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Placental insufficiency contributes to fatty acid metabolism alterations in aged female mouse offspring

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1 # Placental insufficiency contributes to fatty acid metabolism
2 # alterations in aged female mouse offspring

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4 Running title: Long-term metabolic consequences of placental insufficiency

5

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28 **Abstract**

29 Intrauterine growth restriction (IUGR) is an accepted risk factor for metabolic disorders in later
30 life, including obesity and type 2 diabetes. The level of metabolic dysregulation can vary between
31 subjects and is dependent on the severity and the type of IUGR insult. Classical IUGR animal models
32 involve nutritional deprivation of the mother or uterine artery ligation. The latter aims to mimic a
33 placental insufficiency, which is the most frequent cause of IUGR. In this study, we investigated whether
34 IUGR due to placental insufficiency impacts the glucose and lipid homeostasis at advanced age.

35 Placental insufficiency was achieved by deletion of the transcription factor AP-2γ (Tfap2c), which
36 serves as one of the major trophoblast differentiation regulators. TdelT-IUGR mice were obtained by
37 crossing mice with a floxed *Tfap2c* allele and mice with *Cre* recombinase under the control of the Tpbpa
38 promoter. In advanced adulthood (9-12 months) female and male IUGR mice are respectively 20% and
39 12% leaner compared to controls. At this age, IUGR mice have unaffected glucose clearance and lipid
40 parameters (cholesterol, triglycerides and phospholipids) in the liver. However, female IUGR mice have
41 increased plasma free fatty acids (FFAs) (+87%) compared to controls. This is accompanied by increased
42 mRNA levels of fatty acid synthase and endoplasmic reticulum stress markers in white adipose tissue.

43 Taken together, our results suggest that IUGR by placental insufficiency may lead to higher
44 lipogenesis in female mice in advanced adulthood, at least indicated by greater Fasn expression. This
45 effect was sex-specific for the aged IUGR females.

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46 **Introduction**

47 Intrauterine growth restriction (IUGR), also known as fetal growth restriction, is a complex
48 pregnancy-associated condition, characterized by a decreased growth rate of the fetus (21). Around 3-7
49 % of the newborns worldwide are affected by IUGR (12), and a wide range of factors including maternal,
50 fetal, environmental and placental contributions can lead to the onset of IUGR. With regard to the
51 offspring consequences, IUGR poses an immediate risk for perinatal complications and can lead to
52 adaptive long-term consequences that include predisposition to the development of obesity, diabetes
53 mellitus type 2 and metabolic syndrome in later life (13, 16, 26, 41).

54 There is accumulating evidence in human epidemiological studies that the intrauterine
55 environment shapes the fetal organism in response to the available resources *in utero* by adjustment of
56 the fetal growth and its metabolism (17, 27, 29). These changes can persist for a lifetime, a concept
57 known as developmental programming (38). IUGR has been associated with glucose intolerance (19, 36),
58 decreased insulin secretion (23, 30) and increased adiposity (10) in experimental animal studies.
59 Moreover, several animal studies reported morphological changes such as altered beta cell mass,
60 adipogenesis, and lipogenesis in the IUGR fetus (34, 46). In addition, key metabolic factors that regulate
61 glucose metabolism in skeletal muscle, liver, and heart can be permanently modified in the rat fetus
62 exposed to experimental growth restriction (42, 44, 51). Although several studies on nutritional and
63 vascular deprivation have shown that IUGR constrains the metabolic health of the offspring, no data are
64 yet available to what extent IUGR due to placental insufficiency might contribute to the metabolic
65 deterioration at a more advanced age of the offspring.

66 Male and female offspring exhibit different outcomes following IUGR insult. For example, it was
67 reported in several animal studies that males are more susceptible to insulin resistance and obesity in
68 later life (1, 51), although there are studies that report this effect only in females (2, 50). This suggests

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69 that both sex and the perinatal growth status are important in the establishment of an aberrant
70 metabolic status. Furthermore, also differences in adipose tissue gene expression and signaling
71 molecules have been reported to be influenced by the sex, at least in the early stages of life (52).

72 Given the complexity of the IUGR pathophysiology and its implication in offspring's health, long-
73 term animal studies are imperative for a complete understanding of the metabolic phenotype of these
74 subjects. The most widely used models of IUGR are maternal nutritional deprivation or surgical uterine
75 artery occlusion (49). However, these models are accompanied with extensive maternal manipulation
76 that *per se* can contribute to the developmental programming of the offspring's health. An isolated
77 placental insufficiency without major modification of the maternal physiology will allow a better
78 understanding of placental influence on the underlying disease state of the offspring.

79 Recently, we developed a genetic mouse model of placental insufficiency via growth arrest of
80 the junctional zone of the placenta (45). This was accomplished by conditional ablation of transcription
81 factor Tfap2c (transcription factor AP-2γ) in Tpbpa-positive (trophoblast specific protein a) cell lineage at
82 gestational day 14.5. These Tpbpa positive cells give rise to trophoblast cells that comprise the
83 junctional zone in the placenta. The indicated condition resulted in placental growth arrest and IUGR
84 with pups 19% lighter at birth (45). In the present study, we describe the long-term effects of placental
85 insufficiency on the glucose and lipid metabolism in such mice and demonstrate sex-specific differences
86 in the severity of presenting symptoms.

