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1 **Changes in LXR signaling influence early-pregnancy lipogenesis and protect against dysregulated**
2 **fetoplacental lipid homeostasis**

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23 Running Title: LXR modulates pregnancy adaptations in lipid metabolism

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25 **List of Abbreviations**

26

27 **ABCG5/8** ATP-binding cassette G5/8**, ACC1** Acetyl-CoA carboxylase**, ACS1** Acetyl-CoA synthase 1**,**
28 **AP** Advanced pregnancy**, ARL7** ADP-ribosylation factor like 7, **ATGL** Adipose triglyceride
29 lipase**,CD36** Cluster of differentiation**, CYP51** Lanosterol 14α-demethylase, **DGAT1/2** Diglyceride
30 acyltransferase 1 and 2, **DKO** double knockout, **EP** Early pregnancy, **FATP4** fatty acid transport protein,
31 **FABP-pm** plasma membrane fatty acid binding protein, **GDM** Gestational diabetes mellitus, **HMGCR** 3-
32 hydroxy-3methylglutaryl coenzyme A reductase, **HSL** Hormone-sensitive lipase, **LDLR** LDL receptor**,**
33 **LXR** Liver X receptor, **MGL** Monoglycerol lipase, **NPC1L1** Niemann-Pick C1 like 1, **SCD1** Stearoyl-
34 CoA desaturase 1**, SRB1** Scavenger receptor B1, **SREBP1c** Sterol-regulatory element-binding protein 1c,
35 **SREBP2** Sterol-regulatory element-binding protein 2.

36

Abstract

Human pregnancy is associated with enhanced *de novo* lipogenesis in the early stages followed by hyperlipidemia during advanced gestation. Liver X receptors (LXRs) are oxysterol-activated nuclear receptors which stimulate *de novo* lipogenesis and also promote the efflux of cholesterol from extrahepatic tissues followed by its transport back to the liver for biliary excretion. Although LXR is recognized as a master regulator of triglyceride and cholesterol homeostasis it is unknown whether it facilitates the gestational adaptations in lipid metabolism. To address this question, biochemical profiling, protein quantification and gene expression studies were used, and gestational metabolic changes in T0901317-treated wild-type mice and LXRα, $\beta^{-/-}$ mutants were investigated. Here, we show that altered LXR signaling contributes to the enhanced lipogenesis in early pregnancy by increasing the expression of hepatic *Fas* and stearoyl-CoA desaturase 1 (*Scd1*). Both the pharmacological activation of LXR with T0901317 and the genetic ablation of its two isoforms disrupted the increase in hepatic fatty acid biosynthesis and the development of hypertriglyceridemia during early gestation. We also demonstrate that absence of LXR enhances maternal white adipose tissue lipolysis, causing abnormal accumulation of triglycerides, cholesterol and free fatty acids in the fetal liver. Together, these data identify LXR as an important factor in early-pregnancy lipogenesis which is also necessary to protect against abnormalities in fetoplacental lipid homeostasis.

Keywords: nuclear receptors/ LXR, pregnancy, liver, lipid and lipoprotein metabolism, triglycerides

62 **Introduction**

63 Pregnancy is a dynamic state involving profound changes in the hormonal milieu of the mother which
64 then signal adaptations in maternal nutrient metabolism. These adaptations are necessary in order to
65 ensure a continuous supply of essential metabolites to support the growth and the development of the
66 fetus as well as to provide the mother with sufficient energy stores to meet the demands of pregnancy and
67 prepare for lactation (15, 26). Pregnancy-induced changes in energy and nutrient metabolism follow a
68 biphasic pattern. Early pregnancy is mainly anabolic since it is associated with augmented lipid
69 deposition in maternal tissues as a consequence of maternal hyperphagia, increased *de novo* lipogenesis
70 and enhanced insulin sensitivity (15). In contrast, advanced pregnancy is an overall catabolic state
71 involving augmented hydrolysis of stored lipids, hyperlipidemia and insulin resistance (15).

72

73 Liver X receptors LXRα (*NR1H3*) and LXRβ (*NR1H2*) are oxysterol-activated nuclear receptors with key
74 roles in the regulation of lipid metabolism. The two LXR isoforms share considerable sequence
75 homology, but their tissue distribution differs: LXRα is highly expressed in liver, intestine, adipose tissue
76 and macrophages whereas LXRβ is ubiquitously expressed (31). LXR stimulates *de novo* lipogenesis by
77 increasing the expression of sterol-regulatory element-binding protein-1c (SREBP1c), a master
78 transcriptional regulator of fatty acid and triglyceride synthesis (32, 34). LXR also binds directly to the
79 promoters of the lipogenic enzymes fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD1) and
80 acetyl-CoA carboxylase 1 (ACC1) (9, 18, 38). Moreover, LXR plays a pivotal role in the regulation of
81 cholesterol homeostasis: upon activation, it drives the efflux of cholesterol from non-hepatic tissues (e.g.
82 macrophages and enterocytes), stimulates biliary excretion of the sterol in the liver and reduces the
83 intestinal absorption of luminal sterols (27, 30, 32, 40). However, there is limited understanding of how
84 this nuclear receptor controls lipid metabolism in the physiological settings of pregnancy. Detailed
85 knowledge of the molecular mechanisms responsible for lipid homeostasis during pregnancy is important
86 as evidenced by the numerous reports demonstrating that failure to maintain the levels of circulating

87 lipids and lipoproteins within physiological ranges throughout gestation results in maternal dyslipidemia
88 and fetal metabolic complications such as macrosomia and increased risk of type 2 diabetes mellitus in
89 adulthood (19, 23, 39).
90
91 We hypothesized that alterations in LXR activity could contribute to the gestational adaptations in lipid
92 metabolism during pregnancy. Wild-type mice challenged with T0901317 (a potent and highly specific
93 synthetic LXR agonist) throughout gestation and LXRα, $\beta^{-/-}$ mutants were used to examine the role of
94 LXR in pregnancy metabolic adaptations. In the present study, we identify changes in LXR signaling as
95 an important factor that influences early-pregnancy lipogenesis in mice. Our data also suggest that LXR
96 protects against abnormalities in fetoplacental lipid homeostasis during murine pregnancy.
97

