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Elevated maternal linoleic acid reduces circulating leptin concentrations, cholesterol levels and male fetal survival in rat model

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Key words: linoleic acid, fetal development, inflammation, leptin, lipid, sex ratio

Key Points

- Linoleic acid consumption is increasing
- We investigated if elevated linoleic acid in pregnancy was deleterious
- Maternal and fetal body and organ weights were not affected by elevated linoleic acid consumption
- Maternal lipids and leptin were altered due to elevated linoleic acid consumption
- Male offspring numbers were reduced due to elevated linoleic acid consumption

53 ## Abstract

54 Dietary intakes of linoleic acid (LA) have increased dramatically in Western populations,
55 including in women of reproductive age. Pro-inflammatory effects of LA may have
56 detrimental effects on maternal and offspring outcomes. We aimed to investigate if
57 consumption of a maternal diet with elevated LA altered maternal inflammatory or metabolic
58 markers during pregnancy, fetal growth and/or the sex ratio of the offspring. Female Wistar
59 Kyoto rats consumed a diet high in LA (HLA) (6.21% of energy) or a diet low in LA (LLA)
60 (1.44% of energy) for 10 weeks prior to mating and during pregnancy. Pregnant rats were
61 sacrificed at embryonic day 20 (E20). There were no differences in maternal or fetal body
62 weights or organ weights in the HLA group compared to LLA group. There was no
63 difference in maternal circulating cytokine concentrations between dietary groups. In the
64 maternal liver, IL-1α concentrations were significantly lower, and TNF-α and IL-7
65 significantly higher in the HLA group. Total plasma cholesterol, LDL-cholesterol, HDL
66 cholesterol and the total: HDL cholesterol ratio were lower in dams fed the HLA diet. mRNA
67 expression of sterol regulatory element binding transcription factor 1 (*SREBF-1*) and leptin in
68 maternal adipose tissue was lower in the HLA group, as were circulating leptin
69 concentrations. The proportion of male fetuses was lower and circulating prostaglandin E
70 metabolite concentrations were increased in the HLA group. In conclusion, consumption of a
71 maternal diet high in linoleic acid alters cholesterol metabolism and prostaglandin E
72 metabolite concentrations that may contribute to the reduced proportion of males.

73

74

75 Introduction

76 The omega 6 (n-6) polyunsaturated fatty acid (PUFA), linoleic acid (LA; 18:2n-6; cis, cis-
77 9,12-octadecadienoic acid), is an essential fatty acid that can be metabolised to produce γ-
78 linoleic acid and arachidonic acid (AA). LA and AA in particular can then be metabolised
79 into downstream lipid mediators, collectively known as oxylipins, including pro-
80 inflammatory eicosanoids and prostaglandins (Ramsden *et al.*, 2012). In Western societies,
81 LA consumption has increased to three times the recommended daily intake in the last 40
82 years (Naughton *et al.*, 2015). In Australia, LA availability in the diet has increased by
83 120% (Naughton *et al.*, 2015) and in the USA by 158% (Blasbalg *et al.*, 2011), and this has
84 been driven primarily by the increased use of plant-based oils such as corn, safflower,
85 sunflower and soybean in the food supply (Sardesai, 1992). Both LA and the omega 3 (n-3)
86 FA α-linoleic acid (ALA) are metabolised by the same enzymes, FADS1 and FADS2
87 (Simopoulos, 2016). In contrast to LA, ALA is known to have anti-inflammatory effects,
88 and the recommended ratio of consumption of n-6/n-3 PUFA is 1:1–2:1 (Simopoulos,
89 2002). However, in recent years the ratio of n-6/n-3 PUFA consumed in western diets has
90 increased to 16.7:1 (Simopoulos, 2002).

91 The increase in LA intake has resulted in an increased ratio of n-6 to n-3 fatty acids in the
92 diet. Rat studies have demonstrated that consumption of a diet with elevated concentrations
93 of LA increases the production of circulating pro-inflammatory leukotriene and
94 prostaglandins (Ilich *et al.*, 2014) and cytokines including TNF-α and IL-1β (Marchix *et al.*,
95 2015). The high intake of n-6 FA in the western diet is reflected in the fatty acid profile of
96 pregnant women (Ailhaud *et al.*, 2006), however, the effect of LA consumption during
97 pregnancy on the mother are unknown, as is their impact on fetal development. Optimal

98 maternal health during pregnancy is critical for fetal development, and when this does not
99 occur, fetal development can be perturbed leading to an increased risk of disease in later life
100 (Gluckman *et al.*, 2008).

101 While limited studies have investigated the impact of consumption of a diet with altered FA
102 concentrations on fetal outcomes, some studies have provided evidence that this may impact
103 sex ratios in the offspring. In opossums, elevated n-3 consumption increases the number of
104 female offspring (Austad & Sunquist, 1986), while elevated consumption of LA in mice
105 (Fountain *et al.*, 2008) and sheep (Gulliver *et al.*, 2013) decreases the number of male
106 offspring. However, in mice, fish oil enriched with n-3 diet had no effect on sex ratio
107 (Fountain *et al.*, 2008). At this time, despite the pro-inflammatory effects of elevated LA
108 consumption and the changes in sex ratio, the effect of a diet high in LA on fetal
109 development has not yet been determined.

110 Leptin is an adipokine that is essential in the maintenance of body weight through
111 neurological maintenance of satiety (Stern *et al.*, 2016), and this hormone also plays a
112 critical role in fetal growth and development (Briffa *et al.*, 2015). In addition to its effects
113 on inflammatory pathways, excess LA consumption has the potential to influence
114 development by altering leptin concentrations. Studies using primary rat adipocytes have
115 demonstrated that LA reduces leptin secretion (Perez-Matute *et al.*, 2007). A reduction in
116 leptin secretion has the ability to reduce fertility (Rosenbaum & Leibel, 1998). In contrast to
117 the effect of LA on primary adipocytes, in non-pregnant rats, consumption of a diet with
118 elevated PUFA increases circulating leptin (Cha & Jones, 1998). However, at this time the
119 effect of elevated LA consumption prior to and during pregnancy on maternal leptin
120 concentrations is unknown. Further, increased PUFA intake in our diet is demonstrated to

121 lower the cholesterol level, however, the evidence of specific LA effect on blood lipid
122 profile is less clear (Khandelwal *et al.*, 2013).

123 The current study aimed to investigate the effects of elevated maternal LA consumption on
124 a range of maternal inflammatory markers, metabolic parameters, as well as fetal growth in
125 a rodent model. We hypothesised that exposure to elevated maternal concentrations of LA
126 would alter maternal inflammatory markers, the lipid profile, leptin concentrations, fetal
127 growth and fetal sex-ratio.
128

129 # Materials and Methods

130 **Ethical approval, experimental animal model and diet**

131 Wistar Kyoto rats (8 weeks of age; n=8 for diet with low linoleic acid (LLA) and n=10 for
132 diet with high linoleic acid (HLA)) were purchased from the Australian Resource Centre
133 (ARC, WA, Australia) and housed in accordance to the Australian Code of Practice for Care
134 and Use of Animals for Scientific Purpose after ethical approval being granted by the
135 Griffith University Animal Ethics Committee (NSC/01/17/AEC). The investigators
136 understand the ethical principles under which the The Journal of Physiology operates and
137 confirm that this work complies with the journal's animal ethics checklist (Grundy, 2015).

138

139 Rats were housed in individually ventilated cages under 12 hours light-dark cycle at a
140 temperature of 20-22°C and provided with standard food pellets during acclimatisation and
141 tap water *ad libitum* throughout the study. After a week for acclimatization, female rats
142 were randomised to either a control low linoleic acid (LLA: 1.44%) diet or a high linoleic
143 acid (HLA: 6.21%) diet for 10 weeks. These diets were matched for n-3 PUFA and total fat
144 content (Table 1). The HLA diet used in this study is comparable to diet we are consuming
145 in Western society (Jandacek, 2017). After 8 weeks of dietary exposure, vaginal impedance
146 was measured daily for at least two estrous cycles using a rodent vaginal impedance reader
147 (Muromachi Kikai Co. Ltd., Japan). Rats were considered ready for mating after 10 weeks
148 of dietary exposure and when vaginal impedance was greater than 4.5×10^3 Ω and at this
149 time were placed with a Wistar Kyoto male rat overnight. The day after mating was
150 considered embryonic day 1 (E1). The rats were fed the LLA or HLA diet during gestation
151 as well. The female rat was weighed daily and monitored for weight gain during pregnancy.

