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Combined Effects of Ambient $PM_{2.5}$ and Cold Exposure on the Development of Metabolic Disorder

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Abstract: The coexistence of temperature changes and air pollution poses a severe global environmental issue, exacerbating health burdens. The aim of this study was to clarify the combined effects of ambient $PM_{2.5}$ and cold exposure on the development of metabolic disorders. Male C57BL/6 mice were randomly divided into four groups: T_N-FA, T_N-PM, T_C-FA and T_C-PM. The mice were then exposed to concentrated $PM_{2.5}$ or filtered air (FA) under normal (22 °C) or cold (4 °C) environment conditions for 4 weeks. Metabolic-disorder-related indicators, blood pressure, serous lipids, fasting blood glucose and insulin, energy metabolism, mitochondria and protein expression in tissues were detected for comprehensively assessing metabolic disorder. The results showed that, compared to being exposed to $PM_{2.5}$ only, when mice were exposed to both $PM_{2.5}$ and the cold (non-optimal), they exhibited more significant metabolic disorders regarding glucose tolerance ($p < 0.05$), insulin resistance ($p < 0.05$), lipid metabolism, adipocytes ($p < 0.01$) and mitochondrial function. This study suggested that a cold environment might substantially exacerbate $PM_{2.5}$-induced metabolic disorder. The interaction between temperature changes and air pollution implied that implementing the necessary environment-related policies is a critical and complex challenge.

Keywords: $PM_{2.5}$; cold; metabolic disorder; non-optimal temperature

Citation: Liu, Z.; Zhang, J.; Fan, D.; Wang, G.; Wu, B.; Lei, L.; Wang, L.; Zhao, J.; Chen, J. Combined Effects of Ambient $PM_{2.5}$ and Cold Exposure on the Development of Metabolic Disorder. *Atmosphere* **2023**, *14*, 1157. https://doi.org/10.3390/atmos14071157

Academic Editor: Iva Hůnová

Received: 30 May 2023
Revised: 14 July 2023
Accepted: 14 July 2023
Published: 17 July 2023

1. Introduction

The acceleration of modernization has allowed for more convenience and comfort within life experiences but is also accompanied by serious ecological environmental pollution and temperature changes. Severe environmental issues, such as the greenhouse effect, and heat waves and cold waves occur frequently, raising alarms for human beings. The World Health Organization (WHO) has identified air pollution as the single largest environmental health risk factor [1]. Ambient particulate matter (PM) with an aerodynamic diameter of $\leq$2.5 μm (fine particulate matter, $PM_{2.5}$) is known to significantly contribute to many health problems. The Global Burden of Disease (GBD) Study (2019) has shown that PM is still one of the top ten contributing factors to the global burden of diseases [2]. Meanwhile, temperature changes are associated with a series of health problems such as affecting the risk of morbidity and mortality of cardiovascular diseases and infant health [3,4]. Non-optimal temperature has been found to be the main risk factor in GBD (2019), and low temperature accounted for 0.946 million deaths in females [2]. In a realistic context, heavy $PM_{2.5}$ pollution is often accompanied by extreme temperature changes. Cold waves especially occur alongside high $PM_{2.5}$ pollution in winter because of the heating supplies.

Therefore, the coexistence of temperature changes and air pollution not only exacerbates the health burden but also reinforces higher health-protection-related requirements.

Noncommunicable diseases are still the largest and fastest-growing global health burden, even more strongly linked to air pollution and temperature changes. In most noncommunicable diseases, metabolic syndrome is a generic term for the risk factors of type 2 diabetes mellitus (T2DM) and cardiovascular diseases [5]. In the survey analyzing the prevalence of metabolic syndrome in housewives, it has been suggested that household $PM_{2.5}$ is closely related to the prevalence of metabolic syndrome in females [6]. Animal experiments have found that long-term exposure to $PM_{2.5}$ impaired glucose tolerance, induced insulin resistance, decreased energy expenditure and induced the development of liver inflammation accompanied by elevated triglycerides [7–9]. Transcriptome analysis of cold-stimulated mice also found that up-regulated genes were enriched in lipid metabolism, fatty acid metabolism, lipid oxidation and fatty acid oxidation [10–12]. However, in most $PM_{2.5}$-related epidemiological studies, temperature is always controlled as a confounding factor in statistical analysis [13,14], and vice versa. Consequently, there are few studies elucidating the combined effects of $PM_{2.5}$ and cold, excluding some epidemiological investigations focusing on premature mortality [15–18], and hospital admissions for cardiovascular disease or respiratory outcomes [19]. Similarly, there are few corresponding animal studies linking air pollution and cold exposure, and metabolic disorders. Although some animal studies have reported that $PM_{2.5}$ or cold exposure alone was associated with metabolic disorders, the effects and potential mechanisms induced by the combination of the two are still unclear. At the same time, few studies have described metabolic disorders (blood pressure, energy homeostasis, glucose tolerance, insulin resistance, lipids, adipocytokines, inflammation, mitochondria and energy-related protein expression) induced by air pollution and temperature changes comprehensively. Instead, most only observed morbidity, mortality, hospital admissions, insulin, glucose or blood pressure. As such, this study intended to mimic the general population exposed to ambient $PM_{2.5}$ and cold simultaneously through the use of healthy C57BL/6 mice, and explore the combined effects and potential mechanism of these factors on metabolic disorders by measuring various relevant indicators.

