



STUDY PROTOCOL

Study protocol: The INTERMAP China Prospective (ICP) study

[version 1; peer review: 2 approved with reservations]

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Abstract

Background: Unfavourable blood pressure (BP) level is an established risk factor for cardiovascular diseases (CVD), while the exact underlying reasons for unfavourable BP are poorly understood. The INTERMAP China Prospective (ICP) Study is a prospective cohort to investigate the relationship of environmental and nutritional risk factors with key indicators of vascular function including BP, arterial stiffness, and carotid-intima media thickness.

Methods: A total of 839 Chinese participants aged 40-59 years from three diverse regions of China were enrolled in INTERMAP in 1997/98; data collection included repeated BP measurements, 24-hour urine specimens, and 24-hour dietary recalls. In 2015/16, 574 of these 839 persons were re-enrolled along with 208 new participants aged 40-59 years that were randomly selected from the same study villages. Participant's environmental and dietary exposures and health outcomes were assessed in this open cohort study, including BP, 24-hour dietary recalls, personal exposures to air pollution, grip strength, arterial stiffness, carotid-media thickness and plaques, cognitive

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Any reports and responses or comments on the

function, and sleep patterns. Serum and plasma specimens were collected with 24-hour urine specimens.

Discussion: Winter and summer assessments of a comprehensive set of vascular indicators and their environmental and nutritional risk factors were conducted with high precision. We will leverage advances in exposome research to identify biomarkers of exposure to environmental and nutritional risk factors and improve our understanding of the mechanisms and pathways of their hazardous cardiovascular effects. The ICP Study is observational by design, thus subject to several biases including selection bias (e.g., loss to follow-up), information bias (e.g., measurement error), and confounding that we sought to mitigate through our study design and measurements. However, extensive efforts will apply to minimize those limitations (continuous observer training, repeated measurements of BP, standardized methods in data collection and measurements, and on-going quality control).

Keywords

INTERMAP, Nutrition, Air Pollution, Exposome, Blood Pressure, China

article can be found at the end of the article.

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Introduction

Unfavourable blood pressure (BP) level is an established risk factor for cardiovascular disease (CVD)¹, and is the single largest risk factor for disease burden globally². Associations between BP level and CVD are positive, independent, strong, graded, and continuous with no apparent threshold^{1,3,4}. Although adverse levels of BP are highly prevalent in middle and older-aged individuals worldwide⁵, the exact underlying reasons are poorly understood. Environmental and dietary risk factors such as air pollution and sodium intake have emerged as potentially important contributors that warrant further, coordinated investigation^{6–9}. CVD is the leading cause of death in China with 3.97 million deaths attributed to it in 2016¹⁰; previous studies in China show large regional differences in the incidence of CVD^{11–14} and in the prevalence of environmental and dietary risk factors^{6,15–18}. Studies that rigorously quantify exposures to these modifiable risk factors and evaluate their associations with BP and other cardiovascular outcomes may help to explain these geographical variations and inform the development of region-specific interventions to prevent CVD.

The International Study of Macro/Micronutrients and Blood Pressure (INTERMAP)¹⁹ has produced extensive findings on cross-sectional associations between multiple dietary factors (e.g., macro- and micro-nutrients/food groups)^{9,20,21} and urinary metabolites^{22–25} with BP in individuals. The findings from INTERMAP indicate the cumulative effects of individual nutrients may be sizable²⁰ and important in accounting for the high prevalence of adverse BP patterns in populations. However, INTERMAP was cross-sectional and thus causal associations could not be inferred regarding the role of dietary factors in the development of adverse BP or to assess the influence of nutrient intakes on dynamic BP change. Prospective follow up of the INTERMAP participants will enable investigation of patterns in nutrients, food groups, metabolites and diet, and their associations with BP over time. It will also achieve a better understanding of the mechanisms by which diets lead to adverse BP levels.

Since the inception of INTERMAP, substantial epidemiologic and toxicological evidence has linked particle air pollution exposure (e.g., particulate matter with aerodynamic diameters less than 2.5 μm [$\text{PM}_{2.5}$], black carbon) to higher BP and increased risk of CVDs and related mortality. Air pollution from household biomass and coal (i.e., solid fuel) stoves – used by over 600 million Chinese for cooking, heating, and other energy needs²⁶ – has emerged as a potentially important environmental determinant of adverse BP levels and central haemodynamics^{7,8}, but is far less studied compared with urban and traffic-related air pollution. Adding detailed measurements of exposure to air pollution in settings of household solid fuel use to the existing profile of INTERMAP cohort could provide deeper insights into the aetiology of adverse BP patterns by studying their environmental and nutritional risk factors and related interactions.

Methods

The INTERMAP Study was a cross-sectional epidemiological investigation of 4,680 men and women aged 40 to 59 years

from 17 diverse population samples that were surveyed between 1996 and 1999 in China, Japan, the United Kingdom, and United States¹⁹. The main aim of INTERMAP was to investigate the aetiology of adverse BP and its associations with diet. Three participated INTERMAP rural populations in China were selected from geographically diverse regions: two in the north (Pinggu County, Beijing, and Yu County, Shanxi Province) and one in the south (Wuming County, Guangxi Zhuang Autonomous Region) of China. These three sites were chosen to represent low-income areas with environmental and nutritional risk factor patterns that are considered characteristic of rural northern and southern China. The INTERMAP China Prospective (ICP) Study was initiated in 2014, and the prospective follow-up of those 3 INTERMAP populations in China would be one of the most critical tasks.

The aims of ICP Study are to: 1) extend the observations of profiles of CVD risk factors and health outcomes from INTERMAP to present day; 2) produce evidence for region-specific environmental and nutritional interventions to address adverse BP profiles; and 3) leverage advances for identifying biomarkers of air pollution exposure and nutritional risk factors and for understanding the mechanisms and pathways of their effects on CVD. Overall, this cohort study will investigate the transitions in nutrients, food groups, dietary patterns, household energy use, urinary sodium, and vascular indicators between baseline and follow-up visits. Results and findings from ICP Study will provide better insight into the evolution of raised BP and CVD in China, and its underlying environmental and nutritional risk factors.