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87 ## Materials and methods

88 **Animals.** All animal experiments were approved by the institutional animal care committee and
89 were conducted in accordance with the International Guiding Principles for Biomedical Research
90 Involving Animals as announced by the Society for the Study of Reproduction. 129-SV *Tpbpa-Cre*
91 transgenic mice were crossed with female 129 SV *Tfap2c* $^{fl/fl}$ to generate *TpbpaCre: Tfap2c* $^{-/-}$ placentas
92 (referred to as "TdelT-IUGR" later in the text) and *TpbpaCre: Tfap2c* $^{+/+}$ ("controls"). The genotype was
93 determined as previously described (45).

94 In this system, the embryos without the Cre-allele have a functional Tfap2c allele, are
95 considered to be wildtype and develop with a regular placenta. The Cre allele is active in the
96 spongiotrophoblast only and leads to the removal (floxing out) of the 5th exon of Tfap2c in the DNA of
97 spongiotrophoblast cells. Hence, the embryos possessing the Cre-transgene undergo a permanent
98 deletion of Tfap2c in the spongiotrophoblast layer (45). We previously have used laser-microdissection
99 to remove the spongiotrophoblast layer from wildtype and Tfap2c;Tbpba-Cre placentae. We
100 demonstrated that the expression level of Tfap2c drops dramatically in the Tfap2c;Tbpba-Cre placentae
101 (Fig. 2, B in Sharma et al. 2016 (45)). This demonstrated that the Cre-mediated deletion of Tfap2c in the
102 spongiotrophoblast layer is functional and leads to an almost complete absence of Tfap2c transcripts.

103 The offspring (5 female controls, 4 female TdelT-IUGR, 7 male controls, 6 male TdelT-IUGR) was
104 housed in plastic cages with bedding following a 12 hour light/dark cycle in a controlled environment
105 until 9-12 months of age, with access to chow diet and water *ad libitum.* The age distribution was
106 comparable in all groups. Prior to termination, animals were fasted for 8 hours. Blood samples were
107 collected via heart puncture in EDTA coated collection tubes. Plasma was obtained within 40 minutes of
108 termination with centrifugation for 20 minutes at 1000 x g, and stored on -80 ^{o}C until further analysis.

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109 The liver and the adipose tissue were collected and immediately snap frozen and stored at -80 ^{o}C until
110 further analysis.

111 **Intraperitoneal glucose tolerance test (ipGTT).** All animals, 9-12 months of age, were fasted 12
112 hours prior to ipGTT. A glucose bolus (Sigma Aldrich, Zwijndrecht, Netherlands) of 2 g/kg was
113 administered intraperitoneally. Blood glucose levels were assessed by tail bleeding using the OneTouch
114 Ultra glucose meter (Lifescan Benelux, Beerse, Belgium) at 0, 15, 30, 60 and 120 minutes, after glucose
115 administration.

116 **Biochemical plasma analysis.** Plasma total cholesterol, triglycerides, insulin, free fatty acids, and
117 phospholipids were analyzed with commercially available enzymatic kits in duplicates according to
118 manufacturer's recommendations (Roche Diagnostics, Basel, Switzerland, Alpco Diagnostics, Salem, NH
119 and Wako Pure Chemical Industries, Neuss, Germany). Intra-assay coefficient of variation was
120 determined based on calculated concentrations and was below 20%.

121 **Analysis of liver lipid composition.** Liver homogenates were made by homogenization of 100
122 mg snap-frozen liver samples. The Bligh and Dyer procedure was followed as previously described (6).
123 Cholesterol and triglycerides were measured with commercially available kits, and phospholipids were
124 measured as previously described (43).

125 **RNA isolation.** Total RNA was isolated from mouse livers and gonadal adipose tissue using the
126 miniprep DNA/RNA kit (Qiagen, Venlo, the Netherlands). RNA quality and quantity was determined with
127 NanoDrop ND-100 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE). 800 ng of total
128 RNA was used for cDNA conversion with reagents from Invitrogen (Invitrogen, USA), according to the
129 manufacturer's recommendations.

130 **RT-PCR.** Real-time quantitative PCR was carried out using an ABI-Prism 7700 (Applied
131 Biosystems, Foster City, CA) fast system with the following settings: 50°C for 2 min, followed by an initial

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132 denaturation step at 95°C for 10 min, 45 cycles at 95°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec.
133 The experiments were carried out in duplicate for each sample. Multi-exon spanning PCR primers
134 (sequences available at rtprimerdb.org) and fluorogenic probes were designed with the Primer Express
135 Software (Applied Biosystems) and synthesized by Eurogentec (Seraing, Belgium). Serial dilutions of
136 pooled cDNA were prepared to evaluate the performance of the TaqMan probe based qPCR assays. Each
137 dilution was amplified in two replicates. The slope and R2 were then determined from the standard
138 curve with the slope being between -3,1 and -3,5 and R2 higher than 0,993. Several reference genes
139 (Gapdh, Hsp90, bactin) were tested for the best reference gene. mRNA expression levels were
140 calculated relative to the reference gene beta-actin and further normalized to the relative expression of
141 the control group.

142 **Statistical analysis.** Statistical analyses were carried out using Prism 6.0. Data were tested for
143 normal distribution, then presented as box plot with median and min to max whiskers, unless otherwise
144 mentioned. The Mann-Whitney U test was used to assess statistical differences between the groups,
145 except repeated-measures ANOVA was used to assess the significance between time courses for the
146 glucose clearance experiment. Statistical significance for all comparisons was assigned at $p < 0.05$.