2. Materials and Methods

2.1. Animals and Exposure Design

Six-week-old male C57BL/6 mice were purchased from the Experimental Animal Center of the College of Medicine, Fudan University (Shanghai, China). After one-week acclimation at 22 ± 2 °C the mice were randomly divided into four groups, with 10 in each group: T_N-FA, T_N-PM, T_C-FA and T_C-PM. In these groups, T_N and T_C proceeded at a normal (optimal) temperature (22 °C) and cold (non-optimal temperature, 4 °C), respectively. Under the different (T_N and T_C) temperatures, the mice were separately exposed to concentrated $PM_{2.5}$ (PM) or filtered air (FA). The PM and FA exposure were performed using the "Shanghai Meteorological and Environmental Animal Exposure System (Shanghai-METAS)", which can concentrate or filter outdoor $PM_{2.5}$. Then, the concentrated $PM_{2.5}$ or FA entered the chambers, in which the temperature was simultaneously adjusted to T_N or T_C, according to the study design (Figure 1a). We performed several studies linking $PM_{2.5}$ and metabolic disorder/atherosclerosis using Shanghai-METAS [20–22]. In detail, the mice were treated with $PM_{2.5}$/FA under normal (optimal) or cold (optimal) temperature conditions, using METAS for 8 h/day, for a total of 4 weeks. The mice were provided with free access to water and food during the exposure. All of the experiments were approved by the Animal Care and Use Committee of Fudan University and followed appropriate protocols.

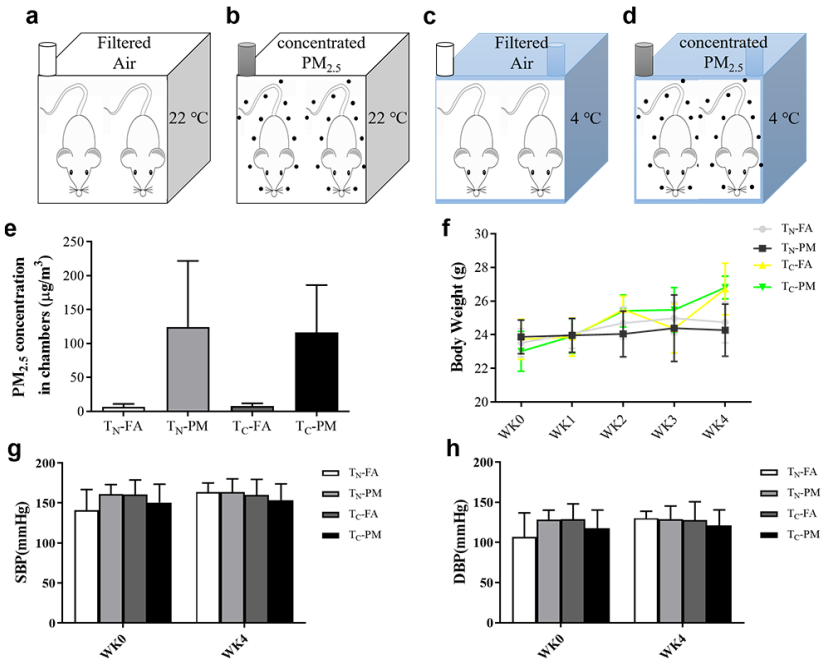

Figure 1. Exposure schematic (**a**–**d**), $PM_{2.5}$ concentrations (**e**), body weight (**f**), and blood pressure of different groups during exposure (**g**,**h**).

2.2. Blood Pressure Measurement

At the beginning and the end of the entire exposure, the blood pressure of the mice was measured using the non-invasive blood pressure acquisition system (CODA, Kent, WA, USA). Before testing, the mice were placed on a heated animal stand by an animal binder for 10 min. Then, a volume pressure recording (VPR) sensor and an O-cuff kit were placed on the tail to measure the systolic blood pressure (SBP) and diastolic blood pressure (DBP). The measurement interval was set to 10 s and every was mouse tested 10 consecutive times.

2.3. Metabolic Cage

At the end of exposure, the mice were placed in metabolic cages (Shanghai Research Center of the Southern model organisms) for 24 h to determine the respiratory exchange ratio (RER), carbon dioxide production (VCO_2), oxygen consumption (VO_2) and heat production in a resting state, at 22 °C. Water and food were freely available. Data were normalized to the body weight.

2.4. Blood Glucose Tolerance and Insulin Sensitivity Measurement

At the end of the 4-week exposure, the mice were fasted for 12 h for intraperitoneal glucose tolerance testing (IPGTT). Blood samples were collected from the vena caudalis to measure the level of blood glucose at 0, 30, 60, 90 and 120 min after the dextrose (2 mg/g body weight) injection, respectively.

For the insulin tolerance test (ITT), insulin (0.5 U/kg) was administered via an intraperitoneal injection after 4.5 h of fasting. The homeostasis model assessment (HOMA-IR) was calculated according to the following equation: [fasting insulin concentration (mU/L) × fasting glucose concentration (mmol/L)]/22.5.