98 **Materials and Methods**

99 *Animal experiments*. C57BL/6 mice were purchased from Harlan Laboratories (UK). LXR wild-type
100 (WT) and LXRα, $\beta^{-/-}$ (LXR DKO; model previously described (27) were maintained on a mixed strain
101 background (C57BL/6: Sv129). All the mice were housed in a temperature and light-controlled
102 environment with 12-hour light/ 12-hour dark cycles. Age-matched mice had free access to water and
103 were fed a standard chow diet or chow supplemented with 0.012% T0901317 (Cayman Chemicals, UK)
104 (34) (T0901317 diet). Pregnant females were sacrificed on days 7, 10, 14 and 18 of gestation. Tissues and
105 sera were collected from chow-fed virgin controls and non-pregnant mice fed with a T0901317 diet for a
106 period of 7, 10, 14 or 18 days. Mice, fasted for 4 hours with free access to water, were sacrificed by CO_2
107 asphyxiation. All studies were conducted in conformity with the Public Health Service (PHS) Policy on
108 Humane Care and Use of Laboratory Animals and were approved by local animal ethics committees in
109 King's College London and were authorised by the Home Office.
110

111 *Lipid analysis*. Lipid extraction was performed as previously described (25). Briefly, frozen tissue
112 fragments were homogenized in Hank's Balanced Salt Solution using TissueLyser II system (Qiagen,
113 UK) for 2 minutes at 30Hz and then centrifuged the samples at 12000 rpm for 15minutes at 4° C. The
114 supernatant was discarded and the pellet was then re-suspended in 500µL of potassium phosphate lysis
115 buffer with Triton-X100. The samples were then sonicated for 8 minutes at 4° C (a 30 seconds sonication
116 phase followed by a 30 seconds resting phase) using a Bioruptor Plus system (Diagenode, Belgium).
117 Following a centrifugation at 10000rpm for 20 minutes at 4°C, the supernatants were transferred to clean
118 glass containers and were stored at -80° C. Serum and tissue extracts were processed and tested in the lab
119 of Dr Eugene Jansen (Laboratory for Health Protection Research, National Institute of Public Health and
120 the Environment, Netherlands). Beckman Synchron LX20 chemistry analyser (Beckman Coulter, UK)
121 and commercially available kits were used for the measurement of total cholesterol (467825, Beckman
122 Coulter, UK), HDL-cholesterol (467820, Beckman Coulter, UK), triglycerides (445850, Beckman

123 Coulter, UK) and free fatty acids (NEFA-HR(2) assay kit, Wako-Diagnostics, USA) ; measurements were
124 normalized to tissue protein content as previously described (25).
125
126 *RNA isolation, cDNA synthesis and quantitative real-time PCR analysis.* Total RNA was extracted using
127 RNeasy Mini Kit (Qiagen, UK) (liver and placenta samples) or RNeasy Lipid Tissue Mini Kit (Qiagen,
128 Manchester, UK) (adipose tissue) and then reverse transcribed into cDNA with random hexamers using
129 SuperScript II Reverse Transcriptase (Thermo Fisher Scientific, UK) in accordance with the
130 manufacturers' protocols. Real-time quantitative PCR reaction were performed on Viia7 system (Thermo
131 Fisher Scientific, UK), in a 384-well assay format using SYBR Green Mastermix (Sigma-Aldrich, UK).
132 Relative mRNA levels were calculated using the comparative Ct method normalized to cyclophilin.
133 Primer sequences are listed in Supplementary Table S1.
134
135 *Western blotting*. Total cell lysates were prepared using RIPA buffer (Sigma-Aldrich, UK) supplemented
136 with a Protease Inhibitor Cocktail (Sigma-Aldrich, UK) as well as Phosphatase Inhibitor Cocktails 2 and
137 3 (Sigma-Aldrich, UK). Samples were separated on a 12% SDS-polyacrylamide gel and transferred to a
138 nitrocellulose membrane. Membranes were hybridized with rabbit anti-ATGL (2138, 1:1000, Cell
139 Signalling Technology, UK), rabbit anti-Phospho-HSL (Ser563) (4139, 1:1000, Cell Signalling
140 Technology), rabbit anti-HSL (4107, 1:1000, Cell Signalling Technology, UK),mouse anti-LXRα
141 (ab41902, 1:1000 dilution; Abcam, UK), rabbit anti-LXRβ (ab28479, 1:500; Abcam, UK) and mouse
142 anti-GAPDH (MAB374, 1:80000; Millipore, UK) antibodies at 4° C overnight to detect expression,
143 followed by 1 hour incubation at room temperature with goat anti-mouse (sc-2005, 1:4000; Santa Cruz
144 Biotechnology, UK) and goat anti-rabbit (P0448, 1:2000, DAKO, UK) peroxidase-conjugated antibodies.
145 Proteins were detected by chemiluminescence (Millipore, UK).
146

147 *Statistical analysis.* All values were expressed as mean +/- SEM for biological replicates. Unpaired two-
148 tailed t-test was used for single comparisons while multiple comparisons were analyzed using multiple
149 measures of ANOVA with Newman-Keuls post-hoc testing.
150

151 **Results**

152 *Alterations in serum and hepatic lipids during mouse pregnancy.* To examine the impact of pregnancy on
153 murine lipid metabolism, serum lipids were profiled in mice at different stages of pregnancy: day 7 post-
154 coitum, corresponding to early pregnancy (EP) and days 10, 14 and 18 post-coitum taken to represent
155 advanced pregnancy (AP). Virgin female mice were used as non-pregnant controls. During pregnancy,
156 serum triglyceride concentrations were unchanged in early pregnancy and then gradually increased
157 starting from day 10 of mouse pregnancy onwards (Table 1). In contrast, total cholesterol levels decreased
158 in early pregnancy (at day 7), and were further reduced in advanced gestation. The same pattern was
159 observed in the abundance of cholesterol in HDL. Serum levels of free fatty acids were not significantly
160 altered during pregnancy. Since the liver functions as a metabolic hub which regulates all domains of
161 lipid homeostasis (2, 34), the temporal changes in hepatic lipid profiles in pregnant mice were examined.
162 In early pregnancy, hepatic triglyceride and cholesterol content was significantly increased whereas the
163 levels of free fatty acids remained unchanged (Table 1). During advanced pregnancy, concentrations of
164 hepatic triglyceride and cholesterol returned to pre-conception levels.

165

166 *Alterations in the hepatic LXR transcriptome during pregnancy.* Throughout pregnancy the expression of
167 LXR target genes involved in hepatic lipogenesis follow a distinct biphasic pattern. In early pregnancy,
168 consistent with the early-gestational increase in hepatic triglycerides, the transcript levels of the lipogenic
169 targets *Fas* and *Scd1* were substantially raised (Figure 1A). In contrast, during advanced pregnancy the
170 mRNA abundance of *Srebp1c, Fas* and *Scd1* was markedly diminished.

