18. Byington RP, Davis BR, Plehn JF, White HD, Baker J, Cobbe SM, et al for the Prospective Pravastatin Pooling (PPP) Project. Reduction of stroke events with pravastatin: The Prospective Pravastatin Pooling (PPP) Project. *Circulation* 2001; **103:** 387–392.
19. Davis BR, Vogt T, Frost PH, Burlando A, Cohen J, Wilson A, et al. for the Systolic Hypertension in the Elderly Program Cooperative Research Group. Risk factors for stroke and type of stroke in persons with isolated systolic hypertension. *Stroke* 1998; **29:** 1333–1340.
20. The Kyushu Lipid Intervention Group. A coronary primary prevention study of Japanese men: Study design and, implementation and baseline data. *J Atheroscler Thromb* 1996; **3:** 95–104.
21. The Kyushu Lipid Intervention Group. Pravastatin use and risk of coronary events and cerebral infarction in Japanese men with moderate hypercholesterolemia: Kyushu Lipid Intervention Study. *J Atheroscler Thromb* 2000; **7:** 110–121.
22. Sasaki J, Arakawa K, Iwashita M, Matsushita Y, Kono S for the Kyushu Lipid Intervention Study (KLIS) Group. Reduction in serum total cholesterol and risks of coronary events and cerebral infarction in Japanese men: The Kyushu Lipid Intervention Study. *Circ J* 2003; **67:** 473–478.
23. Iwashita M, Matsushita Y, Sasaki J, Arakawa K, Kono S for the Kyushu Lipid Intervention Study (KLIS) Group. Relation of serum total cholesterol and other risk factors to risk of coronary events in middle-aged and elderly Japanese men with hypercholesterolemia: The Kyushu Lipid Intervention Study. *Circ J* 2004; **68:** 405–409.
24. Friedewald WJ, Levy RI, Fredrichson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18:** 459–502.
25. The Fukuoka Heart Study Group. Medication for hypercholesterolemia and the risk of nonfatal acute myocardial infarction: A case–control study in Japan. *Circ J* 2002; **66:** 463–468.
26. Piechowski-Jozwiak B, Bogousslavsky J. Cholesterol as a risk factor for stroke: The fugitive? *Stroke* 2004; **35:** 1523–1524.
27. Thrift AG. Cholesterol is associated with stroke, but is not a risk factor. *Stroke* 2004; **35:** 1524–1525.
28. Rosenson RS, Tangney CC. Antiatherothrombotic properties of statins: Implications fro cardiovascular event reduction. *JAMA* 1998; **279:** 1643–1650.
29. Sever PS, Dahlöf B, Poulter NR, Wedel H, Beevers G, Caulfield M, et al for the ASCOT Investigators. Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm (ASCOT-LLA): A multicentre randomised controlled trial. *Lancet* 2003; **361:** 1149–1158.
30. Corvol JC, Bouzamondo A, Sirol M, Hulot JS, Sanchez P, Lechat P. Differential effects of lipid-lowering therapies of stroke prevention. *Arch Intern Med* 2003; **163:** 669–676.
31. Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischemic heart disease, and stroke: Systematic review and meta-analysis. *BMJ* 2003; **326:** 1423–1429.
32. Shepherd J, Blauw GJ, Murphy MB, Bollen ELEM, Buckley BM, Gobbe SM, et al on behalf of the PROSPER study group. Pravastatin in elderly individuals at risk of vascular disease (PROSPER): A randomized controlled trial. *Lancet* 2002; **360:** 1623–1630.
33. Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001; **285:** 2486–2497.
34. Matsuzaki M, Kita T, Mabuchi H, Matsuzawa Y, Nakaya N, Oikawa S, et al and the J-LIT Study Group. Large scale cohort study of the relationship between serum cholesterol and coronary events with low-dose simvastatin therapy in Japanese patients with hypercholesterolemia: Primary Prevention Cohort Study of the Japan Lipid Intervention Trial (J-LIT). *Circ J* 2002; **66:** 1087–1095.
35. Packard CJ, Saito Y. Non-HDL cholesterol as a measure of atherosclerotic risk. *J Atheroscler Thromb* 2003; **11:** 6–11.
36. Steinberg D, Pearson TA, Kuller LH. Alcohol and atherosclerosis. *Ann Intern Med* 1991; **114:** 967–976.
37. Hannuksela ML, Liisanantt MK, Savolainen MJ. Effect of alcohol on lipids and lipoproteins in relation to atherosclerosis. *Crit Rev Clin Lab Sci* 2002; **39:** 225–283.

Appendix 1

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Dietary Patterns and Colorectal Adenomas in Japanese Men

The Self-Defense Forces Health Study

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The role of dietary patterns in colorectal carcinogenesis remains unclear in Asian populations. Using 1999–2002 data, the authors investigated the association between dietary patterns and colorectal adenomas in 1,341 Japanese men who underwent total colonoscopy. Information about diet was obtained using a 74-item food frequency questionnaire prior to the colonoscopy. Three dietary patterns were generated by factor analysis: 1) a high-dairy, high-fruit and -vegetable, high-starch, low-alcohol pattern; 2) an "animal food" pattern; and 3) a Japanese pattern. Logistic regression analysis was used to estimate the odds ratio of having colorectal adenomas with the adjustment for potential confounding variables including body mass index, smoking, alcohol, and leisure-time physical activities. A significant inverse association was found for the high-dairy, high-fruit and -vegetable, high-starch, low-alcohol pattern; the odds ratios for the second, third, and fourth quartiles were 0.97 (95% confidence interval: 0.70, 1.36), 0.71 (95% confidence interval: 0.50, 1.01), and 0.62 (95% confidence interval: 0.43, 0.90), respectively, compared with the lowest (p_{trend} = 0.003). Similar associations were observed for larger adenomas or for each subsite of the colorectum. The Japanese and "animal food" patterns were not clearly associated with colorectal adenomas. A dietary pattern including greater consumption of dairy products and fruits and vegetables with low alcohol consumption may be associated with decreased risk of colorectal adenomas.

adenoma; cross-sectional studies; diet

Abbreviation: DFSA, high-dairy, high-fruit and -vegetable, high-starch, low-alcohol (dietary pattern).

Colorectal cancer is a major cause of cancer deaths in developed countries. Geographic and time-trend analyses, as well as migrant studies, strongly suggest that environmental factors, especially diet, play an important role in the pathogenesis of colorectal cancer (1–3). However, analytical epidemiologic studies have yielded conflicting findings; for example, a body of evidence suggesting a protective role of vegetables or dietary fiber (4) has been either challenged (5–8) or supported (9, 10) by recent large-scale studies. In Japan, colorectal cancer mortality has markedly increased over the last several decades (11) and is now among the highest levels in the world (12). Time-trend analysis has suggested that decreased consumption of dietary fibers (13) or grains (14) may account for the increase in mortality. Yet it is largely unknown which lifestyle changes associated with Westernization or modernization have contributed to the rapid increase of colorectal cancer in Japan, or whether the traditional Japanese diet protects against this type of cancer.

Analysis of dietary patterns has recently drawn a great deal of attention as a method of investigating the role of foods or nutrients in studies of chronic diseases. Approaches of this sort, dealing with a combination of several foods, can overcome problems arising from close intercorrelation and potential effect modifications among numerous foods or nutrients (15). Factor-analysis studies of Western populations have suggested that a certain dietary pattern may be

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predictive of colorectal cancer risk (16–18). Dietary patterns generated by factor analysis, however, are sample specific and may not be applicable to populations having different dietary cultures. While having adopted a Western-style diet, many Japanese still consume large amounts of traditional foods, including rice, fish, and soybean products (19). Thus, dietary patterns among Japanese may differ considerably from those among Western populations.

The aim of the present study was therefore to investigate dietary patterns in relation to the risk of colorectal adenoma, a precursor of colorectal cancer (20, 21), using data from preretirement check-ups among male Self-Defense Forces officials in Japan.

MATERIALS AND METHODS

Study setting

The data used were derived from the Self-Defense Forces Health Study, a cross-sectional survey of male Self-Defense Forces officials who participated in a preretirement health examination at two hospitals (Fukuoka and Kumamoto) in Japan. The study procedure has been described elsewhere (22, 23). In short, all officials undergo a comprehensive health examination before retirement; total colonoscopy is included as a routine procedure. Study questionnaires about health-related lifestyles were distributed prior to colonoscopy to male examinees on the first day of hospital admission for examination. Research assistants checked the questionnaire for unanswered questions and apparently inconsistent answers and, if necessary, sought clarification from the study subjects.

Results of laboratory tests and colonoscopic findings, including histologies for polyp, were extracted from clinical reports. Written informed consent was obtained from study participants. The study protocol has been approved by the ethics committee of Kyushu University.

Study subjects

The present study used data from April 1999 through March 2002. Among 2,390 male Self-Defense Forces officials who underwent the examination, 2,370 (99 percent) agreed to participate in the present study. After excluding men with histories of cancer, stroke, myocardial infarction, coronary revascularization, inflammatory bowel diseases, colorectal surgery, or diabetes mellitus, we kept 2,141 men in the analysis of dietary patterns. Of these, we excluded men who did not receive colonoscopy (n = 57), who underwent partial or unsuccessful colonoscopy (n = 177), or who had colorectal polyp removal prior to the examination (n = 148). Of the remaining 1,759 subjects who completed total colonoscopy, 764 men were identified as having colorectal polyps including hyperplastic nodules. Of these, 476 men had their polyps histologically confirmed: cancer (n = 1), carcinoid (n = 1), adenoma (n = 346), and other histologies (n = 128). Only 29 men had adenomas of 10 mm or larger, and nine had tubulovillous or villous adenomas. The data for the 346 men who had adenoma (case group) and 995 men who were free from any colorectal polyp and cancer (referent group) were analyzed to assess the association between dietary patterns and colorectal adenomas.

Dietary assessment

Information about diet was collected using a food frequency questionnaire designed to assess the average intakes of 74 food items, food groups, and food preparations over the previous year. The questionnaire was an expanded version of a 45-item food frequency questionnaire that was developed on the basis of a published questionnaire (24) and was validated against the 28-day dietary record (25). The expansion of food items was done with reference to food consumption in the National Nutrition Survey (19) and a dietary questionnaire developed elsewhere in Japan (26). Participants were asked to choose from seven response options for most dietary items, ranging from "never/less than one per month" to "two to three times per day." Different response schemes were used for green tea, coffee, and rice (five options) and for alcoholic beverages (six options). Daily consumers of green tea, coffee, or rice were asked about the number of cups or bowls consumed per day. Current drinkers, defined as those who have consumed alcoholic beverages weekly for at least 1 year in their lifetime and who were drinking at the time of the survey, were asked about the frequency of consumption and the amount of consumption per occasion of five alcoholic beverages, that is, sake (a Japanese wine), shochu (a Japanese distilled beverage), beer, whiskey, and wine. The amount of consumption per occasion was used in the estimation of total ethanol intake from these alcoholic beverages, but only the frequency of consumption for each alcoholic beverage was used in the analysis of dietary patterns.

Grouping of food factors

Before the analysis of dietary patterns, intakes of green tea, coffee, or rice were converted into units of cups or bowls per day, while those of other dietary items were quantified in terms of frequency per week. Five dietary questions that overlapped with or were duplicated by others (collective consumption of cooked vegetables, apples, mandarin oranges, other oranges, watermelons) and three questions about food spreads (butter, margarine, and jam/honey) were not used. Furthermore, some foods or food groups similar in nutritional content or culinary use were combined, leaving 39 food items for the purposes of the present study.

Statistical analysis

Dietary patterns were generated by factor analysis (principal components) using SAS PROC FACTOR statistical software (27). Factor analysis is a technique to reduce a number of variables into fewer independent factors. To make interpretation easier, a linear transformation called a "rotation" is normally performed on the initial factor solution. We used an orthogonal rotation procedure (varimax rotation), which maintains the uncorrelated nature of the factors and tries to get the original variables to load high on one of the factors and low on the rest. When factor scores are used as

independent variables in a subsequent regression analysis, this procedure has the advantage over oblique rotation that the analysis is less subject to problems of collinearity. In determining the number of factors to retain, we consider eigenvalue, the scree test, and interpretability. Eleven factors satisfied the criteria for eigenvalues greater than one, and the scree plot showed small breaks in the eigenvalues after factor 5, suggesting three or four factors to retain. Post-rotated factor loadings revealed that three factors well describe distinctive dietary patterns of the study population.