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147 ## Results

148 **Persistent compromised body weight in growth-restricted offspring.** Previously, we reported
149 that ablation of the transcriptional factor Tfap2c in Tpbpa precursor cells leads to growth arrest of the
150 junctional zone in the placenta and results in growth restricted embryos (45). We hypothesized that
151 growth restricted TdelT-IUGR fetuses remain growth restricted in adulthood as well. Approximately at
152 one year of age which roughly corresponds to the advanced human adulthood, female TdelT-IUGR mice
153 were 20% leaner when compared to female wild-type controls, whereas male TdelT-IUGR mice were
154 approximately 12% leaner compared to the controls (Figure 1).

155 **Aged growth restricted mice have normal glucose clearance.** Several studies have shown that
156 growth restriction at birth is associated with increased risk of developing insulin resistance, diabetes and
157 metabolic syndrome in later life (12). To determine whether our aged growth restricted mice have
158 impaired glucose metabolism, we performed intraperitoneal glucose tolerance test (ipGTT). There were
159 subtle significant differences in the glucose levels post glucose injection showing slightly better glucose
160 clearance at 60 minutes in aged TdelT-IUGR females in comparison to the wild-type controls (Figure 2A).
161 However, the area under the curve (AUC) for the aged TdelT-IUGR females only showed a trend towards
162 greater glucose clearance (Figure 2B, p=0,0727). In continuation, we did not observe significant
163 differences between the aged TdelT-IUGR males in comparison to the wild-type controls in the glucose
164 clearance levels (Figure 2C, D).

165 **Aged growth restricted mice show a stable metabolic status with exception to the free fatty**
166 **acids.** To further evaluate the metabolic status of our aged TdelT-IUGR mice, we performed a plasma
167 biochemical profiling from eight hours fasted animals. There were no significant differences in the
168 concentrations of insulin, cholesterol, triglycerides, and phospholipids in plasma between the aged
169 TdelT-IUGR females and female controls and between the aged TdelT-IUGR males and male controls

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170 (Table 1). However, the free fatty acid concentration was significantly greater in the aged TdelT-IUGR
171 females in comparison to controls by 87% (Table 1). This was not observed in the aged TdelT-IUGR males
172 in comparison to male controls. Because of the increment of FFAs in plasma from aged growth restricted
173 females, we asked whether the levels of lipids were also compromised in the liver from aged growth
174 restricted mice. However, we did not find any differences between cholesterol, triglyceride, and
175 phospholipid concentrations in liver homogenates from aged TdelT-IUGR female and male mice in
176 comparison to controls (Table 1).

177 **Aged growth restricted females have greater fatty acid synthase expression in the white**
178 **adipose tissue.** To determine whether growth restriction and this increment in plasma FFAs are due to
179 changes in hepatic mRNA expression, we performed gene expression analysis of several transcription
180 factors and genes important for lipid metabolism. Hepatic mRNA expression of transcription factors
181 *Lxra*, *Srebf1a, Srebf1c, Srebf2*, *Chrebp*; lipogenesis regulators fatty acid synthase *Fasn,* and sterol CoA
182 desaturase 1 (*Scd1*), were not different between the groups (Figure 3A, B). Since white adipose tissue
183 (WAT) is another important organ in the fatty acid metabolism, we also performed white adipose mRNA
184 expression analysis. Expression of *Fasn* showed a 3-fold increase in white adipose tissue of aged TdelT-
185 IUGR females compared to control females (Figure 4A). In agreement with the data from the
186 biochemical analysis of the plasma, we did not observe any changes in *Fasn* expression in the male
187 group (Figure 4B). Furthermore, we did not observe any changes in the hydrolases that regulate lipolysis
188 in the white adipose tissue (Figure 4C, D).

189 **Endoplasmic reticulum stress markers are greater in white adipose tissue from aged growth**
190 **restricted females**. Several studies have reported that lipid composition is important in maintaining
191 endoplasmic reticulum (ER) function (22, 31). Moreover, it was reported that elevated plasma free fatty
192 acids can induce ER stress in the adipose tissue (39). Due to these facts, we hypothesized that increased
193 FFAs might induce ER stress in metabolically active tissues. Therefore, we measured the mRNA

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194 expression of several ER stress markers in the white adipose and the liver tissue. There was a significant
195 increase in the activating transcription factor 4 (*Atf4*) and heat shock protein family A member 5 (*Hspa5*,
196 old name: Grp78) gene expression in the white adipose tissue of aged TdelT-IUGR females in comparison
197 to controls (Figure 5A). As expected, no significant differences in white adipose gene expression were
198 observed in the aged TdelT-IUGR males (Figure 5B).

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199 **Discussion**

200 It has been previously reported that maternal nutrient deprivation and placental insufficiency,
201 including compromised placental oxygen delivery, lead to IUGR and contribute to the altered metabolic
202 status of the offspring. In our study, we show that fetal growth restriction by placental dysfunction
203 results in increased free fatty acids in plasma and greater expression of fatty acid synthase in white
204 adipose tissue, without compromised glucose clearance in later life. Most importantly, these changes
205 showed sex-specific inclination and only the aged growth restricted females showed affected fatty acid
206 metabolism.