171

172 LXR controls hepatic cholesterol homeostasis by regulating the scavenger receptor B1 (SRB1), a
173 membrane-bound glycoprotein which mediates the selective and efficient uptake of cholesteryl esters
174 specifically from HDL, as well as the ATP-binding cassette G5 and G8 (ABCG5 and ABCG8) canalicular
175 half-transporters, which form an obligate functional heterodimer that mediates the efflux of hepatic free

176 cholesterol into bile (3, 21). Similar to the lipogenic genes, the cholesterol efflux genes *Abcg5, Abcg8* and
177 *Srb1* were also significantly downregulated during advanced pregnancy in mouse livers when compared
178 to non-pregnant controls (Figure 1B). In contrast, the mRNA expression of the ATP-binding cassette A1
179 *(Abca1*; an LXR target gene encoding a transmembrane protein, which mediates the transfer of
180 cholesterol and phospholipids to lipid-poor plasma carriers such as apolipoprotein A1 (4)) was
181 significantly raised in early pregnancy and then returned to pre-conception levels during advanced
182 gestation (data not shown).

183

184 In the liver, LXRα is the dominant nuclear receptor isoform (1). Immunoblot analysis revealed that the
185 dynamic alteration in the expression of *bona fide* targets in the liver during pregnancy were not
186 accompanied by any changes in the protein levels of LXRα (data not shown). The protein abundance of
187 LXRβ also remained unaltered thus excluding a potential compensatory role.

188

189 Gene expression analysis revealed that pregnancy has no significant impact on the expression of either
190 *Lxr* isoform or its targets in the duodenum (*Abca1, Abcg5, Abcg8 and* Niemann–pick c1 l1 *(Npc1l1))* and
191 primary blood monocytes (*Abcg1, Srb1, ADP-ribosylation factor like 7 (Arl7) and LDL receptor*
192 *(Ldlr)*)(data not shown) thereby suggesting that changes in LXR activity are unlikely to affect intestinal
193 cholesterol absorption and the reverse transport of sterols from extrahepatic tissues during pregnancy.

194

195 *LXR remains transcriptionally active during pregnancy*. After confirming that changes in the protein
196 availability of LXR could not explain the diminished mRNA levels of hepatic LXR targets during
197 advanced-to-late pregnancy, we tested whether the reduced transcription of these genes could result from
198 loss of LXR function during pregnancy. In order to pharmacologically activate LXR during gestation,
199 pregnant mice were fed standard chow diet or T0901317-supplemented diet for 7, 10, 14 and 18 days

200 following the identification of a copulatory plug. Non-pregnant female mice fed a T0901317 or chow diet
201 for the same number of days were used as controls.
202
203 Comparison of the pregnant T0901317-fed groups versus pregnancy-matched chow-fed groups revealed
204 that the expression of LXR targets involved in lipogenesis (*Srebp1c, Fas* and *Scd1*) and cholesterol efflux
205 (*Abcg5* and *Abcg8*) was markedly increased. These data indicate that LXR is transcriptionally active
206 during pregnancy and pharmacological activation of LXR is able to reverse the reduction in the mRNA
207 levels of key metabolic genes in mouse liver in late pregnancy (Figure 2A and B). The gene expression
208 changes were functionally confirmed by the significant increase in hepatic triglyceride levels and the
209 reduction in hepatic cholesterol concentrations (Figure 2C). Serum lipid profiling revealed that treatment
210 of pregnant mice with T0901317 raises the levels of circulating cholesterol without affecting the levels of
211 circulating triglycerides; these observations are in agreement with previous reports detailing the effect of
212 this synthetic LXR agonist on non-pregnant mice (2, 34).
213
214 Activation of LXR contributes to early-pregnancy lipogenesis. To determine whether the adaptations in
215 lipid metabolism observed during normal pregnancy are entirely LXR dependent and therefore
216 completely reversed by the presence of T0901317, pregnant T0901317 mice were compared against non-
217 pregnant diet-matched controls.
218
219 T0901317 mimics the gestational signal that promotes lipogenesis during early pregnancy. Gene
220 expression analysis showed that the mRNA levels of the lipogenic LXR targets *Fas* and *Scd1* were not
221 further upregulated in T0901317-fed mice in early pregnancy as compared to diet-matched non-pregnant
222 controls (Figure 2A). Also, there were no differences in the hepatic triglyceride content of pregnant and
223 non-pregnant mice challenged with the agonist-supplemented diet for 7 days (Figure 2C).

224 During advanced pregnancy, however, the T0901317 agonist was not able to reverse the gestational
225 changes in triglyceride homeostatic pathways in the liver. Specifically, from day 10 onwards the
226 expression of *Srebp1c, Fas* and *Scd1* was significantly reduced in pregnant mice administered with
227 T0901317 as compared to diet-matched non-pregnant controls (Figure 2A). Interestingly, serum lipid
228 profiling revealed that the presence of T0901317 abrogated the gestational increase in circulating
229 triglycerides during advanced pregnancy (Figure 2D).
230
231 Pharmacological activation of LXR had a limited effect on the gestational adaptations in cholesterol
232 metabolism. Lipid profiling showed that the gestational increase in hepatic cholesterol levels on days 7
233 and 10 of pregnancy were abrogated by the presence of T0901317. In contrast, this synthetic agonist was
234 unable to reverse the gestational changes in the expression of cholesterol homeostatic genes in the liver as
235 evidenced by the fact that the transcript abundance of the cholesterol transporters *Abcg5, Abcg8* and *Srb1*
236 was lower in pregnant T0901317-fed mice as compared to non-pregnant T0901317-fed controls during
237 advanced pregnancy (Figure 2B). Administration of T0901317 to pregnant mice also did not interfere
238 with the pregnancy-associated drop in serum cholesterol as evidenced by the fact that pregnant mice
239 challenged with the agonist-supplemented diet had significantly lower circulating total cholesterol levels
240 from day 10 onwards as compared to diet-matched non-pregnant controls (Figure 2D).
241
242 Overall these results suggest that pharmacological activation of LXR mimics the early-pregnancy increase
243 in lipogenesis. During advanced pregnancy, however, T0901317 appeared unable to compete with the
244 gestational cues that signal the alterations in triglyceride and cholesterol metabolism and therefore the
245 patterns of gestational adaptations in lipid homeostasis were preserved throughout this period.
246
247 *LXR is required for lipogenesis during pregnancy.* To delineate the functional importance of LXR in the
248 control of the gestational adaptations in fatty acid and cholesterol metabolism and also to investigate to

