination of apelin concentrations.

Placental transport of 2-deoxy-D-[³H] glucose

E17 pregnant females (n=10) were anesthetized by isoflurane and 10 min later implanted with a catheter in the jugular vein. An intravenous injection of 50 µCi 2-deoxy-D-[³H] glucose ([³H]-2DG) (NEN LifeScience) with either saline or 600 pmol/kg of [pyr-1]-apelin-13 (Bachem, UK) was performed. After 30 min, placentas and fetuses were rapidly collected, weighed and frozen. Total [³H]-2DG content in placental and fetal homogenates were measured.

Effect of apelin-13 on glycemia of neonates

E21 pregnant females (n=9) were killed by decapitation and fetuses were collected by cesarean section. Neonates were immediately weighted and injected intraperitoneally with either 0.1 ml of saline or different doses of [pyr-1]-apelin-13 (from 1 to 40 nmol/kg). After 30 min, blood glucose was measured. Plasma samples from neonates injected with saline and with 10, 15, 20 and 40 nmol/kg of apelin were also used for insulinemia determination.

In Vivo 2-deoxy-D-[³H] glucose incorporation in neonatal tissues

An intraperitoneal injection of 50 µCi of 2-deoxy-D-[³H] glucose in saline or associated with [pyr-1]-apelin-13 (15 nmol/kg) was performed in rat neonates at birth (n=14). After 30 min, rats were decapitated and 7 selected tissues were collected for [³H]-2DG uptake determination.

Statistical analysis

Results are reported as means ± SEM. Statistical analyses were performed using one way ANOVA and Dunnett's test. A *P* value of less than 0.05 was considered significant.

Results

Plasma and expression levels of apelin and APJ in the rat fetoplacental unit

Maternal plasma apelin concentrations were decreased at E7, increased from E7 to E17 and reduced at term (Fig. 1A). Fetal plasma apelin concentrations were 2-fold higher than maternal levels at E17 while they were similar at E21 (Fig. 1B). Apelin mRNA levels were higher in the Meso-tr compared to placenta at E13 and thereafter reduced until term of gestation in both tissues (Fig. 1C). APJ mRNA levels were similar in these two tissues at E13 (Fig. 1D). In the placenta, APJ expression was increased at E17 vs E13 level and reduced at E21 whereas in the Meso-tr an opposite modulation was observed (Fig. 1D). *Ex-vivo*, rat placentas released increasing quantities of apelin during 24h and this secretion was approximately twice higher at E17 than at E21 (Fig. 2A).

Apelin stimulates transplacental glucose transport and glucose uptake in fetus

Using [³H]-2DG as a tracer, we showed that apelin-13 i.v. administration to E17 females did not significantly affect placental glucose content (Fig. 2B) but significantly increased fetal glucose uptake (Fig. 2C) without affecting placental GLUT1 and GLUT3 mRNA levels (Fig. 2D). In E21 fetuses, tissue distribution of apelin and APJ mRNAs demonstrated high expression levels in lung, heart, brain, kidney, stomach, muscle and testis (Fig. 3A-3B). A modest expression of this system was found in all others E21 tissues investigated (Fig. 3A-3B). Neonates injected with 10-15 nmol/kg of apelin-13 displayed a reduction in glycemia whereas the injection of higher doses of apelin-13 caused an increase in glycemia (Fig. 3C) and a reduction of insulinemia (Fig. 3D). Concomitant administration of apelin-13 (at 15 nmol/kg) and [³H]-2DG demonstrated that apelin has a powerful glucose-lowering effect by enhancing glucose uptake in skeletal muscle and lung (Fig. 3E).

Effects of maternal food restriction on fetal growth and the apelin/APJ system

FR30 reduced both maternal and fetal body weights from E13 to E21 (Fig. 4A-C). FR30 reduced maternal plasma apelin concentrations from E13 to E21 (Fig. 4D) and fetal plasma apelin concentrations at E17 (Fig. 4E). Apelin mRNA levels were up-regulated at E17 in FR30 placentas and Meso-Tr (Fig. 4F-4G). APJ gene-expression was increased in FR30 placentas at E13 and reduced at E21 in FR30 placentas and Meso-Tr (Fig. 4H-4I).

