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Holocaust history is not reflected in telomere homeostasis in survivors and their offspring

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ABSTRACT

Telomeres, nucleoprotein structures at the ends of eukaryotic chromosomes, are crucial for the maintenance of genome integrity. While the lengths of telomeres at birth are determined genetically, many factors including environmental and living conditions affect the telomere lengths during a lifespan. In this context, extreme and long-term stress has been shown to negatively impact telomeres and their protective function, with even offspring being influenced by the stress experienced by parents. Using quantitative PCR, the relative lengths of telomeres of survivors of the Holocaust during World War II and two generations of their offspring were analyzed. These data were related to those of control groups, persons of comparable age without a strong life stress experience. In contrast to previous studies of other stress-exposed groups, the relative lengths of telomeres were comparable in groups of persons exposed to Holocaust-related stress and their progenies, and in control groups. Interestingly, shorter telomeres of Holocaust survivors of the age under 12 in the year 1945 compared to Holocaust survivors of the age above 12 were detected. Our results are discussed with respect to certain exceptionality of persons having been able to cope with an extreme stress more than 70 years ago and living to a very old age.

1. Introduction

Telomeres are nucleoprotein structures delimiting the ends of eukaryotic chromosomes. In humans, telomeres consist of short tandem repeats of the hexamer 5′- TTAGGG – 3′ (Moyzis et al., 1988) of lengths within 5–20 kb (Moyzis et al., 1988). Telomeres distinguish natural chromosome ends from double strand DNA breaks and protect coding parts of the genome from loss due to incomplete replication of chromosome ends, which is related to the inability of DNA polymerase to complete the replication of DNA lagging strands. Thus, telomeres are shortened during each replication (Olovnikov, 1973), and shortening to or below a critical length represents a mark of cellular aging leading to senescence or cell death.

Telomeres within a single cell are heterogeneous in length (Lansdorp et al., 1996), with the shortest telomere determining the prospective initiation of cellular senescence (Hemann et al., 2001; Herbig et al., 2004; Zou et al., 2004; Fumagalli et al., 2012). The rate of telomere shortening differs with age; in humans, telomeres are most rapidly shortened during the first four years of life (Rufer et al., 1999). The telomere length at birth is determined mostly genetically, but also other factors such as environmental conditions, gender and maternal stress during pregnancy may play a role (Slagboom et al., 1994; Martens et al., 2017; Send et al., 2017). Nevertheless, during life, many environmental, social and lifestyle factors including obesity, cigarette smoking, low socioeconomic status and stress may affect telomere lengths as reported in numerous studies (e.g., Robertson et al., 2013; Zannolli et al., 2008; Zhang et al., 2016), though contradictory data have also been presented (Muzzinler et al., 2015; Weischer et al., 2014). Exposure to severe and/or long-lasting stress conditions was shown to be associated with symptoms of premature aging including shorter

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Table 1
Telomere length and characteristics of all participants stratified by stress (Holocaust and its consequences vs. controls).

Parameter	Total (N = 285)	Stress (N = 174)	Control (N = 111)	p-value[a]
Age (years) (min-max)	59 (16–95)	59 (16–95)	59 (16–88)	0.58 MW test
T/S ratio (untransformed)	0.97 (0.07–7.55)	0.92 (0.13–7.55)	1.09 (0.07–6.26)	0.31 MW test
T/S ratio (natural log-transformed)	−0.0055 ± 0.73	−0.020 ± 0.72	0.017 ± 0.75	0.68 Welch *t*-test
G1, n (%)	67 (23.5%)	39 (22.4%)	28 (25.2%)	
Age (years) (min-max)	80 (73–95)	84 (74–95)	79.5 (73–88)	**0.002 MW test**
T/S ratio (untransformed)	0.96 (0.07–5.53)	0.99 (0.32–5.53)	0.88 (0.07–4.91)	0.60 MW test
G2, n (%)	123 (43.2%)	77 (44.3%)	46 (41.4%)	
Age (years) (min-max)	64 (30–73)	64 (30–73)	64 (32–73)	0.89 MW test
T/S ratio (untransformed)	0.86 (0.13–7.55)	0.83 (0.13–7.55)	0.88 (0.17–4.51)	0.90 MW test
G3, n (%)	95 (33.3%)	58 (33.3%)	37 (33.3%)	
Age (years) (min-max)	34 (16–48)	35.5 (16–48)	33 (16–47)	0.11 MW test
T/S ratio (untransformed)	1.18 (0.16–7.47)	1.03 (0.16–7.47)	1.46 (0.39–6.26)	0.069 MW test
Women, n (%)	**176 (61.8%)**	**109 (62.6%)**	**67 (60.4%)**	
Age (years) (min-max)	61.5 (16–95)	59 (16–95)	65 (16–88)	0.74 MW test
T/S ratio (untransformed)	0.82 (0.07–4.91)	0.82 (0.15–4.12)	0.85 (0.07–4.91)	0.88 MW test
T/S ratio (nat. log-transformed)	−0.22 ± 0.66	−0.22 ± 0.59	−0.21 ± 0.77	0.92 Welch *t*-test
G1, n (%)	40 (22.7%)	24 (22.0%)	16 (23.9%)	
Age (years) (min-max)	81 (74–95)	83.5 (75–95)	80 (74–88)	0.054 MW test
T/S ratio (untransformed)	0.78 (0.07–4.91)	0.78 (0.32–1.77)	0.73 (0.07–4.91)	0.60 MW test
G2, n (%)	81 (46.0%)	48 (44.0%)	33 (49.3%)	
Age (years) (min-max)	64 (30–73)	64 (30–73)	66 (32–73)	0.64 MW test
T/S ratio (untransformed)	0.75 (0.15–4.51)	0.76 (0.15–4.12)	0.65 (0.24–4.51)	0.55 MW test
G3, n (%)	55 (31.3%)	37 (33.9%)	18 (26.9%)	
Age (years) (min-max)	33 (16–47)	34 (16–47)	25.5 (16–43)	0.11 MW test
T/S ratio (untransformed)	0.87 (0.19–4.33)	0.85 (0.19–3.76)	1.09 (0.39–4.33)	0.10 MW test
Men, n (%)	**109 (38.2%)**	**65 (37.4%)**	**44 (39.6%)**	
Age (years) (min-max)	59 (17–91)	59 (17–91)	51 (19–85)	0.17 MW test
T/S ratio (untransformed)	1.50 (0.13–7.55)	1.39 (0.13–7.55)	1.53 (0.17–6.26)	0.53 MW test
T/S ratio (nat. log-transformed)	0.34 ± 0.71	0.32 ± 0.78	0.37 ± 0.58	0.73 Welch *t*-test
G1, n (%)	27 (24.8%)	15 (23.1%)	12 (27.3%)	
Age (years) (min-max)	80 (73–91)	84 (74–91)	77 (73–85)	**0.023 MW test**
T/S ratio (untransformed)	1.13 (0.42–5.53)	1.37 (0.64–5.53)	1.02 (0.42–1.90)	0.39 MW test
G2, n (%)	42 (38.5%)	29 (44.6%)	13 (29.5%)	
Age (years) (min-max)	62.5 (36–72)	64 (50–72)	60 (36–72)	0.41 MW test
T/S ratio (untransformed)	1.29 (0.13–7.55)	1.10 (0.13–7.55)	1.50 (0.17–2.89)	0.28 MW test
G3, n (%)	40 (36.7%)	21 (32.3%)	19 (43.2%)	
Age (years) (min-max)	38 (17–48)	40 (17–48)	38 (19–43)	0.21 MW test
T/S ratio (untransformed)	1.73 (0.16–7.47)	1.78 (0.16–7.47)	1.72 (0.85–6.26)	0.80 MW test

