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Supporting Information for:

Adverse organogenesis and predisposed long-term metabolic syndrome from prenatal exposure to fine particulate matter

Guoyao Wu, Jacob Brown, Misti L. Zamora, Alyssa Miller, M. Carey Satterfield, Cynthia J. Meininger, Chelsie B. Steinhauser, Gregory A. Johnson, Robert C. Burghardt, Fuller W. Bazer, Yixin Li, Natalie M. Johnson, Mario J. Molina, Renyi Zhang

To whom correspondence may be addressed. Email: renyi-zhang@geos.tamu.edu; g-wu@tamu.edu; mjmolina@ucsd.edu.

This PDF file includes:

Glossary of Acronyms

ADGD	Adrenal gland
ANOVA	Analysis of variance
BAT	Brown adipose tissue
BN	Brain
BW	Body weight
CA	Clean air
CPC	Condensation particle counter
DMA	Differential mobility analyzer
DNA	Deoxyribonucleic acid
EDL	Extensor digitorum longus
EDTA	Ethylenediaminetetraacetic acid
HFD	High-fat diet
HT	Heart
IE	Intestine
KY	Kidney
LFD	Low-fat diet
LG	lungs
LPM	Liter per minute
MTLN	Mesenteric lymph node
NEFA	Nonesterified fatty acids
NC	No change
NO	Nitric oxide
PA	Polluted air
PG	Pregnancy
PG WAT	Peri-gonads white adipose tissue
PM	Particulate matter
$PM_{2.5}$	Particles with an aerodynamic diameter small than 2.5 μm
PND	Postnatal day
PP	Post parturition
RP WAT	Retroperitoneal white adipose tissue
SEM	Standard error of the mean
SI	Small intestine
SM	Soleus muscle
SN	Spleen
SQ	Subcutaneous
TS	Thymus
WAT	White adipose tissue
WHO	World Health Organization

Supplementary text

The Sprague-Dawley rat is a breed of rats that is widely used in medical and nutritional research (1,2). Inside the chambers, rats (one in each sub-chamber within a unit) had free access to a casein-based diet (1) and drinking water. Once dams were placed into chambers, cages were cleaned every other day to prevent the build-up of ammonia. On Day 18 of gestation, dams were removed from their respective chamber and individually placed into normal cages until parturition (term = ~21 days) to prevent direct exposure of pups to polluted air. Therefore, food intake and water consumption were measured for individual dams. Maternal BW, food intake, and water intake were measured on Days 6, 12, and 18 of gestation, and on the day that they gave birth to pups. At birth (PND 0), pups in each litter were weighed individually, sexed, and culled to eight pups (four males, four females). Within each sex, offspring were selected randomly to be euthanized or sacrificed at later time within the study. All culled pups were euthanized and necropsied to determine weights of organs, including heart, liver, spleen, kidneys, brain, pancreas, intestine, lungs, adrenal glands, brown adipose tissue (BAT), and testes; a sample of each of those tissues was snap-frozen in liquid nitrogen for subsequent analyses. The eight remaining pups were reared on their dams in bedded cages in clean ambient air. At weaning (PND21), four pups (two males and two females) were euthanized and necropsied to determine the weights of organs, i.e., heart, liver, spleen, kidneys, brain, pancreas, small intestine, lungs, adrenal glands, gonads, thymus, extensor digitorum longus (EDL) muscle, soleus muscle, BAT, white adipose tissue (WAT), and stomach, and to collect samples of each of those tissues. Tissue samples were snap-frozen in liquid nitrogen or fixed in paraformaldehyde.

The remaining four pups from each litter were individually housed in bedded cages and placed on either a high-fat (24% fat) or low-fat (4.3% fat) diet (1 male and 1 female on each diet per litter) from PND21 to PND105. Therefore, food intake and water consumption were measured for individual pups. The composition of the low-fat and high-fat diets was reported previously (2). At PND105, all pups were euthanized and necropsied to weigh organs and collect tissue samples as described previously. At each necropsy point, plasma was obtained from whole blood that was collected into in EDTA-coated vacutainer tubes via cardiac aspiration after euthanasia (2). In addition, on PND105, the thoracic aortas from male offspring were obtained for the measurement of endothelium-dependent relaxation as an assessment of vascular function.

Because dams and their post-weaning offspring were housed and fed individually diets with known nutrient composition, food and nutrient intakes as well as water consumption of the animals were measured individually. Based on the previous studies, the high-fat diet was formulated to contain 23% more energy than the low-fat diet, and the ratios of protein, vitamins, minerals and fiber to energy were constant in both diets. Thus, when rats fed the low-fat diet consumed about 23% more food than rats fed the high-fat diet, dietary intakes of energy, protein, vitamins, minerals or fiber per kg body weight did not differ between the low- and high-fat groups. By taking these cautious measures, the consumption of energy, protein and other nutrients by dams or their post-weaning offspring was well controlled in our study.

Analyses of Glucose and Lipids in Plasma and of Lipids in Liver. Concentrations of glucose in plasma were determined using a fluorometric method involving glucose-6-phosphate dehydrogenase and hexokinase (2). Total lipids in the liver, as well as triacylglycerols and nonesterified fatty acids (NEFA) in plasma were measured as described previously (2).

Measurement of Percent Relaxation in Aorta. The percent relaxation of rings from the thoracic aorta was measured in the presence of 10^{-9} to 10^{-5} mol/L acetylcholine as described previously (3). In all rings analyzed, NO-mediated relaxation at the conclusion of the experiment was verified with the use of 100 μmol/L sodium nitroprusside (an NO donor).

Hepatic Histological Analyses. Organs were fixed in 4% paraformaldehyde in phosphate-buffered saline (pH 7.4) for 24 h and then embedded in Paraplast Plus (Oxford Labware, St. Louis, MO). Parafin-embedded tissues were sectioned at (5 μm), deparaffinized, and stained with hematoxylin and eosin for general histomorphological evaluations, as we described previously (49).

Hepatic Fabp1 mRNA Levels. Hepatic mRNA levels of *Fabp1* (fatty acid-binding protein-1) were determined by RT-PCR (4). Primers for *Fabp1* were GAACTTCTCCGGCAAGTACCA (forward) and CATGCACGATTTCTGACACCC (reverse), with the accession number of NM_012556.2 and the product size of 128 bp. Primers for *18S rRNA* (the reference gene) were GCCGCTAGAGGTGAAATTCTTG (forward) and CATTCTTGGCAAATGCTTTCG (reverse), with the accession number of KU_939305 and the product size of 65 bp. We measured the mRNA level of the *Fabp1* gene because it encodes for the major fatty acid-binding protein-1 in the liver. This protein binds long-chain fatty acids, thereby preventing their toxicity. Altered expression of *Fabp1* is associated with metabolic syndrome in humans and animals (5).

Calculations and Statistical Analyses. Organ weights for PND0, PND21, and PND105 were calculated as both absolute and relative weights. Absolute organ weight is the actual weight of the organ, while the relative organ weight is the percentage it represents relative to total BW. All statistical analyses were performed using one-, two- or three-way ANOVA using PROC GLM or PROC MIXED procedures of SAS (SAS Institute). In addition to the main effects, any possible interactions among PM exposure, diet and sex were analyzed. A P-value was ≤ 0.05 was taken to indicate statistical significance.

