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AMPK agonist AICAR ameliorates maternal hepatic lipid metabolism disorder, inflammation, and fibrosis caused by $PM_{2.5}$ exposure during pregnancy

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Liver is an important target organ of ambient fine particulate matter ($PM_{2.5}$). Numerous studies have shown that $PM_{2.5}$ exposure can cause liver lipid metabolism disorders and other liver damage in mammals. However, the impact of $PM_{2.5}$ on liver health during pregnancy, a sensitive life stage, remains understudied, and the underlying mechanisms are also unknown. Given the critical role of adenosine 5′-monophosphate activated protein kinase (AMPK) in regulating lipid metabolism and inflammation, we hypothesize that AMPK activation may mitigate maternal hepatic lipid metabolism disorders, reduce inflammation, and attenuate fibrosis induced by $PM_{2.5}$ exposure during pregnancy. To test this hypothesis, pregnant C57BL/6 mice were randomly assigned to 4 groups: filtered air (FA) + NS (normal saline), $PM_{2.5}$+NS, FA + AICAR (acadesine, an AMPK activator), and $PM_{2.5}$+AICAR. $PM_{2.5}$+NS and $PM_{2.5}$+AICAR groups were continuously exposed to $PM_{2.5}$ with a whole-body $PM_{2.5}$ exposure chamber, while the other two groups were exposed to filtered air in the FA chamber. Simultaneously, the FA + AICAR and $PM_{2.5}$+AICAR groups received intraperitoneal injections of the AMPK agonist AICAR (200 mg/kg·bw per day) from gestational day 13 (GD13) to GD17, while mice in the FA + NS and $PM_{2.5}$+NS groups were administered normal saline injection. We found that gestational $PM_{2.5}$ exposure induced dyslipidemia in pregnant mice, which was alleviated by AICAR treatment. Histopathological analysis showed that the exposure to $PM_{2.5}$ during pregnancy induced hepatic lipid deposition and fibrosis in pregnant mice, and biochemical assays revealed that hepatic triglyceride and cholesterol levels were also significantly increased in pregnant mice after exposure to $PM_{2.5}$, whereas the AICAR treatment ameliorated hepatic lipid deposition and fibrosis induced by the exposure to $PM_{2.5}$ during pregnancy. Furthermore, $PM_{2.5}$ exposure during pregnancy disrupted the expression of key genes and proteins associated with hepatic lipid synthesis, cholesterol synthesis, inflammation, and fibrosis, while treatment with AICAR mitigated these effects. These findings demonstrated that AMPK activation ameliorates hepatic lipid metabolism disorders, reduces inflammation, and attenuates fibrosis caused by $PM_{2.5}$ exposure in mice during pregnancy. AMPK may be a target of action for maternal liver injury induced by $PM_{2.5}$ exposure during pregnancy.

Keywords Fine particulate matter, Gestational exposure, AICAR, Hepatic lipid metabolism, Liver injury

Fine particulate matter ($PM_{2.5}$), a major pollutant in air pollution, is the leading contributor to the global burden of disease in 2021 [1]. Epidemiological studies have shown that prolonged exposure to $PM_{2.5}$ increases the risk of various health conditions, including liver disease[2], respiratory disease[3], cardiovascular disease[4], and other multi-system diseases[5,6]. In particular, the relationship between $PM_{2.5}$ and liver disease has received widespread attention[2,7–9].

The liver is a multifunction organ and plays an important role in lipid metabolism[10]. It is responsible for synthesizing triglycerides (TGs) through fatty acid uptake and *de novo* lipogenesis (DNL), as well as fatty acid

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oxidation, fatty acid transport, and cholesterol metabolism[11]. Impairment of liver lipid metabolism can result in compromised liver function, ultimately causing a cascade of chronic liver diseases such as metabolic associated fatty liver disease (MAFLD), hepatitis, hepatic fibrosis, cirrhosis, and liver cancer[12,13]. Epidemiological studies have found that $PM_{2.5}$ exposure increased the risk of fatty lesions[14], inflammation[15], fibrosis[16], and even cancer[17] in the liver. In animals, numerous studies have shown that both short and long term $PM_{2.5}$ exposure can cause hepatic lipid deposition[18–20]. It was also found that $PM_{2.5}$ exposure induced liver inflammation and fibrosis in mice, which exacerbated the development of MALFD[21]. These findings demonstrate that exposure to $PM_{2.5}$ is a significant risk factor for liver damage, specifically in terms of disorders in liver lipid metabolism, inflammation, and fibrosis. However, there is a scarcity of research on the impact of $PM_{2.5}$ exposure on liver damage in vulnerable populations or sensitive stages of life.

Pregnancy is a special physiological period during which a series of physiological changes occur in the body, especially in the liver, which undergoes adaptive changes in order to ensure the nutrients required for pregnancy and fetal growth and development[22]. During the first and second trimesters, the liver accumulates both cholesterol and TGs to maintain the nutrients necessary for the accelerated development of the fetal in late pregnancy[23,24]. However, it predominantly becomes catabolic, transferring nutrients to the fetus via the placenta, in the third trimester[23,24]. Disturbances in the adaptation of maternal hepatic lipid metabolism can lead to gestational diabetes[25], preterm delivery[26], and other adverse pregnancy outcomes. We recently demonstrated that $PM_{2.5}$ exposure during pregnancy caused aberrant liver lipid metabolism in mice, primarily characterized by the accumulation of TGs and cholesterol esters[27]. Therefore, it is imperative to carry out additional comprehensive studies on the effects of $PM_{2.5}$ exposure during the vulnerable stage of pregnancy on maternal hepatic lipid metabolism abnormalities and the associated implications, as well as to explore potential mechanisms.

Adenosine 5'-monophosphate activated protein kinase (AMPK), a heterotrimeric protein kinase with α, β, and γ subunits, is a key cellular energy sensor. AMPKα (Thr172) modulates the transcription of genes involved in lipid metabolism, making it a crucial target for the treatment of hepatic steatosis[28,29]. AMPK activation has been shown to alleviate hepatic lipid metabolism disorders caused by $PM_{2.5}$ exposure in male mice[30]. However, it is still unknown if AMPK plays a role in the disruption of hepatic lipid metabolism and associated harm induced by exposure to $PM_{2.5}$ during pregnancy.

In this study, we established a mouse model exposure to $PM_{2.5}$ during pregnancy to evaluate the effects of gestational $PM_{2.5}$ exposure on hepatic lipid metabolism, inflammation, and fibrosis in pregnant mice and initially explored the role of AMPK in liver damage caused by $PM_{2.5}$ (Fig. 1A). This study will enhance our understanding of the impact of $PM_{2.5}$ exposure on liver in vulnerable populations and establish a foundation for the protection of these groups.