152 Pregnant females were sacrificed at E20 (term is 22 days). Pregnant females were
153 terminally anaesthetized with an intraperitoneal injection of sodium pentobarbital (60
154 mg/kg).Rats were monitored until abolishment of reflexes and a state of apnoea was
155 observed and regarded as confirmation of humane killing. Maternal blood was immediately
156 collected by cardiac puncture. The fetuses were sacrificed by decapitation and fetal blood
157 was collected from the trunk following decapitation. Blood collected in EDTA and heparin
158 tubes was centrifuged at 5000 x g for 10 minutes to separate plasma. Plasma samples were
159 stored at -80°C. At post mortem, maternal body and organ weights were recorded as was
160 the litter size, fetal body weight and fetal organ weights. Organs weight was expressed as
161 the percentage of weight of dam (total weight of dam minus the sum of fetal and placental
162 weight). Maternal and fetal organs were snap frozen immediately in liquid nitrogen and
163 stored at -80°C for RNA extraction.

164 **Fatty acid analysis in maternal and fetal plasma**

165 The concentrations of fatty acids in maternal and fetal plasma were measured using Gas
166 chromatography (GC) as previously described (Liu *et al.*, 2014). Briefly, 50 µl of plasma
167 sample were spotted onto blood collection paper and dried in air at room temperature. The
168 samples were transesterified with 2ml of 1% (v/v) H_2SO_4 in anhydrous methanol and heated
169 at 70°C for 3 hours. The resultant fatty acid methyl esters (FAME) were extracted into
170 heptanes and injected into a GC (Hewlett-Packard 6890; Palo Alto, CA, USA) for analysis.
171 FAME were quantified by comparing the retention times and peak area values of unknown
172 samples to those of commercial lipid standards (Nu-check prep Inc., MN, USA). The fatty
173 acid contents were expressed as a percentage of total lipids.

174 **Maternal blood analysis**

175 Cytokines, chemokines and growth factors (IL-1α, IL-1β, IL-2, IL-6, TNF-α, IL-4, IL-5,
176 IL-7, IL-10, IL-12 (p70), IL-13, IL-17, IL-18, IFN-γ, RANTES, VEGF and MCP-1) were
177 quantified using the Bio-Plex Pro^{TM} rat cytokine, chemokine, and growth factor assay kit
178 following manufacture's instruction and using the Bio-Plex 200 system (Bio-Rad
179 laboratories, Inc.). All the parameters measured were within detectable ranges. Maternal
180 blood gas concentrations were measured using a Siemens Rapidpoint 500 analyser
181 (Erlangen, Germany), including an onboard calibration cassette, and using ~ 200 μl of
182 EDTA anticoagulated plasma, derived from the venous circulation. Maternal plasma
183 biochemical markers were assessed using an automated chemistry analyser (Integra 400
184 plus, Roche Diagnostics, North Ryde, Australia) in EDTA anticoagulated plasma. All
185 chemistry assays were performed using Roche certified assay kits, which were calibrated
186 using Calibrator for Automated Systems reagent. Quality control material (PreciControl
187 ClinChem Multi 1 and 2; Roche Diagnostics) was run prior to sample analysis to ensure
188 accuracy of results. All analyses were performed in duplicate and averaged to provide the
189 final result.

190 The concentrations of prostaglandin E (PGEM- Cayman chemical, MI, USA) and
191 leukotriene B4 (LTB4- Cayman chemical, MI, USA) in maternal plasma were quantified by
192 ELISA, according to the manufacture's protocols. The ELISA was competitive assay and
193 the absorbance was measured in microplate reader at a wavelength of 414 nm. All the
194 samples were assessed in duplicate and intra assay coefficient of variation was 7.5%.

195 **Sex determination of fetus**

196 DNA was extracted from the tail of each fetus. Fetal tails were incubated in lysis buffer
197 with proteinase K overnight at 55°C prior to heat inactivation. DNA samples were subjected
198 to qPCR amplification of SRY (sex-determining region Y) gene using a commercially

199 available hydrolysis probe (Rn04224592_u1; NM_012772.1; Applied Biosystems) as
200 previously described (Cuffe *et al.*, 2012). Briefly, DNA samples were mixed with
201 appropriate volumes of QuantiNovaTM probe master mix (Qiagen), SRY primer probe
202 assay on demand (Applied Biosystems) and nuclease free water. TaqMan qPCR was
203 performed using a StepOne^{TM} PCR system (Applied Biosystems).

204 **Quantitative polymerase chain reaction (qPCR)**

205 Total RNA was extracted from maternal retroperitoneal white adipose tissues using TRI
206 reagent® (Sigma- Aldrich) following the manufacturer's guidelines. The quantification and
207 evaluation of purity of RNA samples were done by NanoDrop 1000 spectrophotometer
208 (Thermo Fisher Scientific). Reverse transcription of RNA to synthesize complementary
209 DNA was performed using the iScript gDNA clear cDNA synthesis kit (Biorad) following
210 manufacturer's guidelines. Quantitative PCR was performed using QuantiNova SYBR®
211 green master mix (Qiagen) following manufacturer's guidelines, in line with the Minimum
212 Information for Publication of Quantitative Real-Time PCR Experiments (MIQE)
213 guidelines (Bustin *et al.*, 2009). PCR initial heat activation was run for 2 minutes at 95°C,
214 then qPCR reactions were run for 40 cycles of 95°C for 5 seconds (denaturation) and 60°C
215 for 10 seconds (combined annealing/ extension) using StepOne^{TM} real-time PCR systems
216 (Applied Biosystems). Gene expression was quantified using the $2^{-\Delta\Delta Cq}$ method normalised
217 to the geometric mean of β-actin and β-2 microglobulin as reference genes. These reference
218 genes were stable across the treatment groups. All the primers used for this study were
219 KiCqStart^{TM} predesigned primers from Sigma- Aldrich: Leptin (*Lep*; NM_013076), Leptin
220 receptor (*Lepr*; NM_012596), SREBF-1 (*Srebf1*; XM_001075680), β-actin (*Actb*;
221 NM_031144) and β-2 microglobulin (*B2m*; NM_012512).

222 **Statistical analysis**

223 All data are analysed using student's unpaired t test, Mann-Whitney U test or two-way
224 ANOVA followed by Tukey test where appropriate. Normality test was run using D'Agostino
225 & Pearson normality test. Statistical analysis was performed using GraphPad Prism 7. For
226 fetal body weight, fetal organ weight and fetal blood glucose, data were firstly nested for
227 litter and separated by sex and analysed by two way-ANOVA with sex and treatment as
228 factors. For fetal fatty acid profile, blood samples were pooled from each litter. In addition,
229 fetal body weight organ weight and fetal blood glucose were analysed by two-way ANOVA
230 followed by Tukey test using sex and treatment as two factors. Data are expressed as mean±
231 standard error of the mean (SEM) and p value less than 0.05 is considered to be statistically
232 significant.
233

234 ## Results

235 ### Effect of a high maternal linoleic acid diet on maternal weight and estrous cycle length

236 Maternal consumption of HLA for 10 weeks prior to pregnancy and through gestation did
237 not affect body weight either prior to pregnancy or during gestation (Figure 1A-B)
238 compared to LLA controls. Similarly, HLA did not impact food or water consumption
239 either before or during gestation (Figure 1C-D). Female rats that consumed an HLA diet
240 had similar estrous cycle lengths compared to LLA rats prior to pregnancy (Figure 1E).

241 ### Effect of high maternal linoleic acid diet on maternal and fetal fatty acid profile in
242 ### plasma

243 There was no difference in maternal total saturated fatty acid (SFA) and trans fatty acids
244 levels as a proportion of total lipids between the LLA and HLA groups (Table 2). Total
245 monounsaturated fatty acid (MUFA), total n-9, total n-7, total n-3, 18:3n-3 and 22:6n-3
246 levels were significantly decreased in the maternal plasma of rats fed the HLA diet
247 ($p<0.0001$, Table 2). Total n-6 ($p<0.0001$), LA (18:2n-6, $p<0.0001$) and AA (20:4n-6,
248 $p<0.05$) were significantly increased in the maternal plasma of rats fed the HLA (Table 2).
249 In fetal plasma, there was no difference in total trans level between dietary groups (Table
250 3). However, total SFA levels were significantly increased ($p<0.001$) and total MUFA
251 ($p<0.0001$), total n- 9 ($p<0.0001$), total n-7 ($p<0.05$), total n- 3 ($p<0.0001$), 22:5n-3
252 ($p<0.05$) and 22:6n-3 ($p<0.05$) levels were significantly decreased in the plasma of fetuses
253 in the HLA group (Table 4). Total n-6 ($p<0.0001$), LA ($p<0.0001$) and AA ($p<0.01$) in the
254 fetal plasma were significantly increased in the HLA group (Table 3).