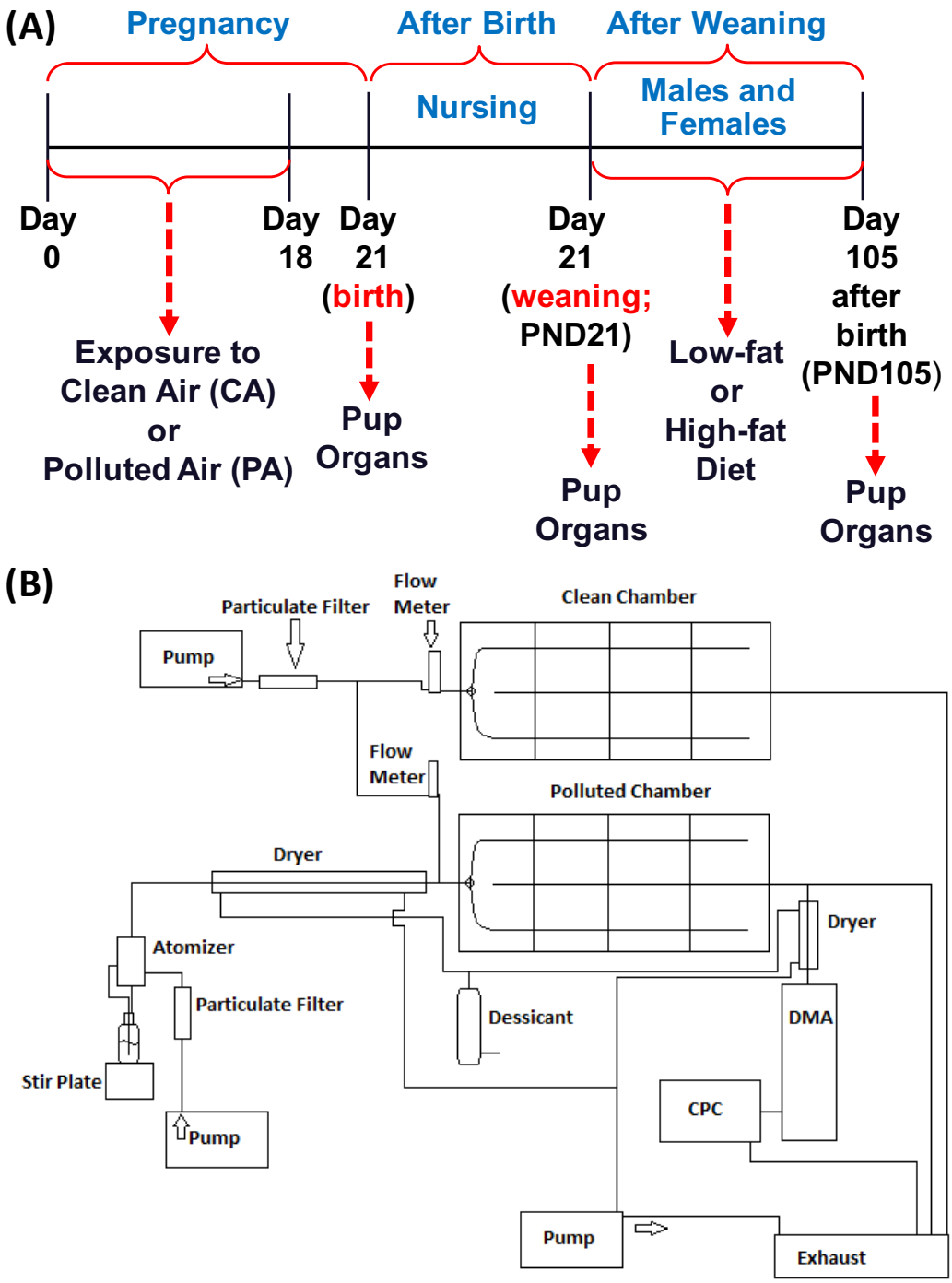

Fig. S1. **Study design and schematic of the dual-animal exposure chambers and instruments.** (A) Study design of the animal exposure model. (B) The dual-animal exposure chambers and instruments. Pure ambient air was pumped into the clean air (CA) chamber, and PM-doped ambient air (polluted air, PA) was pumped into the polluted chamber. The polluted and clean systems were independent of each other. The system flow rate was 55 LPM or 67 chamber turnovers per hour. The polluted chamber aerosols were sampled at a rate of 1 LPM by the DMA and CPC.

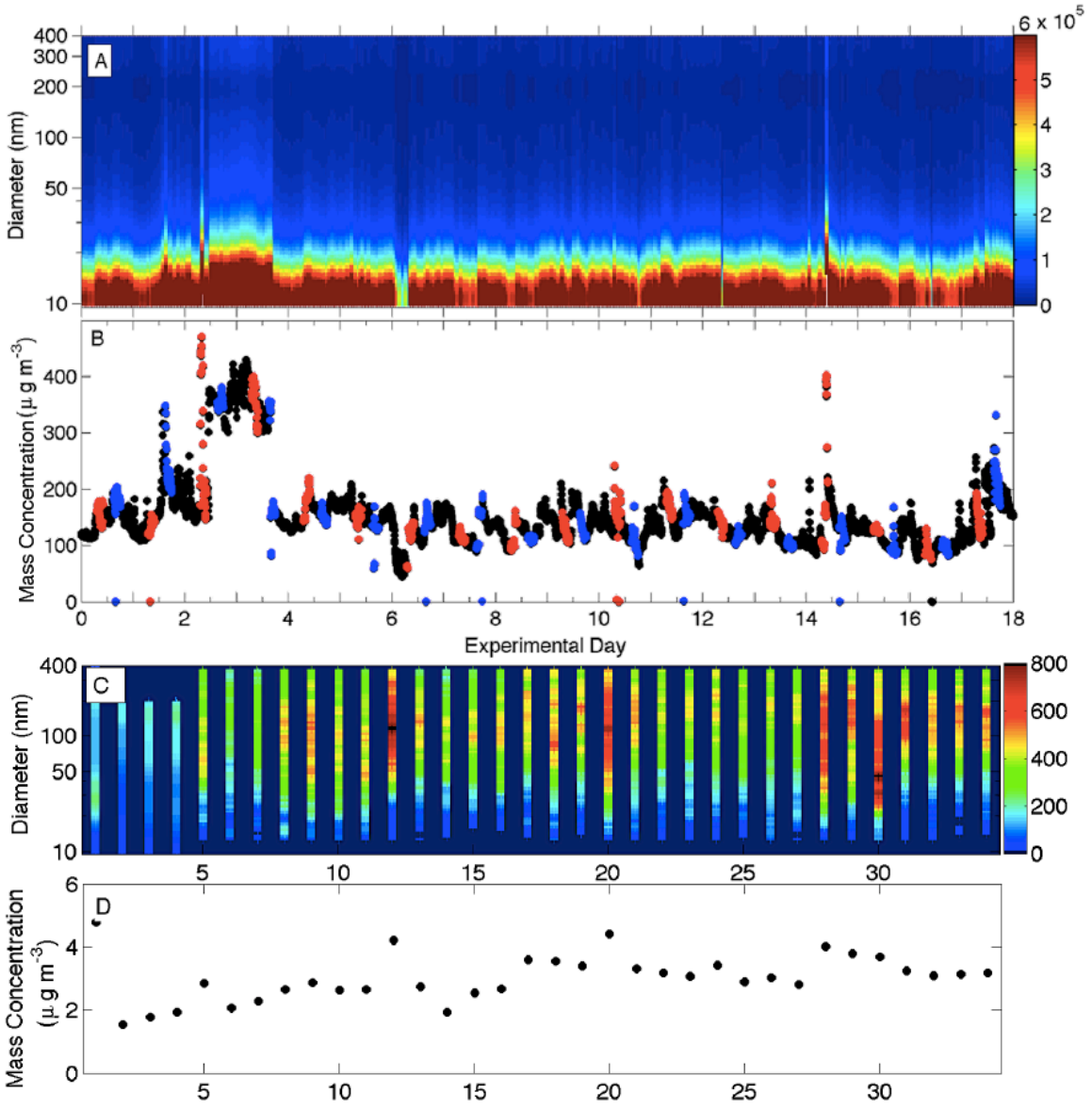

Fig. S2. The properties of particles inside the CA and PA chambers. The number concentration distributions measured by a Differential Mobility Analyzer and the calculated total mass (μg m^{-3}) of ammonium sulfate (A-B) in the PA chamber and (C-D) in the CA chamber. The scans were measured on various days throughout the experiment. The red points indicate the times when daily animal care was performed, and the blue dots indicate the time when machine maintenance or calibration was typically conducted. The surge in the mass concentration in (B) on Days 2-4 was attributable random fluctuations of the aerosol instruments.

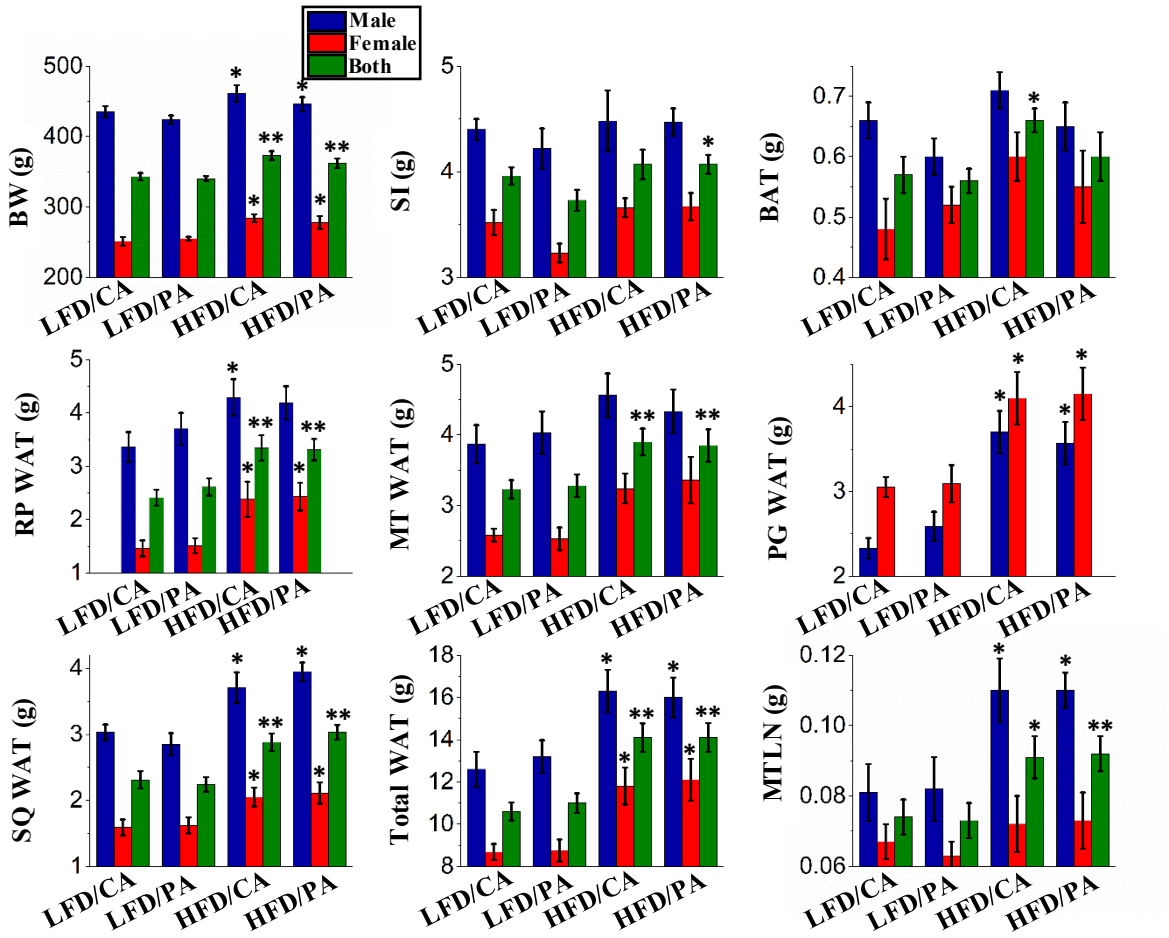

Fig. S3. **Body weights and absolute organ weights at PND105 of offspring from CA dams and PA dams**. Offspring from female Sprague-Dawley rats with exposure to CA or PA between Days 0 and 18 of gestation were fed either a low-fat diet (LFD) or high-fat diet (HFD) between Day 21 (weaning) and Day 105 after birth, and were euthanized on Day 105 for the measurement of body and organ weights. The labeled values correspond to means ± SEM. BAT, brown adipose tissue; BW, body weight; MT, mesenteric; MTLN, mesenteric lymph node; PG, peri-gonad (testes for males or ovaries for females); RP, retroperitoneal; SI, small intestine; SQ, subcutaneous; WAT, white adipose tissue. * $P < 0.05$ vs the corresponding low-fat group. ** $P < 0.01$ vs the corresponding low-fat group. Any of the measured variables in this figure did not differ ($P > 0.05$) between CA and PA groups.