Recruitment and enrolment

The ICP enrolls an open cohort to study the risk factors for CVDs. In 1997–1998, participants of the INTERMAP study were randomly selected from each target population of three rural sites in China. The recruitment programme for each site was stratified by age/gender and approximate 65 persons were randomly selected from each of four age-gender subgroups of population lists: men and women, aged 40–49 and 50–59 years. Selected participants from population lists were then contacted and invited to participate by staff in local collaborating centres. Only one person per household was to be included. Extensive efforts were made to recruit participants who were randomly selected at the beginning. For the ICP study data collection, if substitute participants were needed at follow-up to replace persons who refused to participate or did not satisfactorily complete full data collection, these participants were also invited to participate in a similar manner.

In 2014, we contacted the 680 surviving men and women of INTERMAP participants in China for the enrolment of the ICP Study and 575 of them were enrolled with a response rate of 85%. In addition, to facilitate comparisons of risk factors and biomarkers with INTERMAP data (1997–1998) influenced by aging and enable analyses of temporal trends of risk factors and biomarkers (e.g., urinary sodium) in these populations, new participants were recruited into the open cohort of the ICP Study. We randomly selected and recruited an additional 240 individuals aged 40–59 (matching the age group of INTERMAP in 1997–1998) from each study site

using population lists, and 212 were enrolled (Table 1) and the response rate was 88%. A total of 782 participants completed the full data collection for at least one visit (574 from the INTERMAP Study and 208 from new recruitment), including the health measurements, questionnaires (main questionnaire and fuel and stove use questionnaire, see *Extended data*²⁷), and air pollution exposure monitoring (Table 1). There were five additional participants (one individual from the INTERMAP Study and four individuals newly enrolled into the ICP Study) who only contributed energy use and air pollution exposure data. Thus, the sample size is 787 when the air pollution exposure data are reported in the future.

Ethics approval and consent to participate

INTERMAP was approved by the Institutional Review Board of Northwestern University (STU00204462-CR0002) and the Research Ethics Committee of the Health Research Authority (United Kingdom, #EC3169).

The ICP Study standardised operation protocols (SOPs) were approved by ethical review boards at all investigator institutions including Fu Wai Cardiovascular Hospital (China #2015–650), Imperial College London (United Kingdom #15IC3095), McGill University (Canada #A08-M37-16B), Peking University (China #IRB00001052-15017), and Tsinghua University (China #20140077). All participant signed an informed consent form for prior to enrolment.

Data collection

Data collection of the ICP Study were conducted in 2015–2016 (Shanxi: August 2015 and November 2015; Beijing: December 2015 and September 2016; Guangxi: November 2016), and a follow-up of the ICP cohort is planned for 2020–2021 (Figure 1).

All measurements and biological samples are conducted using the same SOP for both the INTERMAP and ICP Study to ensure comparability and aggregation of data and all research staff must attend a compulsory, hands-on training programme and be certified before data collection. The measurements conducted in INTERMAP and ICP Study are summarized in Table 2. Trained staff collected information on household demographics, education, employment, tobacco smoking, alcohol use, medical history, family history, medication use, and physical activity using a structured questionnaire (*Extended data: Main questionnaire*²⁷)¹⁹.

Visit schedule

Participants visited the clinics four times for INTERMAP in 1997–1998, with at least one visit conducted on a weekend day or equivalent rest day¹⁹. For the first follow-up visit of the ICP Study, participants at Shanxi and Beijing sites completed four clinic visits in 2015–2016, and participants at Guangxi completed two clinic visits in 2016. A second follow-up visit for the ICP Study participants has been planned for 2020–2021.

Table 1. Description of population samples of the INTERMAP-China and INTERMAP China Prospective (ICP) Studies (number and %).

	Pinggu County, Beijing			Yu County, Shanxi			Wuming County, Guangxi			Overall		
	Men	Women	Total	Men	Women	Total	Men	Women	Total	Men	Women	Total
INTERMAP												
Completed data collection (1997–1998)	133	139	272	143	146	289	140	138	278	416	423	839
Deceased (2015)	31 (23.3%)	15 (10.8%)	46 (16.9%)	26 (18.2%)	24 (16.4%)	50 (17.3%)	28 (20.0%)	15 (10.9%)	43 (15.5%)	85 (20.4%)	54 (12.8%)	139 (16.6%)
Lost to follow-up (2015)	0 (0.0%)	2 (1.4%)	2 (0.7%)	4 (2.8%)	4 (2.7%)	8 (2.8%)	6 (4.3%)	4 (2.9%)	10 (3.6%)	10 (2.4%)	10 (2.4%)	20 (2.4%)
Contacted for ICP (2015)	102 (76.7%)	122 (87.8%)	224 (82.4%)	113 (79.0%)	118 (80.8%)	231 (79.9%)	106 (75.7%)	119 (86.2%)	225 (80.9%)	321 (77.2%)	359 (84.9%)	680 (81.0%)
ICP Study												
Total enrolled for ICP (2015–2016)*	108	150	258	133	157	290	109	130	239	350	437	787
Enrolled from INTERMAP	90 (83.3%)	106 (70.7%)	196 (76.0%)	99 (74.4%)	104 (66.2%)	203 (70.0%)	79 (72.5%)	97 (74.6%)	176 (73.6%)	268 (76.6%)	307 (70.3%)	575 (73.1%)
Newly enrolled	18 (16.7%)	44 (29.3%)	62 (24.0%)	34 (25.6%)	53 (33.8%)	87 (30.0%)	30 (27.5%)	33 (25.4%)	63 (26.4%)	82 (23.4%)	130 (29.7%)	212 (26.9%)
Completed all data collection (2015–2016)**	108	149	257	133	157	290	107	128	235	348	434	782

* The number of participants including those 5 additional participants who only contributed energy use and air pollution exposure data.