Discussion

In the present study, we demonstrated that maternal apelinemia is augmented during gestation and that ex-vivo the placenta releases high amount of apelin at E17, a period which coincided with a peak secretion of apelin in both maternal and fetal plasmas. Moreover, we showed that maternal apelin i.v. administration increases the transplacental transport of glucose and that, in neonates, acute apelin i.p. injection has a powerful glucose-lowering effect associated with enhanced glucose uptake in lung and muscle.

We found that maternal apelin levels are decreased during the first week of gestation, increased gradually from E7 to E17 and reduced at term. This decline of apelinemia at term is related to an increase apelin clearance by the placental angiotensin-converting enzyme-related carboxypeptidase-2 (ACE2) which catabolized apelin in late-gestation (15). In fetuses, we observed that apelin levels are twice higher than maternal level at E17 while they are similar at E21. This is consistent with studies in humans that show a similar doubled apelinemia in umbilical cord blood compared to maternal plasma apelin concentrations (16). In newborn babies, a drop in apelin levels was found at neonatal day 1 (16) suggesting that the placenta may be a source of apelin. In accordance, the apelinergic system is expressed in several placental compartments in both rats (15) and humans (17). In our study, we analyzed the gene expression level of this system in rat placentas and in mesometrial triangles from E13 to E21. We observed that placental apelin gene expression is closely correlated to maternal apelin levels pointing out to the potential placental origin of maternal apelin. To test this hypothesis, we studied the apelin release from placenta ex-vivo. We demonstrated that rat placentas are able to release significant amount of apelin and that this secretion is twice higher at E17 than at term (E21). This secretion coincided with a peak secretion of apelin in both maternal and fetal plasmas at E17 suggesting that this organ is a source of circulating apelin. This interpretation is in accordance with data from Van Mieghem *et al.* (15) showing that, in rats, a fetoplacental reduction significantly reduced maternal apelin levels.

Here, we report that the apelinergic system is expressed at the feto-maternal interface. Of note, we observed a drastic increase in APJ placental gene expression between E13 and E17. We postulated that apelin may enhance transplacental nutrients transfer, more especially glucose transport. We found that apelin injection to E17 mothers increased transplacental glucose transport suggesting that maternal apelin levels control fetal glucose supply. This was observed without change in placental glucose transporters expressions. In rat placentas, apelin was detected in perivascular smooth muscle of the labyrinth suggesting that apelin may exert vasoactive action in the placenta (15) as it has been extensively reported for apelin in the periphery (18-20). We propose that apelin may induce a vasodilatation of placental vessels

which would enhance transplacental glucose transfer or that, indirectly, apelin may modulate maternal blood pressure resulting to this enhanced placental transport.

We observed high apelin levels in fetuses suggesting that apelin may be important for fetal development. In E21 fetuses, the apelin and APJ tissue-distribution was closely comparable to adults (5; 6; 21) with higher expressions found in lung, heart, muscle, brain, kidney, stomach and testis. Apelin injection to neonates was able at low dose to reduce glycemia and inversely to increase glycemia at high dose by inhibiting insulin release. These results are in accordance with the hypoglycemic effect of physiologic low doses of apelin in mice (11) and with the inhibitory role of high pharmacologic doses of apelin on insulin release (22). We demonstrated that the hypoglycemic effect of apelin was associated with an increase in lung and muscle glucose uptakes. Similarly, in mice, apelin was shown to exert a powerful glucose-lowering effect associated with enhanced glucose utilization in skeletal muscle (11).

Finally, we studied the apelinergic system in IUGR fetuses from FR30 mothers. FR30 drastically reduced maternal apelin levels from E13 to E21 as well as fetal apelin levels at E17. In accordance, plasma apelin concentrations were found to be reduced by food-restriction in adult rodents and humans (9; 21). In our FR30 model, we found that at the materno-fetal interface, apelin gene expression was up-regulated at E17. This phenomenon may be a compensatory response of IUGR fetuses to increase their glucose supply to improve their growth. All together, our data demonstrate that apelin is a new hormone implicated in fetal and neonatal glucose homeostasis.