255 ### Effect of a high maternal linoleic acid diet on maternal and fetal organ weights

256 There was no significant difference in weight (expressed as % of body weight) of the
257 maternal kidney, brain, adrenal gland, heart or ovaries between LLA and HLA groups
258 (Table 4). The maternal liver weight (% of body weight) was significantly increased in rat
259 fed with diet high in LA ($p<0.01$). There were no significant differences in fetal body
260 weight, fetal blood glucose or the weight of any of the fetal organs measured between LLA
261 and HLA groups (Table 5). Further, there was no significant difference in placental weight
262 and fetal: placental weight ratio between LLA and HLA groups (Table 5). While the total
263 number of fetuses (9.6±1.25 vs 8.88±0.90, $p = 0.635$) and total number of fetal resorptions
264 (1.25±0.36 vs 1.7±0.42, $p = 0.717$) at E20 were not different between HLA and LLA
265 groups, the proportion of fetuses that were male was lower by ~16% in dams fed the HLA
266 diet ($p<0.01$, Figure 2).

267 **Effect of a high maternal linoleic acid diet on cytokines, chemokines and growth**
268 **factors in maternal circulation and liver**

269 There was no significant difference in circulating cytokine, chemokine and growth factor
270 concentrations in the maternal plasma between the HLA and LLA groups (Table 6). In the
271 liver, however, concentrations of tumor necrosis factor alpha (TNF-α) and interleukin-7 (IL-
272 7) were significantly increased ($p<0.05$, Table 7), while concentrations of interleukin 1
273 alpha (IL-1α) were significantly decreased in dams fed the HLA diet ($p<0.05$, Table 7).
274 There was no effect of the HLA diet on the concentrations of other cytokines in the liver
275 (Table 7).

276 **Effect of high maternal linoleic acid on biochemical parameters and blood gas in**
277 **maternal plasma**

278 Maternal plasma total cholesterol, low density lipoprotein - cholesterol (LDL-C) and high
279 density lipoprotein – cholesterol (HDL-C) were all significantly decreased ($p<0.01$, $p<0.05$

280 and $p<0.05$ respectively) in rats fed the HLA diet (Table 8). The ratio of total cholesterol to
281 HDL-C was also significantly decreased in the maternal plasma from the rats fed the HLA
282 diet ($p<0.01$, Table 8). However, there was no difference in glucose, albumin, alanine
283 transaminase (ALT), aspartate transaminase (AST), total bilirubin (TB), triglyceride (TG)
284 or uric acid concentrations in the maternal plasma (Table 8) between the LLA and HLA
285 groups. Maternal plasma PO_2 was significantly increased in the rats fed with HLA diet
286 ($p<0.01$, Table 9), but there was no difference in maternal plasma pH, PCO_2, Na^+, Cl^-,
287 HCO_3^- or osmolality between the dietary groups (Table 9).

288 **Effect of high maternal linoleic acid on leptin and lipid production associated genes**

289 Circulating leptin concentrations were significantly lower in the plasma of rats fed the HLA
290 diet compared with the LLA diet ($p<0.01$, Figure 3A). Relative *leptin* mRNA expression
291 was significantly decreased in the maternal white adipose tissue of rats fed the HLA
292 ($p<0.01$, Figure 3B), however there was no difference in mRNA expression of the *leptin*
293 *receptor* (*Lepr*) (Figure 3C) between dietary groups. Relative mRNA expression of sterol
294 regulatory element binding transcription factor 1 (*SREBF-1*) was significantly decreased in
295 the maternal adipose tissue from the rats fed the HLA compared to the LLA diet ($p<0.01$,
296 Figure 3D). DNA sequencing of PCR products confirmed the appropriate sequence was
297 amplified (data not shown).

298 **Effect of high maternal linoleic acid on prostaglandin E metabolite and leukotriene B_4**
299 **concentration**

300 Circulating prostaglandin E metabolite (PGEM) concentrations were significantly increased
301 in the maternal plasma of rats fed the diet high in LA compared with rats fed the diet low in
302 LA ($p<0.05$, Figure 4A). There was no difference in leukotriene B_4 (LTB_4) concentrations
303 between dietary groups (Figure 4B).

304

305 ## Discussion

306 Maternal nutrition before and during pregnancy can impact fetal growth and subsequent
307 offspring health (Thornburg, 2015). Western diets have become enriched in LA (Marchix *et*
308 *al.*, 2015). While high LA consumption is known to have implications for health (Naughton
309 *et al.*, 2016), little is known about how increased LA intake before and during pregnancy
310 can impact maternal health and fetal growth. In the current study we have shown, using a
311 rodent model, that excess consumption of LA in the maternal diet reduces maternal total
312 cholesterol and leptin concentrations, in the absence of any changes in maternal weight and
313 food consumption. A key finding was that consuming a diet high in LA during pregnancy
314 resulted in a reduced number of male fetuses in late gestation, and an altered inflammatory
315 cytokine profile in the maternal liver. Further, we found that high maternal LA does not
316 affect fetal weight, fetal organ weight and fetal blood glucose. The potential role of diets
317 high in total fat content as well as maternal obesity on fetal growth have been thoroughly
318 explored (Rosario *et al.*, 2015; Howell & Powell, 2017; Musial *et al.*, 2017; Ye *et al.*,
319 2017), however, our study is the first to focus on the effect of consuming high levels of LA
320 with matched total fat and n-3 content on offspring outcomes, and maternal circulating
321 factors that may affect fertility and development.

322 Previous studies have raised concerns that high dietary intake of LA may contribute to
323 inflammation as a result of increased production of LA-derived pro-inflammatory lipoxins,
324 and via synthesis of AA that could be converted to pro-inflammatory eicosanoids, including
325 PGE_2 and LTB_4 (Simopoulos, 2008). In the present study, the total n-6 fatty acid along with
326 LA and AA were elevated in maternal and fetal plasma. Indeed, we demonstrate in the
327 current study that PGEM concentrations were increased in rats consuming a diet high in

328 LA. Previous studies have suggested that exposure to *in utero* inflammation with elevated
329 levels of prostaglandins may be associated with a reduced survival of male embryos
330 (Gulliver *et al.*, 2013), which was observed in the current study. It is important to note,
331 however, not all studies conducted in healthy adult humans have demonstrated that
332 consumption of a high LA diet increase inflammatory markers (Johnson & Fritsche, 2012).
333 In our study, although AA was increased, we found no significant changes in circulating
334 inflammatory cytokines, chemokines or growth factor in maternal plasma. However, TNF-α
335 and IL-7 were significantly increased in the liver of pregnant rats fed with HLA. Relative
336 liver weight was also significantly elevated in HLA group. Hepatic IL-1α is significantly
337 decreased in HLA fed pregnant rats at E20. Increased concentrations of AA in maternal
338 plasma may be associated with increased pro-inflammatory cytokines in the liver. In mice,
339 dietary LA and its oxidized metabolites exacerbates ethanol induced liver injury through
340 hepatic pro-inflammatory responses (Warner *et al.*, 2017). TNF-α has a crucial role in
341 several liver diseases and its circulating levels increase in patients with hepatic failure (Bird
342 *et al.*, 1990). Despite these alterations in markers of hepatic inflammation in our study, there
343 were no changes in serum liver injury markers such as ALT, AST and total bilirubin.

344 A key finding of the current study was that the proportion of male offspring was decreased
345 in dams fed a diet high in LA before and during pregnancy. The effect of maternal n-3 and
346 n-6 FA on offspring sex ratio in different experimental models has been investigated
347 (Fountain *et al.*, 2008; Gulliver *et al.*, 2013). In the mouse, prior to conception, maternal
348 body weight was significantly reduced (Fountain *et al.*, 2008) suggesting that the reduction
349 in body weight may influence the sex ratio of the offspring (Mathews *et al.*, 2008). Of note,
350 the n-6 to n-3 ratio in this study was ~ 150:1 (Fountain *et al.*, 2008), much greater than
351 observed in human populations (Naughton *et al.*, 2015) , or used in the current
352 investigation. In previous studies in sheep, however, the reduction in the number of male

353 lambs born was observed at LA concentrations more similar to the concentrations consumed
354 by the human population (ratio of n-6: n-3 is13.03:1) (Gulliver *et al.*, 2013). In this study,
355 high omega-6 was offered to sheep for 42 days before and 17 days after conception. In
356 developmental programming, early life exposure to maternal perturbations can lead to an
357 increased risk of deleterious developmental consequences, and males are at a relatively
358 greater risk of negative effects than females (Perez-Cerezales *et al.*, 2018). This greater
359 susceptibility of males to deleterious *in utero* effects may account for the decreased number
360 of male offspring. An alternate explanation is that the lower male: female ratio in dams fed
361 the high LA diet is due to a lower number of males being conceived, as a result of effects of
362 the fatty acids on oocyte maturation or the timing of oocyte release (Bilby *et al.*, 2006),
363 prostaglandin synthesis (Thatcher *et al.*, 2001), or vaginal pH at certain times of mating
364 (Pratt *et al.*, 1987), which lead to the selection of X or Y sperm during conception. Further,
365 nutritional requirements such as glucose (Kimura *et al.*, 2005) and total fat (Rosenfeld *et al.*,
366 2003) may alter the sex-ratio of the offspring (Rosenfeld *et al.*, 2003). However, the
367 mechanism describing the effect of a maternal diet high in PUFA on sex ratio of offspring
368 has not been elucidated. Previously, it was proposed that increased *in utero* inflammation
369 associated with elevated prostaglandins may be associated with a reduced survival of male
370 embryos, skewing the sex ratio towards females (Rosenfeld & Roberts, 2004; Gulliver *et*
371 *al.*, 2013). In the present study, the circulating level of PGEM is elevated in the plasma of
372 pregnant rats fed with a diet high in linoleic acid, suggesting the possible mechanism of
373 higher proportion of female fetuses.

