Cross-generational impact of maternal exposure to low level of PM2.5 on kidney health

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Abstract

Inhaled fine and ultrafine particulate matter may affect organs other than the lung, including the kidney. Recent studies have consistently shown the possibility of air pollution in highly polluted countries to be nephrotoxic. However, in countries like Australia, where air quality generally adheres to or remains below the WHO standards, the subtle yet consequential impacts of chronic exposure to seemingly safe levels of traffic $PM_{2.5}$, are a subject of increasing significance. However, how such exposures in the peri-pregnancy period affect kidney health in mothers and the offspring is unclear, which formed the aims of this study. Female Balb/c mice were exposed to $PM_{2.5}$ (5 μg/day) delivered nasally for 6 weeks prior to mating, during gestation and lactation (PM group). In a sub-group, $PM_{2.5}$ was switched to saline from mating until offspring were weaned to model mothers moving to areas with clean air. Kidneys were analysed in dams and adult offspring at 13 weeks of age. $PM_{2.5}$ induced oxidative stress without histological changes in the dam's kidney. However, male PM offspring displayed in-utero underdevelopment, characterised by reduced body weight and kidney-to-body weight at birth compared to control offspring, and lower glomerular numbers, with a marked increase in albuminuria, glomerulosclerosis, inflammation, oxidative stress, and mitochondrial injury. Female PM offspring had delayed postnatal development, lower glomerular numbers, increased glomerulosclerosis and oxidative stress injury markers. Removal of $PM_{2.5}$ from conception was overall protective to the offspring. In conclusion, there is no safe level of ambient $PM_{2.5}$ for kidney health when exposed in-utero. Maternal PM2.5 exposure equally impacts the kidney health of male and female offspring.

Keywords: fetal programming, traffic-derived PM, oxidative stress, mitochondria, CKD

1. Introduction

Air pollution is a pervasive global concern, as over 90% of the world's population lives in areas with polluted air. Epidemiological and experimental studies have well characterised the close association between exposure to high levels of airborne particulate matter (PM) and adverse health outcomes in the respiratory and cardiovascular systems (World Health Organisation, 2018). $PM_{2.5}$ is the most hazardous component of air pollutants due to its small diameter. $PM_{2.5}$ is 2.5 micrometres or less and is, therefore, capable of crossing tissue barriers in the distal airway and alveoli, through which it can rapidly reach distal organs (Chen et al., 2021).

The kidney is one of the recently identified extrapulmonary targets of $PM_{2.5}$. Although experimental findings for $PM_{2.5}$ exposure-related renal injury are scarce, studies have consistently shown nephrotoxic effects of urban air pollution where $PM_{2.5}$ levels are several times above the WHO standards (Bowe et al., 2018; Chan et al., 2019b; Nemmar et al., 2009; Nemmar et al., 2016). Chronic exposure to PM has been associated with reduced kidney function among adults in several populations (Bowe et al., 2019; Chan et al., 2018; Ran et al., 2020; Yang et al., 2017; Zhao et al., 2020). As mentioned above, these studies have predominantly focused on regions with highly polluted air, where $PM_{2.5}$ concentrations surpass the WHO's recommended standard levels. However, in countries like Australia, where air quality generally adheres to or remains below the WHO guidelines, the subtle yet consequential impacts of chronic exposure to seemingly safe levels of $PM_{2.5}$, especially those derived from vehicles along busy roads, have become a subject of increasing significance.

Notably, our previous investigations using mouse models have unveiled concerning findings regarding the effects of exposure to low levels of $PM_{2.5}$, even within the presumed safe range in both the short and long term (Chan et al., 2019a; Wang et al., 2021). Specifically, we observed lung emphysema-like changes in response to chronic exposure, highlighting the potential implications of seemingly innocuous air pollution levels (Wang et al., 2021). In addition, embryonic and fetal development is most sensitive to changes in the in-utero environment, e.g. environmental toxins, which can delay and impair the development of vital organs and increase the susceptibility to chronic diseases after birth (Chen et al., 2021; Chen et al., 2022a; Chen et al., 2022b; Wang et al., 2021). Indeed, we found an increased risk of asthma and liver dysfunction by in-utero exposure to a "safe level" of $PM_{2.5}$ (Wang et al., 2021). This discovery raises a significant research question regarding whether chronic exposure to low-level $PM_{2.5}$, either direct or in-utero, can induce detrimental effects in the other vital organs, such as the kidneys. Given the size of $PM_{2.5}$, systemic absorption is likely, which places fetuses at risk of systemic exposure.

Heavy metals (e.g. lead, cadmium, and arsenic) contained in $PM_{2.5}$ have been associated with renal tubular and interstitial damage (Kim, 2017; Möhner, Pohrt and Gellissen, 2017). Transition metals can attach to glycated proteins, enhancing free radical reactions and exacerbating oxidative stress (Shah et al., 2007). Acute exposure to environmental toxins causes direct renal injury to proximal tubules, while persistent or chronic exposure may result in hypertension, interstitial nephritis, and renal fibrosis (Kim, 2017; Navarro-Moreno et al., 2009; Soderland et al., 2010). Short-term exposure to traffic PM induces acute renal failure via oxidative stress (Waly, Ali and Nemmar, 2013). In rats with pre-existing renal injury, a single high dose (1 mg/kg) of diesel exhaust PM aggravated oxidative stress while reducing SOD activity in the renal cortex (Nemmar et al., 2010). Chronic $PM_{2.5}$ exposure also increases the risk of CKD (Bowe et al., 2017; Bowe et al., 2018). In particular, exposure to $PM_{2.5}$ significantly correlates with the progression to end-stage kidney disease and increased mortality due to renal failure (Bowe et al., 2019; Ran et al., 2020). These studies suggest exposure to ambient $PM_{2.5}$ as a possible risk factor for kidney diseases; however, the nephrotoxic effects, especially in the context of prenatal exposure, remain a knowledge gap. A poor intrauterine environment, such as sub-optimal nutritional status and environmental toxins inhaled by pregnant mothers like cigarette smoke and heavily polluted air, correlates with low birth weight (LBW) in the offspring (Rich et al., 2015; Wang et al., 2002; Wang et al., 2018), while individuals born with LBW had a 70% increased risk of CKD (White et al., 2009). We have shown that maternal smoking during

pregnancy caused low nephron number and progressive renal injury in mouse offspring in conjunction with LBW (Nguyen et al., 2015; Stangenberg et al., 2015a). Mitochondria are particularly susceptible to PM2.5-induced ROS and oxidative stress (Stangenberg et al., 2015a). Aberrant fetal programming of the kidney could be due to abnormal mitochondrial (mt)DNA (Nguyen et al., 2015). Hence, in-utero $PM_{2.5}$ exposure could provoke adverse mitochondrial biogenesis, resulting in impaired mitochondrial functional units (Chen et al., 2021; Nguyen et al., 2015).