Results

Acadesine (AICAR) attenuated maternal dyslipidemia induced by $PM_{2.5}$ exposure during pregnancy

During the exposure period, the average daily concentration of $PM_{2.5}$ in the filtered air (FA) and $PM_{2.5}$ exposure chambers were 4.32 ± 1.32 and 61.31 ± 9.25 $\mu g \cdot m^{-3}$, respectively (Fig. 1B). To assess the metabolic homeostasis of maternal mice during the exposure period, we recorded the daily food intake and body weights of the mice and found that gestational exposure to $PM_{2.5}$ and/or AICAR did not affect food intake and body weight of maternal mice (Fig. 1C and D). Further, we measured serum levels of TGs, total cholesterols (TCs), low-density lipoprotein (LDLs), and high-density lipoproteins (HDLs). We found that exposure to $PM_{2.5}$ during pregnancy had no effect on maternal serum TCs levels (Fig. 1E). However, there was a significant increase in serum TGs and HDLs concentrations following gestational $PM_{2.5}$ exposure (Fig. 1F and G), while a decrease in serum LDLs was observed (Fig. 1H). Meanwhile, the FA-exposed group showed a decrease in LDL levels after administration of AICAR. It was noteworthy that these alterations in TGs and HDLs were significantly alleviated after AICAR treatment (Fig. 1F and G). Given that the liver is crucial for maintaining lipid metabolic homeostasis in the body, we measured liver injury markers glutamic pyruvic transaminase (ALT) and glutamic oxalacetic transaminase (AST) to evaluate potential impacts on hepatic function. It was observed that gestational $PM_{2.5}$ exposure increased the level of AST in the maternal serum, whereas ALT levels remained unaffected (Fig. 1I and J). However, maternal serum AST level was significantly reduced following AICAR treatment compared with $PM_{2.5}$ exposed pregnant mice (Fig. 1I). These findings indicated that AICAR treatment improved gestational $PM_{2.5}$ exposure induced maternal dyslipidemia and abnormal liver function.

AICAR ameliorated $PM_{2.5}$-induced disorders of hepatic TGs metabolism in maternal mice

Exposure to $PM_{2.5}$ and/or AICAR during gestation did not affect the liver weight of maternal mice (Fig. 2A). Nonetheless, $PM_{2.5}$ exposure markedly enlarged the region of hepatic vacuoles and the positive area of Oil Red O staining in the maternal liver; the effects were mitigated following treatment with AICAR (Fig. 2B-D). To further validate the above results, we measured the hepatic TGs levels in mice with a TGs kit and observed an increase in hepatic TGs content following $PM_{2.5}$ exposure, while AICAR treatment decreased hepatic TGs levels in the $PM_{2.5}$ exposure group (Fig. 2E). Then we examined the expression of key genes and proteins involved in lipid metabolism in the liver. In terms of hepatic lipogenesis, gene expression of regulator and rate-limiting enzymes for TGs synthesis, including sterol regulatory element-binding protein 1 (*Srebp1*), fatty acid synthase (*Fas*), and acetyl CoA carboxylase 1 (*Acc1*), was measured (Fig. 2F). We found that $PM_{2.5}$ exposure resulted in elevated expression of genes involved in TGs synthesis, which was significantly attenuated by AICAR treatment (Fig. 2F). In line with this, hepatic protein levels of SREBP1 and FAS, and the activity of ACC1 (inhibition by phosphorylation) showed similar changes (Fig. 2G-J). In addition, *Fas* gene expression was significantly reduced in the livers of FA-exposed pregnant mice after AICAR treatment. Next, we determined the key genes for fatty acid uptake and lipid export, including fatty acid translocase (*Cd36*), fatty acid binding protein 1

