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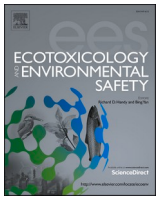

Long-term exposure to air pollution, habitual physical activity and risk of non-alcoholic fatty liver disease: A prospective cohort study

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ABSTRACT

Background: Emerging studies suggest a positive association between air pollution exposure and risk of non-alcoholic fatty liver disease (NAFLD), however, the combined effects of long-term exposure to air pollution, physical activity (PA), and risk of NAFLD is unclear.

Methods: We included 58,026 Taiwan residents who received a standard medical screening program between 2001 and 2016. Levels of fine particulate matter ($PM_{2.5}$) at each participant's residential address were estimated using multiple satellite-based aerosol optical depth data combined with a chemical transport model. PA volume was calculated as hours of metabolic equivalent tasks per week (MET-h/week) based on a standard self-administered questionnaire. Incident NAFLD was defined as the first occurrence of a fatty liver index (FLI) value > 30 or a hepatic steatosis index (HSI) value > 36 in participants without NAFLD at the baseline. Time-varying Cox regression was used to evaluate the combined effects of PA and $PM_{2.5}$.

Results: Exposure to $PM_{2.5}$ was positively associated with NAFLD. A 1 $\mu g/m^3$ increase in $PM_{2.5}$ above 23.5 $\mu g/m^3$ was associated with a hazard ratio (HR) of 1.06 (95% CI: 1.04, 1.09) and 1.05 (95% CI: 1.03, 1.07) for NAFLD identified by FLI and HSI, respectively. Performing PA was inversely associated with NAFLD. Compared with participants in high $PM_{2.5}$ [$\geq$ 27.5 $\mu g/m^3$]–very low PA [$<$ 3.75 MET-h/week] group, low $PM_{2.5}$ [$<$ 23.5 $\mu g/m^3$]–very high PA [$\geq$ 25.50 MET-h/week] group had a 57% (95% CI: 50%, 63%) and 42% (95% CI: 33%, 50%) lower risk of NAFLD defined by FLI and HSI, respectively. We found no evidence of any additive or multiplicative interaction between PA and $PM_{2.5}$.

Conclusion: Long-term $PM_{2.5}$ exposure was positively associated with NAFLD, whereas performing PA was inversely associated with NAFLD. The benefits of PA on NAFLD remained stable in participants exposed to various $PM_{2.5}$ levels.

1. Introduction

As a leading chronic liver disease, non-alcoholic fatty liver disease (NAFLD) is now affecting ~25% of the general population and ~60% of obese individuals globally (Younossi et al., 2016). NAFLD is a multi-system disease impacting the liver and extra-hepatic organs, such as the cardiovascular system (Byrne and Targher, 2015). It is a precursor of cardiometabolic diseases and liver pathological conditions, such as steatohepatitis or hepatocellular carcinoma (Stefan et al., 2019). The burden of NAFLD is not uniform across the globe, and the regions experiencing a rapid surge in obesity are especially concerning, such as Taiwan (Golabi et al., 2021).

Air pollution is a newly recognized emerging risk factor for NAFLD (Guo et al., 2021; Li et al., 2017; Sun et al., 2022; Zheng et al., 2013).

Abbreviations: NAFLD, Non-alcoholic fatty liver disease; FLI, Fatty liver index; HSI, Hepatic steatosis index.