Few studies have investigated the effects of maternal PM exposure on the offspring and whether living in an environment with continuous low-level $PM_{2.5}$ exposure (akin to being close to a busy road) during pregnancy affects the offspring. This research aims to address this gap by investigating the relationship between maternal exposure to a low level of $PM_{2.5}$ and the subsequent renal health of offspring.

2. Materials and Methods

2.1 Animal model

The animal study was approved by the Animal Care and Ethics Committee at the University of Technology Sydney (ETH17-1998) and followed the Australian National Health and Medical Research Council Guide for the care and use of laboratory animals. Female Balb/c mice (8 weeks, Animal Resource Centre, WA, Australia) were divided into 3 groups. Animals were exposed to roadside $PM_{2.5}$ (5µg suspended in 40µl saline, delivered nasally (Chan et al., 2019a) or to saline (40µl of saline, delivered nasally) once per day for 6 weeks before mating and continued during gestation and lactation as we have previously published (Wang et al., 2021). The dose was determined following our previous studies (Chan et al., 2019a; Oliver et al., 2024). A subgroup of $PM_{2.5}$-exposed mice was switched to saline during pregnancy and lactation (Pre-exposure group). The litter size was not significantly different among groups, as we published previously (Wang et al., 2021). The dams were harvested when the pups were weaned, and the pups were not subjected to direct $PM_{2.5}$ exposure. Birth weight and kidney weight were measured in the newborn. Kidneys from the dams and from offspring at 13 weeks of age (representing adulthood) and spot urine samples from adult offspring were analysed.

2.2 ELISA

Spot urine samples were used to calculate the albumin:creatinine ratio (UACR) as a measure of glomeruli function. A mouse albumin ELISA kit (Crystal Chem, IL, United States) was used to determine the urine concentrations of albumin. Creatinine levels were determined using a colourimetric assay kit for creatinine (Cayman Chemical, MI, United States) as previously described (Larkin et al., 2021).

2.3 Histology and immunohistochemistry

Kidneys from the dams and adult offspring were fixed in neutral buffered formalin (10%), embedded in paraffin and sectioned. The kidney structures were examined using hematoxylin and eosin (H&E). Periodic acid-Schiff (PAS) was used to detect polysaccharides suggestive of fibrotic changes in the kidney section (Hui et al., 2017). Paraffin-embedded sections were incubated in 1% w/v periodic acid (15 minutes), followed by Schiff's reagent (15 minutes), Mayer's haematoxylin (2 minutes), and finally Scott's blue (1 minute), with washes between each incubation. Then, the sections were dehydrated in increasing grades of ethanol (1x 95%, 2x 100%) for 3 minutes each, and xylene (2x 3 minutes) and then cover-slipped. The sections were analysed and quantified with ImageJ (National Institutes of Health, MD, USA).

For IHC staining, formalin-fixed paraffin-embedded sections were de-paraffinised and boiled for 20 min in 10 mM citrate buffer (pH 6.0) for epitope retrieval. Sections were washed in TBST buffer and exposed to 0.3% H_2O_2 for 5 min to quench endogenous peroxidases, then blocked with Dako protein block (Dako, Carpinteria, CA, USA) for 10 min and incubated with a rabbit polyclonal antibody against 8-OHdG (1:100, BIOSS, Woburn, MA, USA) overnight as we have previously described (Stangenberg et al., 2015b). The tissues were then incubated with polymer secondary anti-rabbit antibodies (Dako Ref K4003), horseradish peroxidase enzyme, and DAB+ (liquid

DAB+substrate chromogen system, Dako Ref K3468). The sections were counterstained with haematoxylin. Negative controls were prepared by replacing the primary antibodies with rabbit IgG. Quantitation of the positive signals in the images was performed using Image J software (Image J, NIH, USA).

The glomerular number was estimated by counting the developed glomeruli in 8–10 different fields for the same kidney section and then averaged as we have previously demonstrated (Al-Odat et al., 2014). One section was used from each kidney (4-6 randomly selected mice from each group). The glomerular size was measured by assessing the glomerular perimeter using Image J (Image J, NIH, USA) in 8–10 non-overlapping images for the same kidney section and then averaged. Glomerular and tubular structure, in addition to glomerular number and size, were additionally assessed by an independent pathologist in a blinded manner for confirmation.

2.4 Real-time PCR

Total mRNA was extracted from frozen kidney tissue with TriZol reagent (Life Technologies, CA, USA), and the first strand cDNA was generated using M-MLV Reverse Transcriptase, RNase H, Point Mutant Kit (Promega, WI, USA).

Target gene expression was quantified with SYBR® primers (TNF α , Forward-GGTGCCTATGTCTCAGCCTCTT, Reverse- GCCATAGAACTGATGAGAGGGAG; CD68, Forward GGCGGTGGAATACAATGTGTCC, Reverse AGCAGGTCAAGGTGAACAGCTG. Sigma-Aldrich, St. Louis, MO, US) and standardised to housekeeping 18s RNA (Forward GAATAATGGAATAGGACCGCGG, Reverse GGAACTACGACGGTATCTGATC. Sigma-Aldrich). The probes of the target genes and 18s RNA were labelled with FAM® or SYBR®, using SensiFAST™ SYBR® Hi-ROX Kit (Bioline, NSW 2015). The average of the SHAM group was assigned the calibrator against which all other results were expressed as fold changes.

2.5 Western blot

Frozen kidney tissue was homogenised in cell lysis buffer in the Mitochondria Isolation Kit (Thermo Fisher Scientific. Cytosolic and mitochondrial fractions were separated following the manufacturer's protocol. Protein concentration was measured by Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific) and stored at -80°C for further analysis. The same amount of protein was loaded on SDS-PAGE electrophoresis gels (8-13%) and electroblotted to Hybond nitrocellulose membranes (Amersham Pharmacia Biotech). Membranes were incubated with primary antibodies against β-actin (Santa Cruz Biotechnology), Manganese Superoxide Dismutase (MnSOD) (1:2000. Millipore), translocase of the outer membrane 20 (1:2000, TOM20; Abcam), COXIV (1:4000, Cell Signalling), and OXPHOS complex I–V (1:4000, Abcam), followed by washing and incubation with secondary antibody. After washing the membrane, immunoblots were developed using Clarity ECL Substrates (Bio-rad) and visualised on ImageQuant LAS 4000 (Fujifilm, Tokyo, Japan). Membranes were restored by stripping buffer (Thermo Scientific) if necessary. ImageJ (National Institutes of Health) was used for densitometry, and β-actin was used as the housekeeping protein.

