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Low-dose persistent organic pollutants increased telomere length in peripheral leukocytes of healthy Koreans

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Although shortened telomeres have been found in many cancers, elongated telomere length has been observed as an early response after low-dose treatment with various chemical carcinogens *in vitro* and animal experiments, suggesting low-dose exposure to carcinogenic chemicals may function as a tumour promoter at the very early stage of carcinogenesis in humans. This cross-sectional study was performed to examine whether low-dose exposure to persistent organic pollutants (POPs), lipophilic xenobiotics that mainly bioaccumulate in adipose tissue, is associated with telomere length of peripheral blood leukocytes in apparently healthy persons. Telomere length was measured using quantitative polymerase chain reaction method in 84 apparently healthy Koreans. Among various POPs, serum concentrations of organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs) and polybrominated diphenylethers were measured. Most OC pesticides and PCBs were positively and significantly associated with telomere length with correlation coefficients from about +0.25 to +0.35. The strongest associations were observed with *p,p′*-dichlorodiphenyldichloroethylene, PCB99, PCB153, PCB180, PCB183 and PCB187. When we examined adjusted means of telomere length by quintiles of POPs, the steeper increases of telomere length tended to be observed within relatively lower ranges of POPs. Besides serum concentrations of POPs, none of the other variables studied, including age, were associated with telomere length in this study. We found that telomere length was increasing across low doses of exposure to POPs in which the majority of study subjects were found, suggesting that low-dose POPs may act as a tumour promoter in carcinogenesis in humans.

Introduction

Persistent organic pollutants (POPs) are a heterogeneous group of synthetic chemicals sharing a number a common properties, including long-term persistence and diffusion in the environment and bioaccumulation through the food chain (1,2). Recent epidemiological studies in the US general population have reported that low-dose POPs at current exposure levels can be associated with various obesity-related diseases (3,4). POPs have been long suspected as chemicals that can increase the risk of various cancers even though associations between POPs and cancer have been inconsistent in human (5,6).

The telomere is a special functional complex at the end of linear eukaryotic chromosomes, consisting of tandem repeat DNA sequences and associated proteins (7). It is essential for maintaining the integrity and stability of linear eukaryotic genomes (7). Many studies show that the loss of these protective telomere caps leads to genomic instability associated with various age-related disorders including cancers (8).

Although aging has long been recognised as a source for telomere dysfunction and many genes involved in telomere length homeostasis have been identified (9–11), possible effects of environmental factors are just beginning to be understood (12). Twin studies reported that telomere length was largely associated with shared environmental factors rather than genetic factors (12). Among various environmental factors, chemical exposure has been little studied.

Until now, the most widely studied chemical is arsenic, a well-known human carcinogen. Interestingly, arsenic effects on telomere varied depending on exposure dose (13,14). For example, low concentrations of arsenic (0.0001 μM), which are the level most relevant to chronic human exposure, increased telomerase activity and elongated telomere length *in vitro*, while high concentrations of arsenic (1 μM) decreased telomerase activity and telomere length (13,14). In particular, elongated telomere length after exposure to low concentrations of arsenic was observed more clearly after the longer (7 days) exposure than shorter (1 day) exposure; in addition to the low dose, the long exposure would be the more similar to the human exposure pattern in the general environment. Also, low concentrations of arsenic increased *myc* and *ras* oncogenes. The oncogene *c-myc* has been shown to activate telomerase through a variety of sites (15). Therefore, low-dose exposure to arsenic may function as a tumour promoter in carcinogenesis in humans (14). Moreover, the increased telomere activity and the elongated telomere length have been also observed as a rapid response after treatment with various chemical carcinogens (3′-methyl-4-dimethyl-aminoazobenzene, 1-methyl-1-nitrosourethane, diethylnitrosamine, dimethylnitrosamine and aflatoxin B1) in rats (16).

To our best knowledge, there has so far been no study, which examined the association between POPs and telomere length *in vitro* as well as *in vivo*. However, changes in cellular function, such as activation of telomerase, activation of

oncogenes or suppression of suppressor genes after exposure to various chemicals, may be common fundamental features. Therefore, in the absence of specific studies on POPs, we hypothesised that experimental findings related to other chemicals including arsenic and the other chemicals listed above may be similarly observed in associations between POPs and telomere length. This study was performed to examine whether low-dose exposure to organochlorine (OC) pesticides, polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs) is associated with telomere length of peripheral blood leukocytes in an apparently healthy Korean population.

