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Simultaneous exposure of non-diabetics to high levels of dioxins and mercury increases their risk of insulin resistance

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ABSTRACT

Insulin resistance and the defective function of pancreatic β-cells can occur several years before the development of type 2 diabetes. It is necessary to investigate and clarify the integrated effects of moderate-to-high exposure to dioxins and mercury on the pancreatic endocrine function. This cross-sectional study investigated 1449 non-diabetic residents near a deserted pentachlorophenol and chloralkali factory. Metabolic syndrome related factors were measured to examine associations with serum dioxin and blood mercury. We also investigated associations between insulin resistance (HOMA-IR > 75th percentile), defective pancreatic β-cells function (HOMA β-cell > 75th percentile), serum dioxins and blood mercury. After adjusting for confounding factors, we found that insulin resistance increased with serum dioxins ($b = 0.13$, $P < 0.001$) and blood mercury ($b = 0.01$, $P < 0.001$). Moreover, participants with higher serum dioxins or blood mercury were at a significantly increasing risk for insulin resistance ($P_{trend} < 0.001$). The joint highest tertile of serum dioxins and blood mercury was associated with elevated HOMA-IR at 11 times the odds of the joint lowest tertile (AOR 11.00, 95% CI: 4.87, 26.63). We hypothesize that simultaneous exposure to dioxins and mercury heightens the risk of insulin resistance more than does individual exposure.

1. Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are well-known persistent organic pollutants (POPs), which cause insulin resistance with moderate to high exposure levels [1–4]. There has been little detailed analysis of the onset of diabetes in relation to dioxin exposure and whether elevated levels of dioxins contribute to diabetogenesis [5].

Much of what we know about methylmercury toxicity in humans is about its effect on the central nervous system [6–8]. However, patients with documented Minamata disease (methylmercury poisoning) in Japan had an increased incidence of diabetes, and the autopsies of persons who had died of Minamata disease revealed interference with the functioning of the islets of Langerhans cells in the pancreas, which affect insulin production [9]. In addition, in experiments on rats, a single injection of methylmercury injured pancreatic islets, and repeated injections induced a high level of blood glucose [10]. Although not well supported by epidemiological evidence, these studies suggest a link between mercury exposure and diabetes.

Diabetes is referred to as a "lifestyle disease," and its rampant spread is believed to be caused by obesity, increased reliance on the Western dietary pattern diet or getting little or no regular weekly exercise. Although the precise pathogenesis of type 2 diabetes is unknown, insulin resistance is considered the initial pathophysiological sign and may finally lead to fasting hyperglycemia and diabetes [11]. Metabolic syndrome (MetS), a cluster of metabolic risk factors, is a reliable predictor of diabetes and cardiovascular disease. In general, the stigmata of MetS are significantly associated with insulin resistance [12].

Recent studies [13,14] report that there may be more environmental risk factors for diabetes than the traditional ones such as Western fast foods and lifestyle changes. While there are a num-

Abbreviations: MetS, metabolic syndrome; PCP, pentachlorophenol; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; Hg, mercury; PCBs, polychlorinated biphenyls; POPs, persistent organic pollutants; HOMA-IR, homoeostasis model assessment-insulin resistance; TG, triglycerides; FSIGT, frequently sampled insulin-assisted intravenous glucose tolerance test; MetS-TW, MetS criteria for Taiwanese.

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ber of contributing factors, evidence is mounting that diabetes is closely linked with the environment. For example, a study [15] on hyperinsulinemic bisphenol-A (BPA)-treated mice with abnormal glucose and insulin-tolerance test results – which might have been the result of a direct effect of BPA on β-cell insulin content, or a compensatory response caused by insulin resistance – showed that the widespread environmental contaminant BPA, an endocrine-disrupting chemical (EDC) like PCDD/Fs, increases β-cell insulin content. In the 2003–2004 National Health and Nutrition Examination Survey (NHANES) [16], the US general population showed a strong association between higher exposure to BPA and diabetes. We previously [3] found a slight monotonic increase in the risk for insulin resistance across the serum PCDD/F categories, and that groups with serum dioxins higher than 20.5 pg WHO_{98}-TEQ_{DF}/g lipid had a higher risk of insulin resistance than did controls.

