INSULIN RESISTANCE OF CHRONIC HIGH EXPOSURE TO DIOXINS IN RESIDENTS LIVING NEAR A DESERTED PCP FACTORY

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Abstract

The aim of this study was to evaluate the relationship between the serum PCDD/F levels and the insulin resistance of residents living in the vicinity of the deserted PCP factory. Serum PCDD/Fs levels in 299 residents were measured by HRGC/HRMS. Blood biochemistry tests for High-density lipoproteins (HDL), Triglyceride (TG), Glucose, and Insulin were also conducted. Associations between having diabetes mellitus, high insulin resistance (HOMA-IR), high serum PCDD/Fs, and their potential interaction were examined among the study subjects. The average serum PCDD/Fs levels were 74.9 (9.7 to 951.0) pg WHO_{98}-TEQ/g lipid. Significantly higher PCDD/Fs levels and HOMA-IR value were found in diabetic patients than non-diabetic subjects. After adjusting for related confounding factor, HOMA-IR significantly increased with serum PCDD/Fs levels. Moreover, the subjects with high HOMA-IR value and high serum PCDD/Fs level showed a significantly increasing risk of diabetes as compared to subjects with low HOMA-IR value and low serum PCDD/Fs level (adjusted OR=4.35, 95%CI=1.76- 11.16). Thus, accumulated tissue levels of TCDD may place the elderly at increased risk for the development of insulin resistance and diabetes. Long-term health implications should be also promptly delivered to residents with diagnosed glucose and insulin from a disease prevention point of view.

Introduction

PCDD/Fs are well known environmental contaminants. Epidemic studies have suggested an increased risk of diabetes mellitus with high levels of dioxins exposures. In a well-designed study of Air Force veterans who were part of Operation Ranch Hand, the unit responsible for aerial spraying of Agent Orange in Vietnam[1]. Veterans with high blood TCDD levels demonstrated a greater prevalence of diabetes and a shorter time to onset of diabetes, when compared to veterans with low blood TCDD levels. Non-diabetic veterans with high blood TCDD levels were more likely to be hyperinsulinemia, suggesting that the hyperinsulinemia was the result of insulin resistance. A deserted factory, in the An-Nan area of Tainan city in southern Taiwan, had been manufactured pentachlorophenol (PCP) from 1965 to 1979. PCDD/Fs are formed as byproducts in the PCP manufacturing process. The preliminary investigation showed that marine biota in the nearby sea reservoir were seriously contaminated and some of the inhabitants living near the deserted PCP factory had been exposed to high PCDD/F levels through consumption of contaminated seafood[2]. The aim of this study was therefore to evaluate the relationship between serum PCDD/F levels and the insulin resistance of residents living in the vicinity of the deserted PCP factory. Associations between having diabetes mellitus, high insulin resistance (HOMA-IR), high serum PCDD/Fs concentration, and their potential interaction were also examined.

Material and Methods

This cross-sectional study was done in a local clinic near the deserted PCP factory from January to March 2007. Eligibility requirements included living in a targeted neighborhood—Hsien-Gong, Lu-Erh, or Ssu-Tsao Lis for at least 5 years. After signing a consent form, each participant provided 60 mL of venous blood. Blood samples were drawn into chemically clean tubes containing no anti-coagulants, and serum samples were obtained after centrifugation. The serum sample was stored at –70°C until analysis. Serum PCDD/Fs were measured using isotope dilution HRGC/HRMS. The sample enrichment and cleanup procedures used in this study were modified according to the procedures reported by Chang[3]. And the analytical procedure was complied with the protocols defined in USEPA method 1613. Blood biochemistry tests for High-density lipoproteins (HDL), Triglyceride (TG), Glucose, and Insulin were measured in pathology laboratory of National Cheng Kung University Hospital. Information was obtained from the questionnaire including personal characteristics (gender, age, medical history, etc), and life style (alcohol intake and tobacco usage).

In each subject, the degree of insulin resistance was estimated at the baseline by HOMA-IR value according to the method described by Matthews[4]. In particular, an insulin resistance score (HOMA-IR) was computed with the formula: fasting plasma glucose (mg/dL) times fasting serum insulin (mU/l) divided by

22.5. Lower HOMA-IR value indicates high insulin sensitivity, whereas higher HOMA-IR value indicates low insulin sensitivity (insulin resistance). In addition, the European Group for the Study of Insulin Resistance (EGIR) defines the insulin-resistance as a fasting insulin or HOMA-IR (homoeostasis model assessment) score >75 percentile for the reference population. To assess the presence of interaction between insulin resistance and serum PCDD/Fs levels, we compared the odds ratios (ORs) of diabetes mellitus for subjects in each category of jointed to subjects with high or low HOMA-IR value and serum PCDD/Fs. ORs with 95% CI were calculated from multivariate logistic regression analysis after adjustment for age, BMI, triglyceride and smoking status.