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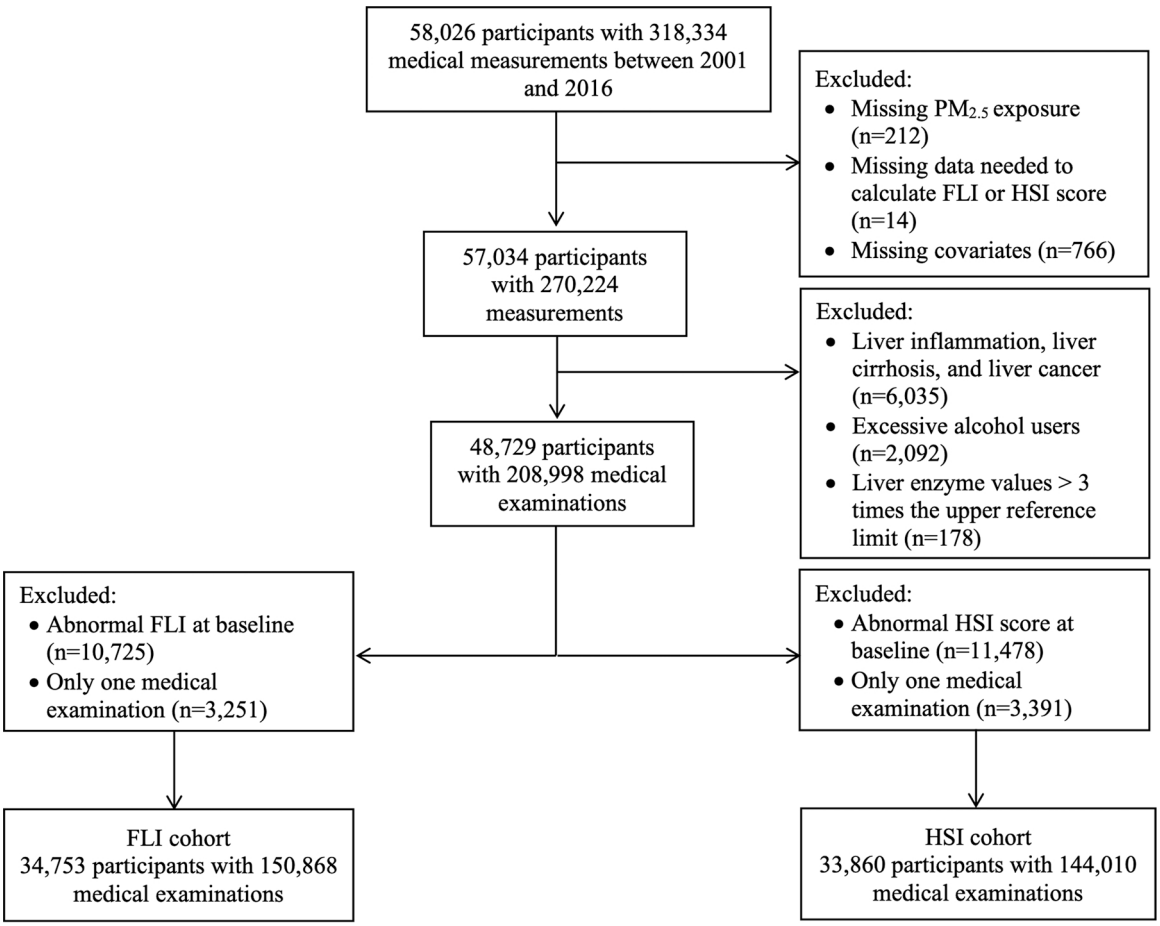

Fig. 1. Flow chart of the study population.

The potential mechanisms underlying the association between air pollution and NAFLD include inflammation, oxidative stress, insulin resistance, and specific molecular and metabolic derangements (Arciello et al., 2013). Although performing physical activity (PA) for NAFLD patients is recommended (Lim et al., 2017), during PA more air pollutants are inhaled with increased respiration rates, exacerbating the adverse effects of air pollution on NAFLD. However, this hypothesis has not been tested.

The risk-benefit assessment of air pollution and PA on NAFLD has become critical given the expected increasing trend in the burden of NAFLD and a high proportion of people residing in areas with poor air quality globally (Golabi et al., 2021). Accordingly, we sought to evaluate the combined effects of long-term exposure to air pollution, PA, and risk of NAFLD in Taiwan.

2. Patients and methods

2.1. Study population

We leveraged a prospective cohort of Taiwan residents who participated in a standard medical screening program in Taiwan (MJ Health Research Foundation, 2021). We selected 58,012 participants who visited the institution at least twice and were followed up at least five years from 2001 to 2016 when $PM_{2.5}$ data were available (Chalasani et al., 2012). Of these, we excluded: a) 992 participants with missing values of $PM_{2.5}$, fatty liver index (FLI), hepatic steatosis index (HSI), or any of the covariates included in the fully adjusted model described below, b) 6213 participants at baseline diagnosed with hepatitis, cirrhosis, liver cancer, or acute liver diseases. Note acute liver disease was defined as alanine aminotransferase test (ALT) > 135 U/L, aspartate aminotransferase test (AST) > 120 U/L, or gamma-glutamyl transferase (GGT) > 165 U/L, c) 2092 participants with excess alcohol intake (EASL, 2016), and d) 13,976 FLI-based cohort participants or 14,869 HSI-based cohort participants either had NAFLD at baseline or underwent only one medical examination. The final analytic sample included 34,753 FLI-based cohort participants or 33,860 HSI-based cohort participants (Fig. 1 **&** Fig S1). We obtained written informed consent from each participant before each medical assessment, and obtained ethical approval for this study from the ethics committee of the Peking University Health Science Center (NO. IRB00001052-20026).

2.2. Habitual physical activity assessment

We used a standard questionnaire to collect information on habitual PA, which includes type of PA, frequency of PA per week, and duration per session during the last four weeks. We classified participants' most frequently performed type of PA into no, light, moderate, medium-vigorous, or high-vigorous. We then assigned a value of standard metabolic equivalent of task (MET) to the intensity of each PA type based on the Ainsworth compendium: < 1.0 (no), 2.5 (light), 4.5 (moderate), 6.5 (medium-vigorous), and 8.5 (high-vigorous PA) (Wen et al., 2011). We calculated the total time spent on PA per week using the product of the hours and frequency spent on PA each week. If a participant reported PA with more than one intensity category, we then assigned a weighted MET to the participant based on the time spent in each category. We finally quantified volume of PA by calculating hours of MET per week (MET-h/week) by multiplying PA intensity and total time spent on PA per week (Sun et al., 2020). We categorized PA volume into very low (<3.75 MET-h/week), low (3.75–7.49 MET-h/week), moderate (7.50–16.49 MET-h/week), high (16.50–25.49 MET-h/week),

and very high (≥25.50 MET-h/week) following classifications in PA guidelines for Americans (Piercy et al., 2018). We used both the continuous and categorical PA for the data analysis.

