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Uteroplacental Insufficiency Increases Visceral Adiposity and Visceral Adipose PPAR γ 2 Expression in Male Rat Offspring Prior to the Onset of Obesity

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Abstract

Uteroplacental insufficiency (UPI) induced intrauterine growth restriction (IUGR) predisposes individuals to adult onset metabolic morbidities, including insulin resistance and cardiovascular disease. An underlying component of the development of these morbidities is adipose dysfunction; specifically a disproportionately abundant visceral adipose tissue. We hypothesize that IUGR will increase rats visceral adiposity and visceral expression of PPAR γ , a key regulator of adipogenesis. To test this hypothesis we employed a well described UPI induced IUGR rat model. Subcutaneous and visceral adipose levels were measured in adolescent control and IUGR rats using MRI. Expression of PPAR γ mRNA and protein, as well as PPAR γ target genes, was measured in neonatal, adolescent and adult rats. UPI induced IUGR increases the relative amount of visceral adipose tissue in male, but not female, adolescent rats in conjunction with an increase in PPAR γ 2mRNA and protein in male visceral adipose. Importantly, these effects are seen prior to the onset of overt obesity. We conclude that increased PPAR γ 2 expression in VAT of IUGR males is associated with increased visceral adiposity. We speculate that the increase in visceral adiposity may contribute to the metabolic morbidities experienced by this population.

Keywords

Intrauterine growth restriction; adipose tissue; PPARgamma; developmental programming; obesity

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Conflict of Interest Statement

None of the authors have any conflicts of interest.

Uteroplacental insufficiency (UPI) is a common cause of intrauterine growth restriction (IUGR) in developed countries (1). Reductions in fetal blood flow result in fetal growth restriction with the fetus failing to achieve its genetic growth potential (2–3). Interestingly, in humans, IUGR leads to an increased risk of *adult onset* metabolic morbidities including insulin resistance and cardiovascular disease (4–6). An important component of the development of the metabolic disorders associated with IUGR is concomitant obesity and adipose tissue dysfunction (7).

In humans, IUGR affects the relative abundance and deposition of adipose tissue. While human IUGR infants are smaller than their appropriately grown counterparts at birth, their *rate* of weight accretion is accelerated in childhood and they tend to acquire more adipose tissue. An important concept is that IUGR adipose tissue appears dysregulated *before* the onset of obesity (8–9). Further, in addition to increased relative amounts of adipose tissue, fat deposition in IUGR children favors formation of visceral adipose tissue (VAT) over subcutaneous adipose tissue (SAT) (10–12). Consistent with the other metabolic sequelae of IUGR, it now appears that VAT and SAT contribute differently to the development of metabolic disease, with VAT being detrimental and SAT conferring protective roles (13–15).

The transcription factor peroxisome proliferator activated receptor gamma2 (PPAR γ 2) is a key regulator of adipose tissue formation and function. The PPAR γ gene gives rise to two protein isoforms, PPAR γ 1 and PPAR γ 2 and achieves tissue specific expression via alternative splicing and promoter usage (16–18). The PPAR γ 1 isoform, expressed in the liver, is reduced in the IUGR rat pups (19). In contrast, the PPAR γ 2 isoform is primarily found in adipose tissue where it increases transcription of numerous genes involved in lipid metabolism and energy homeostasis (20–21). The PPAR γ 2 target genes involved in lipid metabolism include, lysosomal acid lipase (LAL) (22), phosphoenol pyruvate carboxy kinase (PCK1) (23) and adipose differentiation-related protein (ADFP) (24). The transcriptional activating action of PPAR γ 2 is dependent upon ligand binding which induces a conformational change affecting transcription of PPAR γ 2 and PPAR γ 2 target genes (reviewed in (25)). PPAR γ 2 ligands include naturally occurring fatty acids (FA) and their derivatives (26–28); as such, PPAR γ 2 has the potential to serve as a “sensor” of nutritional status with the capacity to influence downstream metabolic outcomes. Interestingly, in the absence of ligand binding, PPAR γ 2 has a repressive action on the transcription of the insulin sensitive glucose transporter, GLUT4 in adipocytes by binding to the promoter and preventing transcription (29). Upon ligand binding, PPAR γ 2 detaches from the GLUT4 promoter alleviating this transrepression (29).

