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Inverse and Direct Effect of Serum DDE Exposure on the Distribution of Leukocyte Telomere Length in Brazilian Adults: The Pró-Saúde Study

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Background: The current literature on associations between organochlorine pesticides and leukocyte telomere length (LTL) is conflicted, showing positive, inverse, or no association, findings that might be related to methodological issues and population characteristics, including the baseline LTL. Alternative exploration of this relationship over the whole LTL distribution may add information to help understand the role of pesticides in telomere shortening or enlargement.

Objective: We evaluated the association between environmental dichlorodiphenyldichloroethylene (DDE) exposure and percentiles of LTL in a sample of adults living in the urban area of Rio de Janeiro, Brazil.

Methods: LTL, serum pesticide concentration, and the covariates were determined cross-sectionally in a sample of 471 adults from the Pró-Saúde Study, a cohort of civil servants at a university campus in Rio de Janeiro, Brazil, conducted from July 2012 to October 2013. The percentiles (5th to 95th) of LTL (outcome variable) were modeled using quantile regression (QR) models with DDE as exposure and adjusted for age, sex, educational level, total body fat mass, total serum lipids, smoking, alcohol intake, and caloric share of *in natura* and ultra-processed foods.

Results: Mean ± standard deviation (SD) LTL and serum DDE were 0.578 ± 0.158 telomere to single-copy gene ratio (T/S ratio) and 0.17 ± 0.34 ng/mL, respectively. Serum DDE was not detected in 44% of the samples. QR coefficients were positive and significant in the first percentiles (up to the 15th percentile) and inverse and significant at the 95th percentile. No significant association was observed between serum DDE and mean LTL ($\beta = -0.001$; $p = 0.93$).

Discussion: DDE exposure predicts some quantiles of LTL distribution, with a positive relationship in the first quantiles and inverse at the highest quantile. This study added new information to help understand the role of pesticides in telomere shortening or enlargement; however, given the few studies and the conflicting results, longitudinal investigations are needed to clarify this association. https://doi.org/10.1289/JHP1033

Introduction

Persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs) can be found in food and environmental samples.[1] OCP compounds had previously been used for agricultural and industrial purposes for a long time. Prohibition of these substances began in the 1970s, but until the 1990s, their trade was still permitted in some countries.[2] In Brazil, they were no longer permitted for use in agricultural applications in 1985 and banned from promotion as a vector control agent in public health campaigns in 1998[3]; the total ban took place in 2009.

The lipophilic nature of the OCPs led to their bioaccumulation in the food chain, and their presence is still detectable in serum samples.[4] Studies have shown that OCP exposure in humans is mainly through ingesting contaminated fatty food,[5] occupational activities, and environmental exposure.[6] The median half-life in adults has been estimated to be 8.6 y for dichlorodiphenyldichloroethylene (DDE) and 11.2 y for polychlorinated biphenyls (PCBs).[7] High levels of OCPs have been reported for various environmental matrixes, such as soil, water, sediment, atmosphere, and biological samples (e.g., human serum, breast milk, cord blood, placenta, and hair).[8]

Telomeres consist of repeated DNA sequences and associated proteins. They are found at the ends of chromosomes and protect the chromosomes against genome instability.[9] Telomeric sequences are gradually lost throughout cell division, therefore, telomere shortening is a continuous process across life.[10] Leukocyte telomere length (LTL), a cellular marker of aging, may predict an individual's health condition and is reported to be associated with various aging-related noncommunicable chronic diseases (NCDs), such as cancers and cardiovascular diseases (CVD).[11–13] Telomeres shorten with age; and this process might be accelerated by exposure to socioeconomic, environmental, and occupational factors that cause oxidative stress and chronic inflammation, such as dietary patterns,[14] education,[15] and POPs.[16] Progressive shortening of telomeres leads to incomplete cell division and incomplete DNA duplication.[10] Therefore, LTL may serve as an indicator of exposure to environmental and occupational chemicals, and LTL shortening may be an additional factor linking such chemicals with their related diseases.