2.3. Long-term air pollution assessment

To estimate $PM_{2.5}$ concentrations, we used the global geographically weighted regression-adjusted satellite-derived $PM_{2.5}$ estimates at 0.01° × 0.01° spatial resolution (Van Donkelaar et al., 2016). These data were derived from atmospheric optical depth (AOD) measurement instruments measured from eight different satellites. AOD value from each source was retrieved, calibrated, simulated, and then translated onto a common 0.01° × 0.01° grid. The resultant estimated $PM_{2.5}$ estimates have been validated and have been used to estimate the adverse health effects of $PM_{2.5}$ (Balakrishnan et al., 2019; Heft-Neal et al., 2018). We assigned each participant's annual $PM_{2.5}$ concentrations from 2000 to 2016 based on his/her residential address. We used a two-year average $PM_{2.5}$ concentration as a proxy for long-term air pollution exposure (Chan et al., 2018; Guo et al., 2020). We grouped all observations into three categories: high $PM_{2.5}$ (upper 15th percentile of the exposure range: ≥27.5 μg/m^3), moderate $PM_{2.5}$ (23.5–27.5 μg/m^3), and low $PM_{2.5}$ (lower than the identified breakpoint in the fully adjusted model described below: <23.5 μg/m^3). In sensitivity analysis, we also used the upper 25th percentile of the $PM_{2.5}$ exposure range (≥26.5 μg/m^3) as the cut-off to categorize high $PM_{2.5}$ group. We used both the continuous and categorical $PM_{2.5}$ exposure for the data analysis.

2.4. Assessment of NAFLD

Imaging or histology is the recommended method to detect hepatic steatosis, however, these methods are costly and are not feasible for fatty liver screening in large population. FLI and HSI are two alternative methods to diagnosis NAFLD, which have been widely used in prior large epidemiological studies (Byambasukh et al., 2019; Gastaldelli et al., 2009; Stefan et al., 2019; Sun et al., 2022). In this study, we defined incident NAFLD as the first occurrence of an FLI value > 30 or an HSI value > 36 in participants without NAFLD at the baseline (Lee et al., 2010; Zhu et al., 2018).

$$FLI = \left(\frac{e^{0.953\times\ln(triglycerides)+0.139\times BMI+0.718\times\ln(GGT)+0.053\times WC-15.745}}{1+e^{0.953\times\ln(triglycerides)+0.139\times BMI+0.718\times\ln(GGT)+0.053\times WC-15.745}}\right)\times 100$$

where triglycerides are measured in mg/dL, GGT in IU/L, BMI in kg/m^2, and waist circumference (WC) in cm.

$$HSI = 8\times\frac{ALT}{AST}ratio + BMI \quad (+2, \quad if \quad DM; +2, \quad if \quad female)$$

where DM represents diabetes mellitus, and ALT is measured in mg/dL, AST in mg/dL, and BMI in kg/m^2.

2.5. Covariates

Clinical examinations were conducted by trained technicians (Supplementary material). Participants at each follow-up were examined WC, blood pressure, body weight, height, serum fasting glucose (FPG), triglyceride, AST, ALT, and GGT. Demographic characteristics, socioeconomic status, lifestyles, and medical history were collected via a standard self-administered questionnaire.

Disease history at each visit was determined based on self-reported physician-diagnosis, supplemented by clinical measurements, which included cancer (self-reported), long-term use of hyperlipidemia drugs (self-reported), cardiovascular diseases (self-reported), hypertension (self-reported and supplemented by the seated systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg), and diabetes mellitus (self-reported and supplemented by fasting blood glucose ≥126 mg/dL).

2.6. Statistical analysis

We used Cox proportional hazards regression with time-varying covariates to estimate the association of long-term exposure to $PM_{2.5}$ and PA with risk of NAFLD stratified by age at follow-up and by calendar year. We selected follow-up time as the timescale and was calculated as the date of recruitment to the first occurrence of NAFLD or the date of the last visit. We found no evidence of departures from the proportional hazards assumption.

We used three models with different combinations of covariates to investigate the association of NAFLD with $PM_{2.5}$ or PA. We first used a minimally adjusted model including age and year of enrollment in the model. We next used a partially adjusted model that additionally adjusted for sex, smoking status, alcohol consumption, educational attainment, fruit intake, occupational exposure to dust or solvent (yes versus no), vegetable intake, fried food intake, sugary drink intake, PA at work, season of the medical examination, and mutually adjusted for PA and $PM_{2.5}$. In the models, we did not adjust for BMI, which has been considered by FLI or HSI. For the same reason, we also did not adjust for sex in the HSI-based models. Finally, we used a fully adjusted model additionally adjusted for long-term use of hyperlipidemia drugs, cardiovascular disease, cancer, and hypertension. Note that all covariates are time varying. For metabolic factors, such as high levels of triglycerides or diabetes, we did not adjust them in the main models as they are more likely to be a mediator in the relationship between long-term exposure to air pollution and NAFLD (Wang et al., 2017).