We thus retained the three dietary patterns and designated them as 1) a high-dairy, high-fruit and -vegetable, high-starch, low-alcohol (DFSA) pattern; 2) an "animal food" pattern; and 3) a Japanese pattern, according to the food items showing high loading (absolute value) with respect to each dietary pattern. We confirmed that these three dietary factors emerged when all 74 food items in our questionnaire were simply included in factor analysis. A factor score for each dietary pattern was calculated by weighting consumption of each food item by the corresponding factor loading and summing the resulting values. This score ranks individuals in terms of how closely they conform to the dietary pattern.

The potential confounding variables considered were hospital (Fukuoka or Kumamoto), age (treated as a continuous variable), parental history of colorectal cancer (absent or present), occupational rank (three categories), body mass index (<22, 22–23.9, 24–25.9, and ≥26 kg/m^2), smoking (lifetime nonsmoker, former smoker, and current smoker using <15, 15–24, or ≥25 cigarettes/day), and leisure-time physical activity, expressed as the sum of metabolic equivalents for each activity multiplied by the corresponding hours of such activity per week (none, <20, 20–39.9, and ≥40 metabolic equivalent-hours). Quartiles of factor scores of each dietary pattern among controls were used for cutoff values. Multiple logistic regression that included terms for the above-mentioned variables was performed to estimate the odds ratio and 95 percent confidence interval of colorectal adenomas according to quartiles of scores for each dietary pattern, taking the lowest quartile group as the referent group. Analyses were repeated for adenomas of 5 mm or larger (n = 140) or according to the location of the lesion (proximal colon including the cecum, ascending colon, liver flexure, transverse colon, and splenic flexure; distal colon including the descending colon and sigmoid colon; and the rectum). Logistic regression analysis was performed using SAS PROC LOGISTIC software (27).

RESULTS

Table 1 shows factor loadings, which are equivalent to simple correlations between the food items and the dietary patterns. A positive loading indicates that the food item is positively associated with the dietary pattern, and a negative loading indicates an inverse association with the dietary pattern. The DFSA dietary pattern was characterized by frequent intake of fermented dairy products, milk, confectionaries, bread, fruits, and vegetables and infrequent intake of shochu, a local alcoholic beverage in the study areas. The "animal food" dietary pattern was characterized by various kinds of animal foods, including red meat, poultry, seafood excluding fish, processed meat and fish products, and fried or broiled foods. The Japanese dietary pattern was characterized by traditional foods in Japan (soybean products, seaweed, pickles, and green tea), vegetables, and fish. The proportion of the total variance explained by the three factors was 24 percent.

TABLE 1. Factor-loading matrix for dietary patterns, Self-Defense Forces Health Study, Japan, 1999–2002*

	DFSA† dietary pattern	"Animal food" dietary pattern	Japanese dietary pattern
Fermented dairy products	0.61	–	–
Confectionaries	0.55	0.18	–
Canned fruits	0.52	–	–
Bread	0.47	–	–0.39
Fruits (not canned)	0.47	–	0.21
Fruit juices	0.47	–	–
Vegetable juice	0.41	–	0.17
Milk	0.40	–	–
Oil dressing	0.33	0.19	0.26
Soda, cola	0.30	0.20	–0.18
Shochu (alcoholic beverage)	–0.40	0.15	0.24
Red meat	–	0.68	–
Poultry	–	0.63	–
Fried foods	0.25	0.49	0.29
Broiled fish/meat	–	0.48	0.32
Seafood (except fish)	–	0.47	0.18
Processed meat	0.17	0.46	–
Processed fish	–	0.41	0.18
Gyoza‡	–	0.40	–
Liver	–	0.38	–
Eggs	–	0.34	0.22
Noodles	–	0.34	–
Soybean products	–	–	0.64
Cooked vegetables	0.36	0.23	0.56
Seaweed	0.27	–	0.55
Raw vegetables	0.45	–	0.52
Pickles	0.19	–	0.51
Green tea	–	–0.15	0.46
Fish	–	0.27	0.38
Potatoes	0.33	0.24	0.35
Garlic	0.20	–	0.32
Variance explained (%)	8.5	7.9	7.7

* Factor loadings are equivalent to simple correlations between the food items and the dietary patterns. Factor loadings less than ±0.15 were indicated by a dash; food items with factor loadings less than ±0.30 for all dietary patterns (rice, mayonnaise, nuts, coffee, wine, beer, whiskey, sake) were omitted.

† DFSA, high-dairy, high-fruit and -vegetable, high-starch, low-alcohol (dietary pattern).

‡ Dumpling with minced pork and vegetable stuffing.

TABLE 2. Dietary patterns in relation to potential confounding variables and alcohol intake among referents, Self-Defense Forces Health Study, Japan, 1999–2002

Dietary patterns	Hospital (% Kumamoto)	Age (mean years)	Rank (% highest)	Parental history of colorectal cancer (%)	Body mass index (mean kg/m²)	Smoking (% current smokers)	Physical activity (median metabolic equivalent-hours)	Alcohol (median ml/day)*
DFSA† dietary pattern								
Quartile 1 (low)	28	52.4	10	6	23.7	45	15	62
Quartile 2, 3	27	52.4	14	4	24.0	38	16	32
Quartile 4 (high)	18	52.4	19	5	23.5	40	16	14
p_{trend}‡	0.01	0.49	<0.01	0.66	0.46	0.26	0.84	<0.01
"Animal food" dietary pattern								
Quartile 1 (low)	30	52.5	15	4	23.6	40	16	14.5
Quartile 2, 3	24	52.4	14	4	23.8	42	16	34
Quartile 4 (high)	22	52.3	13	6	23.9	37	15	49
p_{trend}	0.05	0.23	0.43	0.52	0.15	0.56	0.87	<0.01
Japanese dietary pattern								
Quartile 1 (low)	21	52.4	17	5	23.7	48	9.5	21
Quartile 2, 3	25	52.3	13	4	23.8	39	16	40
Quartile 4 (high)	29	52.5	14	4	23.9	36	19	38
p_{trend}	0.02	0.58	0.51	0.66	0.51	<0.01	<0.01	<0.01

* Estimated from the consumption of five alcoholic beverages: beer, sake, shochu, wine, and whiskey.
† DFSA, high-dairy, high-fruit and -vegetable, high-starch, low-alcohol (dietary pattern).
‡ Mantel-Haenszel chi-squared test for categorical variables and linear regression analysis for continuous variables, assigning to categories of each dietary pattern their median scores (physical activity and alcohol consumption were log transformed).

Table 2 shows the association of dietary patterns with potential confounding variables and alcohol consumption among men free from colorectal polyp or cancer (referent group). Examinees at the Kumamoto hospital had a higher score for the Japanese dietary pattern but lower scores for the DFSA and "animal food" dietary patterns than those at the Fukuoka hospital. This reflects the geographic characteristics of dietary patterns; the southern parts of Kyushu Island, including Kumamoto, are less urbanized than the northern parts, including Fukuoka. Men with a high score for the DFSA dietary pattern tended to have higher occupational positions and consumed smaller amounts of alcohol. Men with high scores for the "animal food" dietary pattern tended to consume greater amounts of alcohol. Men in the upper quartiles of the Japanese dietary pattern tended to be nonsmokers and engaged in higher levels of leisure-time physical activity, and they consumed greater amounts of alcohol.

As shown in table 3, the DFSA dietary pattern was inversely associated with the risk of colorectal adenomas, showing a 40 percent reduced odds ratio among men in the highest quartile of the dietary pattern compared with those in the lowest. This association was slightly more evident for adenomas with a diameter of 5 mm or larger. No apparent association was observed for either the "animal food" dietary pattern or the Japanese dietary pattern.

The DFSA dietary pattern was inversely associated with adenomas at all subsites of the colorectum (table 4). The association was slightly stronger for the proximal colon in terms of the odds ratio of 0.5 for the highest quartile of the dietary pattern score and test for the trend association ($p_{trend} = 0.003$), but the confidence intervals of odds ratios for this site overlapped substantially with those for other sites. The Japanese and "animal food" dietary patterns were not measurably associated with colon adenomas. However, a nonsignificant positive association with rectal adenomas was observed for the Japanese pattern, while a nonsignificant inverse association was found for the "animal food" pattern. The odds ratios for the upper three quartiles combined compared with the lowest were 1.64 (95 percent confidence interval: 0.83, 3.25) and 0.64 (95 percent confidence interval: 0.36, 1.13) for the Japanese pattern and "animal food" pattern, respectively.

DISCUSSION

We investigated the association between major dietary patterns and colorectal adenomas among middle-aged Japanese men. Of the three dietary patterns we identified, the DFSA dietary pattern showed a significant, inverse association with the risk of colorectal adenomas.

Strengths and limitations

Our study had several strengths. Selection bias in terms of study participation was unlikely because of nonselective

TABLE 3. Logistic regression results for the association between dietary patterns and colorectal adenoma, Self-Defense Forces Health Study, Japan, 1999–2002

Dietary pattern	Quartile*							P_{trend}
	1 (low)	2		3		4 (high)		
		Odds ratio†	95% confidence interval	Odds ratio	95% confidence interval	Odds ratio	95% confidence interval	
DFSA‡ dietary pattern								
Adenoma of any size	1.00	0.97	0.70, 1.36	0.71	0.50, 1.01	0.62	0.43, 0.90	0.003
Adenoma of 5 mm or larger	1.00	0.84	0.52, 1.34	0.68	0.41, 1.12	0.59	0.35, 0.996	0.04
"Animal food" dietary pattern								
Adenoma of any size	1.00	0.87	0.61, 1.23	0.91	0.64, 1.28	0.86	0.60, 1.23	0.49
Adenoma of 5 mm or larger	1.00	1.05	0.64, 1.72	0.84	0.50, 1.41	0.98	0.59, 1.63	0.75
Japanese dietary pattern								
Adenoma of any size	1.00	0.96	0.67, 1.38	1.13	0.79, 1.61	1.18	0.83, 1.69	0.26
Adenoma of 5 mm or larger	1.00	1.00	0.59, 1.70	1.11	0.66, 1.86	1.24	0.75, 2.08	0.36

* Among referents.
† Adjusted for hospital, age, parental history of colorectal cancer, occupational rank, body mass index, smoking, and leisure-time physical activity.
‡ DFSA, high-dairy, high-fruit and -vegetable, high-starch, low-alcohol (dietary pattern).

recruitment for the preretirement health examination, which included total colonoscopy as a routine procedure, and high study participation rate. The questionnaire was distributed and collected prior to colonoscopy, and thus recall bias associated with adenoma status was also unlikely. The control series consisted of only subjects who were confirmed via total colonoscopy to be free from any colorectal polyp and cancer, leading to a more valid assessment compared with studies

TABLE 4. Logistic regression results for the association between dietary patterns and colorectal adenoma according to the location of the lesion, Self-Defense Forces Health Study, Japan, 1999–2002

Dietary pattern	Quartile*							P_{trend}
	1 (low)	2		3		4 (high)		
		Odds ratio†	95% confidence interval	Odds ratio	95% confidence interval	Odds ratio	95% confidence interval	
Colon adenoma (n = 299)								
DFSA‡ dietary pattern	1.00	0.93	0.66, 1.32	0.70	0.48, 1.01	0.59	0.40, 0.87	0.003
"Animal food" dietary pattern	1.00	0.93	0.64, 1.35	0.97	0.67, 1.40	0.95	0.65, 1.38	0.85
Japanese dietary pattern	1.00	0.93	0.64, 1.37	1.09	0.76, 1.59	1.11	0.77, 1.62	0.45
Proximal colon adenoma (n = 158)								
DFSA dietary pattern	1.00	1.00	0.64, 1.54	0.67	0.41, 1.09	0.50	0.30, 0.85	0.003
"Animal food" dietary pattern	1.00	0.84	0.51, 1.39	1.08	0.68, 1.73	0.94	0.57, 1.53	0.95
Japanese dietary pattern	1.00	0.87	0.53, 1.42	0.92	0.56, 1.49	1.08	0.67, 1.74	0.70
Distal colon adenoma (n = 171)								
DFSA dietary pattern	1.00	1.00	0.64, 1.54	0.77	0.48, 1.23	0.68	0.42, 1.11	0.08
"Animal food" dietary pattern	1.00	1.01	0.63, 1.62	0.95	0.59, 1.52	1.01	0.63, 1.62	0.97
Japanese dietary pattern	1.00	1.10	0.67, 1.79	1.43	0.90, 2.28	1.21	0.74, 1.96	0.35
Rectal adenoma (n = 63)								
DFSA dietary pattern	1.00	0.94	0.48, 1.84	0.64	0.30, 1.36	0.71	0.34, 1.48	0.26
"Animal food" dietary pattern	1.00	0.66	0.33, 1.34	0.64	0.31, 1.32	0.62	0.30, 1.28	0.22
Japanese dietary pattern	1.00	1.58	0.71, 3.51	1.56	0.70, 3.47	1.79	0.82, 3.92	0.18

* Among referents.
† Adjusted for hospital, age, parental history of colorectal cancer, occupational rank, body mass index, smoking, and leisure-time physical activity.
‡ DFSA, high-dairy, high-fruit and -vegetable, high-starch, low-alcohol (dietary pattern).

based on partial colonoscopy. We controlled for major known or suspected confounding factors. The uniform background of the study subjects in terms of occupation, sex, and age was also advantageous in maintaining comparability between cases and controls, although this uniformity limits the extent to which we may generalize from the present findings.