Particularly concerning pesticide exposure (including POPs and OC compounds), most studies on occupational exposure have shown shorter telomere length in occupationally exposed groups,[17,18] but longer telomere length has also been observed in a few studies in exposed groups.[17,19] Positive and inverse associations have been shown in the same study for different compound exposures.[20] A fewer number of studies have evaluated this relationship in environmentally exposed individuals, with studies also showing positive, inverse, or no association[16,21–23] between serum pesticide concentration and telomere length. One study found that low-dose POP exposures were associated with longer LTL in apparently healthy Korean adults.[19] Compound-specific comparison is limited because of the variety of compounds assessed in different studies. For example, we identified only three studies assessing the relationship of DDE with LTL: *a*) Shin et al.[19] found a positive association at low DDE levels and a negative association at high levels; *b*) Karimi et al.[23] found an inverse association; and *c*) Guzzardi et al.,[22] the only longitudinal study, did not find an association between DDE and LTL.

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This inconsistency might be related to methodological issues and population characteristics, such as age, fat accumulation, exposure level, and other health conditions. Therefore, a variety of studies in different populations and countries (e.g., Latin America) using alternative methodologies are necessary to better explore the association of OCP and LTL. In this context, testing whether serum DDE predicts a range of percentiles of LTL may add information omitted by testing the prediction of mean LTL in a population. Looking at the tail percentiles of the LTL distribution may help to identify associated factors in the part of the population where the condition of interest is of low or high magnitude. Therefore, we aimed to evaluate the association between environmental DDE exposure and percentiles of LTL in a sample of adults living in the urban area of Rio de Janeiro, Brazil.

Methods

Overview

The Pró-Saúde Study is a prospective cohort study conducted in Rio de Janeiro, Brazil, on a university campus with civil servants as participants.[24] The cohort description and design have already been published. In summary, during four waves of data collection (1999, 2001–2002, 2006–2007, and 2012–2013), comprehensive self-administered questionnaires were given to cohort members ($N = 3{,}253$) to examine social determinants of health and health-related behaviors.[25] The protocol for this research study was approved by the Ethics in Research Committee of the Social Medicine Institute at the Rio de Janeiro State University. All participants provided written informed consent.

Study Population

A cross-sectional study was conducted using a subsample of the population assessed in Wave 4 of the Pró-Saúde Study (July 2012–October 2013) data collection. We randomly selected 520 participants from the baseline cohort stratified by sex (male and female), age (<50 vs. ≥50 y), and educational level (less than high school vs. high school or more). An additional data collection including nutritional and socioeconomic variables, body composition, and blood collection for biochemical and genetic analyses, which was performed in the subsample. In this study, we used data from 471 individuals who had blood samples collected and pesticide exposure assessed.

Blood Collection

Blood samples were collected by a trained professional following a 12-h overnight fast, using Vacutainer tubes (Becton, Dickinson & Company). The serum samples were then stored at –80°C. Plasma levels of leptin (Cat. #ZHL-80SK) and adiponectin (Cat. #EZHADP-61K) were measured using a semiautomated chemiluminescent enzyme-labeled immunometric assay (Liaison). The intra-assay coefficients of variation were <6%.