2.6 Statistical methods

Results are expressed as mean ± SEM. Normality was tested. If the data were not normally distributed, then they were log-transformed. The results of the dam, male and female offspring were analysed independently, using one-way ANOVA, followed by Turkey post hoc tests (GraphPad Prism 10, GraphPad, CV, USA). $P < 0.05$ was considered significant.

3. Results

3.1 The effects of maternal PM exposure on anthropometric measurements and UACR

Dams exposed to PM showed significant reductions in body weight ($P < 0.05$) and kidney weight ($P < 0.05$), which were normalised by $PM_{2.5}$ cessation since mating (body weight $P < 0.01$ $PM_{2.5}$ vs Pre-

exposure, Table 1). Kidney to body weight ratio was similar among the three groups. Maternal $PM_{2.5}$ exposure prior to mating for 6 weeks, during gestation and lactation induced reductions in body weight ($P< 0.05$), kidney weight ($P< 0.01$), and kidney/body weight ratio in male offspring at postnatal day 1. Such changes in kidney weight and kidney/body weight ratio were prevented if $PM_{2.5}$ exposure had been removed just before gestation ($P< 0.05$, Table 1). In sharp contrast to male offspring, female offspring showed no difference in body weight, kidney weight or kidney/body weight ratio at day 1; however, their body weight and kidney weight were significantly lower than the SHAM in adulthood ($P < 0.05$, Table 1). At week 13, male offspring showed no significant differences in anthropometric measurements. However, there was a significant increase in UACR in the PM group ($P<0.05$), which was significantly attenuated in the Pre-exposure group ($P < 0.01$). In female offspring, there was also a trend of increased UACR, although this did not reach significance (Table 1).

3.2 The effects of maternal PM exposure on glomerular histology

Dams exposed to $PM_{2.5}$ showed similar number and size of glomeruli (Figure 1A and 1B). Conversely, glomerular numbers in both male and female offspring were significantly reduced by maternal $PM_{2.5}$ exposure (bot sex $P<0.05$ vs SHAM), although kidney weights were normal at 13 weeks (Figure 1C and 1E), which were not reversed by the removal of $PM_{2.5}$ (male $P<0.05$ Pre-exposure vs SHAM). Glomerular size was not different among any offspring. With reduced glomerular number, maternal $PM_{2.5}$ exposure also led to significant increases in markers of glomerulosclerosis in both male and female offspring ($P=0.07$ male $PM_{2.5}$ vs SHAM; $P<0.05$ female $PM_{2.5}$ vs SHAM, Figure 2). The removal of $PM_{2.5}$ exposure at conception reversed glomerulosclerosis to the control levels in both male and female offspring (Figure 2).

3.3. The effects of maternal PM exposure on kidney inflammation

$PM_{2.5}$ exposure in the dams did not significantly increase inflammatory markers, including TNFα and macrophage marker CD68 mRNA expression (Figure 3A, D). At week 13, PM male offspring showed significantly increased TNFα and CD68 expression compared to the SHAM offspring (both $P<0.05$ vs SHAM, Figure 3B, E). Maternal PM removal reduced TNFα expression and CD68 (TNFα $P<0.05$ Pre-exposure vs $PM_{2.5}$, Figure 3B, E). In PM female offspring, both TNFα and CD68 expression was suppressed (CD68 $P<0.05$ vs SHAM, Figure 3C, F). In the female Pre-exposure group, both TNFα and CD68 levels were reversed to surpass the SHAM group levels (CD68 $P<0.01$ vs $PM_{2.5}$, Figure 3C, F).

3.4. The effects of maternal PM exposure on kidney oxidative stress markers and mitochondrial functional markers

To examine oxidative stress in the kidney, we measured the protein levels of MnSOD in both the cytosolic and mitochondrial fractions. As shown in Figure 4, in the dams, MnSOD was significantly reduced by $PM_{2.5}$ exposure in the cytosolic and mitochondrial fractions in the kidneys (both $P<0.05$ vs SHAM, Figure 4A, B); while PM removal only normalised cytosolic MnSOD level ($P<0.05$ vs $PM_{2.5}$, Figure 4A). In line with this, the oxidative stress marker 8-OHdG was also increased in both PM and Pre-exposure dams ($P<0.05$, Pre-exposure vs SHAM, Figure 4C). In the PM male offspring, MnSOD was reduced in the cytosolic fraction ($P<0.01$ vs SHAM), but increased in the mitochondrial fraction ($P<0.01$ vs SHAM), which was not affected by maternal PM removal at conception ($P<0.05$and $P<0.01$ $PM_{2.5}$ vs SHAM for cytosolic and mitochondrial fractions, respectively, Figure 4A, B). The level of 8-OhdG was also increased in male PM offspring, which was normalised in the Pre-exposure group ($P<0.05$ $PM_{2.5}$ vs SHAM, Figure 4C). In the PM female offspring, MnSOD levels in both cytosolic and mitochondrial fractions were similar among groups (Figure 4A, B). However, 8-OhDG level was significantly increased in the female PM offspring and normalised in the Pre-exposure group ($P<0.05$ $PM_{2.5}$ vs SHAM, $P<0.01$ Pre-exposure vs $PM_{2.5}$, Figure 4C).

Mitochondria are highly susceptible to oxidative stress. Mitochondrial dysfunction is one of the key mechanisms in developmental programming. In the dams, kidney COXIV (Cytochrome c oxidase) and substrate transporter TOM20 were not affected by PM2.5 exposure for any duration (Figure 5).

However, COXIV was reduced in both male and female PM offspring (male $P<0.05$ $PM_{2.5}$ vs SHAM; female $P=0.25$ $PM_{2.5}$ vs SHAM), and only COXIV in female kidneys was reversed in the Pre-exposure group ($P<0.01$ vs SHAM, $P<0.001$ vs $PM_{2.5}$ Figure 5). TOM20 level was only reduced in male PM mice ($P<0.05$ vs SHAM), which was not affected by maternal PM removal since conception ($P=0.05$ vs SHAM, Figure 5).

To further examine mitochondrial functional units, we assessed the protein of mitochondrial oxidative phosphorylation complexes (OXPHOS). In the dams, PM exposure led to a significant reduction of complex I level ($P<0.05$ vs SHAM, Figure 6A), without any effects on the other four complexes. In male PM offspring, only complex IV was significantly reduced ($P<0.05$ vs SHAM, Figure 6B), which was not normalised in the Pre-exposure males; However, complex I level was doubled in the Pre-exposure group compared with the SHAM group ($P<0.05$, Figure 6B). In female offspring, OXPHOS complexes were all at similar levels among experimental groups (Figure 6C).

