Original Article

Relationship between dioxin compounds and hepatic disorder with obesity in Japanese adult males

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Abstract

Although exposure to high levels of dioxins was reported to be related to hepatic disorders including glycolipid metabolism abnormalities, the impact of typical low-level environmental dioxin exposure on non-alcoholic fatty liver disease (NAFLD) remains unclear. To examine the relationship between suspected NAFLD and dioxin exposure, we measured the blood levels of 29 dioxin congeners and isomers in 68 adult male volunteers with no history of viral hepatitis or alcoholism. Study participants were recruited from 2006 to 2008 and subjected to a health checkup and a battery of biochemical tests, including measurement of dioxin levels. The participants were determined to be at high risk of NAFLD on the basis of high body mass index (BMI ≥ 25 kg/m^2) and elevated blood levels of alanine aminotransferase (ALT ≥ 36 IU/ℓ). In this study, 11 participants were classified as having NAFLD (16.2% of the total recruited number). A higher concentration of 3,3',4,4'-tetrachlorobiphenyl (PCB77) was detected in the blood of those with suspected NAFLD, with no other significant differences found in levels of other dioxins, compared to the levels in participants who were considered not to be at risk of NAFLD. We used a logistic regression analysis taking into account age, smoking habit, and levels of total cholesterol, triglycerides, high-density lipoprotein cholesterol, and hemoglobin A1c to estimate the impact of elevated blood PCB77 levels on the risk of NAFLD. We observed an increased risk of NAFLD in participants with elevated PCB77 levels, indicating that even low-level exposure to PCB77 may contribute to NAFLD development.

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《**Key words**》 Dioxins, 3,3',4,4'-tetrachlorobiphenyl (PCB77), non-alcoholic fatty liver disease (NAFLD)

Ⅰ. Introduction

There are growing concerns about the adverse effects of toxic chemical compounds in the environment on human health, reflecting the increasing appreciation of their tendency to remain in the tissues and to accumulate over time. These toxic

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compounds, called persistent organic pollutants (POPs), are detected in most humans. Dioxins, a group of POPs known to exhibit deleterious effects on health, include polychloro-dibenzo-*p*-dioxin (PCDD), polychloro-dibenzofran (PCDF), and dioxin-like polychlorinated biphenyl (DL-PCB). While the environmental levels of dioxins and PCBs have been decreasing yearly since the implementation of a ban on their use in Japan in 1973, Ministry of the Environment reported that dioxins were still detected in the Japanese people[1, 2]. The ingestion of fish and other seafood contaminated with dioxins has been reported as a factor underlying the results of the survey. Dioxins tend to accumulate in the adipose tissue and liver[3]. PCDDs and PCDFs are produced as byproducts of incomplete combustion of chlorine-containing materials, and can be released from the chlorine-containing waste and plastics. DL-PCBs are organic chlorine compounds that have been used in the past in a number of electronic and industrial products.

The toxicity of these various dioxins varies among individual congeners and isomers. The toxic equivalency factor (TEF) is often employed to present the relative toxicity of a particular dioxin, by using 2,3,7,8-tetrachloro-dibenzodioxin (TCDD) as a reference (TEF =1)[4].

Short-term exposure of humans to high levels of dioxins may result in skin lesions, such as chloracne, and altered liver function[5–7]. Recently, an increasing number of research has focused on the effects of the exposure to low doses of dioxins and DL-PCBs on human health[8–10]. A number of studies have reported an association of low-level PCB accumulation in the human body with obesity or diabetes[11–14].

Patients with diabetes, as well as those who are overweight or obese, often suffer from fatty liver disease. Fatty liver disease can be categorized into simple fatty liver and non-alcoholic fatty liver disease (NAFLD). The prevalence of NAFLD in Japan is around 14%, increasing in recent years[15]. Furthermore, NAFLD can reportedly cause non-alcoholic steatohepatitis (NASH) or cirrhosis, eventually resulting in liver cancer. The identification of risk factors, effective prevention, and improved early detection of this disorder is therefore a public health priority. A recent study has reported that the exposure to chemical substances is one of the risk factors for NAFLD[8].

The National Health and Nutrition Examination Survey (NHANES) 2003–2004 suggested that exposure to some non-dioxin-like PCBs (NDL-PCBs) or DL-PCBs may potentially cause NAFLD, based on the measured blood levels of these chemicals[16]. However, the relationship between NAFLD and dioxin levels in the Japanese population has not been reported thus far. The increasing prevalence of NAFLD in Japan and growing concern over the potential threat of POPs to human health provide the rationale for investigating the causal relationship between NAFLD and POPs, including dioxin. Therefore, in this report, we aimed to elucidate the relationship between blood dioxin levels measured in Japanese adult male population and NAFLD.