The JMP 5.0 (SAS Institute, Cary, NC) was used for data management and statistical analysis. In addition, the Kruskal-Wallis and Wilcoxon rank-sum test were used to evaluate the PCDD/Fs levels and HOMA-IR value between different demographic characteristics. Since there is a substantial skewing of HOMA-IR value, logarithmic transformation (Log) was applied to subordinate the skew of these values for the analysis.

Results and Discussion

Two hundred and ninety-nine residents (126 men, 173 women), ranging from 33 to 92 years of age were recruited as participants for this study. From Table 1, the average serum PCDD/Fs levels were 74.9 pg WHO_{98}-TEQ/g lipid, ranged from 9.7 to 951.0 pg WHO_{98}-TEQ/g lipid. Serum PCDD/Fs levels were significantly different in different age groups and significantly higher in women (82.2 pg WHO_{98}-TEQ_{DF}/g lipid) than in men (64.9 pg WHO_{98}-TEQ_{DF}/g lipid). Diabetic patients had significantly higher PCDD/Fs levels than non-diabetic subjects. HOMA-IR value was also significantly different in different age and BMI groups and significantly higher in women than in men. Moreover, diabetic patients also had significantly higher Log HOMA-IR value than non-diabetic subjects.

After adjusting for age, gender, BMI, smoking status, Triglyceride, and High-density lipoproteins, HOMA-IR (log transform) significantly increased with serum PCDD/Fs levels (log transform) (R^2=0.28; P=0.021) (table 2). Table 3 showed ORs of diabetes mellitus in 4 groups based on their serum PCDD/Fs levels and HOMA-IR value. In the lower serum PCDD/Fs groups, the higher HOMA-IR value group was related to the increasing risk of diabetes mellitus as compared to the lower HOMA-IR value group after adjustment for age, BMI, triglyceride levels and smoking status (adjusted OR=2.71, 95% CI=0.98- 7.47). However, in the subjects with higher HOMA-IR value and higher serum PCDD/Fs levels, the risk of diabetes mellitus increased significantly compared to the reference group (adjusted OR=4.35, 95% CI=1.76- 11.16).

Subjects in this study had higher average PCDD/Fs concentration (74.9 pg WHO_{98}-TEQ/g lipid) than general populations in Taiwan (19.2 WHO_{98}-TEQ/g lipid), in Spain (male: 12.5 pg I-TEQ/g lipid, females: 14.7 pg I-TEQ/lipid) and in German (13.1-19.1 pg I-TEQ/g lipid) [5-7]. There was a significant association between serum PCDD/Fs levels and HOMA-IR value after the other confounding factors had been considered. Although the mechanism by which TCDD may produce insulin resistance is unclear, there are several possibilities. TCDD is highly soluble in adipose tissue[8] and binds to a cytosolic, high-affinity receptor known as the aryl hydrocarbon (Ah) receptor[9]. TCDD has multiple effects in adipose and other tissues that may be important in glucose metabolism. Moreover, TCDD is also known to increase tumor necrosis factor-α (TNFα) expression in several different cell types[10, 11]. The stimulation of TNFα by TCDD is relevant to insulin resistance and diabetes because of the association between increased adipose tissue TNFα expression and insulin resistance[12]. It is also well recognized that insulin resistance may precede the development of type 2 diabetes under many different conditions[13]. Therefore, the interaction between insulin resistance and serum PCDD/Fs levels was further analyzed in this study. From Table 4, the subjects with high HOMA-IR value and high serum PCDD/Fs levels showed a significantly increasing risk of diabetes as compared to subjects with low HOMA-IR value and low serum PCDD/Fs levels. High TCDD levels were also positively correlated with a number of conditional characteristics of impaired glucose metabolism, including decreased time to onset of diabetes and increased hyperinsulinemia in non-diabetic veterans[1]. The risk of diabetes also increases with age[14]. Thus, accumulated tissue levels of TCDD may let the elderly have an increased risk for the development of insulin resistance, hyperinsulinemia, glucose intolerance, and diabetes. Further study is needed to confirm these findings in other TCDD-exposed subjects. Long-term health implications should be also promptly delivered to residents with diagnosed glucose and insulin from a disease prevention point of view.