374 Dyslipidaemia has been well documented as a risk factor for cardiovascular disease
375 (Nelson, 2013). Increased PUFA intake has been demonstrated to lower cholesterol levels
376 (Khandelwal *et al.*, 2013). The results of our current study suggest that increased LA intake
377 before and during pregnancy results in lower total cholesterol, LDL-cholesterol and HDL-

378 cholesterol in maternal plasma. The cholesterol ratio (total cholesterol/ HDL-cholesterol), a
379 more specific predictor of coronary artery disease than LDL-cholesterol (Mensink *et al.*,
380 2003), is also decreased in maternal plasma with increased LA intake. Thus, while the diet
381 used in the current study appears to have some pro-inflammatory effects, at least within the
382 liver, it may also have cholesterol lowering properties. It must be noted however, that while
383 every care was made to control for total fat and n-3 content, the LLA diet by necessity had a
384 different fatty acid content that may have contributed to this outcome, which is a limitation
385 of our study.

386 Given these changes in cholesterol content, it was also important to characterise alterations
387 in adipose tissue homeostasis. Excess energy is readily stored in adipose tissue in the form
388 of triglycerides (Yu & Ginsberg, 2005). SREBFs are transcription factors that regulates
389 lipid homeostasis by controlling genes involved in cholesterol, triglyceride and fatty acid
390 synthesis (Eberle *et al.*, 2004). The reduction in adipose tissue mRNA expression of
391 *SREBF-1* in rats consuming the high LA diet may have contributed to the reduced
392 cholesterol concentrations in plasma. SREBF-1 is regulated by nutritional condition and
393 plays a central role in nutritional regulation of lipogenesis in liver and adipose tissue
394 (Karasawa *et al.*, 2011). Furthermore, it has also been reported that increased PUFA
395 physiologically inactivates SREBF-1 (Carobbio *et al.*, 2013). Downregulation of *SREBF-1*
396 in adipose tissue along with decreased cholesterol levels in rats fed with HLA before and
397 during pregnancy suggest the role of linoleic acid on the regulation of lipogenesis.

398 Maternal acid base balance during pregnancy is associated with fetal development (Collazos
399 *et al.*, 2017). Very small changes in pH may affect the function of fetal organ systems
400 (Omo-Aghoja, 2014). In cows, negative level of dietary cation-anion difference in maternal
401 diet affected growth and energy metabolism in the calves (Collazos *et al.*, 2017). In our

402 study, there was no difference in maternal plasma pH, PCO_2, Na^+, Cl^-, HCO_3^- or osmolality
403 between dietary groups, however, maternal plasma PO_2 was significantly increased in the
404 rats fed with HLA diet. Previous study in rats demonstrated that arachidonic acid, a PUFA,
405 increases PO_2, suggesting fatty acids role in respiratory control (Erkan *et al.*, 2017).

406

407 Leptin is a key hormone secreted by adipose tissue that plays a central role in regulating
408 energy homeostasis (Park & Ahima, 2015) and lipid metabolism (Yu & Ginsberg, 2005). In
409 the current study, both *leptin* mRNA expression in adipose tissues and circulating leptin
410 concentrations were reduced in dams fed the high LA compared to the low LA diets. These
411 data differ from a previous rodent study, in which an increase in maternal LA consumption
412 from mid-gestation had no effects on maternal leptin concentrations, despite producing a
413 significant decrease in offspring body weights at PN7 (Korotkova *et al.*, 2002). Our
414 findings are, however, consistent with a previous study showing that LA supressed leptin
415 synthesis and secretion in primary rat adipocytes (Perez-Matute *et al.*, 2007). Conversely, a
416 previous study demonstrated that a reduction in essential FA (PUFA) significantly
417 decreased circulating leptin concentrations in the mothers and their offspring (Korotkova *et*
418 *al.*, 2001). As leptin is involved in the regulation of maternal metabolic homeostasis and
419 fetal development, the changes in leptin levels in our study provide important insight into
420 the role of LA in leptin driven maternal metabolic conditions and fetal outcomes.

421 The other intriguing finding in this study was that HLA exposure before and during
422 pregnancy has no effect on maternal SFA, however total SFA was significantly increased in
423 the fetal plasma from HLA group. Total n-3 FA was significantly decreased in both
424 maternal and fetal plasma from HLA group. There is a positive impact of n-3 FA and a
425 negative impact of SFA on fetal insulin sensitivity, which may be involved in programming

426 the susceptibility to metabolic diseases in the offspring (Zhao *et al.*, 2014). Elevated SFA
427 concentration and decreased n-3 FA concentration in the fetal circulation of HLA group
428 suggest the possible negative impact of maternal HLA diet on the health of offspring despite
429 no differences in fetal body weights. In the present study, fetal fatty acid profile was not
430 separated by sex due to the very low plasma volume from fetuses, so plasma samples were
431 required to be pooled, which is a limitation of the study.

432

433 **Conclusion**

434 In conclusion, maternal consumption of elevated LA diet, lowers maternal cholesterol levels
435 possibly as a result of downregulation of *SREBF-1*, a lipogenic gene, in maternal adipose
436 tissue. Maternal consumption of a diet high in LA before and during pregnancy also
437 resulted in upregulation of several inflammatory cytokines in the maternal liver and
438 suppression of leptin synthesis and circulating leptin concentration. In addition, maternal
439 HLA diet affected fetal sex, decreasing the proportion of male fetuses which may be linked
440 with elevated PGEM in the plasma of pregnant rats fed with HLA diet. Hence, with
441 increasing consumption of LA in recent decades, this study highlights the need to further
442 investigate the impact that the increasing consumption of LA in recent years may have on
443 the developing fetus and offspring outcomes.

445 **Additional Information**

446 Competing interests

447 The authors declare no competing interests.

448 Author contributions

449 DHH, NS, JCSC and AJMc drafted and revised the manuscript. NS contributed to the
450 writing and made the figures. Authors contributed to different methods and data analysis
451 and all authors approved the final version of this manuscript and agree to be accountable for
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Figure legends

Figure 1. Effect of HLA diet on maternal body weight, food consumption, water intake and vaginal impedance (estrous cycle). (A-B) There was no difference in body weight between dams fed on LLA and HLA diets either before mating or during pregnancy. (C-D) There was no difference in food consumption and water consumption during pregnancy between the LLA and HLA groups. E) There was no difference in vaginal impedance (estrous cycle) between the LLA and HLA groups. Data expressed as mean ± SEM analysed by student's unpaired t-test or Mann-Whitney U test. n=8 (LLA) and n=10 (HLA).

Figure 2. Effect of the HLA diet on proportion of male fetuses at E20. The proportion of male fetuses was significantly decreased in HLA group. Total number of fetuses was similar between HLA and LLA groups (9.6±1.25 vs 8.88±0.90). Data expressed as mean ± SEM and analysed by student's unpaired t-test. The sample size for dams; n=8 (LLA) and n=10 (HLA). **p<0.01.

Figure 3. Effect of the HLA diet on circulating leptin concentrations and mRNA expression in maternal adipose tissue (A) Circulating leptin concentrations were lower in rats fed the HLA before and during pregnancy. (B) *Leptin* mRNA expression in maternal white adipose tissue was lower in rats fed the HLA. (C) There was no difference in leptin receptor (*Lepr*) mRNA expression in white adipose tissue between the dietary groups. (D) Sterol regulatory element binding transcription factor (*SREBF-1*) mRNA expression in white adipose tissue was significantly reduced in rats fed the HLA diet. Data expressed as mean ± SEM and analysed by student's unpaired t-test or Mann-Whitney U test. n=8 (LLA) and n=10 (HLA), **p<0.01.

