Science of the Total Environment 790 (2021) 148041

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Triiodothyronine ameliorates silica-induced pulmonary inflammation and fibrosis in mice

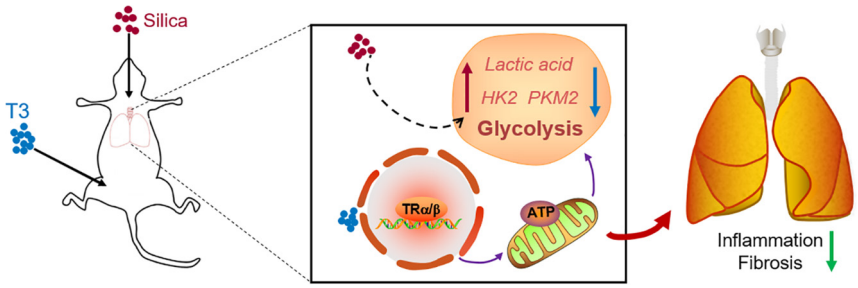
Meng Yang [a,b,1], Dongming Wang [a,c,1], Shiming Gan [a,c], Bin Wang [a,c], Linling Yu [a,c], Yujia Xie [a,c], Lieyang Fan [a,c], Jixuan Ma [a,c], Weihong Chen [a,c,*]

[a] Department of Occupational & Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China
[b] Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430000, China
[c] Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, and State Key Laboratory of Environmental Health (Incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

HIGHLIGHTS

- Thyroid morphology and function could be affected by silica exposure.
- Exogenous supplement T3 alleviates pulmonary inflammation and fibrosis caused by silica.
- T3 exerts its protective effects by inhibiting glycolysis.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 9 February 2021
Received in revised form 21 March 2021
Accepted 21 May 2021
Available online 26 May 2021

Editor: Lotfi Aleya

Keywords:
Triiodothyronine
Silica
Glycolysis
Inflammation
Pulmonary fibrosis

ABSTRACT

Environmental exposure to silica or particles is very common in natural, agricultural and industrial activities. Chronic silica exposure can lead to silicosis, which remains one of the most serious interstitial lung diseases all through the world, while viable therapeutic choices are restricted. Triiodothyronine (T3) has been shown to exert a defensive role in many pulmonary diseases, however, rare data are available regarding the role of T3 on silica-induced injury. We constructed an experimental silicosis mouse model and T3 was intraperitoneally administrated after instillation of silica to observe the effect of T3 on silica-induced lung inflammation and fibrosis. Our results showed that the silicosis mouse model was accompanied by changes in thyroid morphology and function, and T3 supplement reduced silica-induced lung damage, inflammation and collagen deposition. The protective properties of T3 on silica-induced lung injury could be partially mediated through thyroid hormone receptors. And the mechanism by which T3 treatment ameliorated silica-induced fibrosis appeared to be via the reduction of glycolysis. Also, T3 could sufficiently postpone the progression of pulmonary fibrosis in established silicosis. Our findings reveal that administration of T3 could down-regulate the inflammatory response, pulmonary fibrosis and other lung damage caused by silica. The reduction of glycolysis may be one of the mechanisms.

Abbreviations: ATP, adenosine triphosphate; TCA, tricarboxylic acid; HK2, hexokinase 2; HIF-1α, hypoxia-inducible factor 1-α; PKM2, pyruvate kinase isoenzyme M2; TH, thyroid hormone; T3, triiodothyronine; T4, thyroxine; AFC, alveolar fluid clearance; VILI, ventilator-induced lung injury; TR, thyroid hormone receptor; TSH, thyroid-stimulating hormone; BALF, bronchoalveolar lavage fluid; TP, total protein; LDH, lactate dehydrogenase; ACP, acid phosphatase; AKP, alkaline phosphatase; COL1A1, collagen type I alpha 1; COL3A1, collagen type III alpha 1; Fn-1, fibronectin-1.

* Corresponding author at: Department of Occupational and Environmental Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China.
E-mail address: wchen@mails.tjmu.edu.cn (W. Chen).
[1] These authors contributed equally to this article.

https://doi.org/10.1016/j.scitotenv.2021.148041
0048-9697/

1. Introduction

Crystalline silica is one of the most abundant minerals on earth and environmental exposure to silica or particles is very common in natural, agricultural and industrial activities (Yao et al., 2020). According to recent published literatures, more than 30 million workers are exposed to crystalline silica (Lai et al., 2018). Increased mortality from respiratory diseases or cardiovascular diseases is reported to be associated with exposure to silica, making silica exposure a major worldwide public health problem (Leung et al., 2012). Silicosis is characterized by persistent pulmonary inflammation and progressive fibrosis induced by chronic silica exposure. Though anti-inflammatory and anti-fibrotic agents are used clinically, severe adverse outcomes always persecute these silicosis patients (Li et al., 2017). Therefore, it remains urgent to develop new effective drugs in order to prevent this disease or delay its progression.

Metabolic alterations are increasingly recognized as an important pathogenic process in pulmonary inflammation and fibrosis (Zhao et al., 2020). In response to some pro-inflammatory stimuli, lung tissue can transform into a state of increased aerobic glycolysis (McElvaney et al., 2019). This change in metabolism (known as a Warburg effect) causes the rapid low-level production of ATP and yield of lactate through the glycolytic pathway (Liberti and Locasale, 2016). Interestingly, the lung has recently been considered as a metabolically active organ (Breitzig et al., 2018). Thyroid hormones (THs) have broad contributions to energy homeostasis through their effects in many metabolically relevant tissues (Mullur et al., 2014). However, the effects of THs on lung physiology and pathology are rarely studied. A previous study found that T3 administration decreased pro-inflammatory cytokine levels in a mouse model for ventilator-induced lung injury (VILI) (Barca-Mayo et al., 2011). Similarly, another study revealed that T3 treatment improved the survival rate and resolved fibrosis in two experimental pulmonary fibrosis models (Yu et al., 2018). Unfortunately, the potential role of T3 in silica-induced fibrosis and whether aerobic glycolysis is involved in this pathogenesis are still unclear.

Here, we investigate whether silica-injured lung undergoes metabolic reprogramming, especially the transition to increased aerobic glycolysis, and the potential defensive role of T3 in an experimental silicosis model. In brief, we found that a silicosis mice model was often accompanied by changes in thyroid morphology and decrease in hormone secretion, and system administration of T3 could attenuate silica-induced lung damage, inflammation and fibrosis through interaction with thyroid hormone receptors (TRs). Mechanistically, we showed that T3 exerted its protective effects in silica-induced pulmonary inflammation and fibrosis by inhibiting glycolysis. Furthermore, present findings also revealed that T3 was also effective for pulmonary fibrosis in an established silicosis model.

2. Material and methods

2.1. Crystalline silica

Crystalline silica particles were provided by the Chinese Center for Disease Control and Prevention (China CDC). Particle size distribution was as follows: 57 % < 1 μm in diameter, 34% 1–2 μm in diameter, 4.5% 2–3 μm in diameter, 3.0% 3–4 μm in diameter, 1.5% 4–5 μm in diameter. Particulates were ground for 30 min, sterilized at 121 °C for 20 min, dried and suspended in sterile saline. Finally, the suspensions were sonicated for 15 min before use.

2.2. T3

T3 was purchased from Sigma Aldrich (St. Louis, USA) with a purity >95% (HPLC, Radiochemical Purity). T3 was diluted in 5 mL of sterile saline to a final concentration of 12.5, 20, 50, and 100 μg/kg, respectively.

