1 **Replacing smoking with vaping during pregnancy: impacts on metabolic health in mice**

2 Gerard Li[1], Yik L Chan[1,2], Baoming Wang[1,2], Sonia Saad[1,3], Brian G Oliver[1,2], Hui Chen[1]

3 [1] School of Life Sciences, Faculty of Science, University of Technology Sydney, Sydney, NSW

4 2007, Australia

5 [2] Respiratory Cellular and Molecular Biology, Woolcock Institute of Medical Research, The

6 University of Sydney, Sydney, NSW 2037, Australia

7 [3] Kolling Institute of Medical Research, Royal North Shore Hospital, The University of

8 Sydney, Sydney, New South Wales 2065

9 *Corresponding author:*

10 Mr Gerard Li

11 School of Life Sciences, Faculty of Science, University of Technology Sydney, PO Box 123,

12 Broadway, NSW 2007, Australia. Gerard.E.Li@student.uts.edu.au

13 Author contributions:

14 Gerard Li: conceptualisation, validation, formal analysis, investigation, writing-original draft

15 Yik L Chan: investigation, writing- review and editing

16 Baoming Wang: investigation, writing-review and editing

17 Sonia Saad: conceptualisation, writing-review and editing

18 Brian G Oliver: conceptualisation, writing-review and editing, funding acquisition

19 Hui Chen: conceptualisation, writing-review and editing, funding acquisition, supervision

20   **Abstract**

21   Smoking is a significant risk factor for the development of metabolic diseases. Due to social

22   pressures to quit smoking, many pregnant women are vaping as an alternative nicotine source.

23   However, the metabolic consequences of replacing tobacco cigarettes with e-cigarettes during

24   pregnancy are unknown. Therefore, in the mothers and their offspring, we investigated the

25   metabolic and hepatic impacts of replacing cigarette smoke with e-vapour during pregnancy.

26   Female BALB/c mice were either air-exposed or cigarette smoke-exposed (SE) from six weeks

27   before pregnancy until lactation. At mating, a subset of the SE mice were instead exposed to

28   e-vapour. Markers of glucose and lipid metabolism were measured in the livers and plasma,

29   from the mothers and their male offspring (13 weeks). In the SE mothers, plasma insulin levels

30   were reduced, leading to downstream increases in hepatic gluconeogenesis and plasma non-

31   esterified fatty acids (NEFA). In the e-vapour replacement mothers, these changes were not as

32   significant. In the SE offspring, there was impaired glucose tolerance, and increased plasma

33   NEFA and liver triglyceride concentrations. E-vapour replacement restored lipid homeostasis

34   but did not improve glucose tolerance. Therefore, e-cigarette replacement during pregnancy in

35   a low dose setting seems to ameliorate the adverse impact of cigarette smoke exposure on

36   maternal and offspring liver metabolic profile in mice; while future research needs to focus on

37   higher doses to verify such effects.

38   Keywords: maternal smoking; e-cigarette; vaping; pregnancy; glucose tolerance; liver steatosis

39 **Abbreviations**

40	ATGL	Adipose triglyceride lipase
41	AUC	Area under the curve
42	CPT1a	Carnitine palmitoyltransferase Ia
43	FASN	Fatty acid synthase
44	FOXO1	Forkhead box protein O1
45	GLUT	Glucose transporter
46	IPGTT	Intraperitoneal glucose tolerance test
47	NEFA	Non-esterified fatty acid
48	PFK	Phosphofructokinase
49	PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
50	PPAR-γ	Peroxisome proliferator-activated receptor gamma
51	SEM	Standard error of the mean

1. Introduction

Type 2 diabetes is a primary global health concern, affecting approximately 400 million individuals worldwide and contributing to 3.7 million deaths each year [1]. Smoking is a significant risk factor, estimated to increase the risk of Type 2 diabetes by 30-40% [2]. Furthermore, smoking during pregnancy restricts intrauterine resources and primes the foetus to develop insulin resistance [3] and hepatic steatosis [4] later in life. Thus, smoking cessation during pregnancy will optimise the health outcome of the next generation [5]. However, smoking cessation can be difficult to achieve, especially since nicotine replacement therapy is mostly ineffective during pregnancy [6].

