**Title:** Exposure to environmentally persistent free radicals during gestation lowers energy expenditure and impairs skeletal muscle mitochondrial function in adult mice

**Authors:** Erin J. Stephenson1,2,3, Alyse Ragauskas1,2,3, Sridhar Jaligama2,3, JeAnna R. Redd1,2,3, Jyothi Parvathareddy2,,3, Matthew J. Peloquin1,2,3 , Jordy Saravia2,3 and Stephania A. Cormier2,3,, Dave Bridges1,2,3,4

**Author contributions:** Funding (SAC, DB), Conceptualization (SAC, DB), Experimental design (SAC, DB, EJS), Data acquisition (EJS, JP, AR), Technical contributions (EJS, AR, MJP, JP, JRR, SJ), Data analysis (EJS, DB, JS), Data interpretation (EJS, DB), Drafted manuscript (EJS, DB), Edited manuscript (EJS, DB, SAC, SJ), Final approval of manuscript (EJS, DB, SAC, SJ, JS, MJP, JP, JRR, AR)

**Affiliations:**

1Department of Physiology, University of Tennessee Health Science Center, Memphis, Tennessee. 38163. 2Department of Pediatrics, University of Tennessee Health Science Center, Memphis, Tennessee, 38103. 3Children's Foundation Research Institute, Le Bonheur Children's Hospital, Memphis, Tennessee 38103, United States.

4Corresponding author

**Running head:** Gestational EPFR Exposure and energy expenditure

**Address for corresponding author:** email: [dbridge9@uthsc.edu](mailto:dbridge9@uthsc.edu) mail: 894 Union Avenue, Suite 521, Memphis TN 38163

# Abstract

We have investigated the effects of *in utero* exposure to Environmentally Persistent Free Radicals (EPFR’s) on growth, metabolism, energy utilization and skeletal muscle mitochondrial function in a mouse model of diet-induced obesity. Pregnant C57BL6/NHsd dams were treated with either a model particulate bound EPFR (MCP230) or saline. The adult offspring were placed on a high fat diet for 12 weeks, after which we observed a 10.8% increase in their body weight. The increase in body size observed in the MCP230-exposed mice was not associated with increases in food intake, but was associated with a reduction in physical activity and lower energy expenditure when compared to the saline-exposed mice. The reduced energy expenditure in the mice exposed to MCP230 was associated with reductions in skeletal muscle mitochondrial DNA copy number, lower mRNA levels of electron transport genes and reduced citrate synthase activity. These observations suggest that gestational exposure to EPFR’s leads to a reduction in energy expenditure, at least in part, through alterations to mitochondrial metabolism in the skeletal muscle.

# Key words

*In utero* exposure, Environmentally persistent free radicals, Whole-body metabolism, Skeletal muscle, Mitochondria

# Introduction

Obesity is a major global health concern and emerging data supports a role for environmental pollutants in the pathogenesis of obesity and its comorbidities [10–13] [1–4]. Gestational and early-life exposure to combustion-derived particulate matter has been associated with an increased risk of obesity in humans [10–13]. This association is supported by data obtained from animal studies, where the offspring of pregnant female mice, which have been exposed to diesel exhaust *in utero*, are predisposed to weight gain and increased body weight as adults [14]. Furthermore, several studies have linked the exposure to environmentally persistent free radicals (EPFR’s), compounds that result from the incomplete combustion of organic materials, to impaired metabolic health in humans [1–4] and animals [5–9]. Specifically, cross-sectional studies of human subjects who are chronically exposed to combustion derived particulate matter have shown associations with type 2 diabetes and cardiovascular disease [1,3,4], whereas murine models of chronic particulate matter exposure indicate that pollutants lead to elevated adipose tissue inflammation and insulin resistance [6,7,9].

From a mechanistic stand point, exactly how environmental pollutants result in obesity and other metabolic abnormalities is currently unknown. However, mitochondrial deficiencies and structural abnormalities have been observed in adipose tissue [7,8] vascular tissue [15] and cardiac muscle [16] following exposure to pollutants that should contain EPFR’s. The mitochondria are responsible for oxidative cellular energy production, reactive oxygen species production and are the primary site of the antioxidant defense system. Thus, defects in mitochondrial metabolism can have profound effects on whole body metabolic health. The importance of skeletal muscle mitochondrial metabolism for maintaining metabolic health is becoming well recognized [17–19] with deficits in muscle quality and function, particularly during early development [20] being closely linked to many different metabolic disease states [18]. However, the effects of *in utero* exposure to EPFR’s on skeletal muscle mitochondrial function remains to be determined. In this study, we have investigated the effects of *in utero* exposure to EPFR’s on growth, metabolism, energy utilization and skeletal muscle mitochondrial function in a mouse model of diet-induced obesity. We hypothesize that gestational exposure to EPFR’s reduces energy expenditure and results in impairments to the skeletal muscle mitochondria.