To examine the dose-response curve for the association of NAFLD with $PM_{2.5}$ or PA, we alternatively modeled $PM_{2.5}$ or volume of PA using a restricted cubic spline with three knots placed at 10th, 50th, and 90th percentile. When a departure from linearity was detected, we used piecewise Cox proportional hazards regressions to identify potential breakpoints (Sun et al., 2022).

To assess the combined effects of PA and $PM_{2.5}$, we cross-grouped participants into those residing in low (<23.5 μg/m^3), moderate (23.5–27.5 μg/m^3), and high (≥27.5 μg/m^3) $PM_{2.5}$ areas and across PA categories (very low, low, moderate, high, and very high) with reference to participants who performed very low PA in areas of high $PM_{2.5}$ levels. To test the multiplicative interaction, we added a product term between continuous $PM_{2.5}$ and continuous volume of PA in the fully adjusted models, and the p-value of the interaction term< 0.05 indicates a significant interaction. We also tested the additive interaction by calculating the relative excess risk due to interaction (RERI) (Andersson et al., 2005).

To examine the robustness of the combined effects between PA and $PM_{2.5}$, we conducted a secondary analysis to examine the prospective association between changes in PA and risk of NAFLD by air pollution levels. We used the fully adjusted time-varying Cox regression models described above with additionally adjusted for volume of PA immediately preceding visit. We calculated the change in PA (continuous) as the difference in PA volume at a follow-up visit minus the value of the immediately preceding visit. To model the dose-response relationship between changes in PA and NAFLD, we also used a restricted cubic spline with three knots located at 10th, 50th, and 90th percentile of PA changes with a change of 0 MET-h/week as the reference value.

We performed four main sensitivity analyses. First, to exclude the confounding effects of alcohol drinking, we restricted our analysis to never-drinkers, and repeated the main analysis. Second, to examine the potential influence of metabolic factors on the association between $PM_{2.5}$ and NAFLD, we additionally adjusted for the metabolic syndrome in the fully adjusted models (Tan et al., 2004). Third, we also varied $PM_{2.5}$ cut-offs and used the upper 25th percentile of the $PM_{2.5}$ exposure range (≥26.5 μg/m^3) as the cut-off to categorize high $PM_{2.5}$ group. Fourth, NAFLD is an outcome that can be reversed through lifestyle changes including the increase of physical activity. We excluded participants who recovered the next year after the year of diagnosis.

All analyses were performed in R software with "Segmented"

Table 1
Participant baseline characteristics of the fatty liver index (FLI) cohort and hepatic steatosis index (HSI) cohort, 2001–2016.

Characteristics	FLI cohort (n = 34,753)	HSI cohort (n = 33,860)
Age, mean±SD, years	40.4 ± 12.5	41.0 ± 12.9
Male, n (%)	12948 (37.3)	13580 (40.1)
Educational attainment, n (%)		
Lower than high school	5864 (16.9)	5774 (17.1)
High school	8031 (23.1)	7827 (23.1)
College	8621 (24.8)	8392 (24.8)
University	9210 (26.5)	8905 (26.3)
Postgraduate	3027 (8.7)	2962 (8.7)
Cigarette smoking, n (%)		
Never	29678 (85.4)	28368 (83.8)
Former	1366 (3.9)	1490 (4.4)
Current	3709 (10.7)	4002 (11.8)
Alcohol drinking, n (%)		
Never	32126 (92.4)	30860 (91.1)
Former	331 (1.0)	375 (1.1)
Current	2296 (6.6)	2625 (7.8)
BMI, mean ± SD, kg/m^2	21.8 ± 2.6	21.7 ± 2.4
Diabetes, n (%)	750 (2.2)	590 (1.7)
PA volume, MET-h/week, n (%)		
Very low [< 3.75]	15458 (44.5)	14928 (44.1)
Low [3.75–7.49]	8273 (23.8)	7972 (23.5)
Moderate [7.50–16.49]	6601 (19.0)	6487 (19.2)
High [16.50–25.49]	2617 (7.5)	2636 (7.8)
Very high [≥ 25.50]	1804 (5.2)	1837 (5.4)
$PM_{2.5}$ levels, μg/m^3, n (%)		
Low $PM_{2.5}$ [< 23.5]	16115 (46.4)	15647 (46.2)
Moderate $PM_{2.5}$ [23.5–27.5]	15188 (43.7)	14844 (43.8)
High $PM_{2.5}$ [≥ 27.5]	3450 (9.9)	3369 (9.9)
PA at work, n (%)		
Mostly sedentary	22649 (65.2)	22110 (65.3)
Sedentary with occasional walking	9107 (26.2)	8727 (25.8)
Mostly standing or walking	2532 (7.3)	2531 (7.5)
Hard labor	465 (1.3)	492 (1.5)
Vegetable intake, n (%)		
Seldom	5877 (16.9)	5721 (16.9)
Moderate	21260 (61.2)	20782 (61.4)
Frequent	7616 (21.9)	7357 (21.7)
Fruit intake, n (%)		
Seldom	8847 (25.5)	8705 (25.7)
Moderate	20703 (59.6)	20142 (59.5)
Frequent	5203 (15.0)	5013 (14.8)
Sugary drink, n (%)		
Seldom	11021 (31.7)	10831 (32.0)
Moderate	16206 (46.6)	15748 (46.5)
Frequent	7526 (21.7)	7281 (21.5)
Fried food intake, n (%)		
Seldom	11361 (32.7)	11296 (33.4)
Moderate	17968 (51.7)	17417 (51.4)
Frequent	5424 (15.6)	5147 (15.2)
WC, mean ± SD, cm	72.7 ± 7.4	73.0 ± 8.0
Lab test		
FPG, mean ± SD, mg/dL	96.1 ± 14.1	95.9 ± 13.4
ALT, mean ± SD, IU/L	19.9 ± 11.5	18.7 ± 9.2
AST, mean ± SD, IU/L	20.4 ± 6.3	20.4 ± 6.2
GGT, mean ± SD, IU/L	16.2 ± 9.8	17.0 ± 11.8
Triglycerides, mean ± SD, mg/dL	89.6 ± 43.7	97.2 ± 60.7