In our previous studies [3,17–19] on a now-deserted factory that used to manufacture pentachlorophenol (PCP) and produce PCDD/Fs as byproducts, a preliminary investigation [19] showed that the marine biota in the nearby sea reservoir was seriously contaminated with high levels of PCDD/Fs and that residents living in a nearby area had average serum PCDD/F levels 3 times higher than those living in non-polluted areas [17,18]. From 1942 to 1982, this factory also manufactured caustic soda, hydrochloric acid, and liquid chlorine, and discharged into the nearby ecosystem a great deal of mercury-contaminated sludge and wastewater, which methylated and bio-accumulated in aquatic organisms. Preliminary surveys by the Taiwan EPA in 1983 and 2004 showed that the mercury levels of fish collected from the Lu Erh Men River and sea reservoir near the deserted factory would be considered unsafe for human consumption in many countries [20]. In another study [21], we found that the total mercury concentration in the participants consisted of approximately 90% methylmercury, which indicated that the major external exposure route for mercury contamination was eating mercury-contaminated fish and other seafood from the aquatic environment near the deserted chloralkali factory. People living near the deserted factory were co-exposed to PCDD/Fs and mercury from eating contaminated seafood from that reservoir.

Because insulin resistance heightens the risk that MetS sufferers will become pre-diabetic, which can lead to diabetes, we investigated the associations between MetS-related risk factors, serum PCDD/F levels, and blood mercury levels. Insulin resistance and pancreatic β-cell dysfunction can occur several years before the development of type 2 diabetes. We previously showed [3] that serum PCDD/F levels were associated with monotonically increasing insulin resistance in a non-diabetic study population, even after we had adjusted for confounding factors. Little research has been done to examine the hypothesis that high levels of blood mercury are associated with an increased risk of insulin resistance (homoeostasis model assessment-insulin resistance = HOMA-IR) and pancreatic β-cell function (HOMA β-cell) and whether this effect would be greater than the sum of individual components.

2. Experimental

2.1. Study participants

This cross-sectional study was done from July 2005 through December 2007 in a district health center near the deserted PCP factory. The sampling strategies are reported elsewhere [3]. The only recruitment criterion was that the participant had to reside near the factory. The exposure areas were Hsien-Gong, Lu-Erh, and Ssu-Tsao Li, three of the smallest administrative divisions in the study district. All invited participants, chosen from the household registry office, were more than 18 years old when recruited. The 1812 participants consisted of approximately 80% of all invited residents in the local community. We had incorrect addresses for 104 (23.0%) of the 453 non-participants, and 54 (11.9%) were either too ill to participate or else deceased. We received no response to our mailed invitation from the remaining 295 (65.1%), most of whom did not work locally and had changed their permanent address.

After signing an informed consent form, each participant provided 80 mL of venous blood. Participants were asked to fast overnight before the blood samples were drawn. The Human Ethics Committee of National Cheng Kung University Hospital approved both the study protocol and the manner in which informed consent was obtained. Information obtained from the questionnaire included personal characteristics (age, gender, medical history of diabetes, etc.), and lifestyle habits (alcohol intake, tobacco use, eating habits, etc.). Information about the participants' history of diabetes included questions about prior diagnoses of diabetes by a physician, and their current use of insulin and oral hypoglycemic agents. Participants were considered to have diabetes if their fasting plasma glucose was ≥126 mg/dL or they reported a history of physician-diagnosed type 1 or type 2 diabetes.

2.2. Laboratory procedures

In PCDD/F analysis, blood samples were drawn into chemically cleaned tubes without anti-coagulants. The samples were then stored at −70 °C until they were analyzed. We used isotope dilution high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS), as previously described [3,19], to measure seventeen 2,3,7,8-substituted PCDD/Fs in serum samples. All PCDD/Fs were adjusted to the lipid content analyzed from the corresponding samples. Quality assurance/quality control (QA/QC) protocols followed USEPA Method 1613 to ensure positive identification and the quality of the measurements.

In mercury analysis, blood samples were drawn into chemically clean tubes containing heparin, and were stored at 4 °C until they were analyzed. We used a mercury analysis system (MA-2000; Nippon Instruments Corporation, Tokyo, Japan) as previously described [21], in which a 0.1 mL blood sample is thermally decomposed by the heater of the mercury analyzer. Each analytical run consisted of a method blank, a quality control, and six unknown samples for QA/QC. Moreover, data QA was monitored by spiking known amounts of mercury into blood samples and subsequently carrying out the same procedure as described above. One blood sample in every batch was analyzed in duplicate; the relative percentage difference of mercury was 8.0% (0.4–17.2%). A reference material, Standard Reference Materials 966, (National Institute of Standards Technology (NIST), Gaithersburg, MD) was also used for the QA of mercury; the measured concentrations (30.4–31.5 μg/L) met the certified values (29.7–33.1 μg/L). The method detection limit (MDL) of mercury was 0.17 μg/L.

A radioimmunoassay (Coat-A-Count; Siemens Medical Solutions Diagnostic, Los Angeles, CA, USA) was used to measure serum insulin. Coefficients of variation for inter-assay and intra-assay variability for insulin were all below 10%. Blood biochemistry tests for fasting glucose, HDL cholesterol, LDL cholesterol, and triglycerides (TG) were analyzed in the pathology laboratory of National Cheng Kung University Hospital.