Driven by health advice to quit smoking, many pregnant smokers switch to e-cigarettes upon learning of their pregnancy [7], especially since vaping is less stigmatised than smoking [8]. E-cigarettes are marketed as a smoking cessation aid, supposedly delivering inhaled nicotine without the harmful by-products of tobacco combustion [4]. The popularisation of replacement vaping is mostly derived from safety perceptions compared to smoking, among pregnant women [8,9] and even some obstetricians [10]. During pregnancy, ever use of e-cigarettes ranges from 13% to 15% [11,12], making them more prevalent than other forms of nicotine replacement therapy [8,13].

While it is clear that vaping is not safe [14], switching from smoking to vaping may be beneficial among long-term smokers [15]. However, there are no reports on the impacts of switching during pregnancy, due to the recent emergence of e-cigarettes on the market. In mouse models, we have previously shown that intrauterine e-vapour exposure during pregnancy altered inflammatory responses in multiple organs (lungs [16], brain [17,18] and kidneys [19]). Furthermore, replacing tobacco cigarettes with e-cigarettes during pregnancy was less harmful to the brains

75 and kidneys compared to continuous cigarette smoke exposure throughout gestation and

76 lactation [18-20].

77 The increased risk of type 2 diabetes due to *in-utero* cigarette smoke exposure makes it

78 essential to investigate the metabolic impacts of e-cigarette replacement during pregnancy. The

79 liver is a major metabolic hub, contributing to systemic glucose and lipid homeostasis which

80 becomes dysregulated in metabolic diseases, such as type 2 diabetes [21]. Using a Balb/c mouse

81 model, we aimed to investigate the impacts of replacing cigarette smoke with e-cigarette

82 vapour during pregnancy on systemic and hepatic metabolic profiles in both the mothers and

83 their offspring.

84 **2. Methods**

85 **2.1. Animals**

86 The animal experiments were approved by the Animal Care and Ethics Committee of the

87 University of Technology Sydney (ACEC2014-638 and ETH15-0025) and performed

88 according to the Australian National Health & Medical Research Council Guide for the Care

89 and Use of Laboratory Animals. Virgin female BALB/c mice (7 weeks old, Animal Resource

90 Centre, WA, Australia) had ad libitum access to standard laboratory chow and water while

91 housed at 20±2 °C and maintained on a 12-h light, 12-h dark cycle (lights on at 06:00 h).

92 Female breeders were acclimatised for a week prior to the exposure treatments detailed below.

93 Female breeders were either room air exposed (Sham group, n=8) or cigarette smoke exposed

94 (SE group, n=16) to 2 cigarettes (Winfield Red, ≤16 mg tar, ≤1.2 mg nicotine, and ≤15 mg of

95 CO; VIC, Australia) twice daily, 6 weeks before mating and throughout gestation and lactation.

96 In a subset of the SE mice, cigarette smoke was replaced with e-vapour generated from

97 commercial e-liquid (50% propylene glycol/50% vegetable glycerine, tobacco flavour, Vaper

98   Empire, VIC, Australia) containing 18mg/mL nicotine (Replacement group, n=8) from mating

99   until the pups were weaned, as previously described [16]. Aerosols were generated by a human-

100 use e-cigarette (KangerTech NEBOX, 30 Watts, 0.5 Ohms, KangerTech, Shenzen, China) as

101 we have previously published in the same model [16]. Offspring plasma cotinine (a major, stable

102 nicotine metabolite) concentrations were measured in previous studies [16,22] and were similar in

103 the SE and Replacement groups. This nicotine dose represents mothers who are light smokers

104 [23].

105 Dams were removed from their home cages and whole-body exposed to cigarette smoke or e-

106 cigarette vapour. Sham dams were placed in identical exposure chambers without any smoke

107 or vapour. Male breeders and pups were not exposed. Male offspring were weaned at postnatal

108 day 20 and maintained without additional intervention. At 12 weeks of age, an intraperitoneal

109 glucose tolerance test (IPGTT) was performed as previously described [24]. After 5 hours of

110 fasting, baseline blood glucose levels were measured followed by glucose injection (2g/kg, IP).

111 Blood glucose was measured at 15, 30, 60, and 90 minutes post-injection. The area under the

112 curve (AUC) of the blood glucose curve was calculated for each mouse. We euthanised dams

113 (at weaning) and male offspring (at 13 weeks old) after deep anaesthesia (2% isoflurane).