207 In other models of IUGR, offspring usually tend to catch up with the growth trajectories after
208 birth. The underlying mechanism of catch-up growth is not fully understood, although increased food
209 intake and leptin resistance are suggested as plausible mediators (15). This accelerated growth, in
210 infants, usually takes place in a short period during early postnatal life (4). Although it was thought to be
211 a beneficial compensatory mechanism in growth-restricted offspring and infants, it is now considered
212 that catch-up growth is associated with adverse outcomes in later life such as increased insulin
213 resistance, and cardiovascular and metabolic diseases (46, 47). Moreover, experimental models of IUGR
214 that do not show catch up growth are missing, but are crucial for confirmation of the proposed
215 beneficial effect of no-catch up growth for growth restricted infants. Using our TdelT-IUGR mice, we
216 observed that at advanced age growth restricted offspring are leaner, without catch-up growth in early
217 age. Leptin gene expression in WAT of tested animals did not show significant differences between the
218 groups, so this cannot explain the observed changes in body weight, although we cannot exclude
219 possible leptin resistance. Another possible explanation for no-catch up growth is that our aged growth
220 restricted animals were exclusively fed with chow diet and no calory and nutrient excess was introduced

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221 to their diet. It remains to be determined whether exposure to high fat diet (or different food
222 composition) can lead to different phenotype in these offspring.

223 The hormonal status can have sex-specific effects on the metabolic homeostasis. Estrogen is
224 implicated in reduced fatty acid delivery to the liver and decreases circulating levels of TG (40). Estrogen
225 loss, as in menopause or ovariectomy, is associated with adipose tissue accumulation, increased
226 lipogenic gene expression and insulin resistance (3, 33). However, the differences that we observe in
227 energy homeostasis are strictly limited to the aged TdelT-IUGR females (and not in control aged
228 females), so the sex-specific differences cannot be solely assigned to hormonal status differences.

229 Previously, it was reported that experimental IUGR can lead to sex-specific developmental
230 programming (1, 48). Hence our data of sexual dimorphism in aged TdelT-IUGR mice with preferential
231 distortion of white adipose tissue in females further support these results. However, many studies have
232 reported that there is a differential sex-specific susceptibility towards developmental programming,
233 depending on the type of *in utero* insult. While males are more susceptible to certain outcomes after
234 nutritional protein depletion during pregnancies (1, 14) females are more prone to cardiometabolic
235 complications in later life after placental modifications (18, 35). This is in agreement with our results as
236 the major *in utero* insults are based on utero-placental dysfunction rather than maternal nutrient
237 deprivation.

238 Increased plasma free fatty acids are involved in the development of cardiovascular and
239 metabolic diseases (7), majorly via increased hepatic glucose output (8) promoting insulin resistance and
240 type 2 diabetes. In our study, although the aged TdelT-IUGR females showed increased plasma FFAs
241 concentrations, there were no changes in glucose and insulin levels after fasting in comparison to
242 controls. Furthermore, the intraperitoneal glucose tolerance test did not show major differences
243 between the groups, indicating that short-term (within 15-30 minutes) and long-term tolerance (60-120

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244 minutes) to glucose were not affected, despite the increased plasma FFAs. One important characteristic
245 of aged female TdelT-IUGR mice is that they are leaner compared to female controls even though all
246 animals had *ad libitum* access to chow diet. This suggests that the increased FFAs observed act purely as
247 high energy source without affecting the glucose metabolism. In line with this, mRNA levels of *Fasn*
248 were increased in the adipose tissue that accounts for *de novo* lipogenesis. Regardless of the implication
249 of elevated levels of *Fasn* in obesity (5), insulin resistance (5) and cancer cell proliferation (37), *Fasn*
250 primarily acts as anabolic energy storage pathway in response to a nutritional and/or hormonal state
251 (32). TdelT-IUGR mice were not exposed to different nutrient challenges later in life, nor had any
252 increased glucose or insulin levels in advanced age. Still, gene expression data point to increased
253 lipogenesis specifically in the females. However, this needs to be characterized in detail by thorough
254 lipogenesis studies, in terms of lipid turn over and metabolic rates. It is important to mention that a
255 human study from Hudgins et al. showed that liver de novo lipogenesis is nutrient-dependent while *de*
256 *novo* lipogenesis in the adipose tissue is not solely nutrient-dependent (25). It is possible that the fetal
257 growth restriction due to placental insufficiency leads to sex-specific metabolic adaptation of the fetus
258 that later on leads to increased adipose *Fasn* expression and systemic FFAs accumulation. Moreover, it
259 was reported that caloric restriction during pregnancy upregulates the lipogenic regulatory factors in the
260 fetal liver (53) and may contribute to the fatty liver pathophysiology in later life.

261 Increased levels of free fatty acids can induce endoplasmic reticulum stress in several types of
262 organs including the liver and adipose tissue (11, 24). Moreover, ER stress has been reported to
263 contribute to age-associated adipose tissue inflammation (20). We showed that the placental
264 insufficiency in early life leads to increased FFAs only in aged females and is attributed with upregulated
265 ER stress markers only in their white adipose tissue. Which mechanisms mediate this ER stress response
266 in adipocytes is not well known. However, it was proposed that increased ROS production in obese mice
267 can lead to ER stress via oxidation of nascent proteins (28). ER stress itself can lead to increase in

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268 lipolysis and circulating levels of FFAs (9). However, we could not observe upregulated hydrolases in the
269 liver and the adipose tissue to support this. Taken together, this shows that the observed increased
270 levels of FFAs, augments the white adipose tissue ER stress.

271 **Perspectives and Significance**

272 Our study shows that IUGR due to placental insufficiency leads to sex-specific adaptations in
273 adult life. Majorly, this occurs via modulation of the adipose tissue by upregulation of the lipogenic
274 factor *Fasn* and an increase in free fatty acid production, only in the aged IUGR females. These lipogenic
275 changes are accompanied by increased ER stress markers in the white adipose tissue. Hence, these
276 results suggest that while the overall glucose and lipid metabolic parameters are not (yet) compromised,
277 the underlying molecular pathways are affected in the aged female IUGR offspring. Our findings thus on
278 the one hand side strengthen the idea that sex-specific adaptations need to be more and more
279 addressed. On the other hand we offer a model beyond the well characterized nutritional or surgical
280 models of IUGR (49) which facilitates such studies.

