Original Research Article

Mixture Effects of Heavy Metals on Insulin Resistance in Shipyard Welders

Ting-Yao Su [a, b], Kai-Jen Chuang [c, d, e], Hueiwang Anna Jeng [f], Yuan-Ting Hsu [a, g], Hsiao-Chi Chuang [c, h], and Ching-Huang Lai [a, i] *, Chih-Hong Pan [b, i] **.

[a] Graduate Institute of Life Sciences, National Defense Medical Center, Taipei 114, Taiwan.

[b] Institute of Labor, Occupational Safety and Health, Ministry of Labor, Executive Yuan, New Taipei City 221, Taiwan.

[c] School of Public Health, Taipei Medical University, Taipei 110, Taiwan.

[d] School of Medicine, Taipei Medical University, Taipei 110, Taiwan.

[e] Graduate Institute of Medical Sciences, Taipei Medical University, Taipei 110, Taiwan.

[f] School of Community and Environmental Health, College of Health Sciences, Old Dominion University, Norfolk, VA 23529, USA.

[g] National Institute of Environmental Health Sciences, National Health Research Institutes, Miaoli County 350, Taiwan.

[h] School of Respiratory Therapy, Taipei Medical University, Taipei 110, Taiwan.

[i] School of Public Health, National Defense Medical Center, Taipei 114, Taiwan.

* Corresponding author at: Graduate Institute of Life Sciences, National Defense Medical Center, Rm. 4326, 4F., No. 161, Sec. 6, Minquan E. Rd., Neihu Dist., Taipei 114, Taiwan, Tel: +886-2-87923100 Ext. 18464, Fax: +886-2-87926485, Email: lgh@mail.ndmctsgh.edu.tw.

** Corresponding author at: Institute of Labor, Occupational Safety and Health, Ministry of

Labor, Executive Yuan, No. 99, Ln. 407, Hengke Rd., Xizhi Dist., New Taipei 221, Taiwan, Tel:

+886-2-26607600 Ext. 7662, Fax: +886-2-26607731, Email: chpan@mail.ilosh.gov.tw.

Abstract

Certain studies have reported various insulin resistance responses to ambient heavy metal pollution, but few have reported such responses to occupational heavy metal pollution. Even fewer have demonstrated a relationship between mixture effects of heavy metals and insulin resistance in welding workers. Overall, we recruited 53 welders and 48 administrative staff from a shipyard located in northern Taiwan. Personal exposure to heavy metals was monitored for $PM_{2.5}$ and urine. Blood samples from each participant were collected from the antecubital vein after fasting. Urine samples from each participant were collected in the same period as blood samples. The geometric mean levels for chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), and cadmium (Cd) in the $PM_{2.5}$ of the personal breathing zone and urinary Mn of welders were significantly higher than those in administrative staffs. Ambient Cr, Co, Ni, and Cu levels in the $PM_{2.5}$ and urinary Cd were positively related to HOMA2-IR after adjusting for personal covariates ($PM_{2.5}$-Cr: $\beta=0.036$, 95%C.I.: 0.002 to 0.070; $PM_{2.5}$-Co: $\beta=0.040$, 95%C.I.: 0.002 to 0.077; $PM_{2.5}$-Ni: $\beta=0.054$, 95%C.I.: 0.013 to 0.094; $PM_{2.5}$-Cu: $\beta=0.049$, 95%C.I.: 0.010 to 0.088; U-Cd: $\beta=0.209$, 95%C.I.: 0.052 to 0.366, respectively). Our findings indicated the $PM_{2.5}$ metal components and urinary metals were associated with increased insulin resistance in shipyard welders.

Key Words: shipyard welders, $PM_{2.5}$ metal components, urinary metals, insulin resistance.

1. Introduction

Insulin resistance (IR) is a systemic disorder defined as the compromised ability of insulin to regulate insulin-mediated glucose disposal and/or to inhibit hepatic glucose production and adipose tissue lipolysis. IR plays a major pathophysiological role in type 2 diabetes [1]. Long-term IR have been associated with several metabolic abnormalities and major public health problems, including cardiovascular disease and abnormalities, visceral adiposity, endothelial dysfunction kidney disease, hypertension, coronary artery disease, and dyslipidemias [2, 3].

Trace elements deficiencies and excesses were implicated for increasing risks of type 2 diabetes mellitus through interfered with blood glucose homeostasis [4, 5].

Essential metals, such as manganese (Mn), iron (Fe), zinc (Zn), and copper (Cu), are important for the function of various enzymatic systems of human body. For example, Zn is required in the formation and crystallization of insulin, activation of the kinase 3 phosphatidylinositol enzyme, and induction of the translocation of glucose transporter 4 (GLUT4) [6, 7]. However, an excess of intake of the metals could consequently lead to increased risks of insulin resistance. Overload of iron could modify hepatocytes' insulin sensitivity by interfering with insulin receptor and intracellular insulin signaling [8]. Also, studies showed that exposures to copper in excess, could create oxidative stress, which is a factor in the onset and the progression of type 2 diabetes [9].