Abbreviations: FPG=fasting plasma glucose; WC= waist circumference; BMI=-body mass index; FPG=fasting plasma glucose; ALT= alanine aminotransferase test; AST=aspartate aminotransferase test; GGT=gamma-glutamyl transferase; SD=standard deviation; $PM_{2.5}$ =fine particulate matter; MET-h/week=hours of metabolic equivalent tasks per week.

package for the piecewise Cox regression, and "Survival" package for the Cox regression. A two-tailed p-value < 0.05 was considered as statistically significant.

3. Results

We initially included 58,026 participants in the cohort. After

Table 2
Risk of non-alcoholic fatty liver disease associated with long-term exposure to fine particulate matter ($PM_{2.5}$) and physical activity (PA) in Taiwanese population.

Category	Minimally adjusted model[a] HR (95% CI)	Partially adjusted model[b] HR (95% CI)	Fully adjusted model[c] HR (95% CI)
Fatty liver index (FLI)			
PA volume (MET-h/week)			
Very low [< 3.75]	1.00 [Reference]	1.00 [Reference]	1.00 [Reference]
Low [3.75–7.49]	0.88 (0.83, 0.94)	0.87 (0.82, 0.93)	0.87 (0.81, 0.93)
Moderate [7.50–16.49]	0.81 (0.76, 0.87)	0.80 (0.75, 0.85)	0.79 (0.74, 0.85)
High [16.50–25.49]	0.76 (0.69, 0.83)	0.68 (0.61, 0.74)	0.67 (0.61, 0.74)
Very high [≥ 25.50]	0.60 (0.55, 0.66)	0.53 (0.49, 0.59)	0.53 (0.48, 0.58)
Continuous (per 10 MET-h/week)	0.92 (0.90, 0.94)	0.89 (0.88, 0.91)	0.89 (0.88, 0.91)
$PM_{2.5}$ levels (μg/m^3)[d]			
Low $PM_{2.5}$ [< 23.5]	1.00 [Reference]	1.00 [Reference]	1.00 [Reference]
Moderate $PM_{2.5}$ [23.5–27.5]	1.07 (1.01, 1.12)	1.08 (1.02, 1.14)	1.08 (1.02, 1.14)
High $PM_{2.5}$ [≥ 27.5]	1.25 (1.16, 1.34)	1.28 (1.19, 1.38)	1.28 (1.19, 1.38)
Continuous (per 1 μg/m^3 above 23.5 μg/m^3)	1.06 (1.04, 1.08)	1.07 (1.04, 1.09)	1.06 (1.04, 1.09)
Hepatic steatosis index (HSI)			
PA volume (MET-h/week)			
Very low [< 3.75]	1.00 [Reference]	1.00 [Reference]	1.00 [Reference]
Low [3.75–7.49]	1.00 (0.94, 1.06)	1.04 (0.98, 1.11)	1.03 (0.97, 1.10)
Moderate [7.50–16.49]	0.84 (0.79, 0.89)	0.88 (0.83, 0.94)	0.87 (0.82, 0.93)
High [16.50–25.49]	0.70 (0.64, 0.77)	0.74 (0.68, 0.82)	0.73 (0.67, 0.81)
Very high [≥ 25.50]	0.60 (0.55, 0.66)	0.64 (0.58, 0.70)	0.63 (0.57, 0.69)
Continuous (per 10 MET-h/week)	0.91 (0.89, 0.92)	0.92 (0.90, 0.93)	0.91 (0.90, 0.93)
$PM_{2.5}$ levels (μg/m^3)[d]			
Low $PM_{2.5}$ [< 23.5]	1.00 [Reference]	1.00 [Reference]	1.00 [Reference]
Moderate $PM_{2.5}$ [23.5–27.5]	1.07 (1.02, 1.12)	1.08 (1.02, 1.13)	1.08 (1.02, 1.13)
High $PM_{2.5}$ [≥ 27.5]	1.16 (1.08, 1.24)	1.16 (1.08, 1.24)	1.16 (1.09, 1.24)
Continuous (per 1 μg/m^3 above 23.5 μg/m^3)	1.05 (1.03, 1.07)	1.05 (1.03, 1.07)	1.05 (1.03, 1.07)

Abbreviations: HR=hazard ratio; CI=confidence interval; PA=physical activity; MET-h/week=hours of metabolic equivalent tasks per week; $PM_{2.5}$ =fine particulate matter.

