Caloric restriction attenuates C57BL/6J mouse lung injury and systemic toxicity induced by real ambient particulate matter exposure

CURRENT STATUS: UNDER REVIEW

Daochuan Li
Department of Toxicology, School of Public Health, Sun Yat-Sen University

Shen Chen
Department of Toxicology, School of Public Health, Sun Yat-sen University

Qiong Li
Department of Toxicology, School of Public Health, Sun Yat-sen University

Liping Chen
Department of Toxicology, School of Public Health, Sun Yat-sen University

Haiyan Zhang
Department of Toxicology, School of Public Health, Sun Yat-sen University

Huiyao Li
Department of Toxicology, School of Public Health, Sun Yat-sen University

Dianke Yu
Department of Toxicology, School of Public Health, Qingdao University

Rong Zhang
Department of Toxicology, School of Public Health, Hebei Medical University

Yujie Niu
Department of Toxicology, School of Public Health, Hebei Medical University

Shaoyou Lu
Department of Toxicology, School of Public Health, Sun Yat-sen University

Lizhu Ye
Department of Toxicology, School of Public Health, Sun Yat-sen University

Xiaowen Zeng
Department of Toxicology, School of Public Health, Sun Yat-sen University

Guanghui Dong
Department of Toxicology, School of Public Health, Sun Yat-sen University
Rui Chen  
Department of Toxicology and Sanitary Chemistry, School of Public Health, Capital Medical University

Michael Aschner  
Department of Molecular Pharmacology, Albert Einstein College of Medicine

Yuxin Zheng  
Department of Toxicology, School of Public Health, Qingdao University

Wen Chen  
School of Public Health, Sun Yat-sen University  
Corresponding Author  
ORCiD: 0000-0001-5435-1664

DOI:  
10.21203/rs.2.23042/v1

SUBJECT AREAS  
Toxicology

KEYWORDS  
Caloric restriction, particulate matter, pulmonary injury, systemic effects, RNA sequencing, xenobiotic metabolism
Abstract

Background: Caloric restriction (CR) is known to improve health and extend life span in human beings. The effects of CR on adverse health outcomes in response to particulate matter (PM) exposure and the underlying mechanisms have yet to be defined.

Results: Male C57BL/6J mice were fed with CR diet or ad libitum (AL) and exposed to PM for 4 weeks in a real-ambient PM exposure system located at Shijiazhuang, China, with a daily mean concentration (95.77 µg/m³) of PM 2.5. Compared to AL-fed mice, CR-fed mice attenuated PM-induced pulmonary injury and systemic toxicity characterized by reduction in oxidative stress, DNA damage and inflammation. Analysis of RNA sequencing revealed that several pulmonary pathways involved in production of ROS, cytokine production, and inflammatory cell activation were inactivated, while those mediating antioxidant generation and DNA repair were activated in CR-fed mice upon PM exposure. In addition, transcriptome analysis of murine livers revealed that CR led to induction of xenobiotic metabolism and detoxification pathways, corroborated by increased levels of urinary metabolites of polycyclic aromatic hydrocarbons (PAHs) and decreased cytotoxicity measured in an ex vivo assay.

Conclusion: These novel results demonstrate, for the first time, that CR in mice confers resistance against pulmonary injuries and systemic toxicity induced by PM exposure. CR led to activation of xenobiotic metabolism and enhanced detoxification of PM-bound chemicals. These findings provide evidence that dietary intervention may afford therapeutic means to reduce the health risk associated with PM exposure.

Keywords: Caloric restriction, particulate matter, pulmonary injury, systemic effects, RNA sequencing, xenobiotic metabolism.

Introduction
Ambient particulate matter (PM) pollution is among the leading four risk factors contributing to deaths and disability-adjusted life-years (DALYs) in China [1]. Emerging evidence has highlighted the association between PM exposure and respiratory diseases, systemic injuries and multiple extra pulmonary disorders, such as cardiovascular diseases, neurodegenerative diseases, kidney damage, diabetes, colon epithelial injury, etc. [2–8]

Although PM levels have been progressively declining in most regions of China, the adverse health effects attributable to PM exposure will continue to rise over at least the next two decades [9]. Thus, intervention modalities are urgently required against the development of diseases associated with PM exposure.

Both epidemiologic and clinical investigations have established strong evidence that dietary modifications, nutrition, and lifestyle have an impact on the onset of diseases associated with environmental toxic insults [10–12]. Healthy dietary and lifestyles have been shown to lower the intensity of environmental stressors associated with adverse health effects by increasing antioxidant and anti-inflammatory mediators [13, 14]. In contrast, individuals with poor dietary habits, such as high intake of processed foods rich in fat and nutrient imbalance, are more susceptible to hazardous chemical-induced health effects [15]. For example, high fat diet or high caloric intake can alter the biological and metabolic activity of individuals, leading to oxidative damage, inflammation and insulin resistance, all of which increase the health risk associated with environmental exposures [16, 17]. Accordingly, we have posited that modifying dietary habits or nutrient intake would represent a novel approach to protect human beings from health impairment associated with air pollution.

Caloric restriction (CR) is defined as a dietary regimen by reduction of total calorie intake without deprivation of essential nutrients. It has been demonstrated that maintenance of CR is beneficial for health, extending lifespan, reducing weight and the maintenance of
metabolic activation [18]. CR also reduces the incidence of cardiovascular diseases, cancers, immune deficiencies, neurodegeneration and diabetes in humans [19–21]. CR displays health benefits by triggering a series of molecular events, including reduction of oxidative damage, acceleration of autophagy, inhibition of inflammation and decreased DNA damage [18, 22]. CR reduces oxidative stress by decreasing the production of reactive oxygen species (ROS) and enhancing the capacity of antioxidants, such as increased activity of antioxidant response elements (ARE) and the levels of glutathione (GSH) [23]. CR has been shown to decrease the levels of circulating pro-inflammatory cytokines, enhance immune functions, and attenuate DNA damage by activation of key components of the DNA-repair machinery, such as recruitment of base excision repair factors and activation of p53 signaling [22, 24–26]. Moreover, CR could promote anti-tumor effects and inhibit metastases through the induction of autophagy and the modulation of the immune microenvironment [27, 28]. Although CR may impact the reduction in PM-induced pulmonary injuries, the underlying mechanisms and the key pathways involved in regulation of cellular functions and integrity has yet to be elucidated.

Previous studies have reported that CR enhances the metabolic activation and detoxification of chemical carcinogens, such as aflatoxin B$_1$ (AFB$_1$), benzo[a]pyrene (BaP), and 7,12-dimethyl-benz(a)anthracene (DMBA) [29–32]. However, it remains unclear whether the synergistic effects are associated with metabolic activation (primarily phase I enzymes) and detoxification (primarily phase II enzymes), accelerating the clearance of PM-bound chemicals. Moreover, the relationship between the biological effects and specific metabolic pathways has yet to be addressed.

Previously, we established a real-world ambient PM exposure system and showed that high level of PM exposure causes multiple-organ damage in mice [8]. The system we
constructed offers optimized condition to minimize distress and discomfort during the inhalation exposure and replicates a real-ambient PM exposure scenario for experimental animals, resembling the natural state of human exposure to the extent possible. In this study, we examined the effect of CR diet on pulmonary injury and systemic effects upon real-ambient PM exposure and explored the underlying molecular mechanisms of CR-mediated biological effects.