Besides essential metals, toxic metals without any known biological function, such as Cd, As and Pb have been reported to be associated with increases the occurrence of diabetes and increase the risk of metabolic syndrome [10, 11]. For example, chronic exposure to inorganic As at even low to moderate levels could disrupt a number of biochemical processes involved in glucose homeostasis, leading to both decreased insulin-stimulated glucose uptake and decreased glucose-stimulated insulin secretion [12, 13]. The metals could affect hormone states by substitute for essential metals, such as iron, zinc and/or potassium, in biological systems. Additionally, in vitro model showed that metals can catalyze oxidative stress reaction that leads to decrease insulin gene promoter activity and insulin mRNA expression in islet β-cells [4].

Both essential and toxic metals are unambiguously present in the environment. Various pollution sources could result in different metal profiles. Due to traffic emissions, higher concentrations of Cd, Zn, Cr, Ni and Pb occur in ambient air [14]. Industrial process and operations, such as welding, mining, smelters, continues to a prominent source of metals and produce unique metal mixtures. Our biomonitoring studies showed that welding fume heavily include chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), and cadmium (Cd) [references]. Welders experienced significantly higher concentrations of those metals in urine than administrators who didn't work in welding process.

Some epidemiologic studies have investigated the impact of metal exposure from traffic emissions on insulin resistance responses in the general population [references]. The

population-based studies found an association between cadmium and insulin resistance, but this association was not consistent in all studies. Also, most of the studies had measured limited metals. Currently, few have reported such responses to occupational heavy metal pollution despite the concern of health risks to occupational workers. This study aimed to 1) quantify personal exposure to metals in welding fumes; 2) evaluate insulin resistance of the welders and administrative staff; and 3) determine any associations between exposure to metals from welding fumes and insulin resistance of the welders and administrative staff.

2.   Materials and Methods

2.1. Ethics

The Institutional Review Board of Tri-Service General Hospital, National Defense Medical Center in Taiwan approved this study (TSGHIRB 2-106-05-180). All participants provided informed consents voluntarily.

2.2. Study Participants

We recruited 101 health male workers, including of 53 welders and 48 administrative staffs from a shipyard located in northern Taiwan. The welders served as the exposed group that had been chronically exposed to heavy metals; while the administrative staffs served as the reference group. Criteria for the participant selection included males 20-65 years old, >1 year of employment in the plant, no diabetes, and no moderate to severe renal dysfunction. Renal function was estimated using the Taiwanese modification of diet in renal disease (TMDRD) formula [13, 14]. Trained interviewers met the participants between September 2015 and November 2015 to collect their demographic information, including age, work experience, height, weight, waist circumference, number of years of working in the shipyard and health condition, as well as information on their life-style, covering cigarette smoking. Cigarette smoking was deemed positive if either occurred on at least four days per week.

2.3. Exposure Assessment for Metals in Workplace Air

All participants were requested to wear the Personal Environmental Monitor (PEM, SKC

Ltd., Blandford, Dorset, UK.) samplers for $PM_{2.5}$ with polytetrafluoroethylene filters (diameter:

37 mm, pore size: 0.45 μm, Cat. No. 225-17-04, SKC Ltd., Blandford, Dorset, UK.) at a flow

rate of 2.0 L/min during their working hours for monitoring 8 targeted heavy metals, including

Cr, Mn, Fe, Co, Ni, Cu, Zn, and Cd in $PM_{2.5}$. Levels of targeted heavy metals in the personal

breathing zone of shipyard workers were quantified by using inductively coupled plasma mass

spectrometry (ICP-MS, iCAP RQ, Thermo Scientific, Waltham, MA, USA) combined with

microwave-assisted acid digestion, which provides superior detectability for trace elements [15,

16]. The detection limits of Cr, Mn, Fe, Co, Ni, Cu, Zn, and Cd were 5.3 ng/L (101.9 pM), 3.0

ng/L (54.6 pM), 220.3 pM (12.3 ng/L), 6.8 pM (0.4 ng/L), 97.1 pM (5.7 ng/L), 45.6 pM (2.9

ng/L), 273.7 pM (17.9 ng/L), and 6.2 pM (0.7 ng/L) were obtained using seven repeated

analyses of deionized water. The measured levels below lower detection limit were imputed by

using monotone imputation method (IBM SPSS 22.0, IBM Corp., Armonk, NY, USA).