718 Figure 4. Effect of the HLA diet on prostaglandin E metabolite (PGEM) and leukotriene B_4
719 (LTB_4) concentrations in maternal plasma. A) PGEM concentration was significantly
720 increased in the plasma of pregnant rats fed the HLA diet. B) There was no significant
721 difference in LTB_4 concentration in the maternal plasma between the dietary groups. Data
722 expressed as mean ± SEM and analysed by Mann-Whitney U test. n=8 (LLA) and n=10
723 (HLA), $^*p<0.05$.
724

725 **Table 1. Dietary composition of experimental diet**

	LLA	HLA
% of energy derived from		
Carbohydrate	56.8	56.8
Protein	19.4	19.4
Crude fibre	4.7	4.7
AD fibre	4.7	4.7
Others	4.5	4.5
Fat	9	9
SFA	1.36	1.04
MUFA	5.82	1.72
18:2n-6 (LA)	1.44	6.21
18:3n-3 (ALA)	0.36	0.3
n-6/n-3	4.0	20.7

726
727 **LLA:** low linoleic acid; **HLA**: high linoleic acid; **SFA**: saturated fatty acid; **MUFA**:
728 monounsaturated fatty acid; **LA**: linoleic acid; **ALA**: alpha-linolenic acid.
729
730

731 **Table 2. Effect of the high linoleic acid (HLA) diet on fatty acid composition (% of total**
732 **lipids) in maternal plasma.**
733

	LLA	HLA	p-value
Total SFA	33.9±0.32	34.7±0.35	ns
Total Trans	0.15±0.01	0.21±0.02	ns
Total MUFA	28.8±1.46	14.8±0.62	<0.0001
Total n-9	24.9±1.35	12.3±0.61	<0.0001
Total n- 7	3.9±0.10	2.53±0.10	<0.0001
Total n- 3	5.3±0.18	4.06±0.13	<0.0001
18:3n-3	0.4±0.03	0.18±0.02	<0.0001
20:5n-3	0.16±0.02	0.15±0.02	ns
22:5n-3	0.15±0.02	0.27±0.04	<0.05
22:6n-3	4.6±0.17	3.48±0.14	<0.0001
Total n- 6	31.6±1.07	46.05±0.51	<0.0001
18:2n-6 (LA)	12.1±0.30	21.1±1.58	<0.0001
20:4n-6 (AA)	18.7±0.98	22.3±1.19	<0.05

734
735 **LLA:** low linoleic acid; **HLA**: high linoleic acid; **SFA**: saturated fatty acid; **MUFA**:
736 monounsaturated fatty acid; **LA**: linoleic acid; **AA**: arachidonic acid. Data expressed as mean
737 ± SEM and analysed by student's unpaired t-test or Mann-Whitney test. n=8 (LLA) and n=10
738 (HLA).
739
740 **Table 3. Effect of the high linoleic acid (HLA) diet on fatty acid composition (% of total**
741 **lipids) in fetal plasma.**

	LLA	HLA	p-value
Total SFA	41.2±0.23	42.8±0.24	<0.001
Total Trans	0.47±0.02	0.54±0.06	ns
Total MUFA	32.0±0.31	25.8±0.57	<0.0001
Total n- 9	24.7±0.22	18.8±0.47	<0.0001
Total n- 7	7.37±0.10	6.96±0.13	<0.05
Total n- 3	4.53±0.20	3.07±0.10	<0.0001
18:3n-3	0.15±0.01	0.11±0.01	ns
20:5n-3	0.27±0.03	0.2±0.04	ns
22:5n-3	0.3±0.04	0.12±0.05	<0.05
22:6n-3	3.83±0.15	2.58±0.08	<0.0001
Total n- 6	21.4±0.36	27.6±0.69	<0.0001
18:2n-6 (LA)	6.53±0.14	9.53±0.39	<0.0001
20:4n-6 (AA)	12.8±0.23	15.1±0.52	<0.01

742
743 **LLA:** low linoleic acid; **HLA**: high linoleic acid; **SFA**: saturated fatty acid; **MUFA**:
744 monounsaturated fatty acid; **LA**: linoleic acid; **AA**: arachidonic acid. Data expressed as mean
745 ± SEM and analysed by student's unpaired t-test or Mann-Whitney test. n=8 (LLA) and n=9
746 (HLA) (n number represents pooled samples from each litter)
747
748

749
750 **Table 4. Effect of high linoleic acid (HLA) diet on maternal organ weights (% of body**
751 **weight).**
752

	LLA	HLA	p value
Brain weight (% of BW)	0.66±0.018	0.69±0.01	ns
Liver weight (% of BW)	4.21±0.11	4.51±0.05	<0.05
Left kidney weight (% of BW)	0.26±0.004	0.25±0.02	ns
Right kidney weight (% of BW)	0.27±0.004	0.28±0.006	ns
Left adrenal gland weight (% of BW)	0.010±0.0004	0.011±0.0004	ns
Right adrenal gland weight (% of BW)	0.016±0.008	0.011±0.001	ns
Heart weight (% of BW)	0.40±0.028	0.40±0.020	ns
Left ovary weight (% of BW)	0.031±0.004	0.032±0.004	ns
Right ovary weight (% of BW)	0.037±0.005	0.033±0.005	ns

753
754 **BW:** body weight which represent weight of rat excluding total fetal and placental weight.
755 **LLA:** low linoleic acid; **HLA**: high linoleic acid. Data expressed as mean ± SEM and
756 analysed by student's unpaired t-test or Mann-Whitney test. n=8 (LLA) and n=10 (HLA).
757

758
759 **Table 5. Effect of the high linoleic acid (HLA) diet on fetal body weight, placental**
760 **weight, fetal organ weight and fetal blood glucose concentration.**
761

	LLA		HLA				
	Female	**Male**	**Female**	**Male**	**P_{sex}**	**P_{trt}**	**P_{int}**
Body weight (g)	1.745±0.066	1.795±0.067	1.794±0.049	1.815±0.041	ns	ns	ns
Blood glucose (mmol/L)	1.526±0.169	1.427±0.154	1.745±0.153	1.507±0.128	ns	ns	ns
Placental weight (g)	0.357±0.015	0.358±0.014	0.373±0.024	0.353±0.010	ns	ns	ns
Fetal: placenta weight ratio	4.997±0.189	5.013±0.088	4.914±0.199	5.152±0.116	ns	ns	ns
Brain weight (g)	0.104±0.008	0.112±0.003	0.106±0.003	0.108±0.004	ns	ns	ns
Heart weight (g)	0.011±0.001	0.011±0.0009	0.010±0.001	0.011±0.0007	ns	ns	ns
Liver weight (g)	0.110±0.013	0.105±0.010	0.105±0.007	0.097±0.007	ns	ns	ns
Left kidney weight (g)	0.006±0.0009	0.006±0.0003	0.007±0.0004	0.007±0.0003	ns	ns	ns
Right kidney weight (g)	0.006±0.0009	0.005±0.0004	0.007±0.0004	0.006±0.0005	ns	ns	ns
Relative brain weight (g/g)	0.06±0.004	0.063±0.003	0.06±0.002	0.06±0.002	ns	ns	ns
Relative heart weight (g/g)	0.0062±0.0005	0.006±0.0005	0.006±0.0003	0.006±0.0004	ns	ns	ns
Relative liver weight	0.062±0.006	0.058±0.004	0.058±0.003	0.053±0.003	ns	ns	ns
Relative left kidney weight (g/g)	0.004±0.0004	0.003±0.0001	0.004±0.0003	0.004±0.0001	ns	ns	ns
Relative right kidney weight (g/g)	0.004±0.0005	0.003±0.0002	0.004±0.0002	0.003±0.0003	ns	ns	ns

762
763 **LLA:** low linoleic acid; **HLA**: high linoleic acid; BW: body weight; **Psex**: p-value by sex:
764 **Ptrt**: p-value by treatment; **Pint**: p-value by interaction of sex and treatment. Data expressed
765 as mean ± SEM and analysed by a two-way ANOVA test with a Tukey test. n=8 (LLA) and
766 n=9 (HLA) (n number represents number of dams; data were nested for litter and separated
767 by sex).
768

769 **Table 6: Effect of the high linoleic acid (HLA) diet on circulating cytokines, chemokine**
770 **and growth factor concentrations in maternal plasma.**
771