Outcome Assessment

The Qiagen blood kit (Puregene Blood Kit, Qiagen) was used to isolate DNA samples from blood. The next step involved spectrometry (BioDrop DUO, BioDrop) to assess the quality and concentration of DNA samples. Samples were stored at −80°C before analysis. A quantitative real-time polymerase chain reaction (qPCR) was performed to measure the LTL, using a modified method proposed by Cawthon et al.[26–28] We conducted the reactions in triplicate, which included *a*) genomic DNA (1.6 ng), 2 × Rotor-Gene SYBR Green, PCR Master Mix (Qiagen), primers Tel Forward (300 nM—CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT); *b*) Tel Reverse (300 nM—GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCTTACCCT) or primer single gene Hbg1 Forward (300 nM—GCTTCTGACACAACTGTGTTCACTAGC); and *c*) single gene Hbg2 Reverse (700 nM—CACCAACTTCATCCACGTTCACC) (Integrated DNA Technologies), in a 24-μL reaction. For the amplification, a Rotor-Gene Q Real-Time PCR cycler (Qiagen) was used with the following parameters: 95°C for 5 min, 25 cycles (telomere reaction) or 35 cycles (single gene reaction) at 98°C for 7s, and 60°C for 10s. Determination of telomere length was performed for each sample using the telomere to single-copy gene ratio (T/S ratio) based on the ΔCt method (Ct [telomere]/Ct [single gene]). Next, normalization to the mean T/S ratio was performed for each sample, taking the reference sample (r) as $[2^{-(\Delta Ctx - \Delta Ctr)} = 2 - \Delta\Delta Ct]$. The interassay coefficient of variation was <9% for all samples. A random subsample of 10% was drawn to evaluate the LTL to perform a qPCR, as described elsewhere,[29] using a proper commercial kit (Telo TAGGG Telomere Length Assay, Roche Applied Science).

Exposure Assessment

We conducted the chromatographic analyses as previously described.[28] Briefly, plasma samples were brought to room temperature, denatured, and diluted in a mixture of methanol and water in equal parts. Next, we used C18 solid-phase extraction cartridges (SPE; JT Baker) for sample extraction. Each cartridge was eluted with 7 mL of hexane and applied to a florisil SPE. A solution of petroleum ether/hexane (85:15) was used for the elution of the extracts and evaporated under a nitrogen atmosphere. Finally, we resuspended the extracts with 100 μL of hexane and analyzed them using gas chromatography–tandem mass spectrometry (GC-MS/MS) with 5 μL of 1,1′-biphenyl-4,4′dibromine at 1 μg/mL as the internal standard.

The GC-MS/MS analyses were performed on a Thermo Scientific model TSQ 8000 EVO Pesticide Analyzer. The system consisted of a Trace GC 1310 and an AS-1310 autosampler with a programmable temperature vaporization injector operating in splitless mode. It was controlled by Thermo Fisher Xcalibur and TraceFinder (version 5) software. We used a phenylmethyl siloxane column (Agilent DB-5MS; 30 m × 250 μm × 0.25 μm), along with ultrapure helium as the carrier gas set at a constant flow rate of 1 mL/min. The injector temperature was 280°C, and splitless injection occurred for 1 min. The flow was purged at 30 mL/min for 1.2 min, and 2 μL of the sample was injected. We programmed the GC oven temperature ramp to 50°C (2 min) at 10°C/min to 180°C (0 min) at 3°C/min to 230°C (0 min) at 5°C/min to 280°C (0 min), and at 15°C/min to 310°C (7 min), for a total of 50.68 min. The detector temperature was set at 300°C. The compounds were identified by using selected reaction monitoring (SRM) adjusted for retention time. The spectrometric conditions were set based on the National Institute of Standards and Technology (NIST) libraries in the Tracefinder software. However, in some cases, confirmations were performed using Thermo AutoSRM. The transitions were defined based on their high specificity and abundance.

Quality Assurance and Quality Control

The calibration curves were created using spiked plasma. To ensure accuracy, we validated several parameters including linearity, sensitivity, recovery, repeatability, limits of detection (LODs), and quantification (LOQs) based on the US Environmental Protection Agency (EPA) 8081B[29] method and the Brazilian System on Conformity Assessment (INMETRO) guidelines.[30] The calibration curves ranged from 0.2 to 15 ng/mL and showed good linearity for both OCP pesticides and PCBs. The correlation