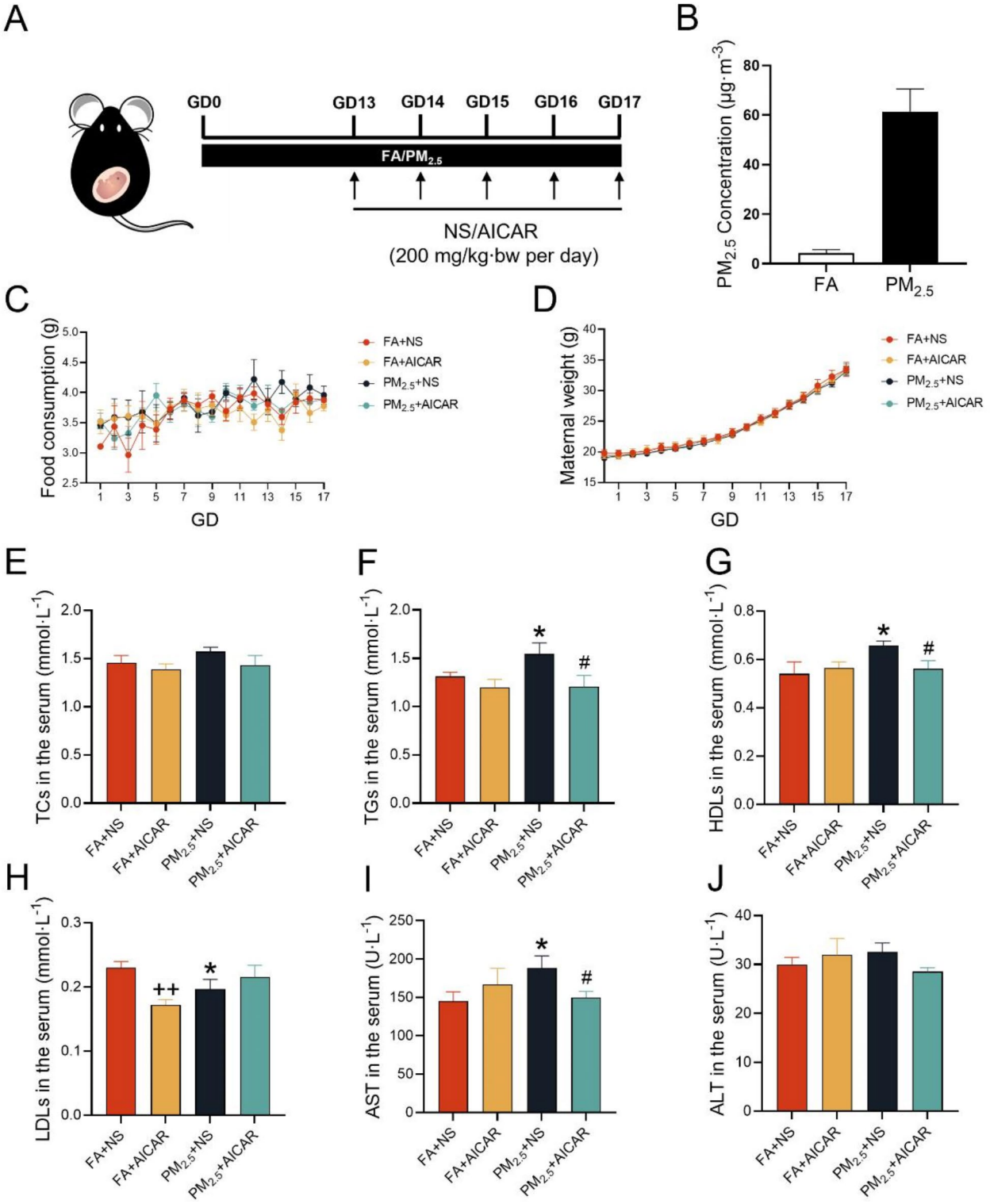

Fig. 1. AICAR ameliorated $PM_{2.5}$-induced dyslipidemia in pregnant mice. (**A**) A schematic depiction of the experimental settings. Pregnant mice exposed to FA or $PM_{2.5}$ were injected intraperitoneally with AICAR from GD13 to GD17. (**B**) Average $PM_{2.5}$ concentrations in the exposure chambers. (**C**) Average daily food intake of pregnant mice. (**D**) Body weight of pregnant mice. (**E–J**) Serum levels of TCs (**E**), TGs (F), HDLs (**G**), LDLs (H), AST (**I**), and ALT (J) in pregnant mice. $n = 6$. $^{++}P < 0.01$, compared to FA + NS group; $^{*}P < 0.05$, compared to FA + NS group; $^{\#}P < 0.05$, compared to $PM_{2.5}$+NS group.

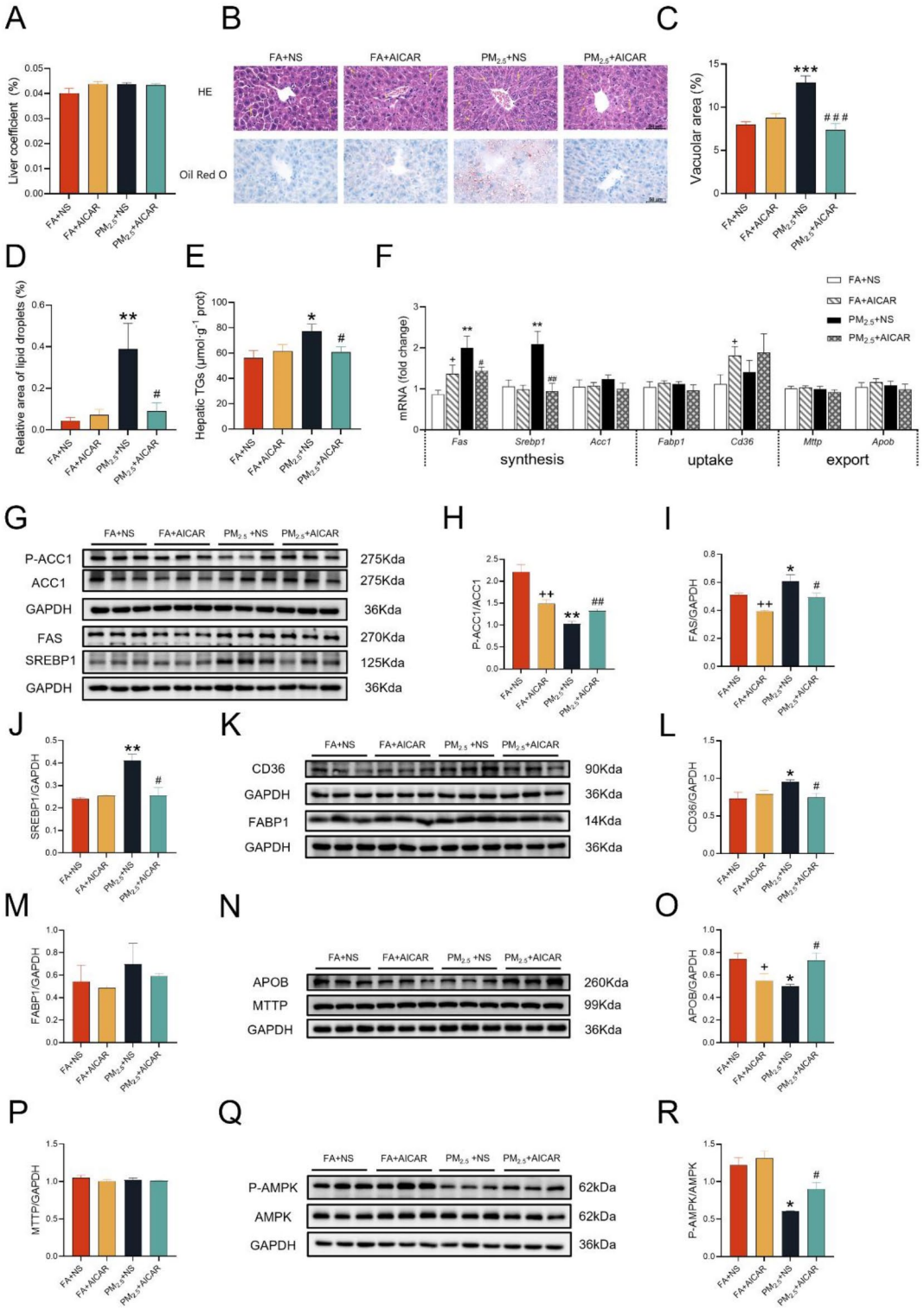

Fig. 2. AICAR alleviated $PM_{2.5}$-induced maternal hepatic TGs metabolism disorder during pregnancy. (**A**). Liver organ coefficients of pregnant mice. (**B–D**). Representative images of H&E and Oil Red O stained liver sections (**B**) and quantitative analysis of H&E-stained liver sections (**C**) and Oil Red O-stained liver sections (**D**). **E.** Hepatic TGs levels of pregnant mice. **F.** qPCR analysis of mRNA expression involved in hepatic lipid metabolism. (**G–J**). Representative bands (**G**) and hepatic protein levels of ACC1 (**H**), FAS (**I**), and SREBP1 (**J**) in pregnant mice. (**K–P**). Representative bands (**K**, **N**) and hepatic protein levels of CD36 (**L**), FABP1 (**M**), APOB (**O**), and MTTP (**P**) in pregnant mice. (**Q–R**). Representative bands (**Q**) and hepatic protein levels of P-AMPK in pregnant mice. $n = 6$ for qPCR; $n = 3$ for western blotting. $^{+}P < 0.05$, $^{++}P < 0.01$, compared to FA + NS group; $^{*}P < 0.05$, $^{**}P < 0.01$, compared to FA + NS group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared to $PM_{2.5}$+NS group.

