**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 , Joan C. Han1,2,3 and Stephania A. Cormier1,2,3,, Dave Bridges1,2,3,4

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**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.

4Corresponding author

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

**Address for corresponding author:** email: [dbridge9@uthsc.edu](mailto:dbridge9@uthsc.edu) mail: Department of Physiology, Room 426. 894 Union Avenue, 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 mitochondria in a mouse model of diet-induced obesity. Pregnant mice were treated with laboratory-generated combustion derived particular matter (MCP230). The adult offspring were placed on a high fat diet for 12 weeks, after which we observed a 9.8% 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. The reduced energy expenditure in mice indirectly 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. Up-regulation of key genes involved in ameliorating oxidative stress was also observed in the muscle of MCP230-exposed mice. These findings suggest that gestational exposure to MCP230 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, Oxidative Stress, 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 (2, 7, 9, 10, 12, 21, 22, 43). Gestational and early-life exposure to combustion-derived particulate matter (PM) has been associated with an increased risk of obesity in humans (9, 12, 17, 21, 22). This association is supported by data obtained from animal studies, where the offspring of pregnant mice, which have been exposed to diesel exhaust *in utero*, are predisposed to weight gain as adults (6). Furthermore, several studies have linked the exposure to combustion-derived PM to impaired metabolic health in humans (2, 7, 10, 43) and animals (27, 28, 41, 52, 53). Specifically, cross-sectional studies of human subjects who are chronically exposed to combustion derived PM have shown associations with type 2 diabetes and cardiovascular disease (2, 7, 43), whereas murine models of chronic PM exposure indicate that pollutants lead to elevated adipose tissue inflammation and insulin resistance (28, 41, 52). Relatively stable radicals with half-lives of ~21 days exist on the surface of airborne PM (13, 15, 29) and these are referred to as Environmentally Persistent Free Radicals (EPFR’s).

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 (52, 53), vascular tissue (50) and cardiac muscle (26) following exposure to combustion derived pollutants that should contain EPFR’s. Mitochondria are responsible for oxidative cellular energy production, endogenous reactive oxygen species production and are an essential component of the antioxidant defense system (REF). Thus, defects in mitochondrial metabolism, particularly in the context of obesity, are likely to have profound effects on energy homeostasis and other metabolic pathways. The importance of skeletal muscle mitochondrial metabolism for maintaining metabolic health is becoming well recognized (20, 35, 39) with deficits in muscle quality and function, particularly during early development (8), being closely linked to later life metabolic disturbances such as impaired growth or insulin resistance (35)(REF). However, the effects of *in utero* exposure to EPFR’s on skeletal muscle mitochondrial quality remains to be determined. In this study, we investigated the effects of *in utero* exposure to EPFR’s on growth, metabolism, energy utilization and skeletal muscle mitochondria in a mouse model of diet-induced obesity. We hypothesized that gestational exposure to EPFR’s reduces energy expenditure and results in mitochondrial impairments in the skeletal muscle.

# Methods and Materials

## MCP230 Preparation

EPFR-containing particles (i.e. MCP230) were generated and characterized by our colleagues as previously described (29). Suspensions of MCP230 and cabosil, a non EPFR-containing amorphous silica particle control, (1mg/ml) were prepared in irrigation saline containing 0.02% tween 80 and the resulting particle suspension was monodispersed by probe sonication.

## Animals, Particulate Exposure 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. 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 (49). Control mice received 50 µl saline or cabosil. Pregnant mice were anesthetized by inhalant anesthetic isoflurane (5%) and placed in a holder and physically supported in an upright position. The suspension was instilled just above the vocal cords while holding the tongue with forceps to prevent swallowing. 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 (HFD), consisting of 45% of calories from fat (Research Diets catalog D12451). Mice were maintained on HFD for 12 weeks. One mouse, an MCP230 treated animal, had malocclusion and was removed from all data analyses. The UTHSC Institutional Animal Care and Use Committee approved all mouse procedures.

## Metabolite Assays

Blood was collected in both the fed and 16- hour fasted state. Blood 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 using a MAGPIX LMX200 system. HOMA-IR was calculated from 16-hour fasting glucose and insulin values.

## Body Composition and Metabolic Cages

Mice were weighed weekly, from approximately ZT10. Body composition was determined non-invasively using an echo-MRI 100. Food intake during the HFD phase was determined on a per-cage level by weighing the food on a weekly basis. For food intake pre-HFD, this was determined by scaled feeders within the CLAMS system.

VO2, 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 determined to be necessary for the mice to acclimate to their new environment. Oxymax software (Columbus Instruments) calculated the volumes of O2, CO2, the respiratory exchange ratio, the ambulatory x- and y-phase physical activity and the food consumption. Heat production was calculated using the Lusk equation (30) via the Oxymax software:

Heat = (3.815 + 1.232 \* RER) \* VO2

## Tissue Collection and Nucleic Acid Preparation

After the 12 week HFD phase, mice were fasted overnight, anesthetized with ketamine/xylazine (180/10 mg/kg, respectively) delivered IP. 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 in a Qiagen Tissue Lyser (30Hz for 5 min). Following careful and complete removal of the RNA-containing aqueous phase and its subsequent column purification (PureLink mRNA kit from Life Technologies), 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 collected and the genomic and mitochondrial 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 TE 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 DNA and primers designed for quantitative RT-PCR were used to assess mRNA transcript levels (Table 1). Briefly, DNA or cDNA from each sample extraction was added to the appropriate working qPCR master mix (containing SYBR Green and the relevant primers at a final concentration of 100 nM each). 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 on a Light Cycler 480. Nucleic acid levels 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*, which was determined to be unaffected by MCP230 treatment in comparison to other commonly used normalization controls, including *Rplp0* and *Gapdh*.

## Preparation of protein lysates and western blotting

Skeletal muscle homogenates were prepared from ~30-50 mg of frozen quadriceps in RIPA buffer (Tris basic [50 mM], Sodium deoxycholate [ 0.25%], NP-40 [1%], NaCl [150 mM], EDTA [1 mM], Na3VO4 [100 µM], NaF [5 mM], Sodium pyrophosphate [10 mM], protease inhibitor cocktail) using stainless steel beads and a Qiagen Tissue Lyser (30Hz for 5 min). Homogenates were centrifuged at 4⁰C for 10 min at 14,000 g, 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 mitochondrial proteins) or 95 ⁰C (for all non-mitochondrial proteins) 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 #ab110413), anti-PGC-1α (Sigma #SAB4200209), anti-phospho AMPKT172 (Cell Signaling #2535S), anti-AMPK (Cell Signaling #2793S), anti-phospho S6KT389 (Cell Signaling #9206), anti-S6K (Cell Signaling #2708), anti-phospho AktS473 (Cell Signaling #4060), anti-Akt (Cell Signaling #9272), anti-LC3 (Cells Signaling #12741) or anti-β-Actin (Proteintech #60008-1-Ig) at 4⁰C. Blots were visualized after a 1 hr incubation with infrared anti-mouse or anti-rabbit secondary antibody, using a LI-COR Odyssey fluorescent western blotting system. Protein expression was quantified using densitometry (Image Studio Lite, LI-COR) and normalized to Akt, which was unchanged by the treatments. LC3 was presented as the ratio of LC3II/LC3I as proscribed in (23).

## 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 (38). Briefly, aliquots of supernatant were added to the appropriate wells of a 96 well microplate containing an assay solution comprised of Tris [72.5 mM], acetyl CoA [0.45 mM] and 5,5’-dithiobis-2-nitrobenzoate (DTNB) [0.1 mM], at a pH of 8.3. After monitoring the plate for possible background activity, activity reactions were initiated by the addition of oxaloacetic acid [0.5 mM] 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

All raw data, and analysis scripts are available at <http://bridgeslab.github.io/ObesityParticulateTreatment> (40). Statistics and calculations were performed using R version 3.1.1 (32). 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 (4)). 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 (18)). Pairwise Student’s *t*, Welch’s *t* or Wilcoxon Rank Sum tests were performed as indicated in the results and figure legends, dependent on normality and homoscedasticity. In cases where cabosil and saline treatment were not significantly different, these data were combined and designated as a single control group. 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 (46). 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 metabolic effects of gestational exposure to EPFR’s, pregnant females were exposed to MCP230 on days 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, male mice were placed on a HFD consisting of 45% of calories from lard, in order to induce obesity (Figure 1A).

As shown in Figure 1B, at 10 weeks of age, mice that were exposed to MCP230 had a 7.6% higher body weight than the saline-exposed mice and remained heavier, gaining more weight throughout the HFD phase (p=3.5 x 10-5). After 12 weeks of HFD, the MCP230-exposed mice were 4.5 g heavier than saline-exposed mice (9.8%, p<0.001; Figure 1B). We assessed body composition after 12 weeks of HFD and observed significant elevations in both fat mass (10.1% increase, p=0.011) and fat-free mass (10.2% 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 throughout the HFD feeding period. 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, p=8.0 x 10-4, Figure 2B). Throughout the 12 week HFD treatment, this corresponds to a 19.2% reduction in total caloric intake. During the metabolic cage experiments, which occurred prior to HFD feeding, the MCP230-exposed mice tended to eat less food per feeding bout, whereas each feeding bout also tended to be shorter in duration; however, neither of these parameters were significantly different (data not shown). There were no differences between groups for the frequency of feeding bouts.