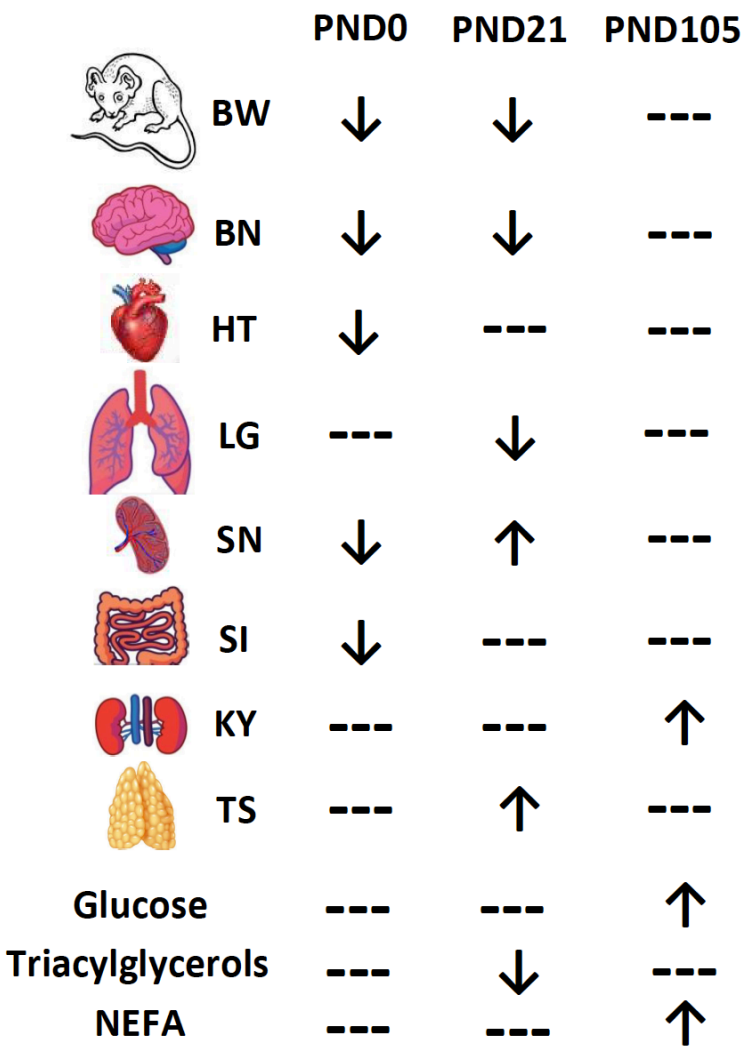

Fig. S4. Adverse organogenesis and predisposed long-term metabolic syndrome. Relative changes in BWs, relative organ weights, and plasma metabolites in offspring of the CA versus PA groups at different postnatal ages. BN, brain; BW, body weight; HT, heart; KY, kidney; LG, lungs; SN, spleen; SI, small intestine; TS, thymus.

SI Appendix table S1. Body weights, food intakes, and water consumption of dams during pregnancy (PG) and post parturition (PP).

Time	Body weight (g)		Time	Food intake (g/kg BW per day)		Water intake (ml/kg BW per day)	
	CA	PA		CA	PA	CA	PA
Day of PG			Days of PG				
0	215 ± 5.6^{c}	219 ± 4.3^{c}	---	---	---	---	---
6	233 ± 5.3^{c}	238 ± 3.9^{c}	0 - 6	102 ± 9.3	103 ± 7.6	133 ± 6.8^{b}	152 ± 11^{b}
12	253 ± 5.4^{b}	259 ± 3.1^{b}	6 - 12	92 ± 6.5	93 ± 6.0	132 ± 8.7^{b}	144 ± 17^{b}
18	314 ± 5.2^{a}	317 ± 4.8^{a}	12 - 18	86 ± 3.2	85 ± 8.9	154 ± 12^{b}	147 ± 7.3^{b}
21†	251 ± 7.4^{b}	257 ± 3.8^{b}	18 – 21	109 ± 8.0	94 ± 5.2	207 ± 18^{a}	193 ± 7.8^{a}
Days PP			Days of PP				
7	273 ± 6.9^{a}	274 ± 6.9^{a}	0 - 7	138 ± 4.6^{c}	136 ± 5.4^{c}	204 ± 19^{c}	207 ± 12^{c}
14	283 ± 6.3^{ab}	283 ± 3.8^{ab}	7 - 14	208 ± 6.9^{b}	206 ± 8.8^{b}	317 ± 17^{b}	314 ± 15^{b}
21	256 ± 4.7^{b}	255 ± 3.7^{b}	14 - 21	247 ± 7.9^{a}	247 ± 7.9^{a}	387 ± 30^{a}	376 ± 26^{a}

Adult female Sprague-Dawley rats received CA or PA between Days 0 and 18 of gestation. During pregnancy, on the day of parturition and on days after parturition, all dams had free access to a casein-based diet and drinking water. The diet contained 17.62% protein and 16,326 kJ energy/kg. Food intakes of individual dams were measured every 6 days in the first 18 days of pregnancy and then measured on the day of parturition. After parturition, food intakes of individual dams were measured every 7 days. Values are means ± SEM, n = 10.

† Immediately after giving birth.

a-c: Means not sharing the same superscript letters within a column (for pregnancy or post-parturition periods) are different ($P < 0.05$).

None of the variables differed ($P > 0.05$) between CA and PA groups.

BW for body weight.

SI Appendix table S2. Absolute and relative weights of organs of dams at weaning (Day 21 after parturition).

Organ	Absolute weight (g)		Relative weight (% of body weight)	
	CA	PA	CA	PA
Heart	1.16 ± 0.06	1.13 ± 0.03	0.45 ± 0.02	0.45 ± 0.01
Liver	13.6 ± 0.35	13.2 ± 0.38	5.32 ± 0.13	5.17 ± 0.11
Spleen	0.56 ± 0.03	0.61 ± 0.03	0.22 ± 0.01	0.24 ± 0.01
Kidneys	2.00 ± 0.07	1.96 ± 0.03	0.78 ± 0.02	0.77 ± 0.01
Brain	1.70 ± 0.02	1.72 ± 0.03	0.67 ± 0.02	0.68 ± 0.01
Pancreas	2.03 ± 0.20	1.92 ± 0.16	0.78 ± 0.07	0.75 ± 0.06
Small intestine	6.60 ± 0.41	7.51 ± 0.46	2.60 ± 0.18	2.95 ± 0.17
Lungs	1.43 ± 0.04	1.55 ± 0.03*	0.56 ± 0.02	0.61 ± 0.01*
Adrenal gland	0.065 ± 0.004	0.069 ± 0.003	0.025 ± 0.001	0.027 ±0.001
Ovaries	0.16 ± 0.01	0.16 ± 0.01	0.064 ± 0.005	0.064 ± 0.004
Thymus	0.14 ± 0.01	0.15 ± 0.01	0.057 ± 0.004	0.059 ± 0.004
EDL muscle	0.085 ± 0.006	0.087 ± 0.005	0.033 ± 0.002	0.034 ± 0.002
Soleus muscle	0.094 ± 0.008	0.089 ± 0.007	0.037 ± 0.003	0.035 ± 0.003
BAT	0.22 ± 0.02	0.22 ± 0.02	0.086 ± 0.006	0.086 ± 0.007
RP WAT	0.46 ± 0.09	0.45 ± 0.07	0.18 ± 0.03	0.18 ± 0.03
Stomach	2.16 ± 0.06	2.02 ± 0.08	0.84 ± 0.02	0.79 ± 0.03

Adult female Sprague-Dawley rats received CA or PA between Days 0 and 18 of gestation. At weaning, the dams were euthanized for the measurement of organ weights. Values are means ± SEM, n = 10.

* $P < 0.05$ vs the corresponding CA group, as analyzed by the unpaired t-test.

BAT, brown adipose tissue; EDL, extensor digitorum longus; RP WAT, retroperitoneal white adipose tissue.

SI Appendix table S3. The litter size and litter weight of pups from CA and PA groups.

Variable	CA	PA
Number of dams (litters)	10	10
Gestation length (days)	22.9 ± 0.15	22.4 ± 0.15*
Total # born dead per litter	1.4 ± 0.16	2.8 ± 0.29**
Total weight born alive per litter (g)	96.8 ± 3.2	87.2 ± 3.1*
Total # born per litter	16.8 ± 0.54	17.0 ± 0.51
Total # born alive per litter	15.4 ± 0.50	14.2 ± 0.42[a]
Average weight born alive[b] (g)	6.29 ± 0.06	6.11 ± 0.06*
# of females born alive per litter	7.60 ± 0.85	7.70 ± 0.40
# of males born alive per litter	7.80 ± 0.93	6.50 ± 0.56
# of males per 100 females born alive	103	84

Adult female Sprague-Dawley rats received CA or PA between Days 0 and 18 of gestation.
* $P < 0.05$ vs the corresponding CA group.
** $P < 0.01$ vs the corresponding CA group.
[a] $P = 0.082$ vs the corresponding CA group.
[b] Based on analysis with litter size (i.e., number of pups per litter) as a covariant, birth weights of live-born pups in CA and PA groups were 6.47 ± 0.06 and 5.92 ± 0.06 g ($P < 0.0001$), respectively.