2.4. Urinary Metals Determination

The urine samples were stored at -80°C until analysis. They were prepared by centrifuging

at 1000 rpm and took out the supernatant, then dilute it 3 times with 1.3% nitric acid (HNO_3),

filtered it through a 0.22μm syringe filter, and store it in a plastic centrifuge tube for measuring

heavy metals. Levels of targeted urinary heavy metals including Cr, Mn, Fe, Co, Ni, Cu, Zn,

and Cd were quantified by using inductively coupled plasma mass spectrometry (ICP-MS,

iCAP RQ, Thermo Scientific, Waltham, MA, USA).

2.5. The Bio-chemical Assays

The blood specimens were collected using the BD Vacutainer system (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), the urine samples were collected using BD centrifuge tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The bio-chemical assays were performed immediately after collection. The analytical methods was the hexokinase method for plasma glucose, the glycerol phosphate dehydrogenase method for serum triglycerides, the catalase elimination method for serum high-density lipoprotein, the Jeffe method for creatinine, the chemiluminescence method for serum insulin, and the high performance liquid chromatography method for HbA1c. The plasma glucose, serum triglycerides, serum high-density lipoprotein, and creatinine were measured using the automated ADVIA Chemistry XPT system (Siemens Healthineers AG, Erlangen, Germany). The serum insulin was determined using the automated ADVIA Centaur XPT system (Siemens Healthineers AG, Erlangen, Germany). The blood HbA1c was quantified using the automated D-100 System (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

2.6. Updated Homeostatic Model Assessment

The parameters of updated Homeostatic Model Assessment (HOMA2) including estimated insulin resistance (IR), pancreatic β cell function (%B) and insulin sensitivity (%S) were obtained using the HOMA2 calculator software developed by the Diabetes Trials Unit

(DTU, the Oxford Centre for Diabetes, Endocrinology and Metabolism), University of Oxford[17].

2.7. Statistical Analysis

Due to the skewness of continuous variables, especially metals (Cr, Mn, Fe, Co, Ni, Cu, Zn, and Cd), and biochemical markers (glucose, triglycerides, high-density lipoprotein, and insulin), descriptive statistics were performed as geometric mean and geometric standard deviation. Furthermore, categorical variables were performed as frequency and percent in descriptive statistics. In inferential statistics, for the purpose to compare the difference of frequency and value of risk factors and outcomes between exposed and reference group, χ^2 test (or fisher's exact test), and Mann-Whitney U test were applied.

All data from shipyard workers were then included in linear mixed-effects regression models to identify significant predictors of workers' plasma glucose AC, serum insulin, blood HbA1c, HOMA2-IR, HOMA2-%B, and HOMA2-%. The subjects' gender, age, BMI, serum triglycerides, urinary cotinine, and urinary creatinine were treated as fixed effects, and each worker was treated as a random effect in the data analysis. The level for statistical significance was set to α=0.05 in all tests. Statistical analysis was conducted using IBM SPSS statistics software for Windows version 22.0 (IBM Corp., Armonk, NY, USA).

3.   Results

3.1.   Characteristics of Study Participants

Table 1 provides the demographic characteristics of for 101 workers by job title in a shipyard. The study population consisted of 53 welders and 48 administrative staff. Their mean age was about 45 years old. Their mean BMI was approximately 25 kg/m^2. There were no statistically significant differences between welders and administrative staff in cardiovascular measurements and urinary cotinine.

3.2.   Comparisons of HOMA2 parameters, PM$_{2.5}$ and Urinary Metals between Exposed and Reference Groups

Table 2 compares the HOMA2 parameters, metals in PM2.5 and urinary metals of the welders and the administrative staff. The GM HOMA-%B of the welders significantly exceeded that of the administrative staff (p=0.044). The GM Cr, Mn, Fe, Co, Ni, Cu, Zn and Cd concentrations of welders were all significantly higher than those of administrative staff. Furthermore, the GM urinary Cr, Mn, Fe, Co, Ni, Cu, Zn and Cd concentrations of welders significantly exceeded those of the administrative staff.

3.3.   . Relationship between Urinary Excretion of Metals and Metals in PM$_{2.5}$

Table 3 demonstrated the Spearman's correlation matrix among metals in PM$_{2.5}$ and urinary metals. Mn in PM$_{2.5}$ was significantly correlated to urinary Mn (ρ=0.225, p<0.05); Fe in PM$_{2.5}$ was significantly correlated to urinary Fe (ρ=0.204, p<0.05).

Table 3 shows the relationship between urinary excretion of metals and metals in $PM_{2.5}$

among 101 shipyard workers Urinary Mn levels was significantly correlated with Cr, Mn, Fe,

Co, Ni, Cu, and Zn levels in $PM_{2.5.}$ Furthermore, urinary Fe levels was significantly correlated

with Cr. Fe, Co, Ni, Cu, Zn and Cd levels in $PM_{2.5}$. Moreover, urinary Ni was positively

correlated with Zn and Cd levels in $PM_{2.5}$ Among all the urinary metals, Co was the only

metal that exhibiting reversely relationship with Cr, Mn, and Fe levels in $PM_{2.5}$.