2.5. Blood Biomarkers

After the mice were sacrificed, the blood samples were collected and centrifuged at 1500 rpm for 20 min. The levels of insulin, interleukin (IL)-6, tumor necrosis factor (TNF)-α, leptin and adiponectin were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Biosource International, Camarillo, CA, USA).

2.6. Serous Lipid Determination

Blood samples were also collected for analyzing serous triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) (NanJing KeyGen Biotech. Inc., Nanjing, Jiangsu, China).

2.7. Histological Analysis and Transmission Electron Microscopy Observation

The inguinal white adipose tissue (WAT) and brown adipose tissue (BAT) were harvested. Part of the WAT and BAT were fixed with 10% neutral buffered formalin for histological morphology analysis (H&E staining). The ultra-structure of BAT was examined via transmission electron microscopy (CM120, Philips, Amsterdam, Holland) to investigate mitochondrial changes. The total mitochondria in 10 micrographs per group were counted, and mitochondrial size and number were analyzed using ImageJ software.

2.8. Western Blot Analysis

The WAT and BAT of mice were homogenized in the lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA) with protease and phosphatase inhibitors (Kangchen Biotech, Beijing, China). The same amounts of proteins were separated by a sodium dodecyl sulfate-polyacrylamide gel, then transferred to PVDF membranes (Bio-Rad, Hercules, CA, USA). The membranes were incubated with primary antibody UCP-1 and HSP90 (Cell Signaling Technology, Boston, MA, USA) overnight at 4 °C, respectively. After being washed, the membranes were incubated with HRP-conjugated secondary antibody (Cell Signaling Technology, Boston, MA, USA). Last, the membranes were detected with enhanced chemiluminescence followed by exposure to X-ray film. The density of the protein bands was calculated using ImageQuant TL8.1 software.

2.9. Statistical Analysis

The results were expressed as the mean ± standard deviation (SD). The data were statistically analyzed using IBM SPSS Statistics 22.0. The normality of each group was tested using the Shapiro–Wilk method and the homogeneity of variance was tested using the Levene method. Pairwise comparisons were performed using the least significance difference (LSD) test. The differences between FA and PM, as well as normal temperature and cold, were analyzed through *t*-tests, and a p-value < 0.05 was considered statistically significant.

3. Results

3.1. $PM_{2.5}$ Concentrations and Body Weight of the Mice

The $PM_{2.5}$ concentrations in PM and FA chambers during exposure are shown in Figure 1e. The average concentrations of $PM_{2.5}$ in the T_N-FA, T_N-PM, T_C-FA and T_C-PM groups during the exposure were 9.28 ± 1.04, 155.47 ± 8.10, 9.50 ± 1.01 and 145.07 ± 8.95 μg/m^3, respectively.

As shown in Figure 1f, the average body weights of the mice in the T_N-FA, T_N-PM, T_C-FA and T_C-PM groups were 23.52 ± 0.84, 23.87 ±1.00, 23.72 ± 1.19 and 23.01 ± 1.18 g before exposure. The baseline body weights of the mice had no significant difference among the four groups. At the end of the 4-week exposure, the average body weights of the mice in the four groups were 24.73 ± 1.22, 24.27 ± 1.55, 26.72 ± 1.54 and 26.81 ± 0.68 g. The $PM_{2.5}$-exposed mice displayed a reduction in the body weight increment.

3.2. Combined Effects of $PM_{2.5}$ and Cold Exposure on Blood Pressure

The baseline, the SBP and DBP among the four groups showed no significant difference. Similarly, after the 4-week exposure, there were also no significant differences in the SBP and DBP between the FA and PM groups, as well as the T_N and T_C groups (Figure 1g,h).

3.3. Cold Exacerbates $PM_{2.5}$-Induced Glucose Tolerance and Insulin Resistance

In both the normal and cold environments, PM exposure significantly increased the level of fasting blood glucose when compared with FA ($p < 0.01$). Meanwhile, the fasting blood glucose in T_C-PM mice was higher than that in the T_N-PM mice (Figure 2a). There was no difference in the fasting insulin among the four groups (Figure 2b). The trends of the HOMA-IR index in these groups were similar to the trends of the fasting blood glucose. PM exposure increased HOMA-IR at normal and cold temperatures (Figure 2c).