SI Appendix table S4. Body weights and relative organ weights of pups at PND0 and PND21 from CA and PA groups.

Maternal exposure	Body weight (BW) or relative organ weight	PND0 (birth)			PND21 (weaning)		
		Males	Females	All rats	Males	Females	All rats
CA	BW (g)	6.39 ± 0.09	6.19 ± 0.07	6.29 ± 0.06	58.0 ± 1.2	54.5 ± 1.2	56.3 ± 0.9
PA	BW (g)	6.18 ± 0.09	6.04 ± 0.08	6.11 ± 0.06*	55.4 ± 1.1	52.3 ± 0.8	53.9 ± 0.7*
Relative organ weight (% of body weight)							
CA	Brain	4.13 ± 0.06	4.41 ± 0.07	4.27 ± 0.05	2.49 ± 0.05	2.57 ± 0.04	2.53 ± 0.03
PA	Brain	3.96 ± 0.05	4.08 ± 0.06	4.02 ± 0.04**	2.55 ± 0.05	2.59 ± 0.04	2.57 ± 0.03
CA	Heart	0.63 ± 0.01	0.67 ± 0.01	0.65 ± 0.01	0.62 ± 0.01	0.63 ± 0.01	0.63 ± 0.01
PA	Heart	0.60 ± 0.02	0.60 ± 0.02	0.60 ± 0.01**	0.63 ± 0.01	0.63 ± 0.01	0.63 ± 0.01
CA	Lungs	1.89 ± 0.04	1.84 ± 0.04	1.87 ± 0.03	1.37 ± 0.07	1.35 ± 0.06	1.36 ± 0.04
PA	Lungs	1.91 ± 0.04	1.95 ± 0.06	1.93 ± 0.04	1.18 ± 0.04	1.32 ± 0.05	1.25 ± 0.03*
CA	Spleen	0.27 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.50 ± 0.01	0.48 ± 0.02	0.49 ± 0.01
PA	Spleen	0.23 ± 0.01	0.22 ± 0.01	0.23 ± 0.01**	0.51 ± 0.02	0.52 ± 0.01	0.52 ± 0.01*
CA	Intestine[a]	4.85 ± 0.06	5.22 ± 0.08	5.04 ± 0.05	3.08 ± 0.11	3.03 ± 0.06	3.06 ± 0.06
PA	Intestine[a]	4.71 ± 0.08	4.69 ± 0.08*	4.70 ± 0.06**	2.99 ± 0.11	3.19 ± 0.12	3.09 ± 0.08
CA	Kidneys	0.89 ± 0.01	0.97 ± 0.02	0.93 ± 0.01	1.12 ± 0.01	1.15 ± 0.02	1.14 ± 0.01
PA	Kidneys	0.86 ± 0.02	0.94 ± 0.01	0.90 ± 0.01	1.11 ± 0.01	1.15 ± 0.01	1.13 ± 0.01
CA	Thymus	---	---	---	0.43 ± 0.02	0.51 ± 0.01	0.47 ± 0.01
PA	Thymus	---	---	---	0.48 ± 0.01	0.54 ± 0.02	0.51 ± 0.01**

Offspring from female Sprague-Dawley rats with exposure to CA or PA between Days 0 and 18 of gestation were euthanized on PND0 and PND 21. Values are means ± SEM. The numbers of pups euthanized at birth were 37, 35, 24, and 34, respectively, for the CA male, CA female, PA male, and PA female groups. The total numbers of live-born pups were 77, 75, 64, and 76, respectively, for the CA male, CA female, PA male, and PA female groups.

[a] Whole intestine for PND0 and the small intestine for PND21.

* $P < 0.05$ vs the corresponding CA group.

** $P < 0.01$ vs the corresponding CA group.

SI Appendix table S5. Absolute organ weights at PND0 (birth) and PND21 (weaning) of pups from CA dams and PA dams.

Maternal exposure	Absolute organ weight	PND0 (birth)			PND21 (weaning)		
		Males	Females	All rats	Males	Females	All rats
CA	Heart	40.3 ± 0.9	38.1 ± 1.1	39.2 ± 0.7	361 ± 10	341 ± 11	351 ± 7.5
PA	Heart	35.7 ± 1.4*	35.9 ± 0.9	35.8 ± 0.8**	347 ± 8.6	331 ± 8.2	339 ± 6.0
CA	Liver	255 ± 4.6	244 ± 4.6	250 ± 3.3	2726 ± 80	2515 ± 73	2621 ± 55
PA	Liver	242 ± 5.4	241 ± 4.7	242 ± 3.6	2532 ± 70*	2369 ± 46	2451 ± 41*
CA	Spleen	17.4 ± 0.8	14.4 ± 0.8	15.9 ± 0.6	290 ± 10	263 ± 12	277 ± 7.9
PA	Spleen	13.8 ± 1.6*	12.7 ± 0.7	13.3 ± 0.8**	281 ± 9.0	273 ± 7.8	277 ± 6.0
CA	Kidneys	57.3 ± 1.6	56.1 ± 1.7	56.7 ± 1.2	652 ± 15	629 ± 19	641 ± 12
PA	Kidneys	53.5 ± 1.6	54.9 ± 1.4	54.2 ± 1.1	617 ± 14	602 ± 12	610 ± 9.3*
CA	Brain	262 ± 4.5	249 ± 6.3	256 ± 3.9	1432 ± 11	1394 ± 14	1413 ± 8.9
PA	Brain	230 ± 7.0**	235 ± 5.4	233 ± 4.4**	1406 ± 14	1353 ± 16	1380 ± 11*
CA	Pancreas	27.4 ± 1.5	28.6 ± 1.4	28.0 ± 1.0	434 ± 38	478 ± 39	456 ± 28
PA	Pancreas	25.9 ± 1.2	25.1 ± 0.8	25.5 ± 0.7*	437 ± 32	402 ± 39	420 ± 25
CA	Intestine[a]	311 ± 8.4	321 ± 19	316 ± 9.8	1786 ± 72	1640 ± 27	1713 ± 35
PA	Intestine[a]	278 ± 12	268 ± 9.3	273 ± 7.6	1662 ± 71	1666 ± 65	1664 ± 49
CA	Lungs	121 ± 4.1	110 ± 3.8	116 ± 2.8	799 ± 47	737 ± 44	768 ± 33
PA	Lungs	121 ± 3.2	113 ± 4.0	117 ± 2.6	652 ± 21*	690 ± 27	671 ± 17**
CA	Adrenal gland	2.90 ± 0.13	2.80 ± 0.20	2.85 ± 0.11	28.4 ± 2.5	31.2 ± 1.7	29.8 ± 1.5
PA	Adrenal gland	3.02 ± 0.33	3.09 ± 0.21	3.06 ± 0.19	26.8 ± 0.9	28.7 ± 1.7	27.8 ± 0.9
CA	Gonads[b]	14.1 ± 0.4	---	---	424 ± 14	38.1 ± 2.8	---
PA	Gonads[b]	12.3 ± 0.6*	---	---	413 ± 12	33.8 ± 2.3	---
CA	BAT	58.1 ± 2.2	61.5 ± 2.8	59.8 ± 1.8	263 ± 10	272 ± 7.8	268 ± 6.4
PA	BAT	56.9 ± 2.7	52.6 ± 2.1*	54.8 ± 1.7*	280 ± 10	254 ± 7.2	267 ± 6.1
CA	RP WAT	---	---	---	55.9 ± 3.6	84.2 ± 7.3	70.1 ± 3.9
PA	RP WAT	---	---	---	52.5 ± 5.5	85.2 ± 4.6	68.9 ± 3.6
CA	Soleus muscle	---	---	---	21.0 ± 1.2	21.3 ± 1.1	21.2 ± 0.8
PA	Soleus muscle	---	---	---	20.0 ± 0.7	18.9 ± 0.8	19.5 ± 0.5
CA	EDL muscle	---	---	---	19.8 ± 0.8	21.7 ± 1.1	20.8 ± 0.7
PA	EDL muscle	---	---	---	19.9 ± 0.7	18.5 ± 0.8	19.2 ± 0.5
CA	Thymus	---	---	---	251 ± 11	277 ± 7.8	264 ± 6.7
PA	Thymus	---	---	---	265 ± 7.4	280 ± 9.0	273 ± 5.9
CA	Stomach	---	---	---	588 ± 14	536 ± 16	562 ± 11
PA	Stomach	---	---	---	556 ± 16	577 ± 19	567 ± 13

Offspring from female Sprague-Dawley rats with exposure to CA or PA between Days 0 and 18 of gestation were euthanized on PND0 and PND 21. Values, expressed as mg, are means ± SEM. The numbers of pups euthanized at birth were 37, 35, 24, and 34, respectively, for the CA male,

CA female, PA male, and PA female groups. The total numbers of live-born pups were 77, 75, 64, and 76, respectively, for the CA male, CA female, PA male, and PA female groups.
[a] Whole intestine for PND0 and small intestine for PND21.
[b] Testes for males or ovaries for females.
* $P < 0.05$ vs the corresponding CA group.
** $P < 0.01$ vs the corresponding CA group.
BAT, brown adipose tissue; EDL, extensor digitorum longus; RP WAT, retroperitoneal white adipose tissue.