	LLA	HLA	p value
IL-1α (pg/ml)	357.4±118.3	242.4±50.86	ns
IL-1β (pg/ml)	248±97.42	136.3±31.22	ns
IL-2 (pg/ml)	3612±1064	3580±861.7	ns
IL-6 (pg/ml)	1057±303.5	998.5±246.7	ns
TNF-α (pg/ml)	1104±354.8	996.4±290.9	ns
IL-4 (pg/ml)	262.8±99	196.5±57.55	ns
IL-5 (pg/ml)	662.9±153.8	642.1±129	ns
IL-7 (pg/ml)	167.2±80.2	132±31.67	ns
IL-10 (pg/ml)	345.8±119.5	281.1±88.64	ns
IL-12 (p70) (pg/ml)	1054±425.3	613.7±145.1	ns
IL-13 (pg/ml)	431±126.8	433.3±98.78	ns
IL-17 (pg/ml)	93.96±36.16	81.24±26.91	ns
IL-18 (pg/ml)	3887±942.1	4114±787.8	ns
IFN-γ (pg/ml)	1040±324.8	876.8±226.3	ns
RANTES (pg/ml)	87.33±13.15	88.2±11.85	ns
VEGF (pg/ml)	454.7±175.9	249.1±56.53	ns
MCP-1 (pg/ml)	335.5±43.69	415.9±55.2	ns

772
773
774 **LLA:** low linoleic acid; **HLA**: high linoleic acid; **IL**: interleukin; **TNF-α:** tumor necrosis
775 factor- alpha; **IFN-γ:** interferon-gamma; RANTES**:** regulated on activation normal T cell
776 expressed and secreted; **VEGF:** vascular endothelial growth factor; **MCP-1**: Monocyte
777 chemoattractant protein-1. Data expressed as mean ± SEM and analysed by student unpaired
778 t-test or Mann-Whitney U test. n=8 (LLA) and n=10 (HLA).
779
780

781 **Table 7: Effect of the high linoleic acid (HLA) diet on circulating cytokines, chemokine**
782 **and growth factor concentrations in maternal liver.**
783

	LLA	HLA	p value
IL-1α (pg/ml)	59.01±7.14	40.69±4.03	<0.05
IL-1β (pg/ml)	473.9±58.44	516.1±55.17	ns
IL-5 (pg/ml)	57.9±6.89	52.1±11.59	ns
IL-6 (pg/ml)	86.65±9.75	67.76±17.92	ns
TNF-α (pg/ml)	2426±208.2	3623±442.4	<0.05
IL-7 (pg/ml)	1565±221.3	4230±968.6	<0.05
IL-10 (pg/ml)	575.7±17.82	535.3±28.19	ns
IL-12 (p70) (pg/ml)	87.27±9.78	65.02±10.68	ns
IL-17 (pg/ml)	9.01±0.98	5.41±1.02	ns
IL-18 (pg/ml)	2079±189.4	1510±247.1	ns
IFN-γ (pg/ml)	196.9±13.52	120.9±24.21	ns
RANTES (pg/ml)	25.64±2.86	21.34±3.28	ns
VEGF (pg/ml)	31.72±3.27	21.63±4.77	ns
MCP-1 (pg/ml)	208.8±16.3	149.3±24.4	ns
G-CSF (pg/ml)	3.36±0.59	3.2±0.88	ns

784
785
786 **LLA:** low linoleic acid; **HLA**: high linoleic acid; **IL**: interleukin; **TNF-α:** tumor necrosis
787 factor- alpha; **IFN-γ:** interferon-gamma; RANTES**:** regulated on activation normal T cell
788 expressed and secreted; **VEGF:** vascular endothelial growth factor; **MCP-1**: Monocyte
789 chemoattractant protein-1; **G-CSF**: Granulocyte-colony stimulating factor. Data expressed as
790 mean ± SEM and analysed by student unpaired t-test or Mann-Whitney U test. n=8 (LLA)
791 and n=10 (HLA).
792
793

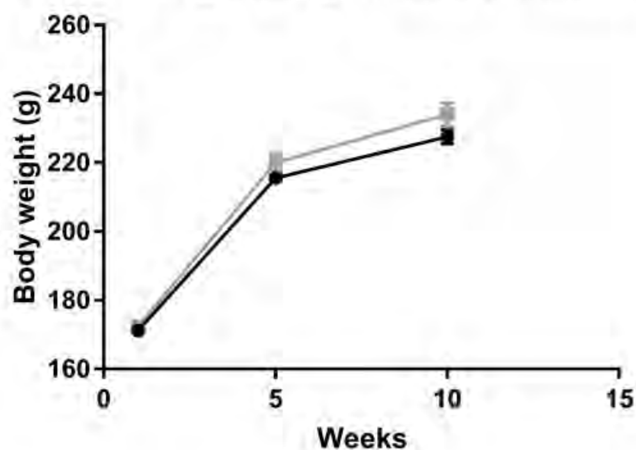
794
795 **Table 8. Effect of the high linoleic acid (HLA) diet on maternal blood biochemical**
796 **markers and blood lipids.**

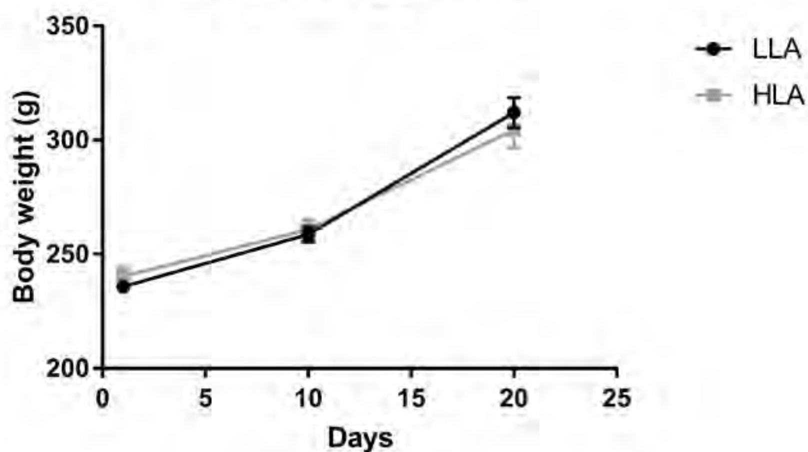
	LLA	HLA	p value
Glucose (mmol/L)	7.35±0.74	7.95±0.47	ns
Albumin (g/L)	43.18±1.51	44.08±2.00	ns
ALT (U/L)	65.15±5.35	53.04±5.30	ns
AST (U/L)	133.8±10.02	115.4±9.64	ns
TB (µmol/L)	1±0.22	1.38±0.24	ns
TC (mmol/L)	2.35±0.03	1.79±0.15	<0.01
TG (mmol/L)	1.28±0.2	0.92±0.05	ns
LDL-C (mmol/L)	0.33±0.007	0.24±0.03	<0.05
HDL-C (mmol/L)	1.76±0.04	1.50±0.12	<0.05
Cholesterol ratio	1.33±0.02	1.19±0.02	<0.01
Uric acid (µmol/L)	56.5±10.59	51.4±7.01	ns

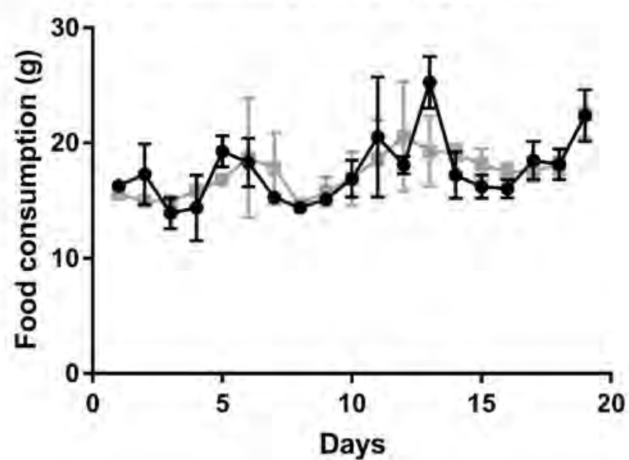
797
798 **LLA:** low linoleic acid; **HLA**: high linoleic acid; **ALT:** alanine transaminase; **AST:**
799 aspartate transaminase**; TB:** total bilirubin**; TC:** total cholesterol; **TG:** triglyceride; **LDL-C:**
800 low density lipoprotein-cholesterol; **HDL-C:** high density lipoprotein-cholesterol. Data
801 expressed as mean ± SEM and analysed by student's t-test or Mann-Whitney U test. n=8
802 (LLA) and n=10 (HLA).
803
804
805 **Table 9. Effect of the high linoleic acid (HLA) diet maternal blood gases.**
806

