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ORIGINAL ARTICLE

Clinical significance of serum adiponectin in Japanese diabetic patients

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Abstract
Introduction To investigate the clinical significance of the serum adiponectin concentration in Japanese diabetic patients.
Methods We examined serum adiponectin levels in 1,541 diabetic patients from 2005 to 2009.
Results The serum level of adiponectin was higher in patients with type 1 diabetes and in those receiving pioglitazone treatment than in other groups. Serum adiponectin was inversely correlated with body mass index, waist circumference, fasting plasma glucose, serum triglycerides, serum uric acid, fasting serum IRI, and HOMA-IR; it was positively correlated with age and HDL cholesterol. Serum adiponectin was significantly lower in diabetic male patients with metabolic syndrome than in those without it, and patients with more cardiometabolic risk factors also had lower adiponectin levels. Serum adiponectin was significantly lower in patients with microangiopathy than in those without (both male and female), and was also significantly lower in male patients with macroangiopathy than in those without it.
Conclusions Measurement of the serum adiponectin in diabetic patients is useful for assessing metabolic status and for evaluating the risk of arteriosclerosis, particularly in males.

Keywords Adiponectin · Metabolic syndrome · Cardiometabolic risk · Diabetes mellitus

Introduction

The number of diabetic patients is increasing in Japan; most have visceral fat obesity with insulin resistance because of their modern westernized lifestyle [1, 2]. In order to study diabetes in Japan, it is therefore necessary to evaluate the effect of visceral fat on glucose metabolism and diabetic complications associated with insulin resistance. Adiponectin is an adipocytokine secreted by adipose tissue that is closely related to insulin resistance and is important in obese diabetic patients [3–5]. A low serum level of adiponectin is reported to be associated with high blood pressure and dyslipidemia, and with deterioration of glycemic control that could lead to diabetic nephropathy and macroangiopathy [6–9]. We have previously reported that serum adiponectin levels in diabetic patients could be increased by lifestyle modification [10]. Therefore, measurement of serum adiponectin concentration could be useful for diabetic patients, and for other lifestyle diseases, both for prediction of the risk of diabetic complications and for judgment of the response to treatment. In order to investigate the clinical importance of the serum adiponectin level, we measured adiponectin in a large group of diabetic patients and analyzed its relationship with various clinical factors.

Parts of this study were presented in oral form at the 53rd Annual Meeting of the Japan Diabetes Society (Okayama, Japan, 27–29 May 2010).

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Materials and methods

The participants in this study were 1,541 adult Japanese patients with diabetes or impaired glucose tolerance who were admitted to Sumitomo Hospital from July 2005 to June 2009. All were admitted for treatment of diabetes or obesity. This study was conducted according to the principles of the Declaration of Helsinki. The study protocol was approved by the ethics committee of our hospital and written informed consent was obtained from every patient.

We compared serum adiponectin levels in relation to the type of diabetes and among different treatments. Subjects were classified as having type 1 or type 2 diabetes or impaired glucose tolerance, according to the criteria published by the Japan Diabetes Society [11]. Because we found serum adiponectin levels were higher in patients with type 1 diabetes than in those with type 2 diabetes or impaired glucose tolerance, we excluded type 1 diabetics from the subsequent assessment. To assess therapeutic modalities, we divided the diabetic patients into 4 treatment groups: insulin, pioglitazone, other oral hypoglycemic agents, and diet alone. There were 296 patients on insulin, including 77 patients with a combination of insulin and oral hypoglycemic agents. Pioglitazone was being used by 128 patients, including 111 patients receiving a combination of pioglitazone and other oral hypoglycemic agents. Other treatment, including biguanides, sulfonylureas, glinide, α-glucosidase inhibitors, and combination therapy, was being given to 664 patients. There were no patients receiving treatment with dipeptidyl peptidase-4 (DPP-4) inhibitors or glucagon-like peptide-1 (GLP-1) analogues.

Because serum adiponectin levels were higher in patients on pioglitazone treatment than in the other groups, we excluded patients receiving pioglitazone from the subsequent assessment. We examined the relationships between serum adiponectin level and metabolic data for patients with or without the metabolic syndrome, patients with or without microangiopathy or macroangiopathy, and among 5 groups classified on the basis of cardiometabolic risk factors.

Clinical evaluation

Height and body weight were measured while patients were wearing lightweight inner clothing without shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. Waist circumference was measured at the umbilicus by our expert nurses. After an overnight fast, blood was obtained to measure biochemical data and serum adiponectin concentration. HbA1c (%) was estimated as the National Glycohemoglobin Standardization Program (NGSP) equivalent value (%), which was calculated as HbA1c (%) = HbA1c (Japan Diabetes Society; JDS) (%) + 0.4%, considering the relationship of HbA1c (JDS) (%), measured with the previous Japanese standard, with HbA1c (NGSP) [11]. Plasma levels of glucose, serum lipids, and uric acid were measured by routine automated laboratory methods. Serum insulin was measured by use of a chemiluminescence immunoassay kit (Fujirebio, Tokyo, Japan). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated to estimate insulin resistance, except in patients being treated with insulin. Estimated glomerular filtration rate (eGFR) was calculated by using the formula $194 \times (\text{serum creatinine}^{-1.094}) \times (\text{age}^{-0.287}) \times F$ (where $F = 1$ if male, and 0.739 if female) [12]. The serum concentration of adiponectin was measured by use of a latex particle-enhanced turbidimetric immunoassay kit (human adiponectin latex kit; Otsuka Pharmaceutical, Tokyo, Japan), as previously reported [13]. Cardiometabolic risk factors were defined as the criteria for diagnosis of metabolic syndrome in Japan [14], which are:

1. a waist circumference ≥85 cm in men and ≥90 cm in women;
2. fasting plasma glucose ≥110 mg/dl;
3. systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg;
4. serum triglycerides ≥150 mg/dl; and
5. serum HDL cholesterol <40 mg/dl.

Metabolic syndrome was diagnosed by use of these criteria [14]. Microangiopathy was diagnosed when at least one of the following criteria was fulfilled: microalbuminuria ≥30 mg/day (nephropathy), simple diabetic retinopathy or more advanced retinopathy, and loss of the Achilles tendon reflex or deterioration of vibration sensation (neuropathy). Macroangiopathy was diagnosed by the presence of at least one of following: a past history of ischemic heart disease or cerebral infarction, a positive exercise test, and cerebral infarction observed by magnetic resonance imaging. Master's double exercise electrocardiograph was performed in all patients without contraindication, and further examinations if needed. For approximately 40% of obese patients, the visceral, subcutaneous, and total fat areas at the umbilicus were measured by computed tomography (CT) (Somatom Definition; Siemens, Munich, Germany) [15].