The present study also features some limitations. For one, the dietary questionnaire has not been validated. However, the former version, including questions and response options similar to those of the present questionnaire, has been validated against 7-day, year-round dietary records (25). Most nutrients and foods demonstrated fairly good correlation between the dietary record and questionnaire; relatively high correlation coefficients of 0.80, 0.77, 0.58, and 0.58 were observed for bread, fruits, dairy products, and pickled vegetables, respectively. Nondifferential misclassification in our dietary assessment could distort risk estimates toward the null. Such a bias may be minimal for the analysis of the DFSA dietary pattern, composed of food items showing good correlation between the dietary record and questionnaire, but this bias could be the reason for the lack of an apparent association with the "animal food" or Japanese dietary pattern.

Limitations of factor analysis arise from the arbitrary decisions (15) involved in selecting and grouping foods for analysis from the questionnaire, in determining the number of factors to retain, in choosing the method of rotation of the initial factors to increase the interpretability of the dietary pattern, and in labeling dietary patterns according to their factor loadings. Masaki et al. (28) identified four major dietary patterns using baseline data of a cohort of male employees in Tokyo. Similar to our study, their study identified a "Western breakfast" dietary pattern and an "animal" dietary pattern, suggesting the existence of dietary patterns common to the Japanese. Our derived dietary patterns accounted for 24 percent of the total variance, which is comparable with a figure observed in a previous study (17) but less than that reported in a Japanese study (28). Caution needs to be exercised when comparing the variance explained across studies, which is determined by various factors including the number of variables in analysis.

Interpretation of findings

A dietary pattern characterized by frequent intakes of dairy products, confectionaries, and fruits and vegetables, as well as by infrequent consumption of shochu, a local alcoholic beverage, was inversely associated with the risk of colorectal adenomas. This dietary pattern seems to consist of relatively healthy selections of foods found in Western countries and includes foods of probably low consumption in Japan. According to the food balance sheet (29), per capita supplies of dairy products in Japan and among developed countries in the year 2001 were 66 kg (181 g/day) and 197 kg (540 g/day), respectively; the corresponding values for fruits were 53 kg (145 g/day) and 83 kg (227 g/day). Although the associations between these foods and colorectal cancer have been inconsistent, the present results are in agreement with the existing body of evidence, including findings from recent studies, indicating that high consumption of dairy products or calcium (30–34) and high consumption of fruits, fruit juices, or fruit fiber (10, 35–38) are each associated with reduced risk of colorectal cancer or adenoma. A positive association of alcohol consumption and colorectal adenomas or cancer has been reported in many studies, including those in Japan (23, 39). In addition to independent effects, there may be complex interactions among food factors constituting the DFSA dietary pattern. For example, fruit juices may enhance calcium absorption (40), and reduced alcohol intake increases the bioavailability of folate (41). The glycemic effects of a high-starch or a high-sugar diet and their contribution to increased risk of colorectal cancer have been suspected, but epidemiologic findings are inconsistent on this point (42, 43). Our finding of an inverse association between the DFSA dietary pattern and colorectal adenomas provides the following suggestions: that a high-starch diet may inhibit, rather than promote, the formation of colorectal adenomas and that the adverse effects of a high-starch diet, if any, may not be so strong as to negate the protective effects of other foods contributing to this dietary pattern on adenoma risk. The inverse association of this dietary pattern with adenoma risk was somewhat stronger for the proximal colon than for other sites. Random error could be an explanation. Alternatively, the dietary pattern may be more closely involved in the formation of adenomas in the proximal colon.

The Japanese dietary pattern was characterized by high consumption of many plant foods, including traditional Japanese foods (soybean products, seaweed, pickles) and vegetables. A diet rich in various plant foods could potentially reduce cancer risk because of their many biologically active chemicals (44). However, the Japanese dietary pattern was not apparently associated with colonic adenomas. In studies of Western populations, inverse associations between similar dietary patterns (designated "prudent" or "healthy" patterns) and colorectal cancer have been unclear (18) or limited to subgroups (17). The lack of such an association in our study may contradict a body of evidence supporting an inverse association between vegetables and colorectal cancer (4) but agree with results of recent prospective, but not all (10, 37), studies reporting no association between vegetables or fiber and colorectal cancer or adenoma (5–8). As most of the adenomas in the present study were small in size and less malignant in nature, the present finding is in line with a hypothesis that vegetables are inversely associated with the progression of colorectal adenomas to cancer but not with the initial appearance of adenomas (38). We found a nonsignificant positive association between the Japanese dietary pattern and the risk of rectal adenomas. Studies in Japan (45–49) have consistently shown that frequent consumption of preserved foods including pickled vegetables and dried/salted fish, typical of the Japanese diet, is associated with increased risk of colorectal cancer; of these studies, three documented a significant association specifically for the rectum (46–48). These preserved foods contain *N*-nitroso compounds (50), which are potent carcinogens (51). Among other foods characterizing the Japanese pattern, broiled fish is a potential source of exposure to carcinogenic heterocyclic amine (52), although we are not aware of any epidemiologic

findings suggesting a relation between broiled fish and colorectal cancer risk.

Meat, especially red meat, processed meat, or meat broiled at high temperature, has been associated with colorectal cancer (53, 54) or adenoma (55). A study in Japan found a significant positive association between intake of animal protein and the risk of colorectal adenoma (56). However, we find no increase in the risk of colorectal adenomas associated with the "animal food" dietary pattern. Besides possible bias due to misclassification in the dietary assessment (as discussed above), the lack of such an association for colon adenomas in our study may be attributable to the moderate consumption of meat in Japan (mean daily intake of total meat: 96 g for men aged 40–49 years (19)). In addition, poultry has contributed to the healthy or prudent dietary patterns in Western populations (16–18). The diversity of animal food sources may dilute the potential carcinogenic effects of a specific animal food. Furthermore, it is possible that moderate intake of animal foods prevents carcinogenesis because these foods provide nutrients such as methionine and folate, which are beneficial in DNA synthesis and DNA methylation (57). In this context, our finding showing an increased risk of rectal adenomas associated with the lowest quartile of the "animal food" pattern may be of note and is consistent with results of certain studies relating to colorectal cancer in Japan (45, 48, 58).

In conclusion, the present results indicate that a dietary pattern characterized by frequent consumption of dairy products, confectionaries, bread, fruits, and vegetables but low intake of local alcoholic beverages is associated with a reduced risk of colorectal adenomas in Japanese men. Nonsignificant associations for rectal adenomas, based on an analysis including only 63 men with adenomas in the rectum, should set a limit to causal inference. However, since the incidence of rectal cancer in Japan has been high among industrial countries (12), the question as to whether a Japanese-style diet or a diet low in animal foods promotes carcinogenesis of the rectum warrants further investigation.

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REFERENCES

1. Buell P, Dunn JE. Cancer mortality among Japanese Issei and Nisei of California. Cancer 1965;18:656–64.
2. Tominaga S. Cancer incidence in Japanese in Japan, Hawaii, and western United States. Natl Cancer Inst Monogr 1985;69: 83–92.
3. Shimizu H, Mack TM, Ross RK, et al. Cancer of the gastrointestinal tract among Japanese and white immigrants in Los Angeles County. J Natl Cancer Inst 1987;78:223–8.
4. Glade MJ. Food, nutrition, and the prevention of cancer: a global perspective. American Institute for Cancer Research/World Cancer Research Fund, American Institute for Cancer Research. Nutrition 1999;15:523–6.
5. Michels KB, Giovannucci E, Joshipura KJ, et al. Prospective study of fruit and vegetable consumption and incidence of colon and rectal cancers. J Natl Cancer Inst 2000;92:1740–52.
6. Fuchs CS, Giovannucci EL, Colditz GA, et al. Dietary fiber and the risk of colorectal cancer and adenoma in women. N Engl J Med 1999;340:169–76.
7. Schatzkin A, Lanza E, Corle D, et al. Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. Polyp Prevention Trial Study Group. N Engl J Med 2000;342:1149–55.
8. Alberts DS, Martinez ME, Roe DJ, et al. Lack of effect of a high-fiber cereal supplement on the recurrence of colorectal adenomas. Phoenix Colon Cancer Prevention Physicians' Network. N Engl J Med 2000;342:1156–62.
9. Bingham SA, Day NE, Luben R, et al. Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet 2003;361:1496–501.
10. Peters U, Sinha R, Chatterjee N, et al. Dietary fibre and colorectal adenoma in a colorectal cancer early detection programme. Lancet 2003;361:1491–5.
11. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labor, and Welfare. Age-adjusted death rates by prefecture. In: Special report on vital statistics, 2000. (In Japanese). Tokyo, Japan: Japan Health and Welfare Statistics Association, 2002.
12. Parkin DM, Whelan SL, Ferlay J, et al, eds. Cancer incidence in five continents. Vol VIII. Lyon, France: International Agency for Research on Cancer, 2002. (IARC publication no. 155).
13. Honda T, Kai I, Ohi G. Fat and dietary fiber intake and colon cancer mortality: a chronological comparison between Japan and the United States. Nutr Cancer 1999;33:95–9.
14. Kono S, Ahn YO. Vegetables, cereals and colon cancer mortality: long-term trend in Japan. Eur J Cancer Prev 2000;9:363–5.
15. Jacques PF, Tucker KL. Are dietary patterns useful for understanding the role of diet in chronic disease? Am J Clin Nutr 2001;73:1–2.
16. Slattery ML, Boucher KM, Caan BJ, et al. Eating patterns and risk of colon cancer. Am J Epidemiol 1998;148:4–16.
17. Terry P, Hu FB, Hansen H, et al. Prospective study of major dietary patterns and colorectal cancer risk in women. Am J Epidemiol 2001;154:1143–9.
18. Fung T, Hu FB, Fuchs C, et al. Major dietary patterns and the risk of colorectal cancer in women. Arch Intern Med 2003;163: 309–14.
19. Bureau of Public Health, Ministry of Health and Welfare. The national nutrition survey in Japan, 1996. (In Japanese). Tokyo, Japan: Daiichi Shuppan, 1998.
20. Hill MJ, Morson BC, Bussey HJ. Aetiology of adenoma-carcinoma sequence in large bowel. Lancet 1978;1:245–7.
21. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525–32.
22. Kono S, Handa K, Hayabuchi H, et al. Obesity, weight gain and risk of colon adenomas in Japanese men. Jpn J Cancer Res 1999;90:805–11.
23. Toyomura K, Yamaguchi K, Kawamoto H, et al. Relation of cigarette smoking and alcohol use to colorectal adenomas by