Leptin concentrations were modestly elevated in serum from MCP230-exposed mice (main effects feeding state, p=0.002, and treatment, p=0.011, by 2-way ANOVA, with post-hoc *t-*test p-values of 0.058 under fasting and p=0.097 under fed conditions, Figure 2C). Elevations in circulating leptin levels are consistent with the increase in fat mass observed in MCP230-exposed mice (Figure 1C). We observed significant serum elevations in both the fasting and fed state for ghrelin (for main effects of feeding state p=0.001, and MCP230 treatment p=6.5 x 10-6, with post-hoc *t-*test p-values of 0.024 in the fasting and p=0.0002 in the fed state; Figure 2D), which is consistent with a reduction in food intake (Figure 2A and B) and reduced energy expenditure (Figure 4A-E) observed in the MCP230-exposed mice (11, 44, 45, 51). Similarly, GLP-1 was elevated in serum from MCP230-exposed mice in both the fasted and fed state (main effects for feeding state p=0.002, and treatment p=3.6 x 10-5, with post-hoc *t-*test p-values of 0.024 in the fasted and p=0.001 in the fed state, Figure 2D), which is also consistent with the MCP230-exposed mice eating less (Figure 2A and B) (3, 47). There was an effect of the feeding state with respect to GIP concentrations (p=6.0 x 10-9 by 2-way ANOVA, Figure 2F) and GIP was elevated in serum from MCP230-exposed mice in the fasted state, although these values did not quite attain statistical significance (p=0.069 by Wilcoxon Rank Sum Test). Although there were main effects of the feeding state for PAI-1 and resistin levels, these were not different between the two treatment groups (data not shown).

We next evaluated the extent of obesity related co-morbidities in these mice after 12 wk of high fat feeding. We observed no differences 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. Calculation of the HOMA-IR revealed that both the saline and MCP230-exposed groups had similar HOMA-IR score (12.77 ± 1.29 vs. 12.14 ± 0.96 for Saline and MCP230, respectively; p=0.74, Figure 3C). Taken together, these data indicate that while HFD did impair insulin sensitivity, there was no difference between these two groups. Consistent with this, we observed no changes in the levels of fasted Akt phosphorylation in muscle tissue (data not shown). With respect to glucagon levels, both feeding state (p=7.3 x 10-5) and MCP230 treatment (p=4.0 x 10-3) increased serum glucagon concentrations. MCP230-exposed mice had elevated glucagon concentrations in the fasted and fed state, although fed state levels did not quite attain statistical significance (32.65%, p=0.009 for fasting and 28.46%, p=0.059 for fed, respectively, by post-hoc Wilcoxon Rank Sum tests; Figure 3D).

## 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 metabolic cages for indirect calorimetry, physical activity monitoring and evaluation of gas exchange rates. As shown in Figures 4A and B, the MCP230 exposed mice had lower oxygen consumption (VO2) in both the light and dark phases (-19.1%, p=0.031 and -16.8%, p=0.019, respectively). This reduction in VO2 translated to a similar reduction in energy expenditure in both the light and dark phase (-18.4%, p=0.032 and -16.4%, p=0.021, respectively; Figures 4C and D). In Figures 4B and 4D, each dot represents the average value for each individual mouse plotted against fat-free mass. Accounting for change in lean mass is necessary due to known associations between this covariate and rates of oxygen consumption (46). 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 4E, compared to the control group, 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 (5). 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 MCP230 (the EPFR) (Figure 4F). These data indicate that particle exposure alone (cabosil) altered substrate preference; but that exposure to the EPFR did not alter substrate preference.

## Skeletal Muscle from MCP230 Treated Mice Have Reduced mtDNA 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, as muscle is the major organ responsible for variations in resting energy expenditure (54). To test this, we first determined mitochondrial DNA (mtDNA) copy number in quadriceps muscle after the 12-week HFD 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 may have reduced skeletal muscle mitochondrial content. Since citrate synthase activity is better associated with skeletal muscle mitochondrial content than mtDNA copy number (and is also a good indicator of tricarboxylic acid cycle activity (25)), 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). Together, reduced mtDNA copy number and lower citrate synthase activity suggest that mice exposed to MCP230 may 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 differences in skeletal muscle mitochondrial electron transport enzymes were present at the protein level, we measured the relative expression of several electron transport chain proteins via western blotting (Figure 5D). We did not observe differences in levels of any of the five oxidative phosphorylation proteins measured in skeletal muscle, nor did we see changes in PGC-1α protein expression (Figure 5D-E) with MCP230 exposure. To test whether the lack of change in mitochondrial protein expression was due to suppression of autophagy, we blotted for processing of LC3, and observed no evidence of decreased autophagy (Figures 5D and F). We did not see alterations in any of the other measured markers of skeletal muscle metabolism and growth (phospho-AktS473, phospho-AMPKT172 or phospho-S6KT389, data not shown).

To test whether reductions in mtDNA copy number and citrate synthase activity were due to lowered mitochondrial biogenesis, we evaluated the expression level of several known mitochondrial biogenesis genes. While we observed increases in the mRNA of *Ppard* and *Ppargc1b* (Figure 6A and C), there were no differences in the expression levels of *Ppargc1a*, *Nrf1*, *Nfe2l2* or *Tfam* (Figure 6B, D-F). In the absence of reduced mitochondrial biogenesis markers, or changes in the levels of oxidative phosoporylation enzymes, the mitochondrial deficits we observe in the skeletal muscle of mice exposed to MCP230 may be a response to oxidative stress, rather than transcriptional downregulation of mitochondrial biogenesis *per se*. In support of this notion, we found robust increases in the mRNA for *Ucp2*, *Sod1*, *Sod2*, *Cat*, *Gpx1* and *Gclm*, enzymes activated in response to oxidative stress (Figure 7).

# Discussion

While epidemiological studies have linked exposure to PM with obesity and its comorbidities, few have evaluated energy expenditure changes in response to acute gestational exposure. In this study, we tested some of the metabolic effects of a limited gestational exposure to a recently realized environmental pollutant that is present in most combustion derived PM – EPFR’s. Each exposure of MCP230 that the mothers received was the equivalent to a human breathing 200 µg/m3 of EPFR, which is similar to what would be inhaled on a typical day in one of the major US cities (36). We noted that pups born from mothers that were acutely exposed to PM grew larger, despite reductions in food intake, and that this was associated with reduced energy expenditure and mitochondrial impairments in skeletal muscle.

One potential explanation for 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 (42, 54)) 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 (33) and exercise performance (14, 31, 34). 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 or some other mechanism. However, our observations of reductions in mtDNA, citrate synthase activity and mRNA transcripts support the hypothesis that gestational exposure to EPFRs can affect skeletal muscle mitochondrial oxidative function, which would contribute to the overall changes we observe in energy expenditure.

The mechanisms by which gestational exposure to EPFRs result in reduced mitochondrial function are not yet clear. Our data are consistent with chronic models of PM2.5 exposure, which show reduced mitochondrial numbers in white adipose tissue (52, 53). Analyses of placental tissues from mothers showed a strong correlation between late-gestational PM10exposure and placental mtDNA content (21). 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 (ROS), or indirectly, via inflammatory processes. Indeed, the bioenergetics proteins in skeletal muscle are highly susceptible to ROS-induced post translational modifications, changes thought to be important for reducing endogenous ROS production and protect against irreversible oxidative damage during periods of cellular stress (24). In line with this concept, Siegel and colleagues (37) have shown that mild oxidative stress *in vivo* impairs skeletal muscle oxidative efficiency and reduces oxidative phosphorylation coupling without altering the expression of key electron transport chain proteins or their respiratory activities *ex vivo*. This suggests that reduced skeletal muscle oxidative capacity in response to oxidative stress is probably not due to down-regulation of the mitochondrial biogenesis pathways or irreversible oxidative damage to bioenergetic proteins. Similar to previous reports on oxidative stress-induced mitochondrial dysfunction (37), we did not observe decreases in upstream regulators of mitochondrial biogenesis, increases in autophagy or changes in mitochondrial protein expression as part of the chronic effects of acute *in utero* MCP230-exposure. We did, however observe marked increases in the transcripts of key enzymes of the antioxidant defense system (*Sod1*, *Sod2*, *Cat*, *Gpx1*), as well as increased expression of *Ucp2*, an uncoupling protein known to be up-regulated as a means to reduce endogenous ROS production (Figure 7; (1, 16)), and increases in both the nuclear receptor *Ppard* and the transcriptional co-regulator *Ppargc1β*, both of which are required for the induction of *Sod1* and *Sod2* (19, 48). Based on our current protocol, mice are exposed to EPFRs after inheritance of maternal mitochondria, indicating that this mitochondrial damage occurs *in situ* in the progeny. It must be emphasized that this exposure is indirect, through the mother, as there is no evidence at present that the conjugated EPFR crosses the placenta to exert its effect on the muscle directly. However, we hypothesize that the changes we observe in the skeletal muscle mitochondria of the MCP230-exposed mice are, at least in part, a consequence of ROS-induced post-translational changes and chronic oxidative stress. Future studies with more direct measurements of mitochondrial function and the oxidative stress response will provide more mechanistic insight into this process.

