

Silicon dioxide nanoparticles inhibit the effects of cold exposure on metabolism and inflammatory responses in brown adipocytes

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Abstract

Objective: Nanoparticles (NPs) in haze are potentially hazardous to health, which is more severe in the winter. Brown adipose tissue (BAT) plays important roles in obesity, insulin resistance, and diabetes. Though the toxicology of NPs has been intensively studied, few studies have been reported on the antagonistic effects between Silicon dioxide(SiO₂) NPs and cold exposure in brown adipocytes. **Materials and methods:** We evaluated changes by quantitative real-time reverse-transcriptase polymerase chain reaction (qRT-PCR) on metabolism genes, plasticity genes and the inflammatory responses genes in brown adipocytes *in vitro*. **Results:** The expression of adipogenic genes *PRDM16*, *Dio2*, *PGC-1 α* and *UCP1* was upregulated upon cold exposure ($P < 0.05$), but downregulated by SiO₂ NPs ($P < 0.05$). The results demonstrated that there was antagonistic effect between SiO₂ NPs and cold exposure on the plasticity genes and metabolism genes in brown adipocytes, where the main effects of SiO₂ NPs or cold exposure on the plasticity genes and metabolism genes were significant ($P < 0.05$). Moreover, the levels of interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α were upregulated by SiO₂ NPs or cold exposure ($P < 0.05$). The factorial analysis indicated that there was also antagonistic effect between SiO₂ NPs and cold exposure on the toxic effects in brown adipocytes, in which the main effects of cold exposure and/or SiO₂ NPs on the toxic effects were significant ($P < 0.05$). **Conclusion:** SiO₂ NPs inhibit the effect of cold exposure on metabolic genes and inflammatory responses genes in brown adipocytes.

Keywords

SiO₂ nanoparticles; cold exposure; metabolic; inflammatory response; brown adipocytes

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1 Introduction

There is a widespread concern on the problem of haze in recent years, owing to its major impact on public health. Ultrafine particulate matter (PM_{0.1}), which is equivalent to nanoparticles (NPs), has been found more harmful to human health^[1]. NPs have been widely used in industry, biomedicine, and commercial applications, which raises a concern about their safety and potential threats to the environment and human health^[2-3]. On an equal-mass basis, NPs induce stronger toxic responses than do larger-sized particles of the same chemical composition^[4]. Inhaled NPs can induce systemic and local tissue toxicological reactions, such as oxidative stress and inflammation responses, enhance blood coagulation, impair

cellular defense and immune function, by different pathways including translocation into the blood circulation thereby to other organs as well^[5-7]. In a word, NPs exposure can cause unexpected remote adverse effects by direct or indirect mechanisms^[8].

Silicon NPs require a detailed understanding on toxicological effects at the nanoscale level because of their widely use in industry and daily life^[9]. Particulate matter, which is a serious air pollution problem especially in winter, is present in the air year-round^[10]. The composition of particulate matter, where silicon accounts for a significant proportion, is complicated and associated with the risk of PM_{2.5}-caused mortality^[11]. Silicon dioxide (SiO₂), one of the main components of haze, is an

important member of the silicon family. SiO₂ NPs (silica) can be transported into the bloodstream after the translocation across biological barriers and become deposited in target organs where they can exert potential toxic effects^[112]. Changes of ambient temperature are the most common survival challenge that all endothermic organisms are facing^[13]. Though the toxic effects of SiO₂ NPs have been intensively studied, investigation on the combined toxic effects of SiO₂ NPs and cold exposure has been sparse. In our previous work, rats co-treated with SiO₂ NPs and cold exposure produced strong systemic inflammatory responses^[14].