Results

Characterization of real ambient PM exposure

Whole-body PM inhalation was conducted in a real-ambient PM exposure system for 4 weeks from Jan 4th to Feb 1st, 2018 in Shijiazhuang, China. Daily PM$_{2.5}$ concentration of ambient air and exposure chamber were monitored (Fig. S1). The daily mean concentration of PM$_{2.5}$ in ambient air was 151.07 µg/m$^3$, which was 4.32-fold higher than the daily average limit of 35 µg/m$^3$ (Air Quality Guidelines of China), and 15.11-fold higher than the daily average limit of 10 µg/m$^3$ (Air Quality Guidelines of the World Health Organization) (Table 1). In the course of 4-week exposure, the number of days exceeded 35 µg/m$^3$ and 150 µg/m$^3$ (moderate degree of air pollution) was 13 days and 14 days. Correspondingly, the mean concentration of PM$_{2.5}$ in the exposure chamber was 95.77 µg/m$^3$, which was 63.40% of the ambient air. The cumulative exposure burden in mice and humans (adults) was estimated at 31.32 µg/mouse and 11,424.36 µg/person, respectively, using the Multiple-Path Particle Dosimetry (MPPD) model (Table 1).
Table 1
The mean PM2.5 concentration and cumulative burden during the exposure period.

| Mean daily PM2.5 concentration (µg/m³) | Estimated cumulative burden a | 35 < PM2.5 ≤ 150 µg/m³ | PM2.5 > 150 µg/m³(Days) |
|---------------------------------------|--------------------------------|--------------------------|--------------------------|
| Chamber                               | Ambient                        | Mouse (µg/mouse)         | Human (µg/person)         | 35 < PM2.5 ≤ 150 µg/m³(Days) | PM2.5 > 150 µg/m³(Days) |
| 95.77                                 | 151.08                         | 31.32                    | 11424.36                 | 13                         | 14                        |

Note: a. Estimated cumulative burden = MV x T x CON x DF. MV: minute ventilation (mL/min); T: total exposure time (min); CON: mean concentration (mg/m³); DF: pulmonary deposition fraction (m³), DF is estimated by MPPD 3.04.

To characterize the chemical composition of PM$_{2.5}$, atmospheric PM$_{2.5}$ was collected daily and quantitative analysis was conducted for polycyclic aromatic hydrocarbons (PAHs), nitro derivatives of PAHs (nitro-PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo dioxins (PCDDs), metal elements and anions (Table S4-S8). As a result, the average concentration of benzo[a]pyrene (BaP), PCDF, PCDD, chromium (Cr) and arsenic (As) far exceeded the daily limit value of Ambient Air Quality Standards of China (Table S9). Thus, the location of this animal study was representative of the heaviest PM exposure areas in China.

CR efficiently protected against mouse pulmonary injury induced by PM exposure

As described in the Methods, we successfully established a mouse model with caloric restriction (CR). The daily caloric intake was reduced by 40% and maintained throughout the experiment. Compared with ad libitum (AL)-fed mice, CR led to a reduction in body weight (BW) by 67.31 ~ 78.43% (Fig. 1A-1B), in subcutaneous fat mass by 23.30 ~ 32.20% (Fig. 1C), and in visceral fat by 43.39 ~ 44.32% (Fig. 1D). Notably, CR conferred mice with enhanced mitochondrial function (Fig. 1F) and absent signs of malnutrition or lean mass loss (Fig. 1E).

To assess the effects of CR on pulmonary injury in response to PM exposure, we conducted histological and bronchoalveolar lavage fluid (BALF) analyses in mice (4 groups, n =
20/group). The histopathological examination revealed that PM exposure induced interstitial infiltration of neutrophils, alveolar septal thickening and alveolar hemorrhage in AL-fed mice, whereas moderate pathologic injury was observed in CR-fed mice (Fig. 2A). As indicated by the pulmonary injury score (Fig. 2B), PM exposure led to a 77% increase of pulmonary injury in AL-fed mice compared to the AF control group, while 45% increase was observed in CR-fed mice. Consistent with the pathological changes, CR remarkably alleviated the pulmonary injury upon PM exposure in terms of total cell number, total protein (TP) content and albumin (ALB) levels, as well as the release of lactate dehydrogenase (LDH) in BALF compared to AL-fed mice (Fig. 2D-2G). In addition, PM exposure led to increased number of TUNEL-positive cells (apoptotic) in AL mice by 73.32%, but no significant change in CR-fed mice (Fig. 2A, 2C). Correspondingly, the level of cleaved caspase-3 was reduced by 97.67% upon PM exposure in CR-fed mice compared to AL-fed mice (Fig. 2H, 2I). Taken together, these observations indicate that CR significantly alleviates pulmonary injury in response to PM exposure.

CR alleviated PM-induced pulmonary oxidative stress, genotoxic damage and inflammatory response.

Next, we assessed the effects of CR on oxidative stress, genotoxicity and inflammatory response upon PM exposure. Compared to the AL-fed mice, the levels of ROS and malondialdehyde (MDA) were lower in the lung tissues and BALF of CR-fed mice upon PM exposure (Fig. 3A-3B). A 21.35 ~ 25.50% decrease in glutathione (GSH) content was observed in AL-fed mice upon PM exposure, but only 13.83 ~ 16.16% decrease was observed in CR-fed mice (Fig. 3C). To determine genotoxicity, we performed immunohistochemistry (IHC) analysis of γH2AX, a marker of the double strand breakage (DSB). As shown in Fig. 3A, γH2AX-positive cells were significantly increased by 78.50% in AL-fed mice after PM exposure, while no significant change was observed in CR-fed mice.
Pulmonary pro-inflammatory and anti-inflammatory effects were determined by IHC staining of F4/80, M1/M2 polarization, and cytokine analysis. The F4/80-positive cells, a marker of macrophage recruitment, were significantly increased by 152.07% and 33.26% in AL-fed and CR-fed mice upon PM exposure, respectively (Fig. 3A). Similar results were obtained when counting the number of macrophages and polymorphonuclear neutrophils (PMNs) in BALF (Fig. 3D-3E). Moreover, we determined the polarization of M1 and M2 macrophages in BALF, which indicated pro-inflammatory and anti-inflammatory effects, respectively. The results showed a significant increase in the proportion of M2 polarized macrophages in CR-fed compared AL-fed mice in response to PM exposure (Fig. 3F, Fig. S2A-D). Consistent results were observed in the mRNA expression of M1 or M2 macrophage markers in BALF cells (Fig. S2E-F). Importantly, the secretion of cytokines (IFN-γ, TNFα, IL-1β, and IL-12p70) in BALF, which is linked to the potency of pro-inflammation and M1 macrophage polarization increased in AL-fed mice. However, the levels of IL-4, IL-10, and TGF-β1, which indicated the anti-inflammatory capacity and M2 macrophage polarization, were notably higher in CR-fed mice exposed to PM. Taken together, these observations demonstrate that CR leads to reduction in oxidative damage, DNA damage, and pro-inflammation, contributing to the protective effects against PM-induced pulmonary injury.