In contrast to previous studies that use chronic pollution models (1, 5, 9, 33, 35), we did not observe any indications that glycemic control was impaired to a greater extent in MCP230-exposed mice compared to the control groups following the HFD, despite differences in fat mass. There were no differences in fasting glucose, insulin, HOMA-IR score (Figure 3A-C) or Akt phosphorylation in muscle tissue (data not shown). We did not measure insulin sensitivity directly, which limits our ability to make strong conclusions about the effects of acute *in utero* PM exposure on insulin sensitivity. That said, our data suggests that the effects of acute gestational particulate exposure may not 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 energy expenditure and a predisposition to elevated body weight when exposed to a HFD. While the mechanisms behind these changes remain to be determined, the finding that limited *in utero* exposure to EPFR’s can affect energy metabolism later in life highlights a need for further research in this area.

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# Disclosures

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

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C**, **Beckham JD**, **Bédard P-A**, **Bednarski PJ**, **Begley TJ**, **Behl C**, **Behrends C**, **Behrens GM**, **Behrns KE**, **Bejarano E**, **Belaid A**, **Belleudi F**, **Bénard G**, **Berchem G**, **Bergamaschi D**, **Bergami M**, **Berkhout B**, **Berliocchi L**, **Bernard A**, **Bernard M**, **Bernassola F**, **Bertolotti A**, **Bess AS**, **Besteiro S**, **Bettuzzi S**, **Bhalla S**, **Bhattacharyya S**, **Bhutia SK**, **Biagosch C**, **Bianchi MW**, **Biard-Piechaczyk M**, **Billes V**, **Bincoletto C**, **Bingol B**, **Bird SW**, **Bitoun M**, **Bjedov I**, **Blackstone C**, **Blanc L**, **Blanco GA**, **Blomhoff HK**, **Boada-Romero E**, **Böckler S**, **Boes M**, **Boesze-Battaglia K**, **Boise LH**, **Bolino A**, **Boman A**, **Bonaldo P**, **Bordi M**, **Bosch J**, **Botana LM**, **Botti J**, **Bou G**, **Bouché M**, **Bouchecareilh M**, **Boucher M-J**, **Boulton ME**, **Bouret SG**, **Boya P**, **Boyer-Guittaut M**, **Bozhkov P V**, **Brady N**, **Braga VM**, **Brancolini C**, **Braus GH**, **Bravo-San Pedro JM**, **Brennan LA**, **Bresnick EH**, **Brest P**, **Bridges D**, **Bringer M-A**, **Brini M**, **Brito GC**, **Brodin B**, **Brookes PS**, **Brown EJ**, **Brown K**, **Broxmeyer HE**, **Bruhat A**, **Brum PC**, **Brumell JH**, **Brunetti-Pierri N**, **Bryson-Richardson RJ**, **Buch S**, **Buchan AM**, **Budak H**, **Bulavin D V**, **Bultman SJ**, **Bultynck G**, **Bumbasirevic V**, **Burelle Y**, **Burke RE**, **Burmeister M**, **Bütikofer P**, **Caberlotto L**, **Cadwell K**, **Cahova M**, **Cai D**, **Cai J**, **Cai Q**, **Calatayud S**, **Camougrand N**, **Campanella M**, **Campbell GR**, **Campbell M**, **Campello S**, **Candau R**, **Caniggia I**, **Cantoni L**, **Cao L**, **Caplan AB**, **Caraglia M**, **Cardinali C**, **Cardoso SM**, **Carew JS**, **Carleton LA**, **Carlin CR**, **Carloni S**, **Carlsson SR**, **Carmona-Gutierrez D**, **Carneiro LA**, **Carnevali O**, **Carra S**, **Carrier A**, **Carroll B**, **Casas C**, **Casas J**, **Cassinelli G**, **Castets P**, **Castro-Obregon S**, **Cavallini G**, **Ceccherini I**, **Cecconi F**, **Cederbaum AI**, **Ceña V**, **Cenci S**, **Cerella C**, **Cervia D**, **Cetrullo S**, **Chaachouay H**, **Chae H-J**, **Chagin AS**, **Chai C-Y**, **Chakrabarti G**, **Chamilos G**, **Chan EY**, **Chan MT**, **Chandra D**, **Chandra P**, **Chang C-P**, **Chang RC-C**, **Chang TY**, **Chatham JC**, **Chatterjee S**, **Chauhan S**, **Che Y**, **Cheetham ME**, **Cheluvappa R**, **Chen C-J**, **Chen G**, **Chen G-C**, **Chen G**, **Chen H**, **Chen JW**, **Chen J-K**, **Chen M**, **Chen M**, **Chen P**, **Chen Q**, **Chen Q**, **Chen S-D**, **Chen S**, **Chen SS-L**, **Chen W**, **Chen W-J**, **Chen WQ**, **Chen W**, **Chen X**, **Chen Y-H**, **Chen Y-G**, **Chen Y**, **Chen Y**, **Chen Y**, **Chen Y-J**, **Chen Y-Q**, **Chen Y**, **Chen Z**, **Chen Z**, **Cheng A**, **Cheng CH**, **Cheng H**, **Cheong H**, **Cherry S**, **Chesney J**, **Cheung CHA**, **Chevet E**, **Chi 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L-L**, **Du L**, **du Toit A**, **Dua P**, **Duan L**, **Duann P**, **Dubey VK**, **Duchen MR**, **Duchosal MA**, **Duez H**, **Dugail I**, **Dumit VI**, **Duncan MC**, **Dunlop EA**, **Dunn WA**, **Dupont N**, **Dupuis L**, **Durán R V**, **Durcan TM**, **Duvezin-Caubet S**, **Duvvuri U**, **Eapen V**, **Ebrahimi-Fakhari D**, **Echard A**, **Eckhart L**, **Edelstein CL**, **Edinger AL**, **Eichinger L**, **Eisenberg T**, **Eisenberg-Lerner A**, **Eissa NT**, **El-Deiry WS**, **El-Khoury V**, **Elazar Z**, **Eldar-Finkelman H**, **Elliott CJ**, **Emanuele E**, **Emmenegger U**, **Engedal N**, **Engelbrecht A-M**, **Engelender S**, **Enserink JM**, **Erdmann R**, **Erenpreisa J**, **Eri R**, **Eriksen JL**, **Erman A**, **Escalante R**, **Eskelinen E-L**, **Espert L**, **Esteban-Martínez L**, **Evans TJ**, **Fabri M**, **Fabrias G**, **Fabrizi C**, **Facchiano A**, **Færgeman NJ**, **Faggioni A**, **Fairlie WD**, **Fan C**, **Fan D**, **Fan J**, **Fang S**, **Fanto M**, **Fanzani A**, **Farkas T**, **Faure M**, **Favier FB**, **Fearnhead H**, **Federici M**, **Fei E**, **Felizardo TC**, **Feng H**, **Feng Y**, **Feng Y**, **Ferguson TA**, **Fernández ÁF**, **Fernandez-Barrena MG**, **Fernandez-Checa JC**, **Fernández-López A**, **Fernandez-Zapico ME**, **Feron O**, **Ferraro E**, **Ferreira-Halder CV**, **Fesus L**, **Feuer R**, **Fiesel FC**, **Filippi-Chiela EC**, **Filomeni G**, **Fimia GM**, **Fingert JH**, **Finkbeiner S**, **Finkel T**, **Fiorito F**, **Fisher PB**, **Flajolet M**, **Flamigni F**, **Florey O**, **Florio S**, **Floto RA**, **Folini M**, **Follo C**, **Fon EA**, **Fornai F**, **Fortunato F**, **Fraldi A**, **Franco R**, **Francois A**, **François A**, **Frankel LB**, **Fraser ID**, **Frey N**, **Freyssenet DG**, **Frezza C**, **Friedman SL**, **Frigo DE**, **Fu D**, **Fuentes JM**, **Fueyo J**, **Fujitani Y**, **Fujiwara Y**, **Fujiya M**, **Fukuda M**, **Fulda S**, **Fusco C**, **Gabryel B**, **Gaestel M**, **Gailly P**, **Gajewska M**, **Galadari S**, **Galili G**, **Galindo I**, **Galindo MF**, **Galliciotti G**, **Galluzzi L**, **Galluzzi L**, **Galy V**, **Gammoh N**, **Gandy S**, **Ganesan AK**, **Ganesan S**, **Ganley IG**, **Gannagé M**, **Gao F-B**, **Gao F**, **Gao J-X**, **García Nannig L**, **García Véscovi E**, **Garcia-Macía M**, **Garcia-Ruiz C**, **Garg AD**, **Garg PK**, **Gargini R**, **Gassen NC**, **Gatica D**, **Gatti E**, **Gavard J**, **Gavathiotis E**, **Ge L**, **Ge P**, **Ge S**, **Gean P-W**, **Gelmetti V**, **Genazzani AA**, **Geng J**, **Genschik P**, **Gerner L**, **Gestwicki JE**, **Gewirtz DA**, **Ghavami S**, **Ghigo E**, **Ghosh D**, **Giammarioli AM**, **Giampieri F**, **Giampietri C**, **Giatromanolaki A**, **Gibbings DJ**, **Gibellini L**, **Gibson SB**, **Ginet V**, **Giordano