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1 **L-carnitine reverses maternal cigarette smoke exposure induced renal**
2 **oxidative stress and mitochondrial dysfunction in mouse offspring**

3

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25 **ABSTRACT**

26 Maternal smoking is associated with metabolic disorders, renal underdevelopment and a predisposi-
27 tion to chronic kidney disease in the offspring, yet the underlying mechanisms are unclear. By ex-
28 posing female Balb/c mice to cigarette smoke for 6 weeks pre-mating, during gestation and lactation,
29 we showed that maternal smoke exposure induced glucose intolerance, renal underdevelopment, in-
30 flammation and albuminuria in male offspring. This was associated with increased renal oxidative
31 stress and mitochondrial dysfunction at birth and in adulthood. Importantly, we demonstrated that
32 dietary supplementation of L-carnitine, an amino acid shown to increase antioxidant defenses and
33 mitochondrial function in numerous diseases, in smoke exposed mothers during pregnancy and lacta-
34 tion significantly reversed the detrimental maternal impacts on kidney pathology in these male off-
35 spring. It increased superoxide dismutase (SOD)2 and glutathione peroxidase (GPx)1, reduced reac-
36 tive oxygen species (ROS) accumulation, and normalized levels of mitochondrial preprotein trans-
37 locases of the outer membrane (TOM20), and oxidative phosphorylation (OXPHOS) complex I–V in
38 the kidney of the mouse progeny following intrauterine cigarette smoke exposure. These findings
39 support the hypothesis that oxidative stress and mitochondrial dysfunction are closely linked to the
40 adverse effects of maternal smoking on male offspring renal pathology. **Our studies suggest**
41 **L-carnitine administration in cigarette smoke exposed mothers mitigates these deleterious renal con-**
42 **sequences.**

43

44 **Key words:** Chronic kidney disease, Reactive oxidative species, mitochondria.

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52 **INTRODUCTION**

53

54 Maternal cigarette smoking during pregnancy is a well-recognized causative factor for intrauterine
55 growth retardation (2), associated with the underdevelopment of fetal/neonatal tissues (24), including
56 kidney (29). We have recently demonstrated that maternal cigarette smoke exposure (SE) decreased
57 kidney weight, delayed nephron formation and maturation, as well as increased urinary albu-
58 min/creatinine ratio at adulthood in the male offspring (1). With the underlying mechanisms not fully
59 understood, we hypothesized that increased oxidative stress and mitochondrial dysfunction are close-
60 ly involved in these adverse kidney outcomes.

61

62 Living organisms are constantly exposed to oxidants from endogenous metabolic processes, such as
63 reactive oxygen species (ROS), a group of oxygen-derived byproducts released during mitochondrial
64 oxidative phosphorylation (OXPHOS) to generate ATP. Oxidative stress occurs when the intracellu-
65 lar antioxidants are unable to counteract the overproduction of ROS, leading to irreversible oxidative
66 modifications to all cellular components, including lipid, protein and DNA, thus affecting cell struc-
67 ture, function, and viability (26). Smoking has been regarded as a major cause of elevated oxidative
68 stress in active and passive smokers (16). Maternal smoking during pregnancy can not only induce
69 severe oxidative stress in the mother, but also the offspring (11, 25), due to the diffusion of free radi-
70 cals and harmful chemicals within cigarette smoke (e.g. nicotine) through the blood-placental barrier
71 into the fetus (18), This impact, however, has been only scarcely studied in neonatal plasma and
72 urine, and rarely in neonatal organs (such as kidney). We hypothesized that maternal cigarette smoke
73 exposure can increase oxidative stress in new born kidneys, which persists until adulthood.

74

75 As the major source of ROS, mitochondrion is the most affected organelle by oxidative stress. As the
76 cellular power house, impaired mitochondria can fatally imperil energy metabolism and cell viability

77 (26). Therefore, oxidative stress associated mitochondrial damage and dysfunction have been impli-
78 cated in a number of diseases such as type 2 diabetes (20), cancer and neurodegenerative disease (9).
79 Importantly, such oxidative damage is likely to result in permanent modifications in mitochondrial
80 DNA (mtDNA), which can be maternally inheritable. This potentially increases the risk of these dis-
81 orders being transmitted to the progeny. Oxidative damage to mtDNA has been found in fetuses and
82 infants whose mothers were exposed to cigarette smoke or nicotine during pregnancy (4, 25), sug-
83 gesting a possible impact of maternal smoking on mitochondrial function in the offspring. However
84 the effect on kidney function has not yet been explored.

