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L-Carnitine reverses maternal cigarette smoke exposure-induced renal oxidative stress and mitochondrial dysfunction in mouse offspring

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¹School of Medical and Molecular Biosciences, Faculty of Science, Centre for Health Technology, University of Technology Sydney, Sydney, New South Wales, Australia; ²Kolling Institute of Medical Research, Royal North Shore Hospital, University of Sydney, Sydney, New South Wales, Australia; and ³MQ BioFocus Research Centre, Macquarie University, Sydney, New South Wales, Australia

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Nguyen LT, Stangenberg S, Chen H, Al-Odat I, Chan YL, Gosnell ME, Anwer AG, Goldys EM, Pollock CA, Saad S. L-Carnitine reverses maternal cigarette smoke exposure-induced renal oxidative stress and mitochondrial dysfunction in mouse offspring. *Am J Physiol Renal Physiol* 308: F689–F696, 2015. First published January 21, 2015; doi:10.1152/ajprenal.00417.2014.—Maternal smoking is associated with metabolic disorders, renal underdevelopment, and a predisposition to chronic kidney disease in offspring, yet the underlying mechanisms are unclear. By exposing female Balb/c mice to cigarette smoke for 6 wk prepartum and during gestation and lactation, we showed that maternal smoke exposure induced glucose intolerance, renal underdevelopment, inflammation, and albuminuria in male offspring. This was associated with increased renal oxidative stress and mitochondrial dysfunction at birth and in adulthood. Importantly, we demonstrated that dietary supplementation of L-carnitine, an amino acid shown to increase antioxidant defenses and mitochondrial function in numerous diseases, in smoke-exposed mothers during pregnancy and lactation significantly reversed the detrimental maternal impacts on kidney pathology in these male offspring. It increased SOD2 and glutathione peroxidase 1, reduced ROS accumulation, and normalized levels of mitochondrial preprotein translocases of the outer membrane, and oxidative phosphorylation complexes I–V in the kidneys of mouse progeny after intrauterine cigarette smoke exposure. These findings support the hypothesis that oxidative stress and mitochondrial dysfunction are closely linked to the adverse effects of maternal smoking on male offspring renal pathology. The results of our study suggest that L-carnitine administration in cigarette smoke-exposed mothers mitigates these deleterious renal consequences.

chronic kidney disease; reactive oxidative species; mitochondria

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

Living organisms are constantly exposed to oxidants from endogenous metabolic processes, such as ROS, a group of O₂-derived byproducts released during mitochondrial oxidative phosphorylation (OXPHOS) to generate ATP. Oxidative stress occurs when intracellular antioxidants are unable to counteract the overproduction of ROS, leading to irreversible oxidative modifications to all cellular components, including lipid, protein, and DNA, thus affecting cell structure, function, and viability (26). Smoking has been regarded as a major cause of elevated oxidative stress in active and passive smokers (16). Maternal smoking during pregnancy cannot only induce severe oxidative stress in the mother but also in offspring (11, 25), due to the diffusion of free radicals and harmful chemicals within cigarette smoke (e.g., nicotine) through the blood-placental barrier into the fetus (18). This impact, however, has been only scarcely studied in neonatal plasma and urine and rarely in neonatal organs (such as the kidney). We hypothesized that maternal cigarette smoke exposure (SE) can increase oxidative stress in newborn kidneys, which persists until adulthood.

As the major source of ROS, the mitochondrion is the most affected organelle by oxidative stress. As the cellular powerhouse, impaired mitochondria can fatally imperil energy metabolism and cell viability (26). Therefore, oxidative stress-associated mitochondrial damage and dysfunction have been implicated in a number of diseases, such as type 2 diabetes (20) as well as cancer and neurodegenerative disease (9). Importantly, such oxidative damage is likely to result in permanent modifications in mitochondrial DNA, which can be maternally inheritable. This potentially increases the risk of these disorders being transmitted to the progeny. Oxidative damage to mitochondrial DNA has been found in fetuses and infants whose mothers were exposed to cigarette smoke or nicotine during pregnancy (4, 25), suggesting a possible impact of maternal smoking on mitochondrial function in offspring. However, the effect on kidney function has not yet been explored.

Carnitine, mainly synthesized in the liver and kidney, is essential for mitochondrial fatty acid metabolism (21). Supplementation of L-carnitine (LC), the active form of carnitine, and its derivatives has been shown to attenuate oxidative stress and mitochondrial dysfunction in diverse conditions, such as age-related disorders and chronic heart failure (15, 19). In patients with end-stage kidney disease requiring dialysis, LC therapy

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has been shown to restore plasma antioxidant/oxidant homeostasis (13). However, as in most studies in patients with end-stage kidney disease, well-established pathology is unlikely to be reversed, independent of the inciting mechanism. The present study aimed to examine the utility of maternal LC supplementation after conception to reverse or ameliorate maternal SE-induced renal oxidative stress and mitochondrial dysfunction in male offspring.