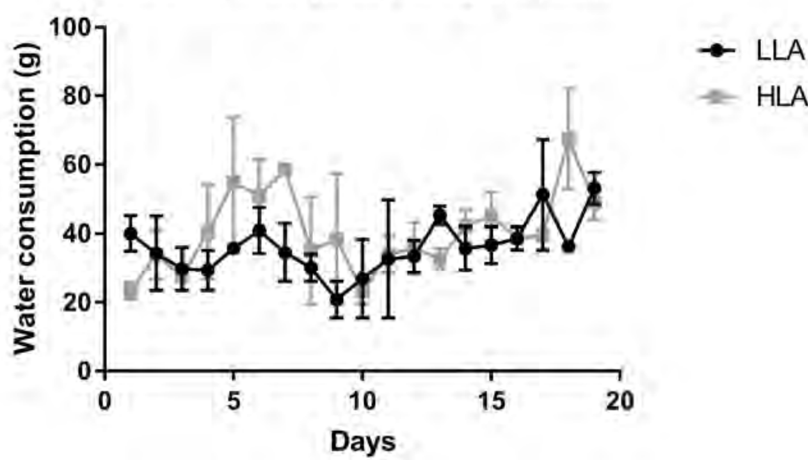
	LLA	HLA	P value
pH	7.41±0.045	7.32±0.059	ns
PCO_2 (mmHg)	47.11±5.04	55.95±7.80	ns
PO_2 (mmHg)	239.7±2.91	262.3±5.05	<0.01
Na^+ (mmol/L)	125.4±0.68	125.4±0.79	ns
Cl^- (mmol/L)	92.5±0.53	93.4±0.4	ns
Lactate (mmol/L)	7.41±0.71	8.38±0.61	ns
HCO_3^- (mmol/L)	28.01±0.77	25.87±0.72	ns
$ctCO_2$ (mmol/L)	29.48±0.84	27.57±0.84	ns
mOsm (mmol/kg)	258.1±1.49	258.8±1.67	ns

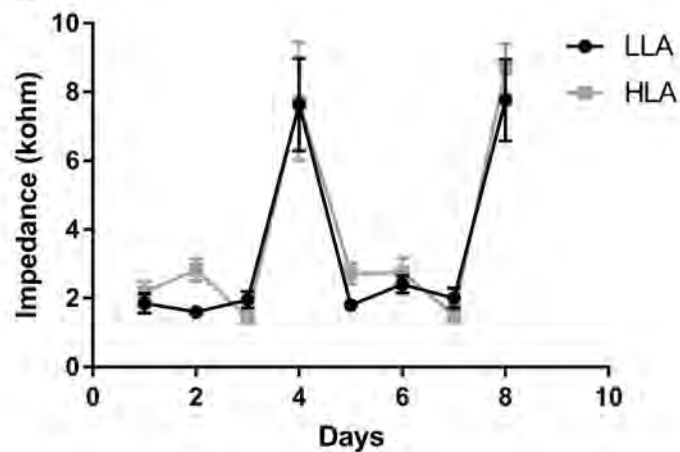
807
808 **LLA:** low linoleic acid; **HLA**: high linoleic acid; **PCO_2:** partial pressure of carbon dioxide**;**
809 **PO_2:** partial pressure of oxygen**; mOsm:** milliosmole. Data expressed as mean ± SEM and
810 analysed by student's t-test or Mann-Whitney U test. n=8 (LLA) and n=10 (HLA).
811
812

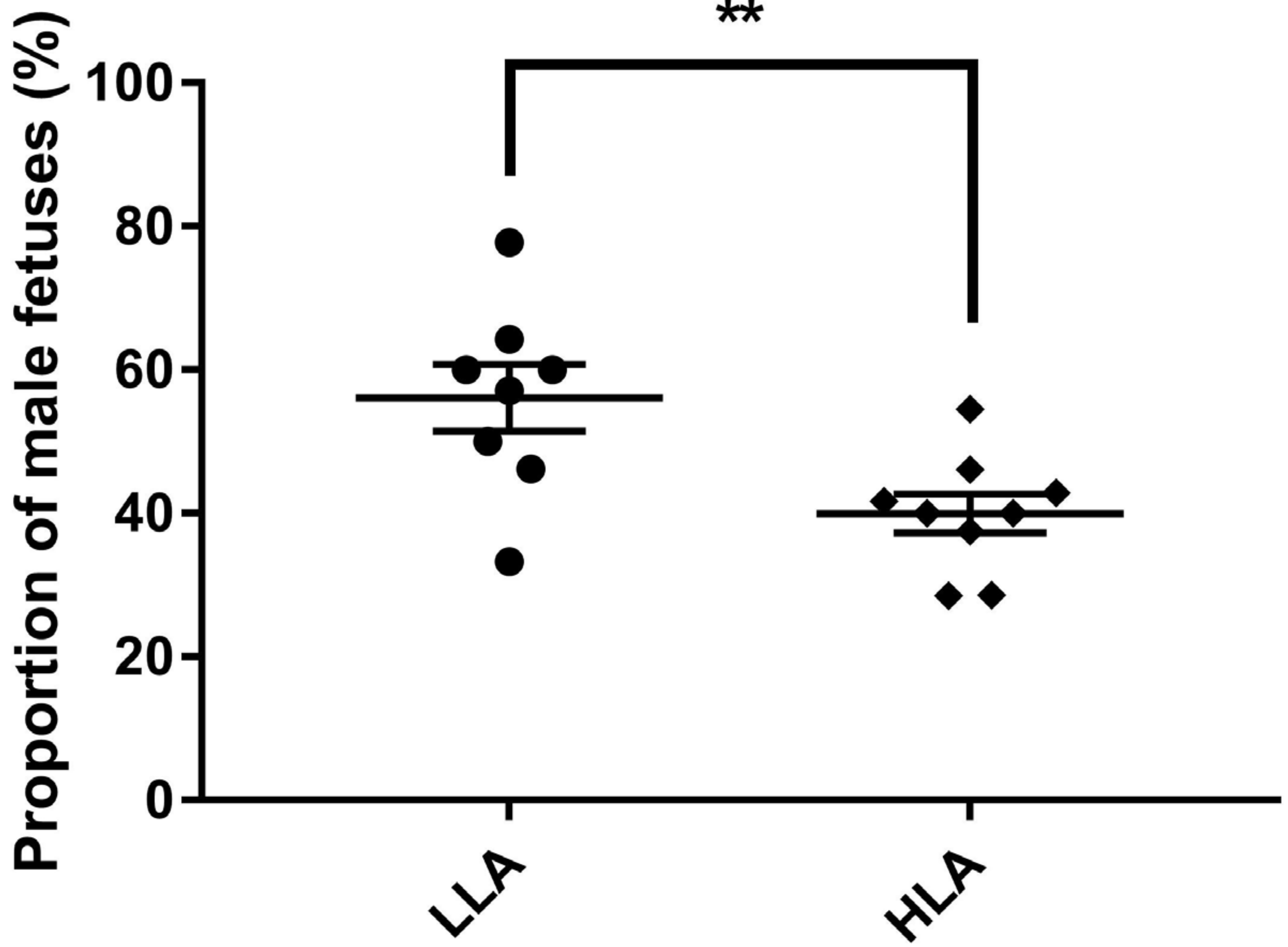

A
Weight during 10 weeks of treatment
Body weight (g)
260
240
220
200
180
160
0
5
10
15
Weeks


B
Weight during gestation
Body weight (g)
350
300
250
200
0
5
10
15
20
25
Days
LLA
HLA

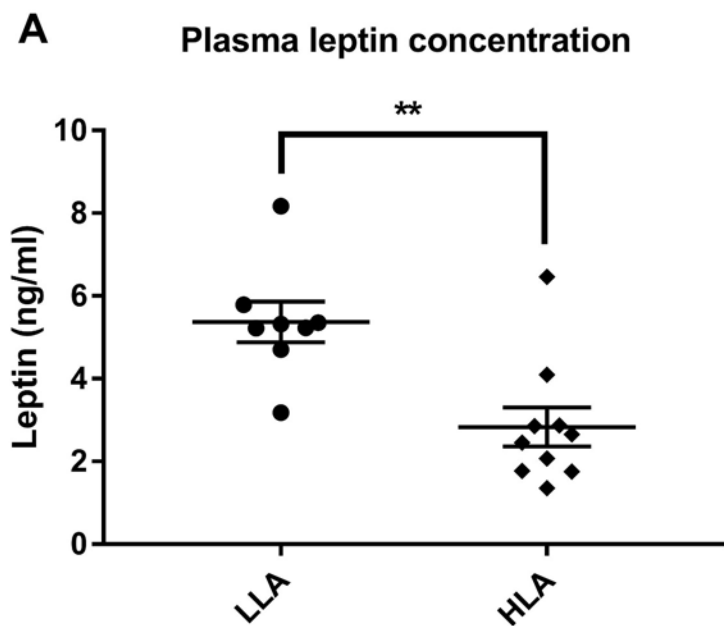

C
Food consumption during gestation
Food consumption (g)
30
20
10
0
0
5
10
15
20
Days