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Table 1 Demographic factors and levels of serum PCDD/Fs of all study subjects (n=299)

Characteristics	Number	PCDD/Fs[1] Mean (range)	Log HOMA-IR Mean (range)
Age (years) †, ¶1, §2			
≤ 60	22	72.9 (9.7- 951.0)	3.25 (1.92- 4.96)
60-69	49	67.5 (14.7- 291.2)	3.57 (2.20- 5.85)
70-79	148	73.7 (10.4- 412.9)	3.35 (-0.15- 6.09)
> 80	80	82.2 (12.5- 555.7)	2.93 (-1.20- 5.29)
Gender‡, ¶1, §2			
Men	126	64.9 (9.7- 555.7)	3.12 (0.95- 5.21)
Women	173	82.2 (13.2- 951.0)	3.37 (-1.20- 6.09)
Smoking status †, ¶1			
Never smoker	183	69.5 (12.5- 412.9)	3.36 (-0.15- 6.09)
Active smoker	76	71.7 (9.7- 555.7)	3.05 (0.95- 4.94)
Passive smoker	40	105.9 (10.4- 951.0)	3.26 (-1.20- 5.82)
BMI†, ¶2			
0-25%（0-22.0）	72	80.0 (12.5- 412.9)	2.60 (-1.20- 4.94)
25-50%（22.0-24.5）	72	62.0 (10.4- 555.7)	3.00 (-0.16- 4.99)
50-75%（24.5-27.3）	72	72.9 (11.9- 951.0)	3.61 (1.87- 5.85)
75-100%（>27.3）	71	84.6 (9.7- 374.1)	3.97 (2.57- 6.09)
Blood pressure (mmHg) ‡, ¶1			
Abnormal[2]	54	66.9 (9.7- 555.7)	3.12 (-1.20- 5.65)
normal	242	77.1 (10.4- 951.0)	3.31 (-0.16- 6.09)
HDL (mg/dL) ‡, §2			
Abnormal[3]	102	82.9 (10.4- 951.0)	3.45 (1.35- 5.96)
normal	197	70.8 (9.7- 555.7)	3.17 (-1.20- 6.09)
Triglyceride (mg/dL) ‡, ¶2			
Abnormal[4]	79	74.6 (13.1- 951.0)	3.75 (1.40- 6.09)
normal	220	75.0 (9.7- 555.7)	3.09 (-1.20- 5.96)
Diabetes mellitus ‡, ¶1, ¶2			
Yes	70	96.5 (13.1- 412.9)	3.78 (0.73- 5.85)
No	229	68.3 (9.7- 951.0)	3.11 (-1.20- 6.09)
Overall	299	74.9 (9.7- 951.0)	3.27 (-1.20- 6.09)

[1]:pg WHO_{98}-TEQ_{DF}/g lipid; [2]:Systolic BP≧ 130 or Diastoloc BP≧ 85 mmHg;
[3]:Men< 40 or Women < 50 mg/dL; [4]:Triglyceride≧ 150 mg/dL
†: Kruskal-Wallis test; ‡:Wilcoxon Rank-Sum test
§1: p<0.05 for PCDD/Fs; §2: p<0.05 for Log HOMA-IR ¶1: p<0.01 for PCDD/Fs; ¶2: p<0.01 for Log HOMA-IR

Table 2 Multivariate linear regression between serum PCDD/Fs levels with HOMA-IR value (R-square=0.28)

Variables	Estimate	P value
Intercept	0.88	0.125
Gender [men]	0.09	0.227
Age	-0.01	**0.046***
BMI	0.11	**<0.001****
Active smokers	-0.34	**0.029***
Passive smokers	0.12	0.574
TG [normal]	-0.21	**0.001****
HDL [normal]	-0.03	0.570
Log PCDD/Fs	0.16	**0.021***

Note: PCDD/Fs levels and HOMA index were log-transformed
*: p<0.05 **: p<0.01

Table 3 Odds ratios (ORs) of Diabetes mellitus with low to high serum PCDD/Fs levels by their corresponding HOMA-IR

PCDD/Fs levels †	Log HOMA-IR ‡	Case (n)	Control (n)	Crude OR	95% CI	Adjusted OR §	95% CI
≤ median	≤ 75% tile	17	96	1	-	1	-
≤ median	> 75% tile	13	20	3.67**	1.53- 8.79	2.71*	0.98- 7.47
> median	≤ 75% tile	22	90	1.38	0.69-2.80	0.94	0.12- 9.79
> median	> 75% tile	18	23	4.42**	1.99-10.00	4.35**	1.76- 11.16

†: Serum PCDD/Fs levels indicate that 1. ≤ median: ≤ 35.5 pg WHO-TEQ/g lipid;

2. > median: >35.5 pg WHO-TEQ/g lipid

‡: Log HOMA-IR indicate that 1. ≤ 75% percentile: ≤ 4.07 ; 2.> 75% percentile: > 4.07

§: Adjusted for age, BMI, TG, and smoking status by multiple logistic regression

*: p<0.05 **: p<0.01