Materials and methods

Study subjects and survey method

A community-based health survey that was performed from June 2006 to December 2006 in Uljin County, South Korea. This county, a geographically small county with a population of 53 042, is located on the shore of the East Sea and the majority of the population is involved in fishery and agriculture. Residents aged ≥40 were passively invited to participate in the survey through a local newspaper and the boards of a community health service center and a local hospital. Study subjects were 90 apparently healthy adults who were randomly selected from 1007 participants as controls in two case–control studies on associations of POPs with diabetes or metabolic syndrome. As telomere length was not measured in six subjects due to lack of specimen, the final sample size was 84. This study was conducted with the approval from the Institutional Review Board at the Kyungpook National University Hospital.

Demographic information and lifestyle factors were determined for all participants by trained interviewers using a standardised questionnaire. A face-to-face interview method was used. Height and body weight were measured using standard methods in light clothes. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Systolic and diastolic blood pressures were measured in the sitting position after 5-min rest. Three blood pressure readings were obtained at 1-min intervals and were averaged.

Blood samples were obtained by venipuncture in the morning after overnight fasting. The serum samples were separated by centrifugation and transferred to contamination-free bottles with Teflon-coated caps. All samples were kept frozen at −70°C until analysis. Fasting glucose, triglycerides and high-density lipoprotein cholesterol were determined by enzymatic methods using ADVIA 1650 (Bayer Inc., Tarrytown, NY, USA).

POPs measurement

POPs were analysed in 2 ml of serum at the laboratory of the School of Environmental Science and Engineering, POSTECH (Pohang, Korea) using an isotope dilution method with gas chromatography–high-resolution mass spectrometry (GC–HRMS). Briefly, the serum samples were spiked with isotopically labelled standards of OC pesticides (ES-5349; Cambridge Isotope Laboratories, Andover, MA, USA), PCBs (P48M-ES; Wellington, Canada) and PBDEs (MBDE-MXFS; Wellington Laboratories Inc., Wellington, Ontario, Canada). Samples were then extracted on C18 solid-phase extraction (SPE) cartridges (Waters, Milford, MA, USA). The eluate was applied to a silica gel/florisil SPE cartridge (Waters) and then eluted with hexane followed by dichloromethane:hexane (1:1 v/v). Custom-made acid silica SPE cartridges were used for additional cleanup of PBDEs analysis. The extracts were evaporated using HyperVap (IEC, Seoul, Korea) and transferred to vials. The vials were stored at −70°C until analysis by GC–HRMS. GC–HRMS measurements were performed on a JMS-800D instrument (JEOL, Tokyo, Japan) interfaced with a 6890N gas chromatography (Agilent Technologies, Santa Clara, CA, USA). PBDEs were measured only among 50 study subjects.

Total lipids were calculated using the short formula (17): total lipids (mg/dl) = 2.27 × total cholesterol + triglycerides + 62.3. We used lipid-standardised concentrations of POPs by dividing wet concentrations of POPs by total lipids throughout the analyses. Among 22 OC pesticides, 34 PCBs and 8 PBDEs measured in this study, we selected 9 OC pesticides, 18 PCBs and 2 PBDEs for which ∼80% of study subjects had concentrations more than the limit of detection (LOD). Samples <LOD were given a half value of each LOD value.