# Methods and Materials

## MCP230 Preparation and Treatment

MCP230 particles were generated and characterized by our colleagues as previously described [21]. Suspensions of MCP230 and cabosil (1mg/ml) were prepared in irrigation saline containing 0.02% tween 80 and the resulting particle suspension was monodispersed by probe sonication. Breeder mice (6 wk of age) were mated and pregnant dams were administered 50 µl of MCP230 particle suspension via oropharyngeal aspiration on days 10 and 17 of gestation, as described earlier [22]. [[1](#_ENREF_1)]Control mice received 50 µl saline or cabosil (amorphous silica). Briefly, mice were anesthetized by inhalant anesthetic isoflurane (5%) and anesthetized mice were placed upright in a holder and physically supported in an upright position. 50 µl of the suspension was instilled just above the vocal cords while holding the tongue with forceps to prevent swallowing.

## Animal Housing and High Fat Diet

C57BL/6NHsd mice were purchased from Harlan (Indianapolis, IN). Mice were maintained in a 12h light/dark cycle room at constant temperature and humidity and allowed unrestricted access to food and water. Offspring were weaned at 4 weeks of age. Male mice were selected for the study and were fed standard rodent chow until 10 weeks of age. At 10 weeks of age, mice were switched from chow to a high fat diet, consisting of 45% of calories from fat (Research Diets catalog D12451). Mice were maintained on high fat diet for 12 weeks. One mouse, a MCP230 treated animal, had a malocclusion and was removed from all data analyses. The UTHSC Institutional Animal Care and Use Committee approved all mouse procedures.

## Metabolite Assays

Glucose was determined using an AccuCheck glucometer. Serum hormone levels were determined using a Bio-Plex pro mouse diabetes multiplex immunoassay, BioRad (#171-F7001M) following the manufacturer’s instructions.

## Body Composition and Metabolic Cages

Mice were weighed weekly, starting at approximately ZT10. Body composition was determined non-invasively using an echo-MRI 100 at approximately ZT10. Food intake during the HFD phase was determined on a per-cage level by weighing the food on a weekly basis. For pre-HFD food intake, this was the sum of food eaten during the time in the metabolic cages, as determined by scaled feeder.

Energy expenditure, ambulatory locomotor activity and respiratory exchange ratios were determined in a home-cage style comprehensive laboratory animal monitoring system (Columbus Instruments). Mice were placed in the cages at approximately ZT10 and monitored for 3-4 days. Data from the first 6 h were discarded as this was the amount of time for the mice to become accustomed to their new single-caged environment. The Oxymax software provided by the vendor calculated the volumes of O2, CO2, the respiratory exchange ratio, the ambulatory x- and y-phase physical activity and the food consumption.

## Tissue Collection and Nucleic Acid Preparation

After the 12 week high fat diet phase, mice were fasted overnight, anesthetized with XXX and euthanized for tissue collection. Immediately after death, quadriceps muscles were carefully dissected out, cleared of any visible adipose and connective tissue and snap frozen in liquid N2. Nucleic acids were isolated from frozen quadriceps samples via Trizol extraction. Following careful and complete removal of the RNA-containing aqueous phase and its subsequent column purification (Life Technologies), genomic DNA extraction buffer (Tris base [1 M], sodium citrate dibasic trihydrate [50 mM], guanidine thiocyanate [4 M]) was added to the tubes containing the remaining Trizol-separated interphase and infranatant. Tubes were shaken vigorously and centrifuged at 12,000 G at room temperature for 30 minutes. The aqueous phase was then collected and the genomic DNA precipitated in isopropanol. Samples were re-spun at 12,000 G at 4°C to pellet the DNA. The DNA pellet was then washed in 70% ethanol, re-spun and, after careful ethanol removal, re-suspended in 1x Tris-EDTA buffer. cDNA was generated from purified RNA using the Applied Biosystems cDNA Synthesis Kit.

## qPCR Analysis of Mitochondrial DNA Copy Number and mRNA Transcripts

## Primers designed for three mitochondrial-encoded gene regions were used to assess mitochondrial DNA (mtDNA) copy number in genomic DNA and primers designed for both mitochondrial- and nuclear-encoded mitochondrial genes were used to assess mRNA transcript levels in cDNA extracted from the quadriceps muscle (Table 1). Briefly, genomic DNA or cDNA from each sample extraction was added to the appropriate working qPCR master mix (containing SYBR Green and the relevant primers). PCR conditions included an activation cycle of 95 ⁰C for 10 min followed by 45 amplification cycles of 15 s at 95 ⁰C, 15 s at 60 ⁰C, and 10 s at 73 ⁰C. Cp values were quantified using Light Cycler 480 software. Values were calculated using the ∆Ct method, with data for mtDNA copy number being normalized to values obtained for a nuclear-encoded genomic locus (*Tsc2*) and mRNA levels being normalized to *Rpl13a*.