Adipose tissues, including white adipose tissue (WAT) and brown adipose tissue (BAT), play important roles in obesity, insulin resistance, and diabetes. The overexpansion of WAT causes obesity, and BAT uncouples oxidation of fatty acids from ATP production. The plasticity of WAT has been demonstrated in cold exposure^[15]. There has been a global resurgence of research interest in BAT since its presence and functionality in humans was confirmed. It is known that the hazard of NPs to human health is more serious in wintertime because of increasing air pollution. However, the combined effects of NPs and cold exposure at the cellular level remained unclear. Our previous studies suggest that SiO₂ NPs in the air inhibit the effect of cold-induced WAT/BAT changes in plasticity and metabolism^[16-17]. The present study aimed to evaluate the combined effects of SiO₂ NPs and cold exposure at the cellular level in brown adipocytes.

The genes, such as *UCP1*, *PRDM16*, *PGC1α* and *Dio2*, are metabolic markers of BAT. *UCP1* is a mitochondrial protein enriched in brown adipocytes. The extraordinary thermogenic potential of brown adipocytes is conferred by *UCP1*. Once activated, *UCP1* uncouples the mitochondrial oxidation of fatty acids from adenosine triphosphate synthesis, thereby initiating heat production^[18-19]. *PRDM16*, a major transcription factor in BAT adipogenesis, plays a key role in triggering brown adipocyte differentiation, mitochondrial biogenesis and expression of *UCP1*^[20-21]. Similar to *PRDM16*, *PGC-1α* is a peroxisome proliferator-activated receptor γ (*PPAR γ*) transcriptional co-activator that confers the brown adipocyte with its energy-burning phenotype. It is directly involved in the stimulation of *UCP1* expression through its ability to co-activate *PPAR γ* ^[22-23]. *Dio2* is expressed by brown adipocytes in order to make triiodothyronine available to sustain the elevated metabolism of BAT^[24-25]. Expression analysis of inflammatory markers in the adipose tissue has implicated macrophages as the primary source of proinflammatory mediators such as tumor necrosis factor (TNF)- α , interleukin (IL)-6, and IL-1 β in adipose tissue^[26-27]. Chronic inflammation, specifically mediated by macrophage activation, plays a crucial role

in the development of obesity-related insulin resistance^[28]. Proinflammatory cytokines induce insulin resistance. TNF- α has been shown to alter adipogenesis^[29]. Adiponectin (ADPN), the most abundant peptide secreted by adipocytes, enhances insulin sensitivity. Leptin, another important adipocyte-derived peptide hormone plays an important role in the regulation of food intake and thermogenesis^[30]. In the present work, the expression of these genes was detected. The results showed that in brown adipocytes, SiO₂ NPs have antagonistic effect with cold exposure on the metabolism genes and plasticity genes, in which the main effects of SiO₂ NPs or cold exposure on the metabolism genes and plasticity genes were significant ($P < 0.05$). Moreover, there was also antagonistic effect between SiO₂ NPs and cold exposure on the toxic effects in brown adipocytes, where the main effects of SiO₂ NPs and/or cold exposure on the toxic effects were significant ($P < 0.05$).

2 Materials and methods

2.1 Sample preparation

SiO₂ NPs (shape, crystal structure; size, 20.2 ± 6.4 nm; composition, SiO₂ > 99.0%), purchased from Shanghai Run He Nano Co. Ltd. (Shanghai, China), were prepared in 2 mg/mL phosphate buffered saline (PBS), followed by ultrasonication at < 50°C for 8 h. Using transmission electron microscopy (JEM-100CX II, JEOL Co., Tokyo, Japan), the shape and size of the SiO₂ NPs were measured (Fig. 1). After sonication, aliquots of the stock suspensions were immediately serially diluted in cell culture medium to obtain the desired test concentrations. The zeta (ζ) potential of SiO₂ NPs (-11.76 ± 0.58 mV) was analyzed by dynamic light scattering (Malvern Zeta sizer Nano ZS, Worcestershire, UK).