Ⅱ. Subjects and Methods

1. Study population

The Center for Preventive Medical Sciences, Chiba University, offers a dioxin medical examination program that aims to assess the impact of dioxins on health by measuring blood dioxin levels, subjecting participants to a general biochemical examination, and surveying the participants' medical histories and lifestyle habits using a questionnaire.

The present cross-sectional study assessed the relationship between NAFLD and the blood dioxin levels. The data collected between 2006 and 2008 were analyzed.

Study population comprised 106 male participants over 20 years of age who consented to undergo a biochemical examination and provide a blood sample for the measurement of blood dioxin levels. Subjects

reporting a history of hepatitis B or C, cirrhosis, hepatocellular carcinoma, or those with high dioxin exposure were excluded. Of the 106 subjects who consented to participate in the survey, data were collected from 68 subjects that met the inclusion and exclusion criteria. The present study was approved by the Ethical Committee of the School of Medicine, Chiba University, and all subjects provided written informed consent prior to participation (Ethics Committee number 907).

2. Measurements of dioxin levels and biochemical parameters

The levels of dioxin congeners and isomers were quantified by measuring the concentrations of 29 isomers of 7 types of PCDDs, 10 types of PCDFs, 4 types of non-ortho PCBs, and 8 types of mono-ortho PCBs. Dioxin concentrations were expressed per fat-weight extracted from the blood samples (fat mass; pg dioxin/g blood lipid). Blood samples (50 mℓ each) were collected from the subjects in pre-washed glass vacuum blood collection tubes. The analytical method used in this study conformed to the technique described in the provisional manual in 2000 by the Japanese Ministry of Health, Labour and Welfare[17]. Approximately 50 g of whole blood with ^{13}C PCDDs/ PCDFs/ DL-PCBs (clean-up spike; Wellington Laboratories, Canada) was hydrolyzed with 2 M KOH/ ethanol at room temperature. The solution containing dioxins was extracted with n-hexane and subjected to purification using a multilayer silica gel column, alumina column, and active carbon-impregnated silica gel column. After extraction and cleanup, the samples were spiked with another set of ^{13}C-labeled compounds (syringe spike; Wellington Laboratories). Levels of PCDDs, PCDFs, and DL-PCBs were analyzed using high-resolution gas chromatography (6890; Agilent Technologies, Santa Clara, CA, USA)/ high-resolution mass spectrometry (Autospec-Ultima; Micromass, Cary, NC, USA) technique (HRGC/ HRMS), with the measurements performed by a specialized testing company (SRL Inc., Tokyo, Japan). Total toxicity of detected dioxins is expressed as a sum of its toxic equivalents (TEQ) using the TEF 2005 index, in accordance with the method proposed by the World Health Organization (WHO)[18]. Levels of dioxin congeners and isomers levels were expressed as the concentration per fat mass in blood (pg dioxin/g lipid) and converted to TEQ concentration per fat mass (pg TEQ/g lipid) using the TEF. General biochemical testing was performed and the outcomes analyzed by SRL Inc.

3. Detection of NAFLD

In the present study, risk of NAFLD was established on the basis of laboratory tests, the Body Mass Index (BMI), and serum alanine aminotransferase levels (ALT), which are an index of hepatic function[15, 16, 19].

Obesity was defined as a BMI ≥ 25 kg/m^2, in accordance with the guidelines of the Japanese Society for the Study of Obesity[20]. Presence of hepatocellular liver injury was defined as ALT ≥ 36 IU/ℓ, according to the guidelines of the Japanese Society of Ningen Dock[21]. In this study, the suspected NAFLD group was defined to include subjects that presented with both BMI ≥ 25 kg/m^2 and ALT ≥ 36 IU/ℓ, with no significant history of alcohol use. Subjects with normal BMI and ALT levels were assigned to the control group (i.e., non-NAFLD).

4. Questionnaire

Drinking and smoking habits were surveyed as background information using a questionnaire. Drinking habits were classified as “drinks often” or “drinks rarely” based on the questionnaire. Smoking habits were classified as “smokes” and “has quit or never smoked” .

