Supporting Information

Early-Life Persistent Vitamin D Deficiency Alters Cardiopulmonary Responses to Particulate Matter-Enhanced Atmospheric Smog in Adult Mice

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Running title: Vitamin D deficiency alters response to smog

Materials and methods

Surgical Implantation of radiotelemeters and data acquisition – Animals were anesthetized using inhaled isoflurane (Isothesia, Butler Animal Health Supply, Dublin OH). Anesthesia was induced by spontaneous breathing of 2.5% isoflurane in pure oxygen at a flow rate of 1 L/min and then maintained by 1.5% isoflurane in pure oxygen at a flow rate of 0.5 L/min; all animals received the analgesic buprenorphrine (0.03 mg/kg, i.p.). Using aseptic technique, each animal was implanted subcutaneously with a radiotelemeter (ETA-F10, Data Sciences International, St Paul, MN); the transmitter was placed under the skin to the right of the midline on the dorsal side. Two electrode leads were then tunneled subcutaneously across the lateral dorsal sides; the distal portions were fixed in positions that approximated those of the lead II of a standard electrocardiogram (ECG). Body heat was maintained both during and immediately after the surgery. Animals were given food and water post-surgery and were housed individually. All animals were allowed 7-10 days to recover from the surgery and reestablish circadian rhythms.

Heart Rate and Electrocardiogram Analysis -Sixty-second ECG segments were recorded every 15 minutes during the pre- and post-exposure periods and every 5 minutes during exposure (baseline and hours 1-4); HR was automatically obtained from the waveforms (Dataquest ART Software, version 3.01, Data Sciences International, St. Paul, MN, USA). ECGAuto software (EMKA Technologies USA, Falls Church VA) was used to visualize individual ECG waveforms, analyze and quantify ECG segment

durations and areas, as well as identify cardiac arrhythmias as previously described. Briefly, using ECGAuto, Pwave, QRS complex, and T-wave were identified for individual ECG waveforms and compiled into a library. Analysis of all experimental ECG waveforms was then based on established libraries. The following parameters were determined for each ECG waveform: PR interval (P_{start}-R), QRS complex duration (Q_{start}-S), ST segment interval (S-T_{end}) and QT interval (Q_{start}-Tend). QT interval was corrected for HR using the correction formula for mice QTc = QT/(RR/100)1/2. Pre-exposure assessments were measured as the exposure time-matched four hours of data from 24 hours before exposure for each animal. Immediately post-exposure assessments were the four hours of data taken immediately post-exposure. Twenty-four post-exposure assessments were the exposure time-matched four hours of data taken 24 hours after exposure.
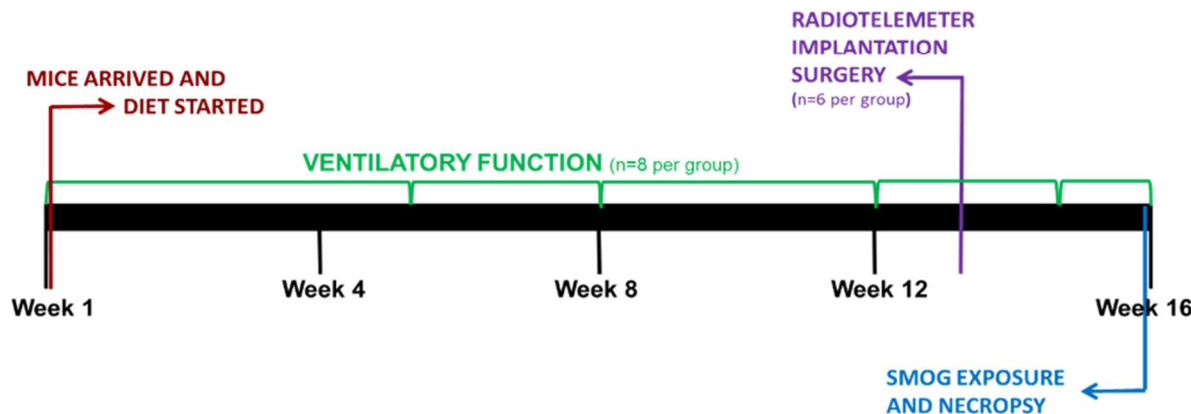
Figures