249 what extent the T0901317-induced effects during pregnancy were signaled directly via LXR, wild-type
250 (WT) and *Lxrα, β* $^{-/-}$ (LXR DKO) were studied at different stages of pregnancy. All the mice in this study
251 were maintained on a C57Bl/6 x Sv129 mixed background.
252
253 In LXR DKO, the gestational adaptations in hepatic lipogenesis during both early and advanced gestation
254 were lost as evidenced by the fact that no significant changes were detected in the expression of *Srebp1c,*
255 *Fas* and *Scd1* in the livers of pregnant mice as compared to non-pregnant controls (Figure 3A). Lipid
256 quantification studies confirmed that in the absence of *Lxrα, β* all of the gestational changes in hepatic
257 triglyceride levels were abolished (Figure 3C). Serum biochemical profiling also showed that the early-to-
258 mid-pregnancy (days 7 and 10) increase in serum triglyceride levels was lost in mice lacking *Lxrα, β*
259 (Figure 3D).
260
261 *LXR is not required for the gestational adaptations in cholesterol metabolism*. Study of WT and LXR
262 DKO at different stages of pregnancy revealed that the absence of *Lxrα, β* had no impact on the
263 gestational adaptations in cholesterol homeostasis. Both WT and LXR DKO mice showed significantly
264 reduced expression of the cholesterol efflux transporters *Abcg5, Abcg8* and *Srb1* on days 14 and 18 of
265 pregnancy (Figure 3B). Even though LXR DKO mice had increased levels of hepatic cholesterol, there
266 were no significant pregnancy-induced changes in cholesterol abundance either in the wild-type mice or
267 in the mutants (Figure 3C). Serum lipid profiling showed that in both WT and LXR DKO mice, the
268 concentrations of total circulating cholesterol are significantly reduced during pregnancy from day 10
269 onwards (Figure 3D).
270
271 *Absence of LXR in the mother causes fetoplacental dyslipidemia.* It is recognized that exposure of fetuses
272 to a dyslipidemic environment *in utero* causes enhanced fetal growth (19, 23, 39). Given that the absence
273 of *Lxrα,* β interferes with maternal lipogenesis during pregnancy, we examined the effect of *Lxrα, β*

274 deficiency on physiology and metabolism of the fetoplacental unit on day 18 of gestation. Our results
275 indicated that LXR DKO mice had increased placental weight accompanied with abnormal accumulation
276 of triglycerides and free fatty acids in this organ (Figure 4A). The *Lxrα, β* heterozygous fetuses also had
277 raised accumulation of cholesterol, triglycerides and free fatty acids in their livers (Figure 4B).
278 Expression analysis revealed that fetuses heterozygous for *Lxrα, β* do not have significant changes in the
279 mRNA levels of lipogenic genes (*Srebp1c, Fas, Scd1, Acc1*, diglyceride acyltransferase 1 and 2 (*Dgat1*
280 *and Dgat2*) and cholesterol biosynthetic genes (sterol-regulatory element-binding protein 2 (*Srebp2*), 3-
281 hydroxy-3methylglutaryl coenzyme A reductase (*Hmgcr*) and lanosterol 14α-demethylase (*Cyp51*) in the
282 liver (Figure 4C). Interestingly, there were no differences in the transcript abundance of *Lxrα*, the
283 dominant isoform of LXR in the liver, in the heterozygous fetuses as compared to the wild-type controls;
284 the expression of *Lxrβ*, however, was significantly downregulated.
285

286 Having determined that the fetoplacental hyperlipidemia detected on day 18 of pregnancy in LXR DKO
287 mice is unlikely to result from the augmented biosynthesis of lipids in the fetal liver, we tested whether
288 this phenotype could arise from the deregulated lipid homeostasis in the mother. Biochemical analysis of
289 maternal serum showed that in LXR DKO mice there was marked increase in the levels of free fatty acids
290 on day 18 of pregnancy (Figure 4D).
291

292 Also, we examined whether the supraphysiological accumulation of free fatty acids in the serum of LXR
293 DKO mice during advanced gestation could result from abnormalities in intestinal lipid absorption.
294 mRNA quantification studies confirmed that *Lxrα, β* deficiency has no significant impact on the duodenal
295 expression of key lipogenic targets (cluster of differentiation (*Cd36*), fatty acid transport protein (*Fatp4*),
296 plasma membrane fatty acid binding protein (*Fabp-pm*), acetyl-CoA synthase 1 (*Acs1*), *Dgat1* and *Dgat2*
297 in mice on day 18 of pregnancy (data not shown). Similarly, there were no differences in the transcript
298 abundance of genes involved in intestinal cholesterol absorption (*Abcg5*, *Abcg8* and *Srb1*) between

299 mutant mice and controls during advanced gestation; the expression of Niemann–pick c1 l1 (*Npc1l1*) was
300 significantly downregulated in the duodena of LXR DKO mice.
301
302 LXR plays a key role in the regulation white adipose tissue metabolism (6), therefore we investigated
303 whether the supraphysiological accumulation of fatty acids in the serum later in pregnancy could result
304 from the deregulated lipolysis in the white adipose tissue of the mutant mice. Our results confirmed that
305 expression of the intracellular lipases monoglycerol lipase (*Mgl*), adipose triglyceride lipase (*Atgl*) and
306 hormone sensitive lipase (*Hsl*) was considerably increased in the maternal white fat depots of LXR DKO
307 mice (Figure 4E). Protein quantification studies confirmed that the levels of ATGL in the adipose tissue
308 of mice deficient in *Lxrα, β* were significantly increased (Figure 4F); no changes in HSL phosphorylation
309 were detected (data not shown).
310

311 **Discussion**

312 In the present study, we have identified that changes in LXR signaling influence early-pregnancy
313 lipogenesis in mice. We have shown that pharmacological activation of LXR disrupts lipid biosynthetic
314 pathways in the liver and prevents early-pregnancy hypertriglyceridemia. We have also demonstrated that
315 absence of LXR deregulates maternal lipid homeostasis and enhances white adipose tissue lipolysis which
316 results in the increased accumulation of lipids in the fetal liver.