MATERIALS AND METHODS

Animal experiments. The study was approved by the Animal Care and Ethics Committee of the University of Sydney. The *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health) were followed. Female Balb/c mice (8 wk) were divided into the following three groups: sham (exposed to air), SE [2 cigarettes twice daily, 6 wk before mating and throughout gestation and lactation, as previously described (1)], and SE + LC [SE mothers supplied with LC (1.5 mM in drinking water) during gestation and lactation]. The LC dose and administration were adapted from a previous study (28). Male breeders and suckling pups stayed in the home cage when mothers were exposed to sham or SE conditions. All offspring studied were male. Offspring were euthanized at *postnatal day 1* (P1), weaning age [*postnatal day 20* (P20)], and mature age (*week 13*). An intraperitoneal glucose tolerance test was performed on *week 12* as previously described (6). Blood, urine, and kidneys were collected for further analysis.

Kidney histology. Kidney samples from male offspring were embedded in paraffin and sectioned in 2- μ m slices. Kidney structure was examined using hematoxylin and eosin and periodic acid-Schiff stain. Glomerular number and size were quantitated as per our previous protocol (1).

Real-time PCR. Kidney total RNA was extracted and purified using TRIzol Reagent (Life Technology), from which cDNA was synthesized using a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany). Real-time PCR was performed using preoptimized SYBR green primers (Sigma-Aldrich) and RT-PCR master mix (Life Technology) to assess mRNA expression levels of macrophage chemoattractant protein (MCP)-1 in the kidney, with 18S rRNA as the housekeeping gene.

ELISA. Levels of urinary albumin and creatinine, serum insulin, and cotinine were measured using a Murine Microalbuminuria ELISA kit (Albuwell M), Creatinine Companion Kit (Exocell), Insulin (Mouse) ELISA Kit (Abnova, Taipei, Taiwan), and cotinine ELISA Kit (Abnova), respectively, as per the manufacturers' instructions.

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

Proteins were electrophoresed and electroblotted to Hybond nitrocellulose membranes (Amersham Pharmacia Biotech). Membranes were incubated in one of the following primary antibodies: anti- β -actin (Santa Cruz Biotechnology), goat anti-glutathione peroxidase (GPx)-1 (R&D Systems), rabbit anti-MnSOD (Millipore), rabbit anti-translocase of the outer membrane 20 (TOM20; Santa Cruz Biotechnology), and mouse anti-OXPHOS complex I-V cocktail (Abcam) and then horseradish peroxidase-conjugated secondary antibody. Blots were developed with Luminata Western HRP Substrates (Millipore) by ImageQuant LAS 4000 (Fujifilm, Tokyo, Japan). Membranes were restored by stripping buffer (Thermo Scientific) afterward. ImageJ (National Institutes of Health) was used for densitometry, and β -actin was used as the housekeeping protein.

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

Confocal microscopy. For ROS detection, CellROX deep red (5 μ M, Molecular Probes) was used, and images were collected at an excitation wavelength of 633 nm and detected in the emission range of 640–680 nm. MitoTracker green FM (200 nM, Molecular Probes) was used to visualize the mitochondria, and images were collected at an excitation wavelength of 458 nm and detected in the emission range of 480–505 nm. Multiple images were taken for over 100 cells in each tissue in 3 replicates of 3 independent samples/group. Morphological features were quantified using a confocal laser scanning microscope (Leica TCS SP2 X, Leica, Wetzlar, Germany). All imaging parameters, including laser intensities, photomultiplier tube voltage, and the pinhole, were kept constant during imaging. The tissue segmentation method was used for data analysis as previously described by Bagett et al. (3) and confirmed using a common threshold units for all images. Data are expressed as mean fluorescent intensities. To calculate the correlation between CellROX and Mitotracker, dual staining using CellROX and Mitotracker was performed, and images were taken sequentially using separate confocal channels over a time not greater than 30 s. The image pixel intensity value correlation was then calculated using Pearson's correlation for all pixels excluding any pairs containing zero values.

Statistical analysis. One-way ANOVA followed by Fisher least-significant-difference post hoc tests was used to determine differences between groups (Prism 6, GraphPad). Data are expressed as means \pm standard errors. *P* values of <0.05 were considered as statistically significant.

RESULTS

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

Maternal LC supplementation normalized renal phenotype and glucose tolerance in SE offspring. There were significant delays in kidney development in SE offspring until adulthood.

Table 1. Body and kidney weights of offspring

	Control Group	SE Group	SE + LC Group
P1			
Body weight, g	1.55 \pm 0.05	1.35 \pm 0.06*	1.58 \pm 0.06†
Kidney weight, g	0.0081 \pm 0.0004	0.0069 \pm 0.0004*	0.0086 \pm 0.0010†
Kidney weight/ body weight, %	0.52 \pm 0.02	0.51 \pm 0.04	0.55 \pm 0.04
P20			
Body weight, g	9.97 \pm 0.16	9.71 \pm 0.14	9.74 \pm 0.43
Kidney weight, g	0.067 \pm 0.001	0.062 \pm 0.003	0.067 \pm 0.002
Kidney weight/ body weight, %	0.67 \pm 0.01	0.062 \pm 0.03	0.70 \pm 0.03
Week 13			
Body weight, g	25.5 \pm 0.3	25.1 \pm 0.6	25.3 \pm 0.3
Kidney weight, g	0.20 \pm 0.01	0.19 \pm 0.01	0.19 \pm 0.01
Kidney weight/ body weight, %	0.77 \pm 0.01	0.76 \pm 0.02	0.77 \pm 0.02