281

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286 **Disclosure**

287 The authors have nothing to disclose.

288 **Acknowledgments**

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430 **Figures legends**

431 **Figure 1. Body weight characterization of aged IUGR offspring.** Female (left) and male (right)
432 body weight at 9-12 months of age. Box plot with median and min to max whiskers of n=4-5 for females
433 and n=6-7 for males,* $p<0.05$, ***$p<0.001$.

434 **Figure 2. Uncompromised glucose tolerance in aged growth-restricted offspring.** (A) Plasma
435 glucose concentrations during ipGTT at 0, 15, 30, 60, 120 min in aged growth restricted females (n=4-5);
436 *$p<0.05$ by two way ANOVA, and representative (B) plasma glucose area under the curve. (C) Plasma
437 glucose concentrations during ipGTT in aged growth restricted males (n=6-7) and representative (D)
438 plasma glucose area under the curve. Data represented as mean ± SD,

439 **Figure 3. Effect of IUGR on hepatic mRNA expression of genes involved in lipid or glucose**
440 **metabolism in advanced age**. Data represented as box plot with median and min to max whiskers; n=4-
441 5 for panel A and n=6-7 for panel B. Abbreviations: Fasn, fatty acid synthase; Pparg, peroxisome
442 proliferator-activated receptor gamma; Lxra, liver x receptor alpha; Chrebp, carbohydrate responsive
443 element binding protein; Srebf1a, Srebf1c, sterol regulatory binding factor 1a and 1c; Ir, insulin receptor;
444 Gck, glucokinase; G6pd, glucose-6-phosphate dehydrogenase.

445 **Figure 4. WAT associated increased Fasn gene expression leveles in aged growth restricted**
446 **females.** mRNA expression levels of several transcription factors and lipogenic regulators in gonadal
447 white adipose tissue in (A) females (n=4-5) and (C) males (n=6-7) and mRNA expression levels of lipolysis
448 regulators in (B) females and (D) males. Data represented as box plot with median and min to max
449 whiskers; ***$p<0.001$. Abbreviations: Fasn, fatty acid synthase; Lxra, liver x receptor alpha; Chrebp,
450 carbohydrate responsive element binding protein; Srebf1a, Srebf1c, Srebf2 sterol regulatory binding
451 factor 1a, 1c and 2; Scd1, stearoyl-CoA desaturase-1; Cpt2, carnitine palmitoyltransferase 2; Atgl,
452 adipose triglyceride lipase; MgII, monoacylglycerol lipase.

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453 **Figure 5. ER stress markers are upregulated in WAT from aged growth restricted females.**
454 mRNA expression levels of several ER stress markers in gonadal white adipose tissue in (A) females (n=4-
455 5) and (B) males (n=6-7). Data represented as box plot with median and min to max whiskers; *p<0.05.
456 Abbreviations: Atf4, activating transcription factor 4; Grp78, 78 kDa glucose-regulated protein; Xbp1s,
457 Xbp1u, X-box binding protein 1 spliced and unspliced**.**

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Table 1. Biochemical characteristics of plasma and liver of aged growth-restricted and control offspring. Data are mean ± SEM; p<0.01 **. Comparison between control and TdelT-IUGR animals per sex.

Parameters	females			males		
	control	TdelT-IUGR	P value	control	TdelT-IUGR	P value
Cholesterol mmol/l	5.63 ± 0.45	5.20 ± 0.53	0.4	5.1 ± 0.97	4.83 ± 0.42	0.73
Triglycerides mmol/l	0.41 ± 0.05	0.44 ± 0.05	0.73	0.7 ± 0.31	0.54 ± 0.12	0.32
Phospholipids mmol/l	1.98 ± 0.42	1.67 ± 0.29	0.4	2.34 ± 0.3	2.49 ± 0.39	0.47
Free fatty acids mmol/l	0.35 ± 0.07	0.66 ± 0.09	0.01**	0.46 ± 0.3	0.36 ± 0.19	0.62
Insulin ng/ml	1.08 ± 0.07	1.09 ± 0.04	1	1.30 ± 0.23	1.14 ± 0.07	0.39
Hepatic cholesterol nmol/mg	3.32 ± 0.21	3.13 ± 0.25	0.55	4.28 ± 0.54	3.42 ± 0.14	0.44
Hepatic triglycerides nmol/mg	4.7 ± 0.94	4.72 ± 1.78	0.91	13.73 ± 5.24	4.02 ± 0.99	0.14
Hepatic phospholipids nmol/mg	28.90 ± 6.67	38.18 ± 4.08	0.55	33.43 ± 2.64	33.14 ± 3.09	0.89

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Figure 1.