A**, **Giorgini F**, **Giovannetti E**, **Girardin SE**, **Gispert S**, **Giuliano S**, **Gladson CL**, **Glavic A**, **Gleave M**, **Godefroy N**, **Gogal RM**, **Gokulan K**, **Goldman GH**, **Goletti D**, **Goligorsky MS**, **Gomes A V**, **Gomes LC**, **Gomez H**, **Gomez-Manzano C**, **Gómez-Sánchez R**, **Gonçalves DA**, **Goncu E**, **Gong Q**, **Gongora C**, **Gonzalez CB**, **Gonzalez-Alegre P**, **Gonzalez-Cabo P**, **González-Polo RA**, **Goping IS**, **Gorbea C**, **Gorbunov N V**, **Goring DR**, **Gorman AM**, **Gorski SM**, **Goruppi S**, **Goto-Yamada S**, **Gotor C**, **Gottlieb RA**, **Gozes I**, **Gozuacik D**, **Graba Y**, **Graef M**, **Granato GE**, **Grant GD**, **Grant S**, **Gravina GL**, **Green DR**, **Greenhough A**, **Greenwood MT**, **Grimaldi B**, **Gros F**, **Grose C**, **Groulx J-F**, **Gruber F**, **Grumati P**, **Grune T**, **Guan J-L**, **Guan K-L**, **Guerra B**, **Guillen C**, **Gulshan K**, **Gunst J**, **Guo C**, **Guo L**, **Guo M**, **Guo W**, **Guo X-G**, **Gust AA**, **Gustafsson ÅB**, **Gutierrez E**, **Gutierrez MG**, **Gwak H-S**, **Haas A**, **Haber JE**, **Hadano S**, **Hagedorn M**, **Hahn DR**, **Halayko AJ**, **Hamacher-Brady A**, **Hamada K**, **Hamai A**, **Hamann A**, **Hamasaki M**, **Hamer I**, **Hamid Q**, **Hammond EM**, **Han F**, **Han W**, **Handa JT**, **Hanover JA**, **Hansen M**, **Harada M**, **Harhaji-Trajkovic L**, **Harper JW**, **Harrath AH**, **Harris AL**, **Harris J**, **Hasler U**, **Hasselblatt P**, **Hasui K**, **Hawley RG**, **Hawley TS**, **He C**, **He CY**, **He F**, **He G**, **He R-R**, **He X-H**, **He Y-W**, **He Y-Y**, **Heath JK**, **Hébert M-J**, **Heinzen RA**, **Helgason GV**, **Hensel M**, **Henske EP**, **Her C**, **Herman PK**, **Hernández A**, **Hernandez C**, **Hernández-Tiedra S**, **Hetz C**, **Hiesinger PR**, **Higaki K**, **Hilfiker S**, **Hill BG**, **Hill JA**, **Hill WD**, **Hino K**, **Hofius D**, **Hofman P**, **Höglinger GU**, **Höhfeld J**, **Holz MK**, **Hong Y**, **Hood DA**, **Hoozemans JJ**, **Hoppe T**, **Hsu C**, **Hsu C-Y**, **Hsu L-C**, **Hu D**, **Hu G**, **Hu H-M**, **Hu H**, **Hu MC**, **Hu Y-C**, **Hu Z-W**, **Hua F**, **Hua Y**, **Huang C**, **Huang H-L**, **Huang K-H**, **Huang K-Y**, **Huang S**, **Huang S**, **Huang W-P**, **Huang Y-R**, **Huang Y**, **Huang Y**, **Huber TB**, **Huebbe P**, **Huh W-K**, **Hulmi JJ**, **Hur GM**, **Hurley JH**, **Husak Z**, **Hussain SN**, **Hussain S**, **Hwang JJ**, **Hwang S**, **Hwang TI**, **Ichihara A**, **Imai Y**, **Imbriano C**, **Inomata M**, **Into T**, **Iovane V**, **Iovanna JL**, **Iozzo R V**, **Ip NY**, **Irazoqui JE**, **Iribarren P**, **Isaka Y**, **Isakovic AJ**, **Ischiropoulos H**, **Isenberg JS**, **Ishaq M**, **Ishida H**, **Ishii I**, **Ishmael JE**, **Isidoro C**, **Isobe K**, **Isono E**, **Issazadeh-Navikas S**, **Itahana K**, **Itakura E**, **Ivanov AI**, **Iyer AK V**, **Izquierdo JM**, **Izumi Y**, **Izzo V**, **Jäättelä M**, **Jaber N**, **Jackson DJ**, **Jackson WT**, **Jacob TG**, **Jacques TS**, **Jagannath C**, **Jain A**, **Jana NR**, **Jang BK**, **Jani A**, **Janji B**, **Jannig PR**, **Jansson PJ**, **Jean S**, **Jendrach M**, **Jeon J-H**, **Jessen N**, **Jeung E-B**, **Jia K**, **Jia L**, **Jiang H**, **Jiang H**, **Jiang L**, **Jiang T**, **Jiang X**, **Jiang X**, **Jiang Y**, **Jiang Y**, **Jiménez A**, **Jin C**, **Jin H**, **Jin L**, **Jin M**, **Jin S**, **Jinwal UK**, **Jo E-K**, **Johansen T**, **Johnson DE**, **Johnson GV**, **Johnson JD**, **Jonasch E**, **Jones C**, **Joosten LA**, **Jordan J**, **Joseph A-M**, **Joseph B**, **Joubert AM**, **Ju D**, **Ju J**, **Juan H-F**, **Juenemann K**, **Juhász G**, **Jung HS**, **Jung JU**, **Jung Y-K**, **Jungbluth H**, **Justice MJ**, **Jutten B**, **Kaakoush NO**, **Kaarniranta K**, **Kaasik A**, **Kabuta T**, **Kaeffer B**, **Kågedal K**, **Kahana A**, **Kajimura S**, **Kakhlon O**, **Kalia M**, **Kalvakolanu D V**, **Kamada Y**, **Kambas K**, **Kaminskyy VO**, **Kampinga HH**, **Kandouz M**, **Kang C**, **Kang R**, **Kang T-C**, **Kanki T**, **Kanneganti T-D**, **Kanno H**, **Kanthasamy AG**, **Kantorow M**, **Kaparakis-Liaskos M**, **Kapuy O**, **Karantza V**, **Karim MR**, **Karmakar P**, **Kaser A**, **Kaushik S**, **Kawula T**, **Kaynar AM**, **Ke P-Y**, **Ke Z-J**, **Kehrl JH**, **Keller KE**, **Kemper JK**, **Kenworthy AK**, **Kepp O**, **Kern A**, **Kesari S**, **Kessel D**, **Ketteler R**, **Kettelhut I do C**, **Khambu B**, **Khan MM**, **Khandelwal VK**, **Khare S**, **Kiang JG**, **Kiger AA**, **Kihara A**, **Kim AL**, **Kim CH**, **Kim DR**, **Kim D-H**, **Kim EK**, **Kim HY**, **Kim H-R**, **Kim J-S**, **Kim JH**, **Kim JC**, **Kim JH**, **Kim KW**, **Kim MD**, **Kim M-M**, **Kim PK**, **Kim SW**, **Kim S-Y**, **Kim Y-S**, **Kim Y**, **Kimchi A**, **Kimmelman AC**, **Kimura T**, **King JS**, **Kirkegaard K**, **Kirkin V**, **Kirshenbaum LA**, **Kishi S**, **Kitajima Y**, **Kitamoto K**, **Kitaoka Y**, **Kitazato K**, **Kley RA**, **Klimecki WT**, **Klinkenberg M**, **Klucken J**, **Knævelsrud H**, **Knecht E**, **Knuppertz L**, **Ko J-L**, **Kobayashi S**, **Koch JC**, **Koechlin-Ramonatxo C**, **Koenig U**, **Koh YH**, **Köhler K**, **Kohlwein SD**, **Koike M**, **Komatsu M**, **Kominami E**, **Kong D**, **Kong HJ**, **Konstantakou EG**, **Kopp BT**, **Korcsmaros T**, **Korhonen L**, **Korolchuk VI**, **Koshkina N V**, **Kou Y**, **Koukourakis MI**, **Koumenis C**, **Kovács AL**, **Kovács T**, **Kovacs WJ**, **Koya D**, **Kraft C**, **Krainc D**, **Kramer H**, **Kravic-Stevovic T**, **Krek W**, **Kretz-Remy C**, **Krick R**, **Krishnamurthy M**, **Kriston-Vizi J**, **Kroemer G**, **Kruer MC**, **Kruger R**, **Ktistakis NT**, **Kuchitsu K**, **Kuhn C**, **Kumar AP**, **Kumar A**, **Kumar A**, **Kumar D**, **Kumar D**, **Kumar R**, **Kumar S**, **Kundu M**, **Kung H-J**, **Kuno A**, **Kuo S-H**, **Kuret J**, **Kurz T**, **Kwok T**, **Kwon TK**, **Kwon YT**, **Kyrmizi I**, **La Spada AR**, **Lafont F**, **Lahm T**, **Lakkaraju A**, **Lam T**, **Lamark T**, **Lancel S**, **Landowski TH**, **Lane DJ**, **Lane JD**, **Lanzi C**, **Lapaquette P**, **Lapierre LR**, **Laporte J**, **Laukkarinen J**, **Laurie GW**, **Lavandero S**, **Lavie L**, **LaVoie MJ**, **Law BYK**, **Law HK**, **Law KB**, **Layfield R**, **Lazo PA**, **Le Cam L**, **Le Roch KG**, **Le Stunff H**, **Leardkamolkarn V**, **Lecuit M**, **Lee B-H**, **Lee C-H**, **Lee EF**, **Lee GM**, **Lee H-J**, **Lee H**, **Lee JK**, **Lee J**, **Lee J**, **Lee JH**, **Lee M**, **Lee M-S**, **Lee PJ**, **Lee SW**, **Lee S-J**, **Lee S-J**, **Lee SY**, **Lee SH**, **Lee SS**, **Lee S-J**, **Lee S**, **Lee Y-R**, **Lee YJ**, **Lee YH**, **Leeuwenburgh C**, **Lefort S**, **Legouis R**, **Lei J**, **Lei Q-Y**, **Leib DA**, **Leibowitz G**, **Lekli I**, **Lemaire SD**, **Lemasters JJ**, **Lemberg MK**, **Lemoine A**, **Leng S**, **Lenz G**, **Lenzi P**, **Lerman LO**, **Lettieri Barbato D**, **Leu JI-J**, **Leung HY**, **Levine B**, **Lewis PA**, **Lezoualc’h F**, **Li C**, **Li F**, **Li F-J**, **Li J**, **Li