Telomere length assessment

Genomic DNA was extracted from peripheral leukocytes using a Gentra Puregene Blood kit (QIAGEN, Valencia, CA, USA). Telomere length was measured using quantitative polymerase chain reaction (PCR) method as described previously (18,19). Briefly, the relative telomere length was determined by PCR through two steps of relative quantification. In the first step, the relative ratio of the telomere (T) repeat copy number to a single gene (S) copy number (T:S ratio) was established for each sample using standard curves. This ratio is proportional to the average telomere length. In the second step, the ratio of each sample was normalised to a reference DNA to standardise the differences between runs. The human β-globin gene was used as the single-copy gene. Telomere PCR and β-globin PCR were always conducted in 384 separate wells and each sample run in triplicate. The PCR primers for the telomeres and the human β-globin were as follows: Tel.1b, 5′-CGG TTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′; Tel.2b, 5′-GGCTTGCCTT ACCCTTACCCTTACCCTTACCCTTACCCT-3′; hbg1, 5′-GCTTCTGACACAACTGTG TTCACTAGC-3′ and hbg2, 5′-CACCAACTT CATCCACGTTCACC-3′. Primers were used at final concentrations of Tel.1b, 100 nM; Tel.2b, 900 nM; hbg1, 300 nM and hbg2, 700 nM. An aliquot of 3.75 ng (3 μl) of template DNA was added to each reaction, which contained 5 μl of SYBR Green PCR Master Mix (QuantiTect SYBR Green PCR; QIAGEN, Seoul, Korea) and 2 μl of a primer mixture. The DNA quantity standards were serial dilutions of a reference DNA sample (the same DNA sample for all runs) to produce five final concentrations (0.4, 0.8, 1.6, 3.2 and 6.4 ng/μl). In each run, a standard curve and a negative control (water) were included. The PCR was performed using a real time PCR instrument (LightCycler 480; Roche, Basel, Switzerland). The thermal cycling profile for the telomere amplification consisted of initial denaturation at 95°C for 10 min followed by 40 cycles of 95°C for 15 sec and 56°C for 1 min and the profile used for the β-globin amplification was 95°C for 10 min followed by 50 cycles of 95°C for 15 sec and 58°C for 1 min. Following amplification, a melting curve was created to confirm the specificity of the reaction. The $R2$ for each standard curve was >0.98. In addition, 10% of the samples were repeated on different plates to assess the T/S reproducibility. The Pearson and Spearman correlation coefficients were 0.89 ($P < 0.01$) and 0.87 ($P < 0.01$), respectively. The interbatch and intrabatch coefficients of variation were 7.5 and 1.7%, respectively.

Statistical analyses

Pearson correlation coefficients between log-transformed serum concentrations of lipid-standardised POPs with telomere length were calculated. We presented both crude and adjusted correlation coefficients. Possible confounders were age (years), sex, cigarette smoking (continuous) and BMI (continuous) and alcohol consumption (continuous). Crude and adjusted means of telomere length by quintiles of serum concentrations of POPs were examined by general linear models. As dose–response relationships were not clearly linear, we presented both P for trend and P for the quadratic term. The quadratic model allows the outcome curve to rise and then fall (as in an inverted U-shape). Also, despite the small sample size, we further categorised the last quintile into three groups (80 to <90%, 90 to <95%, ≥95%) to examine if the associations between POPs and telomere length within the range of relatively high dose of POPs were different from those within the range of low-dose POPs, similar with the experimental findings on arsenic (13,14). All data were analyzed using SAS version 9.1.

Results

Table I shows demographic and clinical characteristics of study subjects. The 84 participants comprised 33 (39.5%) men and 51 (60.5%) women. Mean age (range) was 56.2 years (42–69 years). None of the demographic and health behaviour variables was associated with telomere length (Table II).

Pearson correlation coefficients of various POPs with telomere length were displayed in Table III. In general, the associations between POPs and telomere length became stronger after adjustment for age, BMI, cigarette smoking and alcohol consumption. Among OC pesticides, *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) ($r = +0.31$), heptachlor epoxide ($r = +0.27$), oxychlordane ($r = +0.24$), and *trans*-nonachlor ($r = +0.23$)showed statistically significant correlation coefficients. Ten among 17 PCB congeners were positively and significantly associated with telomere length. The strongest associations were observed with PCB99 ($r = +0.34$), PCB153 ($r = +0.36$), PCB180 ($r = +0.33$), PCB183 ($r = +0.32$) and PCB187 ($r = +0.35$). However, PBDE47 and PBDE99 were not associated with telomere length. When the summary of all

Table I. General Characteristics of study subjects (n = 84)

Characteristics	
Mean ± standard deviation (range)	
Age (years)	56.2 ± 7.0
BMI (kg/m^2)	22.4 ± 2.5
Systolic blood pressure (mmHg)	116.2 ± 12.8
Diastolic blood pressure (mmHg)	71.4 ± 8.2
Fasting glucose (mg/dl)	88.5 ± 8.3
Triglycerides (mg/dl)	87.3 ± 39.0
HDL-cholesterol (mg/dl)	51.4 ± 10.3
Telomere length	2.02 ± 0.32
Percentages (%)	
Male	39.5
BMI ≥ 25 kg/m^2	15.1
Cigarette smoker	25.6
Alcohol drinker	46.5

HDL, high-density lipoprotein.