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Disclosures

No potential conflicts of interest relevant to this article, financial or otherwise are reported.

Figure legends

FIG. 1. Maternal (A) and fetal (B) plasma apelin concentrations and apelin/APJ gene expression at the feto-maternal interface during rat gestation (C, D). **A:** Maternal plasma apelin concentrations during gestation at days E7, 13, 17 and 21. NP: non-pregnant rats. Values are means \pm S.E.M. ($n=5-6$ rats/stage). *, $P < 0.05$; **, $P < 0.01$ E13, E17 and E21 values vs E7 values. ⁽⁺⁾, $P < 0.05$ E7 vs NP values. **B:** Circulating apelin concentrations in mother/fetus pairs at E17 and E21 ($n=6$ litters/stage). ^(##), $P < 0.01$ fetus vs mothers values. ^[+++], $P < 0.01$ E17 fetus vs E21 fetus values. **C, D:** Evolution of apelin and its receptor APJ mRNA levels at E13, E17 and E21 in the rat placenta and mesometrial triangle (Meso. Tr). #, $P < 0.01$; ##, $P < 0.001$ E17 and E21 values vs respective E13 values ($n=6$ male fetuses from 6 independent litters/stage).

FIG. 2. Apelin is released ex-vivo by rat placentas and stimulates transplacental glucose transport. **A:** Ex-vivo apelin secretion during 24-hours of incubation of E17 and E21 rat placentas ($n=12$ placentas from 4 independent litters/stage). Values are means \pm S.E.M. #, $P < 0.001$ vs values at time T0; *, $P < 0.01$; ***, $P < 0.001$ E17 vs E21 values at the same time of incubation. **B:** Changes in placental 2-deoxy-D-[³H] glucose (D-[³H]-2-DG) content and transplacental D-[³H]-2-DG transport to fetus (**C**) at day E17 of gestation in pregnant rats *i.v.* injected with either NaCl (control) or 600 pmol/kg of [pyr-1]-apelin-13 ($n=5$ independent litters/group). *, $P < 0.05$ apelin-injected group vs control. **D:** Placental glucose transporter (Glut1 and Glut3) gene expression level 30 minutes after maternal *i.v.* [pyr-1]-apelin-13 administration at E17 ($n=10$ placentas from 5 independent litters/group).

FIG. 3. Gene expression levels of apelin and APJ in E21 rat tissues (A, B) and effect of apelin on glucose homeostasis in rat neonates. (**A, B:** $n=6$ male fetuses). **C, D:** Effects of *i.p.* administrations of different doses of [pyr-1]-apelin-13 to neonates on glycemia and plasma insulin concentrations ($n=5-7$ rats/treatment groups). Values are means \pm S.E.M. *, $P < 0.05$; ***, $P < 0.001$ apelin-injected group vs NaCl-injected group. (**E**) Effect of a dose of 15 nmol/kg *i.p.* of [pyr-1]-apelin-13 on D-[³H]-2-DG content in rat neonatal tissues. *, $P < 0.05$ apelin-injected group vs control ($n=7$ neonates/group).

FIG. 4. Effect of maternal 70% food restriction (FR30) during gestation on maternal and fetal body weight (A-C), apelinemia (D, E) and on the expression of the apelinergic system at the feto-maternal interface (F-I). E13, E17 and E21: gestational days 13, 17 and 21. (**A-C:** $n=10$ litters/group). Values are means \pm S.E.M. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ FR30 vs control group. **C-F:** Quantification of mRNA levels of the apelin/APJ system in placenta and mesometrial triangle (Meso. Tr). *, $P < 0.05$; **, $P < 0.01$ FR30 vs control group ($n=6$ male fetuses from 6 independent litters/developmental stage).