subsite: the Self-Defense Forces Health Study. Cancer Sci 2004;95:72–6.
24. Shirota T, Yoshizumi F. A study on convenient dietary assessment. (In Japanese). Nippon Koshu Eisei Zasshi 1990;37:100–8.
25. Lee KY, Uchida K, Shirota T, et al. Validity of a self-administered food frequency questionnaire against 7-day dietary records in four seasons. J Nutr Sci Vitaminol 2002;48:467–76.
26. Tokudome S, Ikeda M, Tokudome Y, et al. Development of data-based semi-quantitative food frequency questionnaire for dietary studies in middle-aged Japanese. Jpn J Clin Oncol 1998; 28:679–87.
27. SAS Institute, Inc. SAS/STAT user's guide, version 6. 4th ed. Vol 2. Cary, NC: SAS Institute, Inc, 1989.
28. Masaki M, Sugimori H, Nakamura K, et al. Dietary patterns and stomach cancer among middle-aged male workers in Tokyo. Asian Pac J Cancer Prev 2003;4:61–6.
29. FAOSTAT nutritional data. Food balance sheets. Rome, Italy: Food and Agriculture Organization of the United Nations, 2004. (http://www.fao.org/waicent/portal/statistics_en.asp).
30. Martinez ME, Willett WC. Calcium, vitamin D, and colorectal cancer: a review of the epidemiologic evidence. Cancer Epidemiol Biomarkers Prev 1998;7:163–8.
31. Baron JA, Beach M, Mandel JS, et al. Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. N Engl J Med 1999;340:101–7.
32. Pietinen P, Malila N, Virtanen M, et al. Diet and risk of colorectal cancer in a cohort of Finnish men. Cancer Causes Control 1999;10:387–96.
33. Wu K, Willett WC, Fuchs CS, et al. Calcium intake and risk of colon cancer in women and men. J Natl Cancer Inst 2002;94: 437–46.
34. McCullough ML, Robertson AS, Rodriguez C, et al. Calcium, vitamin D, dairy products, and risk of colorectal cancer in the Cancer Prevention Study II Nutrition Cohort (United States). Cancer Causes Control 2003;14:1–12.
35. Sandler RS, Lyles CM, Peipins LA, et al. Diet and risk of colorectal adenomas: macronutrients, cholesterol, and fiber. J Natl Cancer Inst 1993;85:884–91.
36. Platz EA, Giovannucci E, Rimm EB, et al. Dietary fiber and distal colorectal adenoma in men. Cancer Epidemiol Biomarkers Prev 1997;6:661–70.
37. Terry P, Giovannucci E, Michels KB, et al. Fruits, vegetables, dietary fiber, and risk of colorectal cancer. J Natl Cancer Inst 2001;93:525–33.
38. Smith-Warner SA, Elmer PJ, Fosdick L, et al. Fruits, vegetables, and adenomatous polyps: the Minnesota Cancer Prevention Research Unit case-control study. Am J Epidemiol 2002; 155:1104–13.
39. Otani T, Iwasaki M, Yamamoto S, et al. Alcohol consumption, smoking, and subsequent risk of colorectal cancer in middle-aged and elderly Japanese men and women: Japan Public Health Center-based Prospective Study. Cancer Epidemiol Biomarkers Prev 2003;12:1492–500.
40. Andon MB, Peacock M, Kanerva RL, et al. Calcium absorption from apple and orange juice fortified with calcium citrate malate (CCM). J Am Coll Nutr 1996;15:313–16.
41. Hillman RS, Steinberg SE. The effects of alcohol on folate metabolism. Annu Rev Med 1982;33:345–54.
42. Slattery ML, Benson J, Berry TD, et al. Dietary sugar and colon cancer. Cancer Epidemiol Biomarkers Prev 1997;6:677–85.
43. Terry PD, Jain M, Miller AB, et al. Glycemic load, carbohydrate intake, and risk of colorectal cancer in women: a prospective study. J Natl Cancer Inst 2003;95:914–16.
44. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes Control 1991;2:325–57.
45. Kondo R. Epidemiological study on cancer of the colon and the rectum. (In Japanese). Nagoya Med J 1975;97:93–116.
46. Tajima K, Tominaga S. Dietary habits and gastro-intestinal cancers: a comparative case-control study of stomach and large intestinal cancers in Nagoya, Japan. Jpn J Cancer Res 1985;76: 705–16.
47. Hoshiyama Y, Sekine T, Sasaba T. A case-control study of colorectal cancer and its relation to diet, cigarettes, and alcohol consumption in Saitama Prefecture, Japan. Tohoku J Exp Med 1993;171:153–65.
48. Inoue M, Tajima K, Hirose K, et al. Subsite-specific risk factors for colorectal cancer: a hospital-based case-control study in Japan. Cancer Causes Control 1995;6:14–22.
49. Yang CX, Takezaki T, Hirose K, et al. Fish consumption and colorectal cancer: a case-reference study in Japan. Eur J Cancer Prev 2003;12:109–15.
50. Scanlan RA. Formation and occurrence of nitrosamines in food. Cancer Res 1983;43(suppl):2435s–40s.
51. Tricker AR, Preussmann R. Carcinogenic *N*-nitrosamines in the diet: occurrence, formation, mechanisms and carcinogenic potential. Mutat Res 1991;259:277–89.
52. Ohgaki H, Hasegawa H, Kato T, et al. Carcinogenicities in mice and rats of IQ, MeIQ, and MeIQx. Princess Takamatsu Symp 1985;16:97–105.
53. Sandhu MS, White IR, McPherson K. Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. Cancer Epidemiol Biomarkers Prev 2001;10:439–46.
54. Norat T, Lukanova A, Ferrari P, et al. Meat consumption and colorectal cancer risk: dose-response meta-analysis of epidemiologic studies. Int J Cancer 2002;98:241–56.
55. Yoon H, Benamouzig R, Little J, et al. Systematic review of epidemiological studies on meat, dairy products and egg consumption and risk of colorectal adenomas. Eur J Cancer Prev 2000;9:151–64.
56. Nagata C, Shimizu H, Kametani M, et al. Diet and colorectal adenoma in Japanese males and females. Dis Colon Rectum 2001;44:105–11.
57. Giovannucci E. Epidemiologic studies of folate and colorectal neoplasia: a review. J Nutr 2002;132(suppl):2350s–5s.
58. Kato I, Tominaga S, Matsuura A, et al. A comparative case-control study of colorectal cancer and adenoma. Jpn J Cancer Res 1990;81:1101–8.

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Concentrations of dioxins and related compounds in the blood of Fukuoka residents

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Abstract

Blood samples of 152 residents (male 75 and female 77) aged 20–60 years in Fukuoka, Japan, were analyzed for dioxin toxic compounds of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), non-*ortho* polychlorinated biphenyls (non-*ortho* PCBs) and mono-*ortho* polychlorinated biphenyls (mono-*ortho* PCBs) as well as 35 PCB congeners and 12 chlorinated pesticides by high-resolution gas chromatography/high-resolution mass spectrometry. Average concentrations of dioxin toxic equivalents (TEQ) from PCDDs, PCDFs, non-*ortho* PCBs, mono-*ortho* PCBs and their total in the blood of 152 residents were 10.28, 5.56, 7.75, 4.57 and 28.15 ppt in lipid, respectively. These total TEQ levels were comparable to the blood TEQ levels of normal Japanese ($n = 735$). Lipid basis total TEQ levels progressively increased in the males from 17.1 ppt at age 20s to 47.5 ppt at age 50s and in the females from 19.5 ppt at age 20s to 54.5 ppt at age 50s. Dioxin toxic contribution of PCBs to total TEQ was increased from 31% at age 20s to 55% at age 50s. Average concentrations of total 35 PCBs and total 12 pesticides in the blood of 151 residents were 386 and 1079 ppb in lipid, respectively. Average concentrations (ppb in lipid) of total PCBs and total pesticides also progressively increased in the males from 213 and 444 at age 20s to 868 and 2140 at age 50s, respectively, and in the females from 199 and 588 at age 20s to 550 and 1977 at age 50s, respectively. The levels of TEQ, PCBs, hexachlorobenzene, beta-hexachlorocyclohexane, dieldrin, *p,p'*-dichlorodiphenyldichloroethylene, *p,p'*-dichlorodiphenyltrichloroethane, *trans*-nonachlor and *cis*-nonachlor were positively correlated with each other in most of the combinations, and the highest correlations ($r > 0.8$) were observed between total TEQ and PCBs in both sexes.

Keywords: Human blood; Polychlorinated dibenzo-*p*-dioxins; Polychlorinated dibenzofurans; Polychlorinated biphenyls; Organochlorine pesticides; Concentrations and age

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1. Introduction

Dioxin toxic equivalents (TEQ) have been evaluated from polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and in some cases additionally from non-*ortho* polychlorinated biphenyls (PCBs) in the human blood of American (Patterson et al., 1994; Schecter and Piskac, 2001), German (Päpke, 1998; Wittsiepe et al., 2000), Japanese (Iida et al., 1999a,b; Watanabe et al., 2001), Spanish (Gonzalez et al., 1998), Belgian (Covaci et al., 2002) and other countries. However, mono-*ortho* PCBs have not been included in most of the TEQ evaluation. The World Health Organization (WHO) revised the Tolerable Daily Intake (TDI) to 1–4 pg/kg/day of TEQ consisting of PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs in 1998 (Brouwer et al., 1998). The Japanese government also revised TDI to 4 pg/kg/day of TEQ consisting of the same polychlorinated compounds as WHO in 1999 (Environment Agency, 1999a). Recent studies evaluated TEQs in Japanese blood from all the four groups of chlorinated compounds (Environment Agency, 1999b,c). German milk and blood have been analyzed for the TEQ of PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs in the same samples (Fürst and Päpke, 2002). For understanding the background levels of these chemicals in Japanese, blood samples were collected among residents in Fukuoka City and analyzed for not only TEQ related PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs but also 35 PCB congeners, hexachlorobenzene (HCB), *p,p'*-dichlorodiphenyltrichloroethane (DDT), *p,p'*-dichlorodiphenyldichloroethylene (DDE), *p,p'*-dichlorodiphenyldichloroethane (DDD), beta-hexachlorocyclohexane (β-HCH), dieldrin, *trans*-nonachlor (*t*-nonachlor), *cis*-nonachlor (*c*-nonachlor) and others. The blood samples were also examined for 20 clinical biochemical levels to investigate health effects that may be caused by the chlorinated compounds.

Yusho PCB poisoning occurred in Fukuoka and Nagasaki Prefecture in 1968, and some patients are still keeping body burden of dioxins and PCBs for more than 30 years and are suffering from various symptoms (Masuda, 1996). Data of this study will be helpful to understand the changes of the body burdens in the patients by comparing the blood levels of these chemicals in Yusho patients and the Fukuoka residents.

2. Materials and methods

2.1. Chemicals

Standard chemicals of the PCB congeners listed in Table 3, 10 PCDFs and 7 PCDDs were purchased from Analabs, USA, Chembridge Isotope Laboratories, USA or Wellington Laboratories, Canada. Carbon 13 labeled PCBs, PCDFs and PCDDs were from Chembridge Isotope Laboratories, USA. HCB, β-HCH, dieldrin, DDE, DDD, DDT, *t*-nonachlor, *c*-nonachlor, heptachlor epoxide, *t*-chlordane, *c*-chlordane and oxychlordane were from Wako Pure Chemical Ind. Ltd. Toluene, *n*-hexane, acetone, dichloromethane, ethanol, sodium sulfate, silica gel used were dioxin analysis grade.