Statistical analysis

Statistical analysis was performed by use of Dr. SPSS II for Windows (SPSS, Chicago, IL, USA). Descriptive statistics were calculated. Data on serum adiponectin, fasting plasma glucose (FPG), fasting insulin (IRI), HOMA-IR, and triglycerides (TG) were log-transformed to improve the

Table 1 Clinical characteristics of patients with impaired glucose tolerance, type 2 diabetes, and type 1 diabetes in this study

	B	D2	D1	*p*
n	71	1,428	42	
Age (years)	57 ± 12	63 ± 10††	52 ± 15**	<0.01
Body weight (kg)	79.1 ± 19.6	66.8 ± 14.7††	54.7 ± 9.6**††	<0.01
Body mass index (kg/m^2)	28.9 ± 6.2	25.1 ± 4.5††	20.9 ± 3.4**††	<0.01
Waist (cm)	98.9 ± 14	90.2 ± 11.2††	78.2 ± 9.8**††	<0.01
Fasting plasma glucose (mg/dl)	102 ± 12	150 ± 53††	157 ± 70††	<0.01
HbA1c (%) (NGSP)	6.0 ± 0.5	8.9 ± 1.8††	9.6 ± 2.6††	<0.01
Total cholesterol (mg/dl)	222 ± 31	211 ± 38	206 ± 36	0.044
HDL cholesterol (mg/dl)	58 ± 16	55 ± 15	70 ± 17**††	<0.01
Triglyceride (mg/dl)	191 ± 188	158 ± 125	86 ± 47**††	<0.01
Uric acid (mg/dl)	6.3 ± 1.4	5.4 ± 1.3††	4.5 ± 1.4**††	<0.01
Creatinine (mg/dl)	0.75 ± 0.20	0.72 ± 0.26	0.65 ± 0.16	0.119
eGFR (ml/min/1.73 m^2)	80.0 ± 18.5	85.4 ± 54.6	94.0 ± 22.3	0.399
Adiponectin (μg/ml)	7.0 ± 3.5	8.3 ± 5.7	12.9 ± 7.0**††	<0.01

Mean and SD are shown

B impaired glucose tolerance, *D2* type 2 diabetes, *D1* type 1 diabetes, *eGFR* estimated glomerular filtration rate

** $p < 0.01$ versus type 2 diabetes group, †† $p < 0.01$ versus impaired glucose tolerance group by ANOVA

normality of distribution before analysis of these variables. Pearson's correlation coefficients (and p values) were calculated to assess the associations between serum adiponectin and other variables. Comparison of mean values between multiple groups was performed by ANOVA. When differences between group mean values were significant according to ANOVA, post hoc pairwise group comparisons were conducted by use of the Scheffé test. To compare serum adiponectin levels between patients with or without metabolic syndrome, and patients with or without microangiopathy or macroangiopathy, Student's t test was performed. Results are expressed as mean ± standard deviation (SD) and $p < 0.05$ was considered statistically significant.

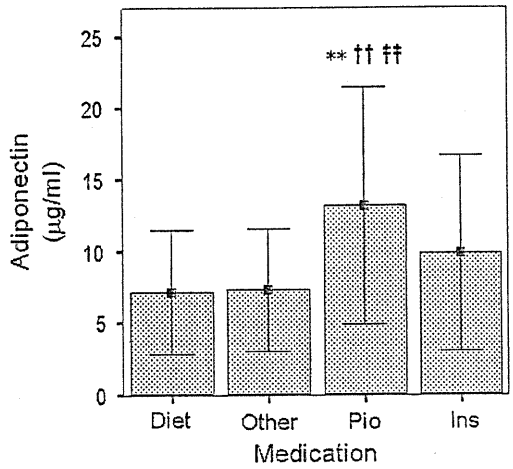

Fig. 1 Serum adiponectin level in 4 groups classified on the basis of medication for diabetes: pioglitazone (*Pio*, $n = 128$), insulin (*Ins*, $n = 296$), other oral hypoglycemic agents (*Other*, $n = 664$), and diet alone (*Diet*, $n = 411$). Mean and SD are shown. **$p < 0.01$ versus insulin group, ††$p < 0.01$ versus other oral hypoglycemic agents group, and ‡‡$p < 0.01$ versus diet alone group by ANOVA

Results

Table 1 presents clinical characteristics of patients with type 1 diabetes, type 2 diabetes, and impaired glucose tolerance. Compared with the impaired glucose tolerance group and the type 2 diabetes group, patients with type 1 diabetes had significantly lower body mass index (BMI), waist circumference, and triglyceride, and higher levels of HDL cholesterol. Serum adiponectin levels were significantly higher in patients with type 1 diabetes than in those with type 2 diabetes and with impaired glucose tolerance ($p < 0.001$), and we obtained the same results separated by sex (data not shown). On the basis of this result, we excluded the patients with type 1 diabetes from subsequent analyses. Figure 1 shows the serum adiponectin levels in 4 groups classified by their medication for diabetes. Serum adiponectin levels were higher in patients receiving pioglitazone treatment than in those on other medications ($p < 0.001$); those on insulin treatment had the second highest levels ($p < 0.001$). In patients receiving a combination of pioglitazone and a sulfonylurea, the serum adiponectin level was 13.2 ± 8.3 μg/ml, and was higher than in those on sulfonylureas alone ($p < 0.001$). In patients receiving biguanide treatment, sulfonylurea treatment, and the combination of a biguanide and a sulfonylurea, serum adiponectin levels were 6.7 ± 2.9, 6.6 ± 3.8, and 7.4 ± 4.4 μg/ml, respectively; these levels were similar to each other. From these results, we excluded patients on pioglitazone treatment from subsequent analyses. Simple correlations between serum adiponectin and metabolic variables are shown in Table 2. Serum levels of adiponectin were inversely correlated with body mass index,

Table 2 Simple correlation between serum adiponectin level and metabolic data

	r	*p*
Age	0.365	<0.01
Body mass index	−0.214	<0.01
Waist circumference	−0.220	<0.01
Fasting plasma glucose	−0.108	<0.01
HbA1c (NGSP)	−0.037	0.177
Total cholesterol	−0.009	0.746
HDL cholesterol	0.348	<0.01
Triglyceride	−0.361	<0.01
Uric acid	−0.206	<0.01
Creatinine	−0.009	0.738
eGFR	−0.089	<0.01
Fasting insulin	−0.322	<0.01
HOMA-IR	−0.338	<0.01
Total fat area	0.004	0.929
Subcutaneous fat area	0.099	0.016
Visceral fat area	−0.182	<0.01

NGSP National Glycohemoglobin Standardization Program, *HDL cholesterol* high-density lipoprotein cholesterol, *eGFR* estimated glomerular filtration rate, *HOMA-IR* homeostasis model assessment of insulin resistance

waist circumference, fasting plasma glucose, serum triglycerides, serum uric acid, fasting serum IRI, and HOMA-IR, whereas they were positively correlated with age and HDL cholesterol. Total cholesterol, HbA1c, and creatinine were not correlated with adiponectin or with total fat area determined by CT; they were inversely correlated with visceral fat area. Serum adiponectin was significantly lower in men who fitted the Japanese criteria for metabolic syndrome than in those who did not (6.2 ± 3.7 vs. 7.9 ± 5.4 μg/ml, $p < 0.001$). Adiponectin levels were also lower in women with metabolic syndrome than in those without, but the difference was not significant (9.1 ± 5.3 vs. 10.0 ± 5.8 μg/ml, $p = 0.102$). Figure 2 shows the serum adiponectin levels for 5 groups classified on the basis of the number of cardiometabolic risk factors. Serum adiponectin levels in patients with 5 cardiometabolic risk factors were significantly lower than in those with 1 ($p < 0.001$), 2 ($p = 0.001$), or 3 risk factors ($p = 0.032$), and were lower than in those with 4 risk factors, but the difference did not reach significance ($p = 0.921$). Clinical characteristics of patients with or without microangiopathy or macroangiopathy are shown in Tables 3 and 4. Adiponectin levels were significantly lower in patients with microangiopathy than in those without, for both males ($p = 0.005$) and females ($p < 0.001$), and levels were also significantly lower in male patients with macroangiopathy than in those without ($p = 0.010$), but not in female patients ($p = 0.643$).

