Pre- and postnatal exposure of mice to concentrated urban $PM_{2.5}$ decreases the number of alveoli and leads to altered lung function at an early stage of life.

Thais de Barros Mendes Lopes, Espen E. Groth, Mariana Veras, Tatiane K. Furuya, Natalia de Souza Xavier Costa, Gabriel Ribeiro Júnior, Fernanda Degobbi Lopes, Francine M. de Almeida, Wellington V. Cardoso, Paulo Hilario Nascimento Saldiva, Roger Chammas, Thais Mauad

Supplementary Material

Pregnancy outcomes

Table S1. Prenatal pregnancy outcomes of CAP-exposed and control dams and the respective *n* of fetuses used in analyses, if any.

Time point	Group	Dam	Fetuses	Resorpt.	*n* stereology	*n* microarray analysis
E14.5	Exposed	#1	13	0	2	3
		#2	7	3	2	1
		#3	2	5	2	
	Control	#1	4	4	3	2
		#2	9	1	2	2
E18.5	Exposed	#1	6	2	2	1
		#2	5	2	2	1
		#3	3	3	1	1
		#4	6	1		1
	Control	#1	3	3	1	
		#2	6			1
		#3	4	2	2	1
		#4	2		1	1
		#5	1	2		1

Resorpt.: Resorptions.

Table S2. Litter size and sex distribution of offspring and *n* of offspring mice used in analyses (at P40) per dam.

Group	Dam	Litter size (m,f)	Dead-born	*n* lung function	*n* BAL	*n* stereology	*n* microarray analysis
Exposed	#1	7 (4,3)		6	3	3	2
	#2	2 (0,2)	1	2	1	1	1
	#3	5 (4,1)		5	5	1	2
Control	#1	4 (0,4)		4	4	2	
	#2	3 (1,2)		2	3	3	2
	#3	3 (1,2)		3	2		1
	#4	6 (4,2)		4	5		2
	#5	3 (2,1)		2	3		

m: males, f: females.

Characterization of $PM_{2.5}$ composition

As previously described (Andrade et al., 2012; de Miranda et al., 2012; Mauad et al., 2008), we assessed the metal elemental composition and black carbon (BC) concentration of ambient air $PM_{2.5}$ during the months of animal exposure. In short, $PM_{2.5}$ was collected in suitable polycarbonate filter membranes. We used X-ray fluorescence spectrometry to measure the concentration of metal trace elements (Na, Al, Si, P, S, K, Ca, Ti, V, Fe, Nu, Cu, Zn, Pb) in a quantitative manner (n=17). Furthermore, a smoke stain reflectometer was used to determine black carbon (BC) concentration (n=15).

Table S3. Ambient air concentration as well as black carbon (BC) and metal trace element content of $PM_{2.5}$ sampled at our exposure site.

Parameter		Mean ± SD	Min	Max
$PM_{2.5}$	($\mu g \cdot m^{-3}$)	19.8 ± 11.4	7.2	52.9
BC	($\mu g \cdot m^{-3}$)	7.6 ± 6.5	1.2	27.0
$BC/PM_{2.5}$	(%)	38.2 ± 15.8	17.3	74.2
Na	($ng \cdot m^{-3}$)	200.9 ± 171.3	0.0	496.6
Mg	($ng \cdot m^{-3}$)	0.2 ± 0.8	0.0	3.1
Al	($ng \cdot m^{-3}$)	90.5 ± 83.6	0.0	301.9
Si	($ng \cdot m^{-3}$)	361.4 ± 206.5	121.2	887.4
P	($ng \cdot m^{-3}$)	33.5 ± 26.5	4.9	106.0
S	($ng \cdot m^{-3}$)	1441.0 ± 988.2	429.4	4465.8
Cl	($ng \cdot m^{-3}$)	25.0 ± 29.6	1.9	123.7
K	($ng \cdot m^{-3}$)	354.9 ± 357.0	41.6	1397.0
Ca	($ng \cdot m^{-3}$)	97.2 ± 52.2	11.0	198.3
Ti	($ng \cdot m^{-3}$)	9.3 ± 7.5	0.7	27.1
V	($ng \cdot m^{-3}$)	0.9 ± 1.2	0.0	4.6
Cr	($ng \cdot m^{-3}$)	0.3 ± 0.7	0.0	2.6
Mn	($ng \cdot m^{-3}$)	4.9 ± 3.0	0.3	11.0
Fe	($ng \cdot m^{-3}$)	182.9 ± 117.5	23.6	426.8
Ni	($ng \cdot m^{-3}$)	0.7 ± 0.6	0.0	1.9
Cu	($ng \cdot m^{-3}$)	6.6 ± 7.4	0.0	28.5
Zn	($ng \cdot m^{-3}$)	66.6 ± 44.7	10.5	169.8
Se	($ng \cdot m^{-3}$)	2.5 ± 3.0	0.0	11.4
Br	($ng \cdot m^{-3}$)	6.8 ± 10.0	0.0	40.8
Pb	($ng \cdot m^{-3}$)	7.8 ± 6.1	0.3	19.3

Lung function in male and female animals

Table S4. Lung function (flexiVent) of exposed and control mice at P40 (males).