K**, **Li L**, **Li M**, **Li Q**, **Li R**, **Li S**, **Li W**, **Li X**, **Li Y**, **Lian J**, **Liang C**, **Liang Q**, **Liao Y**, **Liberal J**, **Liberski PP**, **Lie P**, **Lieberman AP**, **Lim HJ**, **Lim K-L**, **Lim K**, **Lima RT**, **Lin C-S**, **Lin C-F**, **Lin F**, **Lin F**, **Lin F-C**, **Lin K**, **Lin K-H**, **Lin P-H**, **Lin T**, **Lin W-W**, **Lin Y-S**, **Lin Y**, **Linden R**, **Lindholm D**, **Lindqvist LM**, **Lingor P**, **Linkermann A**, **Liotta LA**, **Lipinski MM**, **Lira VA**, **Lisanti MP**, **Liton PB**, **Liu B**, **Liu C**, **Liu C-F**, **Liu F**, **Liu H-J**, **Liu J**, **Liu J-J**, **Liu J-L**, **Liu K**, **Liu L**, **Liu L**, **Liu Q**, **Liu R-Y**, **Liu S**, **Liu S**, **Liu W**, **Liu X-D**, **Liu X**, **Liu X-H**, **Liu X**, **Liu X**, **Liu X**, **Liu Y**, **Liu Y**, **Liu Z**, **Liu Z**, **Liuzzi JP**, **Lizard G**, **Ljujic M**, **Lodhi IJ**, **Logue SE**, **Lokeshwar BL**, **Long YC**, **Lonial S**, **Loos B**, **López-Otín C**, **López-Vicario C**, **Lorente M**, **Lorenzi PL**, **Lõrincz P**, **Los M**, **Lotze MT**, **Lovat PE**, **Lu B**, **Lu B**, **Lu J**, **Lu Q**, **Lu S-M**, **Lu S**, **Lu Y**, **Luciano F**, **Luckhart S**, **Lucocq JM**, **Ludovico P**, **Lugea A**, **Lukacs NW**, **Lum JJ**, **Lund AH**, **Luo H**, **Luo J**, **Luo S**, **Luparello C**, **Lyons T**, **Ma J**, **Ma Y**, **Ma Y**, **Ma Z**, **Machado J**, **Machado-Santelli GM**, **Macian F**, **MacIntosh GC**, **MacKeigan JP**, **Macleod KF**, **MacMicking JD**, **MacMillan-Crow LA**, **Madeo F**, **Madesh M**, **Madrigal-Matute J**, **Maeda A**, **Maeda T**, **Maegawa G**, **Maellaro E**, **Maes H**, **Magariños M**, **Maiese K**, **Maiti TK**, **Maiuri L**, **Maiuri MC**, **Maki CG**, **Malli R**, **Malorni W**, **Maloyan A**, **Mami-Chouaib F**, **Man N**, **Mancias JD**, **Mandelkow E-M**, **Mandell MA**, **Manfredi AA**, **Manié SN**, **Manzoni C**, **Mao K**, **Mao Z**, **Mao Z-W**, **Marambaud P**, **Marconi AM**, **Marelja Z**, **Marfe G**, **Margeta M**, **Margittai E**, **Mari M**, **Mariani F V**, **Marin C**, **Marinelli S**, **Mariño G**, **Markovic I**, **Marquez R**, **Martelli AM**, **Martens S**, **Martin KR**, **Martin SJ**, **Martin S**, **Martin-Acebes MA**, **Martín-Sanz P**, **Martinand-Mari C**, **Martinet W**, **Martinez J**, **Martinez-Lopez N**, **Martinez-Outschoorn U**, **Martínez-Velázquez M**, **Martinez-Vicente M**, **Martins WK**, **Mashima H**, **Mastrianni JA**, **Matarese G**, **Matarrese P**, **Mateo R**, **Matoba S**, **Matsumoto N**, **Matsushita T**, **Matsuura A**, **Matsuzawa T**, **Mattson MP**, **Matus S**, **Maugeri N**, **Mauvezin C**, **Mayer A**, **Maysinger D**, **Mazzolini GD**, **McBrayer MK**, **McCall K**, **McCormick C**, **McInerney GM**, **McIver SC**, **McKenna S**, **McMahon JJ**, **McNeish IA**, **Mechta-Grigoriou F**, **Medema JP**, **Medina DL**, **Megyeri K**, **Mehrpour M**, **Mehta JL**, **Mei Y**, **Meier U-C**, **Meijer AJ**, **Meléndez A**, **Melino G**, **Melino S**, **de Melo EJT**, **Mena MA**, **Meneghini MD**, **Menendez JA**, **Menezes R**, **Meng L**, **Meng L**, **Meng S**, **Menghini R**, **Menko AS**, **Menna-Barreto RF**, **Menon MB**, **Meraz-Ríos MA**, **Merla G**, **Merlini L**, **Merlot AM**, **Meryk A**, **Meschini S**, **Meyer JN**, **Mi M**, **Miao C-Y**, **Micale L**, **Michaeli S**, **Michiels C**, **Migliaccio AR**, **Mihailidou AS**, **Mijaljica D**, **Mikoshiba K**, **Milan E**, **Miller-Fleming L**, **Mills GB**, **Mills IG**, **Minakaki G**, **Minassian BA**, **Ming X-F**, **Minibayeva F**, **Minina EA**, **Mintern JD**, **Minucci S**, **Miranda-Vizuete A**, **Mitchell CH**, **Miyamoto S**, **Miyazawa K**, **Mizushima N**, **Mnich K**, **Mograbi B**, **Mohseni S**, **Moita LF**, **Molinari M**, **Molinari M**, **Møller AB**, **Mollereau B**, **Mollinedo F**, **Mongillo M**, **Monick MM**, **Montagnaro S**, **Montell C**, **Moore DJ**, **Moore MN**, **Mora-Rodriguez R**, **Moreira PI**, **Morel E**, **Morelli MB**, **Moreno S**, **Morgan MJ**, **Moris A**, **Moriyasu Y**, **Morrison JL**, **Morrison LA**, **Morselli E**, **Moscat J**, **Moseley PL**, **Mostowy S**, **Motori E**, **Mottet D**, **Mottram JC**, **Moussa CE-H**, **Mpakou VE**, **Mukhtar H**, **Mulcahy Levy JM**, **Muller S**, **Muñoz-Moreno R**, **Muñoz-Pinedo C**, **Münz C**, **Murphy ME**, **Murray JT**, **Murthy A**, **Mysorekar IU**, **Nabi IR**, **Nabissi M**, **Nader GA**, **Nagahara Y**, **Nagai Y**, **Nagata K**, **Nagelkerke A**, **Nagy P**, **Naidu SR**, **Nair S**, **Nakano H**, **Nakatogawa H**, **Nanjundan M**, **Napolitano G**, **Naqvi NI**, **Nardacci R**, **Narendra DP**, **Narita M**, **Nascimbeni AC**, **Natarajan R**, **Navegantes LC**, **Nawrocki ST**, **Nazarko TY**, **Nazarko VY**, **Neill T**, **Neri LM**, **Netea MG**, **Netea-Maier RT**, **Neves BM**, **Ney PA**, **Nezis IP**, **Nguyen HT**, **Nguyen HP**, **Nicot A-S**, **Nilsen H**, **Nilsson P**, **Nishimura M**, **Nishino I**, **Niso-Santano M**, **Niu H**, **Nixon RA**, **Njar VC**, **Noda T**, **Noegel AA**, **Nolte EM**, **Norberg E**, **Norga KK**, **Noureini SK**, **Notomi S**, **Notterpek L**, **Nowikovsky K**, **Nukina N**, **Nürnberger T**, **O’Donnell VB**, **O’Donovan T**, **O’Dwyer PJ**, **Oehme I**, **Oeste CL**, **Ogawa M**, **Ogretmen B**, **Ogura Y**, **Oh YJ**, **Ohmuraya M**, **Ohshima T**, **Ojha R**, **Okamoto K**, **Okazaki T**, **Oliver FJ**, **Ollinger K**, **Olsson S**, **Orban DP**, **Ordonez P**, **Orhon I**, **Orosz L**, **O’Rourke EJ**, **Orozco H**, **Ortega AL**, **Ortona E**, **Osellame LD**, **Oshima J**, **Oshima S**, **Osiewacz HD**, **Otomo T**, **Otsu K**, **Ou JJ**, **Outeiro TF**, **Ouyang D**, **Ouyang H**, **Overholtzer M**, **Ozbun MA**, **Ozdinler PH**, **Ozpolat B**, **Pacelli C**, **Paganetti P**, **Page G**, **Pages G**, **Pagnini U**, **Pajak B**, **Pak SC**, **Pakos-Zebrucka K**, **Pakpour N**, **Palková Z**, **Palladino F**, **Pallauf K**, **Pallet N**, **Palmieri M**, **Paludan SR**, **Palumbo C**, **Palumbo S**, **Pampliega O**, **Pan H**, **Pan W**, **Panaretakis T**, **Pandey A**, **Pantazopoulou A**, **Papackova Z**, **Papademetrio DL**, **Papassideri I**, **Papini A**, **Parajuli N**, **Pardo J**, **Parekh V V**, **Parenti G**, **Park J-I**, **Park J**, **Park OK**, **Parker R**, **Parlato R**, **Parys JB**, **Parzych KR**, **Pasquet J-M**, **Pasquier B**, **Pasumarthi KB**, **Patschan D**, **Patterson C**, **Pattingre S**, **Pattison S**, **Pause A**, **Pavenstädt H**, **Pavone F**, **Pedrozo Z**, **Peña FJ**, **Peñalva MA**, **Pende M**, **Peng J**, **Penna F**, **Penninger JM**, **Pensalfini A**, **Pepe S**, **Pereira GJ**, **Pereira PC**, **Pérez-de la Cruz V**, **Pérez-Pérez ME**, **Pérez-Rodríguez D**, **Pérez-Sala D**, **Perier C**, **Perl A**, **Perlmutter DH**, **Perrotta I**, **Pervaiz S**, **Pesonen M**, **Pessin JE**, **Peters GJ**, **Petersen M**, **Petrache I**, **Petrof BJ**, **Petrovski G**, **Phang JM**, **Piacentini M**, **Pierdominici M**, **Pierre P**, **Pierrefite-Carle V**, **Pietrocola