PPAR γ 2 levels in the adipose tissue of offspring are affected by maternal nutrient availability as demonstrated by several animal models. In sheep, both maternal nutrient restriction and over-nutrition results in an increase in perirenal adipose PPAR γ expression in offspring (30–31). Gender and adipose depot specific differences in PPAR γ expression have also been reported in response to obesity and a high fat diet (32–34). In a model of IUGR induced via 50% maternal food restriction during gestation, we have previously shown up-regulation of pro-adipogenic transcription factors, including PPAR γ 2, in VAT of male IUGR rats (35).

While it is clearly important to understand the effects of maternal dietary aberrations on the adipose profile of offspring, UPI induced IUGR may be a more representative source of fetal distress in developed countries (1–3). It is however, unknown how UPI induced IUGR alters adipose distribution in the offspring. As previous studies in the rat have been limited to the VAT of male rats, the effects of IUGR on female rats and on SAT are also unknown. Therefore, in this study we have used a well characterized rat model of UPI induced IUGR

to examine the PPAR γ related molecular profile of male and female offspring in VAT and SAT from the early postnatal period through adulthood in the rat. We hypothesized that UPI induced IUGR would increase the relative VAT/SAT ratio in offspring and that PPAR γ 2 mRNA and protein levels, as well as mRNA levels of key downstream targets of PPAR γ 2, would be increased in VAT.

2. Materials and Methods

2.1 Animals

The rat uteroplacental insufficiency model of IUGR has been described in detail previously (36–38). All procedures were approved by the University of Utah Animal Care Committee and are in accordance with the American Physiological Society's guiding principles (39). The surgical procedures have been described previously (40–41). Briefly, on day 19 of gestation, pregnant Sprague-Dawley rats were anesthetized with intraperitoneal xylazine (8 mg/kg) and ketamine (40 mg/kg), and both uterine arteries ligated giving rise to IUGR pups. Control dams underwent identical anesthetic procedures. After recovery, rats were given ad libitum access to food and water.

Dams were allowed to deliver spontaneously and litters randomly culled to 6 pups. Pups remained with, and were fed by, the dam for 7 (d7) or 21 (d21) days. Rats raised to day 60 (d60) were weaned to ad libitum food and water at d21. In d60 females, vaginal smears were used to assess cycle stage and all rats were harvested during estrus (42). Newborn adipose tissue was not examined in this study due to limited availability in newborn IUGR rats and the need to pool samples. For all ages, freshly harvested adipose was flash-frozen in liquid nitrogen, and stored at -80°C . Retroperitoneal adipose tissue was harvested as a representative visceral adipose depot (VAT) and subcutaneous adipose (SAT) was harvested from the right and left hind quarter of the rat. All experiments used tissue from 5–6 pups derived from different litters to ensure adequate litter-to-litter variation.

2.2 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) experiments were conducted using a Bruker Biospec 70/30 instrument (Billerica, MA) and a 72 mm-diameter birdcage radiofrequency (RF) transmit-receive resonator. For each specimen, consecutive multi-slice images (axial view, 1.0 thickness, 4.0 cm field-of-view, and 256×256 matrix size, corresponding to an in-plane resolution of 156 μm) were acquired using a rapid-acquisition-with-relaxation-enhancement (RARE) pulse sequence (4.2 s time-to-repetition, 36 ms time-to-echo, and echo-train-length of 4), both with and without a 1.0 kHz-bandwidth Gaussian pre-saturation RF pulse for fat suppression.

ImageJ macros were created to automate the process of image analysis while still allowing manual intervention at key steps that were less amenable to automation (43). For each animal, a range of paired images (fat/water and water only) were chosen. Duplicates of the fat/water images were converted to binary and used as a mask to clear the areas outside of the body for both paired images. ImageJ's Image Calculator was then used to normalize the fat/water images relative to the water only image by image division (i.e. each pixel value in the fat/water images was divided by the corresponding pixel value in the water only image). ImageJ's Auto Threshold algorithm was then used to select the regions of the resulting normalized image that corresponded to fat depots. A measurement was taken of the total area of fat depots and then after manually defining the boundary of the peritoneal cavity, the area of visceral fat depots was measured.