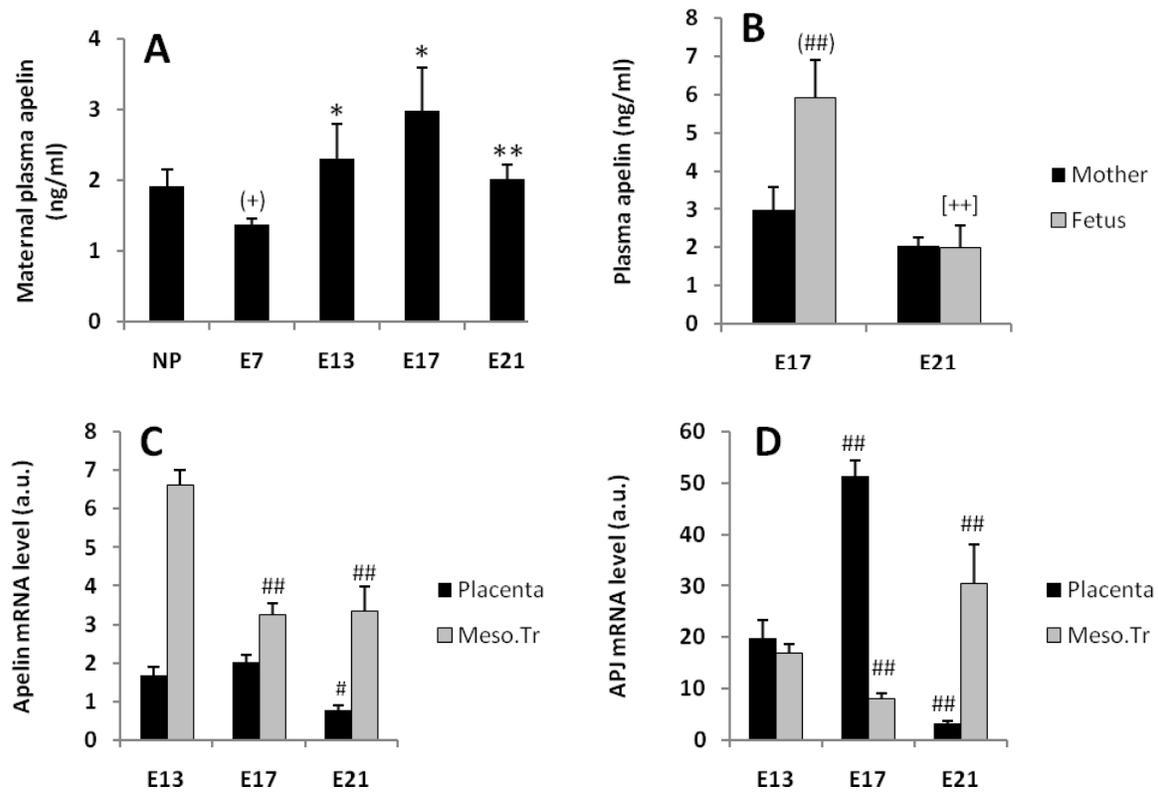


FIGURE 1