2.3. Statistical analysis

PCDD/F concentration is expressed in picograms (1 pg = 10^{-12} g) WHO_{98}-TEQ_{DF}/g lipid. Mercury concentration is expressed as μg/L. We used JMP 5.0 (SAS Institute, Cary, NC, USA) for statistical analysis and the Kruskal–Wallis and Wilcoxon rank-sum tests to evaluate serum PCDD/Fs and mercury between different demographic characteristics.

Type 2 diabetes is characterized by insulin resistance, which decreases the cellular uptake of glucose. Consequently, elevated blood glucose levels reduced the target tissue response to insulin and caused hyperinsulinemia, which manifests early in type 2 diabetes. Therefore, we used the homeostasis model assessment (HOMA) to assess insulin resistance (HOMA-IR) and pancreatic β-cell function (HOMA β-cell). The Matthews et al. method [22] was used to estimate, at the baseline using a HOMA-IR-derived value, the degree of insulin resistance in each participant. Specifically, a HOMA-IR score was calculated using the following formula [23]:

$$\text{HOMA-IR} = [\text{fasting glucose(mmol/L)}] \times \left[\frac{\text{fasting insulin (mU/L)}}{22.5}\right].$$

A lower HOMA-IR indicates high insulin sensitivity, whereas a higher HOMA-IR indicates insulin resistance. In addition, the European Group for the Study of Insulin Resistance [23] defines insulin resistance as a fasting insulin or HOMA-IR score >75th percentile for the reference population. Pancreatic β-cell function was calculated using the following formula:

$$\text{HOMA β-cell} = \frac{20 \times \text{fasting insulin(mU/L)}}{\text{fasting glucose(mmol/L)} - 3.5}.$$

Because HOMA-IR and HOMA β-cell values were substantially skewed, we used a logarithmic transformation (log) to minimize the skew of these values for the analysis. To assess the association between insulin resistance, defective pancreatic β-cell function, serum PCDD/F, and blood mercury levels, we first used multiple linear regressions. Other covariates included age, gender, smoking, physical activity, waist circumference, systolic blood pressure, diastolic blood pressure, and a family history of diabetes. In addition, we hypothesized that higher serum PCDD/F and blood mercury levels elevate the index of insulin resistance and the defective function of pancreatic β-cell (HOMA β-cell) in non-diabetic persons before the development of type 2 diabetes. Therefore, the response variables of interest, measured as a dichotomous variable at the beginning, were "insulin resistance (HOMA-IR >75th percentile)" and "defective function of pancreatic β-cell (HOMA β-cell >75th percentile)". The main exposure variables of interest, serum PCDD/F and blood mercury levels, were divided into tertiles (cutoff levels of tertiles of PCDD/Fs were 15.9 and 30.3 pg WHO_{98}-TEQ_{DF}/g lipid; cutoff levels of tertiles of mercury were 7.2 and 11.4 μg/L).

The definition of MetS in this study is based on the MetS criteria for Taiwanese (MetS-TW), which is modified from the Adult Treatment Panel III [24]: the presence of any three of the following: (1) waist circumference >90 cm in men and >80 cm in women, (2) triglycerides >150 mg/dL, (3) HDL cholesterol <40 mg/dL in men and <50 mg/dL in women, (4) blood pressure >130/85 mmHg, and (5) fasting glucose >100 mg/dL.

3. Results

3.1. Demographic distribution of study participants

Of the initial 1812 study participants recruited, we excluded 37 who had not fasted before blood sampling and 326 who had diabetes, which finally left us with 1449 participants (758 men, 691 women; age range: 25–92 years; mean age: 49.9 years) (Table 1). Excluding those who had not fasted did not significantly change our estimates. The mean serum PCDD/F level was 33.2 pg WHO_{98}-TEQ_{DF}/g lipid (range: 4.5–514.0 pg WHO_{98}-TEQ_{DF}/g lipid). Serum PCDD/F levels were significantly different in the five age groups and significantly higher in women (mean: 37.1; range: 4.7–403.0 pg WHO_{98}-TEQ_{DF}/g lipid; $P < 0.001$) than in men (mean: 29.6; range: 4.5–514.0 pg WHO_{98}-TEQ_{DF}/g lipid). The mean blood mercury level was 10.8 μg/L (range: 0.2–86.8 μg/L). Blood mercury levels were also significantly different in the five age groups and significantly higher in men (mean: 12.7; range: 0.5–86.8 μg/L; $P < 0.001$) than in women (mean: 8.8; range: 0.2–85.7 μg/L) (Table 1). Participants with an abnormally large waist circumference, high systolic blood pressure, and high glucose level had significantly ($P < 0.05$) higher serum PCDD/F and blood mercury levels than those without these factors. In addition, we found no significant differences between different HDL cholesterol groups in serum PCDD/F and blood mercury levels.