5. Statistical analysis

We determined the prevalence of suspected NAFLD (ALT ≥ 36 IU/ℓ and BMI ≥ 25 kg/m^2) in 68 subjects, and used the Mann-Whitney U-test to detect statistically significant differences in ALT levels, BMI elevation, and biochemical parameters. The Mann-Whitney U-test was used to compare the concentrations of each dioxin isomer, ALT levels, and BMI

between the two groups. The Bonferroni method was used to correct for multiple comparisons. The Spearman correlations test was used to assess the correlations between the concentration of each dioxin congeners and ALT levels, with the significance threshold set at $p < 0.1$. Subsequently, concentrations of blood dioxin congeners and isomers that showed significant differences in the Mann-Whitney U-test were categorized into quartiles. The associations of ALT levels and BMI with the exposure level to each dioxin congeners and isomers were evaluated using adjusted odds ratios. Potential confounders included age, smoking habit, total cholesterol levels, triglyceride levels, high-density lipoprotein cholesterol levels, and hemoglobin A1c levels. p values were obtained from multiple logistic regression analysis, with 95% confidence intervals. All analyses were conducted using SPSS (version 19.0; IBM). The level of significance was set at $p < 0.05$.

Ⅲ. Results

The demographics, as well as the drinking and smoking habits of the enrolled subjects are summarized in Table 1. Out of the total 68 recruited subjects, 11 subjects were at risk of NAFLD (16.2%). Significant differences between the suspected NAFLD group and the control group were observed except for the AST and GGTP levels. The blood levels of 29 dioxin congeners and isomers are presented in Table 2. The median blood levels (expressed as concentration in the fat mass of the blood sample) of total PCDDs, total PCDFs, total DL-PCBs, and total dioxins were as follows: total PCDDs, 125.00 (85.50 – 187.50) pg/g lipid; total PCDFs, 13.50 (8.95 – 20.00) pg/g lipid; total non-ortho PCBs, 56.00 (36.25 – 87.25) pg/g lipid; total mono-ortho PCBs: 9,600.00 (6,675.00 – 16,000.00) pg/g lipid; and total dioxins (PCDDs + PCDFs + non-ortho PCBs + mono-ortho PCBs), 9,900.00 (66,800.00–16,000.00) pg/g lipid. The median TEQ values per fat mass for each dioxin congener and isomer were as follows: total PCDDs, 5.06 (3.41–7.76) pg TEQ/g lipid; total PCDFs, 2.14 (1.48–2.98) pg TEQ/g lipid; total non-ortho PCBs, 3.38 (2.14–5.29) pg TEQ/g lipid; total mono-ortho PCBs, 0.29 (0.20–0.49) pg TEQ/g lipid; and total dioxins, 11.51 (7.35–16.25) pg TEQ/g lipid. A comparison of the concentrations of individual dioxin

Table 1 Characteristics of participants

	Total n=68	Suspected NAFLD group n=11(16.2%)	Control n=57 (83.8%)	p-value
Age (year)	44 ± 12.8	37 ± 8.9	45 ± 13.1	0.065
Total cholesterol (mg/dℓ)	195 ± 31.4	206.0 ± 41.2	195.0 ± 29.8	0.947
Triglyceride (mg/dℓ)	111 ± 78.2	128.0 ± 82.2	104.0 ± 78.1	0.127
HDL cholesterol (mg/dℓ)	52 ± 13.5	47.0 ± 10.0	54.0 ± 13.5	0.086
Hemoglobin A1c (%)	5.3 ± 1.2	5.3 ± 1.8	5.3 ± 1.1	0.221
Gamma- glutamyl transpeptidase (IU/ℓ)	34 ± 81.6	66.0 ± 93.2	28.0 ± 82.4	0.018
Aspartate aminotransferase (IU/ℓ)	23 ± 37.6	37.0 ± 89.1	21.0 ± 9.8	<0.001
Alanine aminotransferase (IU/ℓ)	24 ± 24	58.0 ± 143.2	22.0 ± 14.8	<0.001
Body mass index (kg/m^2)	23 ± 3.4	29.1 ± 2.2	23.6 ± 2.9	<0.001
Definite Diabetes (%)	10/68 (14.7%)	0/11 (0.0%)	10/57 (1.8%)	—
Definite Hypertension (%)	9/68 (13.2%)	3/11 (18.2%)	6/57 (10.5%)	—
Smoking habits (%)	35/68 (52.1%)	6/11 (54.5%)	29/57 (50.9%)	—

Catagorical variables shown as actual number (weighted frequency). Continuous variables shown as mean (standard deviation). Suspected NAFLD group: subject with an BMI≥ 25 and ALT level ≥ 36IU/ℓ. Control group (non-NAFLD): subject with no noted elevation in BMI or ALT. The mean were total cholesterol, triglyceride, and hemoglobin A1c, gamma-glutamyl transpeptidase, asparate aminotransferase, alanine aminotransferese in the blood levels.