(*Fabp1*), apolipoprotein B (*Apob*), and microsomal triglyceride transfer protein (*Mttp*) (Fig. 2F). Although $PM_{2.5}$ exposure had no effect on the expression of these genes (Fig. 2F), western blotting analysis of the corresponding proteins revealed that $PM_{2.5}$ exposure upregulated the CD36 protein while downregulated the APOB protein (Fig. 2K-O). These effects were mitigated by treatment with AICAR (Fig. 2K-O). And APOB protein expression was also significantly reduced in the livers of FA-exposed pregnant mice after AICAR treatment. In addition, we found that maternal $PM_{2.5}$ exposure during pregnancy suppressed AMPK phosphorylation in the liver, which was alleviated by AICAR injection (Fig. 2Q and R). These results suggested that $PM_{2.5}$ exposure during pregnancy can affect hepatic TG synthesis, uptake and export, leads to disturbances in maternal hepatic lipid metabolism. Activation of AMPK with AICAR significantly improved the maternal hepatic lipid metabolism disorders caused by $PM_{2.5}$ exposure.

AICAR ameliorated $PM_{2.5}$-induced hepatic cholesterol metabolism disorder in maternal mice

To better understand $PM_{2.5}$-induced disorders of maternal hepatic lipid metabolism and the role of AICAR treatment. We examined hepatic TCs levels in pregnancy mice and found that $PM_{2.5}$ exposure increased TCs levels in maternal liver, which could be significantly reduced by AICAR treatment (Fig. 3A). Further, we examined the expression of key genes and proteins involved in cholesterol metabolism in the liver, including *Srebp2*, 3-hydroxy-3-methylglutaryl coenzyme A reductase (*Hmgcr*), low-density lipoprotein receptor (*Ldlr*), ATP-binding cassette transporter A1 (*Abca1*), ATP binding cassette subfamily G Member (*Abcg1*), Abcg5 and Abcg8. We found that the expression of cholesterol synthesis genes (*Srebp2* and *Hmgcr*) and uptake genes (*Ldlr*) were significantly increased after $PM_{2.5}$ exposure (Fig. 3B), while the expression of cholesterol export genes (*Abca1* and *Abcg1*) was also increased considerably (Fig. 3B). Similarly, the $PM_{2.5}$ exposure-induced adverse effects were significantly ameliorated after administration of AICAR (Fig. 3B). Besides, in FA-exposed pregnant mice treated with AICAR, the expression of most cholesterol metabolism-related genes, with the exception of the *Abcg5* gene, was upregulated in the liver (Fig. 3B). Further we analyzed of proteins expression via western blotting, yielding comparable results (Fig. 3C-H). The above results indicated that $PM_{2.5}$ exposure during pregnancy disrupts maternal hepatic cholesterol metabolism, and AICAR treatment alleviated these disorders.

AICAR attenuated $PM_{2.5}$-induced hepatic inflammation in pregnant mice

When severe fatty lesions occur in the liver, they are often accompanied by inflammation[31]. To test whether $PM_{2.5}$ exposure induced inflammation in the maternal liver, we examined the expression of hepatic inflammatory factors. We found that $PM_{2.5}$ upregulated the expression of tumor necrosis factor-alpha (*Tnfα*) mRNA (Fig. 4A), but had no effect on the mRNA expression of interleukin-1beta (*Il-1β*), and interleukin-6 (*Il-6*) in the maternal liver (Fig. 4A). The AICAR treatment significantly reduced the upregulated maternal hepatic *Tnfα* mRNA caused by $PM_{2.5}$ exposure during pregnancy (Fig. 4A). We further verified the above alteration with western blotting analysis and found that $PM_{2.5}$ exposure increase maternal hepatic IL-1β, TNFα and IL-6 protein levels, which was alleviated by AICAR (Fig. 4B-E). These results collectively indicated that $PM_{2.5}$ exposure during pregnancy significantly impacts the protein expression of inflammatory factors in the maternal liver, and AICAR treatment effectively mitigated these inflammatory responses.

AICAR attenuated $PM_{2.5}$-induced hepatic fibrosis-like changes in pregnant mice

Hepatic lipids deposition and inflammation are important triggers for the development of fibrosis[32]. To investigate whether $PM_{2.5}$ exposure during pregnancy could induce liver fibrosis in pregnant mice, Masson's trichrome staining was performed on the liver sections of pregnant mice. As shown in Fig. 5A and B, we observed liver fibrosis-like changes in the $PM_{2.5}$ exposed group with an enlarge percentage of blue area. However, the effects were mitigated following treatment with AICAR (Fig. 5A and B). For further validation, we examined the expression of key fibrotic genes and proteins in the liver, including α-smooth muscle actin (*α-sma*), collagen type I alpha 1 (*Col1a1*), fibronectin (*Fn*), platelet-derived growth factor receptor beta (*Pdgfrβ*), and transforming growth factor beta (*Tgfβ*) (Fig. 5C). We found that $PM_{2.5}$ exposure upregulated the mRNAs expression of *α-sma*, *Col1a1*, *Fn*, and *Pdgfrβ* in maternal liver, which were significantly reduced by AICAR treatment (Fig. 5C). At the same time, we obtained the same results at the protein level by western blotting (Fig. 5D-G). In addition, α-SMA protein expression in the liver of FA-exposed pregnant mice was also significantly reduced after treatment with AICAR (Fig. 5D and G). These results indicated that AICAR reduced hepatic fibrosis-like changes induced by $PM_{2.5}$ exposure in pregnant mice.

Discussion

In this study, we explored in depth the relationship between gestational $PM_{2.5}$ exposure and maternal hepatic lipid metabolism disorder. Our primary findings revealed that $PM_{2.5}$ exposure during pregnancy inhibited phosphorylation of AMPK in the maternal liver, and activation of AMPK via AICAR attenuated elevated markers of liver injury induced by $PM_{2.5}$ exposure during pregnancy. More importantly, our research demonstrated that AICAR not only attenuated maternal hepatic lipid deposition and disturbance of hepatic lipid metabolism but also alleviated liver inflammation and mitigated hepatic fibrosis-like alterations caused by exposure to $PM_{2.5}$ during pregnancy.