114 Livers were harvested, weighed and then either snap frozen and stored at -80°C, or fixed in

115 10% formalin for further analyses. Liver weights (%) were calculated as a fraction of body

116 weight. Blood was collected via cardiac puncture, and glucose levels were measured (Accu-

117 Chek[(R)], Roche, CA, USA). Plasma was separated and stored at -20°C for further analysis.

118 In the offspring, the average body and liver weight data of each litter was calculated before

119 statistical analysis. One male offspring from each litter (n=8) was used for all further

120 experiments.

121 **2.2. Bioassays**

122 Plasma insulin concentration was measured using an Insulin (mouse) ELISA Kit (Abnova,

123 Taiwan) according to the manufacturer's instructions. Samples were analysed in duplicate, and

124 the intra-assay coefficient of variance was below 10%.

125 Liver lipids were extracted using the Folch method [26], as previously described [24]. Plasma, liver

126 extracts and glycerol standards (Sigma-Aldrich, MO, USA) were incubated with

127 triacylglycerol reagent (Roche Diagnostics, Basel, Switzerland) using an in-house assay [24].

128 Plasma nonesterified free fatty acid (NEFA) concentrations were measured using a NEFA kit

129 (WAKO, Osaka, Japan).

130 **2.3. rt-PCR**

131 Total mRNA was extracted from frozen liver tissue with TriZol reagent (Life Technologies,

132 CA, USA) and first strand cDNA was generated using M-MLV Reverse Transcriptase, RNase

133 H, Point Mutant Kit (Promega, WI, USA). Target gene expression was quantified with

134 manufacturer pre-optimised and validated TaqMan primers and probes (Table 1, Thermo

135 Fisher, CA, USA) and standardised to 18s RNA. The probes of the target genes were labelled

136 with FAM and those for housekeeping 18s RNA were labelled with VIC. The average of the

137 Sham group was assigned the calibrator against which all other results were expressed as fold

138 changes.

139 **2.4. Statistical Analysis**

140 Results are expressed as mean ± standard error of the mean (SEM) and were analysed using

141 one-way ANOVA with Fisher's Least Significant post hoc test if the data were normally

142 distributed. If the data were not normally distributed, they were log transformed to achieve

143 normality of distribution before analysis (GraphPad Prism 7.03, CA, USA). $P < 0.05$ was

144 considered the threshold for statistical significance.

145 **Table 1. TaqMan Probe sequence (Life Technologies, CA, USA) used for rt-PCR.**

Gene	NCBI references	Probe Sequence	ID
ATGL	NM_025802.3	CCAAGACTGAATGGCTGGATGGCAA	Mm00503040_m1
CPT1a	NM_013495.2	TTCCAGGAGAATGCCAGGAGGTCAT	Mm01231183_m1
FASN	NM_007988.3	AGCAATTGTGGATGGAGGTATCAAC	Mm00662319_m1
FOXO1	NM_019739.3	TCGGCGGGCTGGAAGAATTCAATTC	Mm00490671_m1
GLUT2	NM_031197.2	CCGCCTCCCCCGGCGCGCACACACC	Mm00446229_m1
GLUT4	NM_009204.2	TGGCTCTGCTGCTGCTGGAACGGGT	Mm00436615_m1
PFK	NM_008826.4	GCGGTGATGCGCAAGGTATGAATGC	Mm00435587_m1
PGC1a	NR_027710.1	CTGGAACTGCAGGCCTAACTCCTCC	Mm01208835_m1
PPAR-γ	NM_0011273330.1	ATGCTGTTATGGGTGAAACTCTGG	Mm01184322_m1

146 *ATGL:* Adipose triglyceride lipase, *CPT1a*: Carnitine palmitoyltransferase 1a, *FASN*: Fatty

147 acid synthase, *FOXO1*: Forkhead box protein O1, *GLUT2:* Glucose transporter 2, *GLUT4*:

148 Glucose transporter 4, *PFK*: phosphofructokinase, *PGC1a*: Peroxisome proliferator-activated

149 receptor gamma coactivator 1-α, *PPAR-γ*: Peroxisome proliferator-activated receptor gamma.

150 **3. Results**

151 **3.1. Dams**

152 After continuous exposure to tobacco cigarette smoke, smoke exposed (SE) dams had lower

153 body weights (P<0.05 vs Sham, Table 2). Liver weights expressed as a percentage of body

154 weight were higher in the SE dams (P<0.05 vs Sham, Table 2). When the cigarette smoke was

155 replaced by nicotine-containing e-vapour (Replacement), the reduction in body weight was

156   partially prevented, but liver weight remained higher when expressed as a percentage of body

157   weight (P<0.05 vs Sham, Table 2).