Table 2 Concentrations of dioxin congeners and isomers

	Chlorine atoms	TEF 2005	Concentration (pg/g-lipid) (n=68)		Concentration (pg-TEQ/g lipid) (n=68)		Suspected NAFLD (pg/g-lipid) (n=11)		Control (pg/g-lipid) (n=57)		p-value
			Median (25-75%)		Median (25-75%)		Median (25-75%)		Median (25-75%)		
2,3,7,8-TetraCDD	4	1	0.64	0.00 – 0.88	0.64	0.00 – 0.88	0.62	0.54 – 0.97	0.65	0.00 – 0.86	0.34
1,2,3,7,8-PentaCDD	5	1	2.90	2.00 – 4.58	2.90	2.00 – 4.58	3.00	1.90 – 4.30	2.70	2.00 – 4.65	0.89
1,2,3,4,7,8-HexaCDD	6	0.1	1.10	0.76 – 1.50	0.11	0.08 – 0.15	1.10	0.85 – 1.40	1.10	0.70 – 1.50	0.95
1,2,3,6,7,8-HexaCDD	6	0.1	11.00	7.90 – 17.75	1.10	0.79 – 1.78	12.00	8.50 – 16.00	11.00	7.80 – 18.00	0.68
1,2,3,7,8,9-HexaCDD	6	0.1	1.50	1.00 – 2.30	0.15	0.10 – 0.23	2.10	1.20 – 2.30	1.40	0.97 – 2.30	0.44
1,2,3,4,6,7,8-HeptaCDD	7	0.01	7.10	4.95 – 10.00	0.07	0.05 – 0.10	7.50	5.40 – 11.00	7.10	4.90 – 9.95	0.61
OctaCDD	8	0.0003	99.00	62.25 – 150.00	0.03	0.02 – 0.05	110.00	61.00 – 140.00	94.00	62.50 – 165.00	0.83
Total PCDDs			125.00	85.50 – 187.50	5.06	3.41 – 7.76	140.00	76.00 – 170.00	120.00	86.00 – 195.00	0.67
2,3,7,8-TetraCDF	4	0.1	0.30	0.00 – 0.63	0.03	0.00 – 0.06	0.35	0.00 – 0.81	0.30	0.00 – 0.56	0.56
1,2,3,7,8-PentaCDF	5	0.03	0.00	0.00 – 0.00	0.00	0.00 – 0.00	0.00	0.00 – 0.80	0.00	0.00 – 0.00	0.28
2,3,4,7,8-PentaCDF	5	0.3	5.30	3.63 – 7.28	1.59	1.09 – 2.18	6.20	3.60 – 6.40	5.10	3.50 – 7.50	0.71
1,2,3,4,7,8-HexaCDF	6	0.1	2.10	1.30 – 2.68	0.21	0.13 – 0.27	2.40	1.90 – 3.00	1.90	1.30 – 2.60	0.27
1,2,3,6,7,8-HexaCDF	6	0.1	2.60	1.63 – 3.30	0.26	0.16 – 0.33	2.70	2.20 – 3.30	2.60	1.60 – 3.30	0.55
1,2,3,7,8,9-HeptaCDF	6	0.1	0.00	0.00 – 0.00	0.00	0.00 – 0.00	0.00	0.00 – 0.00	0.00	0.00 – 0.00	–
2,3,4,6,7,8-HexaCDF	6	0.1	0.74	0.41 – 1.08	0.07	0.04 – 0.11	0.87	0.57 – 1.50	0.71	0.40 – 1.00	0.43
1,2,3,4,6,7,8-HeptaCDF	7	0.01	2.10	1.40 – 3.10	0.02	0.01 – 0.03	2.90	1.50 – 5.90	2.00	1.40 – 2.90	0.13
1,2,3,4,7,8,9-HeptaCDF	7	0.01	0.00	0.00 – 0.00	0.00	0.00 – 0.00	0.00	0.00 – 0.00	0.00	0.00 – 0.00	–
OctaCDF	8	0.0003	0.00	0.00 – 0.00	0.00	0.00 – 0.00	0.00	0.00 – 0.00	0.00	0.00 – 0.00	–
Total PCDFs			13.50	8.95 – 20.00	2.14	1.48 – 2.98	15.00	12.00 – 24.00	13.00	8.70 – 20.00	0.60
Total (PCDDs+PCDFs)			140.00	97.25 – 210.00	7.31	4.97 – 10.62	160.00	87.00 – 190.00	130.00	97.50 – 210.00	0.71