2.2. Study subjects and blood sampling

A total of 280 men and women aged 20–59 years were randomly selected among residents in an area of Fukuoka City (Located in southwest of Japan, area 340 km^2, 1.315 million inhabitants, economic conditions: the primary 0.8%, the second 16.6% and the tertiary 80.3% industries), and 140 subjects were selected for each sex with 40 subjects in the age classes of 20s and 30s each and 30 subjects in the age classes of 40s and 50s. They were invited to participate in the study by at most three mails, and potential participants were further asked by telephone whether they were eligible on the basis of the criteria used for blood donation by the Japanese Red Cross. Finally 152 subjects (75 men and 77 women, no Yusho patients included) participated in the study. The participation rate was calculated as 58% after exclusion of those who were found to be ineligible ($n = 13$), those who had moved out ($n = 2$), and those with undelivered mail ($n = 5$). The survey was done in October and November in 1999.

Research nurses drew 100 ml of non-fasting venous blood at clinics or hospitals in the study area under physician's supervision. Blood of 90 ml was drawn by using nine glass tubes (10-ml size) treated with heparin; 70 ml were transferred to a glass vehicle provided by ERGO laboratory (Hamburg) and stored at −20 °C until shipment to the Laboratory, and 20 ml was sent to Daiichi College of Pharmaceutical Sciences on the day of blood sampling. The remaining 10 ml was taken into a plastic vacuum tube to separate serum, and the serum was frozen at minus 80 °C until biochemical measurement.

2.3. Analysis of PCDDs, PCDFs, non-ortho PCBs and mono-ortho PCBs

Blood sample, 70 ml, was added with the internal standards of 7 ^{13}C-PCDDs, 10 ^{13}C-PCDFs, 4 ^{13}C-non-*ortho* PCBs and 8 ^{13}C-mono-*ortho* PCBs and mixed with water/ethanol, eluted through silica phase column and extracted with hexane. The hexane solution was evaporated to dryness and remaining lipid was gravimetrically determined. The lipid material was redissolved in hexane

and passed through multi-layer column of sulfuric acid/silica gel, cesium silicate and potassium silicate. The eluate was concentrated and fractionated on a column of carbon on glass fiber. The column was eluted with hexane/dichloromethane for separating mono-*ortho* PCBs, and later reversely eluted with toluene for the fractions of PCDDs, PCDFs and non-*ortho* PCBs. Each fraction was determined for individual congeners by high-resolution gas chromatography/high-resolution mass spectrometer (HRGC/HRMS) in ERGO Laboratory (Masuda et al., 1998).

2.4. Analysis of PCBs and chlorinated pesticides

Blood sample, 10 ml, was extracted with acetone/hexane (2:1) and the extract was concentrated to dryness to determine the lipid weight. The lipid was dissolved in hexane/dichloromethane (1:1), added with internal standards of 12 ^{13}C-PCBs and fractionated on a gel permeation column of Bio-Beads S-X3. The fraction for PCBs and pesticides was analyzed for PCB congeners, HCB, DDT, DDE, DDD, β-HCH, dieldrin, *t*-nonachlor, *c*-nonachlor, heptachlor epoxide, *t*-chlordane, *c*-chlordane and oxychlordane by HRGC/HRMS (Hewlett Packard 5890J gas chromatograph-JEOL SX-102 mass spectrometer) (Mimura et al., 1999).

2.5. Measurement of clinical biochemicals

Frozen serum samples were sent to an external laboratory (SRL, Tokyo), en bloc for determination of clinical biochemicals including thyroxin binding globulin, triiodothyronine, free-triiodothyronine, thyroxine, free-thyroxine, thyroid-stimulating hormone, total cholesterol, triglyceride, creatinine, uric acid, amylase, gamma-glutamyl transpeptidase, cholinesterase, glutamine-oxaloacetic transaminase, glutamic-pyruvic transaminase, albumin, testosterone, free testosterone, luteinizing hormone, follicle-stimulating hormone.

2.6. Statistical analysis

All statistical analyses were carried out using statistical analyses software of StatFlex version 5, 2000, Artech, Osaka. If the levels of the pollutants are below detection limit, half values of the detection limit are used as their concentrations.

3. Results

For estimating the TEQ concentrations, half values of the detection limit were introduced to the spaces of nd (not detectable) and TEQ factors established by WHO in 1998 (Brouwer et al., 1998) were used for calculation. Table 1 displays the concentrations of TEQ from PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs in the blood of Fukuoka residents. All 152 samples were separated into four groups of female aged 20–39, female aged 40–60, male aged 20–39 and male aged 40–60, because the values in the older group were higher than those in the younger group in both sexes. Table 2 lists the TEQ concentrations in the blood of the four groups and their ratio of older/younger groups. Total TEQ concentrations in the older groups are about two times higher than those in the younger groups in both sexes. The TEQ concentration ratios are ranged from 1.2 to 4.0 in PCDD/PCDF congeners and from 1.9 to 3.9 in PCB congeners. Differences of TEQ concentrations between the older group and the younger group are greater in PCB congeners than in PCDD/PCDF congeners. Fig. 1 shows accumulated TEQ concentrations from PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs in the blood by sex and age-class. Total TEQ levels progressively increased with advancing ages 20s to 50s in both sexes. Percentage of the TEQ contributed from non-*ortho* PCBs to total TEQ increased from 18.1% to 35.3% in males and from 17.5% to 37.5% in females when age advanced from 20s to 50s. On the other hand, the TEQ contributed from PCDDs decreased from 46.7% to 28.3% in male and from 46.7% to 28.9% in female when the age changed from 20s to 50s.

Table 3 shows lipid basis concentrations of PCBs and chlorinated pesticides in the blood of 151 Fukuoka residents, data were missing with one female. Total concentrations of PCBs and chlorinated pesticides are 386 and 1079 ppb in lipid, respectively, and are 13 700 and 38 000 times higher than the TEQ concentration, respectively. Table 4 lists the concentrations of 35 PCB congeners and 12 chlorinated pesticides in the four divided groups of age and sex. Total concentrations of PCBs and pesticides of older females are 1.8 and 1.7 times higher than those of younger females, respectively and total concentrations of PCBs and pesticides of older males are 2.6 and 3.1 times higher than those of younger males, respectively. The concentration ratios of older/younger in males are greater than those in females. Concentrations of PCB and pesticide congeners in older groups are mostly higher than those of younger groups except those of PCB #101/90/89, *t*-chlordane and *c*-chlordane in females. Accumulated concentrations of PCBs and nine chlorinated pesticides in 151 residents are shown in Fig. 2. These concentrations also increased with advancing ages. Considerably elevated concentrations of β-HCH were observed at age 50–60 in both sexes.

As shown in Fig. 3, significant positive correlations were observed between TEQ level and age. Fig. 4

Table 1
Concentrations of TEQ from PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs in the blood of Fukuoka residents (total 152: female 77, male 75)

	TEF	Numbers detected	TEQ concentration[a] (pg/g lipid)				
			Mean	SD	Median	Min	Max
Age			36.68	11.83	35.07	20.03	60.03
Fat content (%)			0.34	0.06	0.33	0.22	0.78
2,3,7,8-Tetra-CDD	1	123	1.67	0.91	1.50	0.50	5.00
1,2,3,7,8-Penta-CDD	1	151	5.68	2.28	5.25	1.70	15.00
1,2,3,4,7,8-Hexa-CDD	0.1	120	0.30	0.17	0.26	0.05	0.93
1,2,3,6,7,8-Hexa-CDD	0.1	152	2.05	0.96	1.90	0.44	4.50
1,2,3,7,8,9-Hexa-CDD	0.1	135	0.34	0.19	0.30	0.05	1.00
1,2,3,4,6,7,8-Hepta-CDD	0.01	152	0.20	0.15	0.16	0.05	0.96
OCDD	0.0001	151	0.04	0.05	0.02	0.00	0.42
Total PCDDs TEQ		152	10.28	4.21	9.50	3.49	26.34
2,3,7,8-Tetra-CDF	0.1	21	0.10	0.08	0.10	0.05	0.57
1,2,3,7,8-Penta-CDF	0.05	20	0.03	0.04	0.03	0.01	0.44
2,3,4,7,8-Penta-CDF	0.5	152	4.13	2.22	3.68	1.10	13.00
1,2,3,4,7,8-Hexa-CDF	0.1	151	0.51	0.30	0.46	0.13	2.90
1,2,3,6,7,8-Hexa-CDF	0.1	151	0.43	0.20	0.40	0.19	1.80
1,2,3,7,8,9-Hexa-CDF	0.1	0	0.09	0.07	0.05	0.05	0.50
2,3,4,6,7,8-Hexa-CDF	0.1	109	0.21	0.16	0.18	0.05	1.40
1,2,3,4,6,7,8-Hepta-CDF	0.01	63	0.03	0.04	0.02	0.01	0.39
1,2,3,4,7,8,9-Hepta-CDF	0.01	10	0.01	0.01	0.01	0.01	0.05
OCDF	0.0001	2	0.00	0.00	0.00	0.00	0.00
Total PCDFs TEQ		152	5.56	2.77	5.10	1.82	20.66
3,3′,4,4′-Tetra-CB (#77)	0.0001	1	0.00	0.00	0.00	0.00	0.00
3,4,4′,5-Tetra-CB (#81)	0.0001	58	0.00	0.00	0.00	0.00	0.00
3,3′,4,4′,5-Penta-CB (#126)	0.1	131	7.49	7.71	4.75	0.50	43.00
3,3′,4,4′,5,5′-Hexa-CB (#169)	0.01	152	0.45	0.30	0.37	0.10	1.60
Total non-*ortho* PCBs TEQ		152	7.75	7.93	5.00	0.28	43.98
2,3,3′,4,4′-Penta-CB (#105)	0.0001	151	0.27	0.27	0.18	0.02	1.80
2,3,4,4′,5-Penta-CB (#114)	0.0005	152	0.40	0.33	0.31	0.05	1.90
2,3′,4,4′,5-Penta-CB (#118)	0.0001	151	1.08	0.96	0.77	0.14	6.00
2′,3,4,4′,5-Penta-CB (#123)	0.0001	82	0.03	0.04	0.02	0.00	0.26
2,3,3′,4,4′,5-Hexa-CB (#156)	0.0005	147	2.16	1.57	1.63	0.25	8.00
2,3,3′,4,4′,5′-Hexa-CB (#157)	0.0005	152	0.57	0.42	0.45	0.09	2.65
2,3′,4,4′,5,5′-Hexa-CB (#167)	0.00001	150	0.02	0.01	0.01	0.00	0.08
2,3,3′,4,4′,5,5′-Hepta-CB (#189)	0.0001	147	0.05	0.04	0.04	0.01	0.21
Total mono-*ortho* PCBs TEQ		152	4.57	3.46	3.61	0.75	18.70
Total TEQ		152	28.15	16.98	23.08	9.13	101.96

[a] Half value of detection limit was introduced to nd (not detectable).

illustrates correlations in pairs of major components of the PCDDs, PCDFs, PCBs and pesticides. They were positively correlated with each other and with age. High correlation coefficients were observed for 1,2,3,7,8-penta-CDD, 2,3,4,7,8-penta-CDF, and 2,3,3′,4,4′,5-hexa-CB, very toxic components, and 2,2′,4,4′,5,5′-hexa-CB, the most abundant component among PCBs retained in human.