2.3. Silicosis model

Male C57BL/6 wild type mice 6–8 weeks of age were obtained from Hubei Provincial Center for Disease Control and Prevention. All of the mice were kept in specific-pathogen-free environments. All animal procedures were approved by the Animal Care and Use Committee at Huazhong University of Science and Technology, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The silicosis model was induced as previously described (Li et al., 2019). Simply, the mice were anesthetized by intraperitoneal injection of pentobarbital sodium (1 mL/100 g) and instilled with silica suspensions (containing 2.5 mg silica) 50 μL by intratracheal instillation.

2.4. In vivo treatment

Program 1: Mice were randomly divided into seven groups (n = 8 per group). Saline group: mice were instilled with saline and daily intraperitoneal administration of saline vehicle only; Silica group: mice were instilled with silica and daily intraperitoneal administration of saline vehicle only; T3-treated groups: mice were instilled with silica and daily intraperitoneal administration of T3 at the dose of 12.5, 25, 50, 100 μg/kg, respectively. T3 control group: mice were instilled with saline and daily intraperitoneal administration of T3 (100 μg/kg).

At 7, 28 and 84 days after intratracheal instillation of silica, mice were euthanized with pentobarbital sodium by intraperitoneal injection (1 mL/100 g). The blood samples collected from the eye vein were centrifuged at 3000 rpm for 10 min at 4 °C. The bronchoalveolar lavage fluid (BALF) was collected by lavaged 3 times with 1 mL sterile saline and centrifuged at 1000 rpm for 10 min at 4 °C.

Program 2: Moreover, another experiment mouse model was constructed to assess the effect of T3 on established silicosis. T3 (100 μg/kg) or saline vehicle were administrated by daily intraperitoneal injection 28 days after exposure to silica, and the mice were sacrificed on the 84th day (n = 8 per group). All biological samples were collected as previously described in Program 1.

2.5. Measurement of serum T3, Thyroxine (T4), Free T3 (FT3), Free T4 (FT4) and thyroid-stimulating hormone (TSH) levels

Total serum T3, T4, FT3, FT4 and TSH levels were measured using the ELISA kits from CUSABIO (Wuhan, China), following the manufacturer's instructions.

2.6. Biochemical assays

BALF was obtained as previously described. Total protein (TP) content, lactate dehydrogenase (LDH), alkaline phosphatase (AKP) and acid phosphatase (ACP) activities were measured by using specific commercial assay kits (Nanjing Jiancheng Institute, China) based on the manufacturer's instructions.

2.7. Morphological, histological and immunohistochemistry analysis

The thyroid and left lung of each mouse was removed and fixed with 4% paraformaldehyde for 48 h and then embedded into paraffin. Paraffin sections (4 μm thick) of thyroid or lung were used for hematoxylin and eosin (H&E) staining or Masson staining to evaluate histopathological changes and were photographed using a light microscope (Olympus IX71, Tokyo, Japan) at the magnification of ×200. For immunohistochemical (IHC) analysis, lung sections were incubated with primary antibody α-SMA (1:200, Cell Signaling Technology Inc., Beverly, MA, USA) overnight at 4 °C and then a horseradish peroxidase (HRP) polymer secondary antibody (1:100) were used. Non-immune IgG was used as a negative control.

2.8. Cytokine analysis

Levels of IL-1β, TNF-α, IL-6, and TGF-β1 in BALF were measured using commercially available ELISA Kits (Dakewe Biotech Company, Shenzhen, China) based on the manufacturer's instructions.

2.9. Assay of lung ATP and lactate levels

ATP and lactate levels in lung tissue were measured by using specific commercial assay kits (Nanjing Jiancheng Institute, China) based on the manufacturer's instructions.

2.10. Quantitative PCR analysis

Total RNA was isolated from lung tissue homogenate using the Trizol Reagent (Invitrogen, USA) and was reverse-transcribed to cDNA with the ReverTra Ace qPCR RT kit (TOYOBO, Japan) based on the manufacturer's instructions. The SYBR Green Real-time Master Mix (TOYOBO, Japan) was used to determine the target gene expression. Detail information of specific primer pairs is listed in the Supplementary Table S1. The transcription of GAPDH was measured for normalization and relative mRNA and fold change gene expressions were analyzed by $2^{-\Delta\Delta CT}$ methods.

2.11. Western blotting analysis

The proteins were isolated from lung tissues using RIPA lysis buffer (Beyotime, China) containing 1% phenylmethylsulfonyl fluoride (PMSF, Beyotime, China). Total protein was quantified using the bicinchoninic acid (BCA) method. Equal amounts of protein (40 μg) from each sample were separated on a sodium dodecyl sulfate-polyacrylamide gel and then electrophoresed onto a PVDF membrane. The membranes were blocked with 5% bovine serum albumin (BSA) at room temperature for 1 h, washed and then incubated overnight at 4 °C with the following primary antibodies: hypoxia-inducible factor 1-α (HIF-1α) (1:1000), hexokinase 2 (HK2) (1:1000), pyruvate kinase isoenzyme M2 (PKM2) (1:1000) and COL1A1 (1:2000) (Cell Signaling Technology Inc., Beverly, MA, USA); fibronectin-1 (Fn-1) (1:2000) and β-actin (1:5000) (ProteinTech Group, Chicago, IL, USA). The membranes were then incubated with a secondary antibody (HRP-conjugated goat anti-rabbit) for 1 h. All protein bands were detected using a GeneGnome chemiluminescent imaging system (Syngene, Frederick, USA) and β-actin was used as a loading control.

2.12. Statistical analysis

All numerical data from independent experiments were presented as mean ± standard deviation. One-way ANOVA analysis followed by multiple comparisons using Tukey's honest significant difference (HSD) test. $P < 0.05$ was considered statistically significant. GraphPad Prism 8.0 and R statistical software were performed for graphics and statistical analyses.

3. Results

3.1. The examination of thyroid morphology and function in mice after exposure to silica

Firstly, we performed H&E staining to observe the morphological changes of the thyroid. At each time point after silica exposure, both cubic-shape epithelial cells and rich glial globulin in the follicular cavity were observed in the control groups. In the silica groups, follicular epithelial cells were diffusely proliferating and tended to be flattened, with heterogeneous glial in the follicular cavity. Moreover, in the late stage of silica exposure, some follicles were fused or even vacuolated (Fig. 1A). Then we evaluated the serum T3, T4, FT3, FT4 and TSH at 7, 28, and 84 d after silica instillation, and found that all T3 and T4 levels were significantly reduced in silica groups as compared with control groups (Fig. 1B–F). Although no significant differences were found in TSH levels at 7 d and 28 d between silica and control groups, a significant increase in TSH was observed at 84 d after silica exposure. Overall, these data indicated that silica exposure affected the morphology of the thyroid gland, resulting in decreased hormone secretion of T3 and T4.

3.2. Body weight gain and serum T3 levels after T3 supplementation in silica-exposed mice

To investigate whether supplement of T3 could exert biological effects on the silicosis model, the mice were treated with 12.5, 25, 50 or 100 μg/kg T3 by intraperitoneal injection daily after silica instillation (Fig. 2A). We first observed the effects of administration of T3 on body weight gain in silica-exposed mice. In comparison to the control group, the body weight was reduced in the silica group and all T3-treated groups at 7 d. While no significant changes in body weight were seen in other time points among all groups (Fig. 2B). Then we evaluated the T3 expression after exogenous supplementation and found the reduction of total serum T3 was improved in a dose-dependent manner as compared with the silica group at 7 d. The T3 expressions were drastically increased at high dose (100 μg/kg) group as compared with the silica group at 28 and 84 d (Fig. 2C–E). Simultaneously, no significant changes on metabolism and physical activity indicators including basic metabolic index, vascular and nervous system indicators were observed during the administration period (data not shown).