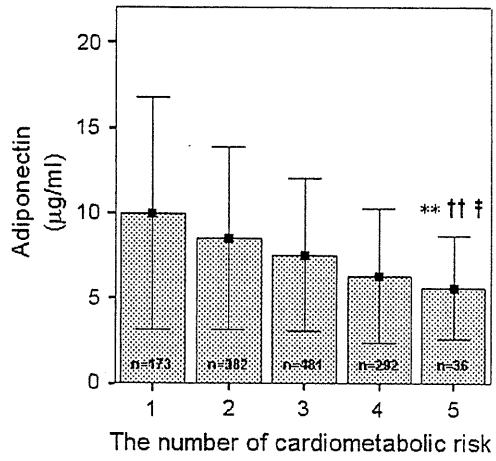

Fig. 2 Serum adiponectin level in 5 groups classified on the basis of the number of cardiometabolic risks. Mean and SD are shown. $^{**}p < 0.01$ versus 1 risk, $^{\dagger\dagger}p < 0.01$ versus 2 risks and $^{\ddagger}p < 0.05$ versus 3 cardiometabolic risks by ANOVA

Discussion

Adiponectin is one of the adipocytokines produced by adipose tissue. It acts to reduce insulin resistance and suppress the progression of arteriosclerosis [4, 5, 16]. An increase of visceral fat reduces adiponectin production, and this decrease may be important in patients with metabolic syndrome. Diabetic patients with excessive visceral fat, high blood pressure, and dyslipidemia (corresponding to metabolic syndrome) have recently been increasing in Japan. At our hospital, diabetic inpatients who fit the criteria for metabolic syndrome have increased to over 50% [17], whereas the other patients are less obese and less resistant to insulin, thus not matching the criteria for metabolic syndrome. Examining serum levels of adiponectin in diabetic patients is expected to be clinically useful, because adiponectin is related to insulin resistance and the progression of arteriosclerosis.

Because diabetes is a heterogeneous syndrome, some subgroups reported to have high adiponectin levels should be excluded. Increased serum levels of adiponectin have been reported In patients with type 1 diabetes, patients on pioglitazone treatment, and patients with chronic renal failure [18–20]. In our study, serum adiponectin levels were higher in patients with type 1 diabetes than in those with type 2 diabetes or impaired glucose tolerance (both males and females), and adiponectin was also higher in patients receiving pioglitazone treatment than in those on other treatments. Two aspects of pioglitazone treatment are relevant to assessment of diabetic complications. Most patients with pioglitazone treatment were insulin-resistant, and thus at high risk of cardiovascular disease, but pioglitazone not only increases serum adiponectin level [19] but

Table 3 Clinical characteristics of patients with or without microangiopathy

	Male		Female	
	Micro (−)	Micro (+)	Micro (−)	Micro (+)
n	442	446	203	235
Age (years)	63 ± 11	59 ± 11**	68 ± 8	64 ± 9††
Body weight (kg)	70.2 ± 14.7	71.4 ± 15.6	59.5 ± 12.8	59.3 ± 11.3
Body mass index (kg/m^2)	25.1 ± 4.4	25.4 ± 5.1	25.4 ± 4.9	25.0 ± 4.5
Waist (cm)	90.4 ± 11.2	90.5 ± 11.7	91.5 ± 12.6	89.6 ± 11.8
Fasting plasma glucose (mg/dl)	157 ± 66	140 ± 42**	154 ± 52	141 ± 43††
HbA1c (%) (NGSP)	9.1 ± 1.7	8.5 ± 1.9**	9.2 ± 1.7	8.4 ± 2.0††
Total cholesterol (mg/dl)	209 ± 39	207 ± 37	220 ± 38	216 ± 34
HDL cholesterol (mg/dl)	54 ± 16	52 ± 13	60 ± 16	60 ± 15
Triglyceride (mg/dl)	177 ± 135	170 ± 146	136 ± 80	129 ± 82
Uric Acid (mg/dl)	5.7 ± 1.4	5.7 ± 1.3	5.1 ± 1.3	4.8 ± 1.1††
Creatinine (mg/dl)	0.83 ± 0.36	0.74 ± 0.14**	0.63 ± 0.19	0.59 ± 0.14††
eGFR (ml/min/1.73 m^2)	83.7 ± 50.2	89.8 ± 66.3	76.6 ± 20.2	83.2 ± 20.9††
Adiponectin (μg/ml)	7.5 ± 5.3	6.5 ± 3.8**	10.4 ± 5.8	8.8 ± 5.3††

Mean and SD are shown

Micro (−) patients without microangiopathy, *Micro* (+) patients with microangiopathy, *eGFR* estimated glomerular filtration rate

** $p < 0.01$ versus male patients without microangiopathy, †† $p < 0.01$ versus female patients without microangiopathy by Student's *t* test

Table 4 Clinical characteristics of patients with or without macroangiopathy

	Male		Female	
	Macro (−)	Macro (+)	Macro (−)	Macro (+)
n	215	674	84	356
Age (years)	66 ± 10	60 ± 11**	68 ± 8	65 ± 9††
Body weight (kg)	68.6 ± 12.8	71.5 ± 15.8*	58.9 ± 11.8	59.5 ± 12.3
Body mass index (kg/m^2)	25.0 ± 3.8	25.3 ± 5.0	25.1 ± 4.6	25.2 ± 4.7
Waist (cm)	90.4 ± 9.9	90.5 ± 11.9	90.7 ± 10.9	90.4 ± 12.4
Fasting plasma glucose (mg/dl)	151 ± 81	148 ± 45	145 ± 45	148 ± 49
HbA1c (%) (NGSP)	8.7 ± 1.7	8.9 ± 1.9	9.0 ± 1.7	8.7 ± 2.0
Total cholesterol (mg/dl)	204 ± 37	209 ± 38	213 ± 31	219 ± 37
HDL cholesterol (mg/dl)	52 ± 16	53 ± 14	57 ± 14	61 ± 16
Triglyceride (mg/dl)	155 ± 91	179 ± 152	148 ± 110	128 ± 72
Uric Acid (mg/dl)	5.7 ± 1.3	5.7 ± 1.3	4.8 ± 1.1	5.0 ± 1.2
Creatinine (mg/dl)	0.82 ± 0.29	0.78 ± 0.27	0.61 ± 0.17	0.61 ± 0.17
eGFR (ml/min/1.73 m^2)	84.0 ± 66.4	87.6 ± 56.3	78.9 ± 18.0	80.4 ± 21.4
Adiponectin (μg/ml)	7.6 ± 4.9	6.8 ± 4.5*	9.2 ± 5.7	9.6 ± 5.6

Mean and SD are shown

Macro (−) patients without macroangiopathy, *Macro* (+) patients with macroangiopathy, *eGFR* estimated glomerular filtration rate

* $p < 0.05$ and ** $p < 0.01$ versus male patients without macroangiopathy, †† $p < 0.01$ versus female patients without macroangiopathy by Student's *t* test

also prevents cardiovascular problems [21]. Thus, we excluded patients with pioglitazone treatment in this study. If these patients were included in the study, similar results were obtained for serum adiponectin with or without micro and macroangiopathy, probably because of the small numbers, 128 patients (data not shown). Because elevation of serum adiponectin was variable and not related to the extent of insulin resistance in these subgroups, we excluded them from subsequent analyses. Patients with chronic renal failure were not included in this study, and the mean serum creatinine level was 0.78 mg/dl for males and 0.59 mg/dl for females.

Patients with type 2 diabetes have both insulin deficiency and insulin resistance, both of which are affected by a variety of genetic and environmental factors. Even in such a complicated metabolic condition as type 2 diabetes, serum levels of adiponectin were inversely correlated with body mass index, waist circumference, fasting IRI, and HOMA-IR, indicating that a low adiponectin level is a good marker of insulin resistance. Adiponectin levels were also inversely correlated with serum triglycerides and uric acid, and positively correlated with HDL cholesterol, suggesting that a low serum level of adiponectin was related to the progression of arteriosclerosis in patients with type 2 diabetes. However, serum adiponectin levels were not correlated with HbA1c or with serum total cholesterol and creatinine concentration.