Table 5 summarizes correlation coefficients between age and the levels of TEQ, PCBs, HCB, β-HCH, dieldrin, DDE, DDT, *t*-nonachlor and *c*-nonachlor in the blood of Fukuoka residents of male 75 and female 77 (or 76). Age was highly correlative with nine retainable chlorinated chemicals in males and females except with dieldrin and DDT in females. Significant positive correlations were recorded between the concentrations of nine

Table 2
Concentrations of TEQ from PCDDs, PCDFs, non-*ortho* PCBs, and mono-*ortho* PCBs in the blood of Fukuoka residents (female: upper line, male: lower line and boldface; age 20–39: left columns, age 40–60: right columns)

	Numbers detected	TEQ concentration[a] (ng/g lipid)					Numbers detected	TEQ concentration[a] (ng/g lipid)					Ratio older/younger
		Age 20–39						Age 40–60					
		Mean	SD	Median	Min	Max		Mean	SD	Median	Min	Max	
Age (females)	53	28.95	5.86	30.03	20.07	39.07	24	49.33	5.74	49.02	40.08	60.03	1.7
Age (males)	**42**	**28.37**	**5.89**	**26.02**	**20.03**	**39.09**	**33**	**50.49**	**5.48**	**50.07**	**40.03**	**60.02**	**1.8**
Fat content (%), females		0.32	0.04	0.32	0.23	0.45		0.34	0.04	0.33	0.27	0.42	1.1
Fat content (%), males		**0.34**	**0.06**	**0.33**	**0.22**	**0.59**		**0.38**	**0.09**	**0.36**	**0.27**	**0.78**	**1.1**
2,3,7,8-Tetra-CDD	41	1.42	0.68	1.40	0.50	3.10	24	2.46	1.13	2.35	1.10	5.00	1.7
	26	**1.22**	**0.68**	**1.00**	**0.50**	**3.50**	**32**	**2.08**	**0.78**	**2.00**	**0.50**	**3.90**	**1.7**
1,2,3,7,8-Penta-CDD	53	5.28	1.82	4.90	2.40	9.50	24	7.16	2.74	6.70	3.40	15.00	1.4
	41	**4.82**	**1.75**	**4.40**	**1.70**	**10.00**	**33**	**6.33**	**2.55**	**6.30**	**1.80**	**12.00**	**1.3**
1,2,3,4,7,8-Hexa-CDD	38	0.28	0.17	0.26	0.05	0.73	23	0.38	0.17	0.32	0.12	0.74	1.3
	26	**0.20**	**0.10**	**0.20**	**0.05**	**0.50**	**33**	**0.40**	**0.19**	**0.37**	**0.16**	**0.93**	**2.0**
1,2,3,6,7,8-Hexa-CDD	53	1.92	0.88	1.70	0.62	4.10	24	2.56	0.93	2.55	1.10	4.50	1.3
	42	**1.61**	**0.83**	**1.35**	**0.44**	**4.10**	**33**	**2.45**	**0.96**	**2.20**	**0.76**	**4.40**	**1.5**
1,2,3,7,8,9-Hexa-CDD	46	0.36	0.18	0.33	0.10	1.00	24	0.47	0.21	0.43	0.17	0.88	1.3
	32	**0.22**	**0.12**	**0.20**	**0.05**	**0.52**	**33**	**0.37**	**0.20**	**0.30**	**0.15**	**0.98**	**1.7**
1,2,3,4,6,7,8-Hepta-CDD	53	0.20	0.10	0.19	0.07	0.69	24	0.30	0.20	0.25	0.09	0.96	1.5
	42	**0.14**	**0.09**	**0.12**	**0.05**	**0.59**	**33**	**0.19**	**0.21**	**0.14**	**0.07**	**0.95**	**1.4**
OCDD	53	0.02	0.01	0.02	0.01	0.07	24	0.08	0.07	0.06	0.01	0.31	3.2
	41	**0.01**	**0.01**	**0.01**	**0.00**	**0.04**	**33**	**0.06**	**0.08**	**0.03**	**0.01**	**0.42**	**4.0**
Total PCDDs	53	9.5	3.3	9.1	4.4	17.8	24	13.4	5.0	12.7	6.2	26.3	1.4
	42	**8.2**	**2.9**	**7.2**	**3.7**	**17.5**	**33**	**11.9**	**4.6**	**11.6**	**3.5**	**23.4**	**1.4**
2,3,7,8-Tetra-CDF	1	0.07	0.04	0.05	0.05	0.20	7	0.13	0.13	0.08	0.05	0.57	[b]
	2	**0.11**	**0.07**	**0.10**	**0.05**	**0.45**	**11**	**0.11**	**0.08**	**0.10**	**0.05**	**0.35**	[b]
1,2,3,7,8-Penta-CDF	4	0.03	0.01	0.03	0.01	0.07	5	0.05	0.09	0.03	0.03	0.44	[b]
	1	**0.03**	**0.02**	**0.03**	**0.03**	**0.13**	**10**	**0.04**	**0.02**	**0.03**	**0.01**	**0.12**	[b]
2,3,4,7,8-Penta-CDF	53	3.4	1.5	3.1	1.2	7.5	24	6.2	2.8	5.1	2.3	13.0	1.8
	42	**2.9**	**1.2**	**2.5**	**1.1**	**6.5**	**33**	**5.4**	**2.2**	**5.0**	**1.5**	**11.0**	**1.9**
1,2,3,4,7,8-Hexa-CDF	53	0.48	0.20	0.46	0.18	1.30	24	0.75	0.54	0.61	0.26	2.90	1.6
	41	**0.40**	**0.18**	**0.35**	**0.13**	**1.00**	**33**	**0.55**	**0.20**	**0.50**	**0.28**	**1.00**	**1.4**
1,2,3,6,7,8-Hexa-CDF	53	0.46	0.20	0.43	0.21	1.00	24	0.55	0.32	0.44	0.27	1.80	1.2
	41	**0.33**	**0.11**	**0.30**	**0.19**	**0.66**	**33**	**0.44**	**0.15**	**0.45**	**0.22**	**0.81**	**1.3**
1,2,3,7,8,9-Hexa-CDF	0	0.11	0.09	0.10	0.05	0.40	0	0.08	0.04	0.08	0.05	0.15	[b]
	0	**0.09**	**0.08**	**0.10**	**0.05**	**0.50**	**0**	**0.07**	**0.04**	**0.05**	**0.05**	**0.20**	[b]

(*continued on next page*)

Table 2 (*continued*)

	Numbers detected	TEQ concentration[a] (ng/g lipid)					Numbers detected	TEQ concentration[a] (ng/g lipid)					Ratio older/younger
		Age 20–39						Age 40–60					
		Mean	SD	Median	Min	Max		Mean	SD	Median	Min	Max	
2,3,4,6,7,8-Hexa-CDF	41	0.20	0.10	0.20	0.05	0.46	16	0.24	0.28	0.20	0.05	1.40	[b]
	32	**0.25**	**0.18**	**0.22**	**0.05**	**1.00**	**20**	**0.15**	**0.09**	**0.15**	**0.05**	**0.38**	[b]
1,2,3,4,6,7,8-Hepta-CDF	31	0.04	0.03	0.04	0.02	0.14	5	0.04	0.08	0.02	0.02	0.39	[b]
	10	**0.03**	**0.03**	**0.02**	**0.01**	**0.20**	**17**	**0.03**	**0.02**	**0.02**	**0.01**	**0.07**	[b]
1,2,3,4,7,8,9-Hepta-CDF	7	0.01	0.01	0.01	0.01	0.05	0	0.01	0.00	0.01	0.01	0.02	[b]
	0	**0.01**	**0.01**	**0.01**	**0.01**	**0.05**	**3**	**0.01**	**0.01**	**0.01**	**0.01**	**0.03**	[b]
OCDF	2	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00	0.00	0.00	[b]
	0	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	[b]
Total PCDFs	53	4.8	1.9	4.3	1.9	9.7	24	8.0	4.0	6.7	3.0	20.7	1.7
	42	**4.1**	**1.7**	**3.5**	**1.8**	**10.2**	**33**	**6.7**	**2.5**	**6.3**	**2.3**	**13.6**	**1.6**
3,3,4,4′-Tetra-CB (#77)	0	0.00	0.00	0.00	0.00	0.00	1	0.00	0.00	0.00	0.00	0.00	[b]
	0	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	[b]
3,4,4′,5-Tetra-CB (#81)	14	0.00	0.00	0.00	0.00	0.00	19	0.00	0.00	0.00	0.00	0.00	[b]
	5	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**20**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	[b]
3,3′,4,4′,5-Penta-CB (#126)	43	4.5	2.8	4.0	1.5	11.0	24	13.6	10.6	9.2	2.1	43.0	3.0
	31	**4.1**	**3.5**	**3.4**	**0.5**	**18.0**	**33**	**11.8**	**9.5**	**9.0**	**1.7**	**41.0**	**2.9**
3,3′,4,4′,5,5′-Hexa-CB (#169)	53	0.30	0.16	0.24	0.10	0.83	24	0.57	0.22	0.53	0.21	0.98	1.9
	42	**0.31**	**0.14**	**0.30**	**0.11**	**0.61**	**33**	**0.78**	**0.37**	**0.79**	**0.20**	**1.60**	**2.5**
Total non-*ortho* PCBs	53	4.6	3.0	3.9	0.3	11.8	24	14.1	10.8	9.7	2.3	44.0	3.0
	42	**4.2**	**3.6**	**3.6**	**0.4**	**18.6**	**33**	**12.6**	**9.8**	**9.7**	**2.0**	**42.6**	**3.0**
2,3,3′,4,4′-Penta-CB (#105)	53	0.19	0.12	0.16	0.06	0.73	24	0.46	0.37	0.30	0.05	1.80	2.4
	41	**0.14**	**0.11**	**0.11**	**0.02**	**0.61**	**33**	**0.43**	**0.33**	**0.31**	**0.06**	**1.30**	**3.1**
2,3,4,4′,5-Penta-CB (#114)	53	0.29	0.18	0.25	0.08	0.95	24	0.66	0.40	0.53	0.23	1.90	2.3
	42	**0.20**	**0.15**	**0.15**	**0.05**	**0.80**	**33**	**0.65**	**0.37**	**0.55**	**0.10**	**1.30**	**3.2**
2,3′,4,4′,5-Penta-CB (#118)	53	0.77	0.49	0.66	0.27	3.00	24	1.77	1.21	1.40	0.32	6.00	2.3
	41	**0.56**	**0.41**	**0.43**	**0.14**	**2.10**	**33**	**1.70**	**1.18**	**1.30**	**0.22**	**4.30**	**3.0**
2′,3,4,4′,5-Penta-CB (#123)	21	0.02	0.01	0.01	0.00	0.07	19	0.04	0.04	0.03	0.01	0.14	2.4
	15	**0.01**	**0.02**	**0.01**	**0.00**	**0.11**	**27**	**0.06**	**0.05**	**0.06**	**0.00**	**0.26**	**3.9**
2,3,3′,4,4′,5-Hexa-CB (#156)	51	1.43	0.81	1.30	0.25	3.95	24	2.99	1.29	2.63	1.25	6.00	2.1
	39	**1.33**	**0.87**	**1.10**	**0.25**	**4.05**	**33**	**3.80**	**1.81**	**3.65**	**0.95**	**8.00**	**2.9**

2,3,3′,4,4′,5′-Hexa-CB (#157)	53	0.38	0.21	0.34	0.14	1.05	24	0.82	0.46	0.68	0.36	2.65	2.1
	42	**0.35**	**0.21**	**0.27**	**0.09**	**0.95**	**33**	**0.97**	**0.45**	**0.90**	**0.22**	**2.00**	**2.8**
2,3′,4,4′,5,5′-Hexa-CB (#167)	52	0.01	0.01	0.01	0.00	0.04	24	0.03	0.01	0.02	0.01	0.08	2.2
	41	**0.01**	**0.01**	**0.01**	**0.00**	**0.03**	**33**	**0.03**	**0.02**	**0.03**	**0.00**	**0.07**	**3.0**
2,3,3′,4,4′,5,5′-Hepta-CB (#189)	51	0.03	0.02	0.03	0.01	0.10	24	0.07	0.03	0.07	0.03	0.13	2.2
	39	**0.03**	**0.02**	**0.03**	**0.01**	**0.09**	**33**	**0.10**	**0.05**	**0.09**	**0.03**	**0.21**	**3.0**
Total mono-*ortho* PCBs	53	3.1	1.7	2.9	1.1	9.1	24	6.8	3.6	5.6	2.3	18.7	2.2
	42	**2.6**	**1.6**	**2.0**	**0.7**	**7.9**	**33**	**7.7**	**4.1**	**6.6**	**1.6**	**16.1**	**3.0**
Total TEQ	53	22.1	8.8	20.4	9.4	46.1	24	42.4	21.6	37.5	14.4	102.0	1.9
	42	**19.2**	**8.0**	**17.7**	**9.1**	**48.6**	**33**	**39.0**	**19.5**	**32.6**	**10.0**	**88.7**	**2.0**

[a] Half value of detection limit was introduced to nd (not detectable).
[b] The ratio was not shown because of many nds (not detectable) where half of the detection limits were introduced.

chlorinated compounds in both males and females with exception for DDE and dieldrin as well as for DDT and β-HCH in females. The highest correlation was between TEQ and PCBs in both sexes.