Count data are presented as n (%), continuous data as mean ± SD or median (range). Gx refers to the generation, G1 being those that lived at the time of WW2.
[a] Continuous data tested by two-sample *t*-test with Welch correction (Welch *t*-test) due to unequal variances or by Mann-Whitney (MW) test.

telomere length, which persisted for a long time period after the stress subsided. This correlation was observed, e.g., for survivors of the Leningrad siege during World War II, with an interrelation with the duration of famine (Rotar et al., 2015), for Israeli veterans having suffered from captivity and confinement during the Yom Kippur War in combination with separation from family and loneliness (Stein et al., 2018), for adults exposed to early life stress combined with self-reported traumatic events (Savolainen et al., 2014), and for children exposed to interpersonal violence and family disruption (Drury et al., 2014). Nevertheless, recent reviews (see, e.g., Mathur et al., 2016; Willis et al., 2018) pointed to only a narrow range of stressor(s) or single specific stress factor (e.g., socioeconomic stress, traumatic life events, work-related stress, neighborhood stress within the selected group of participants) without considering the broader context including evaluation of the intensity, duration and developmental stage (early childhood, pubescence) in which exposure to the stress occurred. As opposing results have been obtained in numerous studies, the longitudinal studies are recommended to convincingly assess the impact of specific psychological stress event on the telomere length. Further, technical factors such as cell type examined and particularities of the measurement of the relative length of telomeres using quantitative PCR as the indispensable methodical approach for these studies may play a role. Last but not least, personal individuality – which is difficult to express objectively – is an important, maybe crucial, factor in the capability of each person to cope with extreme conditions. In this context, resilience, defined as a dynamic process of positive adaptation correlated with distinct personal qualities as optimism, positive affect, self-efficacy and self-esteem (Luthar and Cicchetti, 2000; Lee et al., 2013) was used as a relevant parameter to explain lower level of post-traumatic stress symptoms in the third generation of Holocaust survivors whose fathers were war veterans (Zerach and Solomon, 2016), and lower risk of early mortality in Holocaust survivors compared to groups without this extreme experience (Sagi-Schwartz et al., 2013). Recently, revolutionary hypothesis on specific behavior related to the telomere length was reported (Bateson and Nettle, 2018). Selective adoption hypothesis presented here supposed that either telomere length affected behavior directly or both telomere length and behavior are affected by any third variable. If these interesting hypothesis is supported by other data in future (with convincing examples already presented), then our recent straightforward, one sided view that behavior/living conditions cause telomere attrition will be radically revised.

Here, we present analyses of the relative lengths of telomeres in peripheral blood mononuclear cells (PBMC) of Jewish persons who survived the Holocaust during World War II, and of two generations of their progenies, compared to control groups of people which were not exposed to this extreme stress and its consequences. Holocaust survivors represent a quite exceptional group of people, having been able to oppose enormous physical and psychical hardship, cope with the complicated situation after the war when they suffered from the bereavement of many, sometimes even all, family members, and live to a relatively high age. Therefore, we took advantage of a unique opportunity to examine this specific group in our study.

Table 