85

86 Carnitine, mainly synthesized in the liver and kidney, is essential for mitochondrial fatty acid metab-
87 olism (21). Supplementation of L-carnitine, the active form of carnitine, and its derivatives have
88 been shown to attenuate oxidative stress and mitochondrial dysfunction in diverse conditions, such
89 as age-related disorders and chronic heart failure (15, 19). In patients with end-stage kidney disease
90 requiring dialysis, L-carnitine therapy has been shown to restore plasma antioxidant/oxidant homeo-
91 stasis (13). However, as in most studies in patients with end stage kidney disease, well established
92 pathology is unlikely to be reversed, independent of the inciting mechanism. This study aimed to ex-
93 amine the utility of maternal L-carnitine supplementation post conception to reverse or ameliorate
94 maternal SE-induced renal oxidative stress and mitochondrial dysfunction in male offspring.

95

96 **MATERIALS AND METHODS**

97

98 **Animal experiments**

99 Female Balb/c mice (8 weeks) were divided into three groups: Sham (exposed to air), SE (cigarette
100 smoke exposure, 2 cigarettes twice daily, 6 weeks before mating, throughout gestation and lactation,
101 previous described (1)); SE + LC (SE mothers supplied with L-carnitine (1.5mM in drinking water)
102 during gestation and lactation). L-carnitine dose and administration were adapted from a previous

103 study (28). Male breeders and suckling pups stayed in the home cage when the mothers were sham
104 or cigarette smoke exposed. All the offspring studied were males. Offspring were sacrificed at post-
105 natal day 1 (P1), weaning age (P20), and mature age (Week 13). Intra-peritoneal Glucose Tolerance
106 Test (IPGTT) was performed at week 12 as previous described (6). Blood, urine, and kidneys were
107 collected for further analysis.

108

109 **Kidney histology**

110 Kidney samples from the male offspring were embedded in paraffin and sectioned in 2 μ m slices.
111 Kidney structure was examined using hematoxylin and eosin (H&E) and periodic acid Schiff stain
112 (PAS). Glomerular number and size were quantitated as per our previous protocol (1).

113

114 **Real-time PCR**

115 Kidney total RNA was extracted and purified using TRIzol Reagents (Life Technology, CA, USA),
116 from which cDNA was synthesized using Transcriptor First Strand cDNA Synthesis Kit (Roche Di-
117 agnostics, Mannheim, Germany). Real-time PCR was performed using pre-optimized SYBR Green
118 primers (Sigma-Aldrich) and rt-PCR master mix (Life Technology, CA, USA) to assess the mRNA
119 expression level of macrophage chemoattractant protein (MCP-1) in the kidney, with 18S rRNA as
120 the housekeeping gene.

121

122 **ELISA**

123 The levels of urinary albumin and creatinine, serum insulin and cotinine were measured using Mu-
124 rine Microalbuminuria ELISA kit (Albuwell M, PA, USA), Creatinine Companion Kit (Exocell Inc,
125 PA, USA), Insulin (Mouse) ELISA Kit (Abnova, Taipei, Taiwan), and cotinine ELISA Kit (Abnova,
126 Taipei, Taiwan) respectively as per manufacturer's instructions.

127

128 **Western Blot analysis SOD assay**

129 Frozen kidneys were homogenized in HEPES buffer (20 mM, pH 7.2, containing 1 mM EGTA, 210
130 mM mannitol, 70 mM sucrose). The homogenate was centrifuged to isolate cytosolic and mitochon-
131 drial fractions. Protein concentrations were determined and stored at -80°C for further analysis.

132

133 Proteins were electrophoresed and electro-blotted to Hybond nitrocellulose membranes (Amersham
134 Pharmacia Biotech, New Jersey, USA). The membrane was incubated one of the primary antibodies:
135 anti- β -actin (Santa Cruz Biotechnology, California, USA); goat anti-GPx-1 (R&D System, Minne-
136 apolis, USA); rabbit anti-MnSOD (Millipore, Massachusetts, USA); rabbit anti-TOM20 (Santa Cruz
137 Biotechnology); and mouse anti-OXPHOS complex I-V cocktail (Abcam, Cambridge, UK), and
138 then a horseradish peroxidase (HRP)-conjugated secondary antibody. The blots were developed with
139 Luminata Western HRP Substrates (Millipore) by ImageQuantTM LAS 4000 (Fujifilm, Tokyo, Ja-
140 pan). The membrane was restored by stripping buffer (Thermo Scientific) afterward. ImageJ (Na-
141 tional Institutes of Health) was used for densitometry, and β -actin was used as the house-keeping
142 protein.

143

144 Superoxide Dismutase Assay Kit II (Millipore) was used to measure mitochondrial SOD activity in
145 the isolated mitochondrial proteins according to manufacturer's instruction.