158   Plasma glucose levels were not different among the groups (Table 2). However, plasma insulin

159   levels in the SE dams were decreased compared to the Sham dams (P<0.05, Table 2). There

160   was an increase in the hepatic expression of glucose metabolic markers in the SE dams,

161   including Glucose Transporter (Glut)4 (P<0.05 vs Sham, Figure 1b), Peroxisome Proliferator-

162   Activated Receptor (PPAR)-γ (P<0.01 vs Sham, Figure 1d), PPARG coactivator (PGC)-1α

163   (P<0.01 vs Sham, Figure 1e) and Forkhead box protein O1 (FOXO1, P<0.05 vs Sham, Figure

164   1f). Plasma insulin levels were reversed in the Replacement dams compared to the SE dams

165   (P<0.01, Table 2). While the expression of Glut4 was increased in the Replacement dams

166   compared to the Sham dams (P<0.05, Figure 1b), the expression of other glucose metabolic

167   markers (PPAR-γ, PGC-1α, and FOXO1) were nearly restored to Sham levels.

168   While there were no differences in plasma triglyceride levels, plasma non-esterified fatty acid

169   (NEFA) concentration was increased in the SE dams (P<0.05 vs Sham, Table 2). Liver

170   triglyceride concentration and lipid metabolic markers, fatty acid synthase (FASN), adipose

171   triglyceride lipase (ATGL) and carnitine palmitoyltransferase 1A (CPT1a) were not

172   significantly changed in the SE dams (Table 2, Figure 1g-i). Plasma NEFA levels in the

173   Replacement dams were nearly restored to Sham levels (Table 2), and liver ATGL expression

174   in the Replacement dams was increased compared to the SE dams (P<0.01, Figure 1h).

175 **Table 2.** Parameters of the dams.

	Sham	SE	P (vs Sham)	Replacement	P (vs Sham)	P (vs SE)
Body weight (g)	26.1±0.38	23.1±0.84	P<0.05	24.9±0.51	NS	NS
Liver weight (g)	1.55 ± 0.07	1.53±0.06	NS	1.68±0.11	NS	NS
Liver weight (%)	5.93±0.22	6.62±0.13	P<0.05	6.74±0.32	P<0.05	NS
Blood glucose (mM)	9.42 ± 0.83	8.43 ±0.70	NS	9.98±0.43	NS	NS
Plasma insulin (ng/mL)	0.70 ±0.05	0.50 ± 0.01	P<0.05	0.88 ± 0.11	NS	P<0.01
Liver triglyceride (mg/g liver)	4.0±0.57	4.1±0.71	NS	3.7±0.34	NS	NS
Plasma triglyceride (mg/mL)	1.22±0.21	1.01±0.26	NS	0.93±0.21	NS	NS
Plasma NEFA (mEq/L)	2.1 ± 0.28	3.65 ± 0.32	P<0.05	2.91 ± 0.23	NS	NS

176 Results are expressed as Mean ± SEM, n=8. Data were analysed by one-way ANOVA with Fishers LSD post hoc tests. *P<0.05 vs Sham, ##P<0.01

177 vs SE. NEFA: non-esterified fatty acid; NS: not significant; Replacement: e-vapour replacing SE during gestation; SE: cigarette smoke exposure.