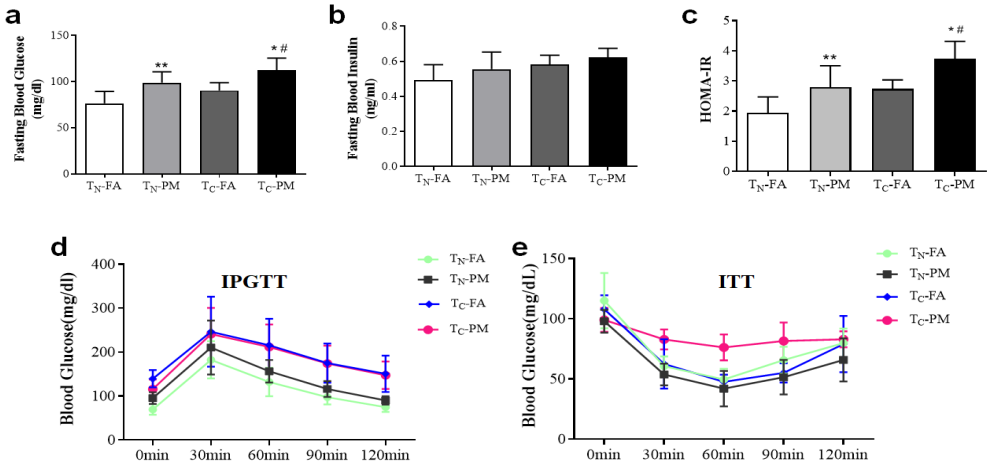

Figure 2. Glucose tolerance and insulin resistance in mice after combined exposure to $PM_{2.5}$ and the cold. (**a**,**b**) Fasting blood glucose and insulin of mice. (**c**) HOMA-IR. (**d**) IPGTT. (**e**) ITT. * $p < 0.05$, ** $p < 0.01$, FA vs. PM; # $p < 0.05$, T_C vs. T_N.

As shown in Figure 2d, in IPGTT, the trends of blood glucose at 30 min, from the highest to the lowest, were T_C-FA, T_C-PM, T_N-PM and T_N-FA mice after a glucose injection. Thirty minutes later, the blood glucose in each group showed a downward trend, but the blood glucose in the T_C-FA and T_C-PM groups remainder at a higher level until 120 min than that in the other groups. In ITT, the blood glucose reached the lowest value at 60 min in the mice after insulin injection (Figure 2e). The trends of blood glucose at 60 min, from the highest to the lowest, were T_C-PM, T_N-FA, T_C-FA and T_N-PM groups. After 60 min, the blood glucose was gradually restored.

3.4. Cold Exacerbates $PM_{2.5}$-Induced Energy Imbalance

As shown in Figure 3, cold exposure could induce an increase in VO_2, VCO_2, RER and heat production, regardless of whether the mice were exposed to $PM_{2.5}$ or not. At a normal temperature, PM exposure could only induce a significant change in heat production, but not the VO_2, VCO_2 and RER. However, at cold temperatures, PM exposure could significantly induce an increase in the VO_2, VCO_2 and RER, and decrease in heat production when compared with the FA exposure ($p < 0.05$). The results indicated that cold exposure absolutely influenced the effects of $PM_{2.5}$ on energy metabolism.

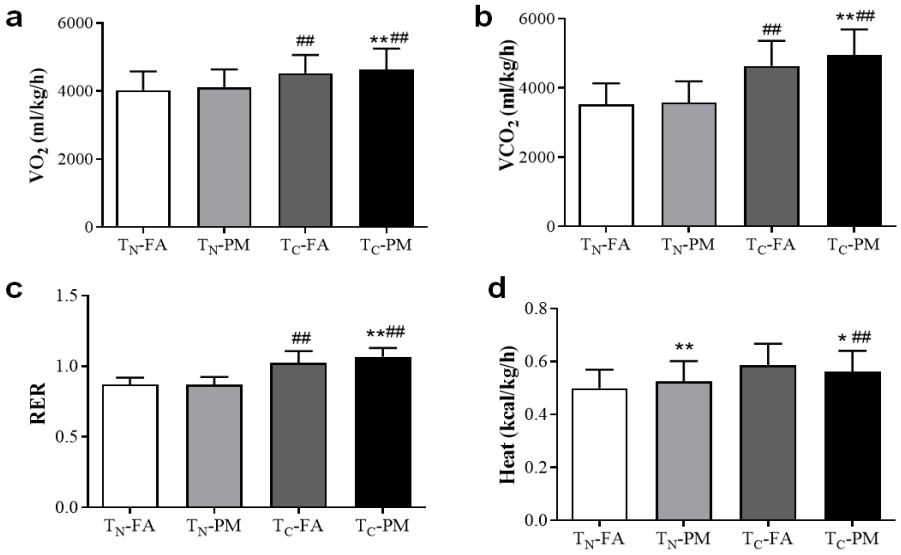

Figure 3. Energy metabolism of mice by metabolic cage. (**a**–**d**) VO_2, VCO_2, RER and heat production in mice after combined exposure to $PM_{2.5}$ and the cold. * $p < 0.05$, ** $p < 0.01$, FA vs. PM; ## $p < 0.01$, T_C vs. T_N.

3.5. Cold Exacerbates $PM_{2.5}$-Induced Pathological and Mitochondria Alteration

A histopathological evaluation of WAT and BAT in each group is shown in Figure 4a and b. Compared with T_N mice, T_C mice displayed severe adipose cell hypertrophy in WAT, regardless of whether the mice were exposed to concentrated $PM_{2.5}$ or not. Cold significantly exacerbated $PM_{2.5}$-induced white adipose cell hypertrophy (Figure 4a). There was no fat accumulation at cold temperatures when compared with normal temperatures, which even displayed smaller brown adipocytes (Figure 4b).