Table II. Spearman correlation coefficients of age and health behaviours with telomere length (n = 84)

Characteristics	Crude	Age-adjusted
Age	−0.11	—
Sex	+0.06	+0.07
BMI	+0.03	+0.02
Cigarette smoking	−0.07	−0.07
Alcohol drinking	+0.09	+0.06
Systolic blood pressure	−0.06	−0.07
Diastolic blood pressure	−0.05	−0.06
Fasting glucose	+0.11	+0.14
Triglycerides	−0.16	−0.15
HDL-cholesterol	+0.06	+0.06

HDL, high-density lipoprotein. $P > 0.05$ for all tabulated values.

POPs was made by summing each rank of serum concentrations of individual POP and examined the association with telomere length, the correlation coefficients was not stronger than those of individual POPs because some POPs were unassociated or were inversely associated with telomere length.

To further clarify the correlation analysis, we examined adjusted means of telomere length by quintiles of several selected POPs that showed significant associations in the Table III. Similar to the results based on correlation analyses, PCBs showed stronger associations than OC pesticides. However, the steeper increases of telomere length tended to be observed within relatively lower ranges of POPs rather than high-dose POPs. For POPs in the fifth quintile, there were plateaus or slight decreases in telomere length. When we further categorised the fifth quintile into three groups and examined associations with telomere length, there were patterns of decreasing telomere length among subjects in the highest fifth percentile of each POP (Table IV). Scatter plotting for two POPs that showed the strongest associations in the correlation analyses were presented in Figure 1. As there was one participant with very low serum concentrations of *p,p′*-DDE and two participants with very low serum concentrations of PCB153, we additionally presented linear plots after excluding these subjects. Even though correlation coefficients became weaker after excluding these subjects, they were still statistically significant. On the other hand, when we checked interactions of 27 individual POP with age, gender and BMI, none of them reached the level of statistical significance.

Table III. Pearson correlation coefficients of log-transformed serum concentrations of persistent organic pollutants with length of telomere

Compounds	Crude	Adjusted[a]
p,p′-DDE	+0.28*	+0.31**
p,p′-DDD	+0.12	+0.15
p,p′-DDT	−0.08	−0.09
Oxychlordane	+0.19	+0.24*
trans-nonachlor	+0.18	+0.23*
Heptachlor epoxide	+0.23*	+0.27*
β-Hexachlorocyclohexane	+0.16	+0.16
Hexachlorobenzene	+0.11	+0.13
Mirex	+0.03	+0.10
PCB74	+0.23*	+0.27*
PCB99	+0.28**	+0.34**
PCB105	+0.16	−0.17
PCB118	+0.23*	+0.26*
PCB138	+0.22*	+0.28*
PCB146	+0.15	+0.21
PCB153	+0.25*	+0.36**
PCB156	+0.09	+0.14
PCB157	+0.08	+0.15
PCB164	+0.18	+0.28*
PCB167	+0.11	+0.14
PCB172	+0.09	+0.18
PCB177	+0.14	+0.20
PCB178	+0.11	+0.24*
PCB180	+0.19	+0.33**
PCB183	+0.24*	+0.32**
PCB187	+0.24*	+0.35**
PBDE47	−0.09	−0.13
PBDE99	−0.04	−0.05

DDD, dichlorodiphenyldichloroethane; DDT, dichlorodiphenyltrichloroethane.
[a]Adjustment for age, sex, BMI, cigarette smoking and alcohol drinking.
*$P < 0.05$, **$P < 0.01$.

Discussion

Within the lower range of POPs in which the majority of study subjects were found, there were positive associations between serum concentrations of POPs and telomere length of peripheral blood leucocyte in apparently healthy Koreans. Among subclasses of POPs, PCBs were more strongly associated with telomere length than were OC pesticides. However, the positive association between POPs and telomere length disappeared in the relatively higher ranges of POPs in which ~5% of the study subjects were found. Besides serum concentrations of POPs, none of the other variables studied, including age, were associated with telomere length in this study, perhaps due to the small sample size, even though a very narrow age range of study subjects may partly contribute to the lack of age dependency in telomere length. Cardiovascular risk factors such as serum lipids or blood pressure were not associated with telomere length, either. Therefore, the statistically significant associations between POPs and telomere length themselves imply the importance of environmental factors such as POPs in determining telomere length compared with conventional risk factors in general population.