3.4. Effects of metals in $PM_{2.5}$ and Urinary Metals on Plasma Glucose, Serum Insulin, and Blood Glycated Hemoglobin

Tables 4 presents the effects of metals in PM2.5 and urinary metals on plasma glucose,

serum insulin, and blood glycated hemoglobin. Urinary Mn, Cu, Zn, Cd, and the summed metals

were five significant and positive predictors of plasma glucose after adjustments were made for

other covariates. However, metals in PM2.5 were not significant predictors of plasma glucose.

Furthermore, Cr, Co, Ni, Cu in $PM_{2.5}$ and urinary Cd were significantly related to increased

serum insulin. However, metals in PM2.5 and urinary metals were not significant predictors of

blood glycated hemoglobin.

3.5. Effects of Metals in $PM_{2.5}$ and Urinary Metals on HOMA2 Parameters

Table 5 indicates the effects of metals in $PM_{2.5}$ and urinary metals on HOMA2 parameters.

Airborne Cr, Co, Ni, Cu in $PM_{2.5}$ and urinary Cd were significant and positive predictors of

insulin resistance after adjustments were made for other covariates. Furthermore, airborne Cr,

Co, Ni, Cu in $PM_{2.5}$ were significant and positive related to pancreatic β cell function. The

HOMA2 insulin sensitivity was the reciprocal of insulin resistance, therefore the regression

coefficients of them were opposite.

4.   Discussion

To the best of our knowledge, the present study is the first study to investigate the effects of occupational metals in $PM_{2.5}$ and urinary Metals on insulin resistance in shipyard welders. The present analysis demonstrated that increased exposure to Cr, Co, Ni, and Cu in $PM_{2.5}$ and urinary Cd were related to the increment of serum insulin and an estimated insulin resistance using the HOMA2 index. These results indicates occupational metals exposure may disrupt the homeostatic of blood glucose and insulin.

The relationship between occupational zinc exposure and the increased risk of diabetes was reported in steel production workers [5], coke oven workers [6], and non-ferrous metals production workers [7, 8]. Cappelletti et al. demonstrated a significant 1.39 times increment of relative risk of diabetes after Mantel-Haenszel method adjusting for age in steel production workers compare to the general population from the same province [5]. Liu et al. observed that urinary Cu, Zn, and Cd were significantly higher in diabetic workers and urinary Mn, Cu, Zn, and Cd were significantly higher in hyperglycemic workers compared with normoglycemic workers in a coke oven worker population. In addition, increased urinary Cu and Zn levels were demonstrated related to increased diabetes odds ratio, and increased urinary Mn, Cu, and Zn were represented related to increased hyperglycemia odds ratio [6]. As defined high-fasting-glucose (high-FPG) as fasting blood glucose equal to 5.2 mM (93.6 mg/dL), A previous epidemiological study indicated that significantly elevated high-FPG odds ratios were observed

14

in the groups with highest quartiles of urinary Ni (>=0.15 μM [9.06 μg/L]) and Zn (>=8.94 μM [584.43μg/L]), meanwhile, the trend tests of quartiles of urinary Ni and Zn on the risks of high-FPG was reached statistical significance level [7]. Yang et al. also observed a significant increment of log-transformed fasting plasma glucose in non-ferrous production workers with urinary zinc over 5.64 μM (369 μg/L), the trend tests of quartiles of urinary zinc on fasting plasma glucose was also reached statistical significance level [8]. Our study provided the statistically significant log-linear relationship between occupational Cr, Co, Ni, and Cu exposure to the homeostasis of serum insulin in shipyard welders, whom were joining galvanized mild steel using arc welding technique, these results may be extrapolated to the population worked in the same scenarios such as steel structure building workers and mild steel pipe welders.

Although epidemiological studies have established the relationship between PM and the increased risk of type 2 DM, study investigated the relationship between inhaled metal particles and urine metals to the underlying mechanisms of diabetes still limited. Chuang et al have observed that intratracheal instilled exposed to nano-sized zinc oxide induces systemic inflammation and oxidative stress in Sprague-Dawley rats [18]. Pan et al have observed that exposed to zinc is upregulating underlying mechanisms involved in glycolysis/gluconegeogenesis pathway in BALB/c mice which were intratracheal instilled nano-sized zinc oxide [19]. Pavanello et al have investigated that steel production workers

occupationally exposed to Fe, Ni, Cu, and Zn in PM_{10} (particulate matters with an aerodynamic

diameters less than 10 μm) is positively related to the elevated expression of micro RNA

(ribonucleic acid) miR-196b [20], which is involved in a post-transcriptional regulation

mechanism of insulin biosynthesis in mice [21].