Parameter		Mean ± SD		*p*
		Exposed	Control	
Weight	(g)	20.1 ± 1.6	23.1 ± 1.5	0.006**
R	(cm $H_2O \cdot s \cdot mL^{-1}$)	0.81 ± 0.17	0.72 ± 0.15	0.35
E	(cm $H_2O \cdot mL^{-1}$)	32.37 ± 6.22	26.83 ± 3.47	0.10
R_n	(cm $H_2O \cdot s \cdot mL^{-1}$)	0.33 ± 0.13	0.25 ± 0.08	0.25
G	(cm $H_2O \cdot mL^{-1}$)	5.51 ± 2.23	5.44 ± 1.18	0.95
H	(cm $H_2O \cdot mL^{-1}$)	30.43 ± 5.61	22.73 ± 2.88	0.02*

n=8 in exposed, *n*=5 in control group. R: dynamic resistance, E: dynamic elastance, R_n: Newtonian resistance, G: tissue damping, H: tissue elastance.

Table S5. Lung function (flexiVent) of exposed and control mice at P40 (females).

Parameter		Mean ± SD		*p*
		Exposed	Control	
Weight	(g)	19.8 ± 1.6	19.5 ± 2.0	0.83
R	(cm $H_2O \cdot s \cdot mL^{-1}$)	0.85 ± 0.11	0.77 ± 0.13	0.31
E	(cm $H_2O \cdot mL^{-1}$)	37.4 ± 9.70	31.10 ± 3.85	0.09
R_n	(cm $H_2O \cdot s \cdot mL^{-1}$)	0.26 ± 0.03	0.30 ± 0.09	0.37
G	(cm $H_2O \cdot mL^{-1}$)	6.72 ± 1.04	6.22 ± 0.96	0.37
H	(cm $H_2O \cdot mL^{-1}$)	35.64 ± 11.00	29.25 ± 4.75	0.13

n=5 in exposed, *n*=10 in control group. R: dynamic resistance, E: dynamic elastance, R_n: Newtonian resistance, G: tissue damping, H: tissue elastance.

Bronchoalveolar lavage

Table S6. Differential cell counts in BAL fluid of exposed and control animals at P40.

Cell type		**Mean ± SD**		***p***
		Exposed	Control	
Neutrophils		0.00	0.08 ± 0.13	0.07
Lymphocytes		0.06 ± 0.07	0.07 ± 0.07	0.65
Eosinophils		0.00	0.00	
Macrophages		4.63 ± 2.10	3.70 ± 1.91	0.26
Total		4.69 ± 2.12	3.85 ± 1.94	0.32
Neutrophils	(%)	0.0	1.9 ± 3.3	0.09
Lymphocytes	(%)	1.2 ± 1.7	1.9 ± 1.9	0.37
Eosinophils	(%)	0.0	0.0	
Macrophages	(%)	98.8 ± 1.7	96.1 ± 4.2	0.09
Epithelial cells		0.33 ± 0.24	0.54 ± 0.51	0.25

Values in 10^4 cells·mL^{-1} if not mentioned differently.

Stereology

Table S7. Stereological measures of lungs of prenatally exposed and control fetuses at E14.5 (pseudoglandular stage).

Parameter		**Mean ± SD**		***p***
		Exposed	Control	
Fetus weight	(g)	0.146 ± 0.060	0.114 ± 0.057	0.40
Lung volume	(mm^3)	0.90 ± 0.43	0.62 ± 0.45	0.32
Volume/weight ratio	($mm^3 \cdot g^{-1}$)	6.36 ± 2.32	4.99 ± 2.56	0.37
V_f of mesenchyme	(%)	49.2 ± 6.5	55.4 ± 6.1	0.14
V_f of epithelial tubes	(%)	36.0 ± 6.1	30.2 ± 6.9	0.17
V_f of vessels	(%)	14.8 ± 1.3	14.4 ± 2.4	0.73
V_t of mesenchyme	(mm^3)	0.43 ± 0.16	0.33 ± 0.22	0.40
V_t of epithelial tubes	(mm^3)	0.34 ± 0.21	0.21 ± 0.18	0.29
V_t of vessels	(mm^3)	0.13 ± 0.07	0.08 ± 0.06	0.25

V_f: volume fraction, V_t: total volume.