3.3. T3 treatment normalizes the silica-induced BALF biochemical changes

Exposure to silica could induce cellular damage in lung tissues and cause changes in biochemical composition. The biochemical changes were assessed in the current study by evaluating TP content, LDH, ACP and AKP activity in BALF of experimental mice. Compared with the control group, silica exposure (single intratracheal instillation) resulted in significantly enhanced levels of the biochemical markers in BALF in all periods of experience, especially at 7 d after instillation. T3 treatment markedly declined the levels of TP, LDH, ACP and AKP as compared with the silica group at all time points of study. At the high dose (100 μg/kg), the effects of T3 on BALF biochemical changes were the most significant. While T3 only treatment group (without silica exposure) exhibited no significant changes in the level of TP, LDH, ACP and AKP in BALF of mice as compared with control group at any studied time interval (Fig. 3).

3.4. T3 treatment reduces silica-induced pulmonary inflammation

To determine whether T3 could be effective on silica-induced inflammation, we first performed H&E staining to observe inflammatory cell infiltration and integrity of lung structure. Notably, compared with the control groups, silica-exposed mice showed severe pulmonary inflammation and pathology damage, whereas T3 treatment reduced lung inflammation and damage in a dose-dependent manner (Fig. 4A). Next, we evaluated levels of IL-1β, IL-6 and TNF-α in BALF, and found silica exposure resulted in significantly elevated levels of these inflammatory cytokines in all periods of experience as compared with the control group. IL-6 and TNF-α levels were increased the most at 7 d after silica instillation, while the IL-1β level increased as the time points progressed (Fig. 4B–J). Importantly, T3 treatment markedly declined the levels of inflammatory cytokines in BALF as compared with the silica group at all time points of study, especially at the high dose of T3 (100 μg/kg). No significant inflammation changes were observed in T3 only treatment group as compared with the control group.

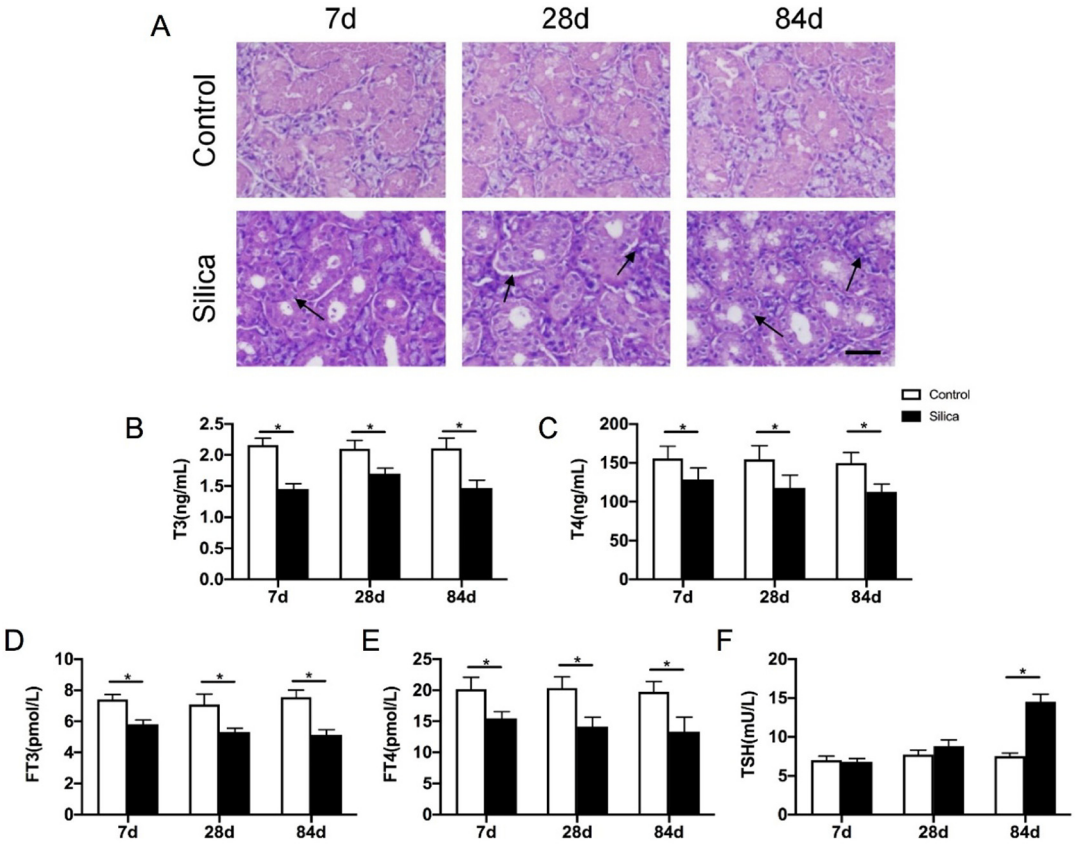


Fig. 1. Effects of silica exposure on thyroid morphology and function in mice. **(A)** The histological changes in thyroid tissues were observed by H&E staining at 7, 28, 84 d after exposure to silica particles. Representative images are shown. Arrows point out pathological changes of thyroid tissue. Magnification ×200. Scale bar indicates 100 μm. **(B–F)** The serum levels of T3, T4, FT3, FT4 and TSH in mice at 7, 28, 84 d after silica instillation. *, $P < 0.05$; Error bar indicates mean ± S.D.

3.5. T3 treatment attenuates silica-induced pulmonary fibrosis

To explore the role of T3 on silica-induced pulmonary fibrosis, we performed Masson staining and results demonstrated reduced collagen deposition in mice treated with T3 (12.5, 25, 50, 100 μg/kg) when compared with the silica group. Also, IHC staining showed that T3 treatment reduced α-SMA deposition in the lungs at 84 d (Fig. 5). Furthermore, attenuated fibrosis was supported by decreased TGF-β1 level in BALF and COL1A1, COL3A1 and Fn-1 mRNA expressions in lung tissues at 7, 28 and 84 d (Fig. 6A–L). Moreover, western blot analysis showed decreased protein levels of Fn-1 and COL1A1 in the lungs at 84 d (Fig. 6M–O). Taken together, these