146

147 **Confocal microscopy**

148 For ROS detection, CellROX Deep Red (5 μ M, Molecular Probes, Australia) was used, and images
149 were collected at 633 nm excitation wavelength and detected in the 640-680 nm emission range. Mi-
150 toTracker Green FM (200nM, Molecular Probes, Australia) was used to visualize the mitochondria,
151 and images were collected at 458 nm excitation wavelength and detected in the 480-505nm emission
152 range. Multiple images were taken for over 100 cells in each tissue in 3 replicates of three independ-
153 ent samples/ each group. Morphological features were quantified using a confocal laser scanning
154 microscope (Leica TCS SP2 X; Leica, Wetzlar, Germany). All imaging parameters including laser

155 intensities, Photomultiplier tubes voltage, pinhole were kept constant during imaging. The tissue
156 segmentation method was used for data analysis as described by Bagett et al. (3) and confirmed
157 using a common threshold units for all the images. Data was expressed as mean fluorescent intensity.
158 To calculate the correlation between CellROX and Mitotracker, dual staining using CellRox and
159 Mitotracker was performed and images were taken sequentially using separate confocal channels
160 over a time not greater than 30 seconds. The image pixel intensity value correlation was then
161 calculated using Pearson's correlation for all pixels excluding any pairs containing zero values.

162

163 **Statistical analysis**

164 One-way ANOVA followed by Fisher Least Significant Difference post hoc tests was used to deter-
165 mine the difference between the groups (Prism 6, GraphPad). Data are expressed as mean \pm SEM. *P*
166 < 0.05 was considered as statistically significant.

167

168 **RESULTS**

169

170 **Maternal L-carnitine (LC) supplementation normalized birth weight and kidney weight in** 171 **smoke exposed (SE) offspring**

172 Body weight and kidney mass were significantly reduced in the SE offspring at birth (P1; *P* < 0.05 ,
173 Table 1). This is consistent with human studies (7, 29), and supports the relevance of this mouse
174 model for studying the effect of maternal SE on renal disorders in the offspring. L-carnitine supple-
175 mentation reversed the phenotype of low birth weight and kidney weight in P1 SE offspring to the
176 control levels (SE + LC group, Table 1).

177

178 **Maternal L-carnitine supplementation normalized renal phenotype and glucose tolerance in** 179 **the SE offspring**

180 There were significant delays in the kidney development in the SE offspring until adulthood. The
181 average number of glomeruli was approximately half of the Control ($P < 0.05$), while glomerular
182 size was increased at weaning ($P < 0.05$) but reduced in adulthood ($P < 0.01$) (Figure 1A, B). This
183 was associated with a significant increase in renal MCP-1 mRNA expression in the SE offspring (P
184 < 0.01 , Figure 1C). Urinary albumin/creatinine ratio (ACR) was also significantly higher in the SE
185 group at week 13. In addition, offspring from SE mothers had normal serum insulin levels (Table 2),
186 but were glucose intolerant (Figure 1D), suggestive of impaired pancreatic insulin secretion.

187

188 In contrast, SE offspring of L-carnitine treated mothers showed an improvement in histological and
189 metabolic parameters. Glomerular number and size were normalised (Figure 1A, B); and glucose
190 tolerance returned to the level of the Control group (Figure 1C, D). Renal MCP-1 mRNA expression
191 and urinary albumin/creatinine ratio was no longer significantly different to that observed in the con-
192 trol animals. Serum cotinine levels were increased in both SE and SE+LC groups, confirming ciga-
193 rette smoke exposure (Table 2).

194

195 **Maternal L-carnitine supplementation alleviated renal oxidative stress in the SE offspring**

196 Manganese Superoxide dismutase (MnSOD) and Glutathione Peroxidase (GPx)1 were measured as
197 representative markers for antioxidative defense, as each is involved in one of the two-step ROS
198 converting reaction ($O_2^- \rightarrow H_2O_2 \rightarrow H_2O + O_2$). In addition, both mitochondrial (mt-) and cytosolic
199 (ct-) fractions were measured to determine which fraction is more susceptible to damage, and wheth-
200 er the changes are due to altered gene expression or protein translocation between cytoplasm and mi-
201 tochondria. At P1, all the measured renal antioxidant markers including mt-MnSOD, ct-MnSOD, mt-
202 GPx-1 and ct-GPx-1 were significantly reduced in the SE offspring by 40% ($P < 0.01$), 50%, 60%
203 and 70% ($P < 0.001$) respectively, suggesting a broad adverse effect of maternal SE on renal antioxi-
204 dant capacity (Figure 2A). However, only ct-MnSOD ($P < 0.05$, Figure 2C) and mt-MnSOD
205 ($P < 0.05$, Figure 2E) were significantly lower than the control levels at P20 and week 13 respectively,

