# A cover page

**The Official Title of the study**: Effect of dietary supplementation with fish oil in alleviating cardiopulmonary hazards associated with air pollution

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**Study protocol**

This trial will be conducted between September 2017 and January 2018. We plan to recruit 70 healthy college students on Fenglin campus of Fudan University in Shanghai, China. We will exclude students who do not live on campus, or have a history of tobacco smoking or alcohol addition, clinically diagnosed cardiovascular and respiratory diseases, or recent infections, or are allergic to omega-3 fatty acids or fish. Eligible participants will be randomly assigned to receive dietary supplementation of either marine-derived fish oil (2.5 g/day) or placebo (sunflower-seed oil), and will be monitored in a double-blinded fashion throughout the study period. We plan to schedule 4 rounds of follow-up visits with an interval of 2 weeks in the last 2 months of the intervention. To expand the daily variation range of $PM_{2.5}$ exposures, in each round we will divide each group to 2 subgroups and arranged health examinations on either Saturday for placebo group or Sunday for fish-oil group at adjacent weeks. There will have identical working conditions and technical procedures between these two assessment days. We will collect data on individual characteristics (i.e., age, gender, height, and weight), time-location information and physical activity at baseline and during the follow-ups. We also plan to ask participants to complete a validated food frequency questionnaire at the start, middle and the end of study period to record the profile of daily dietary nutrients intake. Health measurements will include blood pressure (BP) and lung function, blood samples and skin tape samplings in each round of follow-up.

**Statistical analysis Plan**

Environmental and health data will be linked by the time of health measurements (rounded to the integer hour).

T-test or chi-square test will be applied to compare the difference of characteristics and outcome variables of participant**s** between the fish-oil and placebo groups. Linear mixed-effect models will be applied to evaluate short-term exposure to $PM_{2.5}$ in relation to each of cardiovascular outcomes within each treatment group. We will apply multiple averaging periods preceding the health measurements [i.e., 0–6 hours (h), 0–12 h, 0–24 h, and 0–48 h] to explore the lag patterns that the acute effects of $PM_{2.5}$ might have on individual biomarkers. We will further test the statistical differences between the two treatment groups in their effect estimates of $PM_{2.5}$ on individual biomarkers and calculated the 95% confidence intervals (CI) as

$$(\hat{Q}_1\text{-}\hat{Q}_2) \pm 1.96\sqrt{\hat{SE}_1^{\,2} + \hat{SE}_2^{\,2}},$$

where $\hat{Q}_1$ and $\hat{Q}_2$ are the effect estimates of $PM_{2.5}$ for the two treatment groups, $\hat{SE}_1$and $\hat{SE}_2$ are their respective standard errors.

To test the robustness of our results, we will perform several sensitivity analyses. First, we will repeat analyses by fitting two-pollutant models by adding the present-day concentrations of 4 gaseous pollutants (sulfur dioxide, nitrogen dioxide, carbon monoxide, and ozone) to regression models one at a time. Second, in the basic models, we will further adjust for baseline dietary intakes of nutrients that will show significantly different between the two groups. Finally, to explore the possibly diminishing effects of $PM_{2.5,}$ we will apply longer lag periods (i.e., 0-72 h and 0-96 h)

in supplementary analyses. Additionally, we will adjust for present-day ultraviolet radiation to exclude its confounding effects on skin.

All analyses will be conduct using the "lme4" package of R software. In the within treatment group analyses, we will present the absolute changes in BP (in millimeters mercury) and the percentage changes in other biomarkers and their 95% CIs for each 10-$\mu g/m^3$ increment in $PM_{2.5}$ exposure. To demonstrate potential benefits of fish-oil supplementation, the differences in the effect sizes of the $PM_{2.5}$-biomarker associations between the fish-oil and placebo groups will also be presented.