## Preparation of protein lysates and western blotting

Skeletal muscle homogenates were prepared from ~30-50 mg of frozen quadriceps in RIPA buffer using stainless steel beads and a Qiagen tissue lyser. Homogenates were centrifuged at 4⁰C for 10 min at 14,000G, after which the protein concentration of supernatants was determined by Bradford assay. Lysates of equal protein concentration were prepared in 2x Laemmli buffer containing 2-mercaptoethanol and heated at 37⁰C for 5 min. Proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes for western blotting. After ponceau staining to ensure equal protein loading, membranes were blocked in BSA for 1 hr and incubated overnight in total OXPHOS rodent WB antibody cocktail (Abcam) at 4⁰C. Blots were visualized after a 1 hr incubation with infrared anti-mouse secondary antibody, using a LI-COR Odyssey fluorescent western blotting system. Protein expression was quantified using densitometry (Image Studio Lite, LI-COR).

## Citrate synthase activity

Muscle homogenates were prepared in KCl-EDTA buffer (pH 7.4) from ~10-40 mg of frozen quadriceps. Following 3 freeze-thaw cycles, samples were centrifuged at 4⁰C for 10 min at 1000 G to settle cellular debris. Supernatants were analyzed for citrate synthase activity using a modified method described in [23]. Briefly, aliquots of supernatant were added to the appropriate wells of a 96 well microplate containing an assay solution comprised of 72.5 mM Tris, 0.45 mM acetyl CoA and 0.1 mM 5,5’-dithiobis-2-nitrobenzoate (DTNB) (pH 8.3). After monitoring the plate for possible background activity, activity reactions were initiated by the addition of 0.5 mM oxaloacetic acid to each well. Changes in absorbance at 405 nm were recorded for each well every 9-11 sec over 3 min at room temp. Citrate synthase activity was calculated using the extinction coefficient for DTNB (which is reduced by the CoA-SH released during the cleavage of acetyl-CoA by citrate synthase).

## Statistics

Statistics and calculations were performed using Microsoft Excel and R version 3.1.1 [24]. For longitudinal data, mixed linear models were used and χ2 tests were performed to determine the significance of the MCP230 treatment. Mixed linear models used the R package lme4 (version 1.1-7 [25]). In all cases, normality of the data and models were determined via Shapiro-Wilk Test and equal variance was tested using Levene’s test from the car package (version 2.0-21 [26]). In cases where cabosil and saline treatment were not significantly different, these data were combined and designated as “Control”. For energy expenditure calculations, we performed an ANCOVA analysis with lean body mass and the treatment group as non-interacting covariates and the averaged light or dark VO2 as the responding variable as described in [27]. Statistical significance was designated as a p-value <0.05.

# Results

## Gestational exposure to MCP230 leads to increased body size on a high fat diet

To test the whole-body metabolic effects of gestational exposure to an environmentally persistent free radical (EPFR), we treated pregnant females with two exposures of MCP230 on day 10 and 17 of gestation. As controls, mice were either exposed to cabosil (the non-conjugated particulate without the EPFR group) or saline. After birth, these mice were left with their dams until weaning onto standard rodent chow at 28 days of age. At 10 weeks of age, mice were placed on a high fat diet consisting of 45% of calories from fat (see Figure 1A).

As shown in Figure 1B, at 10 weeks of age, mice that were exposed to MCP230 had a higher body weight than the saline-exposed mice and remained heavier throughout the high fat diet phase. After 12 weeks of high fat diet, the MCP230-exposed mice were 4.5 g heavier than saline-exposed mice (10.3%, p<0.001; Figure 1B). We assessed body composition after 12 weeks of high fat diet and observed significant elevations in both fat mass (10.6% increase, p=0.011) and lean mass (10.8% increase, p=2.2x10-4) in the MCP230-exposed mice (Figures 1C and D). The relative adiposity of these mice, as determined by the percent fat mass, was not different between groups (Figure 1E).

## MCP230-exposed mice have reduced caloric intake and increased serum concentrations of leptin, ghrelin and GLP-1

To determine how energy balance was affected in MCP230-exposed mice, we examined their food intake longitudinally throughout the study. As shown in Figure 2A, all mice tended to eat less food each week, though this did not reach statistical significance. Cumulatively, the MCP230-exposed mice ate less food throughout the diet (-6.3 +/- 1.8 kcal/week/mouse, χ2=11.6, p=8.0 x 10-4, Figure 2B). Throughout the 12 week high fat diet treatment, this corresponds to a 20% reduction in total caloric intake. These data suggest that the increased body mass of MCP230-exposed mice (Figure 1B) was not due to increased caloric intake, as the caloric intake of MCP230-exposed mice was 19.2% less than that of the saline-exposed mice at the end of the high fat diet period (Figure 2.B).