Pregnancy is a special period in a female's life and is particularly sensitive to environmental pollutants. Exposure to pollutants during pregnancy not only causes harm to the developing fetus, but also may affect the health of the mother[33,34]. The short and long-term effects of $PM_{2.5}$ exposure during pregnancy on fetal development have been reported by a large number of studies[35–38]. However, few studies have addressed the potential harm to pregnant mothers. Our study revealed that $PM_{2.5}$ exposure during pregnancy induces maternal dyslipidemia, characterized by increased maternal serum levels of TGs and HDLs, and decreased LDLs levels. Consistent with our findings, $PM_{2.5}$ exposure in male or non-pregnant mice also resulted in elevated serum

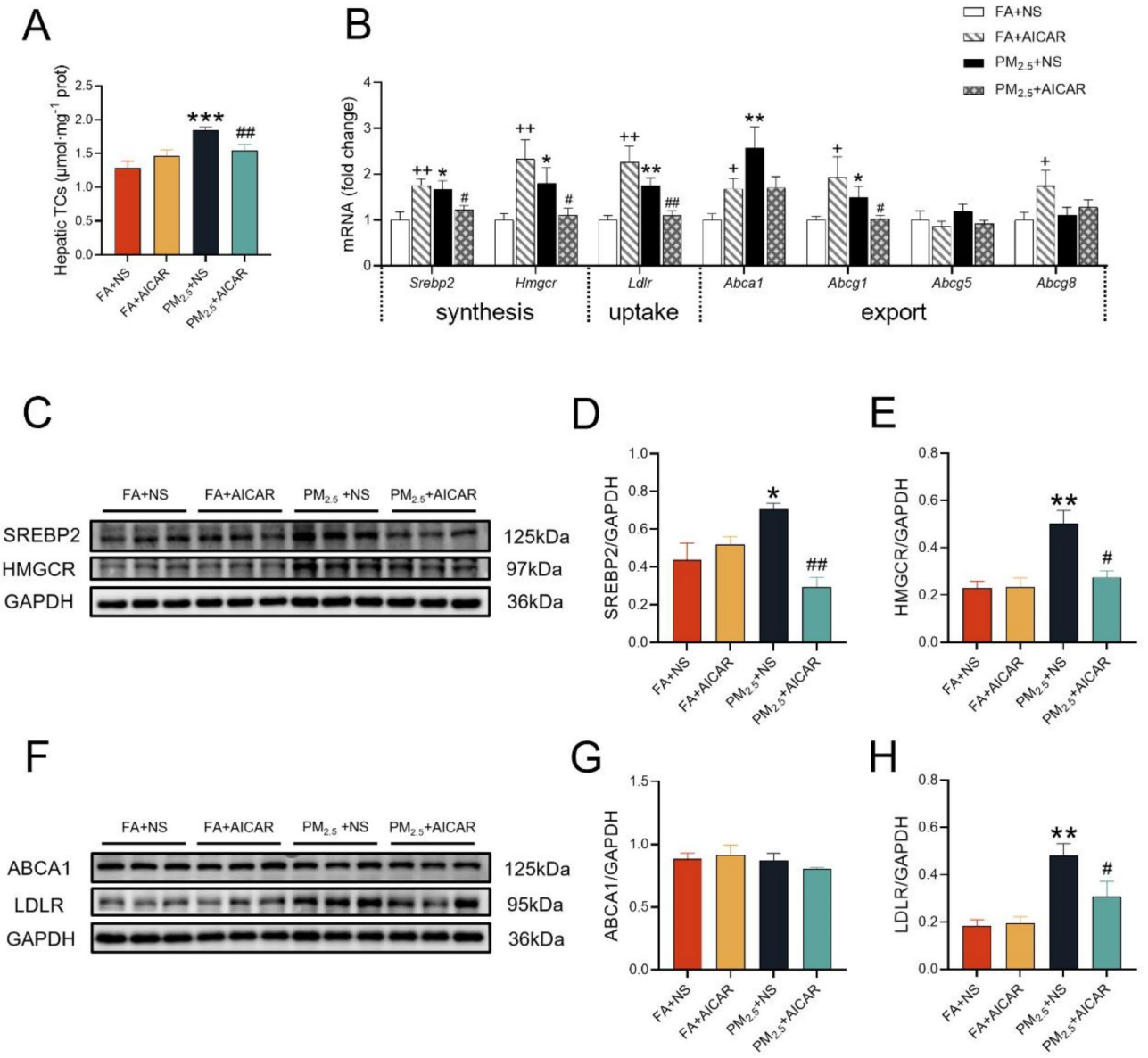

Fig. 3. AICAR alleviated $PM_{2.5}$-induced maternal hepatic cholesterol disorder during pregnancy (**A**). Hepatic TCs levels of pregnant mice **B.** qPCR analysis of mRNA expression involved in in hepatic cholesterol metabolism. **C-E.** Representative bands (**C**) and hepatic protein levels of SREBP2 (**D**) and HMGCR (**E**) in pregnant mice. **F-H.** Representative bands (**F**) and hepatic protein levels of LDLR (**G**) and ABCA1 (H) in pregnant mice. $n = 6$ for qPCR; $n = 3$ for western blotting. $^{+}P < 0.05$, $^{++}P < 0.01$, compared to FA + NS group; $^{*}P < 0.05$, $^{**}P < 0.01$, compared to FA + NS group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared to $PM_{2.5}$+NS group.

lipids levels such as TGs, leading to dyslipidemia[27,39]. Numerous studies have shown that AICAR treatment significantly improves dyslipidemia in mice[40,41]. In the present study, we found that treatment with AICAR also significantly improved the dyslipidemia caused by $PM_{2.5}$ in pregnant mice.

The liver plays an important role in lipid metabolism, serving as the hub for TG and cholesterol synthesis[10,42]. Many studies have demonstrated that $PM_{2.5}$ exposure can lead to disorders in hepatic lipid metabolism in male or non-pregnant mice[19,20,43]. In this study, we found that exposure to $PM_{2.5}$ during pregnancy can also cause disorder in hepatic lipid metabolism in pregnant mice. This was manifested in the increased content of TGs and TCs in the liver, as well as abnormal changes in the expression of key proteins related to TGs and TCs metabolism. These changes ultimately led to hepatic lipid deposition in the pregnant mice. In fact, our mouse liver samples were collected on GD17 (late pregnancy), a period when the liver is primarily engaged in catabolic metabolism, transporting TGs and cholesterol stored during the first and second trimesters of pregnancy to the rapidly growing fetal[23]. Thus, we speculated that abnormal lipid deposition in the livers of maternal mice in late pregnancy due to gestational $PM_{2.5}$ exposure may pose potential risks to both the pregnant mother and the fetus, as reported in previous studies[27,35,44].