SI Appendix table S6. Relative organ weights at PND0 (birth) and PND21 (weaning) of pups from CA dams and PA dams

Maternal exposure	Relative organ weight	PND0 (birth)			PND21 (weaning)		
		Males	Females	All rats	Males	Females	All rats
CA	Liver	4.00 ± 0.01	4.02 ± 0.08	4.01 ± 0.06	4.69 ± 0.07	4.60 ± 0.05	4.65 ± 0.04
PA	Liver	3.92 ± 0.05	3.97 ± 0.05	3.95 ± 0.04	4.56 ± 0.07	4.52 ± 0.05	4.54 ± 0.04
CA	Kidneys	0.89 ± 0.01	0.97 ± 0.02	0.93 ± 0.01	1.12 ± 0.01	1.15 ± 0.02	1.14 ± 0.01
PA	Kidneys	0.86 ± 0.02	0.94 ± 0.01	0.90 ± 0.01	1.11 ± 0.01	1.15 ± 0.01	1.13 ± 0.01
CA	Pancreas	0.43 ± 0.03	0.48 ± 0.03	0.46 ± 0.02	0.74 ± 0.06	0.86 ± 0.06	0.80 ± 0.04
PA	Pancreas	0.44 ± 0.02	0.42 ± 0.01	0.43 ± 0.01	0.78 ± 0.05	0.76 ± 0.07	0.77 ± 0.04
CA	Adrenal gland	0.046 ± 0.002	0.044 ± 0.003	0.045 ± 0.002	0.052 ± 0.005	0.058 ± 0.004	0.055 ± 0.03
PA	Adrenal gland	0.043 ± 0.004	0.046 ± 0.003	0.045 ± 0.003	0.049 ± 0.002	0.055 ± 0.003	0.052 ± 0.02
CA	Gonads[a]	0.22 ± 0.005	---	---	0.73 ± 0.02	0.071 ± 0.006	---
PA	Gonads[a]	0.20 ± 0.007	---	---	0.75 ± 0.02	0.065 ± 0.005	---
CA	BAT	0.90 ± 0.03	0.99 ± 0.04	0.95 ± 0.03	0.46 ± 0.02	0.50 ± 0.01	0.48 ± 0.01
PA	BAT	0.91 ± 0.04	0.86 ± 0.04	0.89 ± 0.03	0.51 ± 0.02	0.48 ± 0.02	0.50 ± 0.01
CA	RP WAT	---	---	---	0.10 ± 0.01	0.15 ± 0.01[§]	0.13 ± 0.01
PA	RP WAT	---	---	---	0.10 ± 0.01	0.16 ± 0.01[§]	0.13 ± 0.01
CA	Soleus muscle	---	---	---	0.037 ± 0.002	0.039 ± 0.002	0.038 ± 0.001
PA	Soleus muscle	---	---	---	0.036 ± 0.001	0.036 ± 0.002	0.036 ± 0.001
CA	EDL muscle	---	---	---	0.035 ± 0.002	0.040 ± 0.002	0.038 ± 0.001
PA	EDL muscle	---	---	---	0.036 ± 0.001	0.036 ± 0.002	0.036 ± 0.001
CA	Stomach	---	---	---	1.02 ± 0.03	0.99 ± 0.03	1.01 ± 0.02
PA	Stomach	---	---	---	1.01 ± 0.03	1.11 ± 0.04	1.06 ± 0.02

Offspring from female Sprague-Dawley rats with exposure to CA or PA between Days 0 and 18 of gestation were euthanized on PND0 and PND 21. Values, expressed as % of body weight, are means ± SEM. The numbers of pups euthanized at birth were 37, 35, 24, and 34, respectively, for the CA male, CA female, PA male, and PA female groups. The total numbers of live-born pubs were 77, 75, 64, and 76, respectively, for the CA male, CA female, PA male, and PA female groups.

[a] Testes for males or ovaries for females.

BAT, brown adipose tissue; EDL, extensor digitorum longus; RP WAT, retroperitoneal white adipose tissue.

SI Appendix table S7. Food intakes of post-weaning pups between PND21 (weaning) and PND 105 (end of study)

Maternal exposure	Week	Low-fat diet			High-fat diet		
		Males	Females	All rats	Males	Females	All rats
		Feed intake (g/kg body weight per day)					
CA	1	92.8 ± 1.5	93.8 ± 1.3	93.3 ± 1.0	88.8 ± 1.3	87.5 ± 1.6	88.2 ± 1.0**
PA	1	89.7 ± 1.2	92.8 ± 1.3	91.3 ± 0.9	86.1 ± 1.7	85.3 ± 0.9	85.7 ± 0.9**
CA	2	88.0 ± 1.6	87.3 ± 1.1	87.7 ± 0.7	73.1 ± 1.1	71.9 ± 1.0	72.5 ± 0.8**
PA	2	89.1 ± 1.9	88.7 ± 1.7	88.9 ± 1.3	75.6 ± 1.4	72.9 ± 1.8	74.3 ± 1.1**
CA	3	83.9 ± 1.6	83.4 ± 1.0	83.7 ± 0.9	69.2 ± 1.1	68.4 ± 1.3	68.8 ± 0.9**
PA	3	85.7 ± 0.9	85.0 ± 1.6	85.4 ± 0.9	71.6 ± 1.1	71.5 ± 1.1	71.6 ± 0.8**
CA	4	71.5 ± 0.8	73.3 ± 0.6	72.4 ± 0.5	58.7 ± 0.5	60.9 ± 0.9	59.8 ± 0.5**
PA	4	72.0 ± 1.1	72.3 ± 1.5	72.2 ± 0.9	60.2 ± 1.1	62.2 ± 1.2	61.2 ± 0.8**
CA	5	63.5 ± 0.7	66.0 ± 1.1	64.8 ± 0.6	53.0 ± 0.8	55.3 ± 1.2	54.2 ± 0.7**
PA	5	66.5 ± 1.1	66.6 ± 1.0	66.6 ± 0.8	55.3 ± 1.1	58.1 ± 0.9	56.7 ± 0.7**
CA	6	59.0 ± 1.3	58.7 ± 1.1	58.9 ± 0.9	48.2 ± 1.1	51.1 ± 1.0	49.7 ± 0.8**
PA	6	60.1 ± 0.9	61.2 ± 1.1	60.7 ± 0.7	49.1 ± 0.9	51.3 ± 0.8	50.2 ± 0.6**
CA	7	55.9 ± 0.7	56.3 ± 1.1	56.1 ± 0.6	45.4 ± 0.6	48.3 ± 1.1	46.9 ± 0.6**
PA	7	55.6 ± 0.7	57.5 ± 1.2	56.6 ± 0.7	46.1 ± 0.6	49.2 ± 0.9	47.7 ± 0.5**
CA	8	51.0 ± 0.7	52.6 ± 1.1	51.8 ± 0.6	42.3 ± 0.6	45.8 ± 0.8	44.1 ± 0.5**
PA	8	51.8 ± 0.9	53.4 ± 0.8	52.6 ± 0.6	42.9 ± 0.9	45.6 ± 0.8	44.3 ± 0.6**
CA	9	46.8 ± 0.9	50.2 ± 1.2	48.5 ± 0.8	39.5 ± 0.9	43.2 ± 1.2	41.4 ± 0.8**
PA	9	48.3 ± 1.3	49.8 ± 1.2	49.1 ± 0.9	39.2 ± 0.8	43.6 ± 1.2	41.4 ± 0.7**
CA	10	45.3 ± 0.6	49.6 ± 0.9	47.5 ± 0.5	38.0 ± 0.7	42.5 ± 0.9	40.3 ± 0.6**
PA	10	47.2 ± 0.6	51.3 ± 0.8	49.3 ± 0.5	39.5 ± 0.7	41.9 ± 0.6	40.7 ± 0.5**
CA	11	43.3 ± 0.7	47.1 ± 1.1	45.2 ± 0.6	35.7 ± 0.9	39.9 ± 0.5	37.8 ± 0.5**
PA	11	43.0 ± 0.9	43.7 ± 0.9	43.4 ± 0.6	34.9 ± 1.1	39.6 ± 1.0	37.3 ± 0.8**
CA	1-11	63.7 ± 1.0	65.3 ± 0.7	64.5 ± 0.6	53.8 ± 0.9	55.9 ± 1.0	54.9 ± 0.7**
PA	1-11	64.5 ± 1.1	65.7 ± 1.2	65.1 ± 0.8	54.6 ± 1.0	56.5 ± 1.1	55.6 ± 0.8**
		Energy intake (kJ/kg body weight per day)					
CA	1-11	1030 ± 16	1055 ± 11	1043 ± 9.6	1069 ± 18	1110 ± 20	1090 ± 14
PA	1-11	1041 ± 18	1061 ± 19	1051 ± 13	1084 ± 20	1121 ± 22	1103 ± 15
		Protein intake (g/kg body weight per day)					
CA	1-11	10.8 ± 0.19	11.1 ± 0.14	11.0 ± 0.12	11.2 ± 0.18	11.7 ± 0.22	11.5 ± 0.14
PA	1-11	11.0 ± 0.21	11.2 ± 0.23	11.1 ± 0.16	11.4 ± 0.20	11.8 ± 0.24	11.6 ± 0.24

Pubs from dams with exposure to CA or PA during gestation were fed a low-fat or high-fat diet between Day 21 (weaning) and Day 105 after birth. The low-fat diet contained 16,155 kJ energy/kg and 169.9 g protein/kg, whereas the high-fat diet contained 19,858 kJ energy/kg and 208.9 g protein/kg. Values are means ± SEM, n = 10.

** $P < 0.01$ vs the corresponding low-fat group.
None of the variables differed ($P > 0.05$) between CA and PA groups.

SI Appendix table S8. Water consumption by post-weaning offspring between PND21 (weaning) and PND 105 (end of study).