Leptin concentrations were modestly elevated in both fasted and fed state serum from MCP230-exposed mice (main effects for both feeding state, p=0.002, and treatment, p=0.011, by 2-way ANOVA, with post-hoc *t-*test p-values of 0.058 for fasting and p=0.097 for fed leptin concentrations, Figure 2C). Elevations in circulating leptin levels are consistent with the increased fat mass observed in MCP230-exposed mice (Figure 1C). We observed significant serum elevations in both the fasting and fed state for the hunger-stimulating hormone Ghrelin (main effects for both feeding state, p=0.001, and treatment, p=6.5 x 10-6, by 2-way ANOVA, with post-hoc *t-*test p-values of 0.024 for fasting and p=0.0002 for fed ghrelin concentrations, Figure 2D), which is consistent with the reduced food intake (Figure 2A and B) and reduced energy expenditure (Figure 4A, B and C) observed in the MCP230-exposed mice [37,40] [38,39]. Similarly, GLP-1 was elevated in serum from MCP230-exposed mice in both the fasting and fed state (main effects for both feeding state, p=0.002, and treatment, p=3.6 x 10-5, by 2-way ANOVA, with post-hoc *t-*test p-values of 0.024 for fasting and p=0.001 for fed GLP-1 concentrations, Figure 2D), which is also consistent with the MCP230-exposed mice eating less (Figure 2A and B) [44,45]. There was a main effect of feeding state for GIP concentrations (p=6.0 x 10-9 by 2-way ANOVA, Figure 3E) and GIP was elevated in serum from MCP230-exposed mice in the fasting state, although these values did not attain statistical significance (p=0.069 for fasted GIP-1 levels by Wilcoxon Rank Sum Test).

We next evaluated the extent of obesity related co-morbidities in these mice. We observed no changes in fasting blood glucose as a result of MCP230 exposure (Figure 3A). As shown in Figure 3B, there was a main effect of feeding state on serum insulin concentrations (p=3.3 x 10-6), however, MCP230 exposure had no effect, suggesting that insulin sensitivity of both the saline and MCP230 exposed mice was similar in response to the high fat diet. A main effect of both feeding state (p=7.3 x 10-5) and treatment (p=4.0 x 10-3) was observed for serum glucagon concentrations, with MCP230-exposed mice having elevated glucagon concentrations in the fasted and fed state, although fed state levels did not quite attain statistical significance (p=0.009 and p=0.059, respectively, by post-hoc *t*-test analysis; Figure 3C).

## MCP230 Mice Have Reduced Energy Expenditure

Since the MCP230 mice did not appear to be larger due to excessive caloric intake, we next examined their energy utilization. To evaluate energy expenditure, we individually housed 9 week old mice (prior to HFD) in individual metabolic cages for indirect calorimetry, physical activity monitoring and evaluation of gas exchange rates. As shown in Figure 4A, the MCP230 exposed mice had lower energy expenditure, as determined by oxygen consumption (VO2). Figure 4B depicts the energy expenditure, as determined by the VO2 for each mouse, plotted against the animal’s fat-free mass. Accounting for change in lean mass is necessary due to known associations between this covariate and rates of oxygen consumption [27]. Based on these calculations, we observed decreased energy expenditure of the MCP230 exposed mice in both the dark (-19.1%, p=0.020) and light (-16.8%, p=0.031) phases.

To determine whether these decreases in energy expenditure were associated with changes in physical activity, we monitored the ambulatory movements of these mice while they were housed in the metabolic cages. As shown in Figure 4C, compared to the control groups, we observed 21.4% (p=0.040) and 26.2% (p=0.0099) reductions in physical activity for the MCP230-exposed mice in the dark and light phases, respectively.

We next evaluated energy substrate preference by analyzing the respiratory exchange ratio of the three groups. When this ratio nears 1, that indicates preference for predominately carbohydrate as fuel and as it nears 0.7 it indicates utilization of mainly lipids [28]. Although there was no difference in the respiratory exchange ratio between MCP230 and cabosil exposed mice, we did observe a significant elevation (carbohydrate preference) in the saline exposed mice during both the light and dark phases relative to mice exposed to either the vehicle control (cabosil) or EPFR (MCP230) (Figure 4D). These data indicate that the unconjugated particle exposure itself (though not the EPFR group) may alter substrate preference in these mice.

## Skeletal Muscle from MCP230 Treated Mice Have Reduced Mitochondrial DNA Copy Number and a Lower Citrate Synthase Activity

Due to the observed reductions in whole-body oxygen consumption and total energy expenditure, we next explored the hypothesis that MCP230 exposed mice have skeletal muscle mitochondrial deficits. To test this, we first determined mtDNA copy number in quadriceps muscle after the 12 week high fat diet phase. Figure 5A demonstrates that MCP230-exposed mice have a marked reduction in mtDNA copy number relative to the saline-exposed mice, as determined using primers designed for three distinct mtDNA-encoded gene regions. Decreases of 61.2%, 68.0% and 51.9% were observed for the mitochondrial D-loop, *mt-Cytb* and *mt-Nd1*, respectively (p=0.039, p=0.031 and p=0.032, respectively) suggesting that MCP230 exposed mice have reduced skeletal muscle mitochondrial content. Since citrate synthase activity is strongly associated with skeletal muscle mitochondrial content [29] and given that mtDNA copy number was reduced with *in utero* MCP230 exposure, we measured citrate synthase activity to further evaluate mitochondrial content and function in the skeletal muscle from MCP230 exposed mice. As shown in Figure 5B, maximal citrate synthase activity was reduced 24.1% in the quadriceps from MCP230 exposed mice (p=0.03). Taken together, reduced mtDNA copy number and lower citrate synthase activity suggest that mice exposed to MCP230 are likely to have reduced mitochondrial oxidative enzyme content and, as a result, reduced skeletal muscle oxidative capacity, which, along with the reduction in physical activity, would likely contribute to the reduced VO2 seen in these mice.