3.2. Association among MetS components, serum PCDD/F levels, and blood mercury levels

Pearson's correlation shows that all MetS components were significantly associated with HOMA-IR ($P < 0.001$ for all) (Table 2). Moreover, serum dioxin levels were significantly associated with HOMA-IR ($r = 0.09$, $P < 0.001$) and moderately-to-strongly associated with systolic blood pressure ($r = 0.35$, $P < 0.001$), fasting glucose ($r = 0.14$, $P < 0.001$), waist circumference ($r = 0.13$, $P < 0.001$), diastolic blood pressure ($r = 0.10$, $P < 0.001$), and HDL cholesterol levels ($r = 0.07$, $P = 0.008$). Blood mercury levels were also significantly associated with HOMA-IR ($r = 0.19$, $P < 0.01$) and moderately-to-strongly associated with waist circumference ($r = 0.27$, $P < 0.001$), fasting glucose ($r = 0.17$, $P < 0.001$), diastolic blood pressure ($r = 0.14$, $P < 0.001$), triglycerides ($r = 0.08$, $P = 0.003$), and systolic blood pressure ($r = 0.07$, $P = 0.006$).

3.3. Multiple regression model

After adjusting for confounding factors, we found that insulin resistance increased with serum PCDD/F and blood mercury levels ($b = 0.13$, $P < 0.001$ for PCDD/Fs, and $b = 0.01$, $P < 0.001$ for Hg, respectively) (Table 3). In addition, HOMA β-cell increased with serum PCDD/F levels ($b = 0.08$, $P = 0.03$) but not with blood mercury levels (Table 3). After we had adjusted for confounding factors, we found that participants with higher serum PCDD/F or blood mercury levels were at a significantly increasing risk for insulin resistance ($P < 0.001$, χ^2 test for the trend) (Table 4). The joint highest tertile of serum PCDD/F and blood mercury levels was associated with elevated HOMA-IR at 11 times the odds of the joint lowest tertile (AOR 11.00, 95% CI: 4.87, 26.63). These data show that serum PCDD/F and blood mercury levels affected the association with insulin resistance. Therefore, we observed an integrated risk of co-exposure to both serum PCDD/Fs and blood mercury, even in non-diabetic participants.

4. Discussion

4.1. Association between MetS components and serum PCDD/F and blood mercury levels

In the present study, we found that serum dioxin and blood mercury levels were moderately to strongly associated with each MetS component, and that PCDD/Fs were associated with HOMA-IR. However, in the NHANES study [25], in which participants were exposed only to background levels of several POPs with different POPs have differing effects on insulin resistance and MetS, organochlorine (OC) pesticides, for example, were strongly correlated with HOMA-IR and positively and significantly associated with four of the five MetS components, but not with high blood pressure. In addition, in the NHANES study, PCDD/Fs were not associated with HOMA-IR or MetS components, except for a small but significant association with high blood pressure. Our findings suggest that dioxins are associated with MetS, and they indicate that the metabolic effect of high-dose exposure to dioxins differs from that of low-dose background exposure. Although we have no

Table 1
Demographic characteristics of all study participants ($N = 1449$).