[a] Models were adjusted for age, and year of enrollment.

[b] Modelswere additionally adjusted for season of measurement, sex, smoking status, alcohol consumption, occupational exposure, educational attainment, vegetable intake,fruit intake, sugar drink, fried food intake, and physical activity at work. HSI takes sex into account, so we did not adjust for it in the models.

[c] Models were additionally adjusted for cancer, long-term use of hyperlipidemia drugs, cardiovascular disease, and hypertension.

[d] The cutoffs for $PM_{2.5}$ levels were theupper 15th percentile of exposure range and the identified breakpoint in the fully adjusted models. The breakpoint of $PM_{2.5}$ exposure were identified by using piece wise Cox proportional hazards regression with "Segmented" R package.

Table 3
The combined effects of fine particulate matter ($PM_{2.5}$) and physical activity (PA) on risk of non-alcoholic fatty liver disease among Taiwanese population.[a]

PA volume (MET-h/week)	High $PM_{2.5}$ [≥ 27.5 μg/m^3][b]	Moderate $PM_{2.5}$ [23.5–27.5 μg/m^3][b]	Low $PM_{2.5}$ [< 23.5 μg/m^3][b]	RERI[c]	*p*-value for interaction[#]
Fatty liver index (FLI)					0.21
Very low [<3.75]	1.00 [Reference]	0.85 (0.76, 0.94)	0.78 (0.70, 0.86)		
Low [3.75–7.49]	0.80 (0.70, 0.93)	0.77 (0.68, 0.87)	0.67 (0.59, 0.76)	0.091 (−0.051, 0.232)	
Moderate [7.50–16.49]	0.80 (0.69, 0.92)	0.68 (0.60, 0.77)	0.61 (0.54, 0.69)	0.032 (−0.110, 0.173)	
High [16.50–25.49]	0.62 (0.50, 0.77)	0.56 (0.48, 0.65)	0.55 (0.47, 0.64)	0.155 (−0.011, 0.320)	
Very high [≥ 25.50]	0.74 (0.60, 0.92)	0.38 (0.32, 0.44)	0.43 (0.37, 0.50)	-0.090 (−0.274, 0.093)	
Hepatic steatosis index (HSI)					0.43
Very low [<3.75]	1.00 [Reference]	0.98 (0.89, 1.08)	0.90 (0.81, 1.00)		
Low [3.75–7.49]	1.10 (0.97, 1.24)	1.01 (0.90, 1.12)	0.91 (0.81, 1.02)	-0.092 (−0.254, 0.070)	
Moderate [7.50–16.49]	0.91 (0.79, 1.04)	0.85 (0.76, 0.95)	0.79 (0.70, 0.88)	-0.020 (−0.172, 0.132)	
High [16.50–25.49]	0.84 (0.69, 1.02)	0.66 (0.57, 0.78)	0.68 (0.58, 0.79)	-0.062 (−0.264, 0.140)	
Very high [≥ 25.50]	0.66 (0.52, 0.84)	0.59 (0.51, 0.69)	0.58 (0.50, 0.67)	0.014 (−0.170, 0.198)	

Abbreviations: HR=hazard ratio; CI=confidence interval; PA=physical activity; MET-h/week=hours of metabolic equivalent tasks per week; $PM_{2.5}$ =fine particulate matter; RERI= relative excess risk due to interaction.

[#] p value of the interaction term by adding a product term between continuous $PM_{2.5}$ and continuous volume of PA in the fully adjusted models.

[a] Models were adjusted for age, year of enrollment, season of measurement, sex, smoking status, alcohol consumption, occupational exposure, educational attainment,vegetable intake, fruit intake, sugar drink, fried food intake, physical activity at work, cancer, long-term use of hyperlipidemia drugs, cardiovascular disease, and hypertension. HSI takes sex into account, so we did not adjust for it in the models.

[b] The cutoffs for $PM_{2.5}$ levels were the upper 15th percentile of exposure range and the identified breakpoint in the fully adjusted models. The breakpoint of $PM_{2.5}$ exposure were identified by using piece wise Cox proportional hazards regression with "Segmented" R package.