Consistent with this hypothesis, mRNA transcript levels for the mitochondrial- and nuclear-encoded electron transport genes *mt-Nd4* (25.2%), *Sdha* (35.9%), *mt-Cytb* (35.4%) and *mt-Co2* (35.1%) were reduced in the quadriceps from MCP230-exposed mice, although not all of these reductions attained statistical significance (p=0.12, p=0.08, p=0.04 and p=0.10, respectively; see Figure 5C). To determine whether similar differences in skeletal muscle mitochondrial electron transport enzymes were also present at the protein level, we measured the relative expression of several electron transport chain proteins via western blotting (Figure 5D). Interestingly, we observed an increase in the relative abundance of the two of the five proteins measured in skeletal muscle from mice exposed to MPC230 compared to the saline-treated mice; NDUFB8 and ATP5A were elevated 66.2% (p=0.042) and 64.2% (p=0.022), respectively, whereas the other three electron transport proteins measured were expressed similarly between the groups (Figure 5E). This finding suggests that functional impairments in the oxidative capacity of the skeletal muscle are likely to be secondary to the ability of the myocyte to generate mitochondrial enzymes at the protein level.

# Discussion

In this study, we have tested the metabolic effects of a limited gestational exposure to EPFR-containing PM. We noted that these mice grew larger, despite reductions in food intake, and that this correlated with reduced energy expenditure and impaired mitochondrial oxidative function in skeletal muscle.

One potential explanation for the reductions in energy expenditure and skeletal muscle mitochondrial function is the observed reduction in physical activity for MCP230-exposed mice. It is also possible that muscle weakness (due to reduced skeletal muscle oxidative capacity [30,31]) could contribute to the reduced physical activity of MCP230-exposed animals. Both of these hypotheses are consistent with cross-sectional studies showing negative associations between ambient air pollutant exposure and leisure time physical activity [32] and exercise performance [33–35]. Our current data are unable to determine whether reduced mitochondrial function is the primary cause of these reductions in energy expenditure or if this observation is secondary to a reduced propensity for physical activity. However, our observations of reductions in mitochondrial DNA, citrate synthase activity and mRNA transcripts support the possibility that gestational exposure to EPFRs directly affects skeletal muscle mitochondrial oxidative function, which may contribute to changes in energy expenditure.

The mechanisms by which gestational exposure to EPFRs result in reduced mitochondrial function are not yet clear. Our data presented here are consistent with chronic models of PM2.5 exposure, which show reduced mitochondrial numbers in white adipose tissue [7,8]. Analyses of placental tissues from mothers showed a strong correlation between late-gestational PM10exposure and placental mitochondrial DNA content [13]. Given the elevated sensitivity of mitochondria to free radicals and oxidative stress, it is reasonable to hypothesize that during development, EPFR-mediated mitochondrial damage may result in chronic decreases in mitochondrial oxidative function, either directly, via reactive oxygen species, or indirectly, via inflammatory processes. Based on our current protocol, mice are treated with EPFRs after inheritance of maternal mitochondria, indicating that this mitochondrial damage occurs *in situ* in the progeny. In contrast to previous studies that use chronic pollution models [1,3,4,6,36], we did not observe any indications that glycemic control was impaired in MCP230 exposed mice (there were no differences in fasting glucose or fasting/fed insulin concentrations relative to the saline or cabosil exposed mice, Figure 3), indicating that the effects of acute gestational particulate exposure are not likely to mimic the effects of chronic exposure, and the risk profiles and mechanisms associated with these exposures may differ.

In conclusion, we have investigated the effects of limited, gestational exposure to combustion-derived pollutants in a mouse model of diet-induced obesity. Our findings show that even brief gestational exposure to environmental pollutants such as EPFR’s can result in chronic changes in growth, metabolism and energy balance. These changes are associated with skeletal muscle mitochondrial deficits and reductions in physical activity, which likely contribute to reduced oxygen consumption and a predisposition to elevated body weight on a high fat diet. While the mechanisms behind these changes remain to be determined, the finding that limited *in utero* exposure to EPFR’s can suppress later life energy metabolism highlights a need for further research.

# Acknowledgements

The authors would like the other members of the Bridges and Cormier, O’Connell and Han labs for helpful discussions and insights.

# Grants

We would like to acknowledge funding from NIH grant 1R01DK107535

Le Bonheur Grant #650700 (to DB) and NIH grants (R01AI090059, R01ES015050, and P42ES013648) to SAC.