coefficients varied from 0.936 to 0.994. Sensitivity was calculated using the curve slope. It varied from 111.655 to 212.082 for DDT and its isomers. Recoveries ranged from 93% to 105% for OCPs and from 87% to 107% for PCBs. LODs and LOQs were calculated from the standard deviation (SD) multiplied by factor *t*, and the LODs ranged from 0.015 to 0.468 ng/mL for OCPs and from 0.2 to 0.36 ng/mL for PCBs. The LOQs ranged from 0.045 to 1.419 ng/mL for OCPs and from 0.05 to 1.08 ng/mL for PCBs. Repeatability at the 2-ng/mL level ranged from 0.5% to 14.9% for within-day variability and from 0.8% to 8.6% for day-to-day variability.

We conducted thorough quality checks for each batch by testing previously spiked plasma to ensure accuracy. We assessed the injection and chromatographic batch conditions by adding an internal standard to all samples and controls. In addition, we performed solvent injections every 10 samples to check for the carryover effect and to ensure the cleanliness of the chromatographic system. For each positive sample, we extensively reviewed three selective reaction monitoring transitions to prevent false positive results. We obtained the relative abundances of the three selected precursor ion–product ion transitions from high purity standards and monitored each pesticide. Each batch included blank samples, quality control samples, and a short calibration curve in spiked plasma. Sample levels were calculated using external standardization through linear regression.

Total Body Fat Mass

Detailed descriptions of the procedures have been published elsewhere.[30,31] Participants' body composition was determined using dual-energy X-ray absorptiometry (DXA; Lunar iDXA, GE Healthcare). The enCore2008 software (version 12.20) was used to determine total fat mass based on whole-body fat analysis. Participants were instructed to wear lightweight clothing without any metal accessories. They were positioned in a dorsal recumbent position and asked to remain motionless throughout the procedure. All scans were administered by the same trained professional at the Interdisciplinary Laboratory for Nutritional Evaluation at the Institute of Nutrition, Rio de Janeiro State University. Daily measurements with the manufacturer-supplied calibration block and weekly measurements with the column calibration block demonstrated a coefficient of variation of <0.7%. Body fat mass was expressed in kilograms.

Alcohol and Dietary Intakes

A validated, retrospective, semiquantitative food frequency questionnaire (FFQ)[32] was used to collect dietary information over the previous 6 months. Energy intake was computed using the Brazilian Table of Food Compositions.[33] The 82 items listed in the FFQ were classified using the NOVA (this is not an acronym) classification according to the extent, nature, and purpose of the food processing into the following categories: *a*) *in natura* and culinary ingredients, *b*) processed foods, and *c*) ultra-processed foods.[34] The FFQ also asked about the frequency of beer, wine, and spirit consumption and the respective serving sizes. The amount of alcohol (in grams) was estimated using the alcoholic percentage of usual brands (5% for beers, 11% for wine, and 40% for spirits). The amount of alcohol intake (in milliliters) was calculated according to the percentage of alcohol in each beverage and then multiplied by the density of ethanol (0.8) to obtain the amount in grams.

Statistical Analysis

Descriptive statistics (mean, SD, and percentiles) were estimated for the LTL and serum DDE in relation to sociodemographic and behavioral variables. We modeled the percentiles (5th to 95th by intervals of 5) of the LTL distribution using quantile regression (QR) models. The exposure variable was the serum DDE concentration and the confounders were age (in years), sex, educational level (fundamental, high school, and higher education), total body fat mass (in kilograms), total serum lipids (in milligrams per deciliter), smoking (current, past, never), alcohol intake (in grams), and caloric share of *in natura* and ultra-processed foods. The confounders were selected based on the available variables and the literature on risk factors for NCD. Given that LTL has been linked to NCD risk,[13] we assumed that NCD predictors were also associated with LTL. Serum DDE concentrations were presented in nanograms per milliliter instead of nanograms per gram of total serum lipids to avoid eventual distortions[35] or overadjustment, given that the regression models were already adjusted for total fat mass. List-wise deletion was adopted for missing data (<5% of observations), leaving 456 valid observations for analysis.