206 without any changes of GPx-1 at either time point, suggesting renal oxidative stress by maternal SE
207 was partially improved as smoke exposure became more remote. The antioxidant activity of renal
208 mt-SOD in the SE offspring was also significantly reduced at P1 ($P < 0.05$, Figure 2B) and Week 13
209 ($P < 0.01$ Figure 2F), but not at P20 (Figure 2D), confirming the impaired mitochondrial ability of
210 ROS clearance in the SE offspring's kidneys at birth and adulthood. L-carnitine treatment signifi-
211 cantly attenuated the reduction of renal MnSOD and GPx-1, in both cytosolic and mitochondrial
212 fractions at P1 ($P < 0.05$, Figure 2A), as well as mt-MnSOD at week 13 ($P < 0.05$, Figure 2E) in the
213 SE offspring. Similarly, it also reversed renal mt-SOD activities at both P1 ($P < 0.01$, Figure 2B),
214 and week 13 ($P < 0.05$, Figure 2F). However, in P20 offspring L-carnitine showed no significant ef-
215 fect (Figure 2C, 2D).

216

217 The levels of total and mitochondrial ROS were measured as markers of oxidative stress. Kidney tis-
218 sues were stained with cell-ROX Red and Mitotracker to identify ROS production and localization.
219 There were marked elevations of renal ROS at week 13 ($P < 0.001$, Figure 3A), which were con-
220 sistent with the observed reductions in MnSOD/GPx-1 expression and activity. The results reflect a
221 dysregulation of renal redox homeostasis in the offspring due to maternal SE. Furthermore, the cor-
222 relation coefficient of cell-ROX Red and Mitotracker was significantly higher in the kidney of the
223 SE offspring at P1 and week 13 ($P < 0.01$, Figure 3B), suggesting that the majority of excessive ROS
224 is likely derived from the mitochondria. Interestingly, maternal SE had no effect on renal ROS or
225 mitochondrial ROS at P20 (Figure 3A and B). Renal mitochondrial ROS level was significantly re-
226 duced by L-carnitine in the SE offspring at P1 and week 13 ($P < 0.001$, Figure 3C). Interestingly, L-
227 carnitine significantly reduced total ROS at P1, P20 and week 13 ($P < 0.01$) and mitochondrial ROS
228 at P1 and week 13 compared to control ($P < 0.001$ and $P < 0.05$ respectively) (Figure 3C).

229

230 **Maternal L-carnitine supplementation reversed renal mitochondrial dysfunction in the SE off-**
231 **spring**

232 To investigate mitochondrial function, we assessed TOM20, a mitochondrial outer membrane recep-
233 tor for translocation of cytosolically synthesized mitochondrial preproteins, and OXPHOS complex-
234 es I – V, the key components of mitochondrial respiratory chain for ATP synthesis. Renal protein
235 levels of TOM20 and OXPHOS Complex I, III, and V were significantly reduced in the SE offspring
236 at P1 ($P < 0.05$, Figure 4A), suggesting impaired mitochondrial protein and ATP synthesis. These
237 markers were restored by P20 (Figure 4B), but again reduced at week 13 (Figure 4C), mirroring the
238 changes of renal mt-SOD in the SE offspring. Maternal L-carnitine supplementation significantly
239 restored renal levels of mitochondrial TOM20, Complex I, II, III, and V at P1 (Figure 4A). However,
240 no impact was observed at P20 (Figure 4B). At week 13, offspring from L-carnitine treated SE
241 mothers had normalized TOM20, Complex I, II, and V ($P < 0.01$, Figure 4C), suggesting a long-term
242 effect of L-carnitine to prevent mitochondrial dysfunction by maternal SE.

243

244 **DISCUSSION**

245 We have previously determined that maternal SE prior to, during gestation and lactation induces re-
246 nal underdevelopment and impaired function in male offspring, although no significant glomerular
247 structural changes and interstitial abnormalities were detected (1). In this study we demonstrated that
248 maternal SE can significantly increase renal oxidative stress and impair mitochondrial function in the
249 offspring at birth and adulthood. Supplementation of L-carnitine from gestation and throughout lac-
250 tation can effectively restore renal oxidative homeostasis and mitochondrial function in the SE off-
251 spring, as well as intrauterine growth retardation.