Figure 4c shows that $PM_{2.5}$ could induce a decrease in BAT weight when compared with FA at a normal temperature. Similarly, cold exposure could induce a decrease in BAT weight in both the $PM_{2.5}$ and FA groups. Figure 4d shows the number and morphology of mitochondria in BAT. Figure 4e shows that the mitochondrial area of BAT in PM groups was significantly smaller than that in FA under T_N or T_C conditions ($p < 0.01$). Meanwhile, compared with T_N groups, the area of BAT mitochondria in the T_C groups was significantly reduced ($p < 0.01$). Neither cold nor $PM_{2.5}$ could induce changes in the number of mitochondria (Figure 4f).

3.6. Cold Influences $PM_{2.5}$-Induced Abnormal Lipid Metabolism

The serum lipid profiles were detected to observe lipid metabolism. As shown in Figure 5, at a normal temperature, $PM_{2.5}$ exposure could induce an increase in the serous TC and LDL, but not in TG and HDL when compared with FA. Most notably, there were no differences in these lipid indicators between T_C-PM mice and T_C-FA mice.

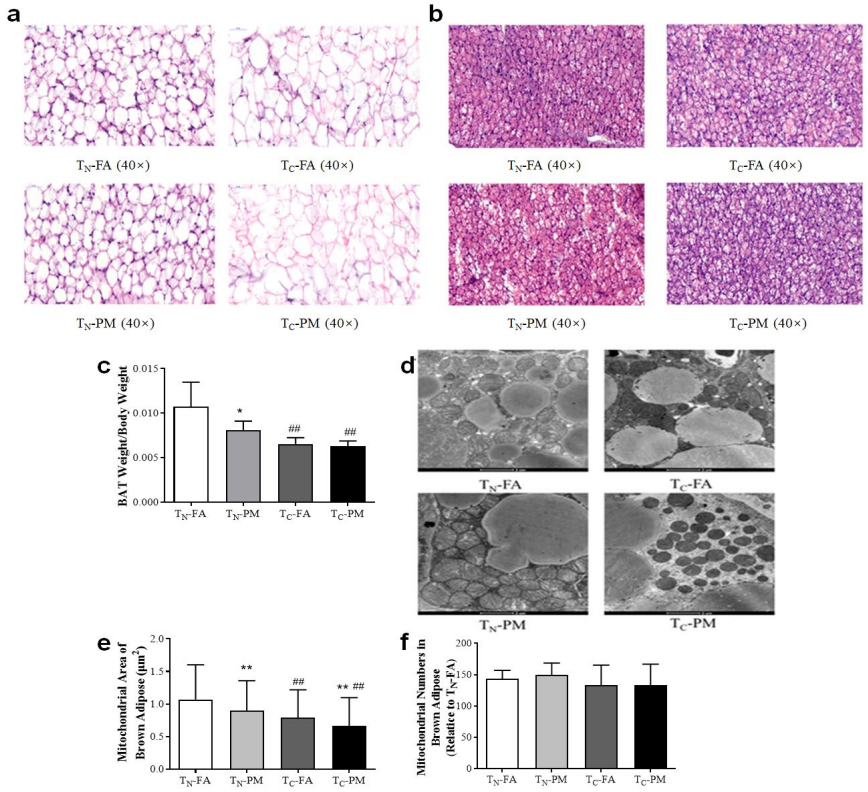

Figure 4. Representative images of the H&E staining of WAT (**a**) and BAT (**b**) in mice after combined exposure to $PM_{2.5}$ and the cold. Number and morphology of mitochondria in BAT of mice via transmission electron microscopy ((TEM, **c**–**f**). (**c**) The ratio of BAT weight to body weight in mice. (**d**) Morphology of mitochondria in BAT. (**e**) Area of mitochondria in BAT. (**f**) Number of mitochondria in BAT. * $p < 0.05$, ** $p < 0.01$, FA vs. PM; $^{\#\#}$ $p < 0.01$, T_C vs. T_N.

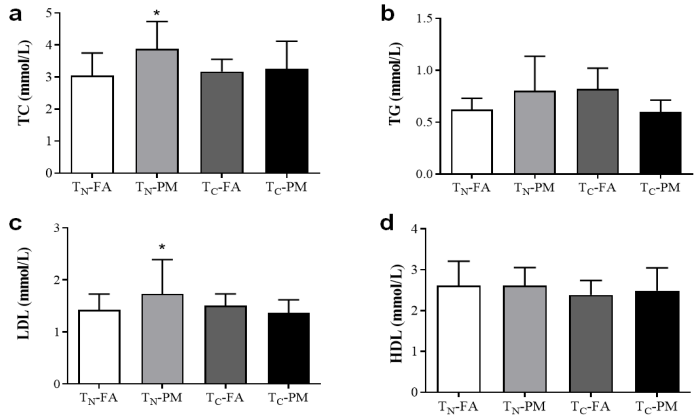

Figure 5. Blood lipids of mice. (**a**–**d**) TC, TG, LDL and HDL in the serum of mice after combined exposure to $PM_{2.5}$ and the cold. * $p < 0.05$, FA vs. PM.