As the concentrations of TEQ, PCBs and pesticides were positively correlated with age in the blood of Fukuoka residents, these concentrations were naturally correlated with levels of biochemicals such as total cholesterol, triglyceride, thyroxin and others of which levels generally change with advancing age. These investigations will be reported elsewhere after careful examination of the levels and ages.

4. Discussion

Total TEQ concentrations in the blood of Fukuoka residents were comprised of non-*ortho* PCBs from 17% to 38%, mono-*ortho* PCBs from 14% to 20% and the both PCBs from 31% to 55% in different age groups from 20s to 50s. Non-*ortho* PCBs and mono-*ortho* PCB constituted 37% of total TEQ in Nose residents, 39% in Saitama residents, and 60% in the six area Japanese (Table 6). In German breast milk and blood, non-*ortho* PCBs and mono-*ortho* PCBs accounted for 51% and 52% of total TEQ, respectively (Fürst and Päpke, 2002). The PCBs in human blood contributed to about a half of total TEQ concentrations and were considered to be important factors for evaluating dioxin toxicity.

One of the important findings in this study is that the levels of TEQ, PCBs and some pesticides in the blood were positively correlated with age. The correlations between TEQ and age have been recently examined in Japan as shown in Table 6. Average TEQ level in the blood of Fukuoka residents was 28 ppt in lipid, and the value was similar to those reported in other three studies in Japan. The increment of TEQ per age, 0.95 ppt, in this study is higher than observed in other two studies, 0.35 and 0.37 ppt per age. Results from the study of Nose and Saitama residents could not be comparable, because the Nose study included the blood samples from a dioxin-polluted area. Time trend of TEQ body burden (lipid base TEQ concentration) has not been examined in Japan except the report of Japanese Environmental Agency (Environment Agency, 1999d). That is, the breast milk samples stored in the laboratory in Osaka from 1973 to 1995 were analyzed for PCDDs, PCDFs and coplanar PCBs. TEQ concentrations in fat base were steadily decreased with the year of sampling from 57 ppt in 1973 to 23 ppt in 1996. Supposing that the milk donors were about 30 years old at the time of milk sampling from 1973 to 1996, they are considered to have been from 56 to 33 of their age in 1999. As shown in Fig. 5, the TEQ levels in milk fat can be applied to the fat base TEQ level in the blood of 77

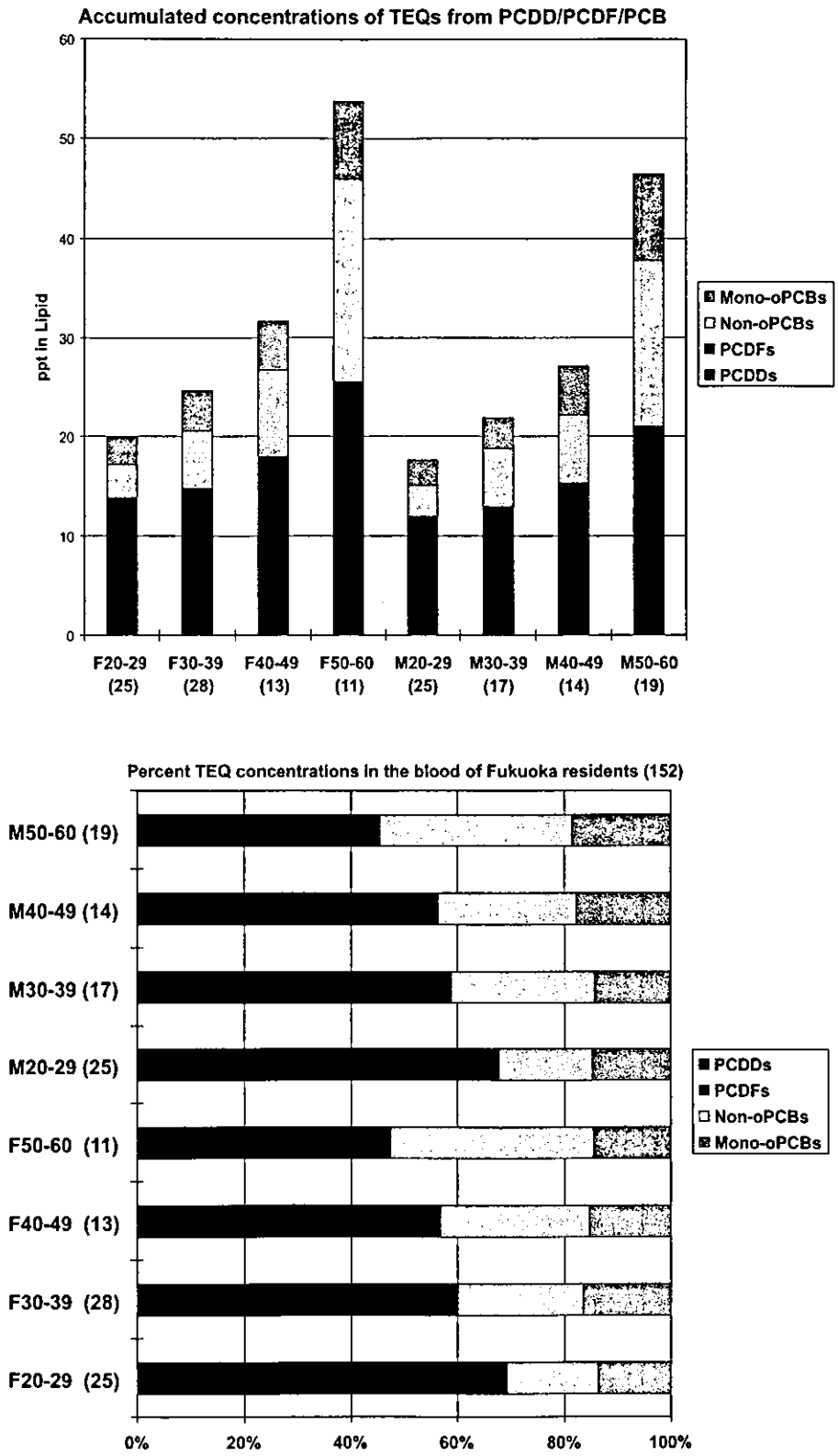

Fig. 1. Accumulated concentrations of TEQs from PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs (upper) and percent of TEQs from PCDDs, PCDFs, non-*ortho* PCBs and mono-*ortho* PCBs (lower) in the blood of 152 residents in Fukuoka. F: female, M: male, range of ages, and number in parentheses.

Table 3
Concentrations of PCB congeners and pesticides in the blood of Fukuoka residents (female 76, male 75)

	Numbers detected in 151	PCB concentration (ng/g lipid)				
		Mean	SD	Median	Min	Max
Age		36.6	11.8	35.1	20.0	60.0
Fat content (%)		0.19	0.04	0.19	0.10	0.29
2,4,4′-TriCB (#28)	151	2.6	2.0	2.0	0.2	11.7
2,2′,5,5′-/2,2′,3,6′-/2,3′,4,6-Tetra-CB (#52/46/69)	148	2.0	1.3	1.9	0.1	7.7
2,4,4′,5-/2,3,4,5-Tetra-CB (#74/61)	151	11.8	11.1	8.1	0.6	64.9
2,3′,4,4′-Tetra-CB (#66)	150	2.2	2.0	1.6	0.3	12.2
2,3,3′,4′-/2,3,4,4′-Tetra-CB (#56/60)	150	1.1	0.7	0.9	0.1	3.7
2,2′,4,5,5′-/2,2′,3,4′,5-/2,2′,3,4,6′-Penta-CB (#101/90/89)	151	5.2	2.5	4.5	1.8	14.0
2,2′,4,4′,5-Penta-CB (#99)	151	10.0	8.1	7.8	1.1	46.1
2,3′,4,4′,5-/2,3,3′,4,5-Penta-CB (#118/106)	151	19.6	15.8	14.6	3.5	81.9
2,3,4,4′,5-Penta-CB (#114)	150	1.3	1.0	1.0	0.1	6.8
2,3,3′,4,4′-/3,3′,4,5,5′-Penta-CB (#105/127)	150	4.4	3.6	3.2	0.5	20.6
2,2′,3,3′,5,5′-Hexa-CB (#133)	135	2.0	1.9	1.4	0.2	10.5
2,2′,3,4′,5,5′-Hexa-CB (#146)	151	9.2	7.5	6.8	1.5	45.4
2,2′,4,4′,5,5′-Hexa-CB (#153)	151	86.8	73.8	65.0	6.6	387.5
2,2′,3,4,4′,5-Hexa-CB (#137)	150	2.3	1.9	1.7	0.2	9.4
2,2′,3,3′,4,5′-Hexa-CB (#130)	151	3.0	1.8	2.6	0.6	10.9
2,3,3′,4′,5′,6-/2,3,3′,4′,5,6-Hexa-CB (#164/163)	151	12.9	9.9	9.9	2.1	48.9
2,2′,3,4,4′,5′-Hexa-CB (#138)	151	42.3	32.2	33.3	7.3	178.7
2,3,3′,4,4′,5-Hexa-CB (#156)	151	9.2	6.7	6.9	0.7	33.2
2,3,3′,4,4′,5′-Hexa-CB (#157)	151	4.2	5.4	3.1	0.6	62.6
2,2′,3,3′,5,5′,6-Hepta-CB (#178)	151	4.4	4.0	2.8	0.6	22.2
2,2′,3,4,4′,5,6′-/2,2′,3,4′,5,5′,6-Hepta-CB (#182/187)	151	21.4	16.6	17.0	3.1	87.8
2,2′,3,4,4′,5′,6-Hepta-CB (#183)	151	6.8	5.7	5.1	0.6	38.8
2,2′,3,3′,4′,5,6-Hepta-CB (#177)	148	4.5	3.7	3.4	0.6	19.9
2,2′,3,3′,4,4′,6-Hepta-CB (#171)	142	1.7	1.3	1.2	0.3	7.8
2,2′,3,3′,4,5,5′-Hepta-CB (#172)	146	2.2	1.9	1.6	0.3	10.9
2,2′,3,4,4′,5,5′-Hepta-CB (#180)	151	64.9	47.4	50.0	9.0	266.5
2,2′,3,3′,4,4′,5-Hepta-CB (#170)	151	20.0	14.2	16.0	3.0	73.9
2,3,3′,4,4′,5,6-Hepta-CB (#190)	149	1.9	1.3	1.5	0.2	7.1
2,3,3′,4,4′,5,5′-Hepta-CB (#189)	131	1.1	0.8	0.8	0.1	5.6
2,2′,3,3′,5,5′,6,6′-OctaCB (#202)	133	1.8	1.6	1.2	0.2	7.2
2,2′,3,3′,4,5,5′,6-OctaCB (#198)	151	6.2	5.1	4.7	0.6	28.0
2,2′,3,4,4′,5,5′,6-OctaCB (#203)	149	5.6	5.9	3.7	0.6	35.6
2,2′,3,3′,4,4′,5,6-OctaCB (#195)	130	1.8	1.4	1.3	0.2	6.9
2,2′,3,3′,4,4′,5,5′-OctaCB (#194)	151	9.0	7.5	6.3	0.7	40.2
2,2′,3,3′,4,4′,5,5′,6-NonaCB (#206)	138	1.8	1.3	1.4	0.3	6.2
Total PCBs	151	386	284	314	64	1433
HCB	151	23.1	15.5	21.6	1.2	84.7
β-BHC	140	564	742	280	59	4830
Heptachlor epoxide	138	5.6	4.3	4.3	0.7	21.7
Dieldrin	150	17.3	31.7	10.3	1.4	274.1
p,p′-DDE	148	364	248	312	40	1598
p,p′-DDD	151	9.4	7.2	7.3	1.0	39.6
p,p′-DDT	149	40	31	28	5	157
t-Chlordane	140	1.3	0.9	1.0	0.1	6.6
c-Chlordane	141	3.8	2.8	3.1	0.1	15.6
t-Nonachlor	151	73	74	50	9	566
c-Nonachlor	148	13.0	13.2	8.5	1.1	67.8
Oxychlordane	128	16.8	15.6	13.8	1.4	99.1
Total pesticides	151	1079	951	762	64	5755