4. Discussion

Kidneys are vulnerable to both acute and chronic injury upon exposure to environmental toxins (Feng et al., 2023; Kim, 2017; Navarro-Moreno et al., 2009; Soderland et al., 2010). Low levels of PM have not previously been considered a concern with respect to kidney health. Here, we observed the impact of low-level $PM_{2.5}$ exposure on oxidative stress in the dams' kidneys without changes in inflammatory and histological markers. Strikingly, such a "mild" environmental factor has shown long-lasting adverse impacts on the offspring's kidneys. We also observed maternal $PM_{2.5}$ exposure induced in-utero underdevelopment in the male offspring but not female offspring. Interestingly, reduced glomerular numbers were observed in both male and female PM offspring, along with induced oxidative stress and mitochondrial injury in adulthood.

The advantage of using an animal model is that we can exclude the potential confounder of postnatal direct $PM_{2.5}$ exposure in the offspring and the genetic variations observed in the general population. As such, any effects observed in the offspring are mostly attributed to maternal effects. Dams exposed to $PM_{2.5}$ only during the preconception period (pre-exposure group) showed significant DNA oxidation in the kidneys, reflected by the increased 8-OHdG levels. There was also a trend of an increase in renal 8-OHdG levels in the dams when they were exposed to $PM_{2.5}$ during the gestation period. This was associated with a significant reduction in mitochondrial levels of the antioxidant marker MnSOD, suggesting increased renal oxidative stress. $PM_{2.5}$ exposure to dams during the preconception or the gestation period significantly reduced mitochondrial OXPHOS complex 1 levels. OXPHOS has a critical role in maintaining cellular homeostasis and is also known to support differentiation processes during embryonic development (Fernández-Vizarra et al., 2022). Although OXPHOS complex 1 was significantly regulated, other complexes, as well as the levels of COX4 and TOM20, were not altered in the dams, suggesting that mitochondrial function was not compromised. Although oxidative stress was increased in the dams, $PM_{2.5}$ exposure did not induce renal inflammation and histological changes. This can potentially be due to the placental ability to synthesise melatonin, which has antioxidant and anti-inflammatory effects, necessary to ensure a stable environment for both the mother and foetus (Joseph et al., 2024). The dams may also have other adaptive mechanisms and the mature kidneys are less vulnerable to oxidative stress than youngsters.

Maternal $PM_{2.5}$ exposure significantly reduced the offspring's birth weight in the male offspring only. A similar effect was observed in male offspring exposed to maternal cigarette smoke during gestation (Sukjamnong et al., 2017). Kidney-to-body weight was also significantly decreased in the male offspring at birth following exposure to $PM_{2.5}$ during gestation, and this was associated with a significant reduction of glomerular number and albuminuria. Removing $PM_{2.5}$ exposure during gestation prevented the development of albuminuria in male offspring, suggesting a protective effect on kidneys.

Previous studies have established sex bias in disease susceptibility, with females less likely to develop certain diseases in contrast to males. This has often been explained by the anti-inflammatory effects of estrogen as a defence mechanism (Chan et al., 2016a). In the kidney, epidemiological findings also support the male sex as a risk factor for the development of kidney diseases in adulthood (Li et al., 2008). A study showed significantly higher levels of renal profibrotic markers in male offspring in response to maternal high fat diet consumption (Nguyen et al., 2017). Our own study on maternal cigarette smoke exposure also showed a male-prominent risk of renal disorders in the offspring (Al-Odat et al., 2014; Chan et al., 2017; Chan et al., 2016a; Chan et al., 2016b). However, maternal exposure to a low level of $PM_{2.5}$ before pregnancy or during gestation seems to affect both sexes, although the changes in renal pathology and renal dysfunction are different between male and female offspring. Both male and female PM offspring displayed smaller glomerular numbers in adulthood; however, only males had in-utero renal underdevelopment, demonstrated by the reduced weight and kidney-to-body weight at birth. Normally, an increased glomerular perimeter is expected when the glomerular number is reduced to compensate for the filtration. However, this was not observed in PM offspring, suggesting impaired renal development without sex discrimination.

Although the urinary albumin to creatinine ratio was not statistically significant in females, the physiological impact can be significant. This can result from glomerular endothelial dysfunction (Satoh, 2012; Strutz, 2009), where proteins are filtered out and reabsorbed in the proximal tubules, causing tubular toxicity (Strutz, 2009). In animal models of high levels of PM exposure, sub-chronic exposure can impair renal function and structure, such as loss of glomerular integrity, glomerular atrophy, loss of epithelial cells, increased Bowman's space, oedema, and tubular dilation and vacuolation (Al Suleimani et al., 2017; Wardoyo, Juswono and Noor, 2018). This can also cause altered renal haemodynamics, haematuria, and albuminuria (Tavera Busso et al., 2018); while long-term PM exposure leads to increased renal inflammation, oxidative stress and DNA damage (Nemmar et al., 2016). PM and related toxicity can induce the release of cytokines and chemokines, contributing to the influx of mononuclear cells, e.g. macrophages (Strutz, 2009), which produce profibrotic cytokines that promote the accumulation of interstitial fibrosis (Black, Lever and Agarwal, 2019; Strutz, 2009). In the long term, this can lead to fibrotic scarring and irreversible chronic kidney disease (Chen et al., 2022a). However, the inflammatory response was only significant in the male offspring. Previous studies have demonstrated that the epigenetic effect of maternal cigarette smoking during gestation is more pronounced in males compared to females. Males are also more susceptible to environmental toxins and more vulnerable to abnormal methylation than females (Murphy et al., 2012). Such differences, as well as differences in early adaptation in males and females, can be responsible for the differences in inflammatory response observed in males and females in our study. Sexual dimorphism in adulthood is explained by either the effect of sex hormones, e.g. the potent anti-inflammatory effects of oestrogen in females, or epigenetic regulation that determines stronger anti-infection capacity of female immune cells (Camporez et al., 2019; Gal-Oz et al., 2019; Shepherd et al., 2021).

The composition of $PM_{2.5}$ includes different types and amounts of heavy metals such as iron (which is mostly abundant), copper, potassium, calcium, zinc, nickel, sodium, manganese, magnesium, chromium and cadmium (Kim et al., 2010). Previous studies have demonstrated the role of fine heavy metal particulates in inducing oxidative stress, DNA damage and inflammatory repones (Karlsson et al., 2006; Karlsson, Nilsson and Möller, 2005; Seaton et al., 2005). Here, we showed increased oxidative stress / oxidative damage in both male and female offspring.