In contrast, these metals has also play important roles in regulating normal physiological

functions involved homeostatic of glucose and insulin. For instance, zinc ions is reported to

target with tyrosine phosphatase 1B, which is a crucial regulator on insulin receptor

phosphorylation [22]. Zinc is also reported to induce glucose transport into cells through

enhancing the insulin signaling pathway [23]. Ahn et al. have found a significantly negative

relationship between serum zinc and HOMA-IR in non-diabetic Korean general population

based on the Korean National Health and Nutrition Examination Survey data [24].

In this study, the urinary metals as one of the exposure assessment biomarkers. Many

metals have been proven to cause chronic kidney disease [25], including Cd, have also been

reported associated with renal tubular dysfunction in welders [26]. Jin et al. found that renal

function affects the urinary metal levels [14], and may resulted in the false estimation of metals

exposure. In addition, Kuo et al. have indicated that renal dysfunction may be a potential

confounder to the risk of diabetes in nickel exposed population due to the significance

difference of urinary Ni and estimated glomerular filtration rate (eGFR) between diabetic

patients and control group in Liu's study [27, 28]. Also, chronic kidney disease (CKD) is a

common comorbid disease of type 2 diabetes mellitus (T2DM) patients [29]. In the present study, participants with eGFR less than 60 mL/min/1.73m^2 were excluded to avoid the confounding of renal dysfunction to the relationship between urinary metals to insulin resistance.

This study has certain limitations. First, the data of dietary essential/micro nutrients intake were lack, possibly confounding the results concerning urinary metal excretion. Furthermore, The other limitation was the lack of data on the exposure to metals outside occupational settings, such as from vehicle traffic emissions. However, the restaurantworkers herein spent >10 h/d at the shipyard, including work and rest periods, but <1 h/d in traffic. The contribution of traffic sources to the metal exposure of shipyard workers is thus assumed to be limited.

5.   Conclusion

Airborne Cr, Co, Ni, Cu in $PM_{2.5}$ and urinary Cd were significant and positive predictors of insulin resistance in a shipyard workers. It is crucial to develop proper preventive measures, including adequate ventilation and the use of personal protection equipment (gloves and respirators) to protect the health of shipyard workers.

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Declaration of competing interest

The authors declare no competing interests.

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Table 1. Characteristics of study participants.

	All participants (n=101)	Exposed group (n=53)	Reference group (n=48)	*p*-Value[a]
	Mean±SD	Mean±SD	Mean±SD	
Age (years)	45.510±12.273	45.655±11.610	45.351±13.089	0.981
Smoking status (n [%])				0.330[b]
Current	38 (37.6)	23 (43.4)	15 (31.3)	
Former	18 (17.8)	10 (18.9)	8 (16.7)	
Never	45 (44.6)	20 (37.7)	25 (52.1)	
U-COT (µM, GM [GSD])	3.024 (6.516)	3.479 (6.999)	2.591 (6.047)	0.353
Family History of T2DM (n [%])				0.126[b]
Yes	14 (13.9)	10 (18.9)	4 (8.3)	
No	87 (86.1)	43 (81.1)	44 (91.7)	
CVD history (n [%])				0.326[b]
Yes	23 (22.8)	10 (18.9)	13 (27.1)	
No	78 (77.2)	43 (81.1)	35 (72.9)	
Weight (kg)	72.812±12.256	70.340±9.479	75.542±14.342	0.069
Height (cm)	169.297±6.128	167.925±5.459	170.813±6.516	0.045
BMI (kg/m^2)	25.311±3.254	24.909±2.785	25.754±3.682	0.373
Waist circumference (cm)	87.599±9.218	85.745±8.008	89.646±10.083	0.054
Waist circumference (n [%])				0.223[b]
>=90	40 (39.6)	18 (34.0)	22 (45.8)	
<90	61 (60.4)	35 (66.0)	26 (54.2)	
SBP (mmHg)	131.901±18.786	131.755±18.405	132.063±19.393	0.881
DBP (mmHg)	80.228±13.057	79.660±12.235	80.854±14.014	0.648
SBP / DBP (n [%])				0.271[b]
>=130 ∨ >=85	51 (50.5)	24 (45.3)	27 (56.3)	
<130 ∧ <85	50 (49.5)	29 (54.7)	21 (43.8)	
S-TG (mM, GM [GSD])	1.253 (1.880)	1.133 (1.734)	1.401 (2.011)	0.096
S-TG (n [%])				0.446[b]
>=1.7	16 (15.8)	7 (13.2)	9 (18.8)	
<1.7	85 (84.2)	46 (86.8)	39 (81.3)	
S-HDL (mM, GM [GSD])	1.253 (1.249)	1.295 (1.247)	1.207 (1.247)	0.091
S-HDL (n [%])				0.817[b]
<1.03	18 (17.8)	9 (17.0)	9 (18.8)	
>=1.03	83 (82.2)	44 (83.0)	39 (81.3)	
U-CREAT (mM, GM [GSD])	9.759 (1.598)	9.486 (1.593)	10.070 (1.609)	0.636
S-CREAT (mM, GM [GSD])	0.079 (1.147)	0.079 (1.153)	0.079 (1.141)	0.639
TMDRD (mL/min/1.73 m^2, GM [GSD])	81.233 (1.166)	81.068 (1.170)	81.416 (1.163)	0.514
TMDRD (n [%])				0.229[b]
>=60 ∧ <90	65 (64.4)	37 (69.8)	28 (58.3)	
>=90	36 (35.6)	16 (30.2)	20 (41.7)	