The effects of CR on regulation of pathways involved in the protective effects towards PM exposure

Transcriptome profiling of lung tissues was performed to elucidate the molecular mechanisms underlying the effects of CR on PM-induced pulmonary injury. Initial analysis identified 2,299 and 1,928 differentially expressed genes (DEGs) based on the comparison between CRC and ALC (AF control group) and between CRE and ALE (PM exposed group). Detailed information of the DEGs is shown in Fig. S3. To address the biological relevance
of the modifications in the gene signature profiles, we employed IPA software to identify critical biological functions and molecular pathways. As a result, IPA analysis identified approximately 500 biological functions, diseases and toxicological outcomes related to the identified DEG profiles. The most related diseases and biological functions predicted were categorized targeting organ injury and abnormalities, inflammatory response, cell death and survival, cell-to-cell signaling and interaction, and respiratory disease (Fig. 4A, 4B). Notably, the biological function changed in CR-fed mice, with or without PM exposure, implicating a reduction in inflammation, accumulation, recruitment and activation of immune cells (leukocyte, monocyte, lymphocyte, neutrophils, etc.), and cytotoxicity (necrosis, apoptosis, cytolysis), concomitant with increased cell viability. These signatures in gene expression correspond to the biochemical and histologic changes that are attributable to CR (Fig. 1-2).

Next, we analyzed canonical pathways associated with the CR-mediated protective effects. We identified 542 and 528 pathways significantly altered between CRC and ALC groups, and between the CRE and ALE groups, respectively. Of the pathways identified, we paid special attention to the those related to inflammatory response, oxidative stress, DNA damage and xenobiotic metabolism (Fig. 4C, 4D). Notably, we found that CR led to a decline in the activity of several pathways, including acute phase response signaling, Th1 pathway, chemokine signaling, and several key molecular pathways (NF-κB, CCR5, STAT3, NFAT, IL-12, HMGB1, TREM1, IL-6, etc.) involved in inflammatory regulation in mice with or without PM exposure. Specifically, pathways mediating antioxidant functions were activated, and those associated with nitric oxide and reactive oxygen generation were inhibited in CR-fed mice with or without PM exposure. In addition, DNA damage checkpoint regulation and GADD45 signaling were activated in CR-fed mice of both the AF control and PM exposure group, indicating that the capacity of DNA repair was enhanced upon CR. The
activation of RXR might be involved in detoxification of xenobiotics. Collectively, the modification in transcriptomic profiles and specific pathway alterations were in agreement with the biological effects of CR, corroborating the notion that CR played an important role in attenuating PM-induced pulmonary injury.

The effects of CR on systemic toxicity induced by PM exposure

Systemic toxicity is implicated in the development of extra pulmonary abnormalities in response to PM exposure. To assess the effects of CR on PM-induced systemic toxicity, we compared oxidative stress levels, genotoxicity and inflammatory responses between AL- and CR-fed mice with or without PM exposure. The results showed that the levels of plasma MDA and urine 8-hydroxydeoxyguanosine (8-OHdG) were increased by 61.02% and 26.58% in AL-fed mice following PM exposure, while no significant change was observed in CR-fed mice (Fig. 5E, 5G). In addition, higher concentration of serum GSH was detected in CR-fed mice, reflecting improved redox status in CR-fed mice (Fig. 5G). To assess the degree of DNA damage, we conducted comet assays in peripheral blood cells and found that the olive tail moments in AL-fed mice were extended by 43%. In contrast, there was no difference between the PM exposed and control groups of CR-fed mice (Fig. 5H).

Moreover, the cell numbers of peripheral white blood cell (WBC), lymphocyte, monocyte, and neutrophil were reduced by 54.96%, 45.74%, 29.68%, 15.20%, respectively, in CR-fed mice compared to those in the AL-fed mice following PM exposure (Fig. 5A-5D). The anti-inflammatory cytokines (IL-4, IL-10 and TGF-β1) were significantly up-regulated, while the pro-inflammatory cytokines (IFN-γ, TNFα, IL-1β, IL-6, and IL-12p70) were slightly induced in CR-fed mice compared to AL-fed mice upon PM exposure (Fig. 5I). We also performed ex vivo assays to examine cytotoxicity in several cell lines, namely Neuro-2A, HBE, THP1, HepG2, HEK, HCT116 using mouse plasma isolated from AL-fed and CR-fed mice with or without PM exposure. Notably, we showed a 16.32 ~ 27.13% and 8.36 ~ 16.28% reduction
in cytotoxicity of AL-fed and CR-fed mice, respectively following PM exposure, implicating a protective role of CR against impairment in extrapulmonary organs (Fig. 5J). Taken together, these findings indicate that CR leads to a decrease in the PM-induced systemic toxicity, which may abate the intensity of extrapulmonary impairment.

CR Enhanced Xenobiotic Metabolism And Detoxification In Mouse Livers

Metabolic activation and detoxification of environmental chemicals play a key role in cellular toxicity and ensuing organ injuries. To examine the effects of CR on xenobiotic metabolism, we performed transcriptome profiling analysis in mouse liver tissues. 2,821 and 2,995 DEGs were identified between CRC and ALC groups and between CRE group and ALE groups, respectively. Detail information on the DEGs is shown in Fig. S4. In addition, 611 and 601 canonical pathways were identified by a comparison between AL-AF and CR-AF mice and between AL-PM and CR-PM mice, respectively. Of the 20 most significantly altered pathways, we found that the activation of NRF2-mediated oxidative stress response and GADD45 signaling were specifically inherent to the CR-fed mice (Fig. 6A-6B), suggesting enhanced capacity of antioxidant and more efficient DNA-repair contribute to the attenuation of PM-induced systemic toxicity. Notably, the activity of metabolic pathways, including xenobiotic metabolism signaling, aryl hydrocarbon receptor (AhR) signaling, NRF2-mediated metabolism, glutathione-mediated detoxification, and Retinoid X receptor (RXR) activation pathways were significantly enhanced in CR-fed mice with or without PM exposure. The identified pathways involved in xenobiotic metabolism are shown in Fig. 6C-F. Particularly, the activation of AhR signaling is associated with increased levels of phase I (CYP1A1, CYP1A2, CYP1B1, ALDH, etc.) and phase II (NQO, UGT, GST, etc.) metabolizing enzymes in CR-fed mice (Fig. 6C). The upregulation of NRF2-mediated metabolism and glutathione-mediated detoxification led to higher levels of phase II metabolizing enzymes (NQO, UGT, GST, etc.) in CR-fed mice (Fig. 6D, 6G). In
addition, we showed that the activated RXR pathway induced by CR might be related to the upregulation of phase I (CYP2C8, ALDH, FMO, etc.) and phase II (UGT, GST, etc.) metabolizing enzymes, as well as transporters (MRP2, MDR1, etc.) (Fig. 6E, 6F). The changes in enzyme activity induced by CR are critical for determining metabolic activation and detoxification of PM-bound chemicals.

To further address the effects of CR on metabolic enzyme activity and the correlation with the removal of toxic chemicals, we examined the levels of metabolic enzymes towards polycyclic aromatic hydrocarbons (PAHs), a major category of PM-enriched chemicals. As shown in Fig. 7A-G, immunoblotting analysis revealed that the phase I (CYP1A1, CYP1A2, and CYP1B1) and phase II (GSTT1, GSTM1, and UGT1A1) metabolizing enzymes involved in PAHs metabolism were all upregulated in the liver of CR-fed mice with or without PM exposure, consistent with the results from the transcriptome analysis. The urinary hydroxylated metabolites of PAHs (OH-PAHs) were quantified by GC-MS. We found that the urinary concentration of 1-OHNap, 2-OHNap 1-OHPhe, 4-OHPhe, 9-OHPhe, 1-OHPyr were significantly higher in the PM exposure group of CR-fed mice than that of AL-fed mice (Fig. 7H-M). Taken together, these findings demonstrate that CR plays an important role in the induction of metabolic activation of PM-bound chemicals, leading to a rapid detoxification of toxicants and likely reduced health risks associated with PM exposure.