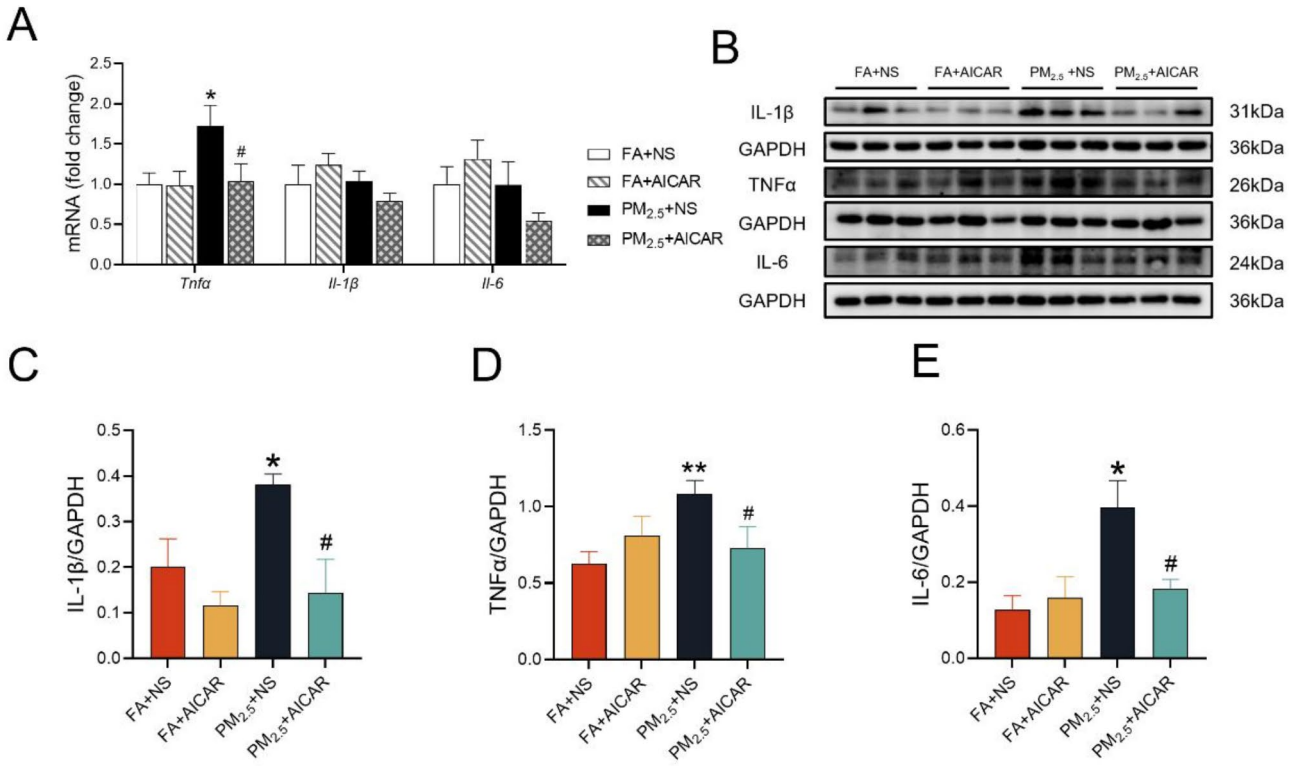

Fig. 4. AICAR alleviated $PM_{2.5}$-induced maternal hepatic inflammation during pregnancy. (**A**). qPCR analysis of mRNA expression of TNFα, IL-1β, and IL-6 in maternal liver. (**B–E**). Representative bands (**B**) and hepatic protein levels of IL-1β (**C**), TNFα (**D**), and IL-6 (**E**) in pregnant mice. $n = 6$ for qPCR; $n = 3$ for western blotting. $^{*}P < 0.05$, compared to FA + NS group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared to $PM_{2.5}$+NS group.

AMPK is a core regulatory molecule in liver lipid metabolism[45]. According to a recent study, male mice with liver lipid metabolic abnormalities caused by $PM_{2.5}$ exposure can benefit from AMPK activation[30]. Similarly, we found that the phosphorylation level of AMPK in the livers of pregnant mice decreased after exposure to $PM_{2.5}$, while treatment with the AMPK agonist AICAR in pregnant mice can alleviate the adverse effects of $PM_{2.5}$ exposure on liver lipid metabolism during pregnancy. While AICAR appears to mitigate some effects of $PM_{2.5}$ exposure, not all observed changes reached statistical significance. Specifically, certain proteins related to lipid metabolism (FABP1 and MTTP, etc.) were more strongly influenced by AICAR than others. We speculate that this may be due to the intrinsic effects of AICAR, such as its role in lipid metabolism, which could differentially impact various metabolic pathways. Therefore, our findings suggested that activation of AMPK by AICAR can partly ameliorate hepatic lipid metabolism disorders in maternal mice caused by $PM_{2.5}$ exposure.

One of the main causes of hepatitis is disturbed hepatic lipid metabolism[31]. When lipids accumulate excessively in hepatocytes, they become lipotoxic to hepatocytes, causing oxidative stress and endoplasmic reticulum stress, which in turn induces inflammation[46]. According to a recent study, persistent exposure to $PM_{2.5}$ exacerbated acute liver damage in mice by creating an inflammatory microenvironment[8]. In the present study, we found that the expression of hepatic inflammatory cytokines such as IL-1β, IL-6 and TNF-α was significantly upregulated after exposure to $PM_{2.5}$ during pregnancy, consistent with previous findings in male mice[9,47]. Studies have demonstrated that inflammatory cytokines activate hepatic stellate cells, leading to increased collagen deposition and thereby triggering fibrogenesis[48]. We found that $PM_{2.5}$ exposure during pregnancy upregulated the expression of key proteins in hepatic fibrosis and causes fibrotic-like changes in the livers of pregnant mice. Interestingly, just as with the liver lipid metabolism disorders mentioned earlier, the inflammation and fibrotic-like changes in the livers of pregnant mice caused by $PM_{2.5}$ exposure were alleviated after AICAR administration. These results were not surprising, as numerous studies have confirmed that the activation of AMPK can improve liver inflammation, fibrosis, and other injuries[28,29,49]. Additionally, consistent with a recent population report that identified an association between gestational exposure to $PM_{2.5}$ and increased levels of the liver injury marker bilirubin in the blood of pregnant women[33], we found that exposure to $PM_{2.5}$ during pregnancy significantly increased the levels of AST, another marker indicative of liver inflammation and injury, in the serum of pregnant mice. And treatment with AICAR ameliorated this alteration. Taken together, maternal liver injury induced by $PM_{2.5}$ exposure during gestation, along with the protective effects of AICAR, were evident at the hepatic tissue level and systemically.