** The number of participants who completed the full data collection for at least one visit.

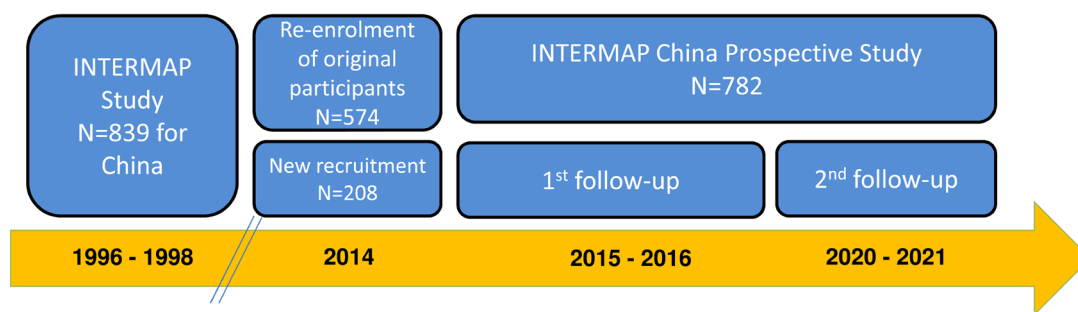


Figure 1. Timeline for data collection of the open cohorts in the INTERMAP and ICP Studies.

Table 2. Measurements in the INTERMAP and INTERMAP China Prospective studies to date, and planned for the future.

	Variables	Measurement or sample collection	Baseline (1997–1998)	First follow-up (2015–2016)	Second follow-up (2020–2021)
INTERMAP and ICP Studies	Demographic variables	Structured questionnaire	Y	Y	
	Lifestyle and behaviour variables	Structured questionnaire	Y	Y	
	Medical history and medication use	Structured questionnaire	Y	Y	Y
	Nutrients, food groups, dietary pattern, dietary supplements	2 to 4, 24-hour dietary recall	Y	Y	
	Anthropometric variables ^a	Scale, measuring tape	Y	Y	Y
	Brachial sitting systolic and diastolic blood pressure	INTERMAP: random zero sphygmomanometer	Y		
		ICP: oscillometric device, Omron HEM-907		Y	Y
	Outdoor temperature	Digital temperature sensor (Tianjianhuayi Inc.)	Y	Y	Y
ICP Study only	Urinary electrolytes, metabolites and biomarkers	Timed 24-hour urine collection	Y	Y	
	Blood biochemistry	Serum		Y	
	Blood metabolites and biomarkers	Serum and plasma, dried blood spots		Y	
	Personal exposure to fine particulate matter (PM _{2.5}) and black carbon	2 to 4 24-hour air samples collected on 37 mm PTFE ^b filters (Zefluor)		Y	
	Cognitive performance	Montreal Cognitive Assessment (MoCA)		Y	Y
	Physical activity	Pedometers, worn for 2-4 24-hour periods		Y	
	Anthropometric variables ^c	Scale, measuring tape		Y	Y
	Grip strength	Jamar J00105 hydraulic hand dynamometer		Y	Y
	Central blood pressure and haemodynamics	Brachial-femoral pulse wave analysis (Vicorder)		Y	Y
	Carotid intima-media thickness and plaques	Ultrasound (Z6 Mindray Medical)		Y	Y
	Mortality information for deceased participants	Verbal autopsy		Y	Y
	Household fuel and stove use patterns	Structured questionnaire	Y ^Δ	Y	Y

^Δ obtained during the first follow-up study during 2015–2016; ^a weight, height; ^b polytetrafluoroethylene ^c added waist, hip, and arm circumference.

to administer questionnaires that evaluate any changes household fuel use or cardiovascular risk factors (i.e., alcohol use), and conduct measurements of height, weight, weight circumference, BP, and carotid intima-media thickness.

Anthropometric measurements

Anthropometric measurements, i.e., weight and height, circumference of waist and hip, to be measured according to standardised procedures (*Extended data*: Supplementary material, Appendix 1²⁷) at each clinic visit by clinical staff of ICP Study, according to SOP^{28,29}.

Blood pressure measurement

Staff conducted measurements of systolic and diastolic BP with seated participants following at least 5 minutes of quiet rest, using a random zero sphygmomanometer¹⁹ for the INTERMAP and an oscillometric device (Omron HEM-907)³⁰ for ICP Study (*Extended data*: Supplementary material, Appendix 2²⁷).

Grip strength measurement

Grip strength to be measured using a Jamar J00105 hydraulic hand dynamometer in each clinic visit of the first and second follow-up of ICP Study following SOP²⁹ (*Extended data*: Supplementary material, Appendix 3²⁷).

Dietary data collection

Four 24-hour dietary recalls were collected by trained staff for all participants, two pairs of visits in two consecutive days (one pair collected in summer and the other in winter during the first follow-up of ICP Study). Each recall ascertained in depth all foods, non-alcoholic and alcoholic beverages, and dietary supplements consumed over the prior 24 hours (*Extended data*: Supplementary material, Appendix 4²⁷). At the first and third visits, information will be obtained on the amount and type of alcoholic beverages consumed each day over the preceding 7 days^{19,31}.

Air pollution exposure measurements

For the first follow-up of the ICP Study, we measured integrated personal exposures to particulate matter <2.5-microns in aerodynamic diameter (PM_{2.5}), collecting PM_{2.5} mass on 37 mm Teflon (PTFE) filters for later gravimetric and optical analysis³². We conducted two consecutive 24-hour personal PM_{2.5} and black carbon (a combustion-related pollutant) exposure measurements in each campaign, according to methods described elsewhere³³. Simultaneous daily (24-hour) measurement of outdoor PM_{2.5} concentrations were conducted using the same air pollution monitors (*Extended data*: Supplementary material, Appendix 5²⁷).

Pulse wave velocity measurements

We measure brachial-femoral pulse wave velocity (PWV), an indicator of arterial stiffness³⁴, and augmentation index, using pulse wave analysis (Vicorder, Smart Medical, Moreton in Marsh, UK) of the first and second follow-up of ICP Study (*Extended data*: Supplementary material, Appendix 6²⁷).

Intima-media thickness measurements

Carotid intima-media thickness (IMT) and carotid plaque are to be measured at the first and second follow-up of ICP Study using a Z6 Ultrasound System (Mindray Medical International Limited, Shenzhen, China) (*Extended data*: Supplementary material, Appendix 7²⁷).