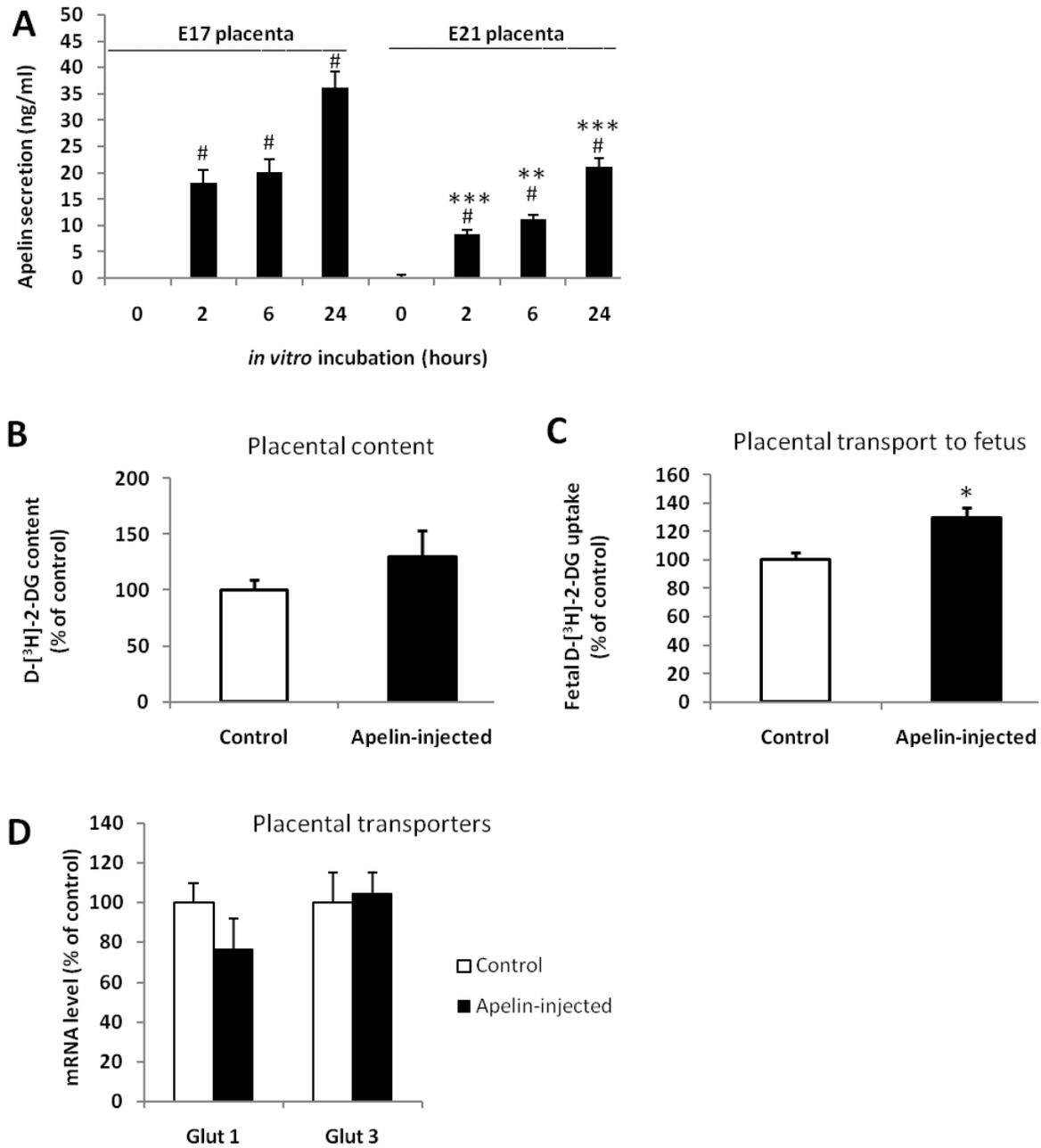


FIGURE 2