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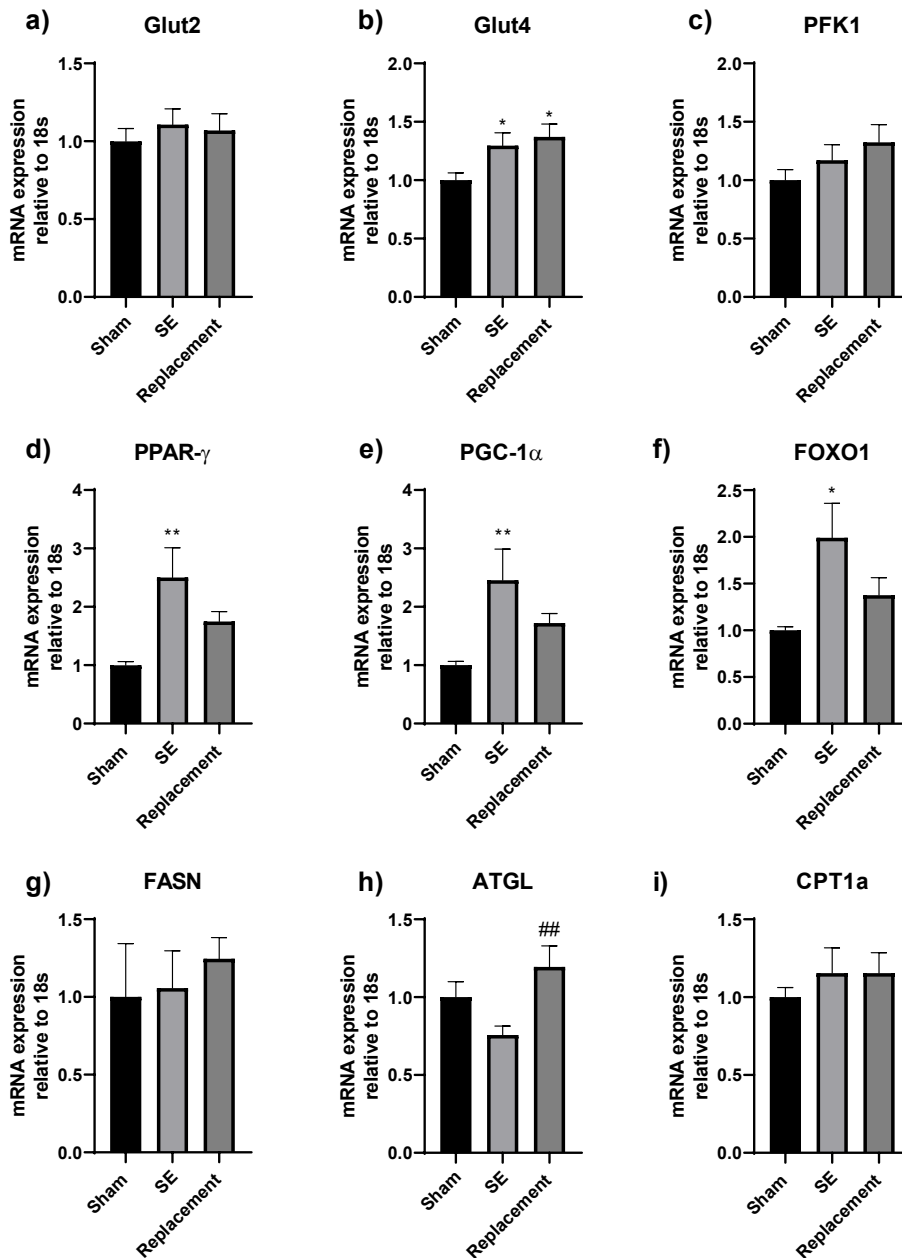
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180   **Figure 1.** Hepatic mRNA expression of glucose metabolic markers (Glut2 (a), Glut4 (b), PFK1

181   (c), PPAR-γ (d), PGC-1α (e), FOXO1 (f)) and lipid metabolic markers (FASN (g), ATGL (h),

182   CPT1a (i)) in the dams. Results are expressed as Mean ± SEM, n=6. Data were analysed by

183   one-way ANOVA with Fishers LSD post hoc tests. *P<0.05, **P<0.01 vs Sham, ##P<0.01 vs

184   SE. Glut: glucose transporter; PFK: Phosphofructokinase; PPAR-γ: Peroxisome proliferator-

185 activated receptor gamma; Peroxisome proliferator-activated receptor gamma coactivator 1-

186 alpha; FOXO1: Forkhead box protein O1; FASN: Fatty acid synthase; ATGL: Adipose

187 triglyceride lipase; CPT1a: Carnitine palmitoyltransferase I; SE: cigarette smoke exposure;

188 Replacement: e-vapour replacing SE during gestation.

3.2. Male offspring (13 weeks old)

190 Adult SE offspring had lower body weights and liver weights ($P<0.01$ vs Sham, Table 3). In

191 contrast, the Replacement offspring had no changes in body ($P<0.01$ vs SE, Table 3) and liver

192 weights ($P<0.05$ vs SE, Table 3).