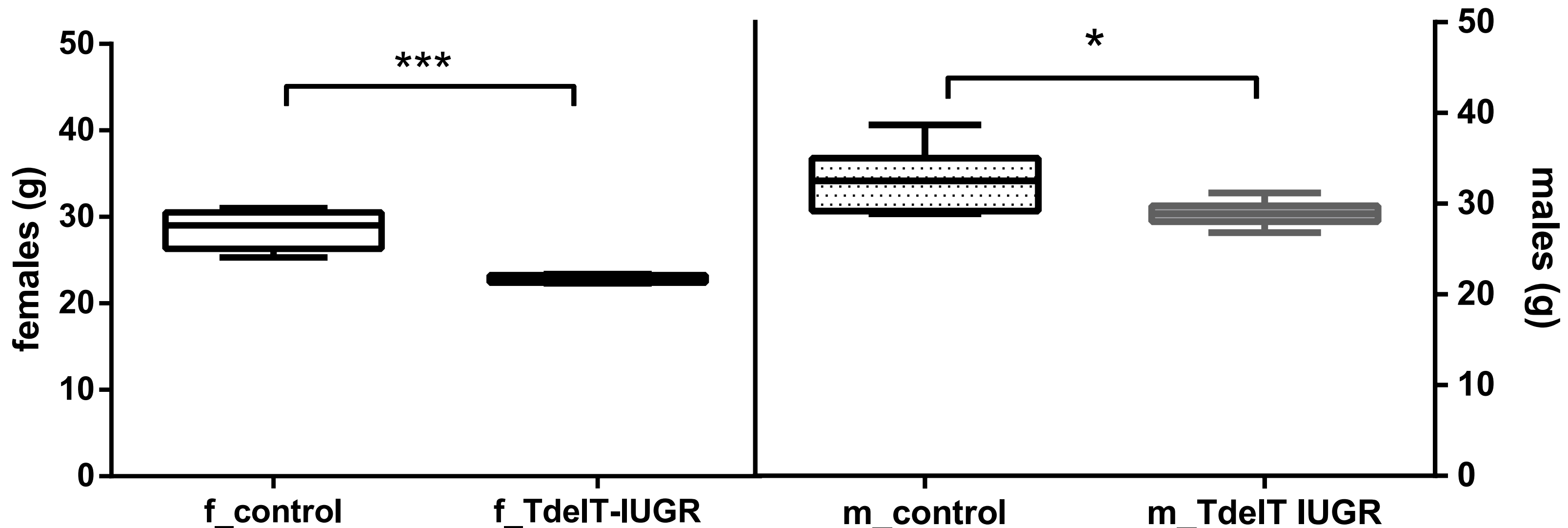

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Figure 2.

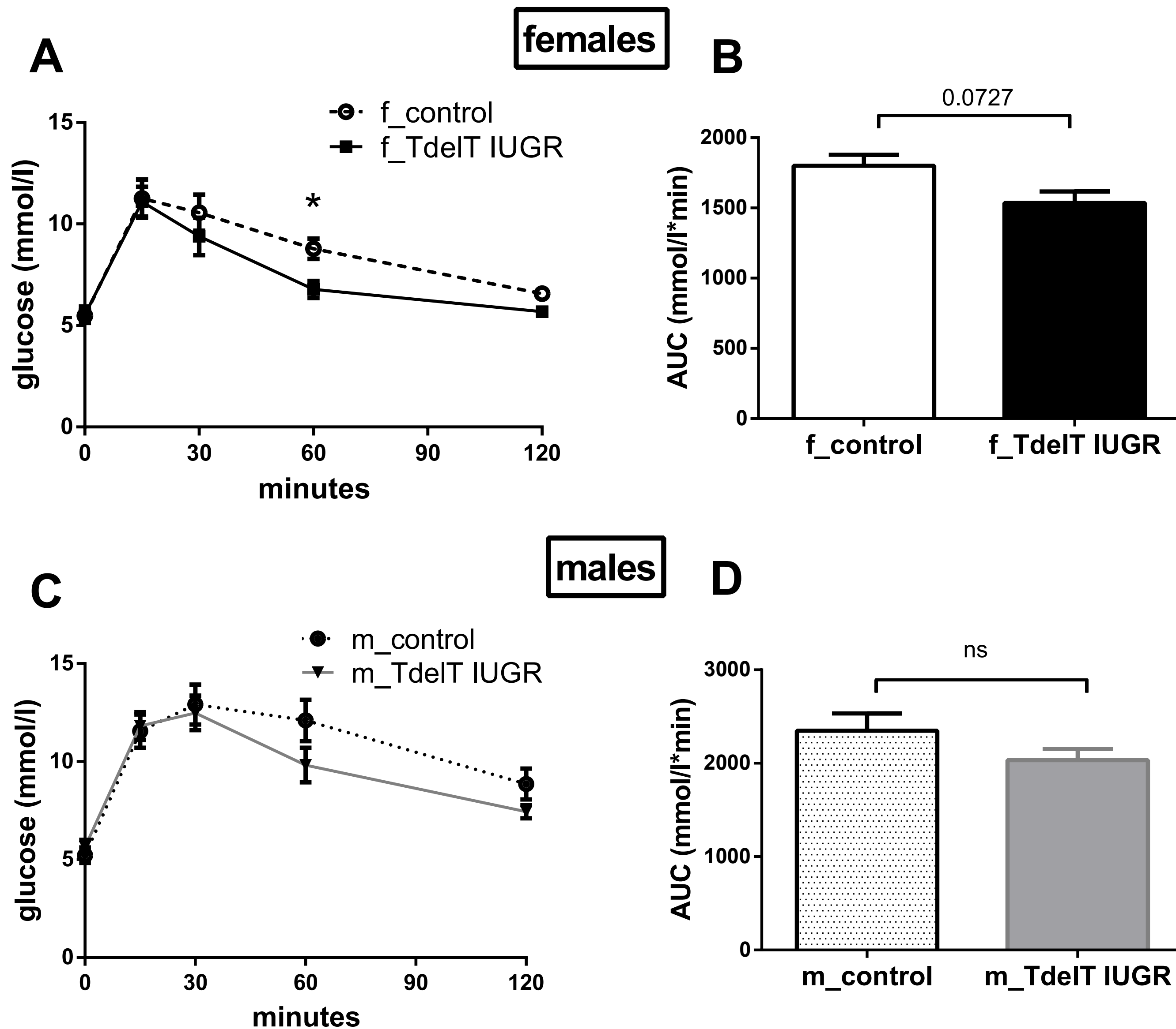

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Figure 3.