3.7. Cold Exacerbates $PM_{2.5}$-Induced Inflammation and Energy Expenditure Inhibition

At a normal temperature, $PM_{2.5}$ exposure had no significant influence on serous TNF-α and IL-6 (Figure 6a,b). However, at a cold temperature, $PM_{2.5}$ exposure could induce an increase in the TNF-α when compared with FA. Therefore, there was a significant difference between T_C-FA and T_C-PM mice.

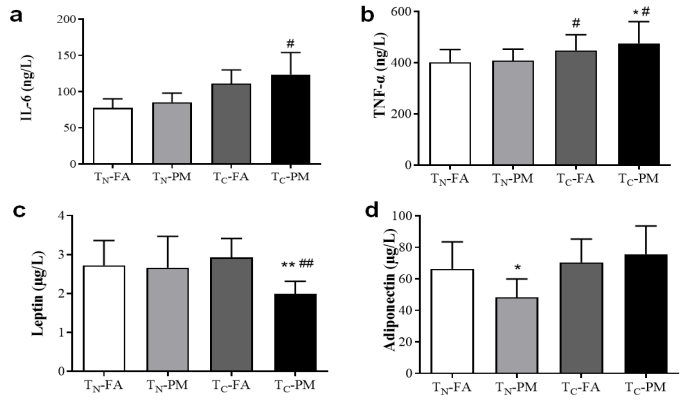

Figure 6. Inflammatory cytokines and adipocytokines in the serum of mice. (**a**–**d**) IL-6, TNF-α, leptin and adiponectin in the serum of mice. * $p < 0.05$, ** $p < 0.01$, FA vs. PM; $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, T_C vs. T_N.

$PM_{2.5}$ did not change the level of leptin at a normal temperature, but it induced a significant decrease in leptin at a cold temperature (Figure 6c). The results suggested that $PM_{2.5}$ markedly inhibited leptin-related energy consumption. $PM_{2.5}$ exposure reduced adiponectin at a normal temperature. However, at a cold temperature, $PM_{2.5}$ exposure had no significant influence on adiponectin (Figure 6d).

3.8. Combined Exposure to $PM_{2.5}$ and the Cold Inhibits Thermogenesis and Energy Expenditure

As shown in Figure 7a,b, compared with that in T_N-FA and T_C-FA, the expression of UCP-1 and HSP90 proteins in the WAT of mice in both the T_N-PM and T_C-PM groups was significantly decreased, indicating that $PM_{2.5}$ could induce a decrease in the UCP-1 and HSP90 proteins in mice. However, the expression of UCP-1 and HSP90 proteins was significantly increased in both T_C-FA and T_C-PM groups compared with the corresponding T_N-FA and T_N-PM, suggesting that cold exposure seemed to induce an increase in UCP-1 and HSP90 expression. The trends of UCP-1 and HSP90 protein expression in BAT were the same as those in WAT (Figure 7c,d).

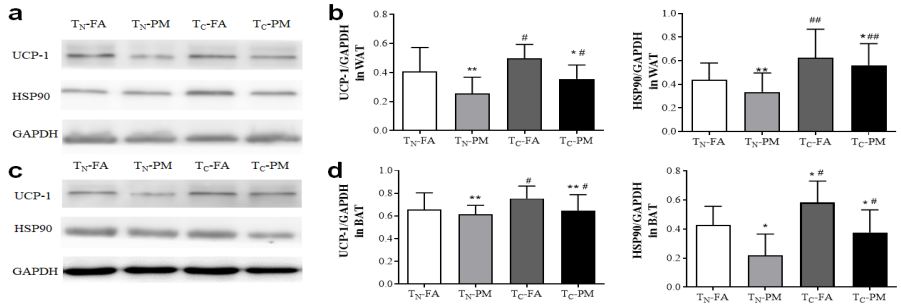

Figure 7. Protein expression of UCP-1 and HSP90 in the WAT (**a**,**b**) and BAT (**c**,**d**) of mice after combined exposure to $PM_{2.5}$ and the cold. * $p < 0.05$, ** $p < 0.01$, FA vs. PM; $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, T_C vs. T_N.

4. Discussion

Temperature changes, air pollution and noncommunicable diseases have become the most serious threats to global health [23,24]. As one of the risk factors for T2DM and cardiovascular diseases, metabolic disorder poses a huge threat to human health. A large number of epidemiological and experimental studies have found that ambient $PM_{2.5}$ exposure played an important role in the occurrence and development of diabetes [25–29]. Although previous studies have shown that both ambient $PM_{2.5}$ and temperature changes could affect blood glucose, blood lipids and blood pressure [30–32], there are few studies

observing the combined effects of air pollution and temperature changes on the development of metabolic disorder, excluding the studies related to asthma and atherosclerotic heart disease mortality [33–35]. Previous studies have reported that temperature changes could not only alter the dispersion of particulate matter but also intensify the formation of secondary pollutants [36]. In order to identify the combined effects of $PM_{2.5}$ and the cold on the development of metabolic disorders, we performed the current animal study. To the best of our knowledge, this study is the first to provide direct evidence related to the combined exposure to $PM_{2.5}$ and the cold exacerbating metabolic disorder within the normal healthy population.