317

318 In this report we demonstrate that LXR targets contribute to early pregnancy lipogenesis as evidenced by
319 increased hepatic expression of *Fas* and *Scd1*, key enzymes involved in fatty acid biosynthesis, on day 7
320 of mouse gestation and concurrent early-pregnancy accrual of hepatic triglycerides. Previous studies have
321 demonstrated that SCD1 is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids
322 which are major components of complex lipids such as diglycerides, triglycerides and cholesteryl esters
323 (22). Also, there is increased mRNA expression of *Scd1* in the fatty livers of mice with genetically-
324 induced obesity (11). Similarly, FAS catalyzes the biosynthesis of saturated fatty acids from simple
325 precursors and as such has been categorized as the "gatekeeper" of *de novo* lipogenesis. Studies in rodents
326 have demonstrated that FAS contributes to the storage of hepatic triglycerides and approximately 11% of
327 the total triglyceride content in the liver is derived from *de novo* lipogenesis (12). FAS has an even more
328 pronounced role in lipogenesis in the context of fatty livers as evidenced by the fact that ob/ob mice have
329 enhanced *Fas* transcript abundance (17). Based on the presence of a molecular environment established as
330 conducive to hepatic lipogenesis and also the direct quantification of the increment of lipogenic products
331 (hepatic triglycerides) we have concluded that there is increased fatty acid biosynthesis in the livers of
332 early-pregnant mice. Moreover, the absence of raised free fatty acid and triglyceride concentrations in the
333 serum of pregnant mice suggests that the hepatic lipids are unlikely to result from dietary lipids and

334 peripheral fats stored in adipose tissue that flow to the liver by way of the plasma free fatty acid pool or
335 from intestinally derived chylomicron remnants.

336 As pregnancy progressed, the transcript abundance of the tested lipogenic LXR targets was substantially
337 reduced and this change was reflected by the decrease in hepatic triglyceride concentrations. During
338 advanced pregnancy, the levels of serum triglycerides increase significantly and this change is not
339 mirrored by an increase in the hepatic triglyceride content. Similarly, the molecular environment of the
340 liver (i.e. the mRNA levels of *Srebp1*c, *Fas* and *Scd1*) suggest reduced hepatic *de novo* lipogenesis in
341 advanced murine pregnancy. These data are in agreement with previous studies (15). Moreover, it has
342 been demonstrated that mice consume progressively greater amounts of food as gestation advances (20),
343 and therefore it is likely that the elevated concentrations of circulating triglycerides result from the
344 increased transfer of intestinally derived dietary lipids. These gestational adaptations occurred while the
345 protein availability of both LXRα and LXRβ remained constant.

346 Having identified *Srebp1c*, *Fas* and *Scd1* as factors involved in the dynamic regulation of triglyceride
347 homeostasis during mouse pregnancy, we aimed to determine whether LXR is involved in control of these
348 adaptations. Administration of the synthetic LXR agonist T0901317 confirmed that LXR is indeed
349 transcriptionally active throughout gestation and therefore it could play a role in the gestational alterations
350 in murine lipid homeostasis. Our data showed that the presence of T0901317 interfered with the early-
351 pregnancy increase in *Fas* and *Scd1* transcription and subsequent triglyceride accumulation.

352 Our results indicate that the pregnancy signals which downregulate the expression of hepatic lipogenic
353 factors during advanced pregnancy are potent enough to compete with T0901317-mediated activation of
354 LXR . At present, there is limited understanding of how different maternal and placental hormones
355 influence metabolic adaptations in the mother. Since the reduction in the expression of hepatic LXR
356 targets parallels the completion of placenta formation it is likely that factors of placental origin signal

357 these adaptations directly, by binding to the LXR receptor itself, or indirectly, by initiating downstream
358 signaling cascades which alter the phosphorylation state of LXR and thereby affect its activity.

359 Deletion of *Lxrα, β* abrogates all the changes in hepatic triglyceride homeostasis during murine
360 pregnancy. This result indicates that during pregnancy LXR is a key factor which relays the signals of
361 reproductive hormones to the lipid metabolic network of the liver. Serum lipid profiling also
362 demonstrated that mice lacking LXR have deregulated lipogenesis early in pregnancy, as evidenced by
363 the fact that the increase in circulating triglycerides observed in wild-type mice on gestational days 7 and
364 10 was lost in the global mutants. Gene expression analysis confirmed that in the absence of LXR, the
365 transcript abundance of lipogenic genes in the liver do not exhibit any significant fluctuation in
366 pregnancy. The results from our study could not exclude the possibility that gestational signals could the
367 expression of lipogenic targets independent of LXR in late pregnancy, as evidenced by the trends for
368 diminished transcript abundance of *Srebp1c*, *Fas* and *Scd1* in LXR DKO mice on gestational days 14 and
369 18.

370 LXR is a master regulator not only of fatty acid and triglyceride metabolism, but also of cholesterol
371 homeostasis (27, 30, 32, 40). Our data demonstrate that gestational adaptations in cholesterol metabolism
372 are independent of LXR. Expression of the cholesterol efflux transporters *Abcg5, Abcg8* and *Srb1* was
373 downregulated during pregnancy independent of T0901317 challenge or *Lxrα, β* deletion. Furthermore
374 circulating total cholesterol concentrations were reduced during advanced pregnancy in all of our
375 experimental models. Moreover, our data suggest that gestational changes in the expression of hepatic
376 *Abca1* are unlikely to contribute to the pregnancy adaptations in cholesterol homeostasis. Also, the
377 expression of this transporter in the duodenum remains unchanged throughout pregnancy (Supplementary
378 Figure 3A). These results are important as although the liver is the major source of plasma HDL,
379 intestinal enterocytes also play a role in the biogenesis of this lipoprotein and 30% of the circulating HDL
380 is derived from the intestine in an ABCA1- dependent manner (5). Thus, we have concluded that changes

381 in LXR signalling do not play a role in the gestational adaptations in cholesterol homeostasis. Further
382 studies are necessary to elucidate the molecular mechanisms that influence cholesterol homeostasis during
383 pregnancy.
384
385 Our data also revealed that defects in maternal lipid metabolism as a consequence of *Lxrα, β* deletion
386 cause fetoplacental dyslipidemia in mice. Previous studies have demonstrated that LXR is expressed in
387 white adipose tissue where it is involved in the regulation of cholesterol, free fatty acid and glucose
388 metabolism (6). *In vivo* studies have reported that pharmacological activation of LXR increases adipose
389 tissue lipolysis and fatty acid β-oxidation (37). We show that mice lacking both LXR isoforms have
390 increased white adipose tissue lipolysis later in pregnancy, as evidenced by the raised maternal serum
391 fatty acid levels and the upregulated expression of intracellular lipases in the white fat of these mice.
392 Moreover, we demonstrated that *Lxrα, β* deficiency increases the protein levels of ATGL (a key lipolytic
393 enzyme which controls energy homeostasis by catalyzing the rate-limiting step in triglyceride catabolism
394 (14)) in the adipose tissue of pregnant mice. Our data suggest that changes in HSL phosphorylation are
395 unlikely to contribute to the enhanced lipolytic response in adipose tissue of the *Lxrα, β* mutant miceOur
396 conclusion that *Lxrα, β* deficiency promotes white fat lipolysis during advanced gestation is consistent
397 with the observed *in vivo* phenotype (i.e increased serum free fatty acid concentrations and reduced
398 adipose tissue mass) as wells as the obtained gene and protein expression data. Furthermore, the precise
399 regulation of whole-body lipid homeostasis depends on the continuous crosstalk between liver and
400 adipose tissue. Therefore, we could speculate that the increase in white fat lipolysis in the pregnant LXR
401 DKO mice could occur as a mechanism to compensate for the defects in hepatic lipogenesis in the mother
402 and protect the fetus from any deficiencies in nutrient supply.
403
404 A key result from this study was that LXR DKO mice have increased placental weight and enhanced fatty
405 acid and triglyceride accumulation in the placenta later in pregnancy. We also showed that fetuses from