Diabetic patients already have diabetes as a metabolic risk factor. Among these patients, patients who have large

waist circumference beyond the criteria adding one or both of high blood pressure and abnormal triglyceride or HDL cholesterol profile are matched to the criteria of metabolic syndrome. In this study, serum adiponectin levels were significantly lower among male diabetic patients with metabolic syndrome than among those without it, and the same trend was seen in female patients. Moreover, serum adiponectin levels were lower in patients with multiple cardiometabolic risk factors, and the lowest adiponectin group had all of the risk factors. Thus, serum adiponectin level could be a valuable marker of the risk of arteriosclerosis in diabetic patients. Similarly, adiponectin levels were lower in patients with diabetic complications, apart from female patients with macroangiopathy. It has been reported that patients with cardiovascular problems have low serum adiponectin levels [9]. Although there was a mixture of primary-prevention high-risk patients and secondary-prevention patients with various interventions in this study, serum levels of adiponectin were lower not only in patients with macroangiopathy but also in male patients with microangiopathy. In this study, serum adiponectin levels were not lower in female diabetic patients with macroangiopathy. Female diabetics had fewer cardiovascular diseases in Japan, fewer metabolic syndromes, and wider changes in serum adiponectin concentrations than males. To elucidate the relationship between serum adiponectin level and the development of macroangiopathy in female diabetic patients in Japan, a follow-up study of these patients will be needed.

Most literature reports suggest that a low level of serum adiponectin is a risk factor for progression to type 2 diabetes or metabolic syndrome in a healthy population [22, 23]. In this study our data imply that the serum adiponectin level is a valuable marker of the risk of arteriosclerosis in diabetic patients who already have an important risk factor, "diabetes". In summary, measurement of the serum adiponectin level is useful for assessment of metabolic status and for evaluation of the risk of arteriosclerosis in diabetic patients.

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ORIGINAL

Congener-specific polychlorinated biphenyls and the prevalence of diabetes in the Saku Control Obesity Program (SCOP)

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Abstract. The prevalence of diabetes is increasing globally. In addition to established risk factors for diabetes, such as diet, inactivity, overweight and obesity, the involvement of persistent organic pollutants, including dioxins and polychlorinated biphenyls (PCBs), has also been suggested to be a possible, but controversial, cause of this epidemic. The present study investigated the association between blood PCB congener levels and the prevalence of diabetes among middle-aged, overweight and obese Japanese participants in the Saku Control Obesity Program. One hundred seventeen participants had their congener-specific PCB levels measured in addition to undergoing routine blood analyses at the time of a medical checkup. Prevalent diabetes was defined according to two methods: definite diabetes was defined as people with an HbA1c level ≥ 6.9% or who were taking medication for diabetes, and all diabetes was defined as people with an HbA1c level ≥ 6.5%, a fasting plasma glucose level ≥ 126 mg/dL, or a history of doctor-diagnosed diabetes. A multiple logistic regression analysis was performed to analyze the association between the PCB levels and the prevalence of diabetes, with adjustments for sex, age, body mass index and total lipids. As a result, PCB 146 and 180 were positively associated and PCB 163/164 and 170 were negatively associated with the prevalence of definite diabetes. The significance of the association of PCB 180 and 163/164 with the prevalence of diabetes persisted regardless of the definition of diabetes or adjustments for total lipids, suggesting the possibility that these parameters may modify the risk of diabetes.

Key words: Diabetes, Polychlorinated biphenyl, Risk factor, Saku Control Obesity Program

DIABETES mellitus is increasing in Japan and other countries worldwide. The global prevalence of diabetes is estimated to be 285 million people among adults aged 20 to 79 years in 2010, and it is projected to increase to 439 million by 2030, accounting for 7.7% of the world population [1].

Well-established risk factors for the development of type 2 diabetes include excessive food consumption, a lack of physical activity, overweight and obesity, aging, smoking, socioeconomic status, and genetic predisposition [2, 3]. In addition to these factors, the involvement of persistent organic pollutants (POPs), such as dioxins and polychlorinated biphenyls (PCBs), in the increasing prevalence of diabetes has also been considered. Observations of occupational or accidental exposure to high levels of POPs have suggested a relationship between POPs and diabetes [4-9]. US Air Force veterans who participated in the spraying of aerial herbicide during the Vietnam War had higher levels of serum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), compared with veterans who performed other tasks, and exhibited a significantly higher prevalence of diabetes, increased use of medicines for diabetes, and a shorter duration until the onset of diabetes [4]. A cross-sec-

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tional study examining the effect of occupational exposure to TCDD among US workers showed a positive association between the TCDD concentration and the risk of diabetes and a higher fasting serum glucose level [5]. In a follow-up study of Italian citizens who were exposed to TCDD as a result of an industrial accident that occurred in 1976, a significantly elevated diabetes-related mortality was observed among women [6-9].

However, the relationship between exposure to low levels of POPs and diabetes is less consistent. A cross-sectional study of a general population in the USA indicated that the prevalence of diabetes, as determined by a fasting plasma glucose (FPG) level ≥ 126 mg/dL, a non-FPG level ≥ 200 mg/dL, or a self-report of diabetes diagnosed by a physician, was associated with the concentrations of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD), 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (OCDD), oxychlordane, *p,p'*-dichlorodiphenyltrichloroethane (DDE), and trans-nonachlor [10]. Another cross-sectional study of a Native American population concluded that the total PCB concentrations of 101 PCB congeners, PCB 153, PCB 74, DDE, and hexachlorobenzene (HCB) were positively associated with diabetes, as defined by an FPG level > 125 mg/dL or the use of hypoglycemic medication [11]. PCB 126, along with polychlorinated dibenzo-*p*-dioxin and *p,p'*-DDT, was considered in a general population in the USA, and a significant association was found with diagnosed diabetes and undiagnosed diabetes, which was defined as an HbA1c level > 6.1% [12]. Among the general population of Japan, a cross-sectional study measured seven polychlorinated dibenzo-*p*-dioxins (PCDDs), ten polychlorinated dibenzofurans (PCDFs) and 12 dioxin-like PCBs and showed that the accumulated toxic equivalents (TEQs) of these compounds were positively correlated with the HbA1c level [13]. In addition, the prevalence of diabetes, as defined by the self-reporting of physician-diagnosed diabetes or an HbA1c level > 6.5%, was positively associated with the accumulated TEQs of dioxin-like PCBs [13].

On the other hand, some studies have not confirmed a positive relationship. A cross-sectional study among Greenland Inuit did not find any association between subclasses of 13 PCB congeners and organochlorine pesticides and the prevalence of diabetes, as detected using a 75-g oral glucose tolerance test (OGTT) or self-reporting [14]. A nested case-controlled study of African-American and white youths showed nonlinear associations between POPs (35 PCB congeners, nine organochlorine pesticides, ten polychlorinated diphenyl ether congeners and one polybrominated biphenyl conger) and the prevalence of diabetes defined by the prescription of hypoglycemic medication or an FPG level ≥ 126 mg/dL at two or more blood tests [15]. A cross-sectional study in Belgium found significantly negative associations between PCB 170, 180, and the PCB total and insulin resistance [16]. Therefore, the effect of low levels of POPs apparently varied among studies, and further investigation is needed to determine whether POP exposure may be a risk factor for diabetes.

In the present study, 13 PCB congeners that are regarded as commonly present among Japanese adults were measured [17], and the associations with diabetes were assessed in middle-aged obese Japanese people without apparent exposure to POPs.