Discussion

Dietary, nutritional and lifestyle interventions have been shown to promote health by lowering toxicant burden in both animals and humans [11, 13]. Growing evidence has established that CR represents an effective intervention against a variety of adverse health outcomes [18, 33]. In this study, we demonstrated that CR confers murine resistance against PM-induced pulmonary injury and systemic toxicity. The afforded protection is characterized by reduction in oxidative stress, genotoxicity, and
inflammatory responses. Transcriptomic analysis further corroborated the efficacy of CR to activate protective molecular pathways. Notably, altered xenobiotic metabolism and detoxification triggered by CR facilitate the metabolic activation and detoxification of PM-bound chemicals such as PAHs. These findings provided novel insight into the mechanism by which CR mediates the protective roles against PM-induced toxicity and suggest that CR might represent a powerful approach for intervention against air pollution-related health injury.

Dietary practices are well-defined modulators of human health and biological effects, thereby having a great impact on disease susceptibility in response to environmental stress [13, 34, 35]. Recent findings indicate that poor dietary habits, such as high intake of caloric carbohydrate- or fat-rich diet contribute to metabolic dysfunction and systemic pro-inflammation [16, 36, 37], leading to increased susceptibility to adverse health effects in response to air pollution [38, 39]. In contrast, chronic reduction of caloric intake, in the absence of malnutrition, has been reported to improve lifespan and have anti-cancer effects [18]. In the present study, we demonstrated that CR attenuated the pulmonary injury and systemic disorders associated with murine exposure to PM. These effects are mediated, at least in part, by the enhancement of the antioxidants, DNA damage repair, and anti-inflammatory capacity in response to PM exposure.

Although previous studies have shown that CR could modulate the redox status [40], maintain the genome integrity [40–42], reduce inflammatory activity, and improve immune function [43, 44], the molecular mechanisms underlying CR-triggered alteration in a variety of biological functions have yet to be determined. With the aid of transcriptional profiling and analysis of pathway perturbation, herein we were able to systematically illustrate the molecular regulatory networks that contribute to CR-induced antagonistic effects against PM exposure-induced damage. Upon CR, pathways involved in antioxidant
functions could be activated to inhibit the generation of ROS and trigger antioxidant enzymatic activity [23]. In this study, analysis of gene expression profiles revealed that multiple key pathways were activated in CR-fed mice with or without PM exposure, corroborating the reduction in nitric oxide and ROS levels, enhanced activity of the antioxidant vitamin C, Nrf2-mediated oxidative stress response, and glutathione-mediated detoxification, which were corresponding to the reduction of oxidative damage in CR-fed mice.

CR facilitates the maintenance of genome integrity by accelerating DNA repair, thus leading promoting cellular survival and longevity [40–42]. In this study, we demonstrate that a major consequence of ambient PM exposure was associated with pulmonary and systemic genotoxicity, which was reversed in CR-fed mice. Previous studies have shown that CR decreased γH2AX foci formation, a marker of DSB in DNA repair deficiency mice, and increased the level and activity of p53 through promoting DNA-dependent protein kinase (DNA-PK) activation, and accelerating cellular DNA repair capacity [26, 45, 46]. Herein, we identified specific pathways, such as GADD45 pathway that was activated by CR and might contribute to the promotion of DNA repair. G2/M DNA damage checkpoint regulator, DNA damage protein 45 (GADD45) signaling is critical in pulmonary and hepatic growth arrest. Activation of GADD45 pathway is involved in stimulation of DNA repair through interaction with cyclin-dependent kinase 1 (Cdk1) [47, 48]. Taken together, CR robustly protects against the PM-induced genotoxicity and promotes genome integrity by improving the DNA repair capacity.

CR is known to inhibit inflammatory responses and to improve immunity [43, 44]. Prior studies have indicated that CR inhibited critical pathways involved in cytokine and chemokine signaling to reduce the level of circulating pro-inflammatory cytokines, such as TNFα, IL-6, and IL-1β [49–54]. Healthy individuals undergoing short-term CR have been
shown to have reduced monocyte metabolic and inflammatory activity and a drastic decrease in circulating and organ monocyte numbers [55, 56]. The recruitment of inflammatory cells in tissues and airway induced by intake of high fat or high caloric diet could be reversed by CR [57, 58]. In this study, our results established that CR could reduce the levels of pulmonary and circulating inflammatory triggers. Notably, we verified that the inhibition of inflammatory pathways, such as NF-κB, CCR5, STAT3, NFAT, IL-12, HMGB1, TREM1, and IL-6 might be involved in CR-mediated anti-inflammatory effects upon PM exposure.

Xenobiotic metabolism plays a vital role in mediating the toxicity of PM exposure [59]. Dietary intake could interact with xenobiotic response elements (XREs) to regulate the expression and activity of metabolizing enzymes, thus modifying the cellular response to xenobiotic stressors [60, 61]. A line of evidence has demonstrated that CR acts as a modulator of XREs and up-regulation of the expression of enzymes involved in phase I, phase II metabolism and xenobiotic transport in mouse liver [29, 58, 62]. CR led to an increase in microsomal enzyme activity of cytochrome P450 (CYP450), modifying phase I metabolism of environmental toxicants [30, 31]. Notably, CR enhanced detoxification of environmental chemicals and reduced the formation of adducts through increasing the activity of phase II metabolizing enzymes, such as glutathione S-transferases (GSTs), uridine diphosphate glucuronosyltransferase (UGTs), and NAD(P)H: quinone oxidoreductase (NQOs) [32, 63, 64]. In addition to the changes in enzyme activity described previously, we also identified that AhR- and Nrf2-mediated metabolism, glutathione-mediated detoxification, and RXR activation might be involved in the metabolism of PM-bound chemicals in mouse liver, thus determining the intensity of systemic toxicity. Notably, the urinary metabolites of PM-bound PAHs were positively associated with the expression of key metabolizing enzymes and decreased cytotoxicity in
CR-fed mice, indicating that the clearance of toxic chemicals in CR-fed mice had been greatly accelerated.

Conclusion
In conclusion, we demonstrated that CR has protective effects against PM-induced pulmonary injury and systemic toxicity by reducing oxidative stress, DNA damage, and inflammatory responses. In addition, CR activates pathways involved in metabolic activation and detoxification of xenobiotics, facilitating the elimination of PM-bound toxic components, such as PAHs. These novel findings provide strong evidence for the significance and necessity of dietary interventions to reduce the health risk in PM-exposure population.