$PM_{2.5}$ has been shown to demonstrate a strong oxidative potency that reaches the fetal circulation (Bové et al., 2019; Leni, Künzi and Geiser, 2020; Li, Xia and Nel, 2008). Intracellular ROS are by-products of ATP synthesis (Nguyen et al., 2015), which are normally scavenged by the endogenous antioxidant system, such as MnSOD, to maintain a balance between antioxidants and prooxidants (Che et al., 2014; Daenen et al., 2019). MnSOD is one of the key antioxidative enzymes involved in oxidative stress in CKD (Nguyen et al., AJP, 2015). Male PM offspring displayed reduced cytosolic MnSOD but increased mitochondrial MnSOD and oxidative stress injury marker 8-OHdG, suggesting

an overall reduction in antioxidant capacity and an adaptive/stress response in the mitochondria. The Tom20 and OXPHOS complex IV were also reduced in male kidneys, suggestive of reduced substrate transportation into mitochondria for ATP synthesis. This may explain the increased mitochondrial MnSOD that normally scavenges ROS that are generated during ATP production mediated by COXIV that was also reduced in male offspring. At the same time, reduced cytosolic MnSOD may suggest oxidative stress induced by unknown mechanisms, such as protein misfolding observed in other organs (Onoda et al., 2020), which is beyond the scope of the current study and requires further investigation. The fact that increased 8-OHdG was observed in female PM offspring despite unchanged levels of MnSOD suggests that other elements of the antioxidant defence may be impaired. Further studies are needed to confirm this hypothesis. Moreover, increased proinflammatory cytokines and oxidative stress are critical contributors to renal diseases (Mihai et al., 2018). Therefore, maternal $PM_{2.5}$ exposure-induced inflammation and oxidative stress may also be accountable for the profibrotic changes in the offspring's kidney, reflected by PAS staining.

The mitochondrion is the powerhouse of the cell, the principal site for ATP synthesis via the OXPHOS complexes, ensuring sufficient vitality to cells and tissues throughout the body. Prolonged $PM_{2.5}$ exposure-associated ROS overproduction can cause mitochondrial depolarisation (Yang et al., 2018). Similar to MnSOD, other mitochondrial markers, COXIV and TOM20, were most affected in the male offspring. These two proteins were still suppressed in the male pre-exposure groups, suggesting permanent impacts of maternal $PM_{2.5}$ exposure on mitochondrial functional units. Such an effect may affect cellular energy suppliers in the offspring, as discussed above. Interestingly, COXIV expression was markedly upregulated in the female pre-exposure group, likely due to an adaptive response. It is unclear if such adaptation would affect the cellular function when the offspring age. As such, it would be interesting to follow these offspring to aged stages. Overall, mitochondrial OXPHOS complexes in male and female offspring, were relatively stable among the three groups.

Although $PM_{2.5}$ exposure during the pre-gestation period did not improve MnSOD levels in the males and induced macrophage infiltration in the females, it mitigated DNA oxidation, kidney function and pathology in both offspring. This study hence demonstrated that continuous exposure to low level of $PM_{2.5}$ from the pre-gestation period and during gestation has detrimental effects on the offspring's kidney but this can be reversed if $PM_{2.5}$ exposure ceased during gestation. Metals present in polluted air or PM can cross the placental membrane and directly affect the foetus (Liu et al., 2021; Reichrtová, Dorociak and Palkovicová, 1998), which might explain the reason for the adverse effect of low $PM_{2.5}$ levels. Additional studies are required to confirm this.

Conclusion

Low levels of $PM_{2.5}$ exposure had a marginal impact on the dams' kidney health, albeit with some increase in inflammatory markers. However, such a "mild" environmental factor exerted long-lasting adverse impacts on the kidney histological integrity in both male and female offspring if exposure occurs in their dams.

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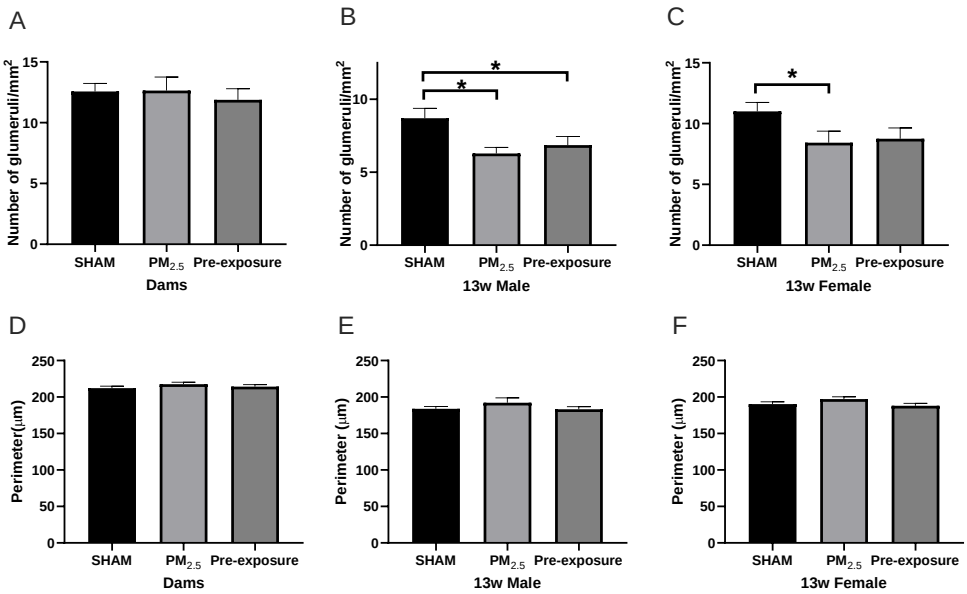
548 Table 1. Anthropometric parameters in the dams and offspring of both sexes