# Disclosures

The authors have no conflicts of interest to disclose. **References**

1. Brook RD, Jerrett M, Brook JR, Bard RL, Finkelstein MM (2008) The relationship between diabetes mellitus and traffic-related air pollution. J Occup Environ Med 50: 32–38. doi:10.1097/JOM.0b013e31815dba70.

2. Cakmak S, Dales R, Leech J, Liu L (2011) The influence of air pollution on cardiovascular and pulmonary function and exercise capacity: Canadian Health Measures Survey (CHMS). Environ Res 111: 1309–1312. doi:10.1016/j.envres.2011.09.016.

3. Andersen ZJ, Raaschou-Nielsen O, Ketzel M, Jensen SS, Hvidberg M, et al. (2012) Diabetes incidence and long-term exposure to air pollution: a cohort study. Diabetes Care 35: 92–98. doi:10.2337/dc11-1155.

4. Thiering E, Cyrys J, Kratzsch J, Meisinger C, Hoffmann B, et al. (2013) Long-term exposure to traffic-related air pollution and insulin resistance in children: results from the GINIplus and LISAplus birth cohorts. Diabetologia 56: 1696–1704. doi:10.1007/s00125-013-2925-x.

5. Li XY, Gilmour PS, Donaldson K, MacNee W (1996) Free radical activity and pro-inflammatory effects of particulate air pollution (PM10) in vivo and in vitro. Thorax 51: 1216–1222. doi:10.1136/thx.51.12.1216.

6. Sun Q, Yue P, Deiuliis J a, Lumeng CN, Kampfrath T, et al. (2009) Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. Circulation 119: 538–546. doi:10.1161/CIRCULATIONAHA.108.799015.

7. Xu X, Liu C, Xu Z, Tzan K, Zhong M, et al. (2011) Long-term exposure to ambient fine particulate pollution induces insulin resistance and mitochondrial alteration in adipose tissue. Toxicol Sci 124: 88–98. doi:10.1093/toxsci/kfr211.

8. Xu Z, Xu X, Zhong M, Hotchkiss IP, Lewandowski RP, et al. (2011) Ambient particulate air pollution induces oxidative stress and alterations of mitochondria and gene expression in brown and white adipose tissues. Part Fibre Toxicol 8: 20. doi:10.1186/1743-8977-8-20.

9. Liu C, Fonken LK, Wang A, Maiseyeu A, Bai Y, et al. (2014) Central IKKβ inhibition prevents air pollution mediated peripheral inflammation and exaggeration of type II diabetes. Part Fibre Toxicol 11: 53. doi:10.1186/s12989-014-0053-5.

10. Crawley HF, While D (1996) Parental smoking and the nutrient intake and food choice of British teenagers aged 16-17 years. J Epidemiol Community Heal 50: 306–312. doi:10.1136/jech.50.3.306.

11. Johnson RK, Wang MQ, Smith MJ, Connolly G (1996) The association between parental smoking and the diet quality of low-income children. Pediatrics 97: 312–317.

12. Burke V, Gracey MP, Milligan RA, Thompson C, Taggart AC, et al. (1998) Parental smoking and risk factors for cardiovascular disease in 10- to 12-year-old children. J Pediatr 133: 206–213.

13. Janssen BG, Munters E, Pieters N, Smeets K, Cox B, et al. (2012) Placental mitochondrial DNA content and particulate air pollution during in utero life. Environ Health Perspect 120: 1346–1352. doi:10.1289/ehp.1104458.

14. Bolton JL, Smith SH, Huff NC, Gilmour MI, Foster WM, et al. (2012) Prenatal air pollution exposure induces neuroinflammation and predisposes offspring to weight gain in adulthood in a sex-specific manner. FASEB J 26: 4743–4754. doi:10.1096/fj.12-210989.

15. Westbrook DG, Anderson PG, Pinkerton KE, Ballinger SW (2010) Perinatal tobacco smoke exposure increases vascular oxidative stress and mitochondrial damage in non-human primates. Cardiovasc Toxicol 10: 216–226. doi:10.1007/s12012-010-9085-8.

16. Li R, Kou X, Geng H, Xie J, Tian J, et al. (2015) Mitochondrial damage: An important mechanism of ambient PM2.5 exposure-induced acute heart injury in rats. J Hazard Mater 287C: 392–401. doi:10.1016/j.jhazmat.2015.02.006.

17. Hoeks J, Schrauwen P (2012) Muscle mitochondria and insulin resistance: a human perspective. Trends Endocrinol Metab 23: 444–450. doi:10.1016/j.tem.2012.05.007.

18. Russell AP, Foletta VC, Snow RJ, Wadley GD (2014) Skeletal muscle mitochondria: A major player in exercise, health and disease. Biochim Biophys Acta - Gen Subj 1840: 1276–1284. doi:10.1016/j.bbagen.2013.11.016.

19. Stephenson EJ, Hawley J a. (2014) Mitochondrial function in metabolic health: A genetic and environmental tug of war. Biochim Biophys Acta - Gen Subj 1840: 1285–1294. doi:10.1016/j.bbagen.2013.12.004.