406 LXR DKO mice have increased free fatty acids, triglycerides and cholesterol in the liver. Given that there
407 was no induction of lipid biosynthetic pathways in the livers of the *Lxrα, β* heterozygous fetuses, it is
408 likely that the fetal hepatic hyperlipidemia is a result of the deregulated maternal lipid homeostasis and
409 impaired placental function. Consistent with this, a recent study has linked maternal hyperlipidemia with
410 placental abnormalities and adverse fetal outcomes in cases of gestational diabetes mellitus (GDM)
411 (35).Moreover, previous studies suggest that maternal adipose tissue lipolytic products are predominantly
412 utilized as ketogenic substrates and the resulting ketone bodies get rapidly transferred to the fetal
413 circulation where they act as lipogenic precursors and energy fuels (15). Maternally-derived free fatty
414 acids are also able to cross the placenta and accumulate in the fetoplacental compartment (16). Our
415 results excluded the possibility that the genetic background of the heterozygous fetuses could contribute
416 to their hyperlipidemic phenotype since the mRNA expression of all fatty acid and cholesterol
417 biosynthetic genes, as well as *Lxrα*, was the same as in the wild-type controls. Previous studies have
418 shown that gestational pathologies such as GDM and obesity, where maternal plasma levels of circulating
419 lipids are supraphysiologically raised, are associated with fetal overgrowth (large-for-gestational age
420 infants) (13, 28, 33, 36). Specifically, obese pregnant women typically have significantly increased levels
421 of circulating triglycerides in combination with reduced HDL-cholesterol levels and hyperglycemia as a
422 consequence of exacerbated peripheral insulin resistance in the third trimester of gestation (29). The
423 transplacental delivery of nutrients is quantitatively and qualitatively altered as a result of the increased
424 total lipid content and the accumulation of macrophages and proinflammatory mediators in the placentas
425 of obese women leading to the birth of infants increased neonatal fa t mass and body fat percentage) (8,
426 24). Moreover, offspring of women with obesity and/or GDM are not only prone to adverse side effects at
427 birth such as preterm delivery (10) and stillbirth (2) but also have a high risk of developing obesity,
428 impaired glucose tolerance, and type 2 diabetes later in life (4, 7). All of these reports underscore the
429 importance of thoroughly understanding the molecular determinants of alterations in maternal and fetal
430 lipid metabolism during pregnancy in order to prevent immediate and long-term metabolic diseases. Our

431 study identifies LXR as an attractive candidate which can be targeted with therapeutic interventions in
432 order to reverse maternal and fetal dyslipidemia.
433
434 In conclusion, we present for the first time evidence that LXR is a factor essential for early-pregnancy
435 lipogenesis in mice. We demonstrate that deletion of LXR not only interferes with the pregnancy
436 adaptations in triglyceride metabolism in the liver but also causes fetoplacental dyslipidemia as a
437 consequence of the raised maternal white fat lipolysis.
438

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441

442 **Disclosures**

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

444

445

Author Contributions

V.N., G.P, E.B. L.B.M. and E.J. performed experiments. V.N analyzed data and drafted original manuscript. S.B and S.A. involved in investigation. V.N., M.P. and C.W. conception and design of research. G.P., M.P. and C.W. edited and revised manuscript. C.W. approved final version of manuscript.

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454

455

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569

570

571 **Table 1: Adaptations in serum and hepatic lipid profiles during mouse pregnancy.** Total
572 triglycerides, free fatty acids, cholesterol and HDL-cholesterol measured in mouse serum and hepatic
573 lipid extracts.
574
575 **Figure 1: Increased expression of lipogenic LXR targets in mouse liver during early pregnancy**. **A**.
576 Lipogenic genes. **B**. Cholesterol homeostatic genes. Results are represented as mean ± SEM (n=6-8) *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, comparison of pregnant groups (day (D) 7-18) versus non-pregnant group
578 (D0). P value determined by one-way ANOVA with Newman-Keuls post-hoc testing.
579
580 **Figure 2: Pharmacological activation of LXR mimics early-pregnancy lipogenesis in mice. A**.
581 Expression of lipogenic genes. **B**. Expression of cholesterol homeostatic genes. **C**. Total cholesterol and
582 triglyceridespregnant T0901317-fed group. Results are represented as mean ± SEM (n=6-8); a $p<0.05$
583 comparison of non-pregnant T0901317-fed groups (day (D) 7-18) versus non-pregnant group D0; b
584 $p<0.05$ comparison of pregnant T0901317-fed groups (D7-18) versus pregnant group D0; c $p<0.05$
585 comparison pregnant T0901317-fed group vs corresponding non-pregnant T0901317-fed group. P value
586 determined by one-way ANOVA with Newman-Keuls post-hoc testing.
587
588 **Figure 3: LXR is required for lipogenesis during early mouse pregnancy. A**. Expression of lipogenic
589 genes. **B**. Expression of cholesterol homeostatic genes. **C**. Total cholesterol and triglycerides measured in
590 hepatic lipid extracts. **D**. Total triglycerides and cholesterol measured in mouse serum. a $p< 0.05$
591 comparison of pregnant wild-type (WT) groups (day (D) 7-18) vs non-pregnant WT controls (D0); b $p< 0.05$ comparison of pregnant LXR DKO groups (D7-18) vs non-pregnant LXR DKO controls (D0); c $p< 0.05$ comparison LXR DKO group vs corresponding WT group. P value determined by one-way ANOVA
594 with Newman-Keuls post-hoc testing.
595