Values are means \pm standard errors; *n* = 6–10. Offspring from the following three groups are shown: sham (mothers exposed to air), smoke exposed (SE; mothers exposed to 2 cigarettes twice daily, 6 wk before mating and throughout gestation and lactation), and SE + LC [SE mothers supplied with L-carnitine (LC; 1.5 mM in drinking water) during gestation and lactation]. P1, *postnatal day 1*; P20, *postnatal day 20*. **P* < 0.05 vs. the control group; †*P* < 0.05 vs. the SE group.

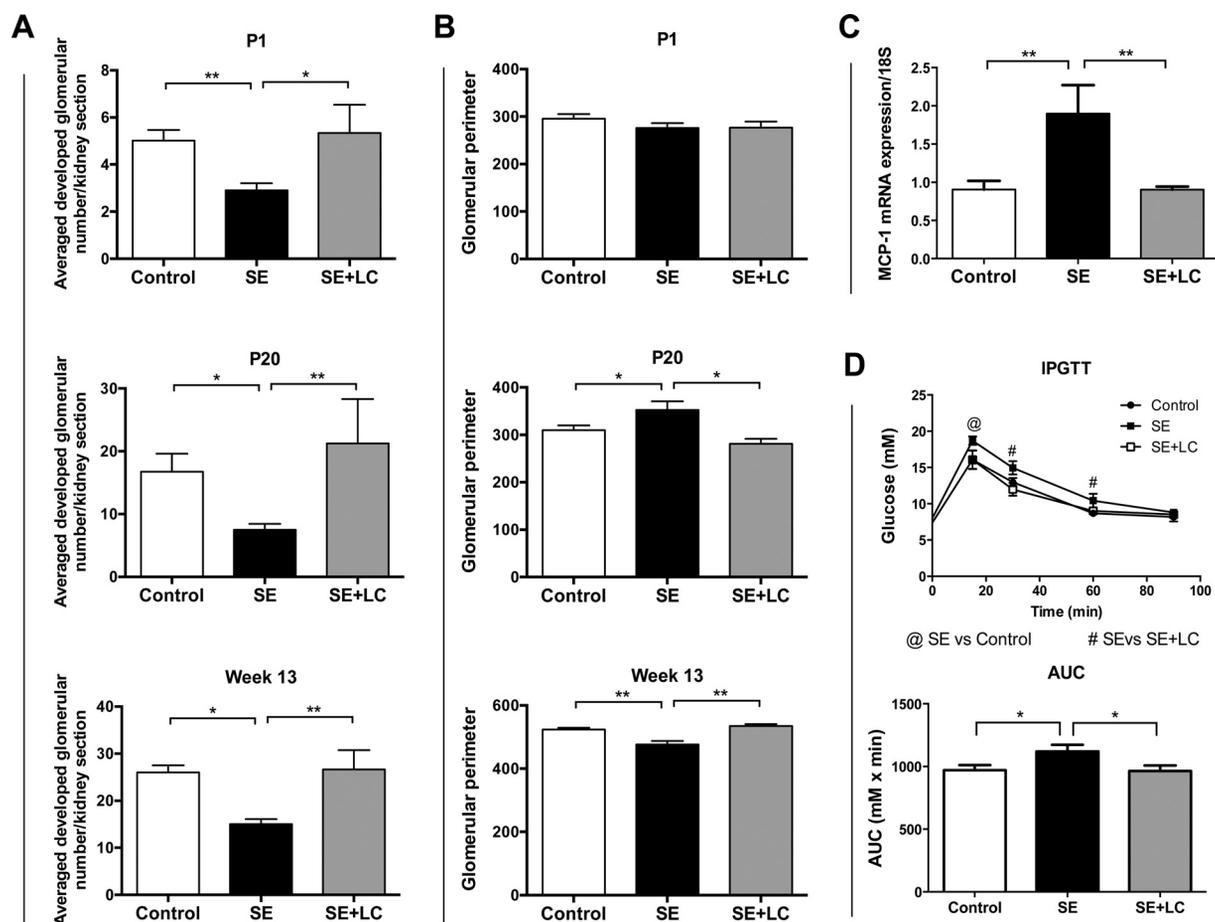


Fig. 1. Impaired renal development, inflammation, and glucose intolerance in male smoke-exposed (SE) offspring. Offspring from the following three groups are shown: sham (mothers exposed to air), SE (mothers exposed to 2 cigarettes twice daily, 6 wk before mating and throughout gestation and lactation), and SE + LC [SE mothers supplied with L-carnitine (LC; 1.5 mM in drinking water) during gestation and lactation]. *A* and *B*: average glomerular number (*A*) and glomerular size (*B*) of offspring kidneys at postnatal day 1 (P1), postnatal day 20 (P20), and week 13. *C*: renal mRNA expression of macrophage chemoattractant protein (MCP)-1 at week 13. *D*: intraperitoneal glucose tolerance test (IPGTT) at week 13. AUC, area under the curve. * $P < 0.05$; ** $P < 0.01$.