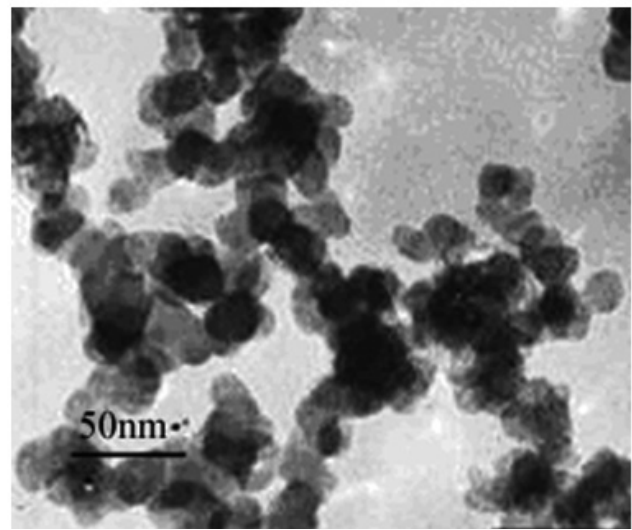


Fig. 1 Image of SiO₂ NPs by transmission electron microscopy

2.2 Cell isolation and culture

Brown adipocytes were isolated from the BAT near scapular area of 1-2-day-old Sprague-Dawley (SD) newborn rats. The parental SD female and male rats were housed in stress free, clean and animal friendly conditions in a standard environment (relative humidity 60% ± 10%, room temperature 21 ± 2°C) with a 12 h dark/light cycle and 10-15 air changes per hour. Food and water were freely accessible. All newborn rats lived with their parental female rats until they were used for experiment. The tissue was cut into 1 mm × 1 mm pieces and digested using 2 mg/mL collagenase and 20 g/L bovine serum albumin (BSA) in Dulbecco's modified eagle medium (DMEM) in a shaking water bath at 37°C for 1 h. After digestion, the digested tissue was filtered through a 100-µm nylon screen, and cells were centrifuged at 300 × g for 5 min. The pellet consisting of precursor cells was washed once in isolation buffer and centrifuged again. Cells were resuspended in 2 mL of culture medium (DMEM containing 25 mmol/L glucose, 20 mmol/L HEPES, 20% FBS, 100 units/mL penicillin/streptomycin), seeded on 35 mm plates, and grown in a humidified atmosphere of 5% CO₂ and 95% air with the medium changed every day. Preadipocytes were grown to confluence for differentiation in culture medium supplemented with 20 nmol/L insulin and 1 nmol/L T3 (differentiation medium). Confluent cells were incubated in differentiation medium further supplemented with 0.125 mmol/L indomethacin, 0.5 µmol/L dexamethasone, and 0.5 mmol/L isobutylmethylxanthine (induction medium) for 24 h. Subsequently, the cells were maintained in differentiation medium for 4-5 days until a fully differentiated phenotype with massive accumulation of multilocular fat droplets had emerged. All experiments involving the care and use of animals were performed in accordance with the guidelines and regulations concerning the ethics of science research in the Institute of Environmental and Operational Medicine and approved by the Ethics Review Board of Institute of Environmental and Operational Medicine (AMMS-04-2020-017). The animals, which were provided by the Laboratory Animal Center of Institute of Environmental and Operational Medicine, were treated humanely and with regard for alleviation of suffering.

2.3 Oil red O staining

After washed twice with PBS, the culture dishes were fixed with 10% buffered formalin at room temperature for at least 1 h. Cells were then stained at room temperature for 2 h with the filtered oil red O solution (0.5 g of oil red O in 100 mL of isopropyl alcohol), washed twice with water, and visualized.