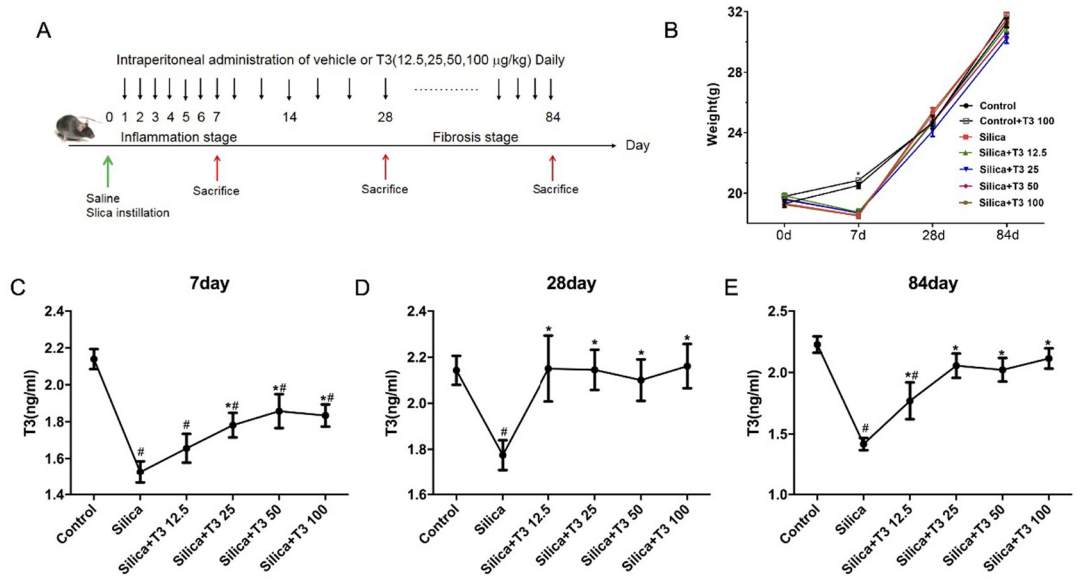


Fig. 2. T3 expression in serum after exogenous supplementation by intraperitoneal injection in mice. **(A)** C57BL/6 mice were intraperitoneal administration of different doses of T3 or vehicle daily after silica instillation. **(B)** Body weight at several time points in mice treated with the different doses of T3. **(C–E)** Concentrations of T3 were measured using ELISA at 7, 28, 84 d after exogenous T3 administration. #, $P < 0.05$ compared with the control group; *, $P < 0.05$ compared with the silica group. Error bar indicates mean ± S.D.

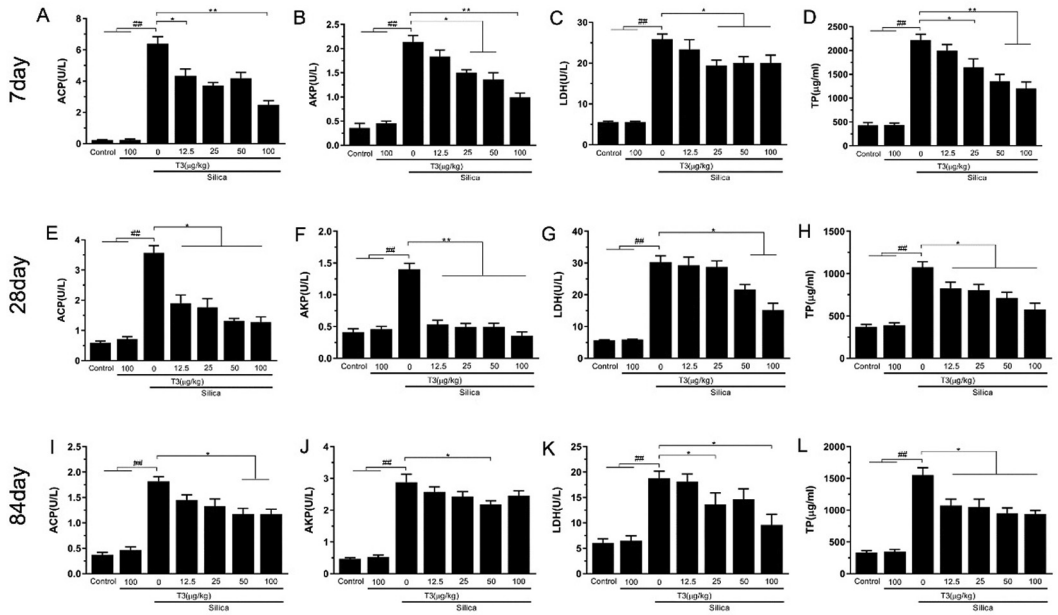


Fig. 3. Effects of T3 treatment on lung injury induced by silica. **(A–D)** ELISA analysis of ACP, AKP, LDH and TP contents in BALF of T3-treated mice for 7 d. **(E–H)** ELISA analysis of ACP, AKP, LDH and TP contents in BALF of T3-treated mice for 28 d. **(I–L)** ELISA analysis of ACP, AKP, LDH and TP contents in BALF of T3-treated mice for 84 d. #, $P < 0.05$; ##, $P < 0.01$ compared with the control group; *, $P < 0.05$; **, $P < 0.01$ compared with the silica group. Error bar indicates mean ± S.D.

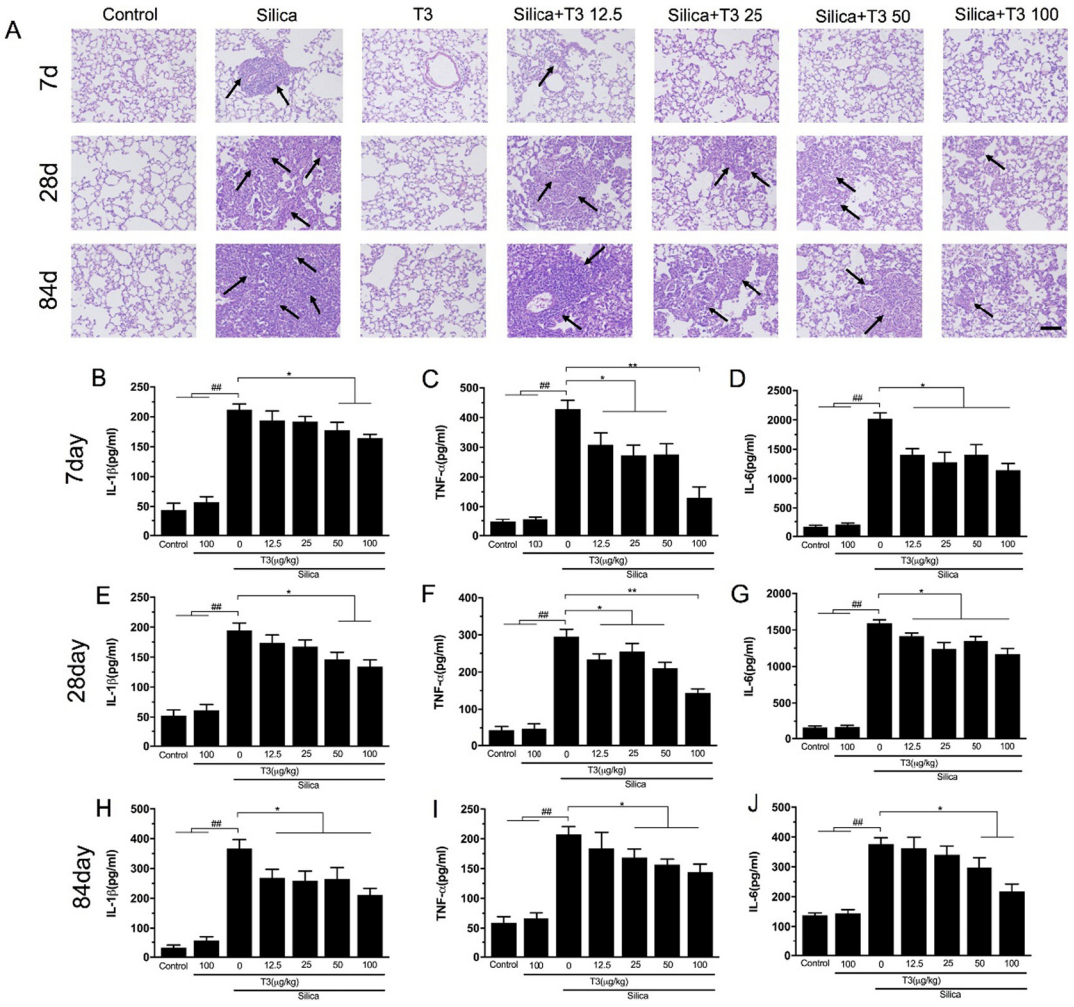


Fig. 4. Silica-induced pulmonary inflammatory responses were alleviated by intraperitoneal administration of T3. **(A)** H&E was used to stain lung sections of mice treated with T3 for 7, 28 and 84 d. Representative images are shown. Arrows point out pathological changes of lung tissue. Magnification ×200. Scale bar indicates 100 μm. **(B–D)** The levels of IL-1β, TNF-α and IL-6 in the BALF from mice for 7 d. **(E–G)** The levels of IL-1β, TNF-α and IL-6 in the BALF from mice for 28 d. **(H–J)** The levels of IL-1β, TNF-α and IL-6 in the BALF from mice for 84 d. #, $P < 0.05$; ##, $P < 0.01$ compared with the control group; *, $P < 0.05$; **, $P < 0.01$ compared with the silica group. Error bar indicates mean ± S.D.