Variables	Number (%)	PCDD/Fs (pg WHO-TEQ/g lipid)	Mercury (Hg) (μg/L)	$P^{\dagger}$	$P^{\ddagger}$
Age (years)				<0.001	<0.001
<40	451 (31.1%)	16.5 (4.5–221.0)	8.6 (0.3–40.5)		
40–50	406 (28.0%)	27.8 (5.3–309.0)	12.8 (0.5–85.7)		
50–59	253 (17.5%)	36.5 (7.3–514.0)	11.4 (1.2–54.4)		
60–69	157 (10.8%)	48.1 (7.3–291.2)	12.3 (0.2–79.4)		
>70	182 (12.6%)	69.0 (10.4–412.9)	9.9 (0.7–86.8)		
Gender				<0.001	<0.001
male	758 (52.3%)	29.6 (4.5–514.0)	12.7 (0.5–86.8)		
female	691 (47.7%)	37.1 (4.7–403.0)	8.8 (0.2–85.7)		
Smoking status				<0.001	<0.001
Never smoked	609 (42.0%)	39.2 (4.7–514.0)	10.3 (0.2–85.7)		
Active smoker	504 (34.8%)	29.1 (4.5–374.1)	12.3 (0.7–86.8)		
Passive smoker	336 (23.2%)	28.3 (5.3–355.2)	9.7 (0.3–45.8)		
Waist circumference (cm)				<0.001	<0.001
men ≤ 90 and women ≤ 80	851 (58.7%)	29.1 (4.5–514.0)	10.0 (0.3–85.7)		
Men > 90 and women > 80	598 (41.3%)	39.0 (4.6–374.1)	12.0 (0.2–86.8)		
Systolic BP (mmHg)				<0.001	0.017
≤135	909 (62.7%)	27.6 (4.5–514.0)	10.5 (0.3–86.8)		
>135	540 (37.3%)	42.4 (4.9–374.1)	11.4 (0.2–79.4)		
Diastolic BP (mmHg)				0.006	<0.001
≤85	1172 (80.9%)	32.5 (4.6–514.0)	10.4 (0.3–86.8)		
>85	277 (19.1%)	36.1 (4.5–374.1)	12.7 (0.2–45.8)		
HDL cholesterol (mg/dl)				0.961	0.053
men ≥ 40 and women ≥ 50	1049 (72.4%)	32.9 (4.5–514.0)	11.0 (0.2–86.8)		
Men < 40 and women < 50	400 (27.6%)	33.8 (4.6–412.9)	10.4 (0.5–45.5)		
Triglycerides (mg/dl)				0.789	0.002
<150	1106 (76.3%)	33.5 (4.5–514.0)	10.5 (0.3–85.7)		
≥150	343 (23.7%)	32.3 (4.6–312.0)	12.0 (0.2–86.8)		
Fasting glucose (mg/dl)				<0.001	<0.001
<100	1131 (78.1%)	31.8 (4.5–514.0)	10.5 (0.2–86.8)		
≥100	318 (21.9%)	38.1 (6.4–412.9)	12.1 (0.7–57.6)		
Insulin (mU/L)				<0.001	<0.001
6–27	746 (51.5%)	34.1 (4.6–403.0)	11.7 (0.2–85.7)		
<6	672 (46.4%)	32.2 (4.5–514.0)	9.6 (0.3– 86.8)		
>27	31 (2.1%)	32.4 (8.5–109.0)	15.2 (2.0–40.5)		

Abbreviations: BP = blood pressure.

† *P*: indicates whether serum PCDD/Fs differed between different demographic characteristics, calculated using the Wilcoxon Rank-Sum test for dichotomous variables and the Kruskal–Wallis test for categorical variables with 3 or more classifications.

‡ *P*: indicates whether blood Hg differed between different demographic characteristics, calculated using the Wilcoxon Rank-Sum test for dichotomous variables and the Kruskal–Wallis test for categorical variables with 3 or more classifications. All tests were 2-sided.

other POPs data for this population, we did not consider exposure to organochlorine pesticides because there were few agricultural activities and organochlorine pesticides sprayed on our study site in the past thirty years.

We also found an association between waist circumference and the concentrations of serum PCDD/Fs and mercury. In several studies [25,26], waist circumference was positively associated with current dioxin-like compound levels. Waist circumference independently contributes to the prediction of non-abdominal, abdominal subcutaneous, and visceral fat [27]. The major site of accumulation of dioxins and the furans in humans at low exposure levels is adipose tissue, and persons with more body fat eliminate dioxins more slowly than those with less body fat [28].

Table 2
Pearson correlation coefficients among Metabolic syndrome components, serum PCDD/F and blood mercury levels ($N = 1449$).

Variable	Age	Waist	Sys BP	Dia BP	HDL	TG	Glucose	Insulin	IR	β-cell	PCDD/Fs
Waist	0.249***	1.000									
Sys BP	0.556***	0.349***	1.000								
Dia BP	0.124***	0.376***	0.569***	1.000							
HDL	0.022	−0.377***	−0.060*	−0.139***	1.000						
TG	−0.012	0.225***	0.088**	0.137***	−0.286***	1.000					
Glucose	0.154***	0.202***	0.173***	0.157***	0.029	0.085**	1.000				
Insulin	0.017	0.434***	0.144***	0.196***	−0.237***	0.162***	0.281***	1.000			
IR	0.027	0.424***	0.152***	0.200***	−0.215***	0.163***	0.386***	0.987***	1.000		
β-cell	−0.024	0.176***	0.048	0.095***	−0.163***	0.058*	−0.154***	0.424***	0.371***	1.000	
PCDD/Fs†	0.600***	0.131***	0.352***	0.096***	0.069**	−0.030	0.143***	0.085**	0.094***	0.008	1.000
Mercury†	0.047	0.273***	0.072**	0.143***	−0.027	0.079**	0.167***	0.180***	0.187***	0.035	0.140***

Abbreviations: Waist = waist circumference; Sys BP = systolic blood pressure; Dia BP = diastolic blood pressure; HDL = high density lipoprotein; TG = triglycerides; IR = HOMA IR; β-cell = HOMA β-cell.