Methods
Animal models
Six-week-old C57BL/6 mice were purchased from the Model Animal Research Center of Nanjing University (Nanjing, Jiangsu, China.). Mice were housed in cages under a 12-hour dark/light cycle with free access to food and water. After 2-week acclimation period, mice were divided into two weight-matched groups and fed with different diets, including ad libitum diet (AL, n = 40) and caloric restriction diet (CR, n = 40) for a period of 4 weeks, respectively. CR was progressive, initiated at 10% restriction during the first week, 25% during the second week, and to 40% for the remainder experimental period, according to the method described previously [45]. The components of AL diet and CR diet were given in Table S1. The food intake and the body weight were recorded daily (Table S2, Fig. 1A-1B). All animal procedures were conducted in accordance with the guidelines of the Animal Care and Protection Committee of Sun Yat-sen University and Hebei Medical University.
Mice were kept in isolated ventilated cages (IVC) and exposed to PM in a real-ambient PM exposure system located at heart of city Shijiazhuang, China, where the annual average concentration of PM2.5 was ranked in the top 5 Chinese cities over the past decade [8]. This system permitted circulating the ambient air into the chambers absence concentrating the ambient PM. The air channels of the control chambers are equipped with a three-layer HEPA filter (air filter, AF), which provides an excellent barrier to block fine ambient PM (PM$_1$ = 0). The concentrations of fine PM in the exposure chamber and ambient air were monitored with Aerodynamic Particle sizer Spectrometer 3321 (TSI Incorporated, Shoreview, MN, USA). Two groups of male mice (n = 20/group, 5 mice/cage) fed with AL or CR diet were exposed to PM for 24 h per day, 7 days per week for 4 weeks, from Jan 4th to Feb 1st, 2018. The other two groups of male mice (n = 20/group, 5 mice/cage) were kept in the control AF chambers (PM$_1$ = 0). At the end of the experiments, mice were sacrificed, and the biological samples were collected for further analyses as described below.

PM Collection, Extraction And Components Analysis

In the course of PM exposure (4 weeks), the ambient PM$_{2.5}$ was collected onto Teflon filters daily at a flow rate of 1.05 m$^3$/min using a High-Volume Air Samples (Thermo Fischer Scientific, Waltham, MA, USA) nearby the PM exposure system. The filters were combined for chemical analysis. Organic components were extracted by Soxhlet extraction for quantification of polycyclic aromatic hydrocarbons (PAHs), nitro- and alkyl- derivatives of PAHs, polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-dioxins (PCDD). Water-soluble fractions were extracted by sonication for analyses of metals and anion species with inductively coupled plasma-mass spectrometry (ICP-MS; ELEMENT2; Thermo Finnegan, San Jose, CA, USA).

BALF Analysis
Bronchoalveolar lavage fluid (BALF) was collected as described previously [8]. The cells and supernatant were separated by centrifugation of BALF at 400 × g for 7 min at 4°C. Total protein, lactate dehydrogenase (LDH) and albumin contents in BALF supernatant were determined by BCA Protein Assay Kit (Beyotime biotechnology, China), LDH release assay kit (Promega, Corporation, Madison, WI, USA) and Albumin Assay kit (Nanjing Jiancheng Bioengineering Institute, China), respectively. The number of cells was determined with a cell counter (Beckman, USA). 1 × 10^4 cells were spread on microscope slides, fixed with 96% ethanol and stained with May-Grünwald-Giemsa. The number of macrophages and polymorphonuclear neutrophils (PMNs) were counted under microscope (Leica, Germany).

Histopathological Analysis

The lung tissues were removed, washed with 0.1 μM phosphate buffered saline (PBS, pH7.4), fixed in 4% formalin for 24 h at room temperature, dehydrated by graded ethanol, and embedded in paraffin. Tissue sections (5 μm) were made and stained with hematoxylin and eosin. The histological examination was performed under a light microscope. The histopathological analysis of lung injury was conducted quantitatively as described previously [65].

TUNEL Staining

Apoptosis of cells in mouse lung was detected by terminal-deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) staining (Beyotime, China) according to the manufacturer’s instructions. For quantification of apoptotic cells, five sections and 20 random fields per section in each group were counted, and the average number of apoptosis per section was calculated.

Detection Of ROS In Mouse Lung

Reactive oxygen species (ROS) levels in the lung tissues were detected with the
fluorescent probe DHE (dihydroethidium; Sigma, USA). Briefly, the frozen sections were incubated with 10 µM DHE at 37°C for 30 min. The slides were viewed under a fluorescence microscope (Leica, Germany). The intensity of DHE was analyzed by ImageJ software.

Immunohistochemistry
Lung sections (5 µm) were mounted onto the slides, deparaffinized and rehydrated, and heated in 0.1 M citrate buffer (pH 5.8) for antigen retrieval. To inactive endogenous peroxidase, we incubated slides with 3% H₂O₂ at RT for 15 min. After blocking with 2% BSA for 30 min at 37°C, the slides were incubated with primary antibodies against γ-H2AX (Abcam, USA), p53 (CST, USA) and F4/80 (CST, USA) overnight at 4 °C. After incubation with the corresponding secondary antibody for an additional 1 h at room temperature in dark, the slides incubated in 10 µg/ml 3, 3′-diaminobenzidine tetrahydrochloride (DAB; Beyotime, China) for 10 min. The nuclei were counterstained with hematoxylin for 10 s. The fluorescent intensity was analyzed using ImageJ software.

Cytokine Analysis
Plasma cytokines, including interferon-γ (IFN-γ), tumor necrosis factor (TNF-α), interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12 (IL-12p70), and transforming growth factor beta (TGF-β1) were measured with an ELISA assay kit (R&D Systems, MN, USA).

Ex vivo assays
Colorimetric cell viability assay (MTS; Promega Corporation, Madison, WI, USA) was used to determinate the effect of the plasma on cytotoxicity of multiple cell lines, Neuro-2A, THP1, HepG2, HEK, HCT116, 16HBE. We seeded the cells in a 96-well plate at a density of 3 × 10^3 and cultured them in a medium containing 10% of fetal bovine serum (FBS) and
1:100 plasma isolated from the mouse blood. Forty-eight h after seeding, 20 µL of MTS reagent were added to each well and incubated for 2 h. The absorbance at 490 nm was determined and the cytotoxicity was presented as fold changes relative to the cell viability of the negative control.

Immunoblotting
Total cellular protein was extracted by RIPA lysis buffer (150 M of NaCl, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, and 50 mM Tris (pH 7.4)) containing protease inhibitors. 20 µg soluble proteins were separated by 8 ~ 12% SDS-PAGE and were transferred onto a nitrocellulose membrane (Pall Corporation, NY, USA). After being blocked with 5% fat free milk, the membranes were incubated with primary antibodies against caspase3, cleaved caspase 3, CYP1A2 (Cell Signaling Technology, MA, USA), CYP1A1, CYP1B1, GSTT1, GSTM1, UGT1A1 (Abcam, UK), and β-actin (Proteintech Group, AL, USA). Immunolabeling was visualized with HRP-conjugated anti-rabbit IgG or anti-mouse IgG (Santa Cruz Biotechnology, CA, USA). The density of the specific bands was quantified using ImageJ software.