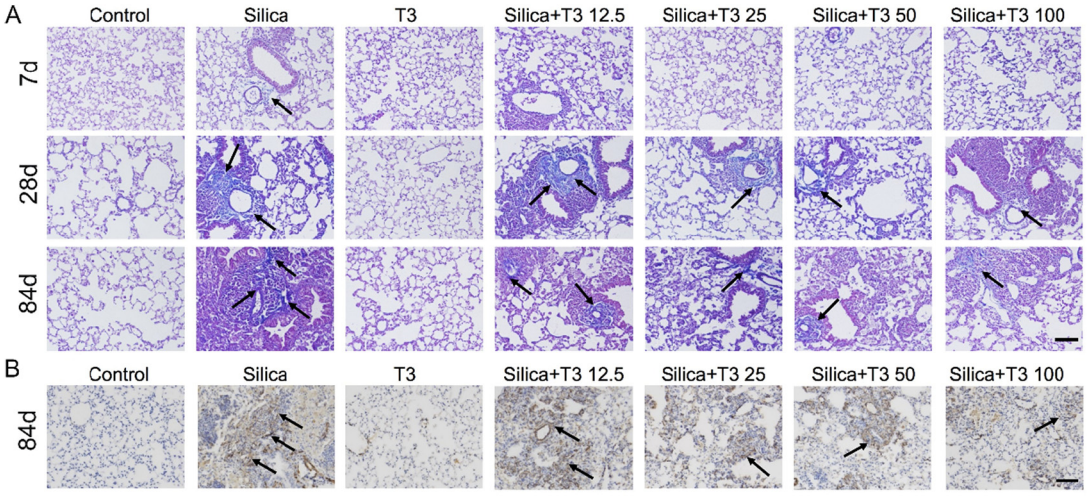


Fig. 5. Pulmonary pathological changes caused by silica were attenuated by treatment with T3. **(A)** Lung sections from mice at 7, 28 and 84 d were treated with Masson stain to visualize collagen deposition. Representative images are shown. Arrows point out pathological changes of lung tissue. Magnification ×200. Scale bar indicates 100 μm. **(B)** The results of IHC staining of α-SMA. Representative images are shown. IgG was used as a control. Arrows point out positive cells of lung tissue. Magnification ×200. Scale bar indicates 100 μm.

data suggested that T3 could protect against silica-induced pulmonary fibrosis.

3.6. Both TRα and TRβ could mediate the effects of T3 on silica-induced lung injury

THs act via binding to their nuclear receptors encoded by separate genes (TRα, TRβ). To investigate the effects of silica particles on expressions of TRs, we chose the dose of 100 μg/kg to examine the changes of TRα, and TRβ after T3 treatment at any studied time interval. Compared with the control groups, silica exposure (single intratracheal instillation) resulted in decreased expression of both TRα and TRβ in all periods of experience, especially at 7 d after instillation. T3 treatment markedly elevated the levels of the TRs as compared with the silica groups at all time points of study. While T3 (100 μg/kg) only treatment groups exhibited no significant changes in the level of TRα and TRβ as compared with the control groups (Fig. S1A–B). These results showed that the protective properties of

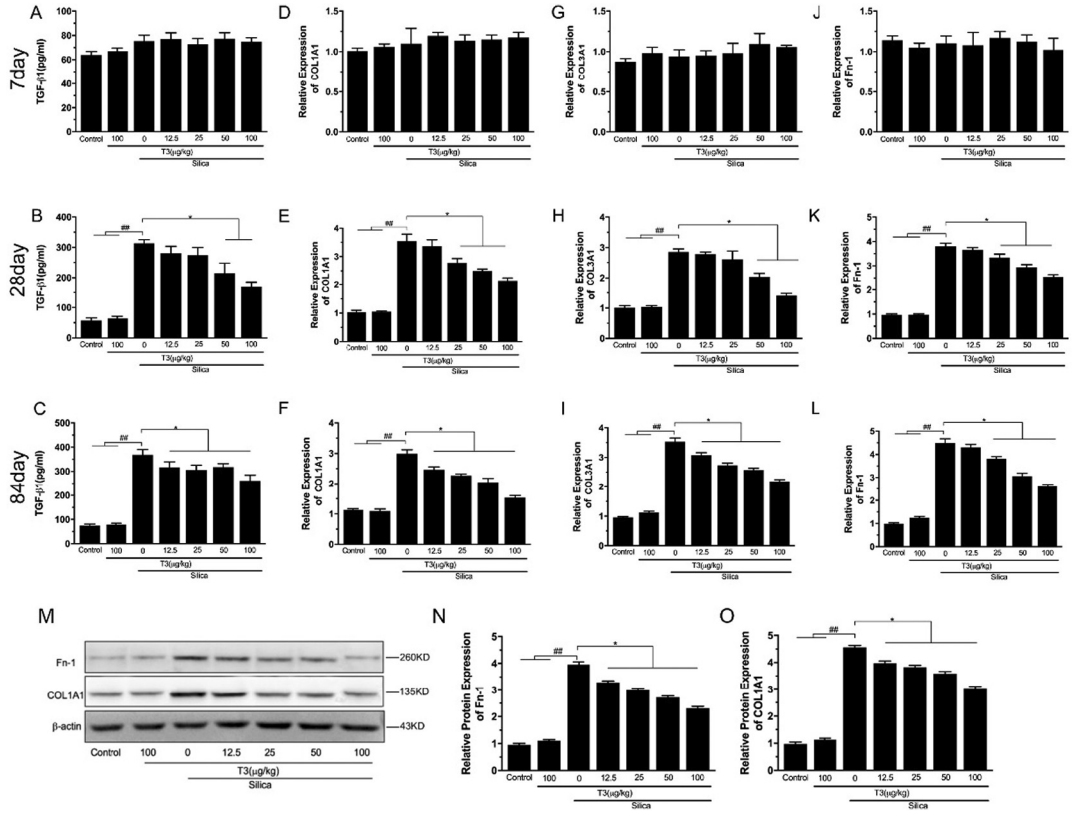


Fig. 6. T3 ameliorated silica-induced pulmonary fibrosis. **(A–C)** The levels of TGF-β1 in the BALF from mice at 7, 28 and 84 d. **(D–F)** Relative mRNA levels of COL1A1 in the lung tissues of mice at 7, 28 and 84 d. **(G–I)** Relative mRNA levels of COL3A1 in the lung tissues of mice at 7, 28 and 84 d. **(J–L)** Relative mRNA levels of Fn-1 in the lung tissues of mice at 7, 28 and 84 d. **(M)** Representative blots of Fn-1 and COL1A1 in the lung tissues of mice at 84 d. **(N–O)** Quantification of relative protein levels of Fn-1 and COL1A1. #, $P < 0.05$; ##, $P < 0.01$ compared with the control group; *, $P < 0.05$; **, $P < 0.01$ compared with the silica group. Error bar indicates mean ± S.D.

T3 on silica-induced lung injury could be partially mediated through TRα and TRβ.