193 The AUC for the IPGTT was increased in the SE offspring ($P<0.05$ vs Sham, Table 3), which

194 is consistent with our previous studies [20,27]. However, there were no changes in fasting blood

195 glucose or plasma insulin levels in the SE offspring. Furthermore, there were no changes in the

196 mRNA expression of glucose metabolic markers, including Glut2, Glut4, PFK, PPAR-γ, PGC-

197 1α ($P=0.056$), and FOXO1 compared to the Sham offspring (Figure 2a-f). Glucose metabolism

198 was impaired (increased AUC of the IPGTT) in the Replacement offspring ($P<0.01$ vs Sham,

199 $P=0.071$ vs SE, Table 3). No changes were found in fasting blood glucose and plasma insulin

200 levels. The gluconeogenesis regulator, FOXO1, was significantly increased compared to the

201 Sham and SE offspring (both $P<0.05$, Table 3, Figure 2f).

202 Liver triglyceride concentrations were increased in the SE offspring ($P<0.05$ vs Sham, Table

203 3), without any changes in plasma triglyceride concentrations. However, plasma NEFA

204 concentrations were increased in the SE offspring ($P<0.01$ vs Sham, Table 3). SE offspring

205 exhibited no changes in the mRNA expression of hepatic lipid metabolic markers, including

206 FASN, ATGL, and CPT1a compared to the Sham offspring (Figure 2g-i). Increased liver

207 triglyceride and plasma NEFA concentrations in the SE offspring were not observed in the

208 Replacement offspring ($P<0.01$ vs SE, Table 3). In the Replacement offspring, mRNA

209 expression of FASN was similar to the Sham offspring level (P<0.05 vs SE offspring, Figure

210 2g). Replacement offspring had increased hepatic expression of ATGL (P<0.01, Figure 2h) and

211 CPT1a (P<0.05, Figure 2i) compared to both Sham and SE offspring.

212 **Table 3.** Parameters of the offspring

	Sham	SE	P (vs Sham)	Replacement	P (vs Sham)	P (vs SE)
Body weight (g)	26.4±0.61	24.42±0.29	P<0.01	25.92±0.29	NS	P<0.01
Liver weight (g)	1.34±0.06	1.077±0.02	P<0.01	1.23±0.03	NS	P<0.05
Liver weight (%)	5.06±0.16	4.40±0.09	P<0.01	4.75±0.11	NS	P<0.05
IPGTT AUC (mM•min)	1146±20	1281±41	P<0.05	1435±69	P<0.01	NS
Blood glucose (mM)	12.58±0.45	11.12±0.44	NS	11.92±0.57	NS	NS
Plasma insulin (ng/mL)	0.50±0.015	0.51±0.016	NS	0.51±0.017	NS	NS
Liver triglyceride (mg/g liver)	3.92±0.45	5.26±0.39	P<0.05	3.65±0.50	NS	P<0.01
Plasma triglyceride (mg/mL)	1.41±0.11	1.52±0.25	NS	1.31±0.09	NS	NS
Plasma NEFA (mEq/L)	4.13±0.47	7.6±0.88	P<0.01	4.42±0.45	NS	P<0.01

213 Results are expressed as Mean ± SEM, n=8. Data were analysed by one-way ANOVA with Fishers LSD post hoc tests. *P<0.05, **P<0.01 vs

214 Sham, #P<0.05, ##P<0.01 vs Replacement. AUC: area under the curve; IPGTT: intraperitoneal glucose tolerance test; NEFA: non-esterified fatty