	Anthropometry	SHAM	$PM_{2.5}$	Pre-exposure
Dams	Body weight (g)	27.2 ± 1.44	24.9 ± 0.53*	28.2 ± 2.46##
	Kidney weight (g)	0.22 ± 0.019	0.19 ±0.014*	0.21 ± 0.016
	Kidney weight/body weight	0.78 ± 0.05	0.78 ±0.069	0.76 ±0.07
Day 1 male	Body weight (g)	1.76±0.226	1.55±0.221*	1.56±0.303*
	Kidney weight (g)	0.0104±0.00057	0.0074±0.00064**	0.0098±0.00108#
	Kidney weight/body weight (%)	0.5929± 0.0318	0.4710±0.0335*	0.6119±0.0415#
13-week male	Body weight (g)	25.7± 1.23	26.6±1.9	26.3±1.5
	Kidney weight (g)	0.23 ± 0.005	0.23±0.007	0.24 ± 0.009
	Kidney weight/body weight (%)	0.90± 0.02	0.88±0.02	0.91±0.02
	Urine albumin / creatinine (µg/mg)	23.9 ± 2.50	64.3 ± 16.7*	14.6 ± 3.9##
Day 1 female	Body weight (g)	1.55±0.208	1.62±0.256	1.49±0.308
	Kidney weight (g)	0.0082±0.00049	0.0081±0.00074	0.0093±0.00072
	Kidney weight/body weight (%)	0.5305±0.0316	0.4957±0.0427	0.6186±0.0388
13-week female	Body weight (g)	21.7±1.91	20.1±1.88*	20.8 ±0.593
	Kidney weight (g)	0.15±0.005	0.14±0.003*	0.15±0.003
	Kidney weight/body weight (%)	0.707±0.020	0.708±0.026	0.735±0.013
	Urine albumin / creatinine (µg/mg)	10 ± 3.39	16 ± 6.46	12 ± 7.02

549 Results are expressed as mean ± SEM and were analysed by one-way ANOVA followed by Turkey
550 post hoc tests. N=9-14. *P< 0.05 SHAM vs $PM_{2.5,}$ SHAM vs Pre-exposure. #P< 0.05, ##P< 0.01 $PM_{2.5}$
551 vs Pre-exposure. P: postnatal day. $PM_{2.5}$: maternal $PM_{2.5}$ exposure prior to mating for 6 weeks, during
552 gestation and lactation. Pre-exposure: maternal exposure to $PM_{2.5}$ for only 6 weeks prior to mating.
553



554
555 Figure 1. Glomerular number (A, B, C) and Glomerulus size (D, E, F) in the dams, 13 weeks old male
556 and female offspring (n=5). Data were analysed by one-way ANOVA followed by Turkey post hoc
557 tests. *P < 0.05. $PM_{2.5}$: dams exposed to $PM_{2.5}$ (5µg/day) prior to mating for 6 weeks, during gestation

and lactation. Pre-exposure: dams exposed to $PM_{2.5}$ for 6 weeks prior to mating only.

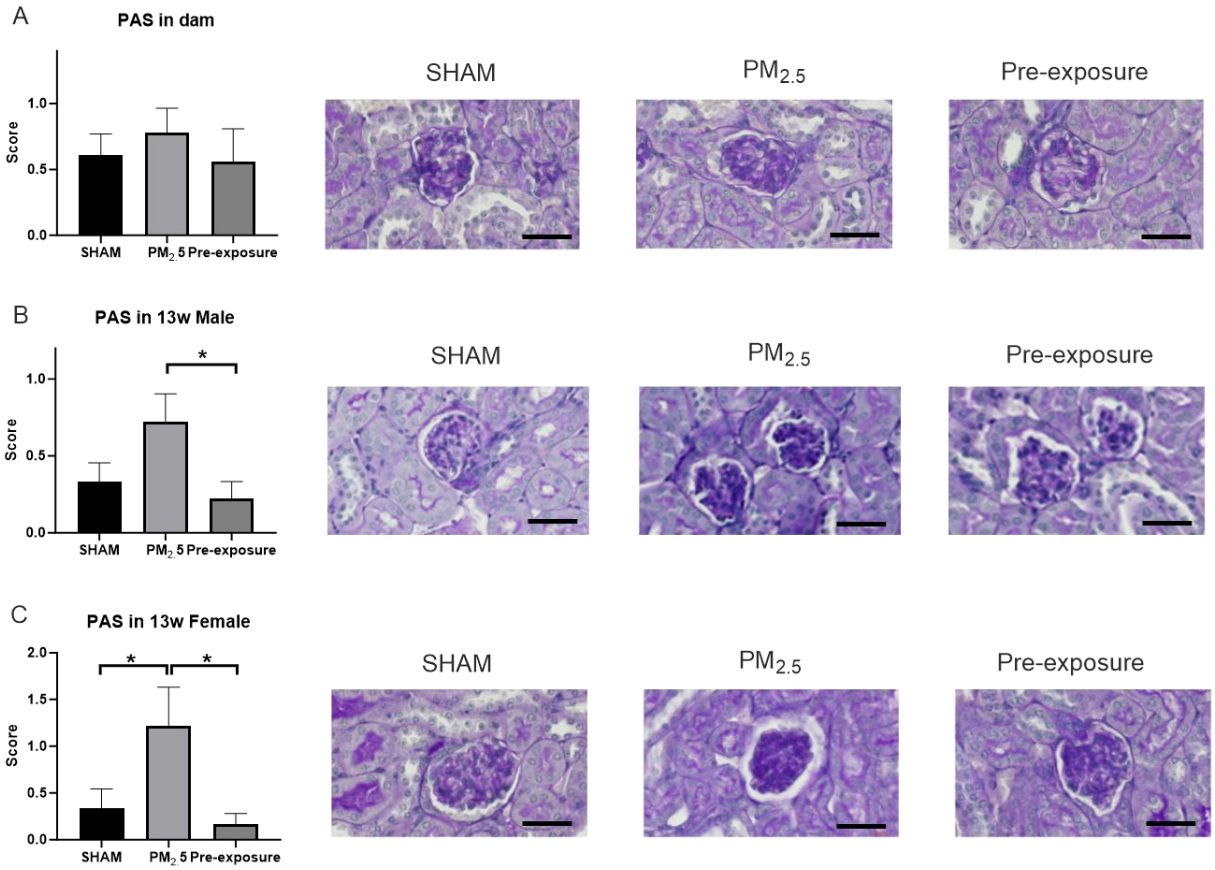


Figure 2. PAS staining of the glomeruli and representative images (mag 10x) in the dam (A), 13 weeks old male (B) and female (C) offspring (n=5). Data were analysed by one-way ANOVA followed by Turkey post hoc tests. *P<0.05. $PM_{2.5}$: dams exposed to $PM_{2.5}$ (5µg/day) prior to mating for 6 weeks, during gestation and lactation. Pre-exposure: dams exposed to $PM_{2.5}$ for 6 weeks prior to mating only. Scale bar is equivalent to 100µm