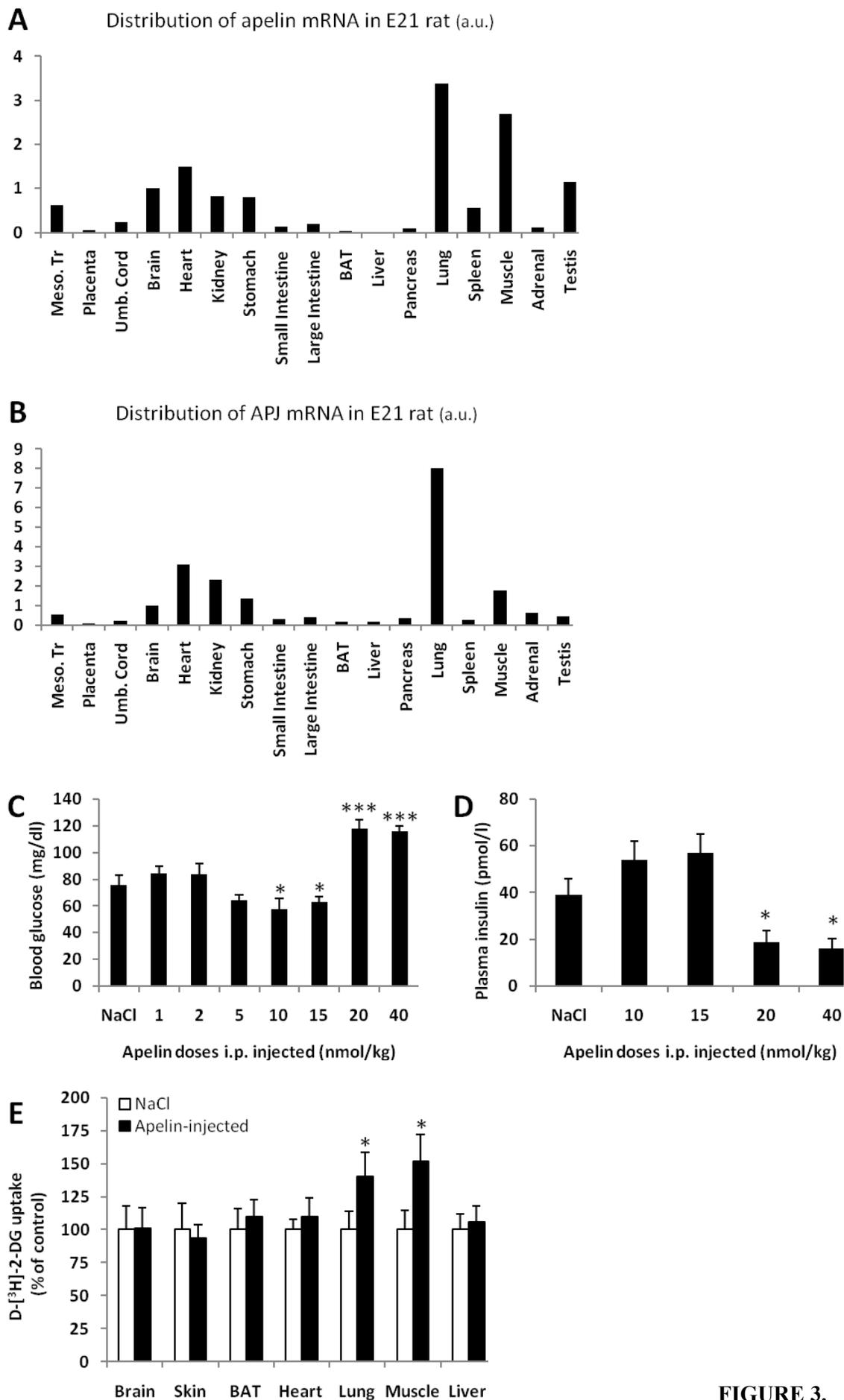


FIGURE 3.