215 acid; NS: not significant; Replacement: e-vapour replacing SE during gestation; SE: cigarette smoke exposure.
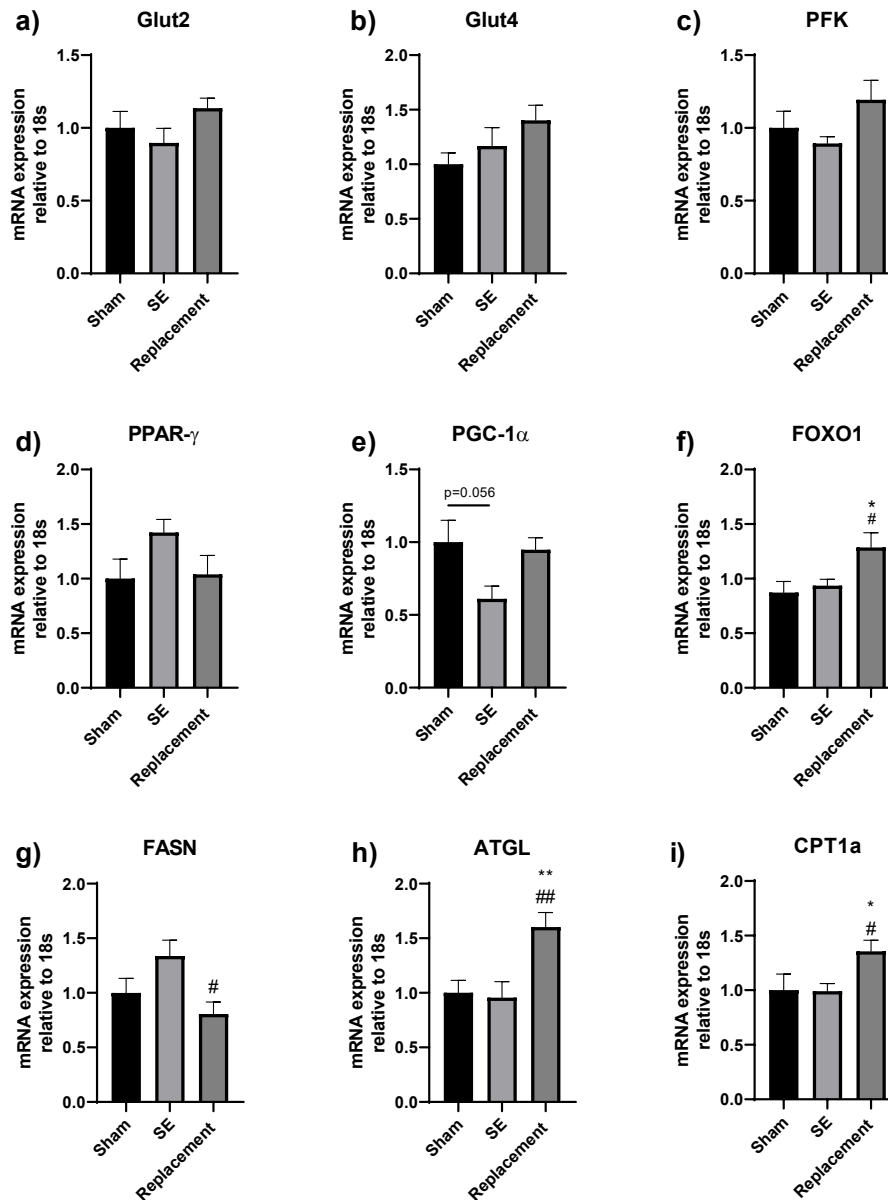
Figure 2. Hepatic mRNA expression of glucose metabolic markers (Glut2 (a), Glut4 (b), PFK1 (c), PPAR-γ (d), PGC-1α (e), FOXO1 (f)) and lipid metabolic markers (FASN (g), ATGL (h), CPT1a (i)) in the male offspring at 13 weeks. Results are expressed as Mean ± SEM, n=6. Data were analysed by one-way ANOVA with Fishers LSD post hoc tests. *P<0.05, **P<0.01 vs Sham, #P<0.05, ##P<0.01 vs SE. Glut: glucose transporter; PFK: Phosphofructokinase; PPAR-γ: Peroxisome proliferator-activated receptor gamma; Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; FOXO1: Forkhead box protein O1; FASN: Fatty acid

224 synthase; ATGL: Adipose triglyceride lipase; CPT1a: Carnitine palmitoyltransferase I; SE:

225 cigarette smoke exposure; Replacement: e-vapour replacing SE during gestation

4. Discussion

227 E-cigarettes are marketed to smokers as a cessation aid or alternative nicotine source. As a

228 result, many smokers switch to vaping during pregnancy due to the stigmatisation of smoking

229 during pregnancy [8], even though the impacts on glucose and lipid metabolism are unknown. In

230 this study, we found that cigarette smoke exposure during pregnancy affects circulating insulin

231 and NEFA levels in the dams and caused glucose intolerance and increased circulating NEFA

232 levels and liver triglyceride concentrations in the offspring. Meanwhile, switching to vaping

233 during pregnancy seems to benefit the dams but did not improve glucose intolerance in the

234 offspring.