[a]Mann-Whitney U test; [b]χ^2 test.

Abbreviations: U-COT (urinary cotinine); T2DM (type 2 diabetes mellitus); CVD (cardiovascular disease); BMI (body mass index); SBP (systolic blood pressure); DBP (diastolic blood pressure); S-TG (serum triglycerides); S-HDL (serum high density lipoprotein); U-CREAT (urinary creatinine); S-CREAT (serum creatinine); TMDRD (Taiwanese modification of diet in renal disease).

Table 2. Comparisons of HOMA2 parameters, $PM_{2.5}$ and urinary metals between exposed and reference groups.

	All participants (n=101)	Exposed group (n=53)	Reference group (n=48)	*p*-Value[a]
	GM (GSD)	GM (GSD)	GM (GSD)	
HOMA2 parameters				
P-FGAC (mM)	5.288 (1.099)	5.317 (1.096)	5.257 (1.103)	0.317
P-FGAC (n [%])				0.746[b]
>=5.6	30 (29.7)	15 (28.3)	15 (31.3)	
<5.6	71 (70.3)	38 (71.7)	33 (68.8)	
S-INSL (pM)	56.253 (1.698)	50.626 (1.528)	63.196 (1.839)	0.107
B-HbA1c (mmol/mol)	35.535 (1.150)	35.071 (1.172)	36.055 (1.123)	0.779
HOMA2-IR	1.228 (1.687)	1.110 (1.526)	1.372 (1.822)	0.107
HOMA2-%B	97.708 (1.446)	89.942 (1.348)	107.065 (1.519)	0.044
HOMA2-%S	81.479 (1.688)	90.134 (1.528)	72.885 (1.823)	0.104
$PM_{2.5}$ metals ($\mu mol/m^3$)				
Cr	0.001 (13.489)	0.002 (9.233)	0.000130 (9.891)	<0.001
Mn	0.033 (37.269)	0.270 (16.392)	0.003 (18.939)	<0.001
Fe	0.209 (23.196)	1.311 (11.234)	0.027 (12.936)	<0.001
Co	0.000052 (10.088)	0.000145 (6.951)	0.000017 (8.765)	<0.001
Ni	0.000366 (7.768)	0.001 (4.859)	0.000142 (8.201)	<0.001
Cu	0.002 (9.016)	0.004 (5.749)	0.001 (8.580)	<0.001
Zn	0.092 (10.922)	0.289 (6.990)	0.026 (9.029)	<0.001
Cd	0.000016 (9.114)	0.000034 (7.328)	0.000007 (8.801)	<0.001
Summed	0.420 (17.571)	2.131 (9.788)	0.070 (10.368)	<0.001
Urinary metals (μM)				
Cr	0.045 (1.423)	0.045 (1.351)	0.044 (1.499)	0.176
Mn	0.046 (1.407)	0.050 (1.445)	0.042 (1.332)	0.014
Fe	1.444 (1.382)	1.491 (1.339)	1.394 (1.426)	0.194
Co	0.010 (1.603)	0.010 (1.557)	0.011 (1.644)	0.268
Ni	0.401 (1.538)	0.395 (1.532)	0.408 (1.551)	0.838
Cu	1.877 (1.395)	1.852 (1.395)	1.906 (1.398)	0.729
Zn	7.337 (1.848)	6.929 (1.917)	7.815 (1.769)	0.422
Cd	0.005 (1.964)	0.005 (2.024)	0.006 (1.909)	0.724
Summed	11.726 (1.521)	11.340 (1.549)	12.168 (1.491)	0.459

[a]Mann-Whitney U test; [b]χ^2 test.