2.3 Real-Time RT PCR

The PPAR γ gene is alternatively processed to produce at least 3 isoforms of PPAR γ mRNA; of these, PPAR γ 2 is predominant in adipose tissue and the only variant examined in this study. Real-time reverse transcriptase PCR was used to evaluate mRNA abundance of adipose PPAR γ 2 as well as PPAR γ target genes, lysosomal acid lipase (LAL), phosphoenol pyruvate carboxy kinase (PCK1), adipose differentiation-related protein (ADFP) and the glucose transporter class 4 (GLUT4). Total RNA was extracted from frozen IUGR and control adipose using the Tissue RNeasy Lipid Tissue kit (Qiagen, DB Biosciences, CA) according to manufactures instruction. Total RNA was quantified using a NanoDrop 3300 Fluorospectrometer (Thermo Scientific, Wilmington, DE) and visualized by gel electrophoresis. cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster CA) from 1 μ g of total RNA. The following Assay-on-demand primer/probe sets were used: PPAR γ 2 - Rn00440940_m1, LAL - Rn00561482_m1, PCK1 - Rn01529013_g1, ADFP - Rn01472318_m1, GLUT4 - Rn00562597_m1 (Applied Biosystems). mRNA levels were determined using the comparative Ct method (44) with GAPDH as an internal control (GAPDH primer and probe sequences; Forward: CAAGATGGTGAAGGTCGGTGT; Reverse: CAAGAGAAGGCAGCCCTGGT; Probe: GCGTCCGATACGGCCAAATCCG). All real-time PCR amplification, data acquisition and analysis were done using the 7900HT Real-time PCR system and SDS Enterprise Software (Applied Biosystems) using a 384-Well Optical Reaction Plate (Applied Biosystems). Taqman Universal PCR Mastermix (Applied Biosystems) was used in a 5 μ L reaction, performed in quadruplicate. Cycle parameters were: 50°C $\times$ 2 min, 95°C $\times$ 10 min, followed by 40 cycles of 95°C $\times$ 15 sec and 60°C $\times$ 60 sec.

2.4 Protein Isolation and Immunoblot

Total protein was isolated from adipose tissue by homogenization in RIPA buffer (50mM Tris-HCl, pH 8.0, 150mM NaCl, 0.5% Na-deoxy-cholate, 1% NP-40 (Igepal), 0.1% SDS) and protease inhibitor cocktail (Roche-Complete Mini), followed by centrifugation at 10,000g, 4°C, 15 minutes. Supernatants were collected and stored at -80°C until use. Protein was assayed in triplicate for protein concentration using the bicinchoninic acid protein assay kit (Pierce).

Immunoblotting was used to determine levels of adipose PPAR γ 2 protein in IUGR and control rats. Immunoblotting was performed as previously described (45–47), with the following specifics; samples containing 30 μ g total protein were run on 10% bis-tris XT Criterion gels (Bio-Rad Laboratories), PVDF membranes were blocked in 5% milk-TBST, primary antibody diluted 1:500 (PPAR γ H-100, sc-7196, Santa Cruz Biotechnology), anti-rabbit secondary antibody conjugated with horseradish peroxidase, (Cell Signaling). Signal was determined using enhanced chemiluminescence (ECL) according to the manufacturer's instructions (Amersham, Little Chalfont, UK).

2.5 Statistics

Data are presented as means $\pm$ SEM. Statistical significance was determined using unpaired t-test with the Statistix 8 software package (Analytical Software, Tallahassee, FL) $p \leq 0.05$ was considered significant.

3 Results

3.1 UPI induced IUGR decreases body weights of rat pups from birth through postnatal d60

Consistent with previously published findings (37), IUGR pups weighed significantly less than control pups from birth through the age examined in this study, d60. Table 1 shows body weights of contemporarily raised male and female IUGR and control offspring at birth (d0), d7, d21 and d60.

3.2 IUGR alters adipose tissue distribution in adolescent rats

Relative levels of VAT (retroperitoneal only) and SAT were quantified in male and female control and IUGR rats at day 21 using an Bruker 7.0 T horizontal-bore MRI scanner (with and without fat-suppression) (Figure 1A). In male rats at d21, IUGR increased the VAT/SAT ratio ($164 \pm 30\%$ ($p=0.005$)), the total amount of VAT/g body weight ($246 \pm 60\%$ ($p=0.002$)) and SAT/g body weight ($148 \pm 9\%$ ($p=0.006$)). In female rats at d21, IUGR did not significantly alter VAT/SAT ratio ($122 \pm 9\%$ ($p=0.3$)), VAT/g body weight ($117 \pm 11\%$ ($p=0.5$)) or SAT/g body weight ($94 \pm 4\%$ ($p=0.6$)) (Figure 1).