Table 4
Concentrations of PCB congeners and pesticides in the blood of Fukuoka residents (female: upper line, male: lower line and boldface; age 20–39: left columns, age 40–60: right columns)

	Number detected	Concentration, ng/g lipid (age 20–39)					Number detected	Concentration, ng/g lipid (age 40–60)					Ratio older/younger
		Mean	SD	Median	Min	Max		Mean	SD	Median	Min	Max	
Age (female)	53	29.0	5.9	30.0	20.1	39.1	23	49.3	5.9	49.0	40.1	60.0	1.7
Age (male)	**42**	**28.4**	**5.9**	**26.0**	**20.0**	**39.1**	**33**	**50.5**	**5.5**	**50.1**	**40.0**	**60.0**	**1.8**
Fat content (%)		0.20	0.05	0.20	0.10	0.29		0.22	0.04	0.21	0.15	0.29	1.1
		0.17	**0.03**	**0.17**	**0.11**	**0.22**		**0.19**	**0.05**	**0.19**	**0.10**	**0.28**	**1.1**
PCB #28	53	2.1	1.3	1.9	0.2	7.6	23	3.5	3.0	2.5	0.8	11.7	1.6
	42	**1.9**	**1.3**	**1.7**	**0.2**	**5.9**	**33**	**3.4**	**2.3**	**2.6**	**0.5**	**8.9**	**1.7**
PCB #52, #46, #69	52	1.7	0.9	1.7	0.1	4.1	23	2.4	1.4	2.1	0.8	6.1	1.4
	41	**1.9**	**1.4**	**1.7**	**0.2**	**7.7**	**32**	**2.4**	**1.4**	**2.1**	**0.1**	**7.0**	**1.2**
PCB #74, #61	53	7.7	5.1	6.9	2.2	31.9	23	20.9	14.5	14.7	6.8	64.9	2.7
	42	**6.2**	**4.5**	**5.2**	**0.6**	**26.3**	**33**	**19.1**	**13.6**	**15.5**	**2.6**	**47.9**	**3.1**
PCB #66	53	1.6	0.9	1.4	0.5	4.9	23	3.0	2.3	1.9	0.9	9.7	1.9
	41	**1.5**	**1.0**	**1.1**	**0.3**	**4.1**	**33**	**3.5**	**3.0**	**2.3**	**0.5**	**12.2**	**2.3**
PCB #56, #60	52	1.0	0.5	0.9	0.2	2.3	23	1.5	0.8	1.3	0.5	3.3	1.5
	42	**0.8**	**0.4**	**0.8**	**0.1**	**1.9**	**33**	**1.5**	**0.9**	**1.2**	**0.3**	**3.7**	**1.8**
PCB #101, #90, #89	53	5.3	2.8	4.4	1.8	12.6	23	4.5	2.3	3.6	2.0	11.1	0.8
	42	**5.0**	**2.0**	**4.5**	**2.2**	**11.1**	**33**	**5.7**	**2.5**	**5.2**	**2.0**	**14.0**	**1.1**
PCB #99	53	7.0	4.2	6.2	1.8	25.0	23	11.1	6.1	10.6	2.3	22.9	1.6
	42	**7.3**	**4.4**	**6.3**	**1.1**	**20.3**	**33**	**17.6**	**11.9**	**13.4**	**3.0**	**46.1**	**2.4**
PCB #118, #106	53	14.5	8.9	12.8	3.7	53.6	23	26.4	16.2	20.4	7.0	81.9	1.8
	42	**12.9**	**9.7**	**9.7**	**3.5**	**58.7**	**33**	**31.7**	**21.2**	**23.7**	**4.5**	**74.6**	**2.5**
PCB #114	52	1.0	0.6	0.8	0.2	2.9	23	1.8	1.0	1.5	0.4	4.4	1.8
	42	**0.8**	**0.8**	**0.5**	**0.1**	**4.3**	**33**	**2.0**	**1.3**	**1.7**	**0.3**	**6.8**	**2.6**
PCB #105, #127	53	3.1	1.8	2.9	0.8	9.1	23	6.1	4.3	4.7	1.2	20.6	2.0
	41	**3.0**	**2.4**	**2.4**	**0.5**	**13.8**	**33**	**6.9**	**4.7**	**4.8**	**0.8**	**16.2**	**2.3**
PCB #133	51	1.1	0.7	0.9	0.2	3.4	17	2.3	1.3	2.0	0.5	4.7	2.1
	34	**1.5**	**0.8**	**1.2**	**0.4**	**3.3**	**33**	**3.9**	**2.6**	**3.5**	**0.6**	**10.5**	**2.7**
PCB #146	53	5.6	2.7	5.1	1.6	13.1	23	10.5	4.5	9.2	3.2	21.9	1.9
	42	**6.2**	**3.0**	**6.0**	**1.5**	**12.5**	**33**	**17.9**	**10.8**	**14.9**	**3.6**	**45.4**	**2.9**
PCB #153	53	52	25	50	14	136	23	97	47	77	42	209	1.8
	42	**60**	**32**	**53**	**7**	**123**	**33**	**170**	**107**	**138**	**27**	**387**	**2.8**
PCB #137	52	1.4	0.7	1.3	0.2	3.7	23	3.0	1.5	3.0	0.6	5.7	2.2
	42	**1.5**	**0.9**	**1.2**	**0.4**	**3.6**	**33**	**4.3**	**2.6**	**3.6**	**0.5**	**9.4**	**2.9**
PCB #130	53	2.4	1.2	2.1	1.0	7.0	23	2.8	1.4	2.8	0.6	6.4	1.2
	42	**2.4**	**1.1**	**2.2**	**0.9**	**6.1**	**33**	**4.8**	**2.3**	**4.7**	**1.2**	**10.9**	**2.0**
PCB #164, #163	53	8.4	4.9	6.6	2.1	23.1	23	14.6	6.6	14.6	3.0	30.4	1.7
	42	**8.9**	**5.1**	**7.9**	**2.3**	**23.8**	**33**	**24.0**	**13.2**	**19.6**	**5.1**	**48.9**	**2.7**

PCB #138	53	28	13	26	8	79	23	47	21	43	14	99	1.7
	42	**30**	**14**	**29**	**7**	**68**	**33**	**77**	**47**	**69**	**13**	**179**	**2.6**
PCB #156	53	6.0	3.2	5.5	1.2	15.1	23	11.0	4.4	11.1	4.7	20.0	1.8
	42	**6.7**	**4.7**	**5.5**	**0.7**	**22.9**	**33**	**16.5**	**8.2**	**13.9**	**3.6**	**33.2**	**2.5**
PCB #157	53	2.6	1.2	2.3	0.6	7.3	23	4.2	1.5	4.1	1.6	7.9	1.6
	42	**4.3**	**9.3**	**2.4**	**1.0**	**62.6**	**33**	**6.5**	**3.6**	**5.9**	**1.5**	**13.3**	**1.5**
PCB #178	53	2.5	1.8	2.1	0.6	7.5	23	5.0	2.5	4.7	1.0	9.7	2.0
	42	**2.8**	**1.8**	**2.3**	**0.7**	**10.0**	**33**	**8.9**	**5.5**	**7.2**	**1.4**	**22.2**	**3.2**
PCB #182, #187	53	14.0	7.9	11.5	3.1	41.0	23	24.8	13.6	21.2	7.9	58.5	1.8
	42	**14.7**	**7.9**	**12.7**	**4.9**	**34.5**	**33**	**39.6**	**21.8**	**37.2**	**6.2**	**87.8**	**2.7**
PCB #183	53	4.4	2.5	3.7	0.6	11.2	23	8.9	4.8	8.3	2.1	18.1	2.0
	42	**4.4**	**2.9**	**3.8**	**1.1**	**12.9**	**33**	**12.1**	**8.2**	**9.8**	**1.6**	**38.8**	**2.8**
PCB #177	53	2.8	1.7	2.3	0.6	8.8	22	5.2	3.1	4.3	1.1	12.7	1.9
	40	**2.9**	**2.0**	**2.3**	**0.6**	**9.1**	**33**	**8.5**	**4.7**	**7.8**	**1.7**	**19.9**	**3.0**
PCB #171	51	1.1	0.7	0.9	0.3	3.7	21	1.8	0.8	1.9	0.5	3.5	1.6
	38	**1.2**	**0.6**	**1.0**	**0.3**	**3.0**	**32**	**3.0**	**1.9**	**2.5**	**0.7**	**7.8**	**2.6**
PCB #172	52	1.4	1.1	1.0	0.3	5.9	23	2.7	1.3	2.6	0.4	5.5	1.9
	39	**1.4**	**1.1**	**1.1**	**0.4**	**4.9**	**32**	**4.3**	**2.5**	**4.1**	**1.0**	**10.9**	**3.1**
PCB #180	53	42	25	36	10	115	23	80	35	77	30	148	1.9
	42	**45**	**25**	**37**	**9**	**122**	**33**	**117**	**60**	**106**	**28**	**267**	**2.6**
PCB #170	53	13.1	7.0	12.3	3.0	36.9	23	24.8	10.5	22.0	5.4	43.1	1.9
	42	**14.4**	**7.5**	**13.4**	**4.4**	**32.0**	**33**	**34.8**	**18.8**	**30.0**	**7.7**	**73.9**	**2.4**
PCB #190	52	1.4	0.9	1.1	0.2	4.4	22	2.3	1.2	2.1	0.6	4.8	1.6
	42	**1.3**	**0.8**	**1.0**	**0.4**	**4.0**	**33**	**3.1**	**1.6**	**2.8**	**0.6**	**7.1**	**2.5**
PCB #189	50	0.8	0.4	0.7	0.2	1.9	18	1.5	1.2	1.4	0.5	5.6	2.0
	34	**0.9**	**0.5**	**0.8**	**0.2**	**2.3**	**29**	**1.7**	**1.0**	**1.6**	**0.1**	**4.6**	**1.9**
PCB #202	44	1.1	0.9	0.8	0.2	4.2	21	2.0	1.4	1.4	0.5	5.0	1.9
	36	**1.3**	**0.9**	**0.9**	**0.3**	**3.6**	**32**	**3.4**	**1.9**	**3.2**	**0.2**	**7.2**	**2.7**
PCB #198	53	4.0	2.8	3.3	0.6	11.9	23	7.4	4.2	6.4	1.2	16.7	1.8
	42	**4.2**	**2.9**	**3.4**	**0.8**	**11.5**	**33**	**11.4**	**6.6**	**10.5**	**2.0**	**28.0**	**2.7**
PCB #203	53	2.8	2.5	2.1	0.6	13.5	23	8.2	5.9	6.6	1.9	27.3	2.9
	40	**3.3**	**2.4**	**2.9**	**0.6**	**13.7**	**33**	**11.0**	**7.9**	**8.8**	**0.8**	**35.6**	**3.4**
PCB #195	48	1.2	0.9	0.9	0.2	4.0	20	2.1	1.3	1.9	0.3	5.8	1.8
	34	**1.1**	**0.9**	**0.8**	**0.2**	**3.8**	**28**	**3.2**	**1.7**	**3.3**	**0.6**	**6.9**	**2.8**
PCB #194	53	5.4	4.2	4.2	0.7	20.2	23	12.0	6.7	10.0	4.3	27.8	2.2
	42	**5.7**	**3.7**	**5.0**	**1.3**	**18.1**	**33**	**17.0**	**9.1**	**14.4**	**5.3**	**40.2**	**3.0**
PCB #206	49	1.2	0.7	1.0	0.3	3.3	20	2.4	1.4	2.1	0.4	5.2	2.0
	39	**1.2**	**0.7**	**1.1**	**0.3**	**3.3**	**30**	**3.0**	**1.6**	**2.5**	**1.0**	**6.2**	**2.4**
Total PCBs	53	252	120	223	69	628	23	462	196	421	169	1005	1.8
	42	**266**	**133**	**243**	**64**	**595**	**33**	**699**	**388**	**562**	**145**	**1433**	**2.6**

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