The average number of glomeruli was approximately half of the control ($P < 0.05$), whereas glomerular size was increased at weaning ($P < 0.05$) but reduced in adulthood ($P < 0.01$; Fig. 1, *A* and *B*). This was associated with a significant increase in renal MCP-1 mRNA expression in SE offspring ($P < 0.01$; Fig. 1*C*). The urinary albumin-to-creatinine ratio was also significantly higher in the SE group at week 13. In addition, offspring from SE mothers had normal serum insulin levels (Table 2) but were glucose intolerant (Fig. 1*D*), suggestive of impaired pancreatic insulin secretion.

In contrast, SE offspring of LC-treated mothers showed an improvement in histological and metabolic parameters. Glomerular number and size were normalized (Fig. 1, *A* and *B*),

Table 2. Blood levels of cotinine and insulin and the urinary albumin-to-creatinine ratio at week 13

	Control Group	SE Group	SE + LC Group
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-to-creatinine ratio	43.0 ± 14.0	104.7 ± 19.6*	81.5 ± 32.5

Values are means ± standard errors; $n = 6-10$. * $P < 0.05$ and † $P < 0.01$ vs. the control group.

and glucose tolerance returned to the level of the control group (Fig. 1, *C* and *D*). Renal MCP-1 mRNA expression and the urinary albumin-to-creatinine ratio were no longer significantly different from those observed in control animals. Serum cotinine levels were increased in both SE and SE + LC groups, confirming cigarette SE (Table 2).

Maternal LC supplementation alleviated renal oxidative stress in SE offspring. MnSOD and GPx-1 were measured as representative markers for antioxidative defense, as each is involved in one of the two-step ROS converting reaction ($O_2^- \rightarrow H_2O_2 \rightarrow H_2O + O_2$). In addition, both mitochondrial and cytosolic fractions were measured to determine which fraction was more susceptible to damage and whether changes were due to altered gene expression or protein translocation between the cytoplasm and mitochondria. At P1, all measured renal antioxidant markers, including mitochondrial MnSOD, cytosolic MnSOD, mitochondrial GPx-1, and cytosolic GPx-1, were significantly reduced in SE offspring by 40% ($P < 0.01$), 50%, 60%, and 70% ($P < 0.001$), respectively, suggesting a broad adverse effect of maternal SE on renal antioxidant capacity (Fig. 2*A*). However, only cytosolic MnSOD ($P < 0.05$; Fig. 2*C*) and mitochondrial MnSOD ($P < 0.05$; Fig. 2*E*) were significantly lower than control levels at P20 and week 13,