2.4 Quantitative real-time reverse-transcriptase polymerase chain reaction (qRT-PCR)

To quantify the relative mRNA levels of the genes of interest

in brown adipocyte, qRT-PCR was performed using primers from SYBR GREEN Gene Expression Assays and the 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Relative expression levels were established using the comparative threshold cycle (CT) method outlined in the manufacturer's instructions. All expression levels were normalized to the expression level of housekeeping gene (*β-actin*), and reported as a relative fold change over the control. Relative mRNA expression was determined by the $\Delta\Delta$ -Ct method using the expression level of the gene encoding the TATA-binding protein levels as the endogenous control. The brown adipocytes for qRT-PCR were cultured at 37°C and at 31°C with the concentrations of SiO₂ NPs 0 and 100 µg/mL, respectively^[31]. The brown adipocytes were divided into four groups, such as the control group(37°C, SiO₂ NPs 0 µg/mL), the SiO₂ NPs group(37°C, SiO₂ NPs 100 µg/mL), the cold exposure group(31°C, SiO₂ NPs 0 µg/mL) and the combined group(31°C, SiO₂ NPs 100 µg/mL). The primer pairs were as follows:

UCP1, 5'-TGTGCAATGACCATGTACACCAA-3' (forward) and 5'-GCACACAAACATGATGACGTTCC-3' (reverse);
PGC-1 α , 5'-CACCGTAAATCTGCGGGATG-3' (forward) and 5'-TATCCATTCTCAAGAGCAGCGAAAG-3' (reverse);
Dio2, 5'-CTGTGGTTGGATGTAGTCACACGA-3' (forward) and 5'-CTTTGCACCAGGACCCAAATG-3' (reverse);
PRDM16, 5'-CAAAGGGAAGCCAGCAGAG-3' (forward) and 5'-GAGGCGGGAAGAAGGAATG-3' (reverse);
TNF- α , 5'-TTCCAATGGGCTTTCGGAAC-3' (forward) and 5'-AGACATCTTCAGCAGCCTTGTGAG-3' (reverse);
IL-1 β , 5'-CCCTGAACTCAACTGTGAAATAGCA-3' (forward) and 5'-CCCAAGTCAAGGGCTTGGAA-3' (reverse);
IL-6, 5'-ATTGTATGAACAGCGATGATGCAC-3' (forward) and 5'-CCAGGTAGAAACGGAACCTCCAGA-3' (reverse);
ADPN, 5'-CAAGGCCGTTCTCTTCACCT-3' (forward) and 5'-CCCCATACACTTGGAGCCAG-3' (reverse);
Leptin, 5'-TGACAAACAGGTTACAGGACCAGA-3' (forward) and 5'-ACGAAACCCGATCCAGTTCA-3' (reverse);
β-actin, 5'-CCTAAGGCCAACCGTGAAAA-3' (forward) and 5'-CAGAGGCATACAGGGACAACAC-3' (reverse).

2.5 Statistical analyses

All quantitative variables are expressed as the mean ± standard error of mean (SEM). Statistical analyses were conducted using the SPSS software (IBM SPSS Statistics version 21.0, IBM Co., New York, USA). Analysis of variance (ANOVA) was used to establish the statistical significance of differences between the experimental groups and control. One-way ANOVA with Bonferroni's correction was used for multiple comparisons. UNIANOVA was used to analyze the main effect and the

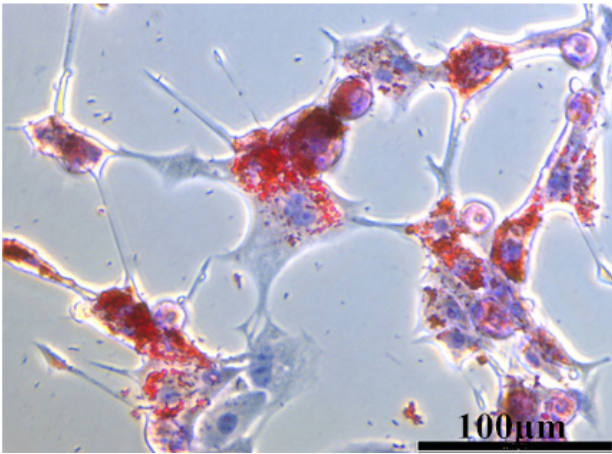


Fig. 2 The oil red O staining of brown adipocytes

The brown adipocytes were isolated from the brown adipose tissue, induced in differentiation medium, and stained with oil red O.

interaction between SiO₂ NPs and cold exposure. *P* values < 0.05 were considered significant and < 0.01 highly significant.