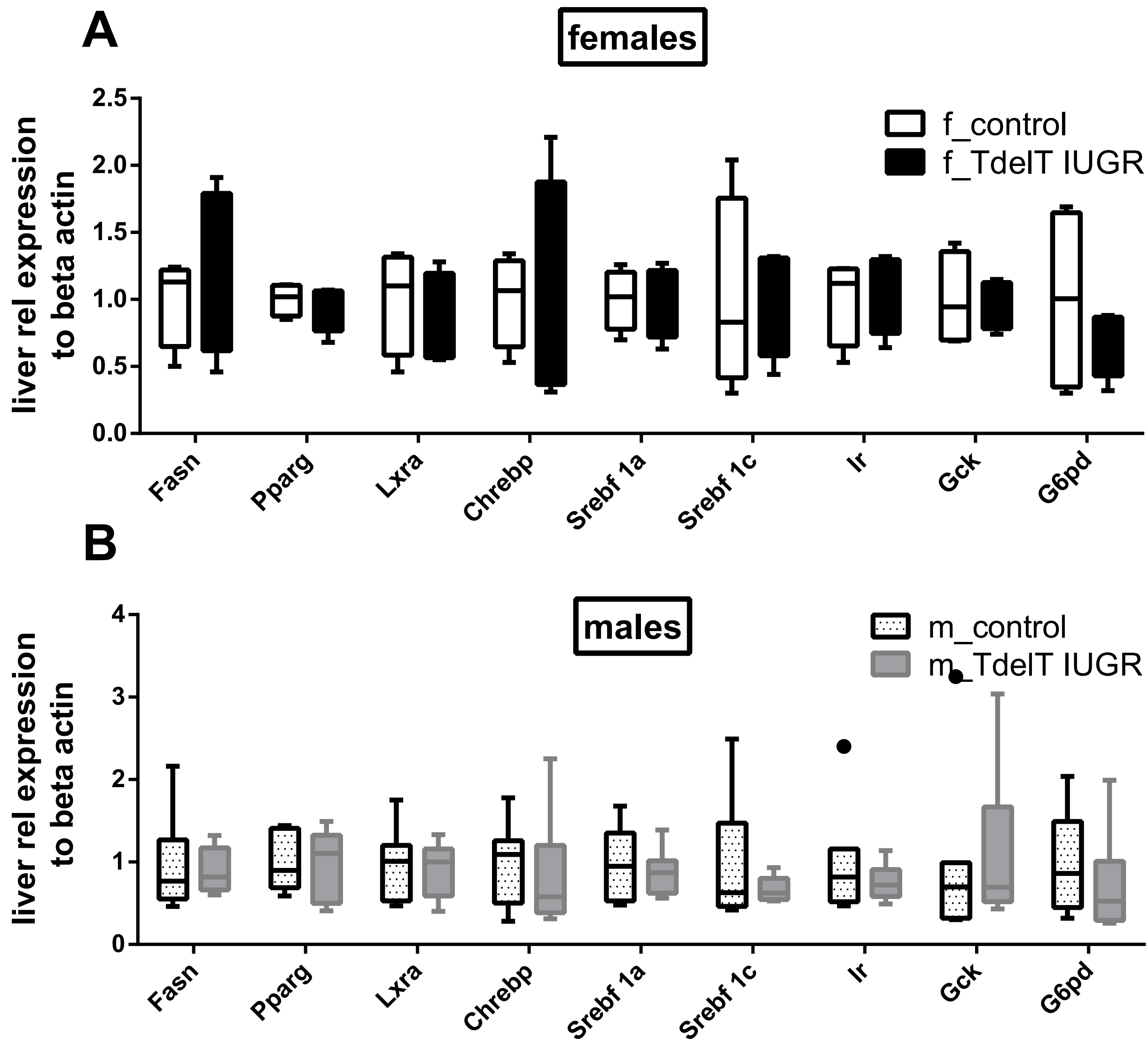

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Figure 4.