Subjects and Methods

Study population

This study was performed among the participants of the Saku Control Obesity Program (SCOP), the details of which have been described previously [18]. Briefly, the program consisted of a randomized intervention trial using cognitive-behavioral treatment at the Saku Health Dock Center. People who had undergone health checkups at the center were registered in the database, and 976 members aged 40 to 64 years who were free of type 1 diabetes or severe diseases such as stroke, cardiovascular disease, advanced cancer or significant renal or hepatic dysfunction and had a body mass index (BMI) in the upper five percentile (28.3 or above at their last medical checkup) were invited. A total of 235 people agreed to participate in the SCOP, and anthropometric measurements, various biomarkers and questionnaires regarding dietary habits, lifestyle, past and present medical history, and family history were obtained at baseline and during a follow-up examination. The participants were randomly assigned to two groups; group A, who participated in a lifestyle intervention program during year 1, and group B, who participated in the same intervention program during year 2. All the procedures were reviewed and approved by the ethical committees of the National Institute of Health and Nutrition and Saku Central Hospital, and written informed consent was obtained from all the participants.

Blood sampling and PCB congener-specific analysis

One hundred seventeen people participated in group A, and each participant provided a whole blood sample at baseline examination. The blood samples were obtained by venipuncture after fasting, and 2 mL of the sample was used for the PCB analysis. The PCB congeners that were measured were PCB 74, 99, 118, 138, 146, 153, 156, 163/164, 170, 180 and 182/187. These congeners reportedly account for 75% of the total amount of detected PCBs among healthy Japanese adults [17]. The PCB congeners were analyzed using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) at Otsuka Pharmaceutical Co. Ltd. (Tokushima, Japan) using a method described in detail elsewhere [17].

The obtained PCB concentrations were measured in units of pg/g blood; however, PCB concentrations are often expressed in terms of lipid weight because of the lipophilic nature of POPs and the invariability of lipid-adjusted PCB values, regardless of fluctuations in serum lipid levels [19, 20]. To correct for the serum total lipid level and obtain the lipid-adjusted PCB value (ng/g lipid), the measured PCB concentration in pg/g blood was divided by the total lipid value, as calculated using the short formula proposed by Phillips *et al.* [19, 20] as follows:

Total lipids (mg/dL)
=(2.27×total cholesterol)+triglycerides+62.3

The resulting value was then multiplied by 10^2 to adjust the unit.

Evaluation of diabetes

Diabetes was diagnosed based on blood test results, prescribed medication, and the medical history. Subjects with an HbA1c level ≥ 6.9% or a prescription for a hypoglycemic medicine were regarded as the 'definite diabetes' group. In addition, a broader definition was also considered and consisted of the combination of a FPG level and HbA1c measurements indicative of diabetes [21], since conducting a 75-g OGTT in all the participants was not practical for the detection of undiagnosed diabetes. Accordingly, subjects with an FPG level ≥ 126 mg/dL, an HbA1c level ≥ 6.5%, a prescription for hypoglycemic medicine, or a history of physician-diagnosed diabetes were defined as the 'all diabetes' group. The value for HbA1c (%) was estimated as an NGSP equivalent value (%) calculated by the formula HbA1c (%) = HbA1c (JDS)(%) + 0.4%, considering the relational expression of HbA1c (JDS)(%) measured by the previous Japanese standard substance and measurement methods and HbA1c (NGSP) [22].

Statistical analyses

The association between exposure variables and the prevalence of diabetes was estimated using odd ratios, 95% confidence intervals, and p values obtained from multiple logistic regression analyses. Three models were applied to analyze the associations between the prevalence of diabetes and the serum PCB concentrations. In the first model, the directly measured wet weight concentration (pg/g blood) of PCBs was applied with age (continuous variable), sex (dichotomous variable) and BMI (continuous variable) as potential confounders. Next, the serum total lipid value was added as an adjustment. In the third model, the lipid-adjusted PCB concentration (ng/g lipid), age, sex and BMI were included in the model.

All analyses were performed using Stata SE 10.1 (StataCorp LP, TX, USA), and p values less than 0.05 were considered statistically significant.

Results

The basic characteristics of the participants are summarized in Table 1. Among the 117 group A members, 59 (50.4%) were male and 58 (49.6%) were female. Fifteen (12.8%) were classified in the definite diabetes group, and 32 (27.4%) were classified in the all diabetes group. No significant differences in the mean values of data for the diabetes and non-diabetes groups were observed except for the HbA1c and FPG levels.

Table 2 summarizes the blood PCB congener levels among the participants. All the congeners were skewed to the right. Mean of their concentration was higher among older age group people for all of the PCB congeners. PCBs that were common within the participants were PCB 153, 180, 138 and 118, and this tendency was similar in other studies measuring these congeners among the population [17, 23].

The results of the multiple logistic regression analyses with definite diabetes as the dependent variable are shown in Table 3. PCB 146 and 180 had a statistically significant positive association with definite diabetes, and PCB 163/164 and 170 had a significantly negative association with definite diabetes when the PCB congeners were adjusted for sex, age and BMI. An additional adjustment for total lipids did not change the significance of these associations. When the analyses were

Table 1 Characteristics of participants.

	Definite DM	Not definite DM	All DM	Not all DM
Number	15	102	32	85
Sex (male/female)	7/8	52/50	17/15	42/43
Age (years)	55.5 ± 6.7	54.0 ± 6.6	55.4 ± 6.0	53.8 ± 6.8
BMI	30.9 ± 3.0	30.3 ± 2.7	30.5 ± 2.7	30.3 ± 2.7
Waist circumference (cm)	101.8 ± 7.7	101.7 ± 7.3	101.4 ± 7.1	101.8 ± 7.5
Abdominal fat (cm^2)	149.4 ± 65.2	136.7 ± 45.1	142.4 ± 51.9	136.8 ± 46.7
HbA1c (%)	8.2 ± 1.9	5.9 ± 0.4	7.3 ± 1.5	5.7 ± 0.3
FPG (mg/dL)	159.7 ± 51.3	103.4 ± 11.4	136.5 ± 42.2	100.9 ± 9.0
Total cholesterol (mg/dL)	207.7 ± 24.4	208.8 ± 34.1	202.8 ± 26.4	210.8 ± 35.0
Triglycerides (mg/dL)	176.7 ± 104.6	145.8 ± 78.5	154.6 ± 80.7	147.9 ± 83.4
HDL cholesterol (mg/dL)	53.9 ± 13.1	52.0 ± 11.9	50.9 ± 12.9	52.8 ± 11.7
LDL cholesterol (mg/dL)	118.5 ± 24.9	128.3 ± 30.9	121.0 ± 25.6	129.3 ± 31.7
Total lipid (mg/dL)	710.4 ± 129.7	682.0 ± 123.5	677.4 ± 110.4	688.8 ± 129.4
Urinary acid (mg/dL)	5.7 ± 1.3	6.0 ± 1.4	5.8 ± 1.2	6.0 ± 1.4
Systolic blood pressure (mmHg)	136.7 ± 12.4	131.6 ± 16.0	134.8 ± 14.6	131.3 ± 16.0
Diastolic blood pressure (mmHg)	80.9 ± 8.5	81.0 ± 13.5	82.9 ± 13.0	80.2 ± 12.9

Definite DM: subjects with an HbA1c level ≥ 6.9% or a prescription for hypoglycemic medicine. All DM: subjects with an FPG level ≥ 126 mg/dL, and HbA1c level ≥ 6.5%, a prescription for hypoglycemic medicine, or a history of diabetes. Data are the mean ± standard deviation. Total lipid was estimated from the measured total cholesterol and triglyceride levels.

Table 2 Concentrations of PCB congeners.