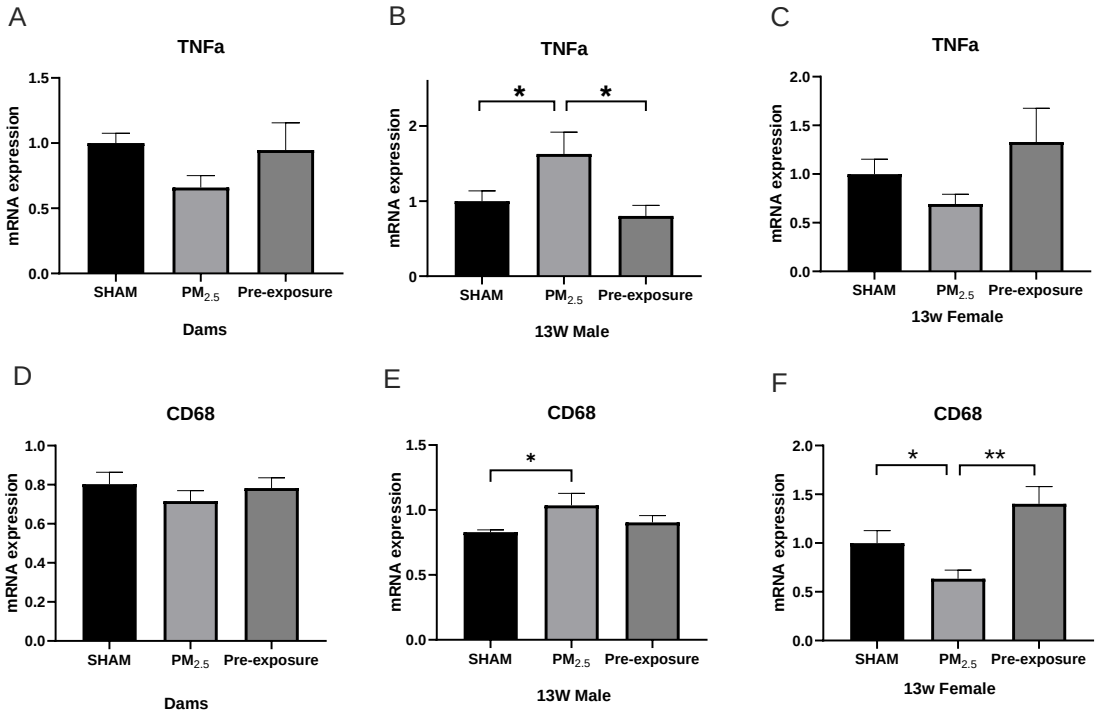


Figure 3. Renal levels of TNFα mRNA (A, B, C) and CD68 mRNA (D, E, F) in dams, 13 weeks old male and female offspring (n=7-8). Data were analysed by one-way ANOVA followed by Turkey post hoc tests. *P < 0.05, **P < 0.01. $PM_{2.5}$: dams exposed to $PM_{2.5}$ (5µg/day) prior to mating for 6 weeks, during gestation and lactation. Pre-exposure: dams exposed to $PM_{2.5}$ for 6 weeks prior to mating only.

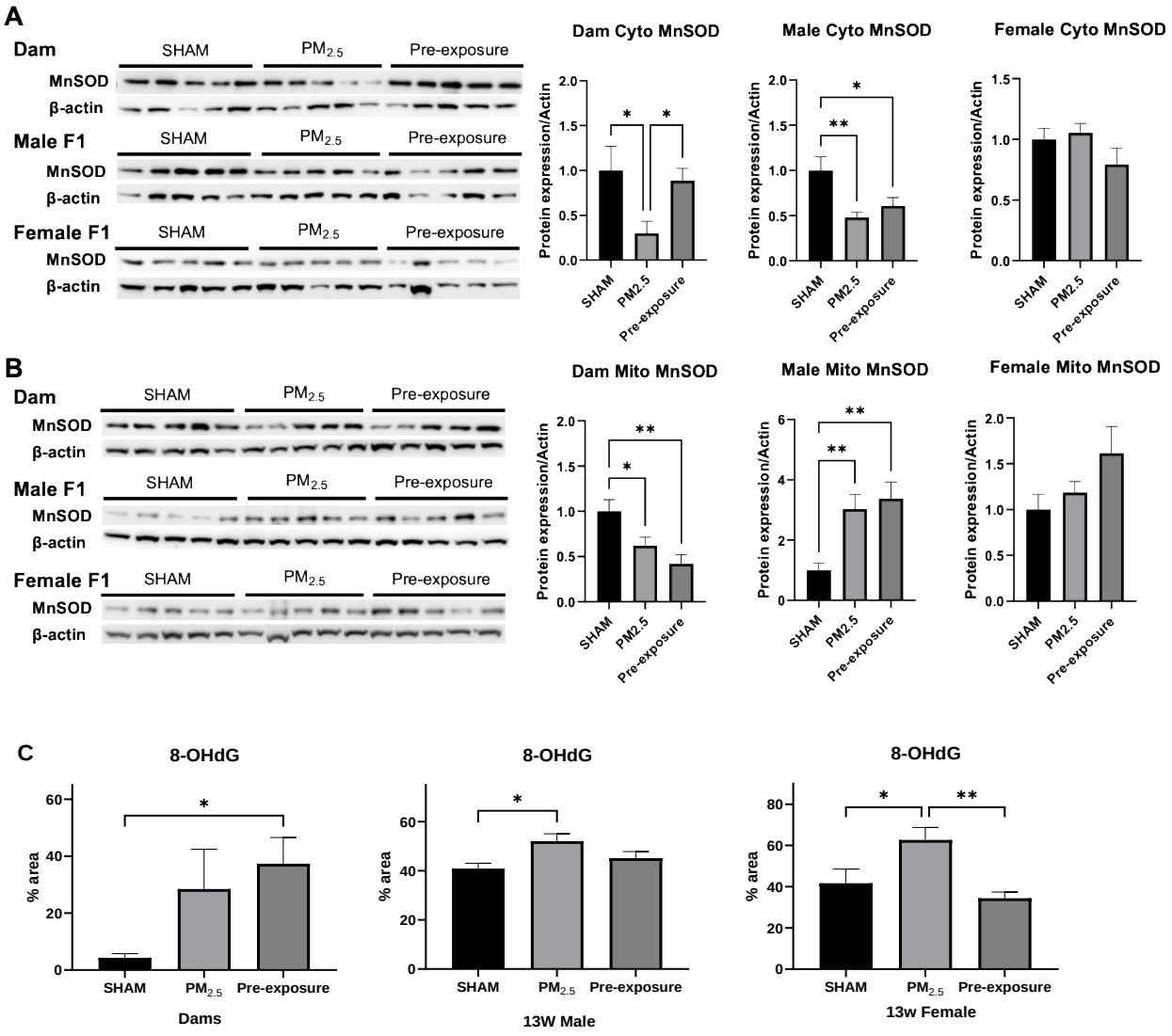


Figure 4. Cytosolic (A) and mitochondrial (B) MnSOD protein levels, as well as 8-OHdG staining in the kidneys in the dam, 13 weeks old male and female offspring (n=5). Data were analysed by one-way ANOVA followed by Turkey post hoc tests. *P<0.05, **P<0.01.