In this study, the average concentration of $PM_{2.5}$ in PM-exposed groups was about 150 $\mu g/m^3$, which was much higher than the upper limit stipulated by the Chinese Air Quality Standard (AQS, GB3095-2012). In addition, the exposure concentration of $PM_{2.5}$ in this study represents the common weather condition in autumn and winter, especially in northern Chinese cities such as Beijing [37,38], which is 10 times the WHO 24 average recommended level. In recent years, our repeated determination of $PM_{2.5}$ components in exposure and control chambers of METAS indicated that the types of chemical components have not changed regardless of the concentration [20–22], and traffic exhausts and industrial emissions are the predominant sources of ambient PM around the exposure system [39]. Therefore, the $PM_{2.5}$ concentration in this study could represent the air pollution condition in most cities in China. In addition, in terms of the temperature setting, this study chose 22 °C and 4 °C to represent the normal temperature and the cold, and there were fewer temperature points. In the future, temperature points can be added to explore the further impact of temperature change curves.

The duration of the current study was 4 weeks, which made for a short-term study design. This study comprehensively detected metabolic-disorder-related indicators, including blood pressure, insulin resistance (fasting insulin, HOMA-IR, ITT), glucose tolerance (fasting blood glucose, IPGTT), energy consumption (VO_2, VCO_2, RER, and heat production), serous lipids profile (TC, TG, LDL and HDL), inflammatory response (IL-6 and TNF-α), leptin, adiponectin and mitochondria function (number and area of mitochondria in BAT), in order to observe the combined effects of $PM_{2.5}$ and cold exposure on the development of metabolic disorders. In the future, we will also conduct long-term exposure studies in order to obtain more comprehensive and reliable conclusions.

4.1. Cold Exacerbates $PM_{2.5}$-Induced Glucose Metabolism Disorders

The present study found that PM and cold exposure had no significant effects on SBP and DBP, which might be associated with the fact that short-term exposure cannot influence blood pressure homeostasis. It was also found that $PM_{2.5}$ could induce an increase in fasting blood glucose under both normal and cold temperatures. The fasting blood glucose in T_C-PM mice was higher than that in the T_N-PM mice, suggesting that the effect of $PM_{2.5}$ on glucose metabolism was exacerbated by cold exposure. Insulin resistance was an important criterion for diagnosing MetS and T2DM. Blood glucose tolerance and insulin sensitivity were often used to determine whether insulin resistance was occurring or not [27,40,41]. The maintenance of blood glucose homeostasis in vivo mainly depends on the insulin reactivity in target tissues. In this study, T_N-PM mice displayed an increase in the HOMA-IR in comparison with T_N-FA mice, indicating the occurrence of insulin resistance. Meanwhile, cold exposure could induce an increase in the HOMA-IR in PM-exposed mice, but not in FA-exposed mice when compared with those at a normal temperature, suggesting that cold exposure might exacerbate the PM-induced insulin resistance. Similarly, IPGTT and ITT showed that $PM_{2.5}$ along with cold exposure could induce serious glucose tolerance and insulin resistance when compared with $PM_{2.5}$ or cold exposure alone, which further confirmed that the cold exacerbates the effects of $PM_{2.5}$.

In the metabolic cage experiment, $PM_{2.5}$ increased heat production under a normal temperature, which was contrary to the long-term $PM_{2.5}$-induced decrease in heat production in our previous study [22]. The reason might be associated with the difference

between long-term and short-term exposure [42,43], and the potential mechanism should be explored in our future study. Importantly, T_C-PM exposure could induce a higher VCO_2 and RER in comparison with T_C-FA, suggesting that there was a higher energy metabolism in PM-exposed mice under cold environment conditions. The higher energy metabolism was consistent with the higher body weight and fasting blood glucose in PM-exposed mice.

4.2. Cold Exacerbates $PM_{2.5}$-Induced Lipid Metabolism Disorders

Circulatory lipids are closely related to the risk of cardiovascular disease. Studies had found that a decrease in LDL and an increase in HDL were associated with a reduction in the incidence of myocardial infarction, unstable angina and sudden cardiac death as early as 1996 [44,45]. Exposure to ultrafine particles could inhibit the protective effects of HDL [46]. A retrospective cohort study also found that intermediate-term exposure to PM10 resulted in an increase in LDL and TG and a decrease in HDL [47]. The present study found that $PM_{2.5}$ exposure could induce an increase in TC under normal temperature conditions when compared with FA exposure, but not in the cold-exposed groups. Cold exposure could induce a lower TC and LDL when compared with that within a normal environment, which might be associated with shivering thermogenesis, increased activity, promoted function of BAT and enhanced plasma metabolism under short-term cold exposure [48,49]. The results might be explained by the function of BAT in regulating triglycerides [11,50]. Interestingly, $PM_{2.5}$ could induce an increase in TC and LDL within a normal environment when compared with FA. Similarly, previous studies, including ours, indicated that $PM_{2.5}$ exposure increased TG and LDL in both ApoE-/- mice [51] and healthy C57BL/6 mice [22]. Curiously, there was no difference in TC and LDL between the $PM_{2.5}$ and FA groups under cold environment conditions, suggesting that cold exposure substantially influenced the effects of $PM_{2.5}$.