Figure S1. Experimental design of diet regimen, electrocardiographic/HRV analysis and photochemical smog exposure.
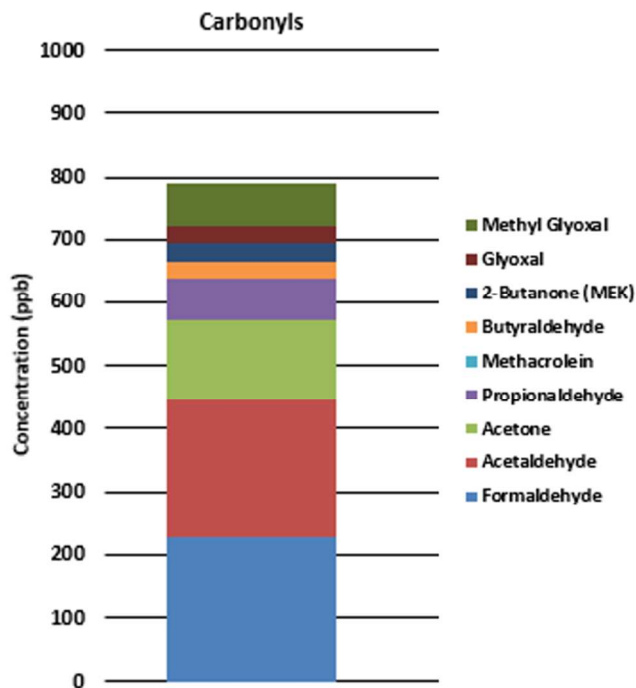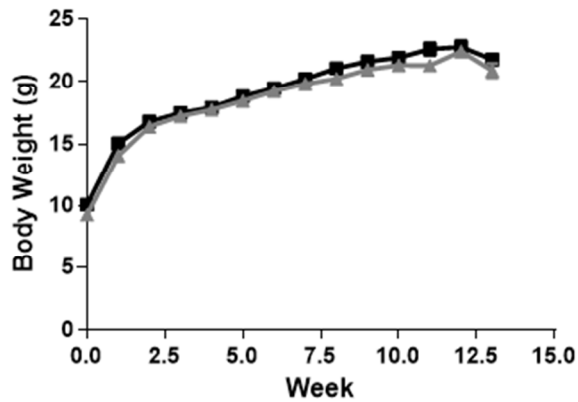

Figure S2. Exposure characteristics of carbonyls in SA-PM atmosphere.
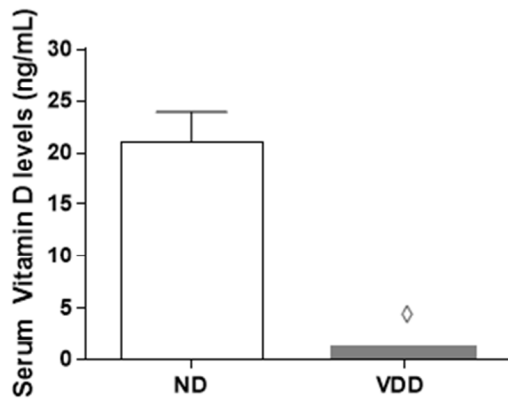
A.

B.

Figure S3. Body weight did not differ and VDD mice did become VDD during diet regimen. A. No significant differences in body weight. **B.** VDD mice had significantly less serum vitamin D levels than the ND mice. $^{\diamond}$Denotes a significant change from ND (p < 0.05). Values represent means ± SEM; n=28 ND mice, n=35 VDD mice.