With this modeling, a regression coefficient was estimated in each of the selected percentiles, representing a linear relationship between the exposure and the outcome at the selected percentile, adjusted for the covariates. The 95% confidence intervals (CIs) were calculated from standard errors estimated from 500 bootstrap replications. The coefficients for each percentile were plotted and considered statistically significant when their 95% CI did not cross the *x*-axis. In addition, the predicted LTL distribution at the 5th and 95th percentiles of pesticide exposure was plotted to show the effect of such change in the exposure over the LTL. We also tested whether serum DDE was associated with mean LTL using a linear regression model. For this analysis, the outcome was converted to the log scale to reach a Gaussian distribution, and the same covariates were included in the linear regression model as the QR. We additionally performed this analysis stratified by sex considering that differences for men vs. women have been previously reported.[22] Analyses were conducted in STATA software (v13, StataCorp) using command sqreg (simultaneous-quantile regression).

Results

Table 1 presents the characteristics of the DDE-detected and non–DDE-detected subgroups. A higher prevalence of DDE detection was observed in females (65% vs. 48% in males) and in current smokers (71% vs. 52% and 61% in never and past smokers, respectively). Serum DDE was not detected in 44% of the sample ($n = 205$). Age and total body fat were significantly higher in the DDE-detected subgroup. Mean LTL was correlated with sex, educational level, and age (Table 2).

The percentile distribution of LTL (as T/S ratio), serum DDE (in nanograms per milliliter), and total body fat mass (in kilograms) are shown in Table 3. The respective sample means ± SDs were 0.578 ± 0.15 T/S ratio, 0.17 ± 0.34 ng/mL, and 28.17 ± 9.85 kg. Dichlorodiphenyltrichloroethane (DDT), a compound that is converted to DDE in the body, was detected in only two individuals.

The QR coefficients across the percentiles of LTL are shown in Figure 1; Excel Table S1. There were positive and statistically significant associations between serum DDE and LTL in the first percentiles (up to the 15th percentile) and an inverse and significant association at the 95th percentile. No significant associations were observed between the 20th and 90th percentile of LTL. In general, there was a visual trend of decreasing the coefficients until they became inverse at the upper tail of the LTL distribution. Figure 2 and Excel Table S2 show the predicted percentiles of LTL at serum DDE = 0 and at serum DDE = 0.78; in both predictions, the covariates were set at their population means. Thus, this figure represents a hypothetical scenario of the effects of a change from 0 to 0.78 in serum DDE on LTL distribution

Table 1. Characteristics of the DDE-detected and non–DDE-detected participant subgroups, Pró-Saúde Study, Rio de Janeiro (Brazil), 2012–2013, $n = 471$ total.

Characteristic	Non-detected [$n = 205$ (44%)]	Detected [$n = 266$ (56%)]	Total	p-Value
Sex [n (%)]				<0.01
Male	117 (52)	106 (48)	223 (47)	
Female	88 (35)	160 (65)	248 (52)	
Educational level [n (%)]				0.68
Middle school	17 (38)	28 (62)	45 (10)	
High school	73 (43)	96 (57)	169 (36)	
College	113 (45)	140 (56)	253 (54)	
Missing	2	2	4	
Smoking [n (%)]				0.02
Current	15 (29)	36 (71)	51 (11)	
Never	135 (48)	145 (52)	280 (60)	
Past	54 (39)	84 (61)	138 (29)	
Missing	1	1	2	
Age (y) (mean ± SD)	50 ± 7.5	53 ± 7.9	51.7 ± 7.9	<0.01
Alcohol intake (g) (mean ± SD)	3.52 ± 9.15	2.70 ± 8.40	3.06 ± 8.74	0.31
In natura foods (%kcal)	58	59	58	0.91
Processed foods (%kcal)	13	13	13	0.82
Ultra-processed foods (%kcal)	27	26	27	0.33
Serum lipids (mg/dL) (mean ± SD)	687 ± 147	679 ± 162	683 ± 156	0.58
Total body fat (kg) (mean ± SD)	26.9 ± 9.9	29.1 ± 9.7	28.1 ± 9.8	0.01
Missing (n)	1	2	3	
LTL (T/S ratio) (mean ± SD)	0.562 ± 0.154	0.591 ± 0.159	0.578 ± 0.158	0.05
Missing (n)	2	4	6	