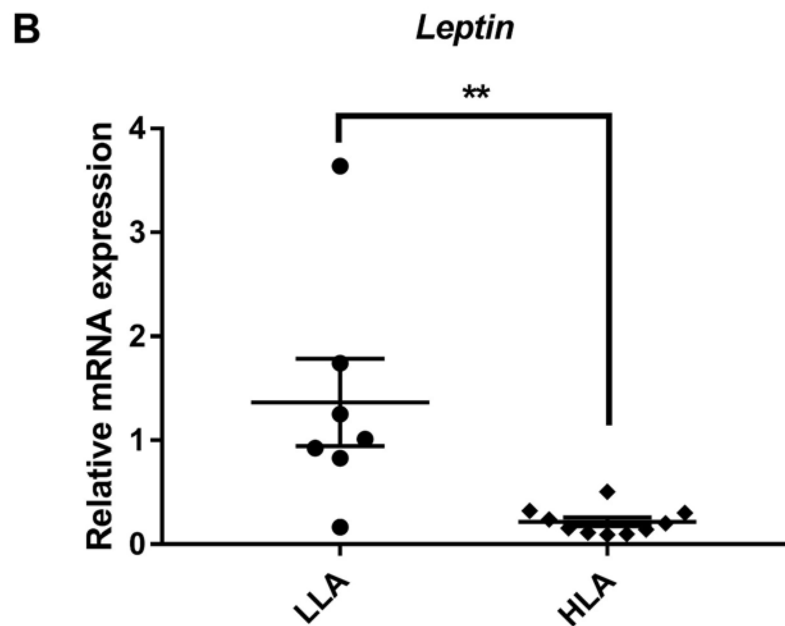

D
Water consumption during gestation
Water consumption (g)
100
80
60
40
20
0
0
5
10
15
20
Days
LLA
HLA

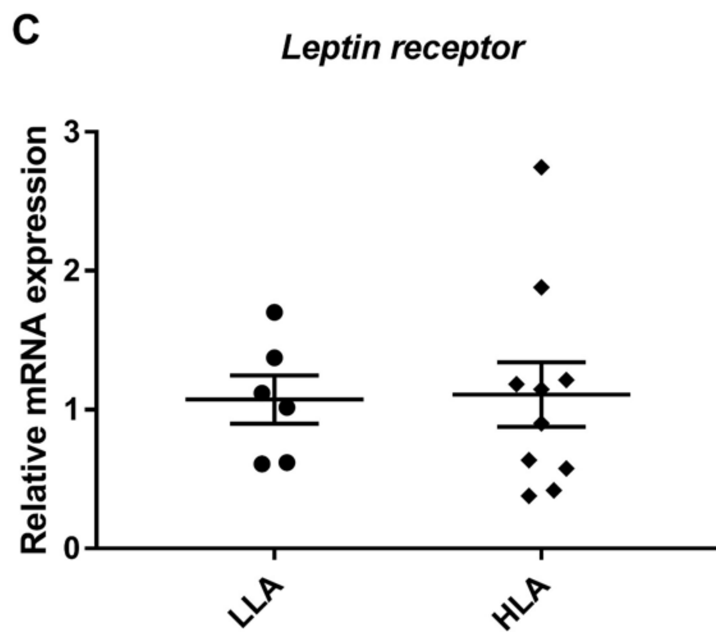

E
Impedance (kohm)
10
8
6
4
2
0
0
2
4
6
8
10
Days
LLA
HLA

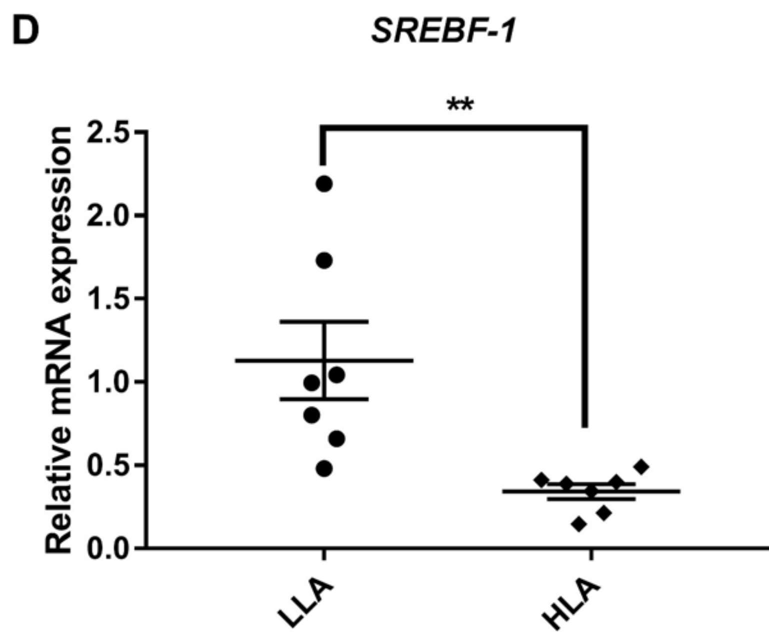


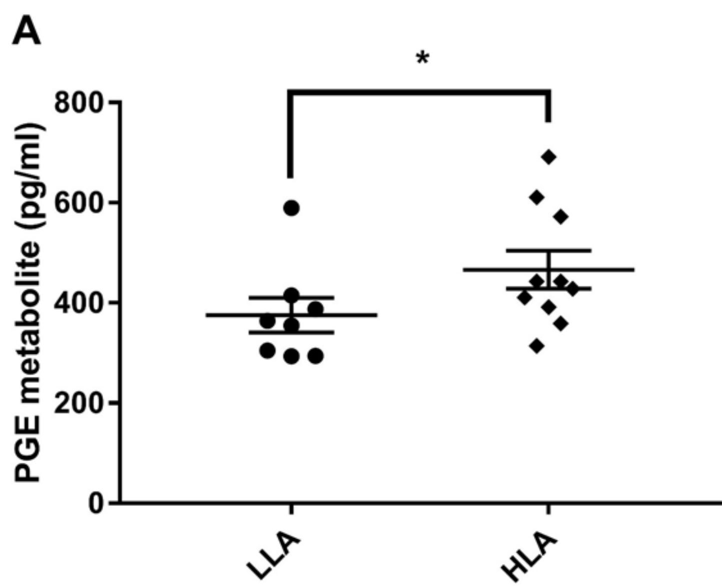
**
Proportion of male fetuses (%)
100
80
60
40
20
0
LLA
HLA

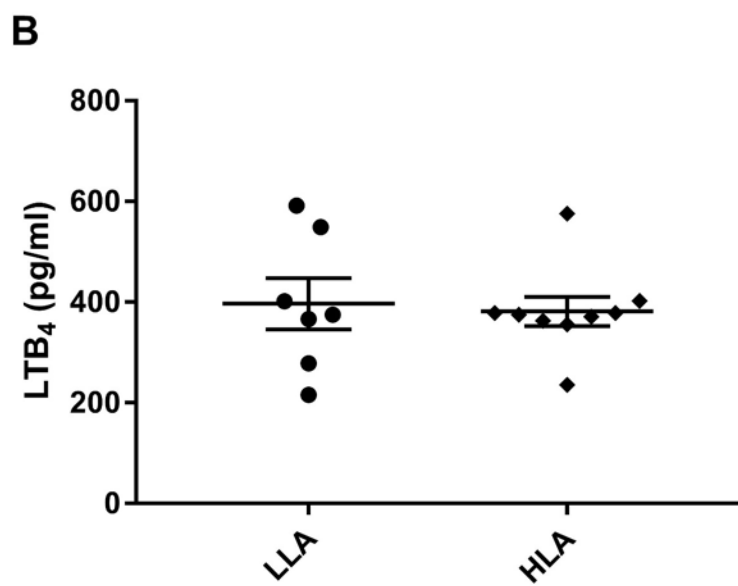

A
Plasma leptin concentration
**
Leptin (ng/ml)
0
2
4
6
8
10
LLA
HLA


B
Leptin
**
Relative mRNA expression
0
1
2
3
4
LLA
HLA


C
Leptin receptor
Relative mRNA expression
0
1
2
3
LLA
HLA


D
SREBF-1
**
Relative mRNA expression
0.0
0.5
1.0
1.5
2.0
2.5
LLA
HLA


A
*
PGE metabolite (pg/ml)
800
600
400
200
0
LLA
HLA


B
LTB4 (pg/ml)
800
600
400
200
0
LLA
HLA