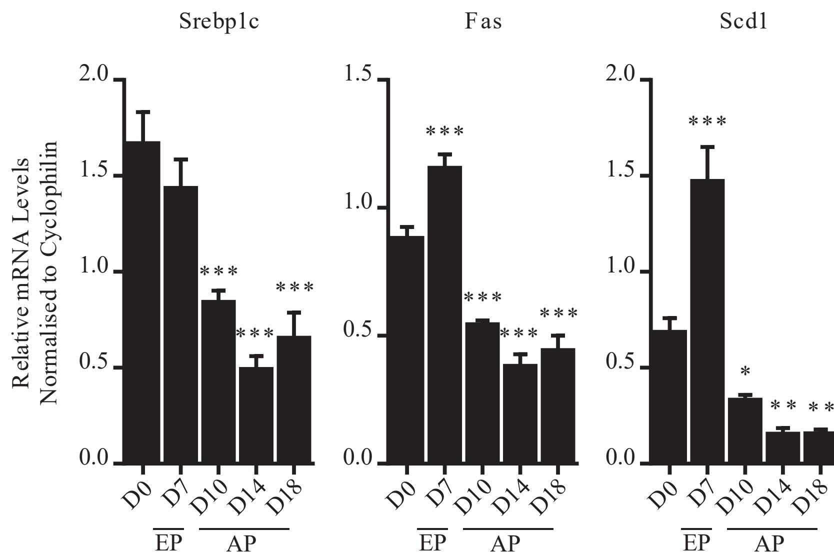
596 **Figure 4: Gestational adaptations in LXR signaling protect against abnormalities in fetoplacental**
597 **lipid metabolism in mice.** **A**. Placenta weight and total cholesterol, triglycerides and free fatty acids
598 measured in mouse placenta. **B**. Total cholesterol, triglycerides and free fatty acids measured in fetal
599 hepatic extracts. **C**. mRNA expression of genes involved in de novo lipogenesis and cholesterol synthesis
600 in fetal liver. D. Levels of free fatty acid in maternal serum. **E**. Expression of intracellular lipases in white
601 adipose tissue. **F**. Protein levels of ATGL in white adipose tissue. Results are represented as mean ± SEM
602 (n=4-8). *$p< 0.05$, **$p < 0.01$, ***$p < 0.001$, comparison of wild-type (WT) vs LXR DKO group. P value
603 determined by unpaired t-test.

Table 1: Adaptations in serum and hepatic lipid profiles during mouse pregnancy

	Non-pregnant	Early Pregnancy	Advanced Pregnancy		
	D0	**D7**	**D10**	**D14**	**D18**
Serum					
Triglycerides (mmol/L)	0.80 ± 0.04	0.76 ± 0.11	1.16 ± 0.06 *	1.34 ± 0.16 **	1.46 ± 0.13 ***
Free fatty acids (mmol/L)	0.81 ± 0.06	0.94 ± 0.09	1.07 ± 0.08	0.82 ± 0.09	0.93 ± 0.03
Total cholesterol (mmol/L)	2.13 ± 0.12	1.80 ± 0.06 ***	0.71 ± 0.05 ***	1.33 ± 0.04 ***	0.97 ± 0.04 ***
HDL-cholesterol (mmol/L)	2.13 ± 0.06	1.70 ± 0.07 ***	0.57 ± 0.05 ***	1.32 ± 0.04 ***	1.05 ± 0.04 ***
Liver					
Triglycerides (µmol/g)	990.11 ± 37.73	1324.50 ± 119.98 *	1280.56 ± 89.90	1058.96 ± 73.03	994.93 ± 143.46
Free fatty acids (µmol/g)	222.63 ± 39.49	202.18 ± 26.80	286.68 ± 29.12	318.29 ± 31.14	203.59 ± 31.52
Cholesterol (µmol/g)	54.93 ± 2.65	109.58 ± 14.45 **	103.03 ± 13.24 **	77.74 ± 5.20	83.31 ± 10.22

Results are represented as mean ± SEM (n=6-8) *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, comparison of pregnant groups (day (D)7-18) versus non-pregnant group (D0). P value determined by one-way ANOVA with Newman-Keuls post-hoc testing.

Figure 1: Increased expression of lipogenic LXR targets in mouse liver during early pregnancy



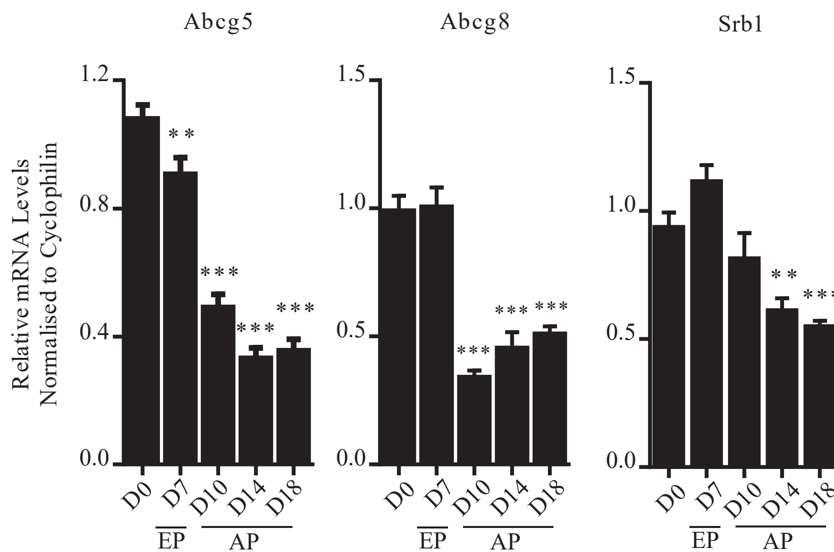


Figure 2: Pharmacological activation of LXR mimics early-pregnancy lipogenesis in mice