252

253 In this study, SE offspring had reduced body weight and kidney weight at birth, which is consistent
254 with human epidemiology studies (5). In addition, maternal smoke exposure induced glucose intoler-
255 ance and albuminuria in the offspring from SE mothers. Moreover, SE offspring showed reduced
256 renal levels of MnSOD and GPx-1, two vital enzymes for intracellular antioxidant defense, especial-
257 ly within the mitochondria. Encoded by genomic DNA, MnSOD is uniquely activated in mitochon-

258 dria and is the only mitochondrial enzyme known to convert O_2^- into H_2O_2 , resulting in ROS dispos-
259 al (27). As such, alternations in MnSOD quantity and activity can directly affect mitochondrial anti-
260 oxidant capacity. Unlike MnSOD, GPx-1 functions to convert H_2O_2 into H_2O and O_2 can also be
261 modulated by several other enzymes such as catalase or peroxiredoxin. However, the reduction of
262 GPx-1 in these studies is evidence of impaired renal antioxidant capacity in the offspring by mater-
263 nal SE. In addition, impaired mitochondrial SOD activities and ROS accumulation provided direct
264 evidence for increased renal oxidative stress due to oxidant/antioxidant imbalance in the SE off-
265 spring.

266 The reduction of mitochondrial functional proteins, including TOM20 and OXPHOS respiratory
267 units correlated with increased oxidative stress. In addition, most of the excessive ROS produced
268 were derived from the mitochondria, as determined by dual-staining of ROS and Mitotracker. This
269 suggests an important interplay between redox imbalance and mitochondrial dysfunction in the ef-
270 fector mechanisms of intrauterine SE on the offspring kidney. It is well-established that increased
271 oxidative stress can impair mitochondrial integrity (26), resulting in impaired mitochondrial prepro-
272 tein import (30), and poor energy metabolism (8). Conversely, mitochondrial dysfunction, such as
273 defects in ATP exportation (12), and/or antioxidant importation may lead to an escalation of oxida-
274 tive stress. This is supported by the reduction of both mt-MnSOD and TOM20 in SE offspring kid-
275 ney at both P1 and Week 13. As the result of this dual effect, a cycle of oxidative stress and mito-
276 chondrial damage/dysfunction is hypothesized in the SE offspring kidney, which might significantly
277 contribute to kidney underdevelopment and/or the onset/progression of renal-related disorders.

278

279 It is surprising that increased renal oxidative stress and mitochondrial dysfunction were detected in
280 the SE offspring both at birth and adulthood yet it was mitigated at weaning. The mechanism of this
281 temporary recovery is unclear, and we can only postulate that it may be due to the protective effect
282 of breast milk, which has been shown to be rich in antioxidants (31). However, this protection was
283 not sustained until adulthood. The persistent impact of maternal SE suggests that the alteration may

284 be related to epigenetic modifications in the offspring kidney that could not be reversed by the pro-
285 tective effects of breastfeeding. This aspect warrants further investigation.

286

287 It is well-reported that L-carnitine supplementation can ameliorate mitochondrial dysfunction and
288 oxidative stress in diverse conditions, including end-stage kidney disease (13). Herein, we showed
289 that this treatment is also able to prevent similar detrimental impacts by maternal SE in the offspring
290 kidney not just immediately at birth, but also in the long term. Several factors could have contributed
291 to this effect. Firstly, maternal plasma L-carnitine levels during pregnancy are lower than normal,
292 which is supposedly linked to inadequate nutrient status (17). Cigarette smoking during pregnancy
293 has been associated with reduced maternal micronutrient intake (22), and hence, is likely to contrib-
294 ute to further reduction of L-carnitine availability in both the mother and fetuses. Thirdly, the kidney
295 being one of the main sites of L-carnitine production is likely to be sensitive to changes in L-
296 carnitine levels. It has been shown that L-carnitine can prevent renal functional deterioration due to
297 ischemic reperfusion injury (23). As L-carnitine is essentially involved in mitochondrial β -oxidation
298 and has important secondary impacts on other metabolic processes, low levels are likely to increase
299 susceptibility to the accumulation of harmful intermediaries (including ROS) and dysregulate energy
300 utilization (21), leading to oxidative stress, and mitochondrial dysfunction. Hence is it unsurprising
301 that maternal L-carnitine supplementation partly reversed the effects of maternal smoke exposure in
302 the offspring's kidney.