3 Results

3.1 SiO₂ NPs antagonize the effect of cold exposure on the metabolism genes and plasticity genes in brown adipocytes

The brown adipocytes, stained with oil red O (Fig. 2), verified that the isolation, differentiation, and culture from BAT were successful. With SiO₂ NPs treatment or cold exposure, the expression of adipogenic genes significantly changed in brown adipocytes (Fig. 3). In Fig. 3A, the expression of PRDM16, a marker gene for brown adipocytes, was downregulated significantly (*P* < 0.05) in the SiO₂ NPs group, but upregulated significantly (*P* < 0.01) in the cold exposure group, compared with the control group. As expected based on the opposing effects between SiO₂ NPs

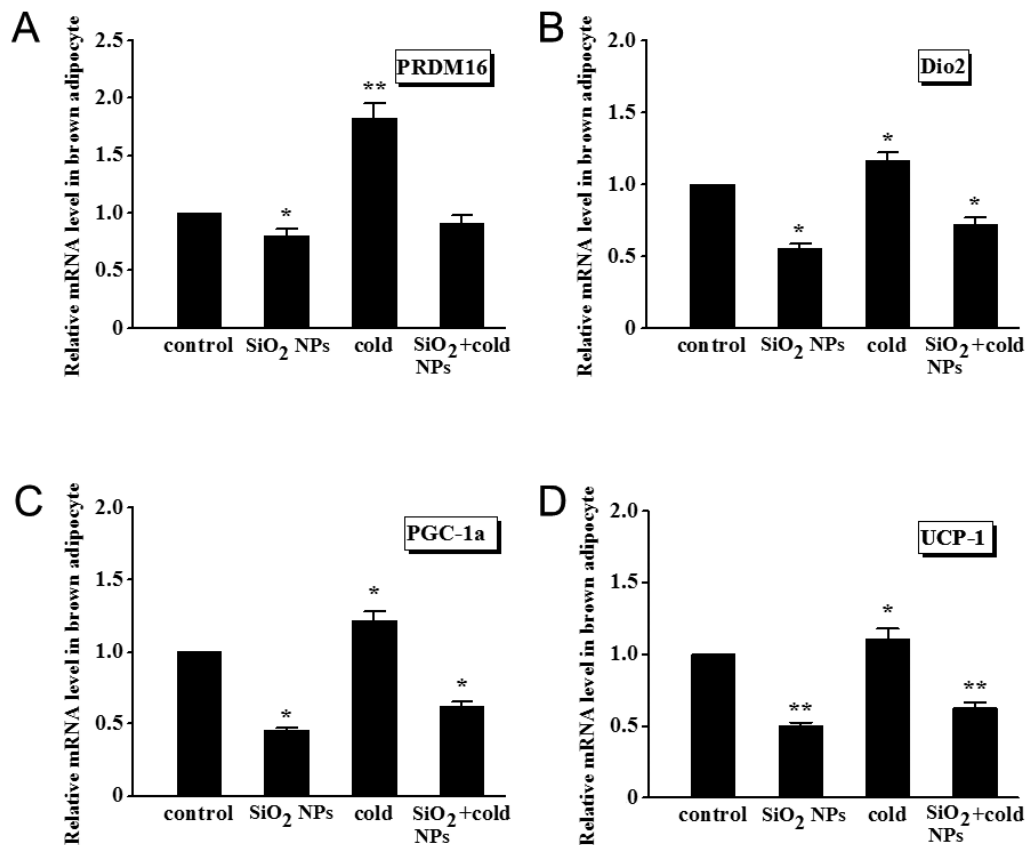


Fig. 3 Adipogenic gene expression determined by qRT-PCR in brown adipocytes

(A) PRDM16. (B) Dio2. (C) PGC-1a. (D) UCP1. Values represent mean \pm SEM, and significant differences compared with controls are indicated by **P* < 0.05, ***P* < 0.01.