RNA Sequencing
For each group, we randomly selected 3 mice for conducting RNA sequencing of mouse lung or liver tissues, 12 mice in total from 4 groups of mice (AL-fed or CR-fed with or without PM exposure). Total RNA samples of mouse lung and liver tissues were extracted using TRIZOL reagents and subjected to RNA sequencing (Beijing Genomics Institute in Shenzhen, China). Briefly, Oligo dT magnetic beads were employed to trap mRNAs with poly A tails, and the mRNAs were fragmented and reversely transcribed to double-stranded cDNA (dscDNA) by random primers. The cDNAs were ligated adaptors and subjected to amplification. The PCR products were then denatured, and single stranded PCR products were cyclized by splint oligos with DNA ligase to construct cDNA library. The
sequencing was performed with BGISEQ-500 platform, generating 23.87 M reads per sample. The average mapping ratio with reference genome was 94.60% and the average mapping ratio with gene was 81.87%. 17,519 genes were detected in this experiment. We set fold-change greater than 1.5 times, P value lesser than 0.05 as the standard to define differentially expressed genes (DEGs). These DEGs were analyzed by Ingenuity Pathway Analysis (IPA) software (Qiagen, Germany). The increased and/or decreased activity of pathways and functions of the DEGs were defined via IPA. Significant differences were defined as P value was less than 0.01 and the absolute value of the z score was greater than 2.

Examination Of Urinary OH-PAHs

Concentration of seven hydroxylated metabolites of PAHs (OH-PAHs), including 1-OHNap, 2-OHNap, 1-OHPhe, 4-OHPhe, 9-OHPhe, 2-OHFlu, 1-OHPyr, in mouse urine were analyzed with a LC-20A high performance liquid chromatography system (HPLC; Shimadzu, Japan) coupled with a Q-Trap 5500 mass spectrometer (MS/MS; AB SCIEX, USA). Five isotopically labeled chemicals were used as internal standards: d8-2-OHNap d9-2-OHFlu, $^{13}$C6-4-OHPhe, $^{13}$C6-1-OHPyr. The concentration was calculated by extrapolating the peak area of the sample from standard sets. Urinary OH-PAHs concentrations were adjusted with the content of urinary creatinine (µg/g Cre).

Statistical analysis

Data are shown as the mean ± S.D. All statistical analysis was performed with SPSS 22.0 statistical software (SPSS Inc., Chicago, IL, USA). Student’s t-test was applied to analyze the difference between two groups and one-way analysis of variance (ANOVA) followed by Bonferroni’s post hoc test was used for comparisons between multiple experimental groups. Differences were considered significant at P < 0.05.
Supporting Information

Supplementary information available online, including Supplementary Materials and Methods, Figure S1 to S4 and Table S1 to S9.

List Of Abbreviations

PM, particulate matter; CR, caloric restriction; ad libitum, AL; IPA, Ingenuity Pathway Analysis; MPPD, Multiple-Path Particle Dosimetry model; PAH, polycyclic aromatic hydrocarbons; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzo dioxins; BALF, bronchoalveolar lavage fluid; LDH, lactate dehydrogenase; TUNEL, terminal-deoxynucleotidyl transferase-mediated nick end labeling; PMN, polymorphonuclear neutrophil; 8-OHdG, 8-hydroxydeoxyguanosine; CYP450, cytochrome P450; UGT, uridine diphosphate glucuronosyltransferase; GST, glutathione S-transferases.

Declarations

Ethics approval and consent to participate

Animals were treated humanely, and all experimental protocols were approved by the Animal Care and Protection Committee of Sun Yat-sen University and Hebei Medical University, China. All the methods in the present study were performed according to approved guidelines.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interest

The authors declare they have no actual or potential competing financial interests.
**Funding**

This work was supported by the Key Major Research Plan of National Natural Science Foundation of China (91943301), Guangdong Provincial Natural Science Foundation Team Project (2018B030312005), National Key Research and Development Project (2018YFE0106900), and National Institute of Environmental Health Sciences (NIEHS) of USA (R01 ES10563, and R01 ES07331).

**Authors’ contribution**

WC, DL, SC and QL designed the study. DL, SC, QL and MA prepared the manuscript. HZ, RZ, YN, HL contributed animal experiments. SC, QL, LC contributed to the pathology analysis and bioassays. SL, LY and XZ contributed to the chemical analysis. DL, DY, GD, RC and YZ contributed to the statistical analysis.

**Acknowledgement**

We thank Dr. Mai, Bixuan, Dr. Chunying Chen, Dr. Jianqing Zhang, and Prof. Yu, Jianzhen for the kind help in analysis of PM$_{2.5}$ compositions.

**References**

1. Zhou, M., et al., *Mortality, morbidity, and risk factors in China and its provinces, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017*. Lancet, 2019. 394(10204): p. 1145-1158.

2. Thurston, G.D., et al., *Workgroup report: workshop on source apportionment of particulate matter health effects--intercomparison of results and implications*. Environ Health Perspect, 2005. 113(12): p. 1768-74.

3. Kirrane, E.F., et al., *A systematic review of cardiovascular responses associated with ambient black carbon and fine particulate matter*. Environ Int, 2019. 127: p. 305-316.

4. Power, M.C., et al., *Traffic-related air pollution and cognitive function in a cohort of*
older men. Environ Health Perspect, 2011. 119(5): p. 682-7.

5. Seltenrich, N., PM2.5 and Kidney Function. Environmental Health Perspectives, 2016. 124(9): p. A168-A168.

6. Eze, I.C., et al., Association between Ambient Air Pollution and Diabetes Mellitus in Europe and North America: Systematic Review and Meta-Analysis. Environmental Health Perspectives, 2015. 123(5): p. 381-389.

7. Li, X., et al., Probiotics Ameliorate Colon Epithelial Injury Induced by Ambient Ultrafine Particles Exposure. Adv Sci (Weinh), 2019. 6(18): p. 1900972.

8. Li, D., et al., Multiple organ injury in male C57BL/6J mice exposed to ambient particulate matter in a real-ambient PM exposure system in Shijiazhuang, China. Environ Pollut, 2019. 248: p. 874-887.

9. Cohen, A.J., et al., Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. Lancet, 2017. 389(10082): p. 1907-1918.

10. Whyand, T., et al., Pollution and respiratory disease: can diet or supplements help? A review. Respiratory Research, 2018. 19.

11. Hennig, B., et al., Using nutrition for intervention and prevention against environmental chemical toxicity and associated diseases. Environmental Health Perspectives, 2007. 115(4): p. 493-495.

12. Kastorini, C.M. and D.B. Panagiotakos, Dietary patterns and prevention of type 2 diabetes: from research to clinical practice; a systematic review. Curr Diabetes Rev, 2009. 5(4): p. 221-7.

13. Hennig, B., et al., Nutrition Can Modulate the Toxicity of Environmental Pollutants: Implications in Risk Assessment and Human Health. Environmental Health Perspectives, 2012. 120(6): p. 771-774.
14. Petriello, M.C., et al., *Modulation of persistent organic pollutant toxicity through nutritional intervention: Emerging opportunities in biomedicine and environmental remediation*. Science of the Total Environment, 2014. **491**: p. 11-16.

15. Holtcamp, W., *Obesogens: an environmental link to obesity*. Environ Health Perspect, 2012. **120**(2): p. a62-8.

16. Duan, Y.H., et al., *Inflammatory Links Between High Fat Diets and Diseases*. Frontiers in Immunology, 2018. **9**.

17. Sung, J., C.T. Ho, and Y. Wang, *Preventive mechanism of bioactive dietary foods on obesity-related inflammation and diseases*. Food Funct, 2018. **9**(12): p. 6081-6095.