303

304 Our data demonstrated that L-carnitine significantly reduced total ROS at all time points compared
305 to control, confirming its role as an anti-oxidant. Although L-carnitine significantly improved anti-
306 oxidant defenses in our study, and reduced total and mitochondrial oxidative stress induced by ma-
307 ternal SE in offspring kidney, it is important to note that there is no evidence of its direct effect on
308 ROS scavenging. Unlike its well-studied role in mitochondrial energy metabolism, the underlying
309 mechanism of its secondary antioxidative effect has not been elucidated (14). Given the high correla-

310 tion between increased oxidative stress and mitochondrial dysfunction in this study, it is likely that
311 L-carnitine increases redox homeostasis through normalizing mitochondrial energy metabolism. The
312 theory is supported by a previous study showing that increasing mitochondrial ATP synthesis is able
313 to normalize ROS production in a diabetic model (10).

314 In conclusion, our study demonstrates that maternal cigarette smoke exposure leads to glucose intol-
315 erance and renal underdevelopment. This was associated with renal oxidative stress and mitochon-
316 drial dysfunction in the offspring at birth and adulthood. Importantly, these defects were significant-
317 ly reversed by the maternal supplementation of L-carnitine during gestation and lactation. This study
318 provides novel insights into abnormalities in mitochondrial function and increased oxidative stress
319 that underpin the adverse effects of maternal SE on renal pathology in the offspring. The studies fur-
320 ther suggest the potential for maternal L-carnitine supplementation to limit the pathomechanistic
321 processes that may predispose to the development of kidney disease in the offspring of smoking
322 mothers.

323

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Figure Captions

Figure 1. Impaired renal development, inflammation, and glucose intolerance in male SE offspring.

(A) Average glomerular number and (B) glomerular size of offspring's kidney at P1, P20, and week 13. (C) Renal mRNA expression of MCP-1 at week 13. (D) Intra-peritoneal Glucose Tolerance Test (IPGTT) at week 13. AUC: area under the curve. * $P < 0.05$; ** $P < 0.01$.

Figure 2. Renal antioxidant capacity in the offspring. (A, C, E) Renal mitochondrial and cytosolic MnSOD and GPx-1 levels at P1, P20, and Week 13, respectively. (B, D, F) Mitochondrial SOD activity at P1, P20, and Week 13, respectively (B, D, F). $n = 4 - 8$. * $P < 0.05$, ** $P < 0.01$.

Figure 3. Confocal laser scanning microscopy images of total and mitochondrial ROS staining in the offspring kidney. (A) Representative confocal images for cell-Rox staining showing total ROS intensity (B) Representative confocal images for Mitotracker and CellRox co-staining showing that most ROS was localized within or within close proximity to the mitochondria. (C) Quantitative representation of Mean Fluorescent Intensity (MFI) for A and B. $n = 3$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs control or as indicated.

Figure 4. Renal TOM20 and OXPHOS complex I – V levels in the offspring of Control, SE mothers and SE mothers with L-carnitine treatment (SE+LC) at P1 (A), P20 (B), and Week 13 (C). $n = 4 - 8$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

463 **Table 1.** Body and kidney weight of the offspring

| P1 | Control | SE | SE + LC |
|-------------------|-----------------|------------------|------------------|
| Body weight (g) | 1.55 ± 0.05 | 1.35 ± 0.06* | 1.58 ± 0.06# |
| Kidney weight (g) | 0.0081 ± 0.0004 | 0.0069 ± 0.0004* | 0.0086 ± 0.0010# |
| Kidney/Body (%) | 0.52 ± 0.02 | 0.51 ± 0.04 | 0.55 ± 0.04 |

| P20 | Control | SE | SE + LC |
|-------------------|----------------|--------------|----------------|
| Body weight (g) | 9.97 ±0.16 | 9.71 ±0.14 | 9.74 ±0.43 |
| Kidney weight (g) | 0.067 ±0.001 | 0.062 ±0.003 | 0.067 ±0.002 |
| Kidney/Body (%) | 0.67± 0.01 | 0.062 ±0.03 | 0.70 ±0.03 |

| Week 13 | Control | SE | SE + LC |
|-------------------|----------------|------------|----------------|
| Body weight (g) | 25.5 ±0.3 | 25.1 ±0.6 | 25.3 ±0.3 |
| Kidney weight (g) | 0.20 ±0.01 | 0.19± 0.01 | 0.19 ±0.01 |
| Kidney/Body (%) | 0.77 ±0.01 | 0.76 ±0.02 | 0.77 ±0.02 |

466 Values are means ± SE; * $P < 0.05$ vs Control; # $P < 0.05$ vs SE; n = 6-10

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477 **Table 2.** Blood levels of Cotinine, Insulin and urinary Albumin/Creatinine ratio

| Week 13 | Control | SE | SE + LC |
|--------------------------|----------------|---------------|----------------|
| Cotinine (ng/ml) | 1.35 ± 0.60 | 3.90 ± 0.42** | 4.48 ± 0.17** |
| Insulin (ng/ml) | 0.53 ± 0.02 | 0.54 ± 0.01 | 0.54 ± 0.01 |
| Albumin/Creatinine ratio | 43.0 ± 14.0 | 104.7 ± 19.6* | 81.5 ± 32.5 |

478 * $P < 0.05$, ** $P < 0.01$ (vs Control), n = 6-10.