3.7. T3 inhibited silica-induced pulmonary fibrosis through reducing glycolysis

Dysfunction of energy metabolism has become a major pathogenic feature of fibrotic diseases. We hypothesized that T3 affected the glycolysis process to reprogram energy metabolism. To test this hypothesis, we evaluated the contents of ATP and lactate in the lungs and found increased lactate and decreased ATP in silica-exposed mice as compared with the control group at 84 d. While T3 (100 μg/kg) treatment reversed this condition (Fig. 7A, B). To certain whether the reduced lactate was associated with an enhanced glycolysis process, we performed qPCR and Western blot analyses to evaluate the mRNA and protein levels of the key glycolysis enzymes (HK2 and PKM2) and HIF-1α in the lungs. As shown in Fig. 7C–I, the mRNA and protein levels of HK2 and HIF-1α were increased in the silica-exposed mice as compared with the control group at 84 d. Mice treated with T3 showed decreased mRNA and protein levels of HK2 and HIF-1α, while no significant changes were found in the level of PKM2 at 84 d between four groups.

3.8. T3 could delay the progression of pulmonary fibrosis caused by silica

The studies above indicated that T3 treatment could attenuate silica-induced pulmonary fibrosis when given at the same time as silica instillation. However, this did not confirm the potential of T3 as a therapeutic agent for reversing pulmonary fibrosis. To test this potential, four weeks after silica exposure, T3 (100 μg/kg) was given to mice daily for eight weeks by intraperitoneal injection (Fig. 8A). Pathological staining results showed that T3 ameliorated silica-induced lung damage and collagen deposition (Fig. 8B, C). Moreover, T3 treatment significantly reduced the protein levels of Fn-1 and type I collagen in silica-exposed mice (Fig. 8D–F). These results revealed that T3 treatment, after four weeks of silica exposure, did partly alleviate the progression of silica-induced lung fibrosis.

4. Discussion

This study attempts to analyze the lung injury caused by silica exposure from the perspective of energy metabolism, and evaluate the intervention effect of TH. It has been known for many years that the TH metabolism may be influenced during illness with decreased serum T3 and T4 (Kannan et al., 2018; Maiden and Torpy, 2019). This condition can be recognized as a useful defense mechanism for the body to resist excessive catabolism in acute phase response (Yu et al., 2018). While chronic and prolonged critical illnesses are also associated with significantly decreased plasma TH levels in recent report (Jacobs et al., 2019). A case-control study in China showed that serum T3 and T4 levels in silicosis were significantly lower than those in healthy controls, and the levels were negatively correlated with disease severity (Nian-guang et al., 2008). In the present study, we found that silica particles could not only markedly reduce the serum T3, T4, FT3 and FT4 levels, but also increased lactate and decreased ATP in a mouse model. Additionally, we also observed pathological changes of the thyroid such as follicular epithelial flatted and diffusely proliferated in silica-induced mice. We observed that silica particles contributed to persistent inflammation and cellular damage in lung tissues, which could destroy the balance of energy metabolism in the lungs and in turn caused lower TH levels (Mancini et al., 2016). Furthermore, our results showed that an elevated TSH level was observed at 84 d after silica exposure, which indicates the pituitary gland could secrete more TSH in a negative feedback manner.

In order to study the role of TH signaling in the pathogenesis of silicosis, T3 (the active form of TH) was supplemented daily to restore the

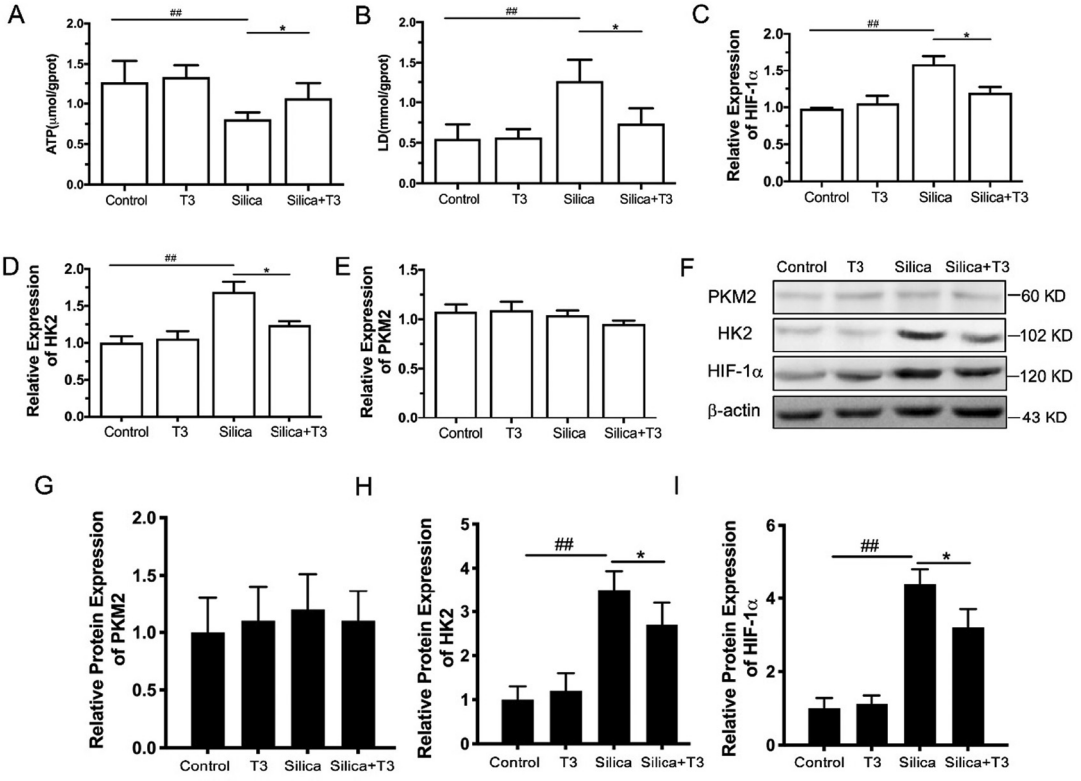


Fig. 7. T3 regulated energy metabolism reprogramming through reducing glycolysis. **(A–B)** ATP and lactate contents in lung tissues from T3-treated mice at 84 d. **(C–E)** Relative mRNA levels of HIF-1α, HK2, and PKM2 in the lung tissues of mice at 84 d. **(F)** Representative blots of HIF-1α, HK2, and PKM2 in the lung tissues of mice at 84 d. **(G–I)** Quantification of relative protein levels of HIF-1α, HK2, and PKM2. #, $P < 0.05$; ##, $P < 0.01$ compared with the control group; *, $P < 0.05$; **, $P < 0.01$ compared with the silica group. Error bar indicates mean ± S.D.