Urine collection and measurements

Timed 24-hour urine samples were collected twice for both studies following the same protocol for both INTERMAP and ICP Study³⁵. For the first follow-up of the ICP Study, spot urine samples were also been collected (*Extended data*: Supplementary material, Appendix 8²⁷).

Blood collection and measurements

We collected dried blood spot samples (five spots) in each ICP measurement campaign (*Extended data*: Supplementary material, Appendix 9²⁷), and fasting whole-blood samples (centrifuged and stored as plasma and serum samples) once during the winter campaigns (*Extended data*: Supplementary material, Appendix 10²⁷).

Other measurements for ICP Study

We assessed physical activity levels of participants during ICP campaigns using a pedometer (Omron HJ-328). We also collected historical (INTERMAP) and current (first follow-up of ICP Study) household energy use including fuel and stove types (to assess historical exposure to air pollution) using structured questionnaires³⁶ (*Extended data*: Fuel and stove use questionnaire²⁷). Sleep patterns were assessed using structured questionnaires (*Extended data*: Main questionnaire²⁷). We measured cognitive function in the Beijing and Shanxi study populations using the Montreal Cognitive Assessment³⁷. For the second follow-up, data on household energy use and cognitive function will be collected.

Causes of mortality

In addition to active follow-up, passive follow-ups will be carried out as well. Medical information of participants is being collected via linkages with their medical records in county hospitals using the health insurance database of the New Rural Cooperative Medical Scheme and the Disease Surveillance Point System managed by the Centre for Disease Prevention and Control³⁸. For deceased participants, mortality data were collected using a verbal autopsy instrument, which was developed and validated in the Population Health Metrics Research Consortium (PHMRC) project³⁹.

Sample and data storage and handling

Air pollution and biological samples were stored immediately in an on-site freezer at -30°C after collection. Air pollution samples were then transferred to the Wisconsin State Laboratory of Hygiene (University of Wisconsin-Madison, Madison, USA) for gravimetric (mass) and black carbon analysis. Dried blood spots, fasting blood samples (both serum and plasma), and urine samples were transferred to a laboratory freezer in Fuwai

Hospital (Beijing, China) for long-term storage at -80°C . All hand-copied primary data (e.g. questionnaires and forms) were entered electronically in duplicate at the end of each field campaign and the original data collection forms were electronically scanned. Electronic data were securely stored in a locked office on two password-protected project computers and hard drives at each site and later transferred to Imperial College London (London, UK), Peking University Clinical Research Institute (Beijing, China), Fuwai Hospital, and McGill University (Montreal, Canada).

Statistical analysis and sample size

The goal of analyses of nutrient/metabolite/environmental exposure-BP associations in INTERMAP and ICP is to estimate associations between average daily nutrient/metabolite/environmental exposure levels of individuals and their average systolic BP and diastolic BP.

For descriptive statistics, means, standard deviations, and medians were computed for nutrients, metabolites, environmental exposure levels and other variables for the three population samples, by gender and age. BP of each individual was the average of eight measurements from the four visits (INTERMAP) or four measurements from two visits in each season (ICP). To examine relationships of dietary, environmental, and other variables to BP, quantile and multiple regression analyses were used, with control for age and gender, then control for other potential confounding variables in sequential linear regression models.

Estimates of power are based on coefficients uncorrected for regression/dilution bias, corresponding to uncorrected partial correlations of nutrients/metabolites with BP. INTERMAP was designed to detect partial correlations of 0.06 or greater; with regression dilution bias resulting from day-to-day variability in nutrient/metabolite levels, an observed partial correlation of 0.06 can be expected to correspond to a true correlation of 0.10 or greater. Regarding the ICP Study, we may not have adequate sample size to detect the association between nutrients and BP in three Chinese populations; however, we should have adequate sample to test other hypotheses as this study generates a considerable amount of both environmental exposure and CVD outcome data. For examining the association between personal exposure to $\text{PM}_{2.5}$ and BP with cross-sectional data collected in 2015–16, we assumed difference of exposure to $\text{PM}_{2.5}$ was 80 ug/m^3 between higher and lower exposure groups and this led to a systolic BP difference of 3.1 mmHg using the predicted dose-response curve from previous study⁷; therefore, nearly 90% power are expected for ICP Study (standard deviation=14 mmHg, $\alpha=0.05$). When comparing BP and urinary sodium differences between north and south sites, we assumed differences were 3.5 mmHg for systolic BP and 80 mmol for urinary sodium⁶, with the sample size in this study, nearly 91% and 99% would be achieved (standard deviation=14 mmHg and 80 mmol, $\alpha=0.05$).

The demographic and health characteristics of ICP participants that completed at least one season of data collection are presented in [Table 3](#).

Discussion

For both INTERMAP and the ICP Study, data collection procedures have been extensively tested and validated to minimise systematic and random error^{20,31,40}. For example, a random 10% of urine samples were split locally and sent to the laboratory with different identification numbers to evaluate the precision of urine analysis²⁰; all dietary recall data were assessed independently by trained staff³¹.

The main strengths of this cohort include:

1. Populations from three different regions enable investigation of the environmental and nutritional risk factors for higher BP and other cardiovascular outcomes across China.
2. Data collection followed the same SOP at baseline and follow-up visits to generate high-quality data, and thus enables comparisons of patterns in diet, household energy use, and other lifestyle factors over time.
3. Comprehensive assessment of environmental and nutritional risk factors in the winter and summer seasons with high precision: ambient air temperature; standardised multi-pass 24-hour dietary recalls; timed 24-hour urine collection; questionnaire-based assessment of current and historical stove and fuel use; multiple 24-hour measurements of personal exposure to air pollution; and blood specimens were also collected for biochemical analyses.
4. Although the sample size of this cohort is relatively small compared with other biobank studies, our high precision in measurement of exposure and key cardiovascular risk factors may lead to less biased associations, and thus contribute new insights in the emerging field of exposome research.