Note: Mean ± SD for the continuous and n (%) for the categorical variables. Chi-square test for the categorical variables, Student's t-test for the continuous variables. Food groups were defined according to the NOVA classification.[34] Missing values were not accounted for to calculate the percentage. %kcal, contribution (percentage) of the group to the total caloric intake; DDE, dichlorodiphenyldichloroethylene; LTL, leukocyte telomere length; SD, standard deviation; T/S ratio, telomere to single-copy gene ratio.

adjusted by the covariates. Among those with lower LTL, the higher the serum DDE exposure, the higher the LTL. On the other hand, among those with higher LTL, the higher the serum DDE, the lower the LTL. No association was observed when stratified by sex (Excel Table S3; Excel Figure S1). When using a linear regression model with the same covariates as used in the QR models, we did not find an association between serum DDE and mean LTL ($\beta = -0.001$; $p = 0.93$).

Discussion

We found that the association of serum DDE with LTL changed in direction and magnitude according to the percentile of LTL. The lowest LTL percentiles were directly associated with DDE. Although we identified an association only at the 95th percentile on the highest tail, there was an apparent trend to increase the strength of the association (in an inverse direction) with the increase of the percentiles.

The relationship between DDE or other pesticide exposure and LTL was unclear. Few studies assessed whether exposure predicts LTL, and the results have been inconsistent. Two cross-sectional studies held in different populations found opposite results. Karimi et al.[23] investigated 300 adults 25–40 years of age in Tehran, Iran, from October 2016 and December 2017. Using a multivariate linear regression model adjusted for age, education, body mass index, smoking, and dietary patterns, they found an inverse relationship between serum DDE and LTL. Of note, the authors observed a mean DDT level of 18.5 ng/g of lipids, whereas in our study DDT was detected in only two individuals. This compound has a short lifespan in the body once it is converted to DDE, but it is expected to be stored for many years (median half-life estimated as 8.6 y[7]). Because DDT has been banned from use in Brazil since 2009, it is surprising that DDT was detected in anyone. In the case of the study by Karimi et al., these results imply a recent, and perhaps continuous exposure to DDT, with consequent conversion to DDE in the body. To what extent this influences the association of DDE and LTL is unknown. The study by Shin et al.[19] investigated 84 apparently healthy South Koreans ≥40 years of age. They found a positive correlation between DDE and LTL adjusted by age, sex, smoking, body mass index, and alcohol consumption. In that study, the mean LTL was 2.02 T/S, much higher than we observed in Brazil (0.57 T/S) and in the study in Tehran (1.13 T/S). Considering that LTL might be an indicator of environmental and occupational chemical exposure and that LTL shortening might be an additional factor that links such chemicals to their related chronic

Table 2. Mean of leukocyte telomere length according to population variables, among participants of the Pró-Saúde Study, Rio de Janeiro (Brazil), 2012–2013, $n = 471$ total.

Characteristic	Telomere length (mean ± SD)	p-Value
Sex		0.01
Male	0.55 ± 0.15	
Female	0.59 ± 0.15	
Educational level		0.02
Middle school	0.53 ± 0.15	
High school	0.60 ± 0.15	
College	0.57 ± 0.15	
Smoking		0.15
Current	0.57 ± 0.17	
Never	0.59 ± 0.15	
Past	0.55 ± 0.16	

Note: SD, standard deviation.