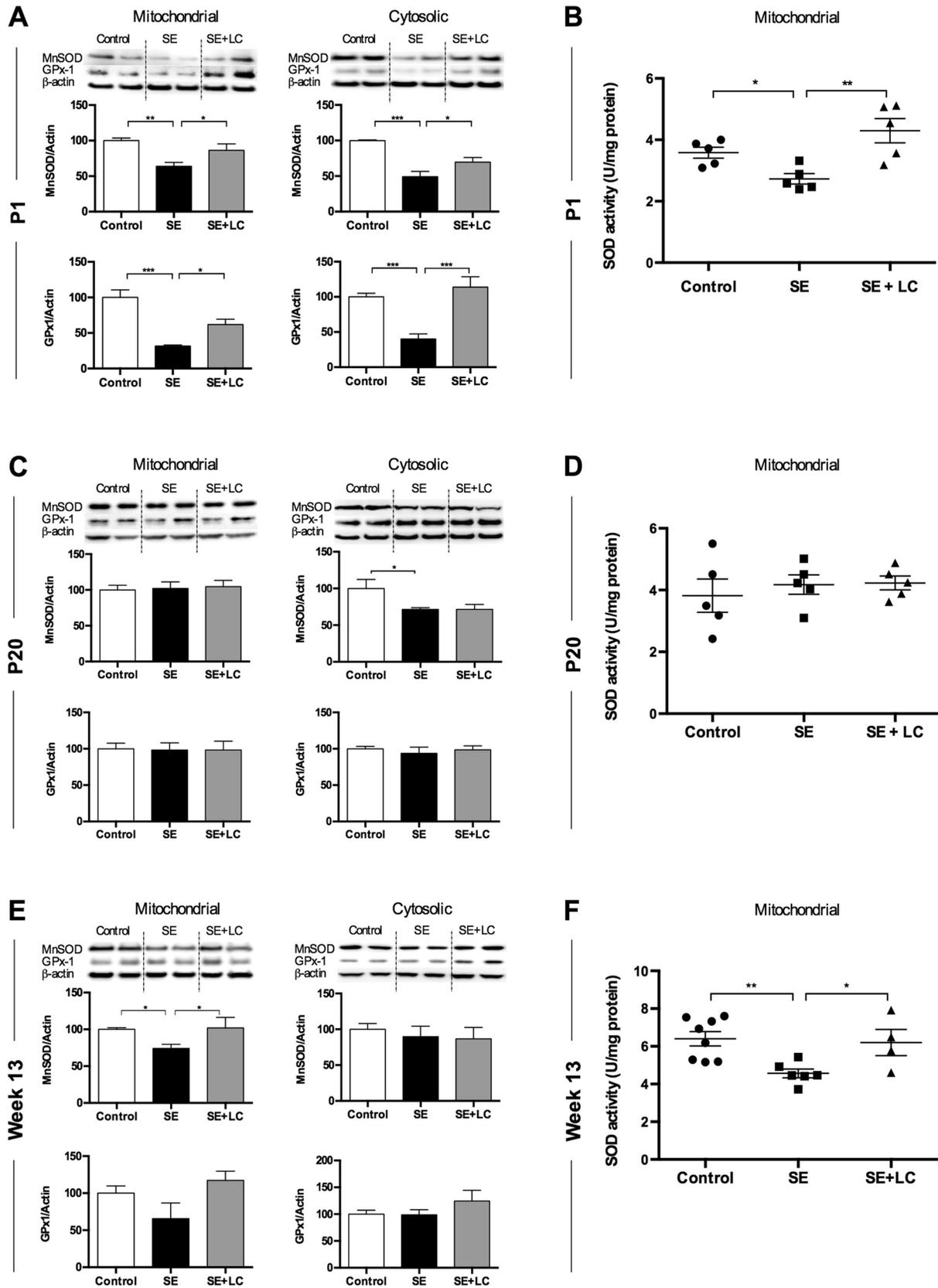


Fig. 2. Renal antioxidant capacity in offspring. *A*, *C*, and *E*: renal mitochondrial and cytosolic MnSOD and glutathione peroxidase (GPx)-1 levels at P1 (*A*), P20 (*C*), and week 13 (*E*). *B*, *D*, and *F*: MnSOD activity at P1 (*B*), P20 (*D*), and week 13 (*F*). $n = 4-8$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

respectively, without any changes of GPx-1 at either time point, suggesting renal oxidative stress by maternal SE was partially improved as SE became more remote. The antioxidant activity of renal mitochondrial SOD in SE offspring was also significantly reduced at P1 ($P < 0.05$; Fig. 2B) and *week 13* ($P < 0.01$; Fig. 2F) but not at P20 (Fig. 2D), confirming the impaired mitochondrial ability of ROS clearance in SE offspring's kidneys at birth and adulthood. LC treatment significantly attenuated the reduction of renal MnSOD and GPx-1 in both cytosolic and mitochondrial fractions at P1 ($P < 0.05$; Fig. 2A) as well as MnSOD in the mitochondrial fraction at *week 13* ($P < 0.05$; Fig. 2E) in SE offspring. Similarly, it also reversed renal mitochondrial SOD activities at both P1 ($P < 0.01$; Fig. 2B) and *week 13* ($P < 0.05$; Fig. 2F). However, in P20 offspring, LC showed no significant effect (Fig. 2, C and D).

Levels of total and mitochondrial ROS were measured as markers of oxidative stress. Kidney tissues were stained with cell-ROX red and Mitotracker to identify ROS production and

localization. There were marked elevations of renal ROS at *week 13* ($P < 0.001$; Fig. 3A), which were consistent with the observed reductions in MnSOD/GPx-1 expression and activity. The results reflect a dysregulation of renal redox homeostasis in offspring due to maternal SE. Furthermore, the correlation coefficient of cell-ROX red and Mitotracker was significantly higher in kidneys of SE offspring at P1 and *week 13* ($P < 0.01$; Fig. 3B), suggesting that the majority of excessive ROS is likely derived from the mitochondria. Interestingly, maternal SE had no effect on renal ROS or mitochondrial ROS at P20 (Fig. 3, A and B). Renal mitochondrial ROS levels were significantly reduced by LC in SE offspring at P1 and *week 13* ($P < 0.001$; Fig. 3C). Interestingly, LC significantly reduced total ROS at P1, P20, and *week 13* ($P < 0.01$) and mitochondrial ROS at P1 and *week 13* compared with controls ($P < 0.001$ and $P < 0.05$, respectively; Fig. 3C).

Maternal LC supplementation reversed renal mitochondrial dysfunction in SE offspring. To investigate mitochondrial function, we assessed TOM20, a mitochondrial outer membrane