As leucocyte telomere length decreases over the adult life course, almost all epidemiological studies have been performed to investigate if short telomere length is associated with unhealthy health behaviours and known risk factors for various diseases or short telomere length at baseline predicts future risk of diseases or death (20–22). Also, shortened telomeres have been found in many cancers although telomerase is activated in most cancers to prevent the telomeres from getting even shorter (9). From this viewpoint, the positive associations between low-dose POPs and telomere length could be awkward to

Table IV. Unadjusted and adjusted[a] length of telomere according to categories of serum concentrations of several selected POPs

		Quintile of serum concentrations of several POPs							P_{trend}	$P_{quadratic}$
		Q1	Q2	Q3	Q4	Q5				
		0–19% (n = 16)	20–39% (n = 17)	40–59% (n = 17)	60–79% (n = 17)	80–89% (n = 9)	90–94% (n = 4)	95–100% (n = 4)		
p,p′-DDE	Conc. (ng/g of lipid)[b]	117	195	334	490	653	905	1244		
	Unadjusted	1.86	1.97	2.11	2.04	2.24	2.11	1.93	0.04	0.05
	Adjusted	1.85	1.95	2.09	2.04	2.29	2.18	1.96	0.02	0.09
Oxychlordane	Conc. (ng/g of lipid)[b]	1.7	5.1	7.3	10.4	13.9	17.0	22.8		
	Unadjusted	1.91	1.98	2.07	2.20	2.03	1.89	1.90	0.43	0.01
	Adjusted	1.88	1.95	2.06	2.23	2.07	1.91	1.90	0.22	<0.01
trans-nonachlor	Conc. (ng/g of lipid)[b]	7.0	11.4	17.4	24.0	35.4	43.33	74.2		
	Unadjusted	1.85	2.02	2.13	2.05	2.19	1.98	1.89	0.20	0.01
	Adjusted	1.84	1.97	2.12	2.09	2.20	1.97	1.99	0.06	0.02
Heptachlor epoxide	Conc. (ng/g of lipid)[b]	2.4	3.7	5.3	8.7	12.8	20.7	30.6		
	Unadjusted	1.88	2.12	1.95	2.03	2.06	2.30	2.08	0.13	0.91
	Adjusted	1.88	2.13	1.90	2.06	2.09	2.27	2.09	0.09	0.84
PCB74	Conc. (ng/g of lipid)[b]	2.8	4.8	6.5	8.9	11.1	12.6	17.2		
	Unadjusted	1.80	2.01	2.15	2.07	1.99	2.39	1.75	0.02	0.01
	Adjusted	1.78	1.98	2.14	2.11	2.03	2.43	1.72	0.01	<0.01
PCB99	Conc. (ng/g of lipid)[b]	3.1	5.6	8.2	12.3	16.6	20.0	29.9		
	Unadjusted	1.86	2.01	2.02	2.13	2.11	2.15	1.97	0.05	0.07
	Adjusted	1.78	2.02	2.00	2.14	2.18	2.30	2.00	<0.01	0.05
PCB153	Conc. (ng/g of lipid)[b]	17.5	32.7	45.7	66.7	89.9	111.3	201.3		
	Unadjusted	1.82	2.05	2.04	2.13	2.12	2.11	1.89	0.07	0.01
	Adjusted	1.75	2.02	2.02	2.15	2.22	2.14	2.01	<0.01	0.01
PCB187	Conc. (ng/g of lipid)[b]	2.9	6.0	8.9	13.6	20.6	27.6	43.8		
	Unadjusted	1.90	1.95	2.06	2.11	2.16	2.22	1.89	0.05	0.07
	Adjusted	1.80	1.96	2.06	2.11	2.25	2.23	2.03	<0.01	0.05

[a]Adjustment for age, sex, BMI, cigarette smoking and alcohol drinking.
[b]Conc.: median concentration of each category of specific POPs.

explain because serum concentrations of POPs were associated with increased risk of various diseases in general population (3,4). However, telomere length may have different implications depending on the stage of carcinogenesis. Telomeres generally shorten with age, so the occurrence of telomere lengthening should also be viewed as possibly adverse. Considering previous experimental studies on carcinogenic chemicals (16), telomere lengthening due to the exposure to carcinogenic chemicals such as POPs may be a very early phenomenon in carcinogenesis. In this sense, when our current findings are evaluated, it is critical to consider that our study was cross sectional and performed among healthy subjects and that our comments on relations to carcinogenesis are therefore speculations that should be pursued in other research.