Age group (Number)		< 50 (27)	50-59 (62)	≥ 60 (28)	All ages (117)		
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Median	Range
PCB 74	pg/g blood	22.4 ± 16.0	33.3 ± 15.9	51.3 ± 25.5	35.1 ± 21.1	28.7	9.4 - 120.8
	ng/g lipid	3.3 ± 2.1	5.0 ± 2.5	7.7 ± 4.5	5.3 ± 3.4	4.4	1.4 - 23.3
PCB 99	pg/g blood	19.2 ± 11.3	24.9 ± 11.2	32.6 ± 21.4	25.4 ± 14.9	21.6	4.7 - 123.7
	ng/g lipid	2.8 ± 1.4	3.7 ± 1.8	5.0 ± 4.1	3.8 ± 2.6	3.4	0.5 - 23.8
PCB 118	pg/g blood	47.9 ± 32.1	59.1 ± 30.4	87.9 ± 52.1	63.4 ± 39.5	54.0	16.1 - 293.8
	ng/g lipid	7.1 ± 4.2	8.8 ± 4.4	13.5 ± 10.0	9.6 ± 6.5	7.9	1.8 - 56.6
PCB 138	pg/g blood	63.3 ± 38.2	77.9 ± 31.9	104.2 ± 68.2	80.9 ± 46.4	73.7	23.2 - 405.6
	ng/g lipid	9.2 ± 4.6	11.7 ± 5.2	16.0 ± 13.3	12.2 ± 8.1	11.0	3.3 - 78.2
PCB 146	pg/g blood	18.8 ± 10.2	23.7 ± 10.6	33.1 ± 22.0	24.8 ± 14.8	21.5	6.0 - 131.8
	ng/g lipid	2.7 ± 1.2	3.6 ± 1.8	5.1 ± 4.3	3.7 ± 2.6	3.3	1.0 - 25.4
PCB 153	pg/g blood	129.6 ± 74.8	168.1 ± 74.1	230.5 ± 158.9	174.1 ± 106.1	154.2	50.9 - 947.5
	ng/g lipid	18.8 ± 9.0	25.4 ± 12.2	35.5 ± 31.0	26.3 ± 18.8	23.2	7.4 - 182.7
PCB 156	pg/g blood	14.0 ± 7.0	17.9 ± 7.6	23.6 ± 13.1	18.3 ± 9.6	16.1	6.2 - 73.3
	ng/g lipid	2.0 ± 0.9	2.7 ± 1.2	3.5 ± 2.4	2.7 ± 1.6	2.5	0.9 - 14.1
PCB 163/164	pg/g blood	30.6 ± 15.5	39.3 ± 17.0	54.0 ± 32.9	40.8 ± 22.9	36.0	10.3 - 200.3
	ng/g lipid	4.5 ± 2.0	5.9 ± 2.8	8.2 ± 6.4	6.1 ± 4.1	5.5	1.6 - 38.6
PCB 170	pg/g blood	16.6 ± 7.6	22.0 ± 8.4	29.3 ± 19.9	22.5 ± 12.7	19.7	7.8 - 110.9
	ng/g lipid	2.4 ± 1.0	3.3 ± 1.3	4.4 ± 3.7	3.4 ± 2.2	3.0	1.1 - 21.4
PCB 180	pg/g blood	63.2 ± 30.1	83.0 ± 30.2	111.7 ± 76.1	85.3 ± 48.1	75.7	28.5 - 446.3
	ng/g lipid	9.2 ± 3.9	12.5 ± 5.1	16.9 ± 14.5	12.8 ± 8.5	11.3	3.9 - 86.0
PCB 182/187	pg/g blood	33.0 ± 20.3	42.3 ± 19.6	58.6 ± 40.5	44.1 ± 27.5	39.3	11.3 - 243.3
	ng/g lipid	4.7 ± 2.5	6.4 ± 3.4	9.0 ± 7.9	6.6 ± 4.9	6.1	1.5 - 46.9
Total PCB	pg/g blood	458.7 ± 249.2	591.3 ± 230.2	817.0 ± 513.9	614.7 ± 345.1	535.5	181.5 - 3097.3
	ng/g lipid	66.8 ± 30.3	89.1 ± 37.8	125.0 ± 100.0	92.5 ± 60.8	82.7	26.9 - 597.1

SD: standard deviation.

Table 3 Risk of definite diabetes according to PCBs.

	No adjustment for lipid[a]		Adjusted for total lipids[b]		Lipid-adjusted PCBs[c]	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
PCB 74	0.92	(0.79-1.07)	0.91	(0.79-1.06)	0.67	(0.25-1.83)
PCB 99	1.09	(0.84-1.41)	1.05	(0.79-1.38)	2.02	(0.36-11.18)
PCB 118	1.00	(0.91-1.09)	1.00	(0.92-1.09)	0.97	(0.52-1.82)
PCB 138	1.02	(0.89-1.16)	1.03	(0.90-1.17)	0.93	(0.39-2.24)
PCB 146	2.36	(1.06-5.24)	2.46	(1.09-5.59)	137	(0.80-23650)
PCB 153	0.93	(0.85-1.01)	0.94	(0.86-1.02)	0.64	(0.37-1.11)
PCB 156	1.60	(0.92-2.77)	1.52	(0.86-2.67)	20.2	(0.57-713)
PCB 163/164	0.60	(0.39-0.91)	0.59	(0.38-0.91)	0.03	(0.002-0.46)
PCB 170	0.45	(0.25-0.79)	0.42	(0.23-0.78)	0.01	(0.0003-0.42)
PCB 180	1.35	(1.10-1.67)	1.39	(1.10-1.76)	5.32	(1.46-19.32)
PCB 182/187	0.95	(0.70-1.28)	0.88	(0.62-1.26)	1.21	(0.15-10.03)

[a] Adjusted for sex, age and BMI. [b] Adjusted for sex, age, BMI and total lipid. Adjusted for sex, age and BMI using lipid-adjusted PCBs. Data are odds ratio (95% confidence interval) per 1 pg/g blood[a,b] or 1 ng/g lipid[c].

Table 4 Risk of all diabetes according to PCBs.

	No adjustment for lipid[a]		Adjusted for total lipids[b]		Lipid-adjusted PCBs[c]	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
PCB 74	1.02	(0.94-1.10)	1.02	(0.94-1.10)	1.16	(0.68-1.98)
PCB 99	1.08	(0.92-1.27)	1.10	(0.94-1.30)	1.70	(0.60-4.80)
PCB 118	0.97	(0.91-1.02)	0.97	(0.91-1.02)	0.79	(0.54-1.15)
PCB 138	1.01	(0.93-1.10)	1.02	(0.93-1.11)	1.17	(0.67-2.06)
PCB 146	1.56	(1.05-2.31)	1.59	(1.08-2.35)	29.2	(1.89-451)
PCB 153	0.96	(0.91-1.01)	0.95	(0.90-1.00)	0.73	(0.51-1.07)
PCB 156	0.93	(0.68-1.27)	0.99	(0.71-1.37)	0.94	(0.12-7.62)
PCB 163/164	0.80	(0.65-0.98)	0.77	(0.62-0.95)	0.14	(0.03-0.58)
PCB 170	0.85	(0.67-1.07)	0.86	(0.69-1.08)	0.40	(0.09-1.91)
PCB 180	1.10	(1.10-1.20)	1.09	(1.01-1.19)	1.76	(1.01-3.08)
PCB 182/187	1.00	(0.85-1.17)	1.05	(0.88-1.25)	1.15	(0.36-3.64)

[a] Adjusted for sex, age and BMI. [b] Adjusted for sex, age, BMI and total lipid. [c] Adjusted for sex, age and BMI using lipid-adjusted PCBs. Data are odds ratio (95% confidence interval) per 1 pg/g blood[a,b] or 1 ng/g lipid[c].

performed using the lipid-adjusted PCB concentrations, the significance of the positive relation between PCB 146 and definite diabetes was attenuated, but the positive association with PCB 180 and the negative association with PCB 163/164 and 170 remained.