20. Brown LD (2014) Endocrine regulation of fetal skeletal muscle growth: impact on future metabolic health. J Endocrinol 221: R13–R29. doi:10.1530/JOE-13-0567.Endocrine.

21. Lomnicki S, Truong H, Vejerano E, Dellinger B (2008) Copper oxide-based model of persistent free radical formation on combustion-derived particulate matter. Environ Sci Technol 42: 4982–4988. doi:10.1021/es071708h.

22. Wang P, Thevenot P, Saravia JS, Ahlert T, Cormier SA (2011) Radical Containing Particles Activate DCs and Enhance Th17 Inflammation in a Mouse Model of Asthma. Am J Respir Cell Mol Biol 45: 977. doi:10.1165/rcmb.2011-00010C.

23. Srere PA (1969) Citrate synthase. Methods in enzymology. pp. 3–11.

24. R Core Team (2013) R: A Language and Environment for Statistical Computing.

25. Bates D, Mächler M, Bolker B, Walker S (2014) Fitting Linear Mixed-Effects Models using lme4. ArXiv 1406.5823: 1–51.

26. Fox J, Weisberg S (2011) An {R} Companion to Applied Regression. Second. Thousand Oaks {CA}: Sage.

27. Tschöp MH, Speakman JR, Arch JRS, Auwerx J, Brüning JC, et al. (2011) A guide to analysis of mouse energy metabolism. Nat Methods 9: 57–63. doi:10.1038/nmeth.1806.

28. Hill A V., Long CNH, Lupton H (1924) Muscular Exercise, Lactic Acid, and the Supply and Utilisation of Oxygen. Proc R Soc B Biol Sci 96: 438–475. doi:10.1098/rspb.1924.0037.

29. Larsen S, Nielsen J, Hansen CN, Nielsen LB, Wibrand F, et al. (2012) Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. J Physiol 590: 3349–3360. doi:10.1113/jphysiol.2012.230185.

30. Zurlo F, Larson K, Bogardus C, Ravussin E (1990) Skeletal muscle metabolism is a major determinant of resting energy expenditure. J Clin Invest 86: 1423.

31. Taivassalo T, Jensen TD, Kennaway N, DiMauro S, Vissing J, et al. (2003) The spectrum of exercise tolerance in mitochondrial myopathies: a study of 40 patients. Brain 126: 413–423. doi:10.1093/brain/awg028.

32. Roberts JD, Voss JD, Knight B (2014) The association of ambient air pollution and physical inactivity in the United States. PLoS One 9: e90143. doi:10.1371/journal.pone.0090143.

33. Marr LC, Ely MR (2010) Effect of air pollution on marathon running performance. Med Sci Sports Exerc 42: 585–591. doi:10.1249/MSS.0b013e3181b84a85.

34. Cutrufello PT, Rundell KW, Smoliga JM, Stylianides GA (2011) Inhaled whole exhaust and its effect on exercise performance and vascular function. Inhal Toxicol 23: 658–667. doi:10.3109/08958378.2011.604106.

35. Rundell KW, Caviston R (2008) Ultrafine and fine particulate matter inhalation decreases exercise performance in healthy subjects. J Strength Cond Res 22: 2–5. doi:10.1519/JSC.0b013e31815ef98b.

36. Chen J-C, Schwartz J (2008) Metabolic syndrome and inflammatory responses to long-term particulate air pollutants. Environ Health Perspect 116: 612–617. doi:10.1289/ehp.10565.

37. Wilson JL, Enriori PJ (2015) Molecular and Cellular Endocrinology A talk between fat tissue , gut , pancreas and brain to control body weight. Mol Cell Endocrinol. doi:10.1016/j.mce.2015.08.022.

38. Tschöp MH, Smiley DL, Heiman ML (2000) Ghrelin induces adiposity in rodents. Nature 407: 908–913. doi:10.1038/35038090.

39. Toshinai K, Mondal MS, Nakazato M, Date Y, Murakami N, et al. (2001) Upregulation of Ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. Biochem Biophys Res Commun 281: 1220–1225. doi:10.1006/bbrc.2001.4518.

40. Cowley M a., Smith RG, Diano S, Tschöp M, Pronchuk N, et al. (2003) The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron 37: 649–661. doi:10.1016/S0896-6273(03)00063-1.

41. Tschöp MH, Weyer C, Tataranni P a, Devanarayan V, Ravussin E, et al. (2001) Circulating ghrelin levels are decreased in human obesity. Diabetes 50: 707–709.

42. Peloquin MJ, Bridges D (2014) Weight Loss in Response to Food Deprivation Predicts The Extent of Diet Induced Obesity in C57BL/6J Mice. bioRxiv. doi:10.1101/004283.

43. Ikezaki A, Hosoda H, Ito K, Iwama S, Miura N, et al. (2002) Fasting Plasma Ghrelin Levels Are Negatively Correlated With Insulin Resistance and PAI-1, but Not With Leptin, in Obese Children and Adolescents. Diabetes 51: 3408–3411. doi:10.2337/diabetes.51.12.3408.