F**, **Pimentel-Muiños FX**, **Pinar M**, **Pineda B**, **Pinkas-Kramarski R**, **Pinti M**, **Pinton P**, **Piperdi B**, **Piret JM**, **Platanias LC**, **Platta HW**, **Plowey ED**, **Pöggeler S**, **Poirot M**, **Polčic P**, **Poletti A**, **Poon AH**, **Popelka H**, **Popova B**, **Poprawa I**, **Poulose SM**, **Poulton J**, **Powers SK**, **Powers T**, **Pozuelo-Rubio M**, **Prak K**, **Prange R**, **Prescott M**, **Priault M**, **Prince S**, **Proia RL**, **Proikas-Cezanne T**, **Prokisch H**, **Promponas VJ**, **Przyklenk K**, **Puertollano R**, **Pugazhenthi S**, **Puglielli L**, **Pujol A**, **Puyal J**, **Pyeon D**, **Qi X**, **Qian W**, **Qin Z-H**, **Qiu Y**, **Qu Z**, **Quadrilatero J**, **Quinn F**, **Raben N**, **Rabinowich H**, **Radogna F**, **Ragusa MJ**, **Rahmani M**, **Raina K**, **Ramanadham S**, **Ramesh R**, **Rami A**, **Randall-Demllo S**, **Randow F**, **Rao H**, **Rao VA**, **Rasmussen BB**, **Rasse TM**, **Ratovitski EA**, **Rautou P-E**, **Ray SK**, **Razani B**, **Reed BH**, **Reggiori F**, **Rehm M**, **Reichert AS**, **Rein T**, **Reiner DJ**, **Reits E**, **Ren J**, **Ren X**, **Renna M**, **Reusch JE**, **Revuelta JL**, **Reyes L**, **Rezaie AR**, **Richards RI**, **Richardson DR**, **Richetta C**, **Riehle MA**, **Rihn BH**, **Rikihisa Y**, **Riley BE**, **Rimbach G**, **Rippo MR**, **Ritis K**, **Rizzi F**, **Rizzo E**, **Roach PJ**, **Robbins J**, **Roberge M**, **Roca G**, **Roccheri MC**, **Rocha S**, **Rodrigues CM**, **Rodríguez CI**, **de Cordoba SR**, **Rodriguez-Muela N**, **Roelofs J**, **Rogov V V**, **Rohn TT**, **Rohrer B**, **Romanelli D**, **Romani L**, **Romano PS**, **Roncero MIG**, **Rosa JL**, **Rosello A**, **Rosen K V**, **Rosenstiel P**, **Rost-Roszkowska M**, **Roth KA**, **Roué G**, **Rouis M**, **Rouschop KM**, **Ruan DT**, **Ruano D**, **Rubinsztein DC**, **Rucker EB**, **Rudich A**, **Rudolf E**, **Rudolf R**, **Ruegg M a.**, **Ruiz-Roldan C**, **Ruparelia AA**, **Rusmini P**, **Russ DW**, **Russo GL**, **Russo G**, **Russo R**, **Rusten TE**, **Ryabovol V**, **Ryan KM**, **Ryter SW**, **Sabatini DM**, **Sacher M**, **Sachse C**, **Sack MN**, **Sadoshima J**, **Saftig P**, **Sagi-Eisenberg R**, **Sahni S**, **Saikumar P**, **Saito T**, **Saitoh T**, **Sakakura K**, **Sakoh-Nakatogawa M**, **Sakuraba Y**, **Salazar-Roa M**, **Salomoni P**, **Saluja AK**, **Salvaterra PM**, **Salvioli R**, **Samali A**, **Sanchez AM**, **Sánchez-Alcázar JA**, **Sanchez-Prieto R**, **Sandri M**, **Sanjuan MA**, **Santaguida S**, **Santambrogio L**, **Santoni G**, **dos Santos CN**, **Saran S**, **Sardiello M**, **Sargent G**, **Sarkar P**, **Sarkar S**, **Sarrias MR**, **Sarwal MM**, **Sasakawa C**, **Sasaki M**, **Sass M**, **Sato K**, **Sato M**, **Satriano J**, **Savaraj N**, **Saveljeva S**, **Schaefer L**, **Schaible UE**, **Scharl M**, **Schatzl HM**, **Schekman R**, **Scheper W**, **Schiavi A**, **Schipper HM**, **Schmeisser H**, **Schmidt J**, **Schmitz I**, **Schneider BE**, **Schneider EM**, **Schneider JL**, **Schon EA**, **Schönenberger MJ**, **Schönthal AH**, **Schorderet DF**, **Schröder B**, **Schuck S**, **Schulze RJ**, **Schwarten M**, **Schwarz TL**, **Sciarretta S**, **Scotto K**, **Scovassi AI**, **Screaton RA**, **Screen M**, **Seca H**, **Sedej S**, **Segatori L**, **Segev N**, **Seglen PO**, **Seguí-Simarro JM**, **Segura-Aguilar J**, **Seki E**, **Sell C**, **Selliez I**, **Semenkovich CF**, **Semenza GL**, **Sen U**, **Serra AL**, **Serrano-Puebla A**, **Sesaki H**, **Setoguchi T**, **Settembre C**, **Shacka JJ**, **Shajahan-Haq AN**, **Shapiro IM**, **Sharma S**, **She H**, **Shen C-KJ**, **Shen C-C**, **Shen H-M**, **Shen S**, **Shen W**, **Sheng R**, **Sheng X**, **Sheng Z-H**, **Shepherd TG**, **Shi J**, **Shi Q**, **Shi Q**, **Shi Y**, **Shibutani S**, **Shibuya K**, **Shidoji Y**, **Shieh J-J**, **Shih C-M**, **Shimada Y**, **Shimizu S**, **Shin DW**, **Shinohara ML**, **Shintani M**, **Shintani T**, **Shioi T**, **Shirabe K**, **Shiri-Sverdlov R**, **Shirihai O**, **Shore GC**, **Shu C-W**, **Shukla D**, **Sibirny AA**, **Sica V**, **Sigurdson CJ**, **Sigurdsson EM**, **Sijwali PS**, **Sikorska B**, **Silveira WA**, **Silvente-Poirot S**, **Silverman GA**, **Simak J**, **Simmet T**, **Simon AK**, **Simon H-U**, **Simone C**, **Simons M**, **Simonsen A**, **Singh R**, **Singh S V**, **Singh SK**, **Sinha D**, **Sinha S**, **Sinicrope FA**, **Sirko A**, **Sirohi K**, **Sishi BJ**, **Sittler A**, **Siu PM**, **Sivridis E**, **Skwarska A**, **Slack R**, **Slaninová I**, **Slavov N**, **Smaili SS**, **Smalley KS**, **Smith DR**, **Soenen SJ**, **Soleimanpour SA**, **Solhaug A**, **Somasundaram K**, **Son JH**, **Sonawane A**, **Song C**, **Song F**, **Song HK**, **Song J-X**, **Song W**, **Soo KY**, **Sood AK**, **Soong TW**, **Soontornniyomkij V**, **Sorice M**, **Sotgia F**, **Soto-Pantoja DR**, **Sotthibundhu A**, **Sousa MJ**, **Spaink HP**, **Span PN**, **Spang A**, **Sparks JD**, **Speck PG**, **Spector SA**, **Spies CD**, **Springer W**, **Clair DS**, **Stacchiotti A**, **Staels B**, **Stang MT**, **Starczynowski DT**, **Starokadomskyy P**, **Steegborn C**, **Steele JW**, **Stefanis L**, **Steffan J**, **Stellrecht CM**, **Stenmark H**, **Stepkowski TM**, **Stern ST**, **Stevens C**, **Stockwell BR**, **Stoka V**, **Storchova Z**, **Stork B**, **Stratoulias V**, **Stravopodis DJ**, **Strnad P**, **Strohecker AM**, **Ström A-L**, **Stromhaug P**, **Stulik J**, **Su Y-X**, **Su Z**, **Subauste CS**, **Subramaniam S**, **Sue CM**, **Suh SW**, **Sui X**, **Sukseree S**, **Sulzer D**, **Sun F-L**, **Sun J**, **Sun J**, **Sun S-Y**, **Sun Y**, **Sun Y**, **Sun Y**, **Sundaramoorthy V**, **Sung J**, **Suzuki H**, **Suzuki K**, **Suzuki N**, **Suzuki T**, **Suzuki YJ**, **Swanson MS**, **Swanton C**, **Swärd K**, **Swarup G**, **Sweeney ST**, **Sylvester PW**, **Szatmari Z**, **Szegezdi E**, **Szlosarek PW**, **Taegtmeyer H**, **Tafani M**, **Taillebourg E**, **Tait SW**, **Takacs-Vellai K**, **Takahashi Y**, **Takáts S**, **Takemura G**, **Takigawa N**, **Talbot NJ**, **Tamagno E**, **Tamburini J**, **Tan C-P**, **Tan L**, **Tan ML**, **Tan M**, **Tan Y-J**, **Tanaka K**, **Tanaka M**, **Tang D**, **Tang D**, **Tang G**, **Tanida I**, **Tanji K**, **Tannous BA**, **Tapia JA**, **Tasset-Cuevas I**, **Tatar M**, **Tavassoly I**, **Tavernarakis N**, **Taylor A**, **Taylor GS**, **Taylor GA**, **Taylor JP**, **Taylor MJ**, **Tchetina E V**, **Tee AR**, **Teixeira-Clerc F**, **Telang S**, **Tencomnao T**, **Teng B-B**, **Teng R-J**, **Terro F**, **Tettamanti G**, **Theiss AL**, **Theron AE**, **Thomas KJ**, **Thomé MP**, **Thomes PG**, **Thorburn A**, **Thorner J**, **Thum T**, **Thumm M**, **Thurston TL**, **Tian L**, **Till A**, **Ting JP**, **Titorenko VI**, **Toker L**, **Toldo S**, **Tooze SA**, **Topisirovic I**, **Torgersen ML**, **Torosantucci L**, **Torriglia A**, **Torrisi MR**, **Tournier C**, **Towns R**, **Trajkovic V**, **Travassos LH**, **Triola G**, **Tripathi DN**, **Trisciuoglio D**, **Troncoso R**, **Trougakos IP**, **Truttmann AC**, **Tsai