Table 3. Percentiles of leukocyte telomere length (LTL), serum DDE, and total body fat in a sample of civil servants, Pró-Saúde Study, Rio de Janeiro (Brazil), 2012–2013, $n = 471$ total.

Percentile	LTL (T/S ratio)	Serum DDE (ng/mL)	Total body fat mass (kg)
5th	0.36	0	14.3
10th	0.4	0	16.4
25th	0.46	0	27.7
50th	0.55	0.07	27.3
75th	0.68	0.18	32.9
90th	0.81	0.47	41.6
95th	0.88	0.78	46.4

Note: DDE, dichlorodiphenyldichloroethylene; LTL, leukocyte telomere length; T/S ratio, telomere to single-copy gene ratio.

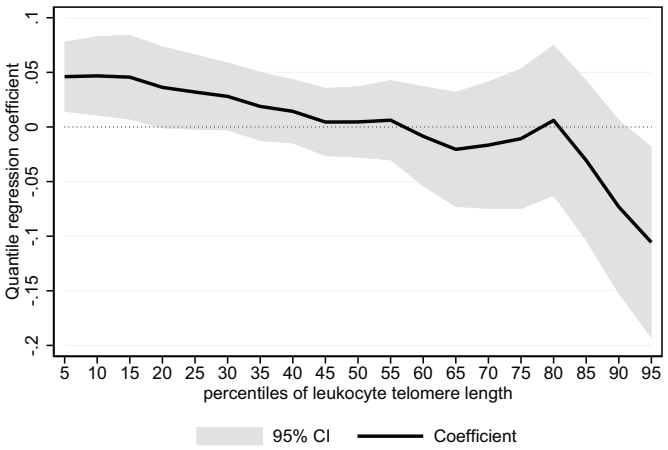

Figure 1. Quantile regression coefficients for the association between serum DDE and LTL, Pró-Saúde Study, Rio de Janeiro (Brazil), 2012–2013, $n = 456$. The model was adjusted for age, education, sex, total body fat mass, total serum lipids, smoking, alcohol intake, and caloric share of *in natura* and ultra-processed foods. Numerical data used to generate the figure are provided in Excel Table S1. Note: DDE, dichlorodiphenyldichloroethylene; LTL, leukocyte telomere length.

diseases, environmental exposure or health conditions in the Korean study population seem to be meaningfully distinct from ours and the study in Tehran, making it difficult to compare and draw any conclusion.

We identified one longitudinal study, which was based on the Helsinki Birth Cohort Study.[22] The authors tested serum DDE, measured in 2003–2004 as predictors of telomere shortening 10 y later in 1,082 individuals born between 1934 and 1944. They did not identify a statistical association between DDE and LTL. That study seems to provide the best evidence so far for this association. Despite being a longitudinal study, the individuals under investigation had been exposed to POPs, including DDE, for several years before the baseline POPs measurement, which included the peak emissions reached progressively after World War II until the late 1970s to late 1980s. The potential effect of DDE on LTL might have happened before 2003, when individuals had accumulated more than 30–40 y of environmental exposure. However, according to the authors, the observed concentrations of POPs were