44. Baggio LL, Huang Q, Brown TJ, Drucker DJ (2004) Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. Gastroenterology 127: 546–558. doi:10.1053/j.gastro.2004.04.063.

45. Turton MD, O’Shea D, Gunn I, Beak SA, Edwards CM, et al. (1996) A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379: 69–72. doi:10.1038/379069a0.

1. Wang P, Thevenot P, Saravia J, Ahlert T, Cormier SA (2011) Radical-containing particles activate dendritic cells and enhance Th17 inflammation in a mouse model of asthma. Am J Respir Cell Mol Biol 45: 977-983.

# Figure Legends

**Figure 1: *In utero* exposure to MCP230 results in increased body size. (**A) Schematic of the experimental design. (B) Body weight throughout the high fat diet phase of the intervention. (C) Absolute body fat, (D) fat-free mass and (E) percent body fat after 12 weeks of high fat diet (ZT12). Data shown is the group mean ± SE. \* indicates p<0.05 via a Student’s *t*-test (C-D). The saline-exposed mice are depicted in black and the MCP230-exposed mice are depicted in grey.

**Figure 2: Gestational exposure to MCP230 causes a reduction in food intake and alters ‘hunger hormone’ concentrations on a high fat diet.** Food intake per mouse was calculated on a (A) weekly and (B) cumulative basis throughout the high fat diet phase of the intervention. MCP230-exposed mice had elevated serum concentrations of (C) leptin, (D) Ghrelin and (E) GLP-1 after access to the high fat diet. (F) Serum GIP tended to be elevated during the fasted state, although this did not attain statistical significance. Fed serum was collected at ZT12. Fasting serum was collected following an overnight fast (~16 hr) at ZT4. Data shown is the group mean ± SE. n=8-14/group. § indicates p<0.05 by mixed linear model, compared by χ2 test. † indicates a main effect for feeding state and ‡ indicates a main effect for MCP230-exposure by 2-way ANOVA. \* indicates p<0.05 via a Wilcoxon Rank Sum Test. The saline-exposed mice are depicted in black and the MCP230-exposed mice are depicted in grey.

**Figure 3: Gestational exposure to MCP230 causes an increase in serum glucagon but does not differentially alter glucose or insulin concentrations following exposure to a high fat diet.** Fasting blood glucose (A), serum insulin (B) and serum glucagon (C) concentrations were determined after a 16h fast at ~ZT4. Fed serum was collected at ZT12 and analyzed for insulin (B) and glucagon (C). Data shown is the group mean ± SE. n=8-14/group. † indicates a main effect for feeding state and ‡ indicates a main effect for MCP230-exposure by 2-way ANOVA. \* indicates p<0.05 via a Wilcoxon Rank Sum Test. The saline-exposed mice are depicted in black and the MCP230-exposed mice are depicted in grey.

**Figure 4: *In utero* exposure to MCP230 reduces energy expenditure and lowers physical activity.** (A) Oxygen consumption rates (VO2) and (B) VO2 analysis, normalized to fat-free mass during both the light and dark phase. Each dot represents the average oxygen consumption of each mouse. (C) Quantification of x-axis ambulatory movement during the light and dark phases. (F) Respiratory exchange ratio of each group. Saline and cabosil groups were not combined for this analysis as there was a significant reduction in the respiratory exchange ratio for both the cabosil- and MCP230- exposed groups. Data shown is either the individual (B) or group mean (A, C and D) ± SE (C and D). n=18, 6 or 14 for MCP230, saline and cabosil groups, respectively. § indicates p<0.05 by ANOVA (B). \*indicates p<0.05 by Student’s *t*-test (C), or Wilcoxon-Rank Sum Test (D). The saline-exposed mice are depicted in black, the cabosil-exposed mice are depicted in white and the MCP230-exposed mice are depicted in grey. Where the saline- and cabosil- exposed groups are combined, these mice are depicted in black and white stripes.

**Figure 4: Exposure to MCP230 *in utero* results in skeletal muscle mitochondrial abnormalities following high fat diet consumption as adults**. mtDNA copy number (A), citrate synthase activity (B) and mRNA levels of oxidative phosphorylation genes (C) were reduced in the quadriceps muscles of mice that were exposed to MCP230 *in utero* and subjected to 12 wk of high fat diet as adults. Quadriceps electron transport chain protein expression was either unchanged, or greater in the MCP230-exposed mice (D and E). Data shown is the group mean ± SE. \*indicates p<0.05 via Student’s *t*-test. n=7-12/group. The saline-exposed mice are depicted in black and the MCP230-exposed mice are depicted in grey.

**Supplementary figure: Fed and fasting PAI-1 and resistin levels.** There was a main effect for feeding state on serum PAI-1 concentrations as measured by 2-way ANOVA, however, there were no differences between the two groups when compared by post-hoc analysis. There were no differences in serum resistin concentrations for either feeding state or treatment.