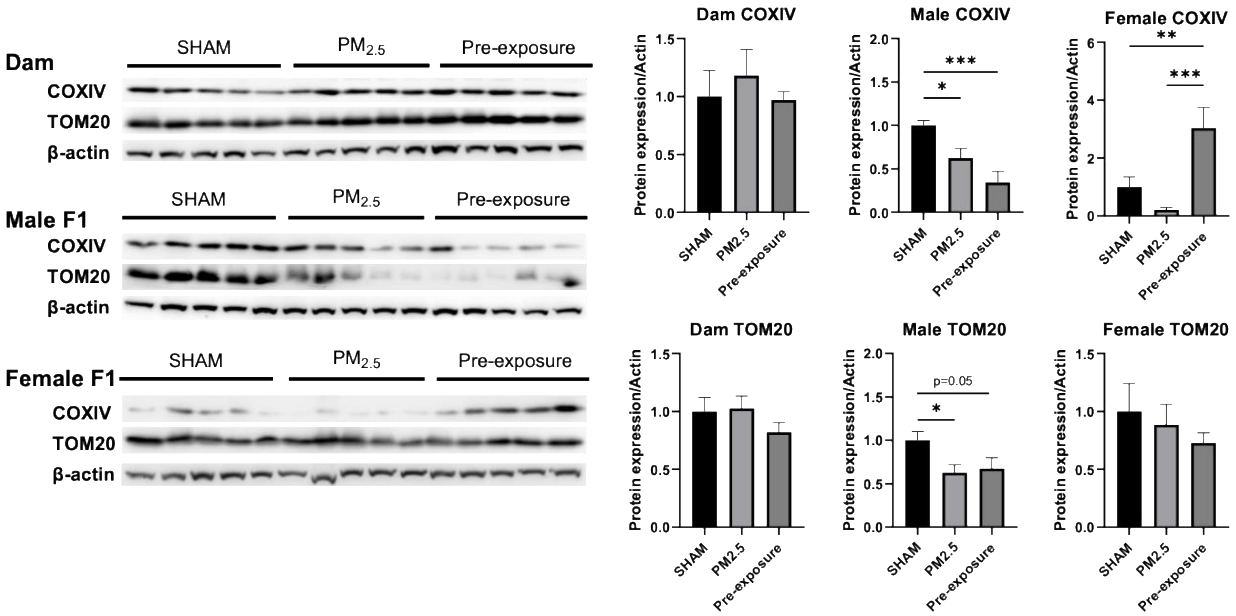


Figure 5. COXIV and TOM20 protein levels in the kidneys in the dam, 13 weeks old male and female offspring (n=5). Data were analysed by one-way ANOVA followed by Turkey post hoc tests. *P<0.05, **P<0.01, ***P<0.001.

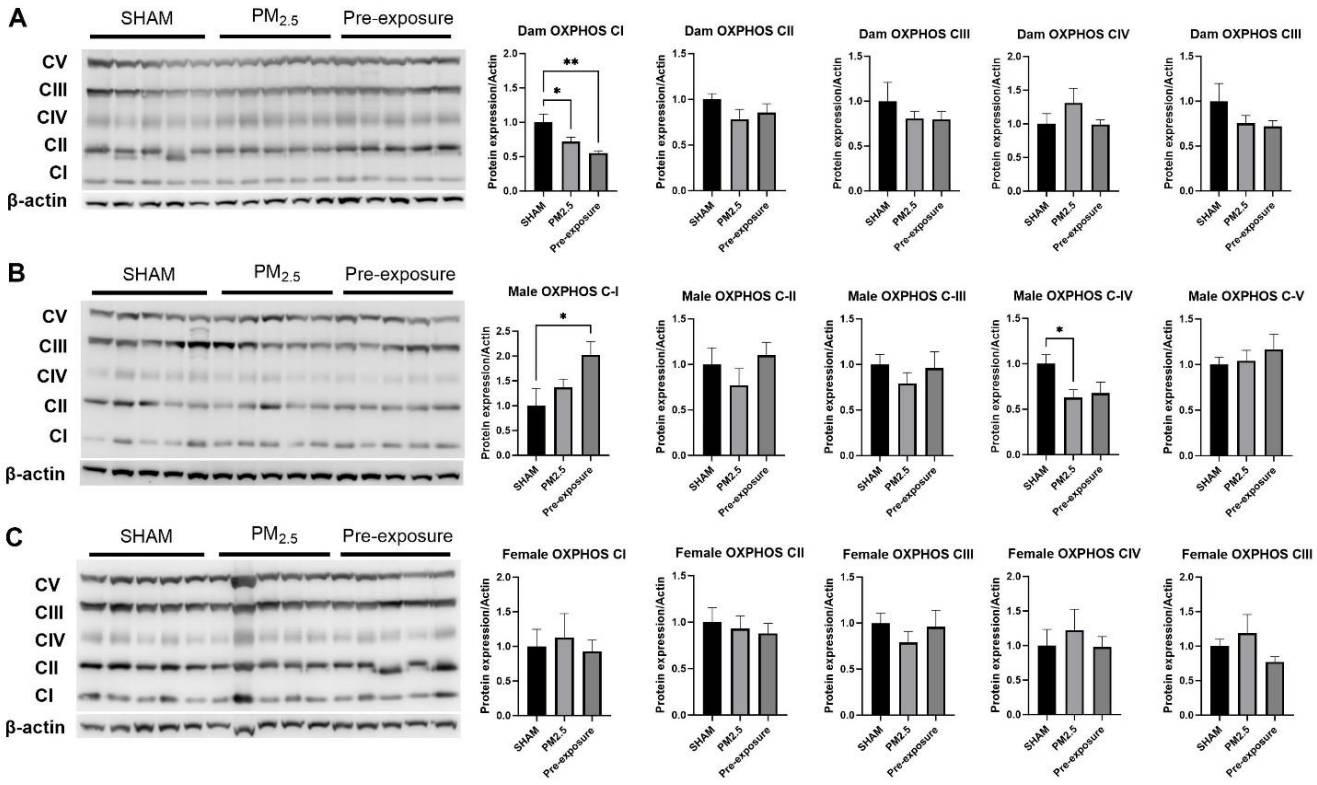


Figure 6. OXPHOS complexes I-V protein levels in the kidneys in the dam (A), 13 weeks old male (B) and female offspring (C) (n=5). Data were analysed by one-way ANOVA followed by Turkey post hoc tests. *P<0.05, **P<0.01.