Maternal exposure	Week	Low-fat diet			High-fat diet		
		Males (n = 10)	Females (n = 10)	All rats (n = 20)	Males (n = 10)	Females (n = 10)	All rats (n = 20)
		mL/kg body weight per day					
CA	1	92.7 ± 1.4	93.9 ± 1.4	93.3 ± 1.0	88.9 ± 1.4	89.1 ± 2.4	89.0 ± 1.4*
PA	1	89.7 ± 1.2	92.9 ± 1.3	91.3 ± 0.89	86.1 ± 1.7	85.3 ± 0.86	85.7 ± 0.91**
CA	2	88.0 ± 1.7	87.3 ± 1.1	87.7 ± 1.0	73.1 ± 1.1	71.9 ± 1.1	72.5 ± 0.79**
PA	2	89.1 ± 1.9	88.7 ± 1.7	88.9 ± 1.3	75.7 ± 1.4	71.3 ± 2.4	73.5 ± 1.4**
CA	3	83.9 ± 1.6	83.4 ± 1.0	83.7 ± 0.93	69.1 ± 1.1	68.4 ± 1.3	68.8 ± 0.86**
PA	3	85.7 ± 0.90	85.0 ± 1.7	85.4 ± 0.93	71.6 ± 1.1	71.4 ± 1.4	71.5 ± 0.89**
CA	4	71.6 ± 0.86	73.3 ± 0.61	72.5 ± 0.53	58.7 ± 0.46	60.9 ± 0.91	59.8 ± 0.49**
PA	4	72.0 ± 1.1	72.3 ± 1.4	72.2 ± 0.89	60.1 ± 1.1	62.1 ± 1.2	61.1 ± 0.82**
CA	5	63.4 ± 0.71	66.0 ± 1.2	64.7 ± 0.68	53.0 ± 0.76	55.3 ± 1.2	54.2 ± 0.70**
PA	5	66.6 ± 1.1	66.7 ± 1.0	66.7 ± 0.75	55.3 ± 1.1	58.1 ± 0.91	56.7 ± 0.72**
CA	6	59.0 ± 1.3	58.7 ± 1.1	58.9 ± 0.86	48.1 ± 1.1	51.1 ± 1.0	49.6 ± 0.75**
PA	6	60.1 ± 0.89	61.3 ± 1.1	60.7 ± 0.71	49.1 ± 0.87	51.3 ± 0.77	50.2 ± 0.59**
CA	7	56.0 ± 0.69	56.3 ± 1.1	56.2 ± 0.64	45.4 ± 0.56	48.3 ± 1.2	46.9 ± 0.63**
PA	7	55.6 ± 0.66	57.6 ± 1.2	56.6 ± 0.67	46.1 ± 0.93	49.3 ± 0.89	47.7 ± 0.65**
CA	8	51.0 ± 0.71	52.6 ± 1.1	51.8 ± 0.65	42.3 ± 0.61	45.9 ± 0.83	44.1 ± 0.51**
PA	8	51.9 ± 0.94	53.4 ± 0.77	52.7 ± 0.61	42.9 ± 0.90	45.6 ± 0.84	44.3 ± 0.62**
CA	9	46.9 ± 0.94	50.1 ± 1.2	48.5 ± 0.76	39.4 ± 0.94	43.1 ± 1.2	41.3 ± 0.76**
PA	9	48.3 ± 1.3	49.9 ± 1.2	49.1 ± 0.89	39.3 ± 0.80	43.6 ± 1.2	41.5 ± 0.71**
CA	10	45.3 ± 0.61	49.6 ± 0.90	47.5 ± 0.54	38.0 ± 0.66	42.6 ± 0.89	40.3 ± 0.55**
PA	10	47.1 ± 0.63	51.3 ± 0.84	49.2 ± 0.53	39.6 ± 0.70	41.9 ± 0.59	40.8 ± 0.46**
CA	11	43.3 ± 0.69	47.1 ± 1.1	45.2 ± 0.64	35.7 ± 0.91	39.9 ± 0.53	37.8 ± 0.51**
PA	11	43.0 ± 0.87	43.7 ± 2.6	43.4 ± 1.2	34.9 ± 1.1	39.7 ± 1.0	37.3 ± 0.75**

Pups from dams with exposure to CA or PA during gestation were fed a low-fat or high-fat diet between Day 21 (weaning) and Day 105 after birth. Values are means ± SEM.
* $P < 0.05$ vs the corresponding low-fat group.
** $P < 0.01$ vs the corresponding low-fat group.
None of the variables differed ($P > 0.05$) between CA and PA groups.

SI Appendix table S9. Body weights and absolute organ weights at PND105 of offspring from CA dams and PA dams.

Maternal exposure	Body weight or absolute organ weight	Low-fat diet			High-fat diet		
		Males (n = 10)	Females (n = 10)	All rats (n = 20)	Males (n = 10)	Females (n = 10)	All rats (n = 20)
CA	Body weight	435 ± 8.2	251 ± 6.3	343 ± 5.2	461 ± 12*	284 ± 5.5*	373 ± 6.3**
PA	Body weight	424 ± 6.0	255 ± 2.7	340 ± 3.6	446 ± 10*	278 ± 9.0*	362 ± 6.8**
CA	SI	4.40 ± 0.10	3.52 ± 0.12	3.96 ± 0.08	4.48 ± 0.29	3.66 ± 0.09	4.07 ± 0.14
PA	SI	4.22 ± 0.19	3.23 ± 0.09	3.73 ± 0.10	4.47 ± 0.13	3.67 ± 0.13	4.07 ± 0.09*
CA	BAT	0.66 ± 0.03	0.48 ± 0.05	0.57 ± 0.03	0.71 ± 0.03	0.60 ± 0.04	0.66 ± 0.02*
PA	BAT	0.60 ± 0.03	0.52 ± 0.03	0.56 ± 0.02	0.65 ± 0.04	0.55 ± 0.06	0.60 ± 0.04
CA	RP WAT	3.36 ± 0.28	1.46 ± 0.15	2.41 ± 0.15	4.29 ± 0.34*	2.38 ± 0.33*	3.34 ± 0.24**
PA	RP WAT	3.70 ± 0.30	1.51 ± 0.14	2.61 ± 0.16	4.19 ± 0.31	2.43 ± 0.26*	3.31 ± 0.20**
CA	MT WAT	3.87 ± 0.27	2.58 ± 0.09	3.23 ± 0.13	4.56 ± 0.31	3.24 ± 0.21	3.90 ± 0.19**
PA	MT WAT	4.03 ± 0.30	2.53 ± 0.16	3.28 ± 0.16	4.33 ± 0.31	3.36 ± 0.33	3.85 ± 0.23**
CA	PG WAT	2.33 ± 0.12	3.05 ± 0.12	---	3.70 ± 0.25*	4.10 ± 0.31*	---
PA	PG WAT	2.59 ± 0.17	3.09 ± 0.22	---	3.57 ± 0.25*	4.15 ± 0.31*	---
CA	SQ WAT	3.03 ± 0.23	1.59 ± 0.12	2.31 ± 0.13	3.71 ± 0.23*	2.05 ± 0.14*	2.88 ± 0.13**
PA	SQ WAT	2.85 ± 0.20	1.62 ± 0.12	2.24 ± 0.11	3.95 ± 0.14*	2.11 ± 0.16*	3.03 ± 0.11**
CA	Total WAT	12.6 ± 0.82	8.68 ± 0.38	10.6 ± 0.43	16.3 ± 1.0*	11.8 ± 0.87*	14.1 ± 0.67**
PA	Total WAT	13.2 ± 0.77	8.75 ± 0.52	11.0 ± 0.46	16.0 ± 0.94*	12.1 ± 0.99*	14.1 ± 0.69**
CA	MTLN	0.081 ± 0.008	0.067 ± 0.005	0.074 ± 0.005	0.11 ± 0.009*	0.072 ± 0.008	0.091 ± 0.006*
PA	MTLN	0.082 ± 0.009	0.063 ± 0.004	0.073 ± 0.005	0.11 ± 0.005*	0.073 ± 0.008	0.092 ± 0.005**

Offspring from dams with exposure to CA or PA during gestation were fed a low-fat or high-fat diet between Day 21 (weaning) and Day 105 after birth, and were euthanized at Day 105 for the measurement of body weight and organ weights. Values, expressed as g, are means ± SEM.

[a] Testes for males or ovaries for females.

* $P < 0.05$ vs the corresponding low-fat group.

** $P < 0.01$ vs the corresponding low-fat group.

BAT, brown adipose tissue; MTLN, mesenteric lymph node; PG, peri-gonad (testes for males or ovaries for females); RP, retroperitoneal; SI, small intestine; SQ, subcutaneous; WAT, white adipose tissue.

SI Appendix table S10. Absolute organ weights of offspring from CA dams and PA dams at PND105.