† PCDD/F levels and mercury were log-transformed.

* $P < 0.05$ (two-tailed test).

** $P < 0.01$ (two-tailed test).

*** $P < 0.001$ (two-tailed test).

Table 3
Multiple regression models using serum PCDD/F and blood mercury levels as independent variables to assess their association to HOMA-IR and HOMA β-cell as dependent variables in non-diabetic participants.

Dependent variable:	HOMA-IR† Model 1		HOMA-IR† Model 2		HOMA β-cell Model 3		HOMA β-cell Model 4	
Independent variable	*b* (SE)	*P*	*b* (SE)	*P*	*b* (SE)	*P*	*b* (SE)	*P*
Age (years)	−0.01 (0.002)	<0.001	−0.01 (0.002)	0.004	−0.01 (0.002)	<0.001	−0.09 (0.002)	<0.001
Gender (men/women)	−0.03 (0.03)	0.311	−0.06 (0.03)	0.041	−0.05 (0.03)	0.102	−0.06 (0.03)	0.031
Smoking (Yes)	0.02 (0.03)	0.381	0.02 (0.03)	0.381	0.02 (0.03)	0.368	0.03 (0.03)	0.353
Family history of Diabetes (Yes)	−0.015 (0.04)	0.683	−0.01 (0.04)	0.774	0.01 (0.04)	0.837	0.01 (0.03)	0.776
Physical activity (Yes)	−0.02 (0.02)	0.393	−0.02 (0.02)	0.419	−0.02 (0.02)	0.477	−0.02 (0.02)	0.500
Waist circumference (cm)	0.03 (0.002)	<0.001	0.03 (0.002)	<0.001	0.03 (0.002)	<0.001	0.03 (0.002)	< 0.001
Systolic BP (mmHg)	0.0003 (0.001)	0.813	0.001 (0.001)	0.731	−0.0004 (0.001)	0.755	−0.0004 (0.001)	0.787
Diastolic BP (mmHg)	0.01 (0.002)	0.003	0.007 (0.002)	0.003	0.004 (0.002)	0.058	0.005 (0.002)	0.055
PCDD/Fs† (pg WHO_{98}-TEQ_{DF}/g lipid)	0.13 (0.04)	<0.001	—	—	0.08 (0.04)	0.030	—	—
Hg (μg/L)	—	—	0.01 (0.003)	<0.001	—	—	0.004 (0.003)	0.138

Abbreviations: SE = standard error.
† PCDD/F levels and HOMA-IR were log-transformed. All tests were 2-sided.

4.2. Serum PCDD/F levels, insulin resistance, and defective pancreatic β-cell function

We found that non-diabetic persons with higher serum PCDD/F levels were at a significant risk of having both insulin resistance and defective pancreatic β-cell function, which supports the hypothesis that PCDD/Fs are involved in the etiology before full-blown type 2 diabetes. Insulin resistance is considered a key player in the pathophysiology of type 2 diabetes [21]. One study [29] reported that insulin resistance can occur several years before the development of type 2 diabetes. In the initial stage of insulin resistance, the normal level of plasma glucose is maintained by the increased secretion of insulin from pancreatic β-cells (hyperinsulinemia). If insulin resistance keeps rising, the compensatory increased insulin secretion becomes insufficient, which causes a rise in plasma glucose levels and, ultimately, type 2 diabetes.

Although the mechanism through which TCDD induces insulin resistance is unclear, there are several possibilities. First, TCDD is highly soluble in adipose tissue and binds to the aryl hydrocarbon (Ah) receptor, a cytosolic, high-affinity receptor [30]. Second, TCDD upregulates Ah-receptor-dependent tumor necrosis factor (TNF)-α expression in several different cell types [31]. Chronic dioxin-induced TNF-α expression may reduce the production of peroxisome-proliferator-activated receptor (PPAR)γ, which suggests that chronic dioxin-induced TNF-α expression causes adult-onset diabetes by inducing insulin resistance [32]. The general population in the U.S.A. showed a "striking" dose-response association between low-level exposure to persistent organic pollutants (POPs) – especially organochlorine pesticides and polychlorinated biphenyl (PCB) congeners – and type 2 diabetes [33]. Moreover, POPs such as organochlorine pesticides and PCBs had already been positively associated with the HOMA-IR model, which suggested an increased risk of diabetes [25]. In victims in a contaminated site, an association between TCDD exposure and hyperinsulinemia was also reported [34] and was suggested to be insulin resistance. We previously reported [3] that serum PCDD/F levels were associated with a monotonically increasing risk of insulin resistance. We found non-diabetic participants with

Table 4
Association between serum PCDD/F, blood mercury levels, and the risk of insulin resistance.