3,3',4,4'-Tetrachlorobiphenyl (PCB77)	**4**	**0.0001**	**0.75**	**0.00 – 1.78**	**0.00**	**0.00 – 0.00**	**1.50**	**0.74 – 2.80**	**0.65**	**0.00 – 1.45**	**0.03 ***
3,4,4',5-Tetrachlorobiphenyl (PCB81)	4	0.0003	0.94	0.65 – 1.80	0.00	0.00 – 0.00	1.30	0.85 – 2.30	0.91	0.63 – 1.55	0.12
3,3',4,4',5-Pentachlorobiphenyl (PCB126)	5	0.1	26.00	14.25 – 40.50	2.60	1.43 – 4.05	30.00	20.00 – 47.00	26.00	14.00 – 40.00	0.38
3,3',4,4',5,5'-Hexachlorobiphenyl (PCB169)	6	0.03	26.50	20.25 – 45.75	0.80	0.61 – 1.37	24.00	20.00 – 32.00	28.00	20.50 – 49.00	0.31
Total non-ortho PCBs			56.00	36.25 – 87.25	3.38	2.14 – 5.29	59.00	47.00 – 71.00	55.00	36.00 – 89.00	0.64
2,3,3',4,4'-Pentachlorobiphenyl (PCB105)	5	0.00003	970.00	590.00 – 1,575.00	0.03	0.02 – 0.05	1,100.00	710.00 – 1,400.00	960.00	575.00 – 1,650.00	0.59
2,3,4,4',5-Pentachlorobiphenyl (PCB114)	5	0.00003	295.00	220.00 – 530.00	0.01	0.01 – 0.02	300.00	240.00 – 330.00	290.00	210.00 – 535.00	0.89
2,3',4,4',5-Pentachlorobiphenyl (PCB118)	5	0.00003	4,850.00	3,050.00 – 7,275.00	0.15	0.09 – 0.22	5,100.00	3,900.00 – 6,600.00	4,600.00	2,950.00 – 7,550.00	0.70
2,3',4,4',5'-Pentachlorobiphenyl (PCB123)	5	0.00003	77.50	47.50 – 120.00	0.00	0.00 – 0.00	93.00	61.00 – 120.00	77.00	47.00 – 125.00	0.63
2,3,3',4,4',5-Hexachlorobiphenyl (PCB156)	6	0.00003	2,200.00	1,600.00 – 4,450.00	0.07	0.05 – 0.13	1,700.00	1,500.00 – 2,400.00	2,500.00	1,600.00 – 4,650.00	0.30
2,3,3',4,4',5'-Hexachlorobiphenyl (PCB157)	6	0.00003	595.00	430.00 – 1,175.00	0.02	0.01 – 0.04	510.00	390.00 – 640.00	640.00	435.00 – 1,250.00	0.38
2,3',4,4',5,5'-Hexachlorobiphenyl (PCB167)	6	0.00003	790.00	532.50 – 1,300.00	0.02	0.02 – 0.04	760.00	650.00 – 870.00	800.00	515.00 – 1,400.00	0.75
2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB189)	**7**	**0.00003**	**305.00**	**210.00 – 545.00**	**0.01**	**0.01 – 0.02**	**220.00**	**160.00 – 270.00**	**320.00**	**210.00 – 565.00**	**0.09 ***
Total mono-ortho PCBs			9,600.00	6,675.00 – 16,000.00	0.29	0.20 – 0.49	9,500.00	8,500.00 – 12,000.00	9,700.00	6,500.00 – 17,000.00	0.90
Total DL-PCBs			9,700.00	6,675.00 – 16,000.00	3.65	2.40 – 5.61	9,600.00	8,500.00 – 12,000.00	9,800.00	6,550.00 – 17,000.00	0.70
Total dioxins (PCDDs + PCDFs + DL-PCBs)			9,900.00	6,800.00 – 16,000.00	11.51	7.35 – 16.25	9,800.00	8,600.00 – 12,000.00	10,000.00	6,600.00 – 17,000.00	0.65