Maternal exposure	Absolute weights of organs	Low-fat diet			High-fat diet		
		Males (n = 10)	Females (n = 10)	All rats (n = 20)	Males (n = 10)	Females (n = 10)	All rats (n = 20)
CA	Heart	1.49 ± 0.04	0.94 ± 0.03	1.22 ± 0.02	1.62 ± 0.05	1.04 ± 0.02	1.33 ± 0.03
PA	Heart	1.52 ± 0.04	0.98 ± 0.03	1.25 ± 0.02	1.57 ± 0.04	1.05 ± 0.04	1.31 ± 0.03
CA	Liver	13.9 ± 0.52	6.52 ± 0.57	10.2 ± 0.39	12.9 ± 0.56	7.50 ± 0.29	10.2 ± 0.30
PA	Liver	13.1 ± 0.63	7.01 ± 0.29	10.1 ± 0.33	12.1 ± 0.47	7.11 ± 0.29	9.61 ± 0.27
CA	Spleen	0.81 ± 0.01	0.57 ± 0.02	0.69 ± 0.01	0.81 ± 0.03	0.62 ± 0.02	0.72 ± 0.02
PA	Spleen	0.84 ± 0.03	0.62 ± 0.02	0.73 ± 0.02	0.78 ± 0.02	0.63 ± 0.03	0.71 ± 0.02
CA	Kidneys	2.69 ± 0.07	1.57 ± 0.01	2.13 ± 0.03	2.75 ± 0.08	1.77 ± 0.05	2.26 ± 0.05
PA	Kidneys	2.72 ± 0.06	1.63 ± 0.03	2.18 ± 0.03	2.80 ± 0.08	1.75 ± 0.05	2.28 ± 0.05
CA	Brain	1.92 ± 0.02	1.75 ± 0.02	1.84 ± 0.01	1.92 ± 0.03	1.81 ± 0.01	1.87 ± 0.01
PA	Brain	1.96 ± 0.01	1.79 ± 0.02	1.88 ± 0.01	1.95 ± 0.04	1.80 ± 0.02	1.88 ± 0.02
CA	Pancreas	1.85 ± 0.05	1.34 ± 0.05	1.60 ± 0.04	1.86 ± 0.08	1.48 ± 0.06	1.67 ± 0.05
PA	Pancreas	1.72 ± 0.06	1.35 ± 0.04	1.54 ± 0.04	1.88 ± 0.07	1.51 ± 0.06	1.70 ± 0.05
CA	Lungs	1.81 ± 0.04	1.35 ± 0.03	1.58 ± 0.03	1.81 ± 0.04	1.45 ± 0.03	1.63 ± 0.02
PA	Lungs	1.82 ± 0.05	1.41 ± 0.05	1.62 ± 0.04	1.81 ± 0.04	1.49 ± 0.04	1.65 ± 0.03
CA	ADGD	0.094 ± 0.004	0.083 ± 0.005	0.089 ± 0.003	0.095 ± 0.004	0.085 ± 0.002	0.090 ± 0.002
PA	ADGD	0.089 ± 0.005	0.082 ± 0.004	0.086 ± 0.003	0.094 ± 0.006	0.089 ± 0.004	0.092 ± 0.004
CA	Gonads[a]	6.04 ± 0.10	0.13 ± 0.01	---	6.26 ± 0.13	0.14 ± 0.01	---
PA	Gonads[a]	5.90 ± 0.30	0.13 ± 0.01	---	6.33 ± 0.09	0.14 ± 0.01	---
CA	SM	0.17 ± 0.005	0.11 ± 0.007	0.14 ± 0.004	0.18 ± 0.009	0.12 ± 0.005	0.15 ± 0.005
PA	SM	0.17 ± 0.005	0.11 ± 0.004	0.14 ± 0.003	0.19 ± 0.009	0.12 ± 0.006	0.16 ± 0.005
CA	EDL	0.17 ± 0.004	0.10 ± 0.004	0.14 ± 0.003	0.18 ± 0.005	0.11 ± 0.003	0.15 ± 0.003
PA	EDL	0.17 ± 0.003	0.10 ± 0.004	0.14 ± 0.002	0.17 ± 0.004	0.11 ± 0.005	0.14 ± 0.003
CA	Thymus	0.60 ± 0.03	0.36 ± 0.01	0.48 ± 0.01	0.53 ± 0.04	0.39 ± 0.02	0.46 ± 0.02
PA	Thymus	0.60 ± 0.03	0.36 ± 0.03	0.48 ± 0.02	0.65 ± 0.05	0.36 ± 0.02	0.51 ± 0.02
CA	Stomach	2.27 ± 0.17	1.63 ± 0.14	1.95 ± 0.11	2.12 ± 0.10	1.73 ± 0.13	1.93 ± 0.08
PA	Stomach	2.07 ± 0.10	1.53 ± 0.06	1.80 ± 0.06	2.10 ± 0.05	1.70 ± 0.09	1.90 ± 0.05

Offspring from dams with exposure to clean or polluted air during gestation were fed a low-fat or high-fat diet between Day 21 (weaning) and Day 105 after birth, and were euthanized at Day 105 for determination of body and organ weights. Values expressed as g, are means ± SEM.

[a] Testes for males or ovaries for females.

ADGD, adrenal gland; EDL, extensor digitorum longus muscle; SM, soleus muscle.

SI Appendix table S11. Relative organ weights of offspring from CA dams and PA dams at PND105.

Maternal exposure	Relative organ weight	Low-fat diet			High-fat diet		
		Males (n = 10)	Females (n = 10)	All rats (n = 20)	Males (n = 10)	Females (n = 10)	All rats (n = 20)
CA	Heart	0.34 ± 0.01	0.37 ± 0.01	0.36 ± 0.01	0.35 ± 0.01	0.37 ± 0.01	0.36 ± 0.01
PA	Heart	0.36 ± 0.01	0.38 ± 0.01	0.37 ± 0.01	0.35 ± 0.01	0.38 ± 0.01	0.37 ± 0.01
CA	Liver	3.20 ± 0.09	2.59 ± 0.20	2.90 ± 0.10	2.80 ± 0.07	2.63 ± 0.06	2.72 ± 0.05
PA	Liver	3.08 ± 0.11	2.75 ± 0.10	2.92 ± 0.08	2.72 ± 0.09	2.56 ± 0.08	2.64 ± 0.06
CA	Spleen	0.19 ± 0.00	0.23 ± 0.01	0.21 ± 0.01	0.18 ± 0.01	0.22 ± 0.01	0.20 ± 0.01
PA	Spleen	0.20 ± 0.01	0.24 ± 0.01	0.22 ± 0.01	0.17 ± 0.01	0.23 ± 0.01	0.20 ± 0.01
CA	Kidneys	0.62 ± 0.01	0.63 ± 0.01	0.63 ± 0.01	0.60 ± 0.01	0.62 ± 0.01	0.61 ± 0.01
PA	Kidneys	0.64 ± 0.01	0.64 ± 0.03	0.64 ± 0.01	0.63 ± 0.01	0.63 ± 0.01	0.63 ± 0.01
CA	Brain	0.44 ± 0.01	0.70 ± 0.01	0.57 ± 0.01	0.42 ± 0.01	0.64 ± 0.01	0.53 ± 0.01
PA	Brain	0.46 ± 0.01	0.70 ± 0.01	0.58 ± 0.01	0.44 ± 0.01	0.65 ± 0.02	0.55 ± 0.01
CA	Pancreas	0.43 ± 0.01	0.53 ± 0.02	0.48 ± 0.01	0.40 ± 0.02	0.52 ± 0.02	0.46 ± 0.01
PA	Pancreas	0.40 ± 0.01	0.53 ± 0.02	0.47 ± 0.01	0.42 ± 0.02	0.55 ± 0.02	0.49 ± 0.01
CA	SI	1.01 ± 0.01	1.41 ± 0.07	1.21 ± 0.03	0.97 ± 0.05	1.29 ± 0.03	1.13 ± 0.03
PA	SI	1.00 ± 0.04	1.26 ± 0.03	1.13 ± 0.03	1.00 ± 0.01	1.32 ± 0.03	1.16 ± 0.01
CA	Lungs	0.42 ± 0.01	0.54 ± 0.01	0.48 ± 0.01	0.39 ± 0.01	0.51 ± 0.01	0.45 ± 0.01
PA	Lungs	0.43 ± 0.01	0.55 ± 0.02	0.49 ± 0.01	0.41 ± 0.01	0.54 ± 0.02	0.48 ± 0.01
CA	ADGD	0.022 ± 0.001	0.033 ± 0.002	0.028 ± 0.001	0.021 ± 0.001	0.030 ± 0.001	0.026 ± 0.001
PA	ADGD	0.021 ± 0.001	0.032 ± 0.001	0.027 ± 0.001	0.021 ± 0.001	0.032 ± 0.002	0.027 ± 0.001
CA	Gonads[a]	1.39 ± 0.03	0.051 ± 0.003	---	1.36 ± 0.02	0.050 ± 0.002	---
PA	Gonads[a]	1.39 ± 0.07	0.051 ± 0.002	---	1.42 ± 0.03	0.050 ± 0.001	---
CA	BAT	0.15 ± 0.01	0.20 ± 0.02	0.18 ± 0.01	0.15 ± 0.01	0.21 ± 0.01	0.18 ± 0.01
PA	BAT	0.17 ± 0.01	0.21 ± 0.01	0.19 ± 0.01	0.14 ± 0.01	0.20 ± 0.02	0.17 ± 0.01
CA	RP WAT	0.77 ± 0.06	0.58 ± 0.05	0.68 ± 0.04	0.93 ± 0.06	0.82 ± 0.10**	0.88 ± 0.06**
PA	RP WAT	0.87 ± 0.06	0.59 ± 0.05	0.73 ± 0.04	0.93 ± 0.06	0.86 ± 0.07**	0.90 ± 0.05**
CA	MT WAT	0.89 ± 0.05	1.03 ± 0.03	0.96 ± 0.03	0.99 ± 0.05	1.13 ± 0.06	1.06 ± 0.04
PA	MT WAT	0.95 ± 0.06	0.99 ± 0.06	0.97 ± 0.04	0.96 ± 0.05	1.19 ± 0.09	1.08 ± 0.05
CA	PG WAT	0.54 ± 0.02	1.22 ± 0.05	---	0.80 ± 0.05	1.43 ± 0.09	---
PA	PG WAT	0.61 ± 0.03	1.21 ± 0.08	---	0.79 ± 0.04	1.48 ± 0.08	---
CA	SQ WAT	0.69 ± 0.05	0.63 ± 0.04	0.66 ± 0.03	0.81 ± 0.05**	0.72 ± 0.04*	0.77 ± 0.03**
PA	SQ WAT	0.67 ± 0.05	0.64 ± 0.05	0.66 ± 0.03	0.88 ± 0.02**	0.75 ± 0.04**	0.82 ± 0.02**
CA	Total WAT	2.88 ± 0.15	3.46 ± 0.14	3.17 ± 0.10	3.52 ± 0.17**	4.11 ± 0.25**	3.82 ± 0.21**
PA	Total WAT	3.09 ± 0.16	3.43 ± 0.19	3.26 ± 0.13	3.58 ± 0.15**	4.29 ± 0.25**	3.94 ± 0.29**
CA	SM	0.039 ± 0.002	0.042 ± 0.002	0.041 ± 0.001	0.038 ± 0.002	0.044 ± 0.002	0.041 ± 0.001
PA	SM	0.040 ± 0.001	0.044 ± 0.001	0.042 ± 0.001	0.043 ± 0.001	0.043 ± 0.002	0.043 ± 0.001
CA	EDL	0.040 ± 0.001	0.042 ± 0.002	0.041 ± 0.001	0.039 ± 0.001	0.040 ± 0.001	0.040 ± 0.001
PA	EDL	0.039 ± 0.001	0.041 ± 0.002	0.040 ± 0.001	0.039 ± 0.001	0.040 ± 0.002	0.040 ± 0.001