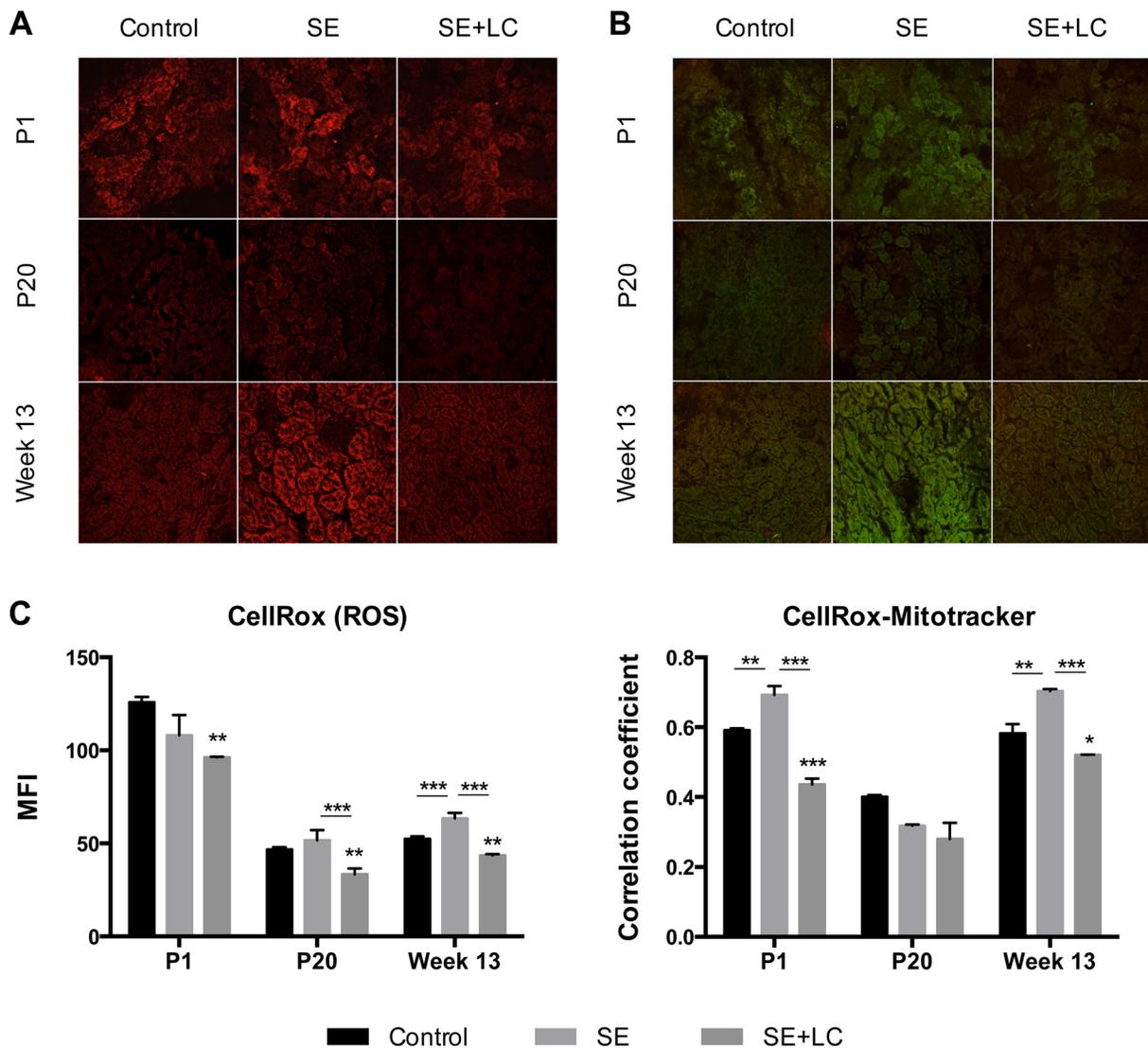


Fig. 3. Confocal laser scanning microscopy images of total and mitochondrial ROS staining in offspring kidneys. *A*: representative confocal images for CellRox staining showing total ROS intensity. *B*: representative confocal images for Mitotracker and CellRox costaining showing that most ROS were localized within or within close proximity to the mitochondria. *C*: quantitative representation of mean fluorescent intensities (MFI) for *A* and *B*. $n = 3$. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ vs. control or as indicated.

receptor for the translocation of cytosolically synthesized mitochondrial preproteins, and OXPHOS complexes I–V, key components of the mitochondrial respiratory chain for ATP synthesis. Renal protein levels of TOM20 and OXPHOS complexes I, III, and V were significantly reduced in SE offspring at P1 ($P < 0.05$; Fig. 4A), suggesting impaired mitochondrial

protein and ATP synthesis. These markers were restored by P20 (Fig. 4B) but again reduced at *week 13* (Fig. 4C), mirroring the changes of renal mitochondrial SOD in SE offspring. Maternal LC supplementation significantly restored renal levels of mitochondrial TOM20 and OXPHOS complexes I–III and V at P1 (Fig. 4A). However, no impact was observed at