On the other hand, broadening the definition of diabetes to include all diabetic participants attenuated the association between the PCB levels and the risk of diabetes (Table 4). PCB 146 and 180 continued to have positive associations with definite diabetes, but the significance of the negative association with PCB 170 was lost and only PCB 163/164 maintained a significant negative association. Adding total lipids as a potential confounder or using the lipid-adjusted PCB values did not change the significant associations with PCB 146, 180 and 163/164.

Discussion

Statistically significant associations between the PCB congener concentrations and the prevalence of diabetes were observed among middle-aged Japanese obese adults. PCB 180 was positively and PCB 163/164 was negatively associated with both the definite diabetes and the all diabetes groups, regardless of adjustments for possible confounding factors. A positive association between PCB 146 and diabetes was observed except in the analysis using lipid-adjusted PCB concentrations in the definite diabetes group, and a negative association for PCB 170 was observed in the definite diabetes group.

To date, information about the relationship between specific PCB congeners and diabetes is limited. Many

epidemiological studies have focused on TCDD, which is known to cause dermal toxicity, neurotoxicity, immunotoxicity and carcinogenicity, as well as PCB 153, which is thought to be correlated with the total amount of PCBs and may be useful as an indicator substance of multifarious PCBs [24]. Recent studies have measured more PCB congeners in detail; however, analyses were performed using the accumulated TEQs [13] or the total/subtotal sum of PCB congeners [14, 25], and differences in the specific effects of PCB congeners have rarely been reported.

Knowledge from experimental studies is also insufficient. The biological pathway is thought to include interactions among aryl hydrocarbon receptor (AhR) [26], peroxisome proliferator-activated receptor (PPAR) [27], and type 4 glucose transporter (GLUT4) [28], but these findings have mainly been obtained for TCDD, and the biological functions of PCB 146, 163/164, 170 and 180 are not known in detail.

Although the results of previous studies have not exactly supported the findings of the present study, the influence of PCBs on diabetes through indirect mechanisms can be considered. Many cross-sectional studies have shown that PCBs correlate positively with BMI, which may increase insulin resistance and the risk of diabetes. A positive relation was observed between BMI and serum level of PCB 180 [29] and the sum of PCB 118, 138, 153 and 180 [30]. Other studies investigated that total PCB concentration of 101 congeners and the concentrations of PCB 74, 99, 153 and 206 were inversely associated with the serum testosterone levels in men [31], and lower testosterone levels are correlated with higher insulin resistance [32] and a higher risk of diabetes [33]. In addition, testosterone replacement for hypogonadal men with diabetes reduced their insulin resistance and improved their control of diabetes [34]. Therefore, PCBs may be partly responsible for the development of diabetes by lowering the testosterone level and increasing insulin resistance, even though PCB 146, 163/164, 170 or 180 was not associated with BMI in the present study.

On the other hand, there were studies showing inverse relationship between PCBs and insulin resistance. There were cross-sectional studies reporting that the serum levels of PCB 118, 138, 153 and 180 [35], PCB 153, 170, 180 and the sum of these PCB congeners and PCB 138 were inversely associated with obesity, while the concentrations of PCB 170, 180 and the sum of the PCBs were correlated negatively with insulin resistance [16].

These findings suggest that PCBs may be positively or negatively associated with insulin resistance and diabetes. As the PCB 170 concentration was especially low among obese people [16], PCB 170 may have a stronger weight reduction effect, compared with the other congeners. The participants of the present study were extremely obese subjects in the Japanese population; therefore, the effect of obesity might have been pronounced.

The strength of the present study is that diabetes was ascertained using a blood test, as a certain proportion of undiagnosed diabetes exists among the general population. In fact, among the participants of this study, there were only nine self-reported physician-diagnosed cases of diabetes, but blood sample measurements enabled the identification of six undiagnosed cases of diabetes using the definite diabetes criteria and 23 cases using the all diabetes criteria.

Nevertheless, the present study also has several limitations. First, because of its cross-sectional design, the cause-effect relation could not be explained. Although the elimination rate of TCDD has been suggested to be unrelated to the presence of diabetes among US veterans [36], implying that the difference in blood TCDD levels occurred before the development of diabetes, the difference in elimination rates among PCB congeners is not well known.

Environmental exposure to PCBs is more complicated than occupational or accidental exposures. Food and inhalation are the main routes of intake, and metabolism and excretion are related to elimination; however, such factors have not been fully explored. Meat, dairy products and fish are the major sources of PCBs, and the consumption of certain types of fish is correlated with certain types of PCBs [25, 37]; however, appropriate data that would enable adjustments to the analyses were not available for the present study population. The possibility that the negative association between PCB congeners and the prevalence of diabetes was due to the protective effect of n-3 polyunsaturated fatty acids contained in fatty fish cannot be denied, considering that the blood levels of dioxins and PCBs are well correlated with fish consumption [38]. The number of parities and lifetime lactation may influence the elimination of PCBs [39], but this information was also not considered. Further studies that include such missing data may clarify the robustness of the present findings.

In conclusion, PCB 180 had a positive associa-

tion and PCB 163/164 had a negative association with the prevalence of diabetes among middle-aged, obese Japanese subjects after adjustments for age, sex, BMI, and total lipids. PCB 146 and 170 may have positive and negative associations, respectively, with the prevalence of diabetes. Further consideration, including the intake and elimination of PCB congeners, may strengthen these findings.

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Obese Japanese Adults with Type 2 Diabetes Have Higher Basal Metabolic Rates than Non-Diabetic Adults

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Summary Several cross-sectional studies in Pima Indians and Caucasians have indicated that obese individuals with type 2 diabetes have a higher basal metabolic rate (BMR) than healthy, obese individuals. However, no study has investigated this comparison in Japanese subjects, who are known to be susceptible to type 2 diabetes due to genetic characteristics. Thirty obese Japanese adults with pre-type 2 diabetes ($n=7$) or type 2 diabetes ($n=13$) or without diabetes ($n=10$) participated in this study. BMR was measured using indirect calorimetry. The relationships between residual BMR (calculated as measured BMR minus BMR adjusted for fat-free mass, fat mass, age, and sex) and biomarkers including fasting glucose, glycosylated hemoglobin (HbA_{1c}), fasting insulin, homeostasis model assessment of insulin resistance (HOMA-R), triglycerides, and free fatty acids were examined using Pearson's correlation. BMR in diabetic subjects adjusted for fat-free mass, fat mass, age, and sex was 7.1% higher than in non-diabetic subjects. BMR in diabetic subjects was also significantly ($p<0.05$) higher than in non-diabetic subjects. There was a significant correlation between residual BMR and fasting glucose ($r=0.391$, $p=0.032$). These results indicate that in the Japanese population, obese subjects with type 2 diabetes have higher BMR compared with obese non-diabetic subjects. The fasting glucose level may contribute to these differences.

Key Words basal metabolic rate, Japanese, obesity, diabetes, predictive equation

As type 2 diabetes and obesity are closely related, the number of patients with type 2 diabetes in Japan has increased as a result of the rise in prevalence of obesity (*1*). In general, the fundamental treatment for type 2 diabetes is improvement in lifestyle such as diet and physical activity, associated with pharmacotherapy (*2*). Control of daily energy balance remains one of the most important treatment principles. Management of daily energy balance is usually conducted by diet control and maintenance of higher levels of physical activity. Accurate assessments of energy intake and energy expenditure are therefore required during treatment of diabetes.