18. Golbidi, S., et al., *Health Benefits of Fasting and Caloric Restriction*. Curr Diab Rep, 2017. **17**(12): p. 123.

19. Bordone, L. and L. Guarente, *Calorie restriction, SIRT1 and metabolism: understanding longevity*. Nat Rev Mol Cell Biol, 2005. **6**(4): p. 298-305.

20. Fontana, L. and L. Partridge, *Promoting Health and Longevity through Diet: From Model Organisms to Humans*. Cell, 2015. **161**(1): p. 106-118.

21. Heilbronn, L.K., et al., *Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial*. JAMA, 2006. **295**(13): p. 1539-48.

22. Madeo, F., et al., *Caloric Restriction Mimetics against Age-Associated Disease: Targets, Mechanisms, and Therapeutic Potential*. Cell Metab, 2019. **29**(3): p. 592-610.

23. Walsh, M.E., Y. Shi, and H. Van Remmen, *The effects of dietary restriction on oxidative stress in rodents*. Free Radic Biol Med, 2014. **66**: p. 88-99.

24. Tang, D.Z., et al., *Dietary restriction improves repopulation but impairs lymphoid differentiation capacity of hematopoietic stem cells in early aging*. Journal of Experimental Medicine, 2016. **213**(4): p. 535-553.
25. Lu, Y.Y., et al., *The signaling pathways that mediate the anti-cancer effects of caloric restriction*. Pharmacological Research, 2019. **141**: p. 512-520.

26. Lopez-Otin, C., et al., *Metabolic Control of Longevity*. Cell, 2016. **166**(4): p. 802-821.

27. Kopeina, G.S., V.V. Senichkin, and B. Zhivotovsky, *Caloric restriction - A promising anti-cancer approach: From molecular mechanisms to clinical trials*. Biochim Biophys Acta Rev Cancer, 2017. **1867**(1): p. 29-41.

28. Le Noci, V., et al., *Local Administration of Caloric Restriction Mimetics to Promote the Immune Control of Lung Metastases*. J Immunol Res, 2019. **2019**: p. 2015892.

29. Fu, Z.D. and C.D. Klaassen, *Short-term calorie restriction feminizes the mRNA profiles of drug metabolizing enzymes and transporters in livers of mice*. Toxicol Appl Pharmacol, 2014. **274**(1): p. 137-46.

30. Chou, M.W., et al., *Effect of caloric restriction on the metabolic activation of xenobiotics*. Mutat Res, 1993. **295**(4-6): p. 223-35.

31. Qin, L.Q., et al., *One-day dietary restriction changes hepatic metabolism and potentiates the hepatotoxicity of carbon tetrachloride and chloroform in rats*. Tohoku J Exp Med, 2007. **212**(4): p. 379-87.

32. Chen, W., et al., *Effect of dietary restriction on glutathione S-transferase activity specific toward aflatoxin B1-8,9-epoxide*. Toxicol Lett, 1995. **78**(3): p. 235-43.

33. Mattison, J.A., et al., *Caloric restriction improves health and survival of rhesus monkeys*. Nat Commun, 2017. **8**: p. 14063.

34. Kang, H.J., et al., *Bioactive food components prevent carcinogenic stress via Nrf2 activation in BRCA1 deficient breast epithelial cells*. Toxicol Lett, 2012. **209**(2): p. 154-60.

35. Ramadass, P., et al., *Dietary flavonoids modulate PCB-induced oxidative stress, CYP1A1 induction, and AhR-DNA binding activity in vascular endothelial cells*. 
36. Janesick, A. and B. Blumberg, *Endocrine disrupting chemicals and the developmental programming of adipogenesis and obesity*. Birth Defects Res C Embryo Today, 2011. 93(1): p. 34-50.

37. Roehrs, M., et al., *Annatto carotenoids attenuate oxidative stress and inflammatory response after high-calorie meal in healthy subjects*. Food Res Int, 2017. 100(Pt 1): p. 771-779.

38. Gotz, A.A., et al., *Comparison of particle-exposure triggered pulmonary and systemic inflammation in mice fed with three different diets*. Part Fibre Toxicol, 2011. 8: p. 30.

39. Kim, J.S., et al., *Associations of air pollution, obesity and cardiometabolic health in young adults: The Meta-AIR study*. Environ Int, 2019. 133(Pt A): p. 105180.

40. Anderson, R.M., et al., *Nicotinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae*. Nature, 2003. 423(6936): p. 181-5.

41. Matt, K., et al., *Influence of calorie reduction on DNA repair capacity of human peripheral blood mononuclear cells*. Mech Ageing Dev, 2016. 154: p. 24-9.

42. Barja, G., *Aging in vertebrates, and the effect of caloric restriction: a mitochondrial free radical production-DNA damage mechanism?* Biol Rev Camb Philos Soc, 2004. 79(2): p. 235-51.

43. Pietrocola, F. and G. Kroemer, *Caloric restriction promotes the stemness and antitumor activity of T lymphocytes*. Oncoimmunology, 2019. 8(10): p. e1616153.

44. Tang, D., et al., *Dietary restriction improves repopulation but impairs lymphoid differentiation capacity of hematopoietic stem cells in early aging*. J Exp Med, 2016. 213(4): p. 535-53.

45. Vermeij, W.P., et al., *Restricted diet delays accelerated ageing and genomic stress in DNA-repair-deficient mice*. Nature, 2016. 537(7620): p. 427-431.
46. Ma, D., et al., *Upregulation of the ALDOA/DNA-PK/p53 pathway by dietary restriction suppresses tumor growth*. Oncogene, 2018. **37**(8): p. 1041-1048.

47. Cretu, A., et al., *Stress sensor Gadd45 genes as therapeutic targets in cancer*. Cancer Ther, 2009. **7**(A): p. 268-276.

48. Niehrs, C. and A. Schafer, *Active DNA demethylation by Gadd45 and DNA repair*. Trends Cell Biol, 2012. **22**(4): p. 220-7.

49. Zhang, N., et al., *Calorie restriction-induced SIRT6 activation delays aging by suppressing NF-kappaB signaling*. Cell Cycle, 2016. **15**(7): p. 1009-18.

50. Makwana, K., et al., *Aging and calorie restriction regulate the expression of miR-125a-5p and its target genes Stat3, Casp2 and Stard13*. Aging (Albany NY), 2017. **9**(7): p. 1825-1843.

51. Noyan, H., et al., *Cardioprotective Signature of Short-Term Caloric Restriction*. PLoS One, 2015. **10**(6): p. e0130658.

52. Aksungar, F.B., A.E. Topkaya, and M. Akyildiz, *Interleukin-6, C-reactive protein and biochemical parameters during prolonged intermittent fasting*. Ann Nutr Metab, 2007. **51**(1): p. 88-95.

53. Faris, M.A., et al., *Intermittent fasting during Ramadan attenuates proinflammatory cytokines and immune cells in healthy subjects*. Nutr Res, 2012. **32**(12): p. 947-55.

54. Most, J., et al., *Significant improvement in cardiometabolic health in healthy nonobese individuals during caloric restriction-induced weight loss and weight loss maintenance*. Am J Physiol Endocrinol Metab, 2018. **314**(4): p. E396-E405.

55. Jordan, S., et al., *Dietary Intake Regulates the Circulating Inflammatory Monocyte Pool*. Cell, 2019. **178**(5): p. 1102-1114 e17.