Suspected NAFLD: subject with an BMI ≥ 25 and ALT level ≥ 36IU/L. Control (non – NAFLD): subject with no noted elevation in BMI or ALT. p-values were calculated by Mann – Whitney tests. *indicates statistically significant change ($p<0.1$).

congeners and isomers measured in the suspected NAFLD group and in the control group revealed a significant difference in 3,3',4,4'-tetrachlorobiphenyl (PCB77) and 2,3,3',4,4',5,5'-heptachlorobiphenyl (PCB 189) levels (Table 2). ALT levels correlated with the blood concentration of PCB77 (Fig 1). BMI values of study participants did not correlate with the blood concentrations of dioxin congeners and isomers (data

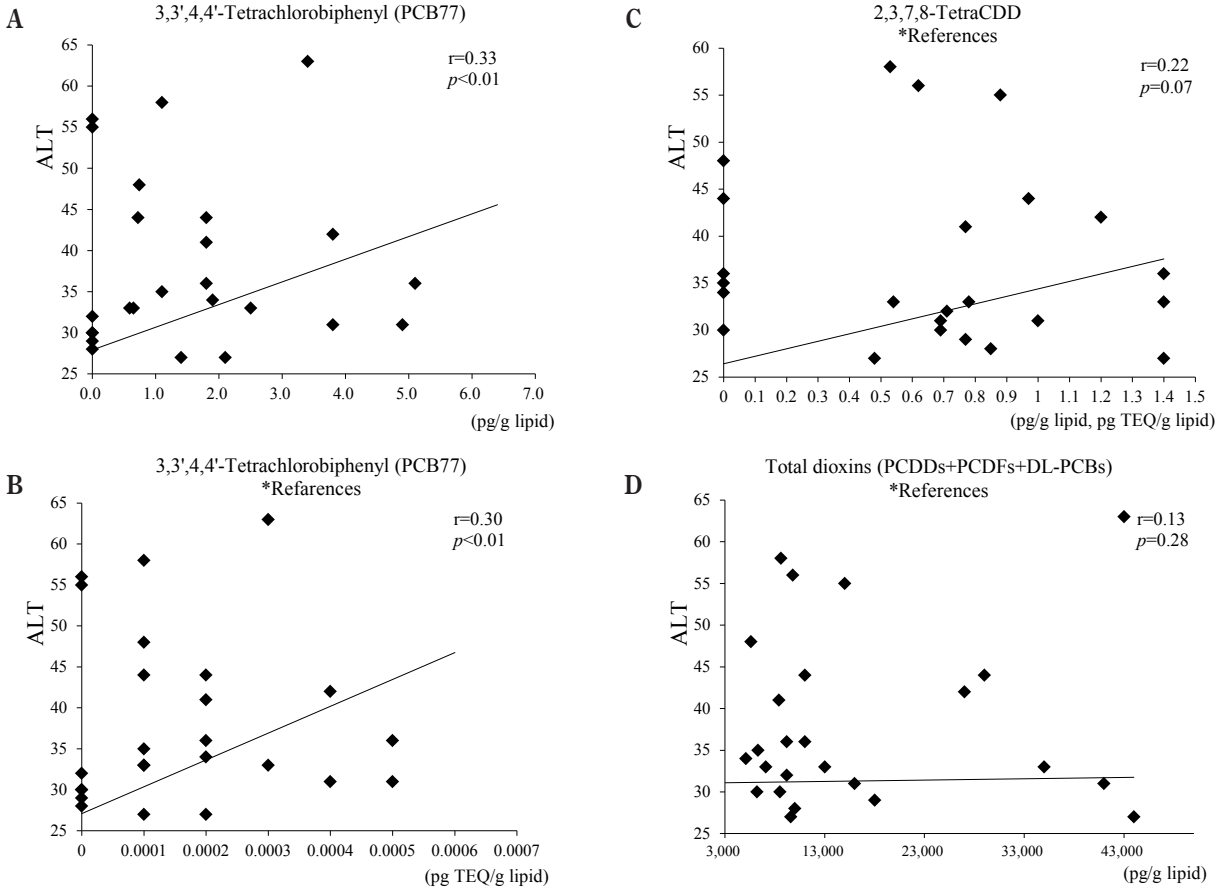

Fig. 1 Correlations of dioxin congeners and ALT

Spearman correlation test. Correlations of blood level of dioxin congeners/isomers and ALT. Data are expressed in concentration per fat mass in blood (A, C, D). Data are expressed in concentration TEQ per fat mass, which is calculated using WHO-TEF (B, C).

Table 3 The prevalence of susupected NFLD and quartiles concentration of DL-PCBs

		Quartile [case/total, median concentration (pg/g-lipid), range]				
	n	First quartile	Second quartile	Third quartile	Fourth quartile	*p*-value
3,3',4,4'-Tetrachlorobiphenyl	68	1/28	2/11	3/12	5/17	< 0.05
(PCB77)		0.00	0.74	1.20	2.80	
		0.00-0.59	0.60-0.97	1.10-1.70	1.80-6.40	
2,3,3',4,4',5,5'-Heptachlorobiphenyl	68	4/16	5/18	0/17	2/17	0.10
(PCB189)		140.00	245.00	400.00	880.00	
		100.00-200.00	210.00-300.00	310.00-530.00	550.00-1,700.00	

The summary values were categorized by cutoff points of 25th, 50th, and 75th values of the sum of ranks. *p-value were calculated by chi-squared test.