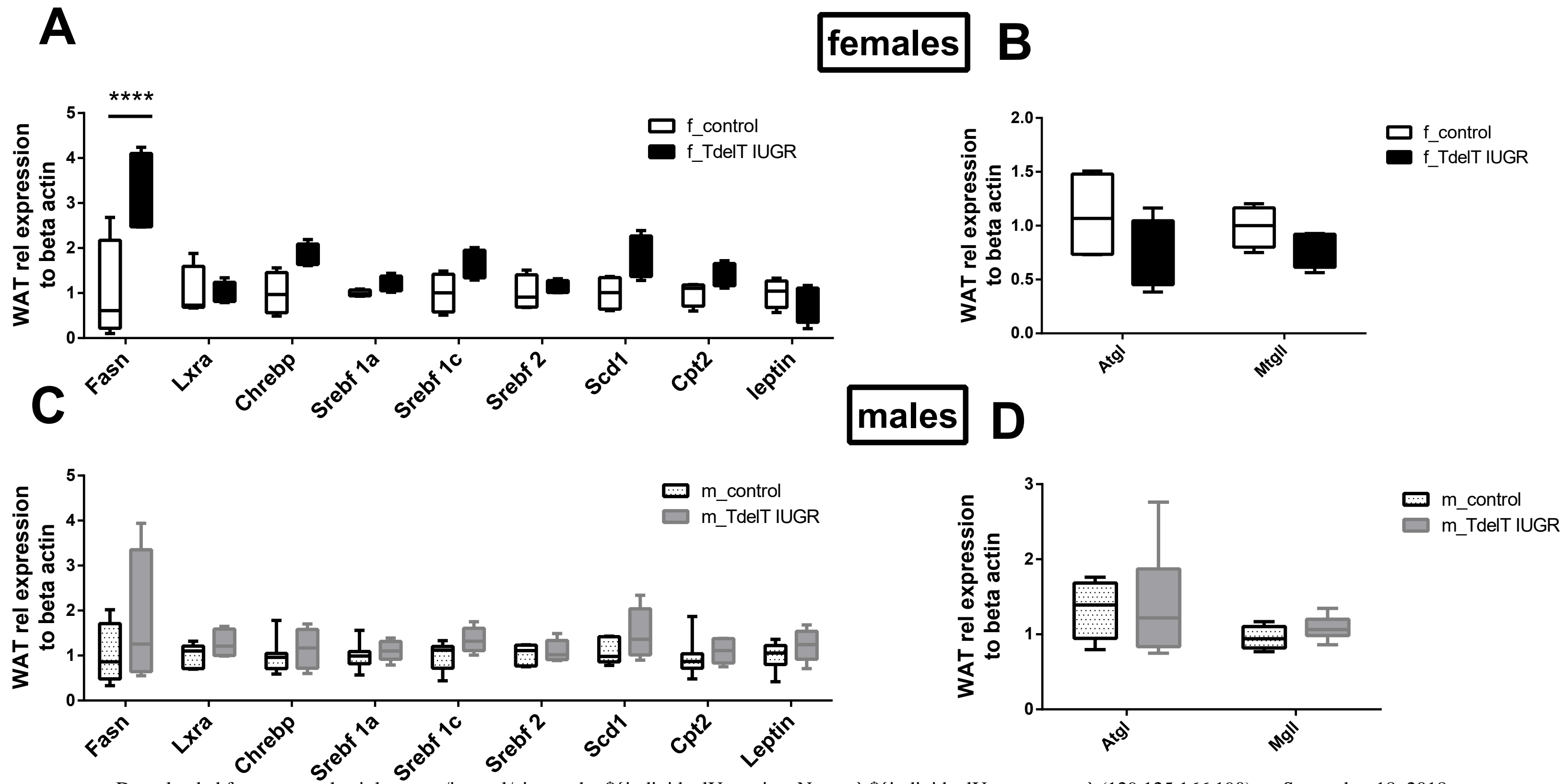

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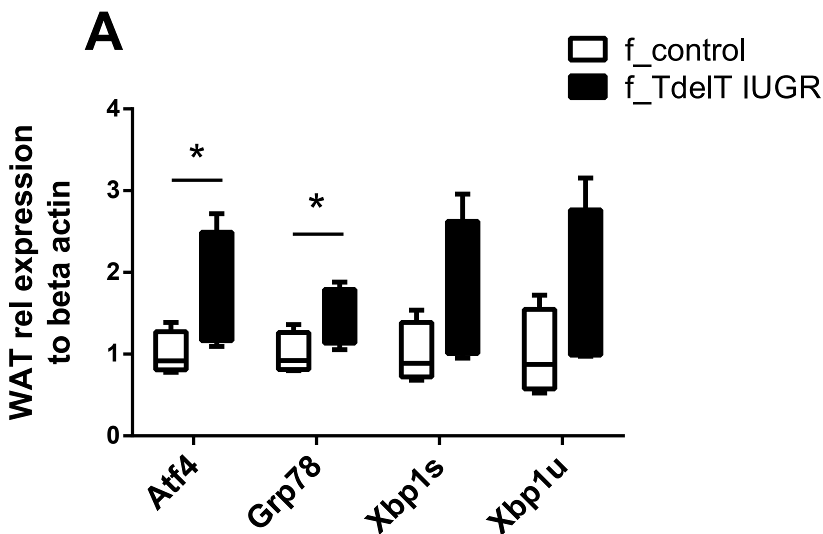
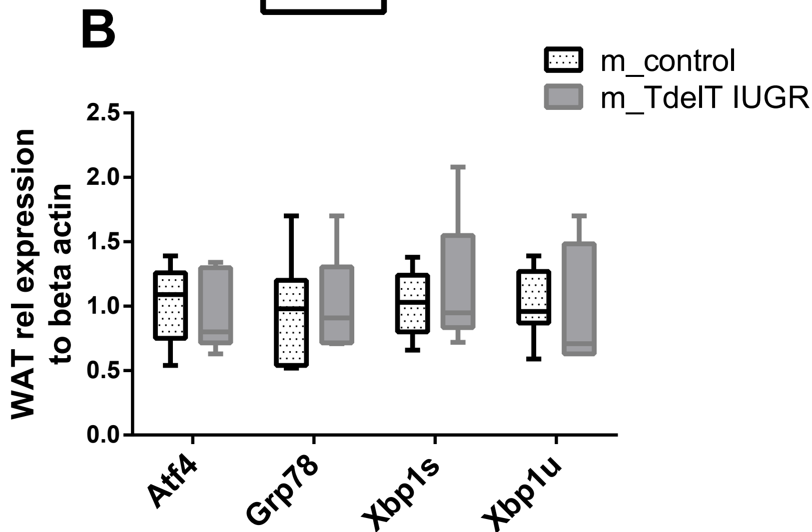
Figure 5.





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