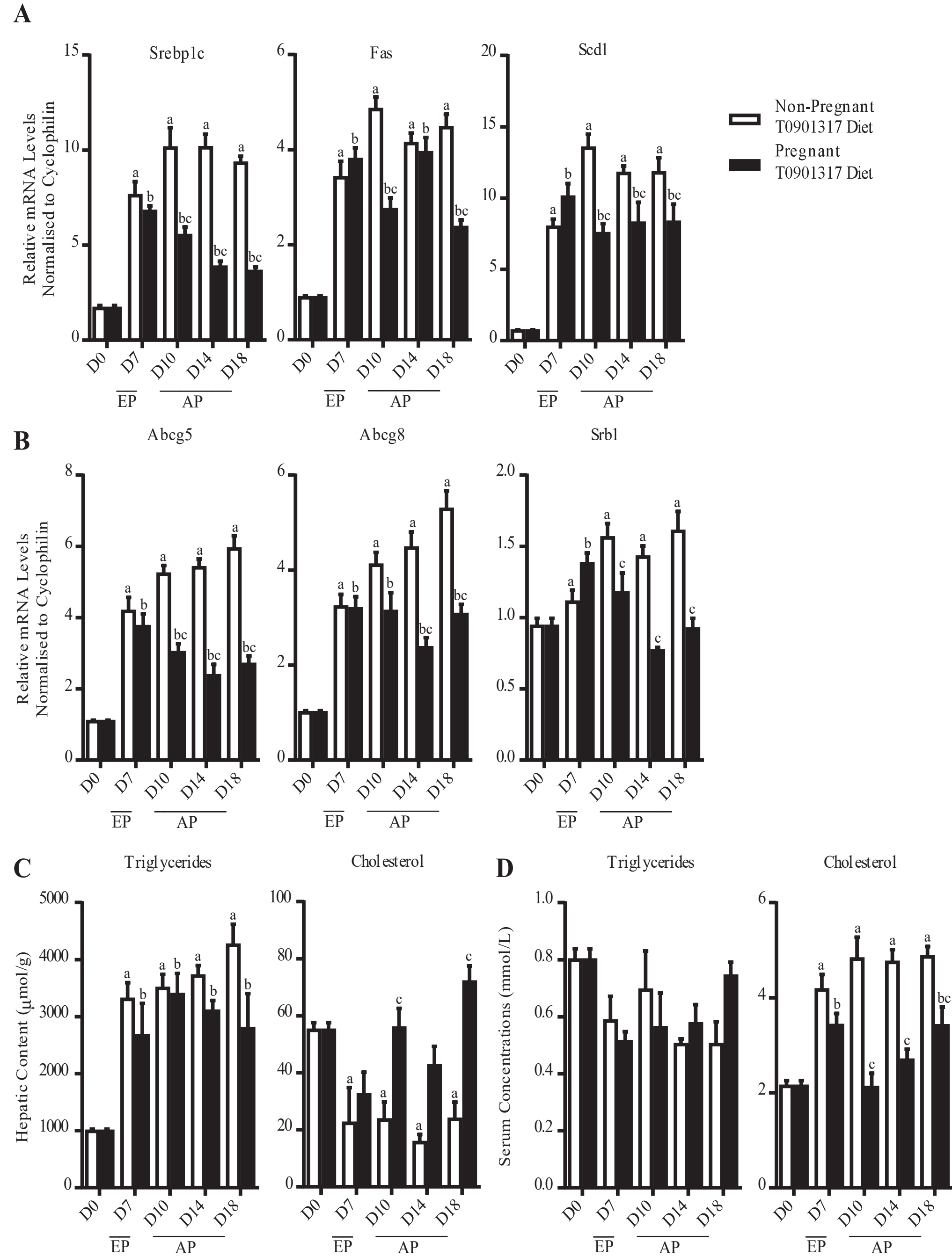
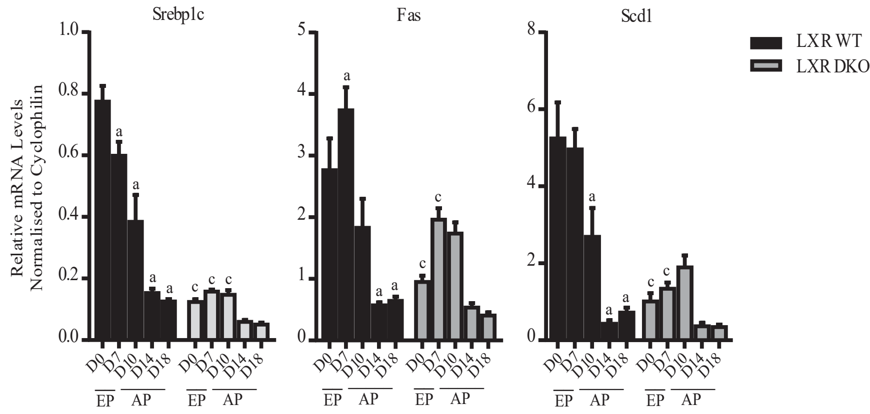
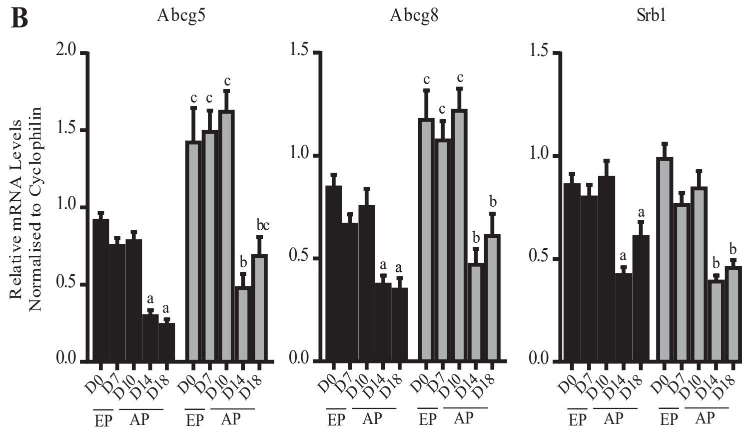
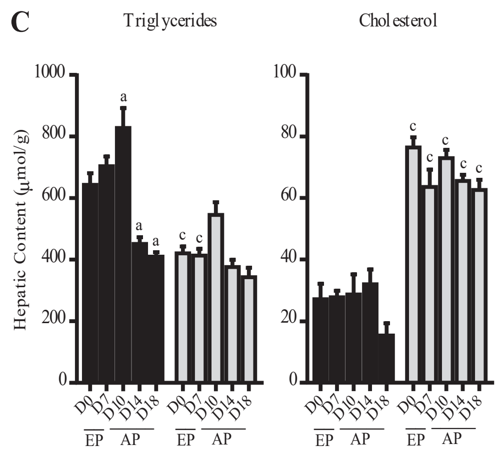


Figure 3: LXR is required for lipogenesis during early mouse pregnancy







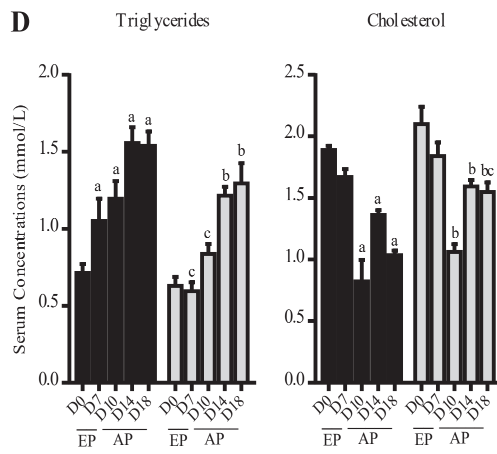


Figure 4: Gestational adaptations in LXR signalling protect against abnormalities in fetoplacental lipid metabolism in mice