Abbreviations: HOMA2 (updated homeostasis model assessment); $PM_{2.5}$ (particulate matters with an aerodynamic diameter less than 2.5 μm); P-FGAC (fasting plasma glucose ante cibum); S-INSL (serum insulin); B-HbA1c (blood glycated hemoglobin); IR (insulin resistance); %B (percentages of β cell function of a normal reference population); %S (percentages of insulin sensitivity of a normal reference population); Cr (chromium); Mn (manganese); Fe (iron); Co (cobalt); Ni (nickel); Cu (copper); Zn (zinc); Cd (cadmium); Summed (the summation of the 8 measured metals).

Table 3. Relationship between urinary excretion of metals and metals in $PM_{2.5}$ among 101 shipyard workers

		Urinary metals (μmol/L)								
		Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Summed metals[a]
	Cr	0.127	0.255*	0.205*	-0.225*	0.170	0.106	0.028	0.118	0.052
	Mn	0.086	0.225*	0.148	-0.206*	0.097	0.036	0.019	0.094	0.027
	Fe	0.137	0.275**	0.204*	-0.230*	0.128	0.065	-0.034	0.118	-0.009
$PM_{2.5}$	Co	0.144	0.262**	0.264**	-0.162	0.160	0.026	-0.017	0.140	-0.002
metals	Ni	0.107	0.225*	0.207*	-0.193	0.148	0.069	0.001	0.069	0.018
$(μmol/m^3)$	Cu	0.168	0.299**	0.262**	-0.187	0.190	0.098	-0.010	0.162	0.030
	Zn	0.193	0.304**	0.227*	-0.116	0.221*	0.056	0.020	0.144	0.051
	Cd	0.112	0.190	0.207*	-0.169	0.208*	0.067	-0.005	0.093	0.012
	Summed metals[a]	0.153	0.287**	0.212*	-0.202*	0.160	0.069	0.000	0.121	0.027

*$p<0.05$,

**$p<0.01$

[a]Summed metals: sum of Cr, Mn, Fe, Co, Ni, Cu, Zn, and Cd.

Table 4. Assessment of multiple linear regression analysis of plasma glucose AC, serum insulin and blood HbA1c using linear mixed-effects regression analysis and and forest plots (n=101).
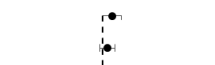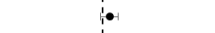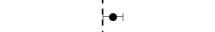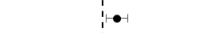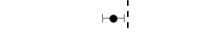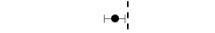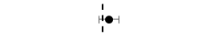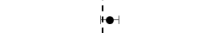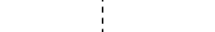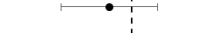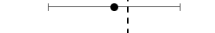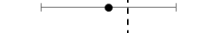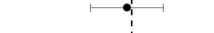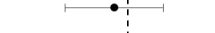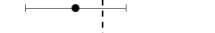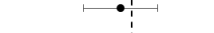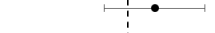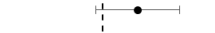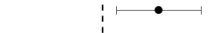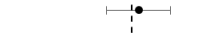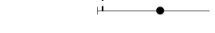
	Plasma glucose AC (mmol/L in Ln scale) β (95% C.I.)	Serum insulin (pmol/L in Ln scale) β (95% C.I.)	Blood HbA1c (mmol/mol in Ln scale) β (95% C.I.)
metals in PM$_{2.5}$ (µmol/m^3 in Ln scale)			
Cr	-0.001 (-0.008, 0.005)	0.037 (0.002, 0.072)*	0.004 (-0.007, 0.015)
Mn	0.002 (-0.004, 0.007)	0.018 (-0.011, 0.046)	0.003 (-0.006, 0.012)
Fe	0.001 (-0.005, 0.008)	0.027 (-0.005, 0.059)	0.004 (-0.006, 0.014)
Co	-0.004 (-0.011, 0.004)	0.042 (0.004, 0.080)*	0.007 (-0.005, 0.020)
Ni	-0.000155 (-0.008, 0.008)	0.055 (0.014, 0.096)**	0.007 (-0.006, 0.021)
Cu	0.000004 (-0.008, 0.008)	0.050 (0.011, 0.090)*	0.004 (-0.008, 0.017)
Zn	0.001 (-0.006, 0.008)	0.020 (-0.018, 0.059)	0.002 (-0.010, 0.014)
Cd	-0.004 (-0.011, 0.003)	0.026 (-0.011, 0.064)	0.007 (-0.005, 0.019)
Summed metals[a]	0.001 (-0.005, 0.008)	0.027 (-0.008, 0.061)	0.003 (-0.008, 0.014)
Urinary metals (µmol/L in Ln scale)			
Cr	0.015 (-0.030, 0.059)	-0.108 (-0.339, 0.122)	-0.062 (-0.134, 0.010)
Mn	0.054 (0.008, 0.101)*	0.037 (-0.210, 0.283)	-0.027 (-0.105, 0.051)
Fe	0.022 (-0.026, 0.071)	0.066 (-0.186, 0.319)	-0.076 (-0.154, 0.003)
Co	0.025 (-0.011, 0.060)	0.046 (-0.140, 0.231)	-0.018 (-0.077, 0.040)
Ni	-0.013 (-0.050, 0.023)	-0.098 (-0.287, 0.092)	-0.017 (-0.077, 0.043)
Cu	0.048 (0.002, 0.094)*	0.111 (-0.131, 0.353)	0.020 (-0.056, 0.097)
Zn	0.058 (0.030, 0.087)***	0.117 (-0.040, 0.275)	0.031 (-0.019, 0.081)
Cd	0.055 (0.026, 0.085)***	0.199 (0.039, 0.359)*	0.025 (-0.026, 0.077)
Summed metals[a]	0.091 (0.049, 0.134)***	0.192 (-0.043, 0.428)	0.034 (-0.041, 0.109)