CA	Thymus	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.13 ± 0.01
PA	Thymus	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.14 ± 0.01
CA	MTLN	0.019 ± 0.002	0.027 ± 0.002	0.023 ± 0.001	0.024 ± 0.002	0.025 ± 0.003	0.025 ± 0.002
PA	MTLN	0.019 ± 0.002	0.025 ± 0.004	0.022 ± 0.002	0.024 ± 0.001	0.027 ± 0.003	0.026 ± 0.001
CA	Stomach	0.52 ± 0.03	0.65 ± 0.04	0.59 ± 0.02	0.46 ± 0.02	0.61 ± 0.04	0.54 ± 0.02
PA	Stomach	0.49 ± 0.02	0.60 ± 0.02	0.55 ± 0.01	0.47 ± 0.01	0.61 ± 0.02	0.54 ± 0.01

Offspring from dams with exposure to CA or PA during gestation were fed a low-fat or high-fat diet between Day 21 (weaning) and Day 105 after birth, and were euthanized at Day 105 for determination of body and organ weights. Values, expressed as % of body weight, are means ± SEM. Prenatal exposure to air PA increased ($P < 0.01$) the relative weights of kidneys by 3%, as compared to the CA group (0.635 ± 0.005 % vs 0.616 ± 0.005 %, n = 40).

[a] Testes for males or ovaries for females.

* $P < 0.05$ vs the corresponding low-fat group.

** $P < 0.01$ vs the corresponding low-fat group.

ADGD, adrenal gland; BAT, brown adipose tissue; EDL, extensor digitorum longus muscle; MT, mesenteric; MTLN, mesenteric lymph node; PG, peri-gonad (testes for males or ovaries for females); RP, retroperitoneal; SI, small intestine; SM, soleus muscle; SQ, subcutaneous; WAT, white adipose tissue.

SI Appendix table S12. Concentrations of glucose, triacylglycerols and nonesterfied fatty acids in plasma of pups from CA dams and PA dams at PND21.

Maternal exposure	Males (n = 20)	Females (n = 20)	All rats (n = 40)
	Concentrations of glucose in plasma (mM)		
CA	5.31 ± 0.33	5.49 ± 0.47	5.40 ± 0.27
PA	5.57 ± 0.39	5.12 ± 0.45	5.35 ± 0.30
	Concentrations of triacylglycerols in plasma (mg/dL)		
CA	122 ± 6.2	122 ± 7.1	122 ± 4.8
PA	91.4 ± 8.1**	85.1 ± 5.5**	88.3 ± 4.9**
	Concentrations of nonesterfied fatty acids in plasma (μM)		
CA	320 ± 19	326 ± 25	323 ± 16
PA	289 ± 24	294 ± 13	292 ± 13

Offspring from female Sprague-Dawley rats with exposure to CA or PA between Days 0 and 18 of gestation were euthanized on PND 21. Values are means ± SEM, with the number of animals indicated in parenthesis.
** $P < 0.01$ vs the corresponding CA group.

SI Appendix table S13. The concentrations of hepatic lipids, as well as glucose, triacylglycerols and nonesterfied fatty acids in plasma of offspring from CA dams and PA dams at PND105 after consuming a low-fat or high-fat diet between PND21 and PND105.

Maternal exposure	Low-fat diet			High-fat diet			All rats
	Males (n = 10)	Females (n = 10)	Both sexes (n = 20)	Males (n = 10)	Females (n = 10)	Both sexes (n = 20)	(n = 40)
			Hepatic lipids (mg/g wet tissue)				
CA	14.1 ± 1.9	14.7 ± 2.3	14.4 ± 1.5	25.6 ± 3.1	25.5 ± 2.1	25.6 ± 1.9†	20.0 ± 1.2
PA	15.5 ± 2.0	31.2 ± 1.6*	23.4 ± 1.3	28.4 ± 1.9	26.4 ± 2.2	27.4 ± 1.5†	25.4 ± 0.98*
			Concentrations of glucose in plasma (mM)				
CA	7.63 ± 0.50	7.02 ± 0.31	7.33 ± 0.29	7.14 ± 0.42	7.07 ± 0.48	7.11 ± 0.64	7.22 ± 0.21
PA	8.22 ± 0.92	7.47 ± 0.71	7.85 ± 0.58	8.06 ± 0.70	8.38 ± 0.45	8.22 ± 0.41	8.03 ± 0.35**
			Concentrations of triacylglycerols in plasma (mg/dL)				
CA	70.1 ± 5.7	51.6 ± 3.2	60.9 ± 3.1	68.1 ± 8.5	43.4 ± 5.7	55.8 ± 5.1	58.3 ± 2.9
PA	77.8 ± 6.6	52.1 ± 3.7	65.0 ± 3.7	70.7 ± 9.3	43.1 ± 3.4	56.9 ± 4.5	60.9 ± 2.9
			Concentrations of nonesterfied fatty acids in plasma (μM)				
CA	248 ± 19	258 ± 30	253 ± 18	314 ± 9	261 ± 27	288 ± 13	270 ± 11
PA	315 ± 28	300 ± 21	308 ± 25	294 ± 16	346 ± 38	320 ± 19	314 ± 13*

Offspring from dams with exposure to CA or PA during gestation were fed a low-fat or high-fat diet between Day 21 (weaning) and Day 105 after birth. On PND105, rats were euthanized to obtain livers and plasma was prepared from blood for metabolite analyses. Values are means ± SEM, with the number of animals indicated in the parenthesis.

* $P < 0.05$ vs the corresponding CA group.

** $P < 0.01$ vs the corresponding CA group.

† $P < 0.01$ vs the corresponding low-fat group.

SI Appendix table S14. Hepatic mRNA *Fabp1* levels in pups from control dams and air-polluted dams at PND21 and PND105

Maternal exposure	Diet	Males	Females	All rats
			PND21	
CA	Nursed by dams	1.21 ± 0.12 (7)	1.28 ± 0.28 (7)	1.24 ± 0.14 (14)
PA	Nursed by dams	1.00 ± 0.17 (7)	1.09 ± 0.20 (9)	1.05 ± 0.18 (16)
			PND105	
CA	Low fat	1.27 ± 0.29 (6)	1.49 ± 0.25 (9)	1.38 ± 0.19 (15)
	High fat	1.37 ± 0.40 (9)	2.20 ± 0.39 (9)	1.78 ± 0.28 (18)
PA	Low fat	0.67 ± 0.11 (7)	1.21 ± 0.23 (8)	0.94 ± 0.12 (15)
	High fat	1.11 ± 0.20 (9)	0.90 ± 0.12 ** (9)	1.01 ± 0.11* (18)

Offspring from female Sprague-Dawley rats with exposure to CA or PA between Days 0 and 18 of gestation were euthanized on PND21 (weaning) for the collection of livers. Remaining rats in each litter were fed a low-fat or high-fat diet between PND21 and PND105, and were euthanized on PND105 for the collection of livers.

Values (ratios of *Fabp1* mRNA to *18S rRNA* mRNA) are means ± SEM, with the number of animals indicated in the parenthesis. On PND105, values for the CA group (n = 33) and PA group (n = 33) were 1.58 ± 0.15 and 0.97 ± 0.15, respectively ($P < 0.01$).

* $P < 0.05$ vs the corresponding CA group.

** $P < 0.01$ vs the corresponding CA group.

SI Appendix table S15. A summary of changes in absolute body weights, relative organ weights, and plasma metabolites in offspring from CA dams versus PA dams at different postnatal ages.

Variable	Birth (Day 0)	Weaning (Day 21)	Adult (Day 105)
Body weight (BW)	↓	↓	NC
Relative organ weight (% of body weight)			
Heart (HT)	↓	NC	NC
Spleen (SN)	↓	↑	NC
Kidneys (KY)	NC	NC	↑
Brain (BN)	↓	↓	NC
Small intestine (SI)	↓	NC	NC
Lung (LG)	NC	↓	NC
Thymus (TS)	---	↑	NC
Plasma metabolites			
Glucose	---	NC	↑
Triacylglycerols	---	↓	NC
NEFA	---	NC	↑

Offspring from female Sprague-Dawley rats with exposure to CA or PA between Days 0 and 18 of gestation were euthanized on PND0, PND21 (weaning), and PND105 for the measurement of body and organ weights.
NC, no change; NEFA, nonesterfied fatty acids.
↓ - Decrease in offspring from the PA dams, compared with offspring from the CA dams.
↑ - Increase in offspring from the PA dams, compared with offspring from the CA dams.

References

1. Wu G, Flynn NE, Flynn SP, Jolly CA, Davis PK (1999) Dietary protein or arginine deficiency impairs constitutive and inducible nitric oxide synthesis by young rats. *J Nutr* 129:1347-1354.
2. Jobgen WJ, et al. (2009) Dietary L-arginine supplementation reduces white-fat gain and enhances skeletal muscle and brown fat masses in diet-induced obese rats. *J Nutr* 139:230-237.
3. Wu G, et al. (2007) Dietary supplementation with watermelon pomace juice enhances arginine availability and ameliorates the metabolic syndrome in Zucker diabetic fatty rats. *J Nutr* 137:2680-2685.
4. Dunlap KA, et al. (2008) Progesterone and placentation increase secreted phosphoprotein one (SPP1 or osteopontin) in uterine glands and stroma for histotrophic and hematotrophic support of ovine pregnancy. *Biol Reprod* 79:983-90.
5. Wang G, Bonkovsky HL, de Lemos A, Burczynski FJ (2015) Recent insights into the biological functions of liver fatty acid binding protein 1. *J Lipid Res* 56:2238-47.