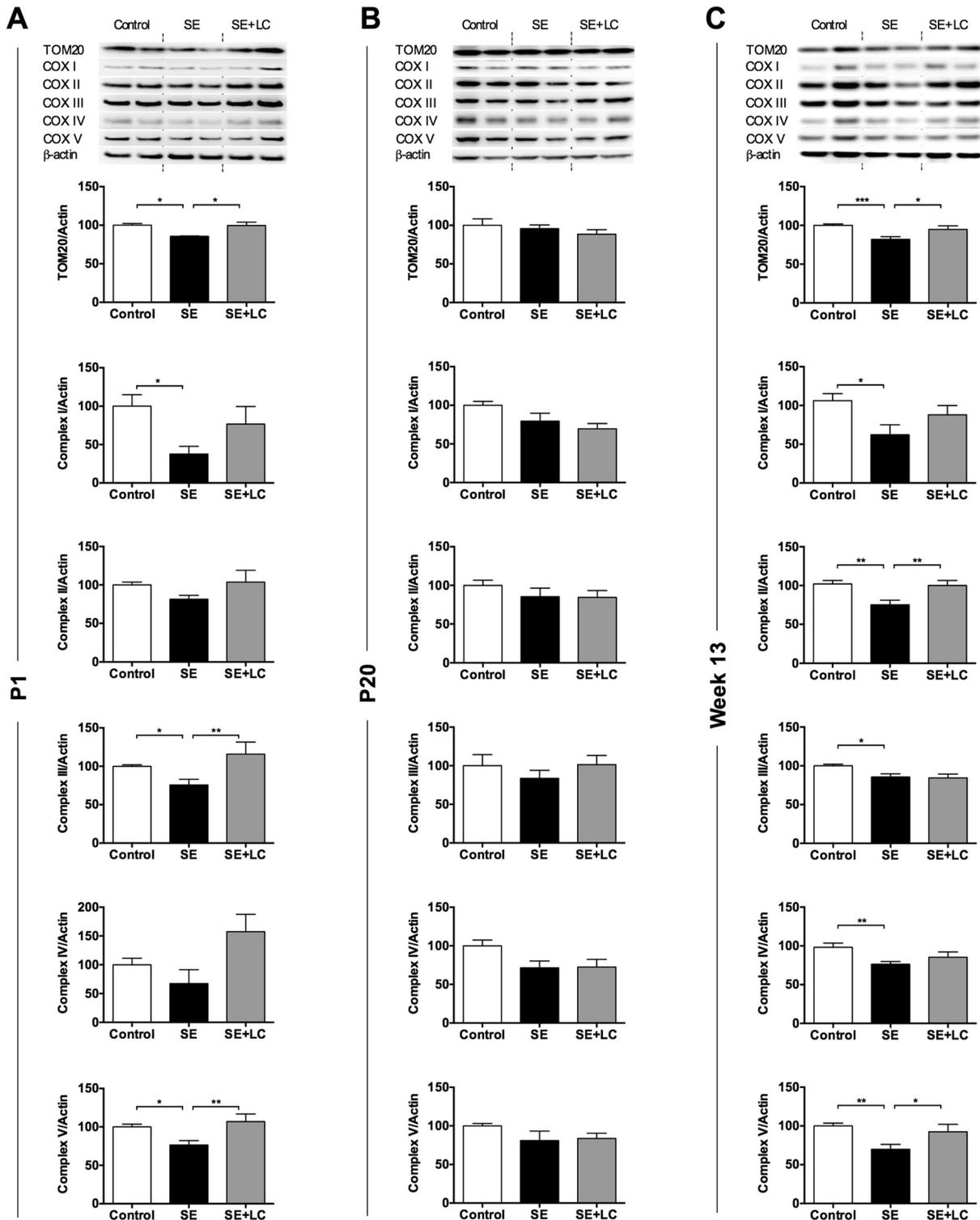


Fig. 4. Renal translocase of the outer membrane 20 (TOM20) and oxidative phosphorylation (OXPHOS) complex I–V (COX I–V) levels in offspring from control, SE, and SE + LC groups at P1 (A), P20 (B), and *week 13* (C). $n = 4–8$. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

P20 (Fig. 4B). At *week 13*, offspring from LC-treated SE mothers had normalized TOM20 and OXPHOS complexes I, II, and V ($P < 0.01$; Fig. 4C), suggesting a long-term effect of LC to prevent mitochondrial dysfunction by maternal SE.

DISCUSSION

We have previously determined that maternal SE before and during gestation and lactation induces renal underdevelopment and impaired function in male offspring, although no significant glomerular structural changes and interstitial abnormalities were detected (1). In the present study, we demonstrated that maternal SE can significantly increase renal oxidative stress and impair mitochondrial function in offspring at birth and adulthood. Supplementation of LC from gestation and throughout lactation can effectively restore renal oxidative homeostasis and mitochondrial function in SE offspring as well as intrauterine growth retardation.

In the present study, SE offspring had reduced body weight and kidney weight at birth, which is consistent with results from a human epidemiology study (5). In addition, maternal SE induced glucose intolerance and albuminuria in offspring from SE mothers. Moreover, SE offspring showed reduced renal levels of MnSOD and GPx-1, two vital enzymes for intracellular antioxidant defense, especially within the mitochondria. Encoded by genomic DNA, MnSOD is uniquely activated in mitochondria and is the only mitochondrial enzyme known to convert O_2^- into H_2O_2 , resulting in ROS disposal (27). As such, alternations in MnSOD quantity and activity can directly affect mitochondrial antioxidant capacity. Unlike MnSOD, GPx-1 functions to convert H_2O_2 into H_2O and O_2 and can also be modulated by several other enzymes, such as catalase or peroxiredoxin. However, the reduction of GPx-1 in these studies is evidence of impaired renal antioxidant capacity in offspring by maternal SE. In addition, impaired mitochondrial SOD activities and ROS accumulation provided direct evidence for increased renal oxidative stress due to oxidant/antioxidant imbalance in SE offspring.