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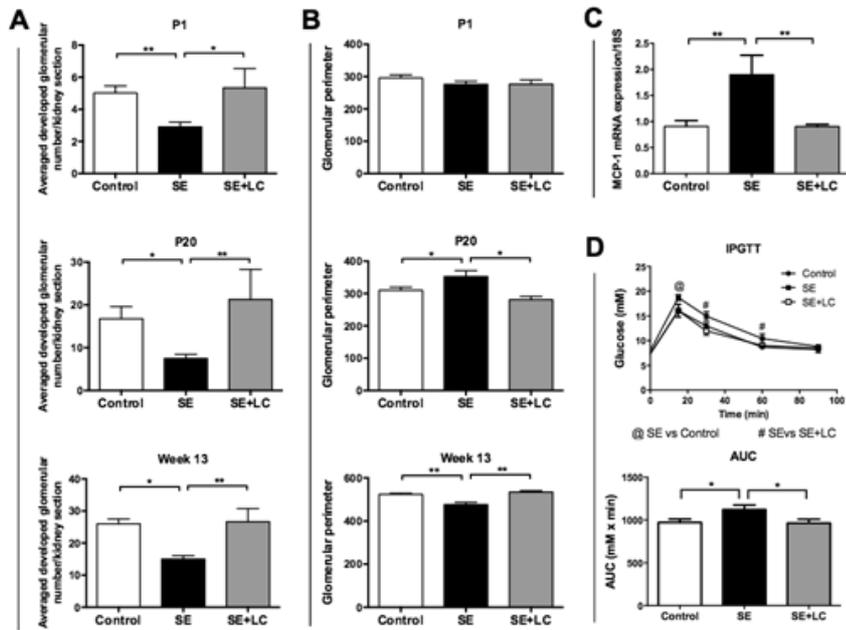
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Figure 1



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526 Figure 2

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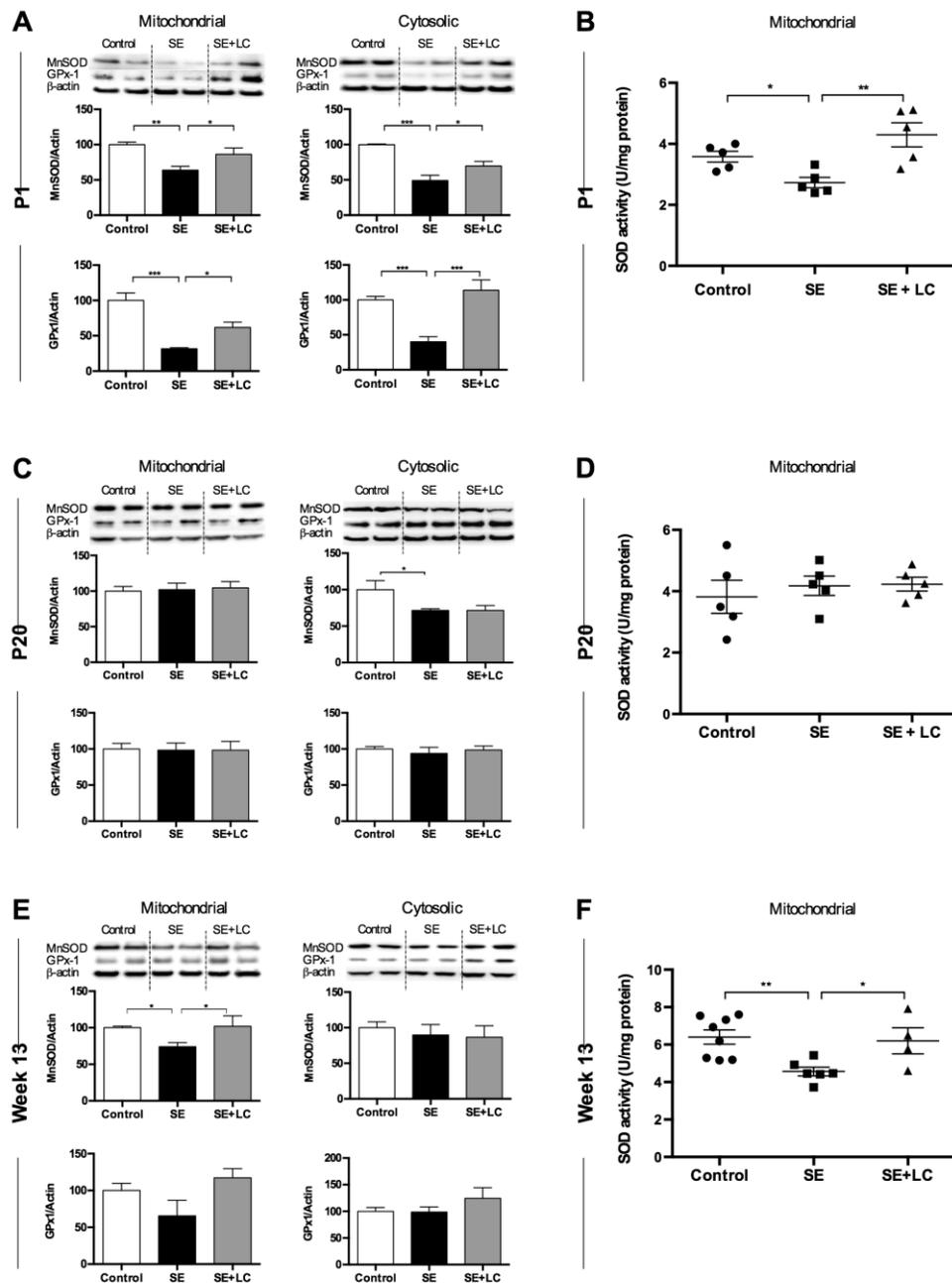
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552 Figure 3