3.3 IUGR increases PPAR γ 2 mRNA and protein levels in VAT in the male rat

The effect of IUGR on PPAR γ 2 mRNA in male and female rat adipose was evaluated by real-time reverse transcriptase (RT) PCR. PPAR γ 2 mRNA transcript levels were measured relative to GAPDH in control and IUGR in VAT and SAT at d7, d21 and d60 ($n=6$). Adipose was not examined at birth as tissue was limiting in IUGR rat pups. As estrous cycling of d60 females could alter expression of adipose genes, vaginal smears were used to assess cycle stage and all females were harvested during estrus (42). In male rats, IUGR significantly increased PPAR γ 2 mRNA in VAT of males at d7, d21 and d60; IUGR $209 \pm 38\%$ ($p=0.03$) of controls at d7, $326 \pm 59\%$ ($p=0.05$) at d21 and $282 \pm 33\%$ ($p=0.03$) at d60. In contrast, IUGR decreased PPAR γ 2 mRNA levels in female VAT at d21 ($49 \pm 9\%$ ($p=0.05$)). PPAR γ 2 mRNA was unaffected by IUGR in SAT of either gender (Figure 2). Data is IUGR as a percent of control $\pm$ SEM.

The effect of IUGR on abundance of PPAR γ 2 protein was assessed by immunoblot. Levels of PPAR γ 2 relative to GAPDH were measured in control and IUGR SAT and VAT at d7, d21 and d60 in male and female rats ($n=6$). In accordance with mRNA data, IUGR significantly increased PPAR γ 2 protein levels in VAT of male rats at d21 and d60 (d21– $254 \pm 5\%$ ($p=0.002$) and d60 – $152 \pm 10\%$ ($p=0.03$)). In contrast to the mRNA data, levels of PPAR γ 2 protein in male IUGR VAT were unchanged from control levels ($115 \pm 10\%$, ($p=0.36$)) at d7. There was no significant difference in PPAR γ 2 protein levels in male SAT or any female depot (Figure 3).

3.4 IUGR alters mRNA levels of PPAR γ responsive genes in male VAT

In order to assess potential downstream effects of the altered PPAR γ 2 protein levels, mRNA transcripts of three genes transcriptionally regulated by PPAR γ 2 were examined. Levels of lysosomal acid lipase (LAL), phosphoenol pyruvate carboxy kinase (PCK1) and adipose differentiation-related protein (ADFP) mRNA were measured in control and IUGR VAT and SAT using real-time RT PCR ($n=6$). Consistent with these genes being responsive to PPAR γ 2 protein, IUGR increased mRNA levels of LAL, PCK1 and ADFP at d21 and d60 in male VAT (LAL, d21 – $146 \pm 17\%$ ($p=0.05$), d60 – $166 \pm 22\%$ ($p=0.03$); PCK1, d21 – $256 \pm 54\%$ ($p=0.05$), d60 – $297 \pm 37\%$ ($p=0.01$); ADFP, d21 – $185 \pm 24\%$ ($p=0.03$), d60 – $168 \pm 16\%$ ($p=0.01$)). There were no significant differences in mRNA levels of any of these genes in male VAT at d7 or female VAT at any time point. In SAT, IUGR increased mRNA levels of

LAL at d60 in males (630 ± 109 , ($p=0.05$)) and decreased mRNA levels of ADFP in both males and females at d60 (male -54 ± 6 ($p=0.02$), female 39 ± 12 ($p=0.01$)) (Figure 4).

As GLUT 4 transcription is repressed by unliganded PPAR γ , mRNA levels of GLUT4 were also measured in control and IUGR VAT and SAT using real-time RT-PCR ($n=6$). Again consistent with the observed levels of PPAR γ 2, IUGR decreased GLUT4 mRNA in VAT of d7 and d21 male rats (d7 $-65 \pm 5\%$ ($p=0.01$), d21 -46 ± 15 ($p=0.01$)), with no change in female VAT. Interestingly, IUGR also decreased GLUT4 mRNA in both male and female SAT at d60 (male -66 ± 11 ($p=0.02$), female 35 ± 9 ($p=0.001$)) with no changes at d7 or d21 (Figure 5).

Discussion

The most significant finding of this study is that UPI induced IUGR increases the relative amount of visceral adipose tissue (VAT) in male, but not female, adolescent rats in conjunction with an increase in abundance of the pro-adipogenic factor, PPAR γ 2. Importantly, this change occurs *prior* to the onset of overt obesity. These findings support a programmed dysregulation of PPAR γ 2 expression in VAT of IUGR males associated with increased visceral adiposity; both factors potentially contributing to the associated metabolic disease observed in this population.