235 Here, we confirm the negative impacts of cigarette smoke exposure on glucose and lipid

236 metabolism in the dams, which mostly did not occur in the e-vapour replacement group. Direct

237 exposure to cigarette smoke resulted in a decrease in plasma insulin concentrations, consistent

238 with the adverse impact of smoking on β-cell function [28]. Reduced insulin signalling usually

239 increases PGC-1α, which promotes hepatic gluconeogenesis through the activation of the

240 transcription factor FOXO1 [29]. In addition, reduced insulin signalling can increase lipolysis in

241 adipose tissue, resulting in elevated plasma NEFA concentrations [30], which we observed in the

242 SE dams. Therefore, direct exposure to tobacco cigarette smoke can result in insulin deficiency,

243 leading to downstream increases in gluconeogenesis and lipolysis, causing plasma NEFA to

244 increase.

245 However, when tobacco cigarette smoke was replaced by e-vapour, plasma insulin

246 concentrations were restored, with normalised hepatic gluconeogenesis and plasma NEFA

247   concentrations. In human smokers, e-cigarette replacement has been shown to improve their

248   lung function, oral health and cardiovascular outcomes [15,31]. Therefore, replacing e-cigarettes

249   with tobacco cigarettes may benefit the regulation of hepatic glucose and lipid metabolism in

250   the direct user.

251   Previously, we found that replacing cigarette smoke with nicotine-containing e-vapour during

252   pregnancy can normalise brain metabolic regulators in the offspring [18]. However, the metabolic

253   impacts in the offspring are unknown. Intrauterine exposure to cigarette smoke impaired

254   glucose tolerance in adult offspring, which is consistent with our previous studies and effects

255   in humans [27,32]. Increased liver triglyceride concentration and plasma NEFA concentrations

256   were also increased in the SE offspring, which is commonly associated with low birth weight

257   [33], a major effect of maternal smoking [4]. However, only the main metabolic regulator PGC1α

258   was reduced in the SE offspring, which may account for glucose intolerance, increased plasma

259   NEFA and liver triglyceride accumulation. Although plasma insulin concentration and liver

260   gluconeogenesis marker, FOXO1, were not changed, we cannot rule out the possibility of

261   impaired insulin release in response to a postprandial glucose surge.

262   Meanwhile, in the Replacement offspring, hepatic triglyceride and plasma NEFA

263   concentrations were restored to normal levels. This was likely due to decreased de-novo

264   lipogenesis (normalised FASN expression) and increased lipolysis and fatty acid β-oxidation

265   (increased ATGL and CPT-1α expression) within the liver. However, glucose intolerance was

266   not improved in the Replacement offspring. Thus, replacing tobacco cigarettes with e-cigarettes

267   during pregnancy restored hepatic lipid metabolism, but did not reduce the risk of type 2

268   diabetes in the offspring. E-cigarette vapour contains toxins in lower quantities than cigarette

269   smoke, likely leading to reduced inflammatory responses [34]. Therefore, it is not surprising that

270   e-cigarette replacement during pregnancy was not as detrimental as tobacco cigarette smoke.

271   This is the first study to report the metabolic consequences of intrauterine e-vapour exposure,

272   but there are some limitations. Since only male offspring were used in this study, the impacts

273   on female offspring are unknown. We also used a whole-body exposure protocol which may

274   result in oral exposure through grooming. Although we report differences in mRNA expression

275   in this study, the impact at the protein level is unknown and should be investigated in future

276   studies. This study used a low exposure regime (nicotine exposure equivalent to light smokers),

277   and future studies should investigate the impact of higher doses of both cigarette smoke and e-

278   vapour. Furthermore, this study did not examine the impacts of complete smoking cessation

279   during pregnancy, which may provide an additional benefit compared to e-cigarette

280   replacement.

281   In conclusion, replacing tobacco cigarette smoke with e-vapour benefited maternal metabolic

282   outcomes. In the offspring, e-cigarette replacement improved lipid metabolism but not glucose

283   homeostasis. Therefore, e-cigarettes may be an alternative nicotine source among pregnant

284   women who are unable to quit smoking by other means. However, e-cigarette vaping still has

285   other health risks, which was highlighted by the recent vaping associated deaths in the US [14].

286   Furthermore, other issues must also be considered regarding vaping, including dual-use and

287   youth uptake [35].

Competing interests

299   The authors declare that they have no conflicts of interest.

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