Table 4 Risk of suspected NAFLD according to DL-PCBs

A

	p-value	OR	95% CI
Total cholesterol	0.633	1.01	0.98 − 1.04
HDL cholesterol	0.370	1.03	0.96 − 1.11
Triglyceride	0.210	0.99	0.99 − 1.00
HbA1c_NGSP	0.250	0.72	0.42 − 1.25
3,3',4,4'-Tetrachlorobiphenyl (PCB77)	0.005	3.19	1.40 − 7.20
2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB189)	0.022	2.38	0.14 − 0.86

B

	p-value	OR	95% CI
Smoking habit	0.514	1.87	0.29 − 12.26
Age	0.180	1.10	0.96 − 1.27
Total cholesterol	0.970	1.00	0.97 − 1.03
HDL cholesterol	0.647	1.02	0.94 − 1.10
Triglyceride	0.170	0.99	0.98 − 1.00
HbA1c_NGSP	0.652	0.87	0.49 − 1.57
3,3',4,4'-Tetrachlorobiphenyl (PCB77)	0.002	4.08	1.67 − 9.98
2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB189)	0.679	0.75	0.19 − 3.00

A; Adjusted for total cholesterol, high-density lipoprotein cholesterol level, triglycerides, hemoglobin A1c. **B**; Adjusted for total cholesterol, high-density lipoprotein cholesterol level, triglycerides, hemoglobin A1c, age and smoking. Data are adjusted odds ratio (OR) and 95% confidence interval (95% CI).

not shown).

Concentrations of blood PCB77 and PCB189 were categorized into quartiles (Table 3). The multiple logistic regression analysis showed that higher blood levels of blood PCB77 and PCB189 were associated with suspected NAFLD (Table 4-A). Furthermore, the adjustment of results for age and smoking habit using multiple logistic regression analyses showed a relationship between elevated blood PCB77 levels and the risk of suspected NAFLD (Table 4-B).

Ⅳ. Discussion

The prevalence rate of suspected NAFLD in this study was consistent with the incidence of this hepatic disorder in a general Japanese population, as reported from the findings of a study using medical examinations[15]. Among the dioxin congeners and isomers measured in this study, PCB77 showed a correlation with ALT levels, suggesting that increased levels of PCB77 exposure may contribute to the risk of NAFLD.

The observed relationship is consistent with the findings of previous studies, suggesting that exposure to PCBs, which have been banned since 1973, proceeds primarily from the diet, with the compounds accumulating in the human body. No other correlation between other concentrations of dioxin compounds and ALT levels could be found. The blood levels of PCB77 in the NAFLD group were significantly higher in comparison to the control group, whereas no significant difference was observed in the concentrations of other dioxin congeners and isomers. The relationship between elevated PCB77 and NAFLD remains significant when the confounding factors, including age and smoking habit, are taken into consideration in comparing the two groups. Although some published reports have suggested a relationship between combined exposure to DL-PCB (PCB126)/NDL-PCBs (PCB153) or their increased levels in the blood and suspected NAFLD, those studies did not

measure PCB77 levels. Our report is, therefore, the first to describe the relationship between the blood levels of PCB77 and NAFLD. On the other hand, this study design has several potential problems which have to be mentioned. Firstly, the exact specify of ALT for hepatic disorder in this study is unknown because liver biopsies were not conducted. Although, elevation of hepatic transaminases in patient with NAFLD are characterized by predominance of ALT, recent research indicate that patients with BMI below 25 often showed normal ALT levels[22, 23]. Actually, lower laboratory values of ALT have been used in some previous studies to obtain higher sensitivity[24, 25]. Previous studies that described the impact of chemicals on human health included patients with normal and boundary ALT levels to investigate the relationship between particular chemicals and biochemical parameters. This study adopted the similar methodology with previous studies, and the finding indicated that subjects with lower blood level of PCBs showed the tendency of lower levels of ALT[15, 26]. For this reason, it is inferred that the measurement of blood levels of PCBs and ALT could possibly be helpful in future diagnosing NAFLD, if not a definitive.

The biological half-life of DL-PCBs varies depending on the number and the binding site of chlorine atoms in the toxin molecule[27]. The liver and adipose tissues may be the principal target organs for the toxicity of DL-PCBs, since these compounds tend to accumulate in the hepatic fatty tissues. Rats fed a diet supplemented with PCBs were reported to exhibit increased liver weight[28, 29]. Administration of PCB77 was reported to worsen fatty liver disease and affect the genes involved in apoptosis, inflammation, and oxidative stress in hepatocytes in mice[30, 31]. Miyazaki et al. reported that an increase in hydroxylated metabolites of PCB77 decreases the levels of thyroid hormone in rodents[32]. Furthermore, insufficient production of thyroid hormone was shown to be a risk factor of NAFLD, with some reports suggesting a relationship between the two clinical conditions[33]. Although some animal studies have reported an association between PCB77 and NAFLD, the relationship in the human body has not been reported. As mentioned above, although the relationship between a number of PCBs and human NAFLD has previously been suggested, there is no published report describing the relationship with PCB77. The findings of this study raise the possibility that PCB77 may be one of the triggers of NAFLD.