Although there has been no previous experimental study on POPs, the increased telomere length within very low levels of POPs along with the deceleration of the increasing pattern of telomere length with high levels of POPs may mirror a phenomenon that was observed in experimental studies on arsenic, a well-known carcinogen (13). In fact, despite the small sample size, we categorised the last quintile into three groups to examine if the associations between POPs and telomere length within the range of relatively high dose of POPs were different from those within the range of low-dose POPs. As the ranges of the last quintile of POPs were very wide (for example, the range of last quintile of p.p′-DDE was 582–1501 ng/g lipid, while the whole range from first quintile to fourth quintile was 6.8–566 ng/g lipid), the further categorisation of the last quintile into several groups provides biological information about whether high-dose POPs showed different associations from those of low-dose POPs, like arsenic. Experimental studies on arsenic have reported increased telomerase activity and elongated telomere length when a very low dose of arsenic was used to treat HL-60 (promyelocytic leukaemia cells) and HaCa T cells (human epidermal keratinoctyes) *in vitro* (13). On the other hand, high-dose arsenic decreased telomerase activity and telomere length through generation of oxidative stress and induced apoptosis (13). As elongated telomere length promoted cellular proliferation, these experimental findings were interpreted as meaning that very low dose of arsenic may lead to tumour progression or tumorigenesis (13). The molecular mechanism for increased telomerase activity and telomere length after treatment using low-dose arsenic remain unclear, although up-regulation of heat shock proteins and some cell growth factors due to low-dose arsenic are possible mechanisms (23,24). Telomerase activation was also observed in very early stages of *in vitro* carcinogenesis with treatment with other chemical carcinogens (16,25). On the other hand, there can be other mechanisms that can affect telomere length, including increase