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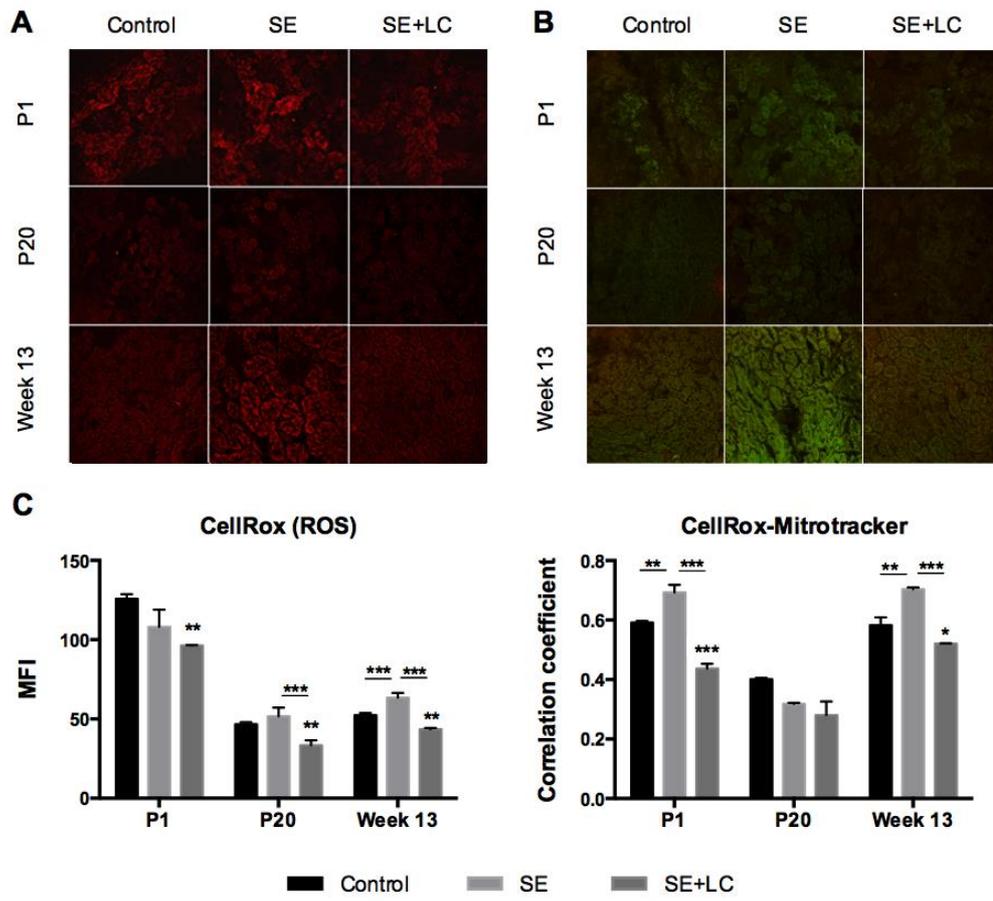
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578 Figure 4

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