It is well established that VAT is more closely associated with metabolic disease than SAT and the male IUGR rat has an increased VAT/SAT ratio in early adolescence, specifically at postnatal day 21. In the rat, this corresponds to the weaning age when the IUGR rat still weighs ~25% less than the control rat. In other words IUGR male rats have a disproportionate amount of VAT before the onset of overt obesity. In addition to the higher VAT/SAT ratio, male IUGR rats have an increased amount of both VAT and VAT per gram body weight, indicating an overall increase in adipose tissue, presumably associated with increased lipid storage. This shift toward increased visceral adiposity in male IUGR rats is consistent with the adult onset metabolic disease and altered adipose properties demonstrated in other animal models of IUGR (48–50). Interestingly, often the alterations in adipose properties display a gender-specific bias with males having a more severely affected phenotype (48–49).

Adipose properties are dependent upon the transcription factor, PPAR γ 2. As a key regulator of adipogenesis, PPAR γ levels dictate the propensity of pre-adipocytes to be converted to mature adipocytes. Our observation of increased expression of PPAR γ 2 in IUGR male VAT is consistent with an increased propensity for adipocyte differentiation and increased lipid storage potential in the visceral adipose. Equally importantly, SAT did not display any changes in expression levels. This study parallels our findings in the maternal food restriction model of IUGR (35), indicating that the mechanisms involved in programming the altered gene expression may be model independent. In the male IUGR rat this provides the conditions for the growth restricted newborn to expand visceral adipose stores during catch up growth, without an increase in SAT; thus setting the stage for a disproportionate increase in metabolically detrimental VAT.

In addition to controlling adipogenesis, PPAR γ 2 regulates the expression of genes involved in adipose tissue function. As such, the effect of increasing PPAR γ levels in adipocytes will also have far reaching consequences, beyond just that of adipocyte differentiation. PPAR γ directly regulates the transcription of numerous genes involved in lipid metabolism and storage. The increased protein levels of PPAR γ observed in this study are sufficient to result in increased transcription of downstream target genes, as shown by the increased mRNA levels LAL, PCK1 and ADFP. The discordance between changes in d60 male LAP and male

and female ADFP mRNA levels in SAT and lack of changes in PPAR γ 2 protein levels may reflect a change in responsiveness to PPAR γ or be the result of regulation by other metabolic influences. Collectively, activation of PPAR γ 2 target genes may result in alterations in the processing of lipid by adipose tissue.

PPAR γ can also influence adipose glucose uptake via transcriptional regulation of the insulin responsive transporter GLUT4. In contrast to the, direct transcriptional activating actions described above, in the absence of ligand, PPAR γ represses the transcription of GLUT4 (29). Our observations of IUGR induced decreased GLUT4 mRNA in VAT of males at d7 and d21 is consistent with the observed increase in PPAR γ . Interestingly, the IUGR induced decrease in GLUT4 mRNA in SAT of both males and females at d60 may contribute to the insulin resistance observed in these animals, as glucose clearance by SAT is substantial. While we have demonstrated that the mRNA levels of PPAR γ 2 responsive genes are altered in association with PPAR γ , this study does not address levels or activities of PPAR γ co-regulators, such as NcoR1 or SMART and they may play a part in IUGR induced dysregulation of adipose (51).

Interestingly, female IUGR rats displayed no alterations in adipose PPAR γ 2 levels or early changes in adipose deposition. In this model, female rats still develop insulin resistance, hypertension and obesity, albeit later than the males. This is the first report that IUGR females are not subject to the early life alterations in PPAR γ 2 related programming that male rats experience. It is likely that adipose dysregulation proceeds by a different mechanism in female IUGR rats than in male IUGR rats. While the exact mechanisms remain unknown, a gender specific response to PPAR γ polymorphisms has been demonstrated in human obesity, with males having increased BMI in conjunction with PPAR γ 2 polymorphisms with no effect of the polymorphism in females (52–53). This suggests that females may utilize other pathways to regulate adipose deposition, potentially those related to sex-steroids.