K-J**, **Tschan MP**, **Tseng Y-H**, **Tsukuba T**, **Tsung A**, **Tsvetkov AS**, **Tu S**, **Tuan H-Y**, **Tucci M**, **Tumbarello DA**, **Turk B**, **Turk V**, **Turner RF**, **Tveita AA**, **Tyagi SC**, **Ubukata M**, **Uchiyama Y**, **Udelnow A**, **Ueno T**, **Umekawa M**, **Umemiya-Shirafuji R**, **Underwood BR**, **Ungermann C**, **Ureshino RP**, **Ushioda R**, **Uversky VN**, **Uzcátegui NL**, **Vaccari T**, **Vaccaro MI**, **Váchová L**, **Vakifahmetoglu-Norberg H**, **Valdor R**, **Valente EM**, **Vallette F**, **Valverde AM**, **Van den Berghe G**, **Van Den Bosch L**, **van den Brink GR**, **van der Goot FG**, **van der Klei IJ**, **van der Laan LJ**, **van Doorn WG**, **van Egmond M**, **van Golen KL**, **Van Kaer L**, **van Lookeren Campagne M**, **Vandenabeele P**, **Vandenberghe W**, **Vanhorebeek I**, **Varela-Nieto I**, **Vasconcelos MH**, **Vasko R**, **Vavvas DG**, **Vega-Naredo I**, **Velasco G**, **Velentzas AD**, **Velentzas PD**, **Vellai T**, **Vellenga E**, **Vendelbo MH**, **Venkatachalam K**, **Ventura N**, **Ventura S**, **Veras PS**, **Verdier M**, **Vertessy BG**, **Viale A**, **Vidal M**, **Vieira H LA**, **Vierstra RD**, **Vigneswaran N**, **Vij N**, **Vila M**, **Villar M**, **Villar VH**, **Villarroya J**, **Vindis C**, **Viola G**, **Viscomi MT**, **Vitale G**, **Vogl DT**, **Voitsekhovskaja O V**, **von Haefen C**, **von Schwarzenberg K**, **Voth DE**, **Vouret-Craviari V**, **Vuori K**, **Vyas JM**, **Waeber C**, **Walker CL**, **Walker MJ**, **Walter J**, **Wan L**, **Wan X**, **Wang B**, **Wang C**, **Wang C-Y**, **Wang C**, **Wang C**, **Wang C**, **Wang D**, **Wang F**, **Wang F**, **Wang G**, **Wang H**, **Wang H**, **Wang H-G**, **Wang H**, **Wang H-D**, **Wang J**, **Wang J**, **Wang M**, **Wang M-Q**, **Wang P-Y**, **Wang P**, **Wang RC**, **Wang S**, **Wang T-F**, **Wang X**, **Wang X**, **Wang X-W**, **Wang X**, **Wang X**, **Wang Y**, **Wang Y**, **Wang Y**, **Wang Y-J**, **Wang Y**, **Wang Y**, **Wang YT**, **Wang Y**, **Wang Z-N**, **Wappner P**, **Ward C**, **Ward DM**, **Warnes G**, **Watada H**, **Watanabe Y**, **Watase K**, **Weaver TE**, **Weekes CD**, **Wei J**, **Weide T**, **Weihl CC**, **Weindl G**, **Weis SN**, **Wen L**, **Wen X**, **Wen Y**, **Westermann B**, **Weyand CM**, **White AR**, **White E**, **Whitton JL**, **Whitworth AJ**, **Wiels J**, **Wild F**, **Wildenberg ME**, **Wileman T**, **Wilkinson DS**, **Wilkinson S**, **Willbold D**, **Williams C**, **Williams K**, **Williamson PR**, **Winklhofer KF**, **Witkin SS**, **Wohlgemuth SE**, **Wollert T**, **Wolvetang EJ**, **Wong E**, **Wong GW**, **Wong RW**, **Wong VKW**, **Woodcock EA**, **Wright KL**, **Wu C**, **Wu D**, **Wu GS**, **Wu J**, **Wu J**, **Wu M**, **Wu M**, **Wu S**, **Wu WK**, **Wu Y**, **Wu Z**, **Xavier CP**, **Xavier RJ**, **Xia G-X**, **Xia T**, **Xia W**, **Xia Y**, **Xiao H**, **Xiao J**, **Xiao S**, **Xiao W**, **Xie C-M**, **Xie Z**, **Xie Z**, **Xilouri M**, **Xiong Y**, **Xu C**, **Xu C**, **Xu F**, **Xu H**, **Xu H**, **Xu J**, **Xu J**, **Xu J**, **Xu L**, **Xu X**, **Xu Y**, **Xu Y**, **Xu Z-X**, **Xu Z**, **Xue Y**, **Yamada T**, **Yamamoto A**, **Yamanaka K**, **Yamashina S**, **Yamashiro S**, **Yan B**, **Yan B**, **Yan X**, **Yan Z**, **Yanagi Y**, **Yang D-S**, **Yang J-M**, **Yang L**, **Yang M**, **Yang P-M**, **Yang P**, **Yang Q**, **Yang W**, **Yang WY**, **Yang X**, **Yang Y**, **Yang Y**, **Yang Z**, **Yang Z**, **Yao M-C**, **Yao PJ**, **Yao X**, **Yao Z**, **Yao Z**, **Yasui LS**, **Ye M**, **Yedvobnick B**, **Yeganeh B**, **Yeh ES**, **Yeyati PL**, **Yi F**, **Yi L**, **Yin X-M**, **Yip CK**, **Yoo Y-M**, **Yoo YH**, **Yoon S-Y**, **Yoshida K-I**, **Yoshimori T**, **Young KH**, **Yu H**, **Yu JJ**, **Yu J-T**, **Yu J**, **Yu L**, **Yu WH**, **Yu X-F**, **Yu Z**, **Yuan J**, **Yuan Z-M**, **Yue BY**, **Yue J**, **Yue Z**, **Zacks DN**, **Zacksenhaus E**, **Zaffaroni N**, **Zaglia T**, **Zakeri Z**, **Zecchini V**, **Zeng J**, **Zeng M**, **Zeng Q**, **Zervos AS**, **Zhang DD**, **Zhang F**, **Zhang G**, **Zhang G-C**, **Zhang H**, **Zhang H**, **Zhang H**, **Zhang J**, **Zhang J**, **Zhang J**, **Zhang J**, **Zhang L**, **Zhang L**, **Zhang L**, **Zhang M-Y**, **Zhang X**, **Zhang XD**, **Zhang Y**, **Zhang Y**, **Zhang Y**, **Zhang Y**, **Zhang Y**, **Zhao M**, **Zhao W-L**, **Zhao X**, **Zhao YG**, **Zhao Y**, **Zhao Y**, **Zhao Y**, **Zhao Z**, **Zhao ZJ**, **Zheng D**, **Zheng X-L**, **Zheng X**, **Zhivotovsky B**, **Zhong Q**, **Zhou G-Z**, **Zhou G**, **Zhou H**, **Zhou S-F**, **Zhou X**, **Zhu H**, **Zhu H**, **Zhu W-G**, **Zhu W**, **Zhu X-F**, **Zhu Y**, **Zhuang S-M**, **Zhuang X**, **Ziparo E**, **Zois CE**, **Zoladek T**, **Zong W-X**, **Zorzano A**, **Zughaier SM**. 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# 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 mixed linear model, compared by χ2 test (B), whereas \* 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 (B). † indicates a main effect for feeding state (C-F) and ‡ indicates a main effect for MCP230-exposure by 2-way ANOVA (C-E). \* indicates p<0.05 via a Wilcoxon Rank Sum Test (D-E). 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.** (A)Fasting blood glucose, (B) serum insulin, (C) HOMA-IR and (D) serum glucagon concentrations were determined after a 16h fast at ~ZT4. Fed serum was collected at ZT12 and analyzed for insulin (B) and glucagon (D). Data shown is the group mean ± SE. n=8-14/group. † indicates a main effect for feeding state (B and D) and ‡ indicates a main effect for MCP230-exposure by 2-way ANOVA (D). \* indicates p<0.05 via a Wilcoxon Rank Sum Test (D). 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) O2 consumption rates (VO2) and (B) VO2 analysis, normalized to fat-free mass during both the light and dark phase. Each dot represents the average O2 consumption of each mouse. (C) Time course of energy expenditure and (D) energy expenditure normalized to fat-free mass during both the light and dark phase. Each dot represents the average energy expenditure of each mouse. (E) Quantification of 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, D) or group mean (A, C, E and F) ± SE (E and F). n=18, 6 or 14 for MCP230, saline and cabosil groups, respectively. § indicates p<0.05 by ANCOVA (B). \*indicates p<0.05 by Student’s *t*-test (E), or Wilcoxon-Rank Sum Test (F). 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 5: Exposure to MCP230 *in utero* results in skeletal muscle mitochondrial abnormalities following high fat diet consumption as adults**. (A) mtDNA copy number, (B) citrate synthase activity and (C) mRNA levels of oxidative phosphorylation genes were reduced in the quadriceps muscles of mice that were indirectly exposed to MCP230 *in utero* and subjected to 12 wk of high fat diet as adults. Quadriceps PGC-1α, LC3 and select electron transport chain protein expression was unchanged in the MCP230-exposed mice (D, representative blots and E-F, relative quantification). 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.