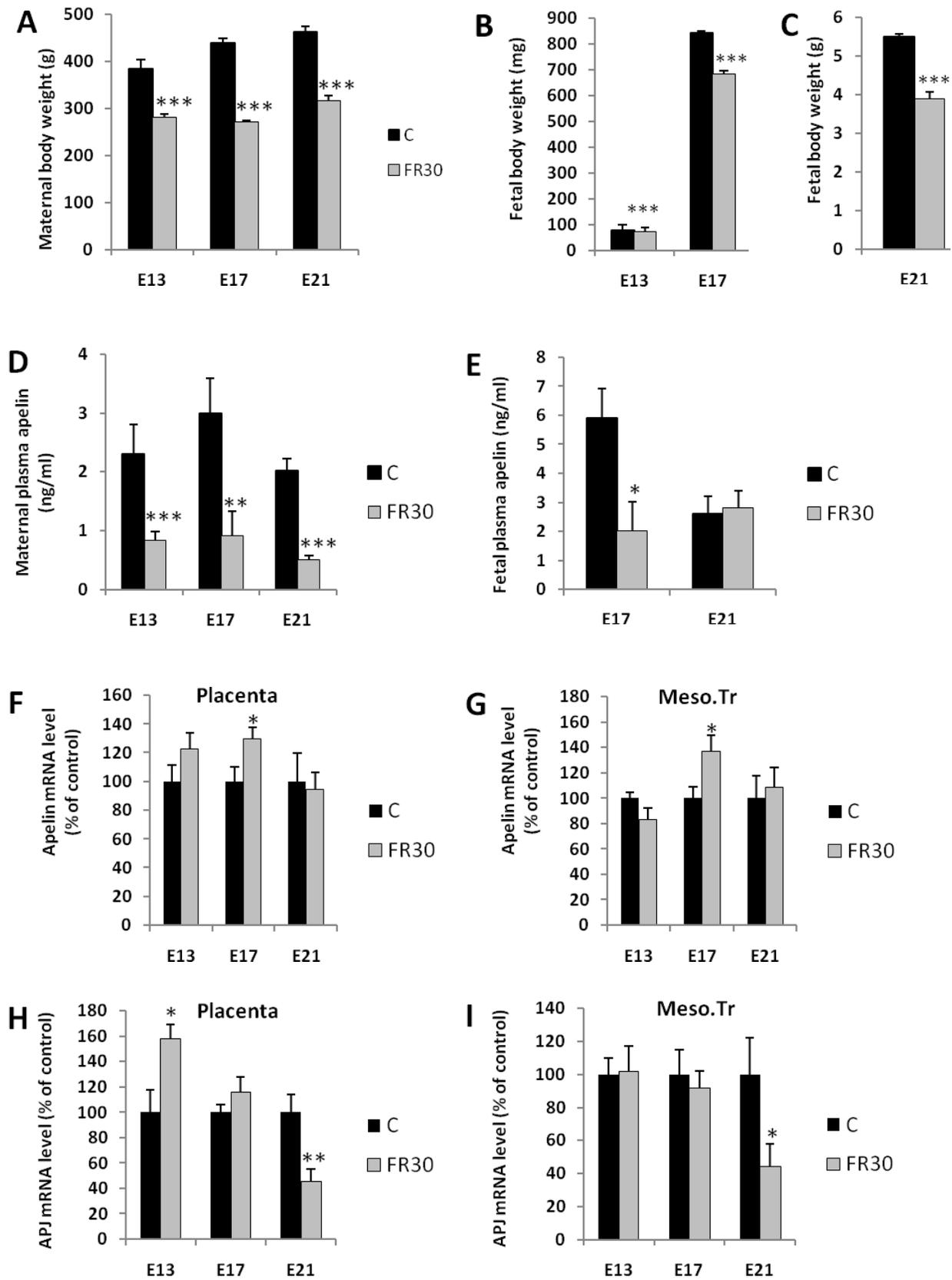


FIGURE 4.

Supplemental table 1: Parameters of the primers used for qRT-PCR

Gene	Primer		Amplification temperature (°C)	Amplicon size	Genbank
	Sequence (F)	Sequence (R)			
<i>Gapdh</i>	5'-TGA CTC TAC CCA CGG CAA GTT-3'	5'-CTT CCC ATT CTC AGC CTT GAC T-3'	60	52	NM_017008
<i>Ppib</i>	5'-TTT CGG CAA AGT TCT GGA AGG CA-3'	5'-CTT GCC ACA GTC TAC AAT GAT CAC A-3'	60	109	NM_022536
<i>Hprt1</i>	5'-GTG TCA TCA GCG AAA GTG GA-3'	5'-ATG GCC ACA GGA CTA GAA CG -3'	60	123	NM_012583
<i>Aplnr</i>	5'-CCT TCA TCA TCA TGC TGA CCT GTT A-3'	5'-GGT GGT AAG GCA TCC AGC ACA G-3'	64	159	NM_031349
<i>Apln</i>	5'-ATG AAT CTG AGT TTC TGC GTG CA-3'	5'-GAG GCA GCA TCA GTG GCA CTC C-3'	64	85	NM_031612
<i>Slc2a1</i>	5'-AGC AAG AAG GTG ACG GGC C-3'	5'-CGG TGG TTC CAT GTT TGA TT-3'	64	134	NM_138827
<i>Slc2a3</i>	5'-GAC CAA GCG ACG GAG ATC-3'	5'-AGA GCT CCA GCA CAG TGA CC-3'	64	124	NM_017102

Gapdh, glyceraldehyde-3-phosphate dehydrogenase; *Ppib* (CycloB), peptidylprolyl isomerase B; *Hprt1*, hypoxanthine phosphoribosyltransferase 1; *Aplnr*, apelin receptor; *Apln*, apelin; *Slc2a1*, solute carrier family2, facilitated glucose transporter member 1 (Glut1); *Slc2a3*, solute carrier family2, facilitated glucose transporter member 3 (Glut3)