The reduction of mitochondrial functional proteins, including TOM20 and OXPHOS respiratory units, correlated with increased oxidative stress. In addition, most of the excessive ROS produced were derived from the mitochondria, as determined by dual staining of ROS and Mitotracker. This suggests an important interplay between redox imbalance and mitochondrial dysfunction in the effector mechanisms of intrauterine SE on offspring kidneys. It is well established that increased oxidative stress can impair mitochondrial integrity (26), resulting in impaired mitochondrial preprotein import (30) and poor energy metabolism (8). Conversely, mitochondrial dysfunction, such as defects in ATP exportation (12) and/or antioxidant importation, may lead to an escalation of oxidative stress. This is supported by the reduction of both mitochondrial MnSOD and TOM20 in SE offspring kidneys at both P1 and *week 13*. As the result of this dual effect, a cycle of oxidative stress and mitochondrial damage/dysfunction is hypothesized in SE offspring kidneys, which might significantly contribute to kidney underdevelopment and/or the onset/progression of renal-related disorders.

It is surprising that increased renal oxidative stress and mitochondrial dysfunction were detected in SE offspring both at birth and adulthood yet was mitigated at weaning. The

mechanism of this temporary recovery is unclear, and we can only postulate that it may be due to the protective effect of breast milk, which has been shown to be rich in antioxidants (31). However, this protection was not sustained until adulthood. The persistent impact of maternal SE suggests that the alteration may be related to epigenetic modifications in offspring kidneys that could not be reversed by the protective effects of breastfeeding. This aspect warrants further investigation.

It is well reported that LC supplementation can ameliorate mitochondrial dysfunction and oxidative stress in diverse conditions, including end-stage kidney disease (13). Here, we showed that this treatment is also able to prevent similar detrimental impacts by maternal SE in offspring kidneys not just immediately at birth but also in the long term. Several factors could have contributed to this effect. First, maternal plasma LC levels during pregnancy are lower than normal, which is supposedly linked to inadequate nutrient status (17). Cigarette smoking during pregnancy has been associated with reduced maternal micronutrient intake (22) and, hence, is likely to contribute to the further reduction of LC availability in both the mother and fetuses. Third, the kidney, being one of the main sites of LC production, is likely to be sensitive to changes in LC levels. It has been shown that LC can prevent renal functional deterioration due to ischemia-reperfusion injury (23). As LC is essentially involved in mitochondrial β -oxidation and has important secondary impacts on other metabolic processes, low levels are likely to increase susceptibility to the accumulation of harmful intermediaries (including ROS) and dysregulate energy utilization (21), leading to oxidative stress and mitochondrial dysfunction. Hence, it is unsurprising that maternal LC supplementation partly reversed the effects of maternal SE in offspring kidneys.

Our data demonstrated that LC significantly reduced total ROS at all time points compared with control, confirming its role as an antioxidant. Although LC significantly improved antioxidant defenses in our study and reduced total and mitochondrial oxidative stress induced by maternal SE in offspring kidneys, it is important to note that there is no evidence of its direct effect on ROS scavenging. Unlike its well-studied role in mitochondrial energy metabolism, the underlying mechanism of its secondary antioxidative effect has not been elucidated (14). Given the high correlation between increased oxidative stress and mitochondrial dysfunction in this study, it is likely that LC increases redox homeostasis through normalization of mitochondrial energy metabolism. The theory is supported by a previous study (10) showing that increasing mitochondrial ATP synthesis is able to normalize ROS production in a diabetic model.

In conclusion, our study demonstrates that maternal cigarette SE leads to glucose intolerance and renal underdevelopment. This was associated with renal oxidative stress and mitochondrial dysfunction in offspring at birth and adulthood. Importantly, these defects were significantly reversed by the maternal supplementation of LC during gestation and lactation. This study provides novel insights into abnormalities in mitochondrial function and increased oxidative stress that underpin the adverse effects of maternal SE on renal pathology in offspring. The study further suggests the potential for maternal LC supplementation to limit the pathomechanistic processes that

may predispose to the development of kidney disease in offspring of smoking mothers.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: L.T.N., S. Stangenberg, I.A.-O., Y.L.C., M.E.G., and A.G.A. performed experiments; L.T.N., Y.L.C., M.E.G., and S. Saad analyzed data; L.T.N., S. Stangenberg, H.C., E.M.G., C.A.P., and S. Saad interpreted results of experiments; L.T.N., S. Stangenberg, and S. Saad prepared figures; L.T.N. drafted manuscript; L.T.N., S. Stangenberg, H.C., I.A.-O., Y.L.C., M.E.G., A.G.A., E.M.G., C.A.P., and S. Saad approved final version of manuscript; H.C. and S. Saad conception and design of research; C.A.P. and S. Saad edited and revised manuscript.

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