In this study, there was no significant difference in serous IL-6 and TNF-α between PM and FA mice under normal temperature conditions. However, at a cold temperature, $PM_{2.5}$ could induce an increase in the TNF-α, suggesting that cold exposure definitely promoted $PM_{2.5}$-induced systemic inflammation. Adipose tissue can secrete fatty acids, leptin and adiponectin. In this study, $PM_{2.5}$ exposure could induce a decrease in adiponectin, but not leptin at a normal temperature. In contrast, at a cold temperature, $PM_{2.5}$ exposure could induce a decrease in leptin and had no significant effects on adiponectin. The results signified that $PM_{2.5}$ combined with cold exposure could induce a reduction in energy consumption and promotion of adipocyte synthesis. Adiponectin is an important thermogenic regulator, which is necessary to maintain the body temperature at low temperatures [52]. Thus, T_C mice secreted higher adiponectin than T_N mice. Adiponectin decreased in obese and T2D patients and the reduction in adiponectin was related to a decrease in insulin sensitivity and glucose tolerance [53]. In the current study, T_N-PM mice had a lower level of adiponectin compared with T_N-FA mice, which indicated that $PM_{2.5}$ might impair glucose metabolism and insulin sensitivity.

Mitochondria are important organelles in energy metabolism. In BAT, $PM_{2.5}$ exposure had no significant effect on mitochondrial quantity under both normal and cold temperature conditions. In the T_N and T_C groups, $PM_{2.5}$ significantly reduced the mitochondrial area. A previous study found that long-term $PM_{2.5}$ exposure decreased mitochondrial counts in visceral adipose and mitochondrial size in interscapular adipose depots [54], which was consistent with the decrease in these indicators in T_N-PM mice in our study. According to the metabolism of mice, VO_2, VCO_2, RER and heat production in T_C mice were significantly higher than those in T_N mice, and VO_2, VCO_2, RER and heat production in T_C-PM mice were significantly higher than those in T_C-FA mice. The results suggested that the changes in the area of mitochondria in BAT would be a compensatory change in order to maintain physical function when short-term-exposed to a combination of the cold and $PM_{2.5}$.

4.3. Cold Exacerbates $PM_{2.5}$-Induced Protein Metabolism Disorders

UCP-1 is a specific expression protein in BAT. Under cold conditions, it can uncouple mitochondrial oxidation and phosphorylation, metabolize fatty acids and generate energy, thereby maintaining the body temperature. A previous study found that cold exposure could induce the expression of BAT thermogenesis genes and increase the protein levels of UCP1 and PGC1α [49]. HSP90 is one of the proteins in the heat shock protein family, and its main function is to maintain the structure of intracellular proteins [55]. HSP90 plays a critical role in both the regulation of normal cellular homeostasis and the stress response [56,57]. Previous studies indicated that Hsp90 genes performed a vital role in cold acclimation [58,59] and ambient $PM_{2.5}$ exposure [60]. In the present study, the expression of UCP-1 and HSP90 was increased in both WAT and BAT during cold exposure when compared with the corresponding normal temperature. More importantly, $PM_{2.5}$ exposure could induce the down-regulation of UCP-1 and HSP90 expression, regardless of whether the mice were exposed to a normal or cold temperature. The UCP-1 alteration might in turn impair the BAT structure and function [61,62].

5. Conclusions

This study showed that the combined exposure to $PM_{2.5}$ and the cold might contribute to the development of metabolic disorders within the healthy population. During short-term exposure, cold exposure might exacerbate $PM_{2.5}$-related metabolic disorders, which are potentially associated with changes in energy homeostasis, glucose metabolism, insulin resistance and mitochondrial function. In the future, we will explore the mechanism of metabolic disorders induced by co-exposure to $PM_{2.5}$ and cold temperatures. The results demonstrated that the combined effects of $PM_{2.5}$ pollution and temperature changes could be a great threat to people's health. Due to the combined effects of air pollution and temperature changes, and the health issue of noncommunicable diseases, a study focusing on air pollution and temperature changes is critical. The government and health authorities have taken into account estimating both the temperature- and air-pollution-related health problems in order to set up an adequate policy to face the temperature and pollution challenges.

Author Contributions: Conceptualization, Z.L., J.Z. (Jia Zhang), J.Z. (Jinzhuo Zhao) and J.C.; investigation, Z.L., J.Z. (Jia Zhang), D.F., G.W., B.W., L.L. and L.W.; writing—original draft preparation, Z.L. and J.Z. (Jia Zhang); writing—review and editing, J.Z. (Jinzhuo Zhao) and J.C.; supervision, J.Z. (Jinzhuo Zhao) and J.C.; funding acquisition, J.Z. (Jinzhuo Zhao) and J.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key Research and Development Program of China, grant number 2016YFC0206600", and the National Natural Science Foundation of China, grant number 91843301 and 91743202.

Institutional Review Board Statement: The animal study protocol was approved by the Fudan University Animal Care and Use Committee (protocol code 201902063S, 27 February 2019).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author (Jinzhuo Zhao) upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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