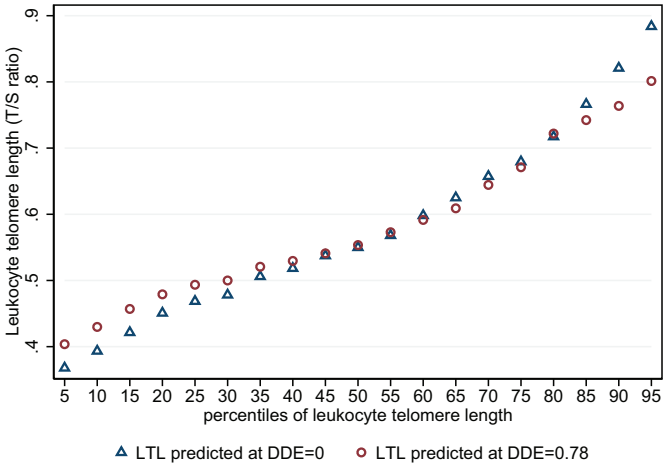

Figure 2. Predicted percentiles of LTL conditioned to the DDE exposure and other covariates, Pró-Saúde Study, Rio de Janeiro (Brazil), 2012–2013, $n = 456$. Numerical data used to generate the figure are provided in Excel Table S2. Note: DDE, dichlorodiphenyldichloroethylene; LTL, leukocyte telomere length; T/S ratio, telomere to single-copy gene ratio.

similar to, or not very different from, concentrations found in surveys conducted in some European countries and in the United States, including in younger people and teenagers.[36,37] The absence of a correlation between serum DDE and LTL, despite the long half-life of DDE, is consistent with studies on DNA integrity in human sperm,[38,39] which shows that PCB 153, but not DDE, might enhance DNA fragmentation. However, controversial results are also observed for PCB and LTL congeners association.[21–23,40] Unfortunately, we did not examine other POPs, such as PCBs.

We cannot affirm that our study has meaningful methodologic differences from the others. Despite having a distinct population setting, which may imply distinct exposure time and individuals' characteristics concerning chronic disease risks, the studies performed adjustments for confounding, used proper assessment of blood samples, and included both sexes and older adults. In fact, from our multivariate linear regression model, we conclude that DDE exposure does not predict the mean LTL, as also concluded the authors in two of the three studies mentioned above. Thus, our study cannot overcome the limitations of the previous studies, nor can it clarify the inconsistencies among them. In fact, our study looked at this question from a different perspective, providing alternative answers not addressed so far. The QR estimates the conditional quantiles of the outcome variable distribution. In other words, a QR coefficient expresses how much a particular quantile of the outcome distribution shifts by a 1-unit increase in the predictor (exposure) variable. Explanatory variables of interest may not have an association only with the outcome mean but also with lower or upper parts of the outcome distribution. In this sense, when modeling only the mean, as is done in linear regression, important information on the exposure–outcome association may be missed.[41] Special applications include when the associations of explanatory variables with the extreme values of an outcome distribution are of particular interest. In this sense, the hypothesis tested here is that, although there might be a weak or null association of DDE with the mean LTL, an association of DDE on other parts of the LTL distribution may exist. In addition, it can be that the relationship with the mean is not reproducible in the same magnitude and direction throughout the percentiles.

We identified, perhaps for the first time, that the association between serum DDE and LTL may have opposite directions according to the quantile of LTL. Specifically, the lowest LTL percentiles (up to p15) are higher in individuals with higher serum DDE compared with those with lower DDE concentrations. On the other hand, in the highest percentile evaluated (p95), LTL is lower in individuals with higher serum DDE. This finding suggests that people may be affected in different ways by DDE exposure.

The limitations of this study are typical of limitations in similar studies. The cross-sectional design does not allow us to infer temporality, that is, we do not know if the telomere shortening or enlargement happened before or after the metabolic consequences of DDE exposure. Sample size impacts the detection of statistically significant associations. If we had a much larger sample size, detecting an association in the quantiles where it was not detected (in the 20th to 90th percentiles) would be more likely. As a consequence, DDE exposure would be a predictor for several quantiles of LTL distribution; how many of these quantiles would have a statistically direct or statistically inverse association with DDE exposure is unknown.

In conclusion, we identified associations between DDE and some quantiles of LTL distribution, with a direct relationship in the first quantiles and inverse relationship at the highest quantile. We did not find an association of serum DDE with mean LTL. This study added new information to help understand the role of pesticides in telomere shortening or enlargement; however, given

the few studies and the conflicting results, further investigations are needed to clarify this association.

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The datasets analyzed in the present study are not yet publicly available but can be obtained upon reasonable request from the corresponding author.

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