The most common PCBs products in Japan include "Kanechlor 300" a manufactured by Kanegafuchi Co. Ltd., while Monsanto Chemical Company's "Aroclors" accounts for the largest share of PCBs worldwide[34–36]. These products contain high concentrations of DL-PCBs, especially PCB77 and PCB126[37]. DL-PCBs are transferred through water, air, and soil to living organisms and accumulate in their tissues[29]. The biotransfer factor or bioaccumulation factor is particularly high in fish and cattle, with these factors shown to be determined by the number of chlorine atoms in the toxin molecule[37, 38]. The beef biotransfer factor of PCB126 was reported to be higher than that of PCB77[39], while the bioaccumulation factor of PCB77 and PCB 126 in fish are reportedly of similar magnitudes[40]. The amounts of DL-PCBs accumulated in the living body can therefore vary according to the frequencies and amounts of food ingested. According to previous reports, annual per-capita consumption of beef is higher in the United States than in Japan, while fish consumption is higher in Japan[41, 42]. Additionally, the biological half-life of PCB126 in blood is longer than that of PCB77[27]. Unlike one previous study that detected an association with PCB126, the relationship with the pathogenesis of suspected NAFLD was only seen with PCB77 in this study, likely reflecting the differences in the kinetics of PCB126 and PCB77 in the blood, or in the frequency and amount of ingested food. Since the current methods of measuring dioxin congeners and isomers are too expensive to be widely used in

clinical practice as an evaluation index of NAFLD, it is difficult to conclusively determine the relationship between NAFLD and dioxins. However, an approach to measuring dioxins using packed column-electron capture detector/gas chromatograph or GC/NICI-MS requires a smaller amount of blood and can be performed at lower cost, as compared to HRGC/HRMS. Since the GC/NICI-MS method uses a capillary column and therefore has strong potential for detecting PCB77 separately, leading to growing interest in this technique and recent efforts to establish its validity[43]. Since more detailed data could be obtained with the increased versatility of novel methods for measuring dioxin level, it should be possible for us to examine the relationship between NAFLD and PCB77 in more depth in future studies. Despite the fact that investigating causes of NAFLD and therapeutic methods for this disease are urgently required due to the worldwide increase in the number of patients, previous studies have not showed the pathogenesis of NAFLD. In this regard, even though the number of subjects is small, this study is of clinical significance because it has shed new light on pathogenesis. Taking these circumstances into consideration, although to conclude the causal relationship between PCBs and NAFLD from the result of this study is impossible because of the limited numbers of subjects, this study is the first to suggest the relationship of PCBs and NAFLD in Japanese population, and it is significant that the finding identified chemicals as one factor of NAFLD. With the goal of establishing a practical method for reducing PCB77 accumulation in both the environment and the human body, social education aimed to reduce PCB77 exposure through diet or other routes will become an increasingly important aspect of environmental prevention medicine.

Conflicts of interest statement

The authors declare that there are no conflicts of interest associated with the present study.

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日本人の肥満成人男性における肝機能障害とダイオキシン類の関連性

要約

近年、ダイオキシン類の曝露が糖・脂質代謝異常や肝機能障害発症の一因であることが複数の報告で示唆されている。しかし、一般環境レベルの低濃度ダイオキシン類曝露と非アルコール性脂肪性肝疾患（NAFLD）の関連性は未だ不明である。我々は68名の一般成人で、ダイオキシン様ポリ塩化ビフェニルを含む29種類の血中ダイオキシン類濃度と生化学パラメータを用いて、血中ダイオキシン類濃度と NAFLD 罹患の関連性を調査した。11名の対象者が NAFLD の罹患疑いに分類された。NAFLD の罹患疑いの対象者は、血中ダイオキシン類濃度のうち3,3',4,4'- tetrachlorobiphenyl（PCB77）が有意に高かった。NAFLD の罹患疑いの対象者は年齢や喫煙、総コレステロール、中性脂肪、HDL コレステロール、HbA1c で補正をおこなっても、PCB77の寄与率が高かった。

本結果から、PCB77の低レベル曝露が NAFLD 発症の一因となる可能性を示唆した。

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《**キーワード**》ダイオキシン類、3,3',4,4'- テトラクロロビフェニル（PCB77）、非アルコール性脂肪性肝疾患（NAFLD）