and cold exposure, co-treatment with two rendered a loss of the effects seen with SiO₂ NPs or cold exposure alone. Similar pattern of changes of the mRNA levels of *Dio2*, *PGC-1α* and *UCP1* genes was consistently observed. Specifically, *Dio2*, a gene critical for thermogenesis in response to dropping temperature, sympathetic stimulation, or overfeeding in BAT, was downregulated ($P < 0.05$)

by SiO₂ NPs alone but upregulated ($P < 0.05$) by cold exposure, whereas SiO₂ NPs/cold exposure combination produced relatively moderate but statistically significant ($P < 0.05$) effect with a smaller magnitude of downregulation compared to SiO₂ NPs alone and less upregulation compared to cold exposure alone. As illustrated in Fig. 3C and 3D, the expression of *PGC-1α* and

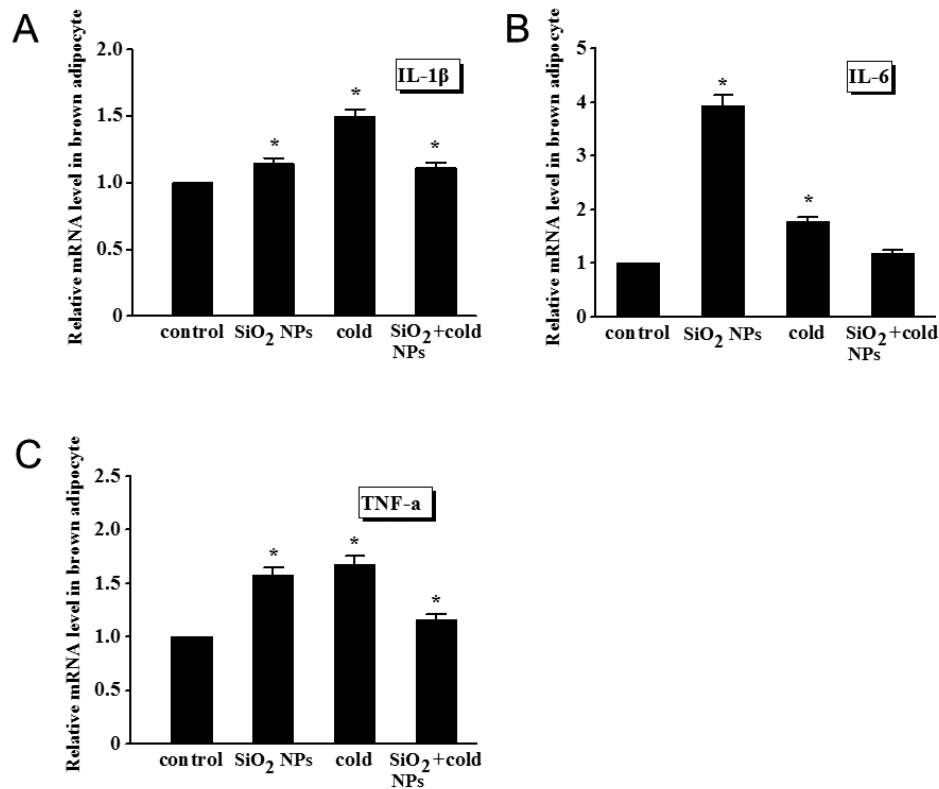


Fig. 4 Expression of inflammatory cytokines determined by qRT-PCR in brown adipocytes

(A) IL-1β. (B) IL-6. (C) TNF-α. Values represent mean ± SEM, and significant differences compared with controls are indicated by * $P < 0.05$, ** $P < 0.01$.