It is interesting to consider the potential consequences of increases in VAT relative to SAT in the context of IUGR. Generally, the dysregulation of adipose tissue is associated with insulin resistance, lipid abnormalities, increased inflammation and cardiovascular disease; all characteristics of the adult IUGR phenotype. It has been proposed that the ability to increase SAT during conditions of energy excess allows provide a storage site for lipid. In the case where the more metabolically active VAT expands, particularly without a concurrent increase in SAT, the consequences are twofold. Firstly, the potential reservoir for lipid storage is inadequate and, if SAT is unable to expand appropriately, lipid is ectopically deposited in liver and skeletal muscle. We have previously shown increased ectopically deposited free fatty acids in adult IUGR rats (54). Secondly, inflammatory mediators secreted by visceral adipose, such as TNF α , are upregulated in the visceral adipose of IUGR males in this model (55) and contribute to insulin resistance when in excess. Further, human adults as young as 25 years of age demonstrate adipose tissue insulin resistance with decreased insulin-stimulated glucose uptake occurring unusually early (56).

An important limitation of this study is the use of a rodent model. While general adipogenic mechanisms are similar between the rodent and human, patterns of adipose deposition in the rat may not totally concordant with the human. A further limitation is the use of only retroperitoneal adipose tissue to represent VAT. This depot was chosen for a number of reasons. Firstly, the retroperitoneal depot is clearly defined in all ages examined, allowing for consistent evaluation of the same depot. Secondly it is not a particularly vascular adipose depot, minimizing contamination of red cell PPAR γ and finally sufficient tissue is available even in the smaller d7 rats. It may be however, that other visceral depots such as the peri-renal or mesenteric adipose have altered gene expression patterns. This may

be particularly important in IUGR female rats. Finally, future in-vitro studies of primary adipocyte cultures from control and IUGR will be helpful to examine the proliferative capacity of these cells as well as the effect of PPAR γ activation on glucose uptake.

We conclude that IUGR affects adipose depots in a gender and depot specific manner, with a preferential increase in the visceral adipose activity and quantity in male IUGR rats. We speculate that, the IUGR induced increase in PPAR γ 2 in VAT, *prior* to the onset of obesity, likely predisposes IUGR males to a greater increase in visceral adiposity and thus increased metabolic disease.

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Figure 1.

IUGR increases the amount of adipose and alters adipose distribution in the male rat. A) Representative MRI images of control and IUGR male and female d21 rat. VAT is visceral adipose tissue (retroperitoneal depot) and SAT is subcutaneous adipose tissue. Scans show abdominal cross sections and arrows indicate VAT and SAT. B) Quantification of IUGR male and female adipose depots relative to control. Data is expressed as VAT/SAT ratio, VAT/gram body weight and SAT/gram body weight. $n=4$, $**p \leq 0.01$.

Figure 2.

IUGR increases visceral PPAR γ 2 mRNA levels by day 7 in males, with the increase continuing through day 60. In contrast, female visceral PPAR γ 2 mRNA levels are significantly decreased at day 21. There is no IUGR induced changes in subcutaneous PPAR γ 2 in either gender. * $p \leq 0.05$, $n=6$

Figure 3.

IUGR increases visceral PPAR γ 2 protein levels at by day 21 in males, with the increase continuing through day 60. In contrast, female visceral PPAR γ 2 protein levels are decreased at day 21. There is no IUGR induced changes in subcutaneous PPAR γ 2 protein in either gender. * $p \leq 0.05$, $n=6$

Figure 4.

IUGR increases levels of PPAR γ 2 target genes LAL, PCK1 and ADFP in the visceral adipose tissue of male rats. In contrast, there are no changes in target gene mRNA in male subcutaneous or any female depot except subcutaneous at d60. * $p \leq 0.05$, $n=6$

Figure 5.

IUGR decreases levels of GLUT4 mRNA in the visceral adipose tissue of male rats at d7 and d21. In contrast, at d60, IUGR decreases subcutaneous GLUT4 mRNA in both male and female rats.. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, $n=6$

Table 1

Body weights of control and IUGR rats from birth (d0) to postnatal day 60 (d60).

Postnatal Age	Control Weight (g) ♂	IUGR Weight (g) ♂	Control Weight (g) ♀	IUGR Weight (g) ♀
d0	4.4 ± 0.1	3.4 ± 0.1 *	4.2 ± 0.1	3.2 ± 0.1 *
d7	20.3 ± 0.6	16.6 ± 0.5 *	19.9 ± 0.5	15.8 ± 0.5 *
d21	62.6 ± 1.4	54.3 ± 1.2 *	60.9 ± 1.3	51.6 ± 1.4 *
d60	225.4 ± 8.3	200.0 ± 14.8 *	374.7 ± 9.3	346.5 ± 21.8 *

* ≤ p 0.05 compared to gender and aged matched control. Value at each age for each group obtained from average weight of rats from 20 litters.