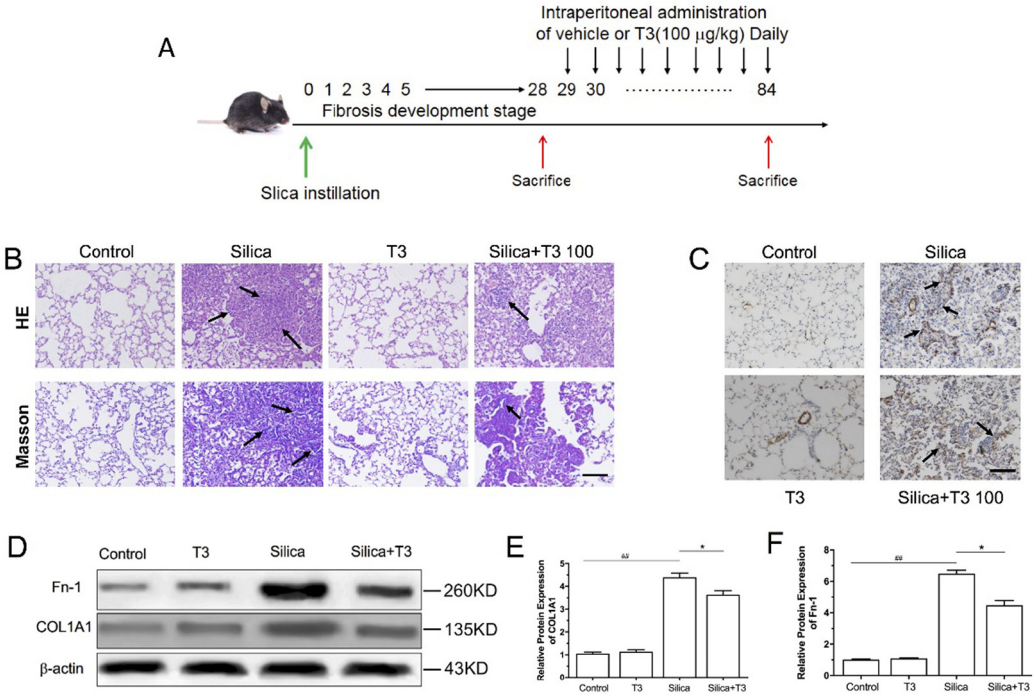


Fig. 8. T3 could delay the progression of pulmonary fibrosis caused by silica. **(A)** A mouse model of silicosis was conducted and the mice were intraperitoneal administration of T3 (100 μg/kg) or vehicle daily 28 days after silica exposure. **(B)** H&E and Masson staining of lung sections from mice at 84 d after silica instillation. Representative images are shown. Arrows point out pathological changes of lung tissue. Magnification ×200. Scale bar indicates 100 μm. **(C)** The results of IHC staining of α-SMA. Representative images are shown. IgG was used as a control. Arrows point out positive cells of lung tissue. Magnification ×200. Scale bar indicates 100 μm. **(D–F)** Representative blots and quantification of Fn-1 and COL1A1. #, $P < 0.05$; ##, $P < 0.01$ compared with the control group; *, $P < 0.05$; **, $P < 0.01$ compared with the silica group. Error bar indicates mean ± S.D.

serum T3 level. Our results showed that the T3 level was elevated after T3 administration in silica-exposed mice. While the T3 level in all treatment groups did not exceed the control group even in group with the highest T3 supplement at all time points. Moreover, no significant differences in basal metabolic rates and the vascular and nervous system were observed between T3-treated groups and control groups. In another word, the supplemented T3 of 12.5– 100 μg/kg in present study did not cause additional side effects.

When silica particles deposit in the pulmonary alveolus, they could injury the lung epithelium and blood-air barrier (Nho, 2020). In our study, the activities of LDH, ACP and AKP and the levels of TP were used as the specific markers of cell damage. Our data showed that all these indicators were increased in silica-exposed groups. Intriguingly, the decrease of these markers showed a T3-dependent trend to a certain extent after given T3 administration. These results suggested that T3 might alleviate cell damage in a dose-dependent manner when the T3 is in an appropriate dosage range.

Inhalation and ingestion of silica lead to an inflammatory response characterized by the release of cytokines, such as IL-1β, TNF-α, and IL-6 (Gan et al., 2020). These inflammation mediators exert an important role in the development of silicosis. Our results showed that silica-exposed mice treated with T3 had decreased pulmonary inflammation, and reduced levels of IL-1β, TNF-α, and IL-6 in BALF at all time points, especially when T3 is administrated at the dose of 100 μg/kg. Consistent with our findings, previous studies have shown that T3 treatment significantly reduced the pro-inflammatory markers IL-1β and TNF-α in mice after VILI (Barca-Mayo et al., 2011). Another study indicated that T3 administration could restore the expression of NLRP3 and IL-1β in ischemia-reperfusion induced mice (Vargas and Videla, 2017).

Chronic or long-term pulmonary injuries and inflammation induced by silica exposure could develop pulmonary fibrosis (Yang et al., 2019). Our findings showed that T3 could attenuate the pulmonary pathology and collagen deposition, and decreased mRNA levels of COL1A1 and COL3A1 and Fn-1 in silica-exposed mice lungs. Moreover, the protein levels of Collagen I and Fn-1 in silica-induced mice were also decreased after T3 treatment. Similarly, T3 treatment also blunted established pulmonary fibrosis in a silicosis mouse model. Consistent with the effects of T3 on the liver and skin fibrosis (Yu et al., 2018), our results strengthened the effects of T3 in fibrotic disease, especially particle-induced fibrosis.

THs exert a wide range of effects on development, growth, and cell differentiation through both genomic and non-genomic mechanisms (Davis et al., 2016; Montesinos and Pellizas, 2019). The important genomic actions of the THs are mediated by binding to nuclear TRs, encoded by two genes TRα and TRβ (Mendoza and Hollenberg, 2017). Our results showed that the mRNA levels of TRs were decreased at all time points in silica groups, while this condition was reverted after T3 administration. These results suggested that the anti-fibrotic properties may be potentially mediated through the interaction of T3 with TRs. However, future studies are needed to confirm the exact role of TRs and their downstream signaling.

Apart from the genomic actions, TH could also affect key metabolic pathways which control energy balance by regulating energy storage and expenditure[14]. Mitochondrial respiration and glycolysis are the two main energy sources that fuel biological functions in lung tissue (Wanka et al., 2012). Evidence demonstrated that inhibition of mitochondrial respiration leads to cell metabolism adaptation by increasing glycolysis and vice versa (Yoshida et al., 2013). A recent study found that the fibroblasts from the lungs of fibrotic mice or patients with idiopathic pulmonary fibrosis were both metabolically reprogrammed to maintain a high glycolytic activity (Xie et al., 2015). Consistently, our results showed that the silica exposure reduced the ATP and elevated the lactate level in the lung tissue, which indicated a switch from TCA to glycolysis. Additionally, HIF-1α is an important transcriptional factor that drives glycolysis and a key regulator of the expression of glycolytic enzymes (Zhou et al., 2018). And specific glycolytic enzymes, namely HK2 and PKM2, have been demonstrated to favour aerobic glycolysis (Zhang et al., 2018). The up-regulated levels of HIF-1α and HK2 in the current

study also confirmed the enhanced glycolysis in silica-induced mice. Importantly, the glycolysis was reduced after T3 treatment through partly reversed the ATP and lactate levels and decreased the expression of glycolytic enzymes. While the level of PKM2 was no significant change among these groups, which might indicate that the HK2 played a more important role in the progress of silica-induced pulmonary fibrosis. Cumulative evidence manifested that inhibition of glycolysis was very effective in reversing the profibrotic M2-like phenotype of alveolar macrophages (Viola et al., 2019). In addition, it has been also reported that augmentation of glycolytic activity could enhance M1 polarization in other tissues, which suggested that metabolic pathways that control macrophage behavior may be varied in different contexts or tissues (Viola et al., 2019). Overall, more in-depth research is needed to further reveal the role of glycolysis in silica-induced pulmonary fibrosis.

In this study, we show for the first time that Warburg metabolism occurs in silica-exposed mice, accompanied by decreased serum TH concentrations. T3 administrated systemically could partly reverse this shift and attenuate pulmonary damage, inflammation and fibrosis in a silicosis mouse model. Our findings inform potential treatment strategies utilizing T3 for silica-induced pulmonary inflammation and subsequent progression of fibrosis.