Table S8. Stereological measures of lungs of prenatally exposed and control fetuses at E18.5 (saccular stage).

Parameter		**Mean ± SD**		***p***
		Exposed	Control	
Lung weight	(g)	0.022 ± 0.004	0.027 ± 0.005	0.19
Lung volume	(mm^3)	15.37 ± 6.91	23.00 ± 12.22	0.27
Volume/weight ratio	($mm^3 \cdot g^{-1}$)	685.99 ± 312.35	832.96 ± 387.59	0.55
V_f of mesenchyme	(%)	64.0 ± 5.9	63.4 ± 4.6	0.86
V_f of airways	(%)	8.1 ± 3.8	9.3 ± 1.0	0.56
V_f of vessels	(%)	5.3 ± 3.5	4.6 ± 2.4	0.73
V_f of saccules	(%)	22.6 ± 3.3	22.8 ± 5.4	0.95
V_t of mesenchyme	(mm^3)	10.05 ± 4.94	14.41 ± 7.64	0.33
V_t of airways	(mm^3)	1.22 ± 0.78	2.05 ± 0.94	0.19
V_t of vessels	(mm^3)	0.66 ± 0.23	0.900 ± 0.53	0.38
V_t of saccules	(mm^3)	3.44 ± 1.63	5.65 ± 3.49	0.25
S_D of saccules	(mm^{-1})	1.28 ± 0.27	1.05 ± 0.05	0.15
S_A of saccules	(mm^2)	20.28 ± 10.589	23.93 ± 12.60	0.65

V_f: volume fraction, V_t: total volume, S_D: surface density, S_A: surface area.

Microarray analysis

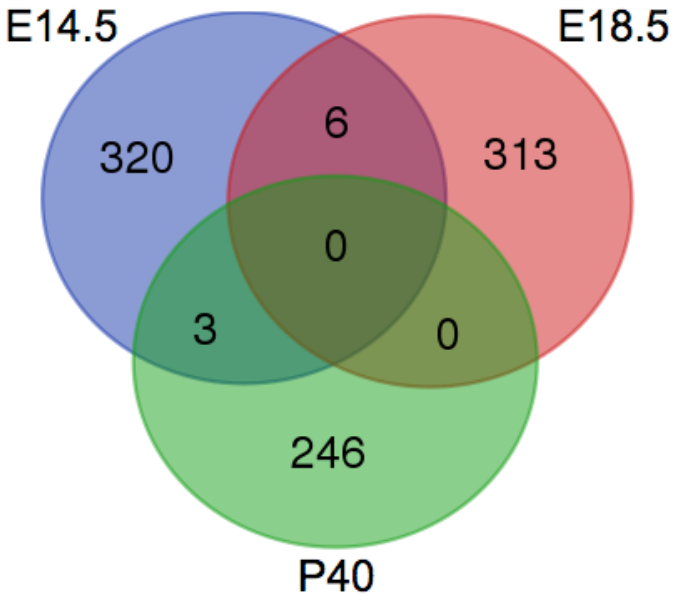

Figure S1. Venn diagram of DEGs at E14.5, E18.5 and P40.

Table S9. DEGs E14.5 ∩ E18.5.

Gene Symbol	Gene Description	E14.5		E18.5	
		log_2 FC	*p*	log_2 FC	*p*
C87198	expressed sequence C87198	0.74	0.0009	-0.62	0.0098
Ccr2	chemokine (C-C motif) receptor 2	0.78	0.0016	-0.47	0.0055
G3bp2	GTPase activating protein (SH3 domain) binding protein 2	-0.08	0.0038	0.26	0.0076
Cyyr1	cysteine and tyrosine-rich protein 1	0.54	0.0013	-0.48	0.0092
Trpc1	transient receptor potential cation channel, subfamily C, member 1	0.34	0.00005	-0.39	0.0068
LOC105243101	keratin-associated protein 5-4-like	-0.34	0.0098	0.68	0.0100

Table S10. DEGs E14.5 ∩ P40.

Gene Symbol	Gene Description	E14.5		P40	
		log_2 FC	*p*	log_2 FC	*p*
Rxfp2	relaxin/insulin-like family peptide receptor 2	-0.86	0.0041	-0.32	0.0029
Fopnl	Fgfr1op N-terminal like	0.15	0.0012	-0.25	0.0097
Olfr1371	olfactory receptor 1371	0.25	0.0019	0.23	0.00005

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