This research, while shedding light on the protective role of AICAR against $PM_{2.5}$-induced liver injury in pregnancy, is not without its limitations. Firstly, the long-term implications of this treatment on maternal liver health and fetal growth and development remain uncertain. Besides, pregnancy is a critical phase, and the

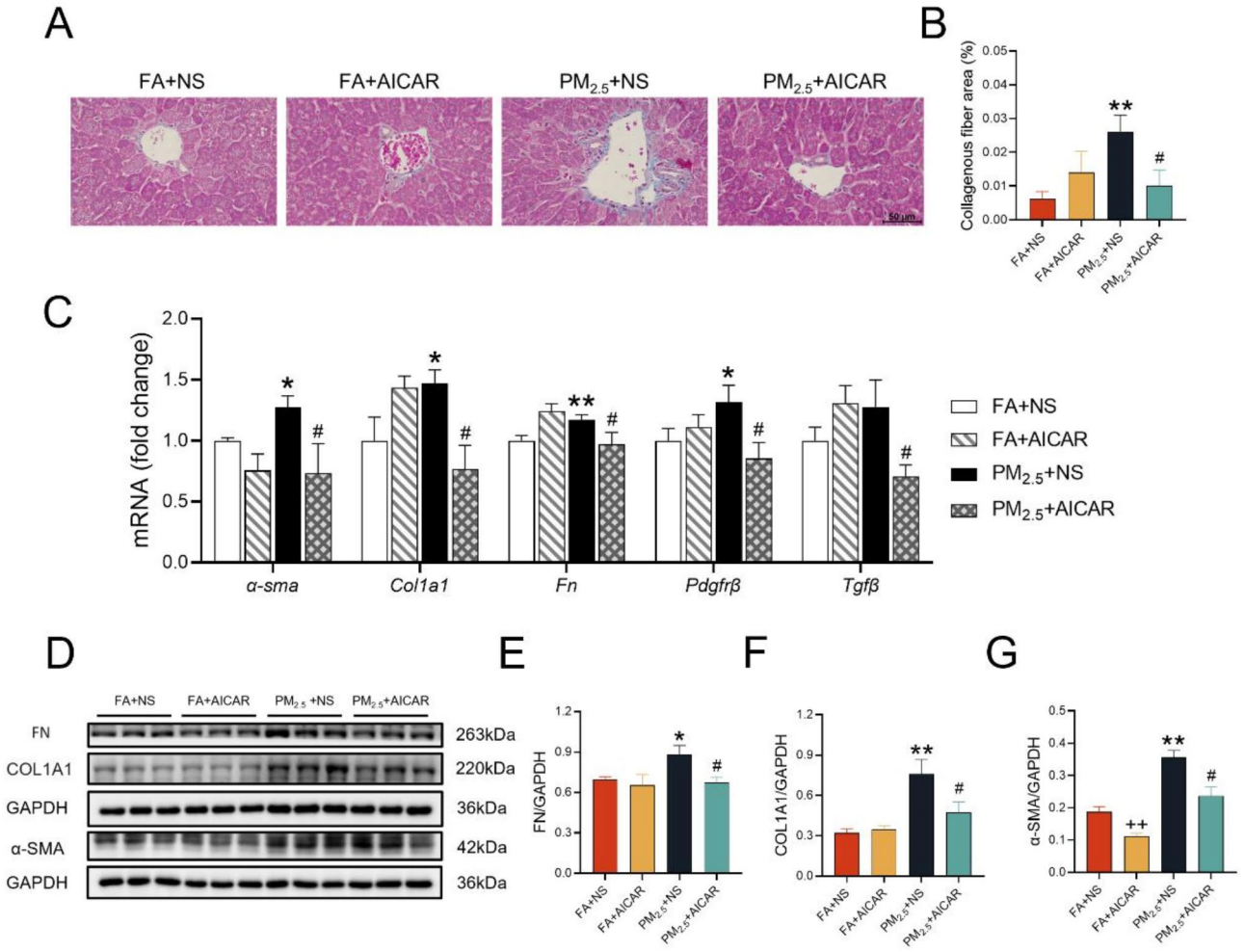

Fig. 5. AICAR alleviated $PM_{2.5}$-induced maternal hepatic fibrosis-like changes during pregnancy. (**A–B**). Representative images of Masson staining liver sections and quantitative analysis of Masson staining liver sections. (**C**). qPCR analysis of mRNA expression involved in hepatic fibrosis. (**D-G**) Representative bands (**D**) and hepatic protein levels of FN (**E**), COL1A1 (**F**), and α-SMA (**G**) in pregnant mice. $n = 6$ for qPCR; $n = 3$ for western blotting. $^{++}P < 0.01$, compared to FA + NS group; $^{*}P < 0.05$, $^{**}P < 0.01$, compared to FA + NS group. $^{\#}P < 0.05$, compared to $PM_{2.5}$+NS group.

judicious use of medications is imperative to prevent any adverse impact on fetal development[50]. Additionally, while AICAR showed promising results in this study, determining the optimal therapeutic dose through dose-response experiments is essential to maximize efficacy and minimize potential side effects. Moreover, our conclusion that AMPK activation by AICAR ameliorates liver injury is based on the observed improvements in lipid metabolism and inflammation. However, the study lacks a detailed investigation of the upstream and downstream targets of AMPK, which are crucial for understanding the complete mechanistic pathway. The specificity of AICAR as an AMPK agonist is also a consideration, as its effects may not be entirely selective. Future studies should incorporate complementary approaches, such as using AMPK inhibitors or knockout models, to validate the AMPK-dependent effects and provide a more definitive understanding of the underlying mechanisms.