Covariates include group (exposed/reference), age (>45 years/<=45 years), waist (cm), serum triglycerides (mmol/L in Ln scale), urinary cotinine (µmol/L in Ln scale), and urinary creatinine (mmol/L in Ln scale).

*$p<0.05$, **$p<0.01$, ***$p<0.001$

[a]Summed metals: sum of Cr, Mn, Fe, Co, Ni, Cu, Zn, and Cd.

Table 5. Assessment of multiple linear regression analysis of HOMA2-IR, HOMA2-%B, and HOMA2-%S using linear mixed-effects regression analysis and and forest plots (n=101).

	HOMA2-IR in Ln scale β (95% C.I.)	HOMA2-%B in Ln scale β (95% C.I.)	HOMA2-%S in Ln scale β (95% C.I.)
PM$_{2.5}$ metals (µmol/m^3 in Ln scale)			
Cr	0.036 (0.002, 0.070)*	0.028 (0.003, 0.053)*	-0.036 (-0.070, -0.002)*
Mn	0.018 (-0.010, 0.046)	0.008 (-0.012, 0.029)	-0.018 (-0.046, 0.010)
Fe	0.027 (-0.005, 0.058)	0.016 (-0.008, 0.039)	-0.027 (-0.059, 0.006)
Co	0.040 (0.002, 0.077)*	0.036 (0.008, 0.063)*	-0.040 (-0.077, -0.002)*
Ni	0.054 (0.013, 0.094)**	0.038 (0.008, 0.068)*	-0.053 (-0.094, -0.013)*
Cu	0.049 (0.010, 0.088)*	0.034 (0.006, 0.063)*	-0.049 (-0.088, -0.010)*
Zn	0.020 (-0.018, 0.058)	0.012 (-0.016, 0.040)	-0.020 (-0.058, 0.018)
Cd	0.025 (-0.013, 0.062)	0.026 (-0.001, 0.053)	-0.024 (-0.062, 0.013)
Summed metals[a]	0.026 (-0.008, 0.061)	0.016 (-0.009, 0.041)	-0.026 (-0.061, 0.008)
Urinary metals (µmol/L in Ln scale)			
Cr	-0.101 (-0.328, 0.126)	-0.104 (-0.270, 0.062)	0.100 (-0.127, 0.328)
Mn	0.050 (-0.193, 0.293)	-0.083 (-0.261, 0.095)	-0.051 (-0.294, 0.193)
Fe	0.071 (-0.178, 0.320)	0.001 (-0.183, 0.184)	-0.072 (-0.321, 0.178)
Co	0.051 (-0.131, 0.234)	-0.018 (-0.153, 0.116)	-0.051 (-0.234, 0.132)
Ni	-0.100 (-0.286, 0.087)	-0.041 (-0.178, 0.097)	0.100 (-0.087, 0.286)
Cu	0.120 (-0.118, 0.359)	-0.018 (-0.194, 0.158)	-0.121 (-0.360, 0.118)
Zn	0.131 (-0.024, 0.286)	-0.036 (-0.151, 0.079)	-0.130 (-0.286, 0.025)
Cd	0.209 (0.052, 0.366)**	0.025 (-0.094, 0.144)	-0.209 (-0.366, -0.052)**
Summed metals[a]	0.213 (-0.019, 0.444)	-0.050 (-0.222, 0.123)	-0.212 (-0.444, 0.019)

Covariates include group (exposed/reference), age (>45 years/<=45 years), waist (cm), serum triglycerides (mmol/L in Ln scale), urinary cotinine (µmol/L in Ln scale), and urinary creatinine (mmol/L in Ln scale).

*p<0.05, **p<0.01, ***p<0.001

[a]Summed metals: sum of Cr, Mn, Fe, Co, Ni, Cu, Zn, and Cd.