**Figure 6: Indirect exposure to MCP230 *in utero* is not associated with reductions in the mRNA of upstream regulators of mitochondrial biogenesis** (A) *Ppard* and (C) *Ppargc1b* mRNA was elevated in the MCP230-exposed mice, whereas (B) *Ppargc1a*, (D) *Nrf1*, (E) *Nfe2l2* and (F) *Tfam* mRNA were not different*.*  Data shown is the group mean ± SE. \*indicates p<0.05 via Student’s *t*-test (A) or Wilcoxon-Rank Sum Test (C). n=7-12/group. The saline-exposed mice are depicted in black and the MCP230-exposed mice are depicted in grey.

**Figure 7: The antioxidant defense system is upregulated in the quadriceps of MCP230-exposed mice.** Data shown is the group mean ± SE. \*indicates p<0.05 via Student’s *t*-test (*Cat*), Welch’s *t*-test (*Sod1*, *Sod2*, *Gpx1*) or Wilcoxon-Rank Sum Test (*Ucp2*, *Gclm*). n=7-12/group. The saline-exposed mice are depicted in black and the MCP230-exposed mice are depicted in grey.

**Tables**

**Table 1.** Mitochondrial DNA copy number and relative gene expression were determined using the following primer sequences. *Tsc2* and *Rpl13a* were used for normalization of genomic DNA and mRNA respectively.

|  |  |  |
| --- | --- | --- |
| **Region/gene** | **Forward primer** | **Reverse primer** |
| **d-Loop** | GGC CCA TTA AAC TTG GGG GT | TTC TTC ACC GTA GGT GCG TC |
| ***mt-Nd1*** | CGT CCC CAT TCT AAT CGC CA | ATG GCG TCT GCA AAT GGT TG |
| ***mt-Cytb*** | CTT CAT GTC GGA CGA GGC TT | CCT CAT GGA AGG ACG TAG CC |
| ***mt-Nd4*** | TAA TCG CAC ATG GCC TCA CA | GCT GTG GAT CCG TTC GTA GT |
| ***Sdha*** | TCT TCG CTG GTG TGG ATG TC | CTT CAG CAC CTG TCC CTT GT |
| ***mt-Co2*** | AAC CGA GTC GTT CTG CCA AT | CTA GGG AGG GGA CTG CTC AT |
| ***Ppard*** | ACA TGG AAT GTC GGG TGT GC | CGG AAG AAG CCC TTG CAC C |
| ***Ppargc1a*** | TGA TGT GAA TGA CTT GGA TAC AGA CA | GCT CAT TGT TGT ACT GGT TGG ATA TG |
| ***Ppargc1b*** | TTG TAG AGT GCC AGG TGC TG | GTG TAT CTG GGC CAA CGG AA |
| ***Nrf1*** | AGA AAC GGA AAC GGC CTC AT | GGC TCT GAG TTT CCG AAG CA |
| ***Nfe2l2*** | TGG ACT TGG AGT TGC CAC C | TCT TGC CTC CAA AGG ATG TCA |
| ***Tfam*** | TCG CAT CCC CTC GTC TAT CA | AGT TTT GCA TCT GGG TGT TTA GC |
| ***Ucp2*** | TGC GGT CCG GAC ACA ATA G | GCC TCC AAG GTC AAG CTT CT |
| ***Ucp3*** | ACA AAG GAT TTG TGC CCT CC | TCA AAA CGG AGA TTC CCG CA |
| ***Sod1*** | GGA ACC ATC CAC TTC GAG CA | CCC ATG CTG GCC TTC AGT TA |
| ***Sod2*** | TTC TGG ACA AAC CTG AGC CC | GTC ACG CTT GAT AGC CTC CA |
| ***Cat*** | CAC TGA CGA GAT GGC ACA CT | TGT GGA GAA TCG AAC GGC AA |
| ***Gpx1*** | TTC GGA CAC CAG GAG AAT GG | TAA AGA GCG GGT GAG CCT TC |
| ***Gclm*** | TGG AGT TCC CAA ATC AGC CC | CAA CTC CAA GGA CGG AGC AT |
| ***Tsc2*** | AAG AAG CCT CTT CTG CTA CC | CAG CTC CGA CCA TGA AGT G |
| ***Rpl13a*** | GGA GTC CGT TGG TCT TGA GG | GGC CAA GAT GCA CTA TCG GA |