

SUPPLEMENTARY MATERIALS

Modelling the impact of chronic cigarette smoke exposure in obese mice: metabolic, pulmonary, intestinal, and cardiac issues

Emilie Dubois-Deruy^{1#}, Gaëlle Rémy^{2#}, Jeanne Alard², Gwenola Kervoaze², Maggy Chwastyniak¹, Morgane Baron², Delphine Beury², Léa Siegwald², Ségolène Caboche², David Hot², Philippe Gosset², Corinne Granette², Florence Pinet¹, Isabelle Wolowczuk² and Muriel Pichavant^{2*}

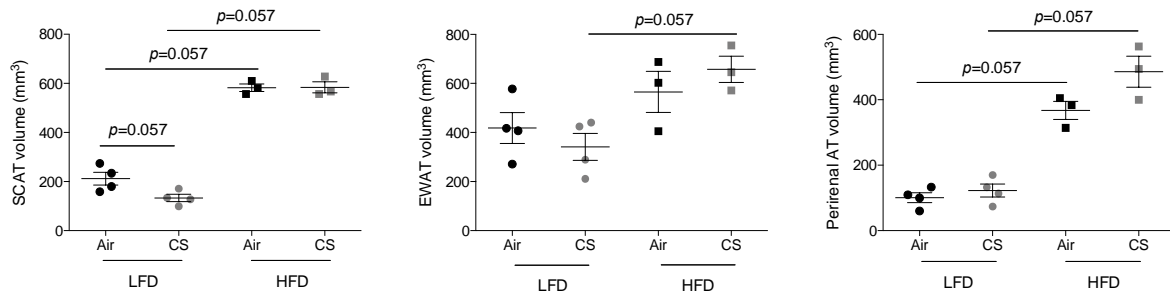


Figure S1: Fat depot distribution in lean and obese mice chronically exposed to cigarette smoke. Magnetic resonance imaging (MRI) was used to draw longitudinal slices of lean (LFD) and obese (HFD) mice exposed to Air or to CS. The manual labeling of the different fat depots was performed using the segmentation tool ITK-SNAP, and SCAT, EWAT and peri-renal adipose tissue volumes were calculated using the implementation of ITK-SNAP (n = 3-4 mice per group). Data are expressed as individual and mean ± SEM values.

Table S1: Disease scoring of lung remodeling.

Lesion distribution	No Lesion	1 to 25% of parenchyma	26 to 50% of parenchyma	51 to 75% of parenchyma	> 75% of parenchyma
Score	0	1	2	3	4
Alveolar wall thickness	< 1 erythrocyte	1 to 2 erythrocytes	3 to 5 erythrocytes	6 to 10 erythrocytes	> 10 erythrocytes
Score	0	1	2	3	4
Hyaline membrane	no			yes	
Score	0			1	
Alveolar inflammation (PNN)	Absent	< 10 / HP field	10 to 20 / HP field	21 to 50 / HP field	> 50 / HP field
Score	0	1	2	3	4
Alveolar suppuration	no			yes	
Score	0			1	
Bronchiolar epithelium necrosis	no			yes	
Score	0			1	
Peribronchiolar	Absent	< 10 / HP	10 to 20 /	21 to 50 /	> 50 / HP

inflammation, PNN		field	HP field	HP field	field
Score	0	1	2	3	4
Peribronchiolar inflammation, mononuclear cells	Absent	< 10 / HP field	10 to 20 / HP field	21 to 50 / HP field	> 50 / HP field
Score	0	1	2	3	4
Vasculitis	no			yes	
Score	0			1	
Fibrinoid necrosis of vascular wall	no			yes	
Score	0			1	

Randomly selected areas of lung sections were analyzed (see Figure S2a).

The score calculated from the distribution and type of lesions is highlighted in grey. HP: high power (400x).

Table S2: Relative abundance of caecal bacteria in lean (LFD) and obese (HFD) mice exposed or not (Air) to cigarette smoke (CS).

Taxonomy	LFD		HFD			P value
	Air	CS	Air	CS		
Actinobacteria phylum	4.86 ± 0.85^a	2.32 ± 0.74^{a,b}	2.21 ± 0.80	0.16 ± 0.07^b		0.038 ^a , 0.028 ^b
Coriobacteriaceae Family	0.00 ± 0.00	0.00 ± 0.00	1.86 ± 0.63	0.00 ± 0.00		0.033
Bifidobacteriaceae Family						
<i>Bifidobacterium</i> Genus	4.70 ± 0.87^a	2.22 ± 0.68^b	0.38 ± 0.22^a	0.00 ± 0.00^b		0.029 ^a , 0.015 ^b
Bacteroidetes phylum	14.25 ± 3.80	8.40 ± 1.54	12.44 ± 6.78	10.84 ± 3.77		
Deferribacteres phylum	0.00 ± 0.00^a	0.94 ± 0.26^{a,c}	0.00 ± 0.00^b	0.90 ± 0.28^b		0.038 ^a , 0.038 ^b , 0.009 ^c
Firmicutes phylum	52.43 ± 3.08	65.14 ± 3.69^a	63.89 ± 6.75	66.75 ± 4.01		0.041 ^a
Clostridia Class	26.17 ± 2.02	33.25 ± 2.46	37.81 ± 0.05	42.28 ± 2.52		
Ruminococcaceae Family	4.40 ± 0.52	4.16 ± 0.35^a	3.51 ± 0.28	4.70 ± 0.87		0.002 ^a
Clostridiaceae Family	2.49 ± 1.41	5.77 ± 0.98^{a, b}	7.66 ± 1.43	9.01 ± 0.76^b		0.002 ^a , 0.026 ^b
Peptostreptococcaceae Family	1.10 ± 0.70^a	4.34 ± 0.46^{a, b, c}	5.09 ± 0.74	6.06 ± 0.38^c		0.019 ^a , 0.004 ^b , 0.015 ^c
Proteobacteria phylum	20.07 ± 2.11	15.46 ± 2.01^a	10.61 ± 1.90	13.60 ± 1.02		0.004 ^a
Deltaproteobacteria Class						
Desulfovibrionaceae Family	6.42 ± 0.86^a	4.18 ± 0.20^{a, b}	3.79 ± 0.73	4.47 ± 0.35		0.019 ^a , 0.002 ^b
Desulfovibrio genus	3.71 ± 0.34^{a, d}	2.70 ± 0.06^{a, c, e}	0.00 ± 0.00^{b, d}	1.62 ± 0.23^{b, e}		0.016 ^a , 0.010 ^b , 0.004 ^c , 0.029 ^d , 0.009 ^e
Tenericutes phylum	0.16 ± 0.16	0.19 ± 0.09	0.21 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
TM7 phylum	1.87 ± 0.13^a	2.76 ± 0.21^{a, b}	1.09 ± 0.63	2.59 ± 0.40		0.010 ^a , 0.041 ^b
Unclassified phylum	4.74 ± 0.48	3.98 ± 0.16	5.10 ± 0.30	4.87 ± 0.29	4.96 ± 0.31	0.015

Data are expressed as mean ± SEM values. Data significantly different between groups are marked in bold. *P* values are only indicated for significantly different data with an uppercase letter corresponding to the group analyzed.