Tables

Diet	Age (weeks)	Breathing Frequency (f)	Tidal Volume (mL)	Inspiratory Time (msec)	Expiratory Time (msec)	Minute Volume (mL/min)	Ventilatory Timing (penh)
ND	3	474.2±0.6	0.13±0.0	51.69±0.1	83.46±0.1	6.42±0.0	-
	8	475.5±0.5 *	0.23±0.0 *	56.01±0.1 *	76.28±0.1 *	10.73±0.0 *	-
	11	435.2±0.4 *	0.27±0.0 *	64.01±0.1 *	78.29±0.1 *	11.42±0.0 *	-
	15	423.6±0.3 *	0.28±0.0 *	66.26±0.1 *	79.48±0.1 *	11.76±0.0 *	-
	Pre-Exp.	432.8±0.6	0.29±0.0	61.97±0.1	81.69±0.2	12.37±0.0	1.26±0.0
VDD	3	503.0±0.5 ◊	0.15±0.0 ◊	50.40±0.1 ◊	74.72±0.1 ◊	7.41±0.0 ◊	-
	8	483.5±0.5 *◊	0.23±0.0 *◊	54.57±0.1 *◊	75.29±0.1*◊	10.56±0.0 *◊	-
	11	439.5±0.4 *◊	0.24±0.0 *◊	62.35±0.1 *◊	79.56±0.1 *◊	10.55±0.0 *◊	-
	15	426.7±0.41 *◊	0.26±0.0 *◊	65.17±0.1 *◊	80.86±0.1 *◊	10.64±0.0 *◊	-
	Pre-Exp.	435.5±0.6	0.3±0.0	61.75±0.1	81.98±0.2	12.24±0.0	1.54±0.0

Table S1. VDD induced changes in ventilatory function parameters during diet regimen. *Denotes a significant change from 3-week assessment ($p < 0.05$). ◊Denotes a significant change from ND ($p < 0.05$). Values represent means ± SE.

Diet	Exposure	Timing	PR (ms)	QRS (ms)	QTcB (ms)
ND	Filtered Air	**Pre-Exposure**	37.21±0.28	10.38±0.11	56.23±0.65
		Exposure	36.62±0.10 §	7.66±0.14	57.16±0.55
		Immediately Post-Exposure	37.42±0.22	10.85±0.09	59.24±0.67
		24 Hr Post-Exposure	37.58±0.23	10.55±0.06	55.16±0.68
	SA-PM	**Pre-Exposure**	37.22±0.34	10.40±0.08	56.21±0.80
		Exposure	39.07±0.08 ‡	6.43±0.15 ‡	56.53±0.47
		Immediately Post-Exposure	36.27±0.30 ‡	10.60±0.07	54.82±0.79 ‡
		24 Hr Post-Exposure	36.06±0.25 ‡	10.34±0.06	55.11±0.86
VDD	Filtered Air	**Pre-Exposure**	37.27±0.45	10.39±0.10	55.20±0.71
		Exposure	36.49±0.10 §	6.31±0.14 §◊	54.96±0.56
		Immediately Post-Exposure	36.49±0.24 ◊	10.40±0.08 ◊	54.82±0.44 ◊
		24 Hr Post-Exposure	36.31±0.21 ◊	10.42±0.09	55.02±0.57
	SA-PM	**Pre-Exposure**	37.34±0.22	10.38±0.10	56.37±0.46
		Exposure	37.46±0.08 ‡◊	5.35±0.14 ◊	54.13±0.47 ◊
		Immediately Post-Exposure	38.11±0.23 ‡◊	10.46±0.08	54.63±0.41
		24 Hr Post-Exposure	37.15±0.20 ‡◊	10.01±0.06 ‡◊	53.37±0.46

Table S2. SA-PM exposure and VDD induced alterations in electrocardiogram in adult mice. *Denotes a significant change from pre-exposure ($p < 0.05$). †Denotes a significant change from immediately-post exposure ($p < 0.05$).◊Denotes a significant change from ND ($p < 0.05$). ‡Denotes a significant change from filtered air exposure ($p < 0.05$). §Denotes a significant change from baseline of exposure ($p < 0.05$). Values represent means ± SE.

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