Conclusion

This study investigated how $PM_{2.5}$ exposure during pregnancy affected the maternal liver. According to our findings, pregnant mice exposed to $PM_{2.5}$ had abnormal hepatic TGs and TCs metabolism, which resulted in hepatic lipid accumulation and subsequently promoted hepatic inflammation and fibrosis. Furthermore, the negative effects of $PM_{2.5}$ exposure during pregnancy can be mitigated by the treatment of the AMPK activator AICAR. This study offers toxicological proof of how exposure to $PM_{2.5}$ affects liver function during pregnancy and identifies potential treatment targets.

Materials and methods

Animals and treatment

Male and female C57BL/6 mice (6–7 weeks old) were acquired from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Mice were adaptively fed for 2 weeks in a constant temperature environment (22 ± 2 °C) with 12 h of alternating light and dark, and mice had free access to standard rodent feed and water. After adaptive feeding, male and female mice were housed together in a ratio of 1:2 and kept overnight starting at 8:00 p.m. At 8:00 a.m. the next morning, mice that were checked for vaginal plugs were documented as being on gestational day (GD) 0. Pregnant mice were randomly divided into 4 groups: FA + NS, $PM_{2.5}$+NS, FA + AICAR, and $PM_{2.5}$+AICAR, with 6 pregnant mice in each group. Two chambers, FA chamber and $PM_{2.5}$ chamber, were used in this study. The FA chamber was equipped with a high-efficiency particulate air (HEPA) filter, ensuring the exclusion of all particulate matter from the ambient air. The $PM_{2.5}$ chamber was filled with concentrated ambient $PM_{2.5}$ dispersed in filtered air. Mice in $PM_{2.5}$+NS and $PM_{2.5}$+AICAR groups were continuously exposed to $PM_{2.5}$ with a whole-body $PM_{2.5}$ exposure chamber on the campus of Zhejiang Chinese Medical University, located in Binjiang District, Hangzhou City, Zhejiang Province, China. Mice in the other two groups were exposed to filtered air in the FA chamber. The pregnant mice were exposed to FA or $PM_{2.5}$ 12 h a day from 8:00 a.m. to 8:00 p.m. from GD0 to GD17. Simultaneously, the FA + AICAR and $PM_{2.5}$+AICAR groups received AICAR (200 mg/kg·bw per day) through intraperitoneal injection for 5 days starting on GD13, while mice in the FA + NS and $PM_{2.5}$+NS groups were administered normal saline injection (Fig. 1A). We selected GD13-GD17 for AICAR intervention was based on the developmental equivalence between mouse and human pregnancy stages. This period is analogous to the third trimester in human pregnancy, which is also a time of rapid fetal growth and significant metabolic adaptations in the mother. All pregnant mice were euthanized by cervical dislocation at GD17. The average daily food intake was determined by recording the total amount of food consumed by each cage of mice and dividing by the number of mice per cage. This calculation was performed daily throughout the exposure period. $PM_{2.5}$ samples were collected on Teflon filters (Pall Life Sciences, Ann Arbor, Michigan, USA) during the exposure period and weighed in a temperature- and humidity-controlled room using an Excellence Plus XP microbalance (Mettler Toledo, Greifensee, Switzerland) to calculate $PM_{2.5}$ concentration in the exposure chamber. The AICAR was purchased from MedChemExpress (cat# HY-13417, New Jersey, USA). The animal study protocol was approved by the Animal Ethical and Welfare Committee of Zhejiang Chinese Medical University (Approval number: IACUC-20241125-21), and all methods were performed in accordance with the relevant guidelines and regulations. Furthermore, all animal experimental procedures strictly adhered to the ARRIVE Guidelines.

Histological analysis

Fresh liver tissues were collected and fixed in paraformaldehyde solution and shaker fixed for 48 h, followed by dehydration and embedding. The embedded wax blocks were cut and stained with HE or Masson's trichrome stain. In addition, some fresh liver tissues were collected for OCT embedding, and subsequently sectioned into 7 μm slices using a microtome, and the frozen sections were fixed and stained with Oil Red O. All sections were observed using a Leica DM6B microscope. For each section, five non-adjacent fields of view were randomly chosen and photographed under the microscope. The images obtained were then analyzed using the ImageJ Fiji software.

Biochemical analysis

The blood of mice was collected and centrifuged at 3000 rpm to take the upper layer of serum. The serums were sent to the Zhejiang Chinese Medical University Laboratory Animal Research Center for analysis of serum biochemical parameters, including TGs, TCs, HDLs, LDLs, ALT, and AST.

Hepatic TGs and TCs detection

The hepatic levels of TGs and TCs were measured using a TG assay kit (cat# E1013, Applygen Technologies Inc., Beijing, China) and a TC detection kit (cat# BC1985, Solarbio Science & Technology Co., Ltd., Beijing, China), respectively, following the manufacturer's instructions. Protein levels of the liver extracts were detected with a BCA protein assay kit (cat# P0011, Beyotime Biotechnology, Shanghai, China) to calibrate the levels of TGs and TCs.

RNAs isolation and qPCR

Total RNAs from liver tissue were extracted with RNAiso Plus reagent (cat# 9109, Takara, Japan) according to the manufacturer's instructions and then reverse transcribed into cDNAs using a reverse transcription kit (cat# R423-01, Nanjing Vazyme Medical Technology Co. Ltd., Nanjing, China). cDNAs were configured into qPCR reaction mixes according to the Taq Pro Universal SYBR qPCR Master Mix instructions. The QuantStudio Q7 Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA) was used to perform real-time qPCR, and the relative gene expression levels were calculated using the $2^{-\Delta\Delta CT}$ method following normalization to β-actin. qPCR primers used were listed in Table S1 in Supplementary Materials.

Protein extraction and Western blotting

Whole lysates from liver tissues were extracted with RIPA buffer (cat# AR0102-100, Beyotime Biotechnology, Shanghai, China) containing a protease inhibitor cocktail. The obtained proteins were separated by SDS-PAGE gel and then transferred to immobilon-P polyvinylidene difluoride (PVDF) membranes. The obtained PVDF membranes were incubated overnight at 4 °C with a specific primary antibody, and the next day they were incubated with a secondary antibody at room temperature before being imaged for signal intensity by the

ChemiDoc Imaging System (Bio-Rad, Hercules, California, USA). The detailed information of the antibodies was summarized in Table S2 in Supplementary Materials.

Statistical analysis

All statistical analyses were performed with GraphPad Prism 9.5 software. Quantified data were presented as the mean ± standard error of the mean (SEM). Multi-group data was analyzed by one-way ANOVA followed by Fisher's LSD testing. *P*-values lower than 0.05 were considered significantly different.

Data availability

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

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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