The ICP Study is observational by design, thus subject to several biases that we sought to mitigate. To minimize the potential for selection bias, our participants were randomly selected from village rosters and we achieved high participation rates at baseline. We maintained the cohort through home visits by dedicating staff at each site and were able to follow up 84% of living INTERMAP participants. We also collected verbal autopsy reports for deceased participants to investigate the influence of survivor bias. To reduce the impact of information bias arising from measurement error, we conducted repeated measurements of cardiovascular outcomes (e.g., BP) dietary and environmental exposure variables, and urinary/blood biomarkers using validated instruments and adhered to strict quality assurance and quality control practices during data collection. We also collected detailed information on the

Table 3. Characteristics of participants in the INTERMAP China Prospective (ICP) Study at follow-up in 2015–2016 (N=782).

Variables as Mean (SD) or n (%)	Enrolled from INTERMAP		Independent enrolment	
	Men	Women	Men	Women
	(n=268)	(n=306)	(n=80)	(n=128)
Age (years), mean (SD)	66.6 (5.9)	67.0 (5.6)	51.4 (6.4)	52.7 (5.0)
Education, n (%)				
None	20 (7.5)	92 (30.1)	0 (0.0)	8 (6.3)
Primary school	133 (49.6)	157 (51.3)	12 (15.0)	22 (17.2)
Secondary school	114 (42.5)	57 (18.6)	66 (82.5)	96 (75.0)
College	1 (0.4)	0 (0)	2 (2.5)	2 (1.6)
Annual household income, n (%)				
<¥20,000 (3,150 USD)	124 (46.3)	116 (37.9)	28 (35.0)	37 (28.9)
¥20,000 – ¥35,000 (3,150 – 5,500 USD)	31 (11.6)	58 (19.0)	19 (23.8)	34 (26.6)
>¥35,000 (5,500 USD)	86 (32.1)	90 (29.4)	25 (31.3)	44 (34.4)
Not disclosed	27 (10.1)	42 (13.7)	8 (10.0)	13 (10.2)
Occupation, n (%)				
Agriculture	159 (59.3)	160 (52.3)	41 (51.3)	87 (68.0)
Housekeeping	5 (1.9)	48 (15.7)	3 (3.8)	15 (11.7)
Factory worker	6 (2.2)	2 (0.7)	6 (7.5)	0 (0)
Manager	3 (1.1)	0 (0)	6 (7.5)	1 (0.8)
Retired	84 (31.3)	93 (30.4)	4 (5.0)	14 (10.9)
Other occupations ^a	11 (4.1)	3 (1.1)	20 (25.0)	11 (8.6)
Smoking status, n (%)				
Current	117 (43.7)	7 (2.3)	55 (68.8)	2 (1.6)
Past	108 (40.3)	7 (2.3)	17 (21.3)	0 (0.0)
Never	43 (16.0)	292 (95.4)	8 (10.0)	126 (98.4)
Alcohol consumption, n (%)				
Never ^b	100 (37.3)	269 (87.9)	17 (21.3)	103 (80.5)
Occasional ^c	67 (25.0)	30 (9.8)	29 (36.3)	23 (18.0)
Regular ^d	101 (37.7)	7 (2.3)	34 (42.5)	2 (1.6)
Currently taking anti-hypertensive medication, n (%)	85 (31.7)	148 (48.4)	22 (27.5)	36 (28.1)
Body mass index (kg/m ²)	24.2 (3.4)	25.6 (3.9)	25.9 (4.2)	26.2 (4.0)
Current household fuel use, n (%)				
At least 1 solid fuel ^e for cooking	161 (62.9) ^f	185 (64.0) ^g	42 (34.7)	79 (61.7)
At least 1 solid fuel for heating	147 (57.4) ^f	159 (55.0) ^g	39 (48.8)	81 (63.3)
At least 1 clean fuel ^h for cooking	234 (91.4) ^f	262 (90.7) ^g	77 (96.3)	123 (96.1)
At least 1 clean fuel for heating	113 (44.1) ^f	115 (39.8) ^g	33 (41.3)	65 (50.8)

^a This category includes service and service workers, professionals and technicians, self-employed worker, unemployment, and other occupations that were not specified.

^b This includes people who quit drinking alcohol; ^c less than once per week; ^d at least once per week;

^e Solid fuels defined as coal, charcoal, wood, and crop residues;

^f N=256 as denominator; ^g N=289 as denominator;

^h Clean fuels defined as liquefied petroleum gas, biogas, natural gas, and electricity.

major risk factors for BP and cardiovascular risk at each visit, including physical activity, tobacco smoke, and alcohol consumption that can be evaluated as potential confounders in the statistical analysis.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Extended data

Open Science Framework: Study Protocol: The INTERMAP China Prospective (ICP) Study. <https://doi.org/10.17605/OSF.IO/7P3JB>²⁷.

This project contains the following extended data:

- Fuel and stove use questionnaire (this folder contains the fuel and stove use questionnaires used for each region).
- Main questionnaire

- ICP Protocol Paper Extended data_Supplementary Materials v1 (this file contains appendices 1–10).

Reporting guidelines

Open Science Framework. STROBE checklist for ‘Study protocol: The INTERMAP China Prospective (ICP) Study’. <https://doi.org/10.17605/OSF.IO/7P3JB>²⁷.

Extended data and the reporting guidelines checklist are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Acknowledgements

We thank all INTERMAP and ICP staff at local, national, and international centres for their invaluable efforts. A partial listing of these colleagues is given in Reference 18 for INTERMAP Study. We thank every participant in the study for their invaluable contributions. The PhD work of L.Y. was supported by Lee Family Scholarship. F.K. and P.E. acknowledge support from the Medical Research Council and Public Health England for the MRC-PHE Centre for Environment and Health (MR/L01341X/1).