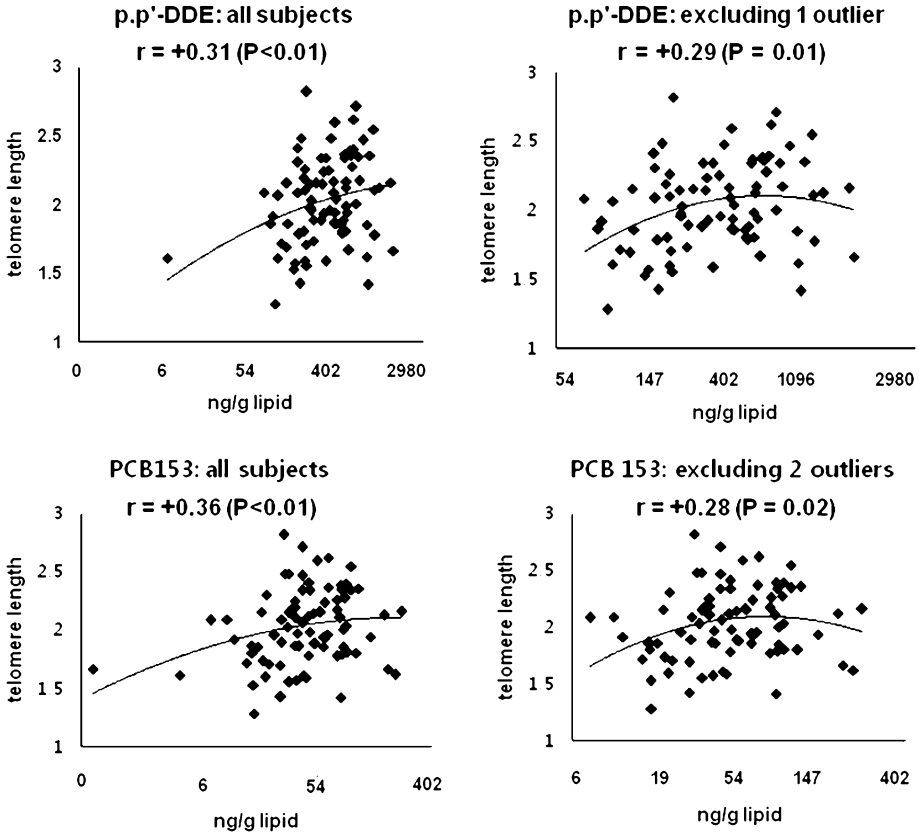

Fig. 1. Scatter plots of telomere length versus serum concentrations of *p,p′*-DDE and PCB153. Due to skewed distributions, log scale of serum concentrations was used for plotting. As there was one participant with very low serum concentrations of *p,p′*-DDE and two participants with very low serum concentrations of PCB153, we additionally presented scatter plots after excluding these subjects. Correlation coefficients were adjusted for age, sex, BMI, cigarette smoking and alcohol drinking.

in telomerase activity, decrease in proliferation rate (which could decrease telomere shortening rates due to cell division) and changes in white blood cell composition (26).

Therefore, the positive associations between low-dose POPs and telomere length observed in this study are worthy of note. Although there is no direct evidence based on experimental studies, our finding on the associations between POPs and telomere length may suggest a role for POPs as tumour promoters in carcinogenesis in humans. In fact, PCBs have been confirmed as tumour promoters in rodents after initiation with a genotoxic carcinogen even though technical PCB mixtures and individual congeners were inactive in most genotoxicity assays (27). Some OC pesticides have also been suspected as tumour initiators or promoters in experimental studies (28). Despite experimental evidence, however, most epidemiologic studies to date have not consistently supported an association exposure to POPs and human cancer (5,6). These inconsistent results may be related to low-dose effects of POPs in carcinogenesis. Our telomere findings imply that, if POPs are really involved in carcinogenesis as tumour promoters, low-dose POPs may be more important than high-dose POPs. Under this kind of dose–response relation, the failure to select a reference group with very low dose of POPs, excluding the lowest active doses of POPs, could lead to invalid estimates of association. Depending on POPs concentrations in the reference group and the gradient of risk within the reference group, estimates of association could vary from inverse to positive, depending on the reference group and the study. Invalid results may be most commonly observed in epidemiological studies with occupationally or accidentally high-exposure subjects because general populations with meaningful low dose of POPs tend to be used as the reference group in this kind of study. Our results about the association between background exposure to POPs and telomere length suggest that the issue of the association between POPs and cancers need to be revisited, focusing on low-dose effects.

As we measured telomere length in peripheral leukocytes, our findings may be also more relevant to hematopoietic cancers than cancers in other sites. In fact, serum concentrations of POPs have shown more consistent associations with hematopoietic cancers such as leukaemia or lymphoma than other cancers (29,30) despite the general inconsistency of the associations between POPs and cancer in human. Also, among subclasses of POPs, PCBs showed stronger associations with hematopoietic cancers than did OC pesticides (31,32), similar to the current findings of the stronger associations of PCBs with telomere length than OC pesticides.

This study has several limitations. First of all, this study is cross sectional and reverse causality is possible, although it is unlikely that telomere length affects serum concentrations of POPs. While we do not actually know that longer telomere lengths in the midrange of POPs exposures are the result of telomere lengthening, we have no alternative explanation for why the individuals in these exposure groups would have had longer telomeres throughout their lifetimes. Second, as the study size was small, some associations may be chance findings. Third, as people are simultaneously exposed to a variety of POP mixtures mainly through food, we cannot rule

out a possibility that other pollutants that are correlated with POPs may contribute to these findings. Also, it is difficult to point to specific POPs as causally contributing to the telomere length based only on epidemiological study. Finally, as our study subjects were the control group in case–control studies with outcomes of type 2 diabetes or metabolic syndrome, they were healthier than the entire population. However, as the study purpose was to enhance understanding of the association between POPs and telomere length among study subjects, the representative of the study sample is not a critical issue.

In conclusion, we found that low-dose exposure to POPs was associated with increased telomere length in peripheral leucocyte in apparently healthy Koreans. However, within POPs concentrations over certain levels, POPs were associated with decreased telomere length. All these findings suggest that POPs may alter telomere length in a complex way, and therefore long-term health effects of POPs may differ depending on doses of POPs.

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