[c] RERI indicates the additive interaction between low and high $PM_{2.5}$ group and PA volume groups.

excluding participants with missing covariates and those diagnosed with liver diseases or NAFLD at baseline or those who drank excess alcohol or underwent only one medical examination, we included 34,753 participants for the FLI-based cohort and 33,860 participants for the HSI-based cohort in the final analytic sample (Fig. 1 & Fig S1). Compared with excluded participants, the final analytic participants

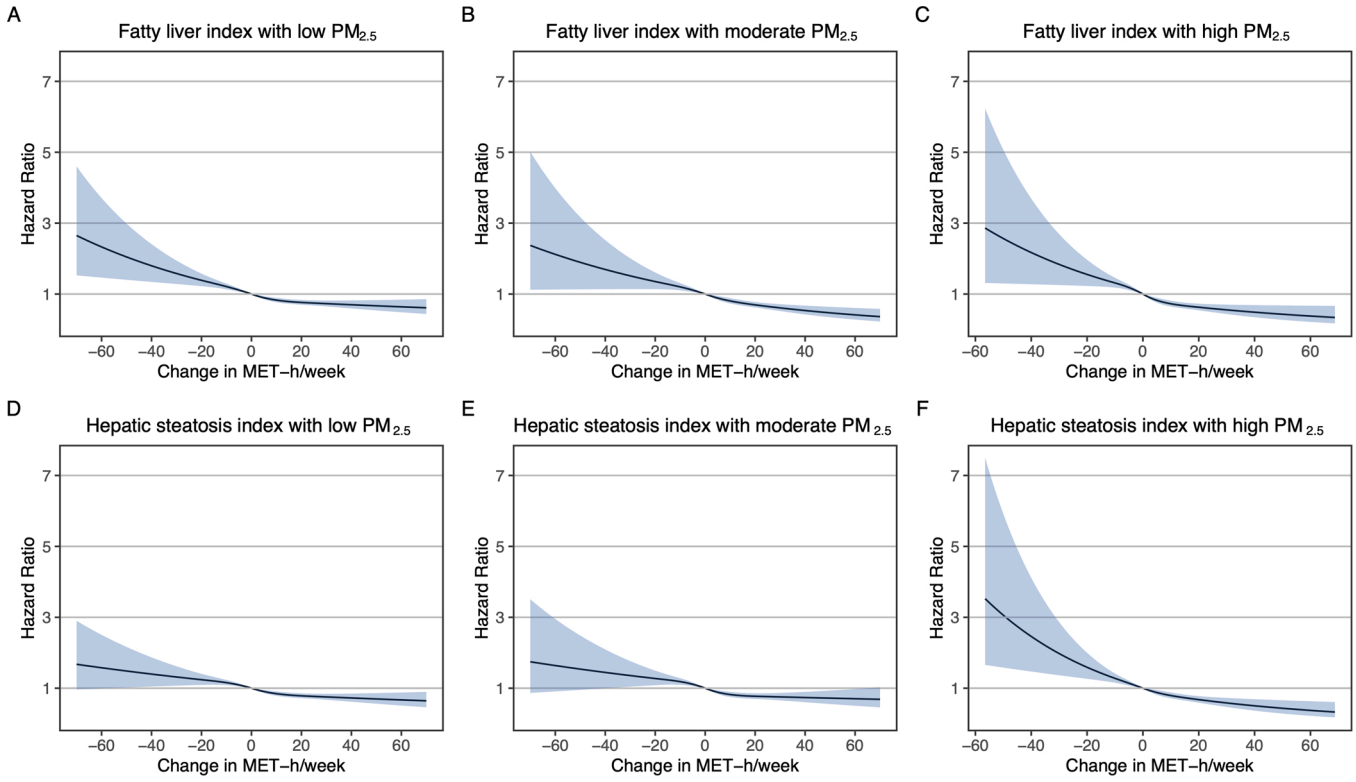

Fig. 2. Association of the change in physical activity (PA) with non-alcoholic fatty liver disease by fine particulate matter ($PM_{2.5}$) levels among Taiwanese participants.

were younger with healthier lifestyles (Table S1).

Participants were more likely to be females, never smokers, never drinkers, performed low level of habitual PA, and done mostly sedentary PA at work (Table 1). Between 2001 and 2016, we identified 6873 incident NAFLD over 247,970 person-years and 7659 incident NAFLD over 235,505 person-years of follow-up for the FLI- and HSI-based cohort.

We found a monotonic association between PA and risk of NAFLD, with lower risk associated with higher volume of PA (Fig S2). In contrast, we observed a nonlinear relationship (p-value for deviation from linearity<0.001) for $PM_{2.5}$ with an estimated breakpoint of 23.5 $\mu g/m^3$ (Fig S3).

In the fully adjusted models, each 10 MET-h/week increase in the volume of PA was associated with an HR of 0.89 (95% CI: 0.88, 0.91) and 0.91 (95% CI: 0.90, 0.93) in risk of NAFLD defined by FLI and HSI, respectively. We found performing higher volume of PA was inversely associated with NAFLD compared with physically inactive participants. For $PM_{2.5}$, the HR per 1 $\mu g/m^3$ increase in $PM_{2.5}$ above 23.5 $\mu g/m^3$ was 1.06 (95% CI: 1.04, 1.09) for NAFLD defined by FLI and 1.05 (95% CI: 1.03, 1.07) for NAFLD defined by HSI. Participants residing in high $PM_{2.5}$ regions were associated with a higher risk of NAFLD. For example, compared with low $PM_{2.5}$ area participants, the HR of NAFLD defined by FLI was 1.08 (95% CI: 1.02, 1.14) for participants in moderate $PM_{2.5}$ areas and 1.28 (95% CI: 1.19, 1.38) for those in high $PM_{2.5}$ areas (Table 2).