Several cross-sectional studies have examined whether or not individuals with type 2 diabetes have a higher basal metabolic rate (BMR). Previous studies in Pima Indians (*3*) and Caucasians (*4*) using calorimetry showed obese subjects with type 2 diabetes had 5.2% and 6.9% higher BMR, adjusted for body composition, compared with their respective non-diabetic counterparts. Although the physiological mechanisms responsible for the increased BMR in individuals with type 2 diabetes are poorly understood, several mechanisms have been proposed to explain this change in BMR. These include increases in protein turnover (*5*), futile substrate cycling (*6*), gluconeogenesis (*7*), plasma glucagon (*8*), and sympathetic nervous system activity (*3*). As Japanese people are susceptible to type 2 diabetes (*9*), mainly due to a lower ability to secrete insulin than Caucasians (*10*), this genetic characteristic may provide different results in BMR than similar studies in Pima Indians or Caucasians (*3*, *4*). However, no study has examined whether BMR is higher in Japanese subjects with type 2 diabetes compared to subjects without diabetes.

As BMR may be different between individuals with non-diabetes, pre-diabetes or diabetes, some adjustments may be necessary when BMR is calculated in these groups. As the majority of clinical facilities are unable to carry out indirect calorimetry, BMR is usually estimated from predictive equations using data including age, sex, height, and weight (*11*). Previous studies indicate that predictive equations derived mainly from measurements in Caucasian subjects tend to overestimate BMR in both Asians (*11*, *12*) and Caucasians (*11*, *13–17*). We recently developed new predictive equations for BMR in the Japanese population (*18*). One of these equations was shown to be the best predictor of BMR amongst several predictive equations in healthy Japanese subjects (*19*). However, no study has investigated the validity of several of these published equations in Japanese subjects with type 2 diabetes.

The purpose of the present study was therefore to compare BMR between subjects with non-diabetes, pre-diabetes or diabetes in the obese Japanese population.

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Table 1. Physical characteristics and metabolic parameters in subjects with non-diabetes, pre-diabetes, or diabetes.

	Non-diabetes (n=10)		Pre-diabetes (n=7)		Diabetes (n=13)		ANOVA p value
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	
Male/female	5/5		3/4		8/5		
Age (y)	54±3	51–59	53±3	50–57	53±3	50–59	0.760
Height (cm)	165.3±10.0	151.5–179.2	162.9±7.7	155.0–174.0	165.3±8.7	152.0–176.6	0.826
Weight (kg)	81.1±7.2	69.3–93.5	82.0±10.7	67.0–97.7	87.4±12.4	70.2–116.5	0.317
Body mass index (kg/m^2)	29.7±1.7	27.7–33.2	30.9±3.3	27.9–38.0	32.0±3.5	28.1–39.2	0.211
Body fat (%)	33.7±7.4	24.0–45.4	36.3±8.9	23.4–45.6	36.5±6.6	24.0–46.0	0.627
FM (kg)	26.9±4.5	21.2–34.5	29.5±8.0	20.9–44.6	31.7±6.2	23.1–45.7	0.206
FFM (kg)	54.1±10.2	41.6–71.1	52.4±11.4	40.8–68.5	55.8±11.2	41.6–73.0	0.808
Fasting glucose (mg/dL)	99±5	92–109	111±10	90–121	130±17[a,b]	100–168	<0.001
$\mathrm{Log_e}$ $\mathrm{HbA_{1c}}$	1.8±0.0	1.7–1.8	1.8±0.1	1.7–1.9	1.9±0.2[a]	1.6–2.5	0.016
[$\mathrm{HbA_{1c}}$ (%)]	5.9	5.7–6.0	5.9	5.4–6.4	7.1	5.0–12.6	
$\mathrm{Log_e}$ fasting insulin	1.8±0.4	1.0–2.3	2.5±0.7[a]	1.5–3.3	2.3±0.6	1.5–3.5	0.019
[Fasting insulin (μU/mL)]	6.5	2.8–10.1	14.9	4.4–27.4	12.1	4.3–33.8	
HOMA-R	1.6±0.6	0.7–2.5	4.1±2.4	1.3–8.2	3.8±2.7	1.5–11.8	0.030
Triglycerides (mg/dL)	149±66	76–268	221±119	87–410	153±89	51–384	0.221
Free fatty acid (mEq/L)	0.4±0.2	0.1–0.7	0.5±0.1	0.4–0.7	0.5±0.2	0.3–1.0	0.339

FM: fat mass. FFM: fat-free mass. $\mathrm{HbA_{1c}}$: glycosylated hemoglobin. $\mathrm{HbA_{1c}}$ and fasting insulin were log transformed. HOMA-R=fasting insulin×fasting glucose/405. Differences between the non-diabetes, pre-diabetes and diabetes groups were evaluated by one-way ANOVA and Bonferroni post hoc test. [a] $p<0.05$ vs. non-diabetes, [b] $p<0.05$ vs. pre-diabetes.

The second aim of the study was to examine the validity of several predictive equations for BMR in these subjects.

MATERIALS AND METHODS

Subjects. The subjects in the study were 50- to 59-y-old obese subjects who resided in Saku City (Nagano Prefecture in Japan). The subjects were selected randomly from participants in the Saku Control Obesity Program (SCOP). The details of SCOP are described elsewhere (*20*). Thirty obese Japanese adults without diabetes (n=10), or with pre-type 2 diabetes (n=7) or type 2 diabetes (n=13) participated in this study. Two diabetic patients were treated by diet and exercise prescription, and one diabetic patient by metformin or glibenclamide therapy. Another diabetic patient who had experienced a diabetes patient education program in the past was included also, whereas those on insulin therapy were excluded. The subjects were instructed to eat a usual diet and carry out normal, but not vigorous physical activity beginning 1 d before the measurements. All the investigations were carried out in the Saku Central Hospital. This study was conducted according to the guidelines of the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethical Committee of the National Institute of Health and Nutrition in Tokyo, Japan and the Ethical Committee of the Saku Central Hospital. The study protocol was explained to the subjects prior to enrollment, and all the subjects signed an informed consent form.

Anthropometric and body composition. The physical characteristics of the subjects are summarized in Table 1. Body weight was measured to the nearest 0.1 kg and body height to the nearest 0.1 cm using an automatic scale (Tanita, BF-220, Tokyo, Japan). The measurements were performed in light clothing and underwear. The light clothing was then weighed and subtracted from the total to obtain body weight with minimal clothing (underwear). Body mass index (BMI: kg/m^2) was calculated as body weight (kg) divided by square of body height (m^2). Percentage body fat was measured using a bioelectrical impedance technique (Tanita, BF-220). Fat-free mass (FFM) and fat mass (FM) were calculated from percentage body fat and body weight.

Measurements of BMR. The subjects came to the hospital in the early morning and were asked to minimize walking prior to the laboratory visit and BMR measurement. In the majority of previous studies, especially in those using the dietary reference intakes for Japanese (Japan-DRI), Schofield, or the Food and Agriculture Organization of the United Nations/World Health Organization/United Nations University, the subjects also came to the laboratory in the early morning (*21*). BMR was measured in the post-absorptive state at least 12 h after the last meal. Measurements were performed in a room at a constant temperature of approximately 25°C. After entering the hospital, the subjects rested in the supine position wearing a face mask for at least 30 min. Two samples of expired air were collected in Douglas bags over two 10-min periods, and the mean of the two values used in the analyses.

The expired air was sampled and the O_2 and CO_2 concentrations measured using a gas analyzer (Arco System, AR-1, Kashiwa, Japan) with a galvanic O_2 sensor and an infrared CO_2 sensor. Prior to each of the consecutive measurements, the gas analyzer was calibrated using atmospheric air. The volume of expired air was