Variables	Total	HOMA-IR			HOMA β-cell		
		No. (%)	OR	95% CI	No. (%)	OR	95% CI
Age (years)							
<40	451	68 (15.1)	1	Referent	86 (19.1)	1	Referent
40–60	659	190 (28.8)	2.19	1.49, 3.22	191 (29.0)	1.56	1.09, 2.25
>60	339	79 (23.3)	1.36	0.78, 2.37	78 (23.0)	0.89	0.52, 1.51
Gender							
men	758	200 (26.4)	1	Referent	204 (26.9)	1	Referent
women	691	137 (19.8)	0.72	0.55, 0.94	151 (21.9)	0.78	0.60, 1.01
Waist circumference (cm)							
men ≤ 90 and women ≤ 80	851	110 (12.9)	1	Referent	134 (15.7)	1	Referent
men ≤ 90 and women ≤ 80	598	227 (38.0)	3.97	3.01, 5.23	221 (37.0)	3.27	2.51, 4.27
Diastolic BP (mmHg)							
≤85	1172	230 (19.6)	1	Referent	262 (22.4)	1	Referent
>85	277	107 (38.6)	1.83	1.36, 2.48	93 (33.6)	1.29	0.95, 1.75
Dioxin†/mercury‡							
1st tertile/1st tertile	207	12 (5.8)	1	Referent	32 (2.2)	1	Referent
1st tertile/2nd tertile	153	23 (15.0)	2.64	1.20, 6.07	31 (2.1)	1.23	0.68, 2.23
1st tertile/3rd tertile	119	33 (27.7)	4.94	2.23, 11.69	33 (2.3)	1.51	0.78, 2.93
2nd tertile/1st tertile	150	25 (16.7)	4.89	2.09, 12.20	29 (2.0)	1.20	0.63, 2.27
2nd tertile/2nd tertile	154	36 (23.4)	4.34	1.98, 10.07	33 (2.3)	1.05	0.54, 2.03
2nd tertile/3rd tertile	183	65 (35.5)	6.42	3.15, 14.03	57 (3.9)	1.77	0.94, 3.39
3rd tertile/1st tertile	126	28 (22.2)	6.19	2.21, 18.08	28 (1.9)	1.45	0.62, 3.42
3rd tertile/2nd tertile	174	44 (25.3)	8.62	3.57, 21.93	55 (3.8)	1.97	0.95, 4.22
3rd tertile/3rd tertile	183	71 (38.8)	11.00	4.87, 26.63	57 (3.9)	1.54	0.77, 3.11

Abbreviations: OR = odds ratio; CI = confidence interval.
† Serum PCDD/F levels indicate: 1st tertile: <15.9 pg WHO_{98}-TEQ_{DF}/g lipid; 2nd tertile: 15.9 ≤ serum PCDD/F levels < 30.3 pg WHO_{98}-TEQ_{DF}/g lipid; 3rd tertile: 30.3 pg WHO_{98}-TEQ_{DF}/g lipid ≤ serum PCDD/F levels.
‡ Blood Hg level indicates: 1st tertile: <7.2 μg/L; 2nd tertile: 7.2 ≤ blood Hg level < 11.4 μg/L; 3rd tertile: 11.4 μg/L ≤ blood Hg level.

serum PCDD/Fs over 20.5 pg WHO_{98}-TEQ_{DF}/g lipid had a more significant health risk of insulin resistance (OR = 2.7–5.0) than did reference groups.

4.3. Blood mercury levels, insulin resistance, and defective pancreatic β-cell function

In the present study, participants with higher blood mercury levels were also at a significant risk of having insulin resistance, which suggests that mercury is also involved in the etiology of type 2 diabetes.