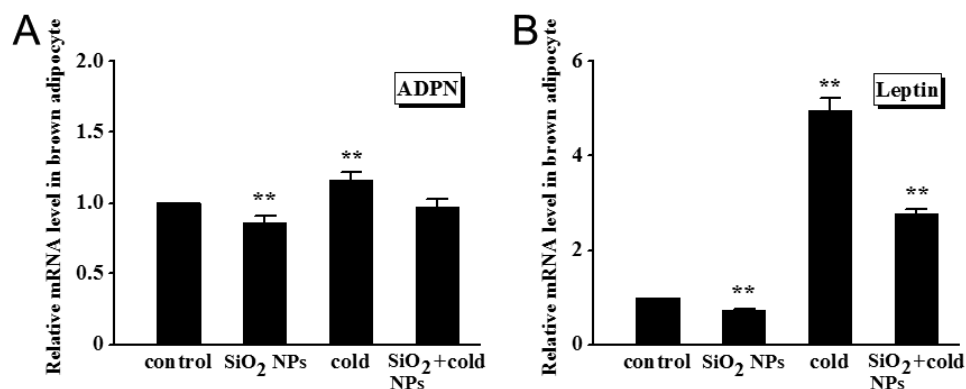


Fig. 5 Effect of cold exposure or/and SiO₂ NPs on the expression of secretion-related genes in brown adipocytes

(A) ADPN. (B) Leptin. Values represent mean ± SEM, and significant differences compared with controls are indicated by ** $P < 0.01$.

UCP1 was significantly downregulated by SiO₂ NPs alone (*UCP1*: $P < 0.01$; *PGC-1 α* : $P < 0.05$) but increased ($P < 0.05$) by cold exposure alone, and the effects of SiO₂ NPs or cold exposure alone were neutralized by their combination (*UCP1*: $P < 0.01$, *PGC-1 α* : $P < 0.05$). The factorial analysis showed that there were antagonistic effects between SiO₂ NPs and cold exposure on the plasticity genes and metabolism genes in brown adipocytes, where the main effect of SiO₂ NPs or cold exposure on the plasticity genes and metabolism genes were significant ($P < 0.05$).

3.2 SiO₂ NPs antagonize the effect of cold exposure on the inflammatory response genes in brown adipocytes

After SiO₂ NPs or cold exposure, the expression of proinflammatory cytokine genes changed significantly in brown adipocytes (Fig. 4). The expression of *IL-1 β* , *IL-6* and *TNF- α* was remarkably ($P < 0.05$) upregulated by either SiO₂ NPs alone or cold exposure alone; yet, co-treatment with SiO₂ NPs and cold exposure markedly mitigated the upregulation of these proinflammatory cytokine genes. The factorial analysis indicated that the main effects of SiO₂ NPs and/or cold exposure on the inflammatory responses genes were significant ($P < 0.05$). Moreover, SiO₂ NPs and cold exposure have mutually antagonistic effects on the expression of proinflammatory genes in brown adipocytes.

3.3 Antagonistic effects between SiO₂ NPs and cold exposure on the expression of secretion genes in brown adipocytes

Fig. 5A demonstrated that the expression of *ADPN* gene, which correlates with adipocyte differentiation and modulates a number of metabolic processes, was decreased significantly ($P < 0.01$) by SiO₂ NPs alone, but increased significantly ($P < 0.01$) by cold exposure, compared with non-treated control. Similarly, leptin, which plays a pivotal role in inflammatory responses and in the regulation of energy expenditure and appetite, was considerably decreased ($P < 0.01$) by SiO₂ NPs alone but increased ($P < 0.01$) by cold exposure (Fig. 5B). As anticipated, SiO₂ NPs/cold exposure combination compromised the effects produced by SiO₂ NPs alone or cold exposure alone. There were antagonistic effect between SiO₂ NPs and cold exposure on leptin and ADPN.