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Abu Mohammed Naser Titu

Emory Global Diabetes Research Center, Rollins School of Public Health, Emory University, Atlanta, GA, USA

This is a protocol paper that describes the rationale and methodologies of the ICP study. The authors have described the study population, objectives, numbers of visits, and data collection methods. They have listed the exposure and outcomes of the study. I have several comments and suggestions:

1. The mean age of the study population seems high, particularly those who were enrolled in the INTERMAP study. Age is an important biological risk factor for many CVD outcomes in this study. The study findings should not be generalizable for other age groups. The authors may explicitly mention age in the study title and papers published from this study.
2. In the "Introduction" and "Methods" sections, the authors have mentioned the environmental factors. I think they are considering only air pollution as environmental exposure, mainly due to household energy consumption. I was wondering whether authors need to consider whether variables such as temperature, humidity, rainfall as the environmental exposure since they can collect these data from secondary sources.
3. I think the statistical analysis plans need more elaboration. How will the repeated measures be accounted for? What are the strategies for adjusting p-values for multiple hypotheses testing to avoid false discovery? Should we adjust the models for seasonality or ambient temperature? Evidence from China suggests there is a higher mean blood pressure of the population in winter compared to mean blood pressure in summer.
4. In the first follow-up, spot urine samples will be collected. There are many controversies regarding the utility of spot urine samples to evaluate daily sodium intake. The authors may consider the collection of 24-hour urine sodium. Even a single measurement of 24-hour is not sufficient.
5. For outcomes (e.g., blood pressure), I feel there is a huge gap between two follow-up visits.

Exposure might change between the period.

6. The authors may list what lifestyle variables will be collected?

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Partly

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Environmental and cardiovascular epidemiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 23 Mar 2020

Queenie Chan

Dear Dr Naser,

Thank you for reviewing our manuscript and providing those valuable comments. Please find out reply to your suggestions listed below:

1) The mean age of the study population seems high, particularly those who were enrolled in the INTERMAP study. Age is an important biological risk factor for many CVD outcomes in this study. The study findings should not be generalizable for other age groups. The authors may explicitly mention age in the study title and papers published from this study.

We have revised the Background in the Abstract as recommended to emphasize the age group and added "*among middle-aged and older men and women*", and we plan to emphasize the age groups in the papers published from this study.

2) In the "Introduction" and "Methods" sections, the authors have mentioned the environmental factors. I think they are considering only air pollution as environmental exposure, mainly due to household energy consumption. I was wondering whether authors need to consider whether variables such as temperature, humidity, rainfall as the environmental exposure since they can collect these data from secondary sources.

Thank you for the suggestion. Actually as temperature is quite an important risk factor to high BP, we have measured ambient temperature and indoor temperature before BP measurement as indicated in the Table 2, regarding relative humidity, and other pollutants, we are going to find and collect these data from secondary potential available sources as suggested. We have added "*Data on other pollutants (PM₁₀ and gaseous pollutants) and meteorological parameters (e.g., ambient temperature, relative humidity, etc) are going to be collected from the nearest air quality monitoring stations in each site.*" in the Methods section.

3) I think the statistical analysis plans need more elaboration. How will the repeated measures be accounted for? What are the strategies for adjusting p-values for multiple hypotheses testing to avoid false discovery? Should we adjust the models for seasonality or ambient temperature? Evidence from China suggests there is a higher mean blood pressure of the population in winter compared to mean blood pressure in summer.

We have revised as suggested in the section of Methods (Statistical analysis and sample size): "*As for repeated measurements in different seasons, mixed-effect models with random intercepts at individual level will be used to adjust for time-variant factors (e.g., ambient air temperature, physical activity, etc) as well as time-invariant factors (e.g., age, years of education, etc). Estimate of random effect at individual level would have accounted for having measurements in both winter and summer. Bonferroni correction will be applied for multiple testing for statistical significance in our modelling.*"

4) In the first follow-up, spot urine samples will be collected. There are many controversies regarding the utility of spot urine samples to evaluate daily sodium intake. The authors may consider the collection of 24-hour urine sodium. Even a single measurement of 24-hour is not sufficient.

In the section of urine collection and measurements, "timed 24-hour urine samples were collected twice for both studies following the same protocol for both INTERMAP and ICP Study", we have collected multiple 24-hour urine samples during baseline (INTERMAP) and the 1st follow-up survey (ICP, 1st follow-up). We agree that the utility of spot urine might not reflect the true levels of urinary metabolites. We are working on analysis to evaluate the bias of estimate of 24-hour urinary sodium using 24-hour urine samples and spot urine samples both collected in our study.

5) For outcomes (e.g., blood pressure), I feel there is a huge gap between two follow-up visits. Exposure might change between the period.

Indeed, the exposure might change after the enrollment of participants at baseline. This is the challenge that a lot of cohort studies have to face, and regular follow-up visits should be arranged to capture the changes in exposure metrics. We do realize the gap between the baseline and the follow-up, we have several measures in place to improve our study.

(1) Regarding the dietary pattern, we also collected food frequency data in the main questionnaire to measure dietary pattern and eating behaviours for a longer time.

(2) Regarding the air pollution exposure in long term, as we have edited in the text, models

are planned to be built with integrating multiple sources of data, including stationary ambient outdoor measurements in long term, personal air pollution monitoring, and housing characteristics (e.g. energy and stove use) from questionnaire data, to estimate the environmental exposure (PM 2.5 and black carbon) of individuals in long term.

(3) Besides the cross-sectional analyses we have done for baseline survey, regarding the BP change between baseline and follow-up, we could still study the BP change with age, and see what factors might predict those changes. Some exposure metrics might change a lot, but some might stay the same as the baseline, e.g. education, and some metrics for SES etc.

6) The authors may list what lifestyle variables will be collected?

Regarding the lifestyle variables collected, we have revised as suggested in the section of other measurements for ICP Study: "*Lifestyle and behaviour variables, including cigarette smoking and second-hand smoking, physical activity and sleep patterns alcohol consumption, and eating behaviours were assessed using structured questionnaires (Extended data: Main questionnaire ²⁷).*

A revised version has been submitted online, and we look forward to your response.

Best regards,
Dr Queenie Chan

Competing Interests: No competing interests were disclosed.

Reviewer Report 30 October 2019

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Akira Sekikawa

Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh,
Pittsburgh, PA, USA

Summary

The paper describes the protocol of the INTERMAP China Prospective (ICP) study which is to investigate the relationship of environmental and nutritional risk factors. A total of 839 Chinese participants aged 40-59 years from three diverse regions of China were enrolled in INTERMAP in 1997/98. In 2015/16, 574 of these 839 persons were re-enrolled along with 208 new participants aged 40-59 years that were randomly selected from the same study villages. Participant's environmental and dietary exposures and health outcomes were assessed in this open cohort study, including BP, 24-hour dietary recalls, personal exposures to air pollution, grip strength,

arterial stiffness, carotid-media thickness and plaques, cognitive function, and sleep patterns. Serum and plasma specimens were collected with 24-hour urine specimens. The second follow-up will be performed in 2020/21.