CRediT authorship contribution statement

Meng Yang, Shiming Gan, Yujia Xie and Lieyang Fan performed the experiments. All authors interpreted the results. Meng Yang and Weihong Chen designed the study. Meng Yang and Dongming Wang drafted the manuscript. Weihong Chen, Dongming Wang, Bin Wang, Linling Yu and Jixuan Ma proofed the manuscript.

Declaration of competing interest

We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by grants of the National Natural Science Foundation of China (81872593 and 81903291).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.148041.

References

Barca-Mayo, O., Liao, X.H., DiCosmo, C., Dumitrescu, A., Moreno-Vinasco, L., Wade, M.S., et al., 2011. Role of type 2 deiodinase in response to acute lung injury (ALI) in mice. Proc. Natl. Acad. Sci. U. S. A. 108, E1321–E1329.

Breitzig, M.T., Alleyn, M.D., Lockey, R.F., Kolliputi, N., 2018. Thyroid hormone: a resurgent treatment for an emergent concern. Am. J. Phys. Lung Cell. Mol. Phys. 315, L945–l950.

Davis, P.J., Goglia, F., Leonard, J.L., 2016. Nongenomic actions of thyroid hormone. Nat. Rev. Endocrinol. 12, 111–121.

Gan, S., Yang, M., Fan, L., Xie, L., Xu, Y., Wang, B., et al., 2020. Triiodothyronine attenuates silica-induced oxidative stress, inflammation, and apoptosis via thyroid hormone receptor α in differentiated THP-1 macrophages. Chem. Res. Toxicol. 33, 1256–1265.

Jacobs, A., Derese, I., Vander Perre, S., van Puffelen, E., Verstraete, S., Pauwels, L., et al., 2019. Non-thyroidal illness syndrome in critically ill children: prognostic value and impact of nutritional management. Thyroid 29, 480–492.

Kannan, L., Shaw, P.A., Morley, M.P., Brandimarto, J., Fang, J.C., Sweitzer, N.K., et al., 2018. Thyroid dysfunction in heart failure and cardiovascular outcomes. Circ. Heart Fail. 11, e005266.

Lai, H., Liu, Y., Zhou, M., Shi, T., Zhou, Y., Weng, S., et al., 2018. Combined effect of silica dust exposure and cigarette smoking on total and cause-specific mortality in iron miners: a cohort study. Environ. Health 17, 46.

Leung, C.C., Yu, I.T., Chen, W., 2012. Silicosis. Lancet 379, 2008–2018.

Li, C., Lu, Y., Du, S., Li, S., Zhang, Y., Liu, F., et al., 2017. Dioscin exerts protective effects against crystalline silica-induced pulmonary fibrosis in mice. Theranostics 7, 4255–4275.

Li, W., Xie, L., Ma, J., Yang, M., Wang, B., Xu, Y., et al., 2019. Genetic loss of Gas6/Mer pathway attenuates silica-induced lung inflammation and fibrosis in mice. Toxicol. Lett. 313, 178–187.

Liberti, M.V., Locasale, J.W., 2016. The Warburg Effect: how does it benefit cancer cells? Trends Biochem. Sci. 41, 211–218.

Maiden, M.J., Torpy, D.J., 2019. Thyroid hormones in critical illness. Crit. Care Clin. 35, 375–388.

Mancini, A., Di Segni, C., Raimondo, S., Olivieri, G., Silvestrini, A., Meucci, E., et al., 2016. Thyroid hormones, oxidative stress, and inflammation. Mediat. Inflamm. 2016, 6757154.

McElvaney, O.J., Zaslona, Z., Becker-Flegler, K., Palsson-McDermott, E.M., Boland, F., Gunaratnam, C., et al., 2019. Specific inhibition of the NLRP3 inflammasome as an antiinflammatory strategy in cystic fibrosis. Am. J. Respir. Crit. Care Med. 200, 1381–1391.

Mendoza, A., Hollenberg, A.N., 2017. New insights into thyroid hormone action. Pharmacol. Ther. 173, 135–145.

Montesinos, M.D.M., Pellizas, C.G., 2019. Thyroid hormone action on innate immunity. Front. Endocrinol. (Lausanne) 10, 350.

Mullur, R., Liu, Y.Y., Brent, G.A., 2014. Thyroid hormone regulation of metabolism. Physiol. Rev. 94, 355–382.

Nho, R., 2020. Pathological effects of nano-sized particles on the respiratory system. Nanomedicine 29, 102242.

Nian-guang, C., Keng-keng, C., Qiu-ying, L., 2008. Detection and analysis of serum thyroxine level in patients with gem silicosis. China Trop. Med. 8, 583–585.

Vargas, R., Videla, L.A., 2017. Thyroid hormone suppresses ischemia-reperfusion-induced liver NLRP3 inflammasome activation: role of AMP-activated protein kinase. Immunol. Lett. 184, 92–97.

Viola, A., Munari, F., Sánchez-Rodríguez, R., Scolaro, T., Castegna, A., 2019. The metabolic signature of macrophage responses. Front. Immunol. 10, 1462.

Wanka, C., Steinbach, J.P., Rieger, J., 2012. Tp53-induced glycolysis and apoptosis regulator (TIGAR) protects glioma cells from starvation-induced cell death by up-regulating respiration and improving cellular redox homeostasis. J. Biol. Chem. 287, 33436–33446.

Xie, N., Tan, Z., Banerjee, S., Cui, H., Ge, J., Liu, R.M., et al., 2015. Glycolytic reprogramming in myofibroblast differentiation and lung fibrosis. Am. J. Respir. Crit. Care Med. 192, 1462–1474.

Yang, M., Qian, X., Wang, N., Ding, Y., Li, H., Zhao, Y., et al., 2019. Inhibition of MARCO ameliorates silica-induced pulmonary fibrosis by regulating epithelial-mesenchymal transition. Toxicol. Lett. 301, 64–72.

Yao, W., Yang, P., Qi, Y., Jin, L., Zhao, A., Ding, M., et al., 2020. Transcriptome analysis reveals a protective role of liver X receptor alpha against silica particle-induced experimental silicosis. Sci. Total Environ. 747, 141531.

Yoshida, S., Tsutsumi, S., Muhlebach, G., Sourbier, C., Lee, M.J., Lee, S., et al., 2013. Molecular chaperone TRAP1 regulates a metabolic switch between mitochondrial respiration and aerobic glycolysis. Proc. Natl. Acad. Sci. U. S. A. 110, E1604–E1612.

Yu, G., Tzouvelekis, A., Wang, R., Herazo-Maya, J.D., Ibarra, G.H., Srivastava, A., et al., 2018. Thyroid hormone inhibits lung fibrosis in mice by improving epithelial mitochondrial function. Nat. Med. 24, 39–49.

Zhang, H.S., Du, G.Y., Zhang, Z.G., Zhou, Z., Sun, H.L., Yu, X.Y., et al., 2018. NRF2 facilitates breast cancer cell growth via HIF1α-mediated metabolic reprogramming. Int. J. Biochem. Cell Biol. 95, 85–92.

Zhao, X., Kwan, J.Y.Y., Yip, K., Liu, P.P., Liu, F.F., 2020. Targeting metabolic dysregulation for fibrosis therapy. Nat. Rev. Drug Discov. 19, 57–75.

Zhou, L., Wang, Y., Zhou, M., Zhang, Y., Wang, P., Li, X., et al., 2018. HOXA9 inhibits HIF-1α-mediated glycolysis through interacting with CRIP2 to repress cutaneous squamous cell carcinoma development. Nat. Commun. 9, 1480.