4 Discussion

Hazy weather is common in the northern part of China literally for a year round, especially in wintertime. NPs are the main hazardous pollutants in atmospheric particulate matter and when in combination with other factors such as cold their detrimental effects on human health can be seriously exacerbated. The inhaled NPs can reach many organs through blood circulation, such as the spleen, liver, brain and kidney^[7], and their impact

on weight control in relation to brown/white adipose tissues has been intensively studied^[32-33]. It was suggested that NPs can move across tissue and cellular barriers and interact with DNA^[34]. Our previous investigation found that SiO₂ NPs inhibit the weight lost and the increase of BAT following cold exposure^[17], suggesting that SiO₂ NPs impose damaging effects on adipose tissue remodeling. To the best of our knowledge, the present study is the first to assess the effects of SiO₂ NPs and cold exposure on gene expression in brown adipocytes. The adipogenic genes including *PRDM16*, *Dio2*, *PGC-1 α* and *UCP1* were significantly downregulated by SiO₂ NPs relative to the control levels, indicating that SiO₂ NPs could inhibit the increase of the brown adipocytes. Notably, there seemed to be antagonistic effects between cold exposure and SiO₂ NPs on the expression of adipogenic genes in brown adipocytes. The finding is consistent with the results of our previous studies that SiO₂ NPs inhibited the cold-induced metabolism activation in white adipocytes and SD rats^[16-17].

Cold exposure, which exacerbates asthma and chronic obstructive pulmonary disease (COPD), is a major environmental factor^[35]. As an evidence of inflammation of the heart, kidney and blood vessel caused by chronic cold exposure, the mRNA levels of *TNF- α* and *IL-6* increase significantly^[36]. The present study showed that the mRNA levels of *IL-6*, *IL-1 β* and *TNF- α* were upregulated by cold exposure, suggesting that an inflammatory response may be induced by cold exposure, as well as, in brown adipocytes. These findings were consistent with the results from one of our previous studies showing that cold exposure and SiO₂ NPs induced a strong inflammatory response in BAT^[14]. Multiple hormones and cytokines can influence instinctual behavior and metabolic pathways^[37-38]. A study showed that *IL-6* and *TNF- α* inhibited lipoprotein lipase, and *TNF- α* induced uncoupling protein expression and stimulated hormone-sensitive lipase^[39]. *IL-1 β* and *IL-6* can also reduce the activity of adipose tissue lipoprotein lipase^[40]. In the present study, the expression of proinflammatory cytokines was increased by cold exposure and SiO₂ NPs, which likely influences their secretion and metabolism in brown adipocytes. BAT and WAT have important roles in the development of therapeutics for diabetes and obesity^[41]. Meanwhile, the problem of haze in winter is particularly prominent. The present work underlines the potential role of SiO₂ NPs exposure in plasticity genes and metabolism genes in brown adipocytes. It suggested that SiO₂ NPs inhibit the effect of cold exposure on metabolism genes and plasticity genes.

In summary, wide spread attention has been attracted on the potential hazard of SiO₂ NPs from haze in winter. The work about the combined effect of cold exposure and SiO₂ NPs at the cellular level on brown adipocytes has been scarce. Here

we reported the antagonistic effect between cold exposure and SiO₂ NPs on the expression of metabolism- and plasticity-related genes in brown adipocytes. Moreover, SiO₂ NPs and cold exposure both induce an inflammatory response in brown adipocytes. These results will contribute to the further understanding the effects of a combination of haze and winter conditions at the cellular level.

Acknowledgments

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Author Contribution

Zhang Y Q and Yang D F designed the study and drafted the manuscript. Zhang L and Li X performed data analysis and data

interpretation. Wu S, Zhang G Y and Wei X D performed all the experiments. All participated in the writing and approved the final version of the manuscript.

Ethnical Approval

All experiments involving the care and use of animals were performed in accordance with the guidelines and regulations concerning the ethics of science research in the Institute of Environmental and Operational Medicine and approved by the Ethics Review Board of Institute of Environmental and Operational Medicine (AMMS-04-2020-017).

Conflicts of interest

Yang D F is an Editorial Board Member of the journal. The article was subjected to the journal's standard procedures, with peer review handled independently of this member and his research groups. There are no conflicts of interest to declare.

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