The study is a follow-up of well-examined cohorts from the INTERMAP China Study. The reviewer has only minor comments which are meant to be constructive.

1) Second follow-up

In the text, the second follow-up was described as one important component whereas it was not described in the abstract. This component should be added in the abstract.

2) Exposome Research

Exposome research is one key term. However, very few was described or discussed in the text. Add more description/discussion on exposome.

3) Study aim

The third aim of the study was described as 'leverage advances for identifying biomarkers of air pollution exposure and nutritional risk factors and for understanding the mechanisms and pathways of their effects on CVD.' Since the study was not designed and powered to monitor CVD morbidity and mortality, this should be effects on BP?

4) Air pollution exposure measurements

The study collected two consecutive 24-hour personal PM2.5 and black carbon. Questions are (1) China has an air pollution-monitoring system which is zip-code based, thus the investigators should be able to calculate zip-code based past exposures to air pollution in each participant from the existing database. Air pollution has seasonality and large day-to-day variations, thus estimate of past cumulative measures appears to represent exposure to air pollution than two consecutive 24-hour personal exposure. (2) There are many other pollutants that may potentially be associated with blood pressure than PM2.5 and black carbon.

5) Pulse wave velocity measurements

The study evaluated arterial stiffness using brachial-femoral PWV. Measurement of arterial stiffness is important in hypertension research partly because some studies show that arterial stiffness precedes incident hypertension whereas other studies show the opposite. These studies typically use either carotid-femoral PWV (regarded as the gold standard) or brachial-ankle PWV. More recently some studies have started to report using cardio-ankle vascular index. Considering these backgrounds, it would be better to provide some references for brachial-femoral PWV in terms of (1) difference among this and other widely-used techniques, and (2) how to manage quality control of the measurements throughout the study period across the different sites. Though the reviewer has read the Supplemental material, it was not described well.

6) Intima-media thickness measurements

In the protocol or in supplemental material, quality control procedures of the measurements should be clearly described. How to monitor intra-examiner, inter-examiner variations, throughout the study period and study sites and to what extent, the variation would be permitted.

7) Cardiovascular outcome/CVD outcome

Throughout the text, a term 'cardiovascular outcome' or 'CVD outcome' is used five times. In some

places, it referred to BP and other places it referred to outcome other than BP. Please clarify.

8) The sample size of this cohort is relatively small compared with other biobank studies. Biobank was used only once in the text. In the reference, UK biobank was referenced. China has many biobank studies, e.g., Kddorie Biobank, Shanghai Zhangjing Biobank. Either drop the term 'biobank' or define biobanks with some examples in China.

9) Kidney function

The initial INTERMAP study recruited subjects aged 40-59 where the kidney function can be assumed normal. The mean age of the follow-up cohort is 65 years old, thus mention on kidney function (whether it would be assessed or not, if assessed, how it would be assessed, or if not, why) is necessary.

10) Other nutrients/metabolites other than sodium/salt

The INTERMAP was developed to examine the association of macro- and micro-nutrients with blood pressure after the INTERSALT study. Thus, the focus shifted from salt to other micro-nutrients as well as macro-nutrients. In fact, as the author referenced, the INTERMAP and INTERMAP China reported some urinary metabolites and nutrients and their association with blood pressure. Adding a little description of this aspect would strengthen the paper.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Partly

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: cardiovascular epidemiology, arterial stiffness, subclinical atherosclerosis, international studies, nutrients, dementia

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 23 Mar 2020

Queenie Chan

Dear Dr Sekikawa,

Thank you for reviewing our manuscript and providing those valuable comments.

Please find our responds to your suggestions listed below:

1) Second follow-up "In the text, the second follow-up was described as one important component whereas it was not described in the abstract. This component should be added in the abstract."

We have revised the Abstract as suggested: "*A second follow-up visit for the ICP Study participants has been planned for 2020-2021.*" has been added in our abstract.

2) Exposome Research "Exposome research is one key term. However, very few was described or discussed in the text. Add more description/discussion on exposome."

We have added a paragraph as suggested at the beginning of the section of Discussion: "*More complete and precise exposure assessment in epidemiological studies is critically needed nowadays. The exposome refers to the totality of exposure across the life course, integrating both the external (e.g., environmental sensors) and internal exposure (e.g., biomarkers and -omics), and it could help elucidate the link between exposure, underlying biological mechanisms and onset of diseases. From the perspective of exposome research, both the INTERMAP and the ICP Study used a range of diverse tools to measure exposure of participants in the cohort. Portable monitors with environmental sensors were used to measure external personal exposure to air pollutants of PM 2.5 and black carbon, and high-throughput omics technologies combined with conventional tools (e.g. dietary recall and questionnaires) were used to detect specific metabolites of foods and nutrients in urine specimens to improve exposure assessment. For example, with the 24-hour urine collected at the baseline of INTERMAP, a proton nuclear magnetic resonance (1H NMR) spectroscopy-based metabolome-wide association approach 24 was used to identify urinary metabolites that discriminated between southern and northern Chinese participants and those population-level biomarkers may also be associated with differences in BP and CVD risk in populations.*"

3) Study aim "The third aim of the study was described as 'leverage advances for identifying biomarkers of air pollution exposure and nutritional risk factors and for understanding the mechanisms and pathways of their effects on CVD.' Since the study was not designed and powered to monitor CVD morbidity and mortality, this should be effects on BP?"

We have revised the aim to use the "BP" as the primary outcome for this study is BP. As Dr Sekikawa, pointed out, the study may not have adequate statistical power to monitor CVD morbidity and mortality, we may only explore the associations between the sets of exposure metrics and some outcomes of CVD outcomes (e.g., non-fatal CVD events).