The combined effects of PA volume and $PM_{2.5}$ were shown in Table 3 with the very low PA-high air pollution participants as the joint reference group. Our analyses indicate that participants with the low-$PM_{2.5}$ and very high-PA group had the lowest risk of NAFLD. PA was inversely associated with NAFLD, irrespective of air pollution levels. We found no evidence of any multiplicative or additive interaction between PA and $PM_{2.5}$. Sensitivity analyses excluding those excess drinkers, additionally adjusted for metabolic syndrome, used varied $PM_{2.5}$ cutoffs to categorize $PM_{2.5}$ groups, or excluded those participants who recovered the next year after the year of diagnosis did not materially change our findings (Table S2-Table S5).

In the secondary analysis, we examined the changes in PA and risk of NAFLD by levels of $PM_{2.5}$. We found a reduced risk of NAFLD among participants who increased PA during follow-up, whereas decreasing PA levels were associated with a higher risk of NAFLD (Fig. 2).

4. Discussion

In this large prospective cohort study in Taiwan, habitual PA was inversely associated with NAFLD, and long-term exposure to $PM_{2.5}$ was positively associated with NAFLD. We found no evidence of any interaction between PA and $PM_{2.5}$ on NAFLD, which was supported by the secondary analysis examining the association between changes in PA and risk of NAFLD by $PM_{2.5}$ levels.

The evidence of the adverse effects of air pollution on risk of NAFLD is primarily from animal studies (Tan et al., 2009; Zheng et al., 2013), which might have limited clinical relevance due to the higher concentrations of air pollution in the experimental settings than that in the real world. For example, male C57BL/6 mice exposure to $PM_{2.5}$ for six weeks exhibited higher levels of hepatic steatosis and fibrosis compared with mice exposed to filter air (Tan et al., 2009). Among mice exposed to $PM_{2.5}$ for ten weeks, mice showed a nonalcoholic steatohepatitis-like phenotype, such as hepatic steatosis and fibrosis (Zheng et al., 2013). The key novelty of this study is that we provide evidence from human population to confirm that long-term exposure to $PM_{2.5}$ was positively associated with NAFLD (Guo et al., 2021; Sun et al., 2022). Our findings are consistent with the Framingham Heart Study (Li et al., 2017).

To our knowledge, our study is the first to investigate the interaction between PA and $PM_{2.5}$ exposure on risk of NAFLD. We found no evidence of any multiplicative or additive interaction between PA and $PM_{2.5}$. These findings are novel and have important clinical recommendations regarding PA, given that PA is regarded as a foundation for managing NAFLD, and a large proportion of the world population lives in poor air quality regions. Our finding suggests that people could still benefit from PA even they reside in relatively high polluted areas.

4.1. Strengths and limitations

This study has several limitations. First, we used FLI and HSI to define NAFLD. Although these two markers cannot be used to determine the severity of steatosis (Fedchuk et al., 2014), they are among the best-validated markers for steatosis, especially in large-scale screening (Bedogni et al., 2010; Lee et al., 2010; Zhu et al., 2018). Second, we used $PM_{2.5}$ concentrations at the residence as a proxy for air pollution exposure without considering participants' time-activity patterns, which might introduce some degrees of exposure misclassification. However, we expect this exposure misclassification to be non-differential and tend to bias our estimates towards the null hypothesis of no association. Third, we are unclear where the participants performed their PA. However, ~93% of Taiwanese residents reported that they performed their most frequent PA outdoors (Department of Physical Education Ministry of Education, 2021). Fourth, the study participants are generally well-educated with relatively healthier lifestyles, the results may not be generalizable to other locations beyond Taiwan.

Our study has several notable advantages. First, our study is the first to investigate the interaction between PA and long-term exposure to $PM_{2.5}$ on risk of NAFLD. Findings from our study fill this knowledge gap and provide evidence for people residing in high polluted areas to manage their NAFLD through PA. Second, to confirm the robustness of the combined effects of $PM_{2.5}$ and PA, we used an alternative PA and examined whether the benefits of changes in PA were modified by levels of air pollution. We also used two noninvasive markers (i.e., FLI and HSI) to define NAFLD. The consistent results from these analyses confirmed the robustness of our findings.

5. Conclusions

In a prospective cohort study in Taiwan, we found that long-term exposure to air pollution was positively associated with NAFLD and performing PA was inversely associated with NAFLD. The benefits of PA on NAFLD remain, irrespective of exposure to various levels of $PM_{2.5}$. These findings indicate that PA is still a recommended strategy to manage NAFLD, even for people residing in relatively polluted areas.

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Declaration of Competing Interest

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

CRediT authorship contribution statement

Shengzhi Sun: Conceptualization, Formal analysis, Methodology, **Qingqing Yang:** Formal analysis, Methodology, **Qingxin Zhou:** Resources, **Feng Sun:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, **All authors**: Writing – editing.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.113440.

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