56. Fabbiano, S., et al., *Caloric Restriction Leads to Browning of White Adipose Tissue through Type 2 Immune Signaling*. Cell Metabolism, 2016. **24**(3): p. 434-446.
57. Antunes, M.M., et al., Adipose tissue is less responsive to food restriction anti-inflammatory effects than liver, muscle, and brain in mice. Brazilian Journal of Medical and Biological Research, 2019. 52(1).

58. Younas, H., et al., Caloric restriction prevents the development of airway hyperresponsiveness in mice on a high fat diet. Sci Rep, 2019. 9(1): p. 279.

59. Feng, S., et al., The health effects of ambient PM2.5 and potential mechanisms. Ecotoxicol Environ Saf, 2016. 128: p. 67-74.

60. Xu, C., C.Y. Li, and A.N. Kong, Induction of phase I, II and III drug metabolism/transport by xenobiotics. Arch Pharm Res, 2005. 28(3): p. 249-68.

61. Murray, M., Altered CYP expression and function in response to dietary factors: potential roles in disease pathogenesis. Curr Drug Metab, 2006. 7(1): p. 67-81.

62. Corton, J.C. and H.M. Brown-Borg, Peroxisome proliferator-activated receptor gamma coactivator 1 in caloric restriction and other models of longevity. J Gerontol A Biol Sci Med Sci, 2005. 60(12): p. 1494-509.

63. Chen, W., et al., Effect of dietary restriction on benzo[a]pyrene (BaP) metabolic activation and pulmonary BaP-DNA adduct formation in mouse. Drug Chem Toxicol, 1996. 19(1-2): p. 21-39.

64. Diaz-Ruiz, A., et al., Benefits of Caloric Restriction in Longevity and Chemical-Induced Tumorigenesis Are Transmitted Independent of NQO1. J Gerontol A Biol Sci Med Sci, 2019. 74(2): p. 155-162.

65. Matute-Bello, G., et al., An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Biol, 2011. 44(5): p. 725-38.

Figures
Characteristics of mice fed with CR diet. Prior to PM exposure, we fed the mice with AL and CR diet for 3 weeks and maintained the dietary pattern throughout a 4-week period. (A) Body weight change relative to time. The average body weight gain (B), the subcutaneous fat coefficient (C), the visceral fat coefficient (D), the quadriceps coefficient (E), and the liver ATP content (F) in the course of the experiment in AL-fed and CR-fed mice with or without PM exposure. The data are expressed as the mean ± SD. &P < 0.05 CR-FA vs. AL-FA, #P < 0.05 CR-PM vs. AL-PM.
Figure 2
CR protects against PM-induced pulmonary injury. Al-fed and CR-fed mice were exposed to PM for four weeks. (A) Representative images of H&E staining (magnification, 200×) and TUNEL staining of lung tissues (magnification, 400×). The typical pathological changes, including neutrophil infiltration (★), alveolar septal thickening (◆), alveolar hemorrhage (▲), were indicated. The lung injury scores (B), the number of tunnel positive cells (C) in mouse lung tissues. The
total cell number (D), the lactate dehydrogenase (LDH) (E), the total protein (TP) (F), albumin (ALB) (G) in mouse bronchoalveolar lavage fluid (BALF). (H-I)

Immunoblotting analysis shows the levels of cleaved caspase-3 expression in the lysates of mouse lung tissues. The data are expressed as the mean ± SD. *P < 0.05 (AF vs. PM), &P < 0.05 (CR-AF vs. AL-AF), #P < 0.05 (CR-PM vs. AL-PM).
Figure 3

CR led to attenuation of PM-induced oxidative stress, DNA damage and inflammatory response in mouse lung. (A) Representative images and quantitation of DHE (magnification, 200×), γH2AX, F4/80 staining (magnification, 400×). The contents of MDA (B) and GSH (C) in mouse lung tissue and BALF. The number of macrophages (D) and polymorphonuclear neutrophils (PMN) (E) in BALF. (F) The M1 and M2 macrophage polarization of BALF cells. (G) The heatmap shows the levels of cytokines in BALF, including IFNγ, TNFα, IL-6, IL1-β, IL12p70, IL-4, IL-10, and TGF-β1. The data are expressed as the mean ± SD, *P < 0.05 (AF vs. PM), &P < 0.05 (CR-AF vs. AL-AF), #P < 0.05 (CR-PM vs. AL-PM).
Signature of gene expression and functional alternations altered by CR in mouse lung tissues. Ingenuity Pathway Analysis (IPA) reveals differentially expressed genes (DEGs) in mouse lung tissues of AL-fed and CR-fed mice with or without PM exposure. The comparison of the categories of disease and toxicological effects involved in organ injury and abnormalities, respiratory disease, inflammation, cell death and survival between CR-AF and AL-AF mice (A) and between CR-PM and AL-PM mice (B). The comparison of the key canonical pathways involved in inflammatory response, oxidative stress, DNA damage, and xenobiotic metabolism between CR-AF and AL-AF (C) and between CR-PM and AL-PM mice (D).
Effects of CR on PM-induced systemic toxic effects. The cell counts of white blood cells (WBC) (A), lymphocytes (B), monocytes (C), and neutrophils (D) in mouse peripheral blood. The contents of MDA (E) and GSH (F) in mouse plasma. (G) The concentration of 8-OHdG in mouse urine. (H) The olive tail moment of peripheral blood cells (Comet assay). (I) The heatmap shows the levels of cytokines in mouse plasma, including IFNγ, TNFα, IL-6, IL1-β, IL12p70, IL-4, IL-10, and TGF-β1. (J) Cytotoxicity of mouse plasma on different human cell lines, including Neuro-2A, HBE, THP1, HepG2HEK, and HCT116 were determined in ex vivo experiment. The data are expressed as the mean ± SD, *P < 0.05 (AF vs. PM), &P < 0.05 (CR-AF vs. AL-AF), #P < 0.05 (CR-PM vs. AL-PM).
Figure 6

Perturbation of canonical pathways were reversed by CR in mouse livers.
Comparison of the 20 most significant canonical pathways identified by IPA analysis between CR-AF and AL-AF mice (A) and between CR-PM and AL-PM mice (B). Molecular pathways involved in xenobiotic metabolism include AhR signaling (C), NRF2-mediated oxidative stress response (D), RXR activation (E-F), and glutathione-mediated detoxification (G). Red circles indicate an increased expression, and green colors indicate a decreased expression in CR-fed mice with or without PM exposure.
CR enhances the capacity of xenobiotic metabolism and detoxification of PM-bound PAHs. (A) The induction of CYP1A1, CYP1A2, CYP1B1, GSTT1, GSTM1, and UGT1A1 in mouse liver tissues. The quantitative analysis of CYP1A1 (B), CYP1A2
(C), CYP1B1 (D), GSTT1 (E), GSTM1 (F), and UGT1A1(G) in AL-fed and CR-fed mice with or without PM exposure. The concentration of urinary hydroxylated metabolites of PAHs (OH-PAHs), 1-OHNap (H), 2-OHNap (I), 1-OHPhe (J), 4-OHPhe (K), 9-OHPhe (L), 2-OHFlu (M), and 1-OHPyr (N). The data are expressed as the mean ± SD, *P < 0.05 (AF vs. PM), &P < 0.05 (CR-AF vs. AL-AF), #P < 0.05 (CR-PM vs. AL-PM).

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

Supplementary materials (final).docx