4) Air pollution exposure measurements "The study collected two consecutive 24-hour personal PM_{2.5} and black carbon. Questions are (1) China has an air pollution-monitoring system which is zip-code based, thus the investigators should be able to calculate zip-code based past exposures to air pollution in each participant from the existing database. Air pollution has seasonality and large day-to-day variations, thus estimate of past cumulative measures appears to represent exposure to air pollution than two consecutive 24-hour

personal exposure. (2) There are many other pollutants that may potentially be associated with blood pressure than PM_{2.5} and black carbon."

Thank you for sharing your insights. Indeed, two consecutive 24-hour personal PM_{2.5} and black carbon exposure measurements in each campaign could not represent the long-term exposure of individual, but we may have to discuss the value of this exposure metric in different scenarios. If we study acute effects of exposure to PM_{2.5} on BP, the exposure assessment used here would be useful. But if we try to study chronic effects on health outcomes, the exposure assessment should take into account cumulative exposure in long term as suggested. The data obtained from stationary ambient air pollution monitors could be useful to estimate the long-term exposure and we should definitely try to obtain such information. As we cannot only rely on the data of stationary ambient monitoring here, because in those deprived areas of developing countries, the household air pollution (e.g. from burning of solid fuel and environmental tobacco smoking) might be a main source of exposure to PM_{2.5}. Personal exposure levels are usually much higher than ambient exposure levels in those areas. Thus, the estimated air pollution exposure only using ambient monitored data may underestimate the true exposure in our cohort. A possible resolution is integrating multiple sources of data, including both stationary ambient outdoor measurements in long term, personal air pollution monitoring, and housing characteristics (e.g. energy and stove use) from questionnaire data, and building models to estimate the environmental exposure of individuals in long term.

Regarding the second question, for other pollutants that may potentially associated with BP, as we did not collect any data for those pollutants from personal air monitoring (especially gaseous pollutants), we may only be able to do such analyses if we can obtain more reliable data from stationary ambient monitors.

We have added some key information in the related section (air pollution exposure measurements) in Methods: *"The acute effects of PM_{2.5} and black carbon on BP to be investigated. Regarding the long-term effects of air pollution, models are planned to be built with multiple sources of data, including stationary ambient outdoor measurements, personal air pollution monitoring, and housing characteristics (e.g., energy and stove use) from questionnaire data, to estimate the environmental exposure (PM_{2.5} and black carbon) of individuals. Data on other pollutants (PM₁₀ and gaseous pollutants) and meteorological parameters (e.g., ambient temperature, relative humidity, etc) are going to be collected from the nearest air quality monitoring stations in each site."*

5) Pulse wave velocity measurements "The study evaluated arterial stiffness using brachial-femoral PWV. Measurement of arterial stiffness is important in hypertension research partly because some studies show that arterial stiffness precedes incident hypertension whereas other studies show the opposite. These studies typically use either carotid-femoral PWV (regarded as the gold standard) or brachial-ankle PWV. More recently some studies have started to report using cardio-ankle vascular index. Considering these backgrounds, it would be better to provide some references for brachial-femoral PWV in terms of (1) difference among this and other widely-used techniques, and (2) how to manage quality control of the measurements throughout the study period across the different sites. Though the reviewer has read the Supplemental material, it was not described well."

Thank you for the suggestion and we have revised the supplemental material and added more details.

6) Intima-media thickness measurements "In the protocol or in supplemental material, quality control procedures of the measurements should be clearly described. How to monitor intra-examiner, inter-examiner variations, throughout the study period and study sites and to what extent, the variation would be permitted."

We have revised as suggested in the supplemental material about the procedures of quality control.

7) Cardiovascular outcome/CVD outcome "Throughout the text, a term 'cardiovascular outcome' or 'CVD outcome' is used five times. In some places, it referred to BP and other places it referred to outcome other than BP. Please clarify."

We have revised those places and added more descriptions as recommended to be more precise in the text.

8) The sample size of this cohort is relatively small compared with other biobank studies. Biobank was used only once in the text. In the reference, UK biobank was referenced. China has many biobank studies, e.g., Kddorie Biobank, Shanghai Zhangjing Biobank. Either drop the term 'biobank' or define biobanks with some examples in China.

We have dropped the term 'biobank' here, as we did not mean to compare with any specific large biobank studies here.

9) Kidney function "The initial INTERMAP study recruited subjects aged 40-59 where the kidney function can be assumed normal. The mean age of the follow-up cohort is 65 years old, thus mention on kidney function (whether it would be assessed or not, if assessed, how it would be assessed, or if not, why) is necessary."

We have revised as suggested in the section of Methods: "*Urinary sodium and potassium excretion rates and other urinary metabolites (e.g., hippurate, formate) were measured. Urinary albumin has been planned to be measured as a marker of kidney disease.*" and "*Fasting blood glucose, lipid profiles, serum creatinine, and estimated glomerular filtration rate were measured.*"

10) Other nutrients/metabolites other than sodium/salt: "The INTERMAP was developed to examine the association of macro- and micro-nutrients with blood pressure after the INTERSALT study. Thus, the focus shifted from salt to other micro-nutrients as well as macro-nutrients. In fact, as the author referenced, the INTERMAP and INTERMAP China reported some urinary metabolites and nutrients and their association with blood pressure. Adding a little description of this aspect would strengthen the paper."

We have revised as suggested in the section of Introduction: "*The focus of INTERMAP is on elucidating effects of other dietary factors and urinary metabolites than high salt and suboptimal*

potassium on BP of individuals. For instance, dietary alanine was higher in people who predominantly consume animal than vegetable diet, which was consistent with the findings of a direct association of urinary alanine excretion and BP in the INTERMAP cohort²²."

We have also added an extra example in the section of Discussion: "*For example, with the 24-hour urine collected at the baseline of INTERMAP, a proton nuclear magnetic resonance (¹H NMR) spectroscopy-based metabolome-wide association approach²⁴ was used to identify urinary metabolites that discriminated between southern and northern Chinese participants and those population-level biomarkers may also be associated with differences in BP and CVD risk in populations.*"

A revised version has been submitted, and we look forward to your response.

Thank you.
Dr Queenie Chan

Competing Interests: No competing interests were disclosed.