Oxidative stress is involved in the progression of insulin resistance and of pancreatic β-cell dysfunction [35]. Shenker et al. [36] reported that mercury induces apoptosis in human T lymphocytes, and hypothesized both that the target organelle was the mitochondrion and that inducing oxidative stress activated apoptotic pathways. Their findings suggest that methylmercury induces oxidative-stress-regulated pancreatic β-cell cytotoxicity through a mitochondrial apoptotic pathway that activates caspase-3 in response to the mitochondrial release of cytochrome c. In an *in vitro* study [37], treatment with methylmercury—at about the same levels as people would consume in fish under the U.S. Food and Drug Administration's recommended limits—decreased insulin secretion and initiated apoptosis in HIT-T15 cells and isolated mouse islets. This finding clearly indicated that methylmercury-induced oxidative stress causes pancreatic β-cell apoptosis and dysfunction [37]. In an *in vivo* study, 2 or 4 weeks of oral exposure to low-dose mercury increased plasma lipid peroxidation levels, decreased plasma insulin levels, and elevated blood glucose and glucose intolerance. *N*-acetyl-L-cysteine (NAC; a ROS scavenger) prevented these mercury-induced responses [38]. These findings show that low-dose mercury-induced oxidative stress and PI3K activation cause Akt signaling-related pancreatic β-cell dysfunction, which also indicates that oxidative stress is involved in the toxic mechanism in mercury-induced hypoinsulinemia and hyperglycemia.

4.4. Limitations

The present study has several limitations. HOMA is a commonly used method to assess insulin resistance and β-cell function; it requires only fasting glucose and insulin levels and, in large-scale epidemiologic studies, is thus considered an alternative to the complicated "gold standard" methods. HOMA-IR is correlated with the hyperinsulinemic glucose clamp and minimal-model measures of insulin action and β-cell function [39]. However, others [40] have concluded that HOMA-IR may detect age-related insulin resistance in large populations of older people, but that dynamic testing (e.g., the Frequently Sampled Intravenous Glucose Tolerance Test (FSIGT)) seems necessary to quantify diminished insulin secretion in older people. Although we were unable to verify at what age our participants began to be exposed to PCDD/Fs and mercury, and the duration of their exposure, or to establish an association between PCDD/Fs and type 2 diabetes, we did identify the non-negligible roles of exposure to PCDD/Fs, mercury, and insulin resistance. Although rare epidemiological studies support the association between mercury exposure and diabetes, we did observe that participants – even those without diabetes – co-exposed to higher levels of PCDD/Fs and mercury had a much greater risk (AOR 11.00, 95% CI: 4.87, 26.63) of having insulin resistance. These associations seem to be stronger than those in our previous study [3], in which we did not evaluate mercury exposure as another risk factor for insulin resistance. Dioxins and mercury, two of the chemicals often found in fish, can both cause pancreatic endocrine dysfunction. Experimental and clinical pathological evidence suggests that mercury, persistent organic pollutants, BPA, and dioxins, interfere with the functioning of the cells in the islets of Langerhans of the pancreas [41]. However, individual cases of adverse health effects from heavy consumption of fish containing moderate to high levels of dioxins and mercury have been reported only rarely.

In addition, most toxicity testing has been done on a chemical-by-chemical basis, often by exposing animals to a range of concentrations to find the maximum dose that causes no harm. This, however, does not gauge the effects of complex mixtures of toxic chemicals in the human body. Although in low doses a chemical may not be deemed harmful, the effects of mixtures could be additive or synergistic, that is, greater than the sum of the individual components. Although we still need to confirm whether mercury and dioxins act through the same mechanisms or whether the additive effects are discernable under different exposure doses. This is also a problem for related regulatory agencies because governments generally do not take into account the additive effects of simultaneous exposure to different chemicals. Moreover, what is not well known is the cumulative risk posed by exposure to multiple environmental toxins such as mercury and dioxins, which are consumed in a wide variety of seafood.

5. Conclusions

In our large-scale study with a great deal of variation in PCDD/F and mercury co-exposure, we found that PCDD/Fs and mercury affected HOMA-IR and HOMA β-cell function, respectively. Participants – even those without diabetes – co-exposed to higher levels of PCDD/Fs and mercury had a much greater risk (AOR 11.00) of having insulin resistance, which persisted even after we had adjusted for the effect of the other explanatory variables. Our results have important ramifications for public policy, whatever the pathophysiologic mechanism that underlies the integrated risk between serum PCDD/Fs and mercury and their association with insulin resistance.

Whether the increasing risk of insulin resistance leads to a higher prevalence of diabetes deserves more attention and follow-up. In humans, PCDD/Fs and mercury are easily accumulated by eating fish and other seafood. It should concern us that the current tolerable daily intake (TDI) for PCDD/Fs and recommended limits for methylmercury may underestimate the integrated risk.

In summary, we found a significant association between serum PCDD/F levels, blood mercury levels, and insulin resistance after adjusting for confounding factors, even in persons without diabetes. Accumulated dioxins and mercury may both heighten the risk of developing insulin resistance more than individual exposure. Further study is needed to confirm these findings in other persons co-exposed to high levels of PCDD/Fs and mercury. Information about the long-term health implications of insulin resistance should be promptly delivered to those at risk for MetS, and to those with a history of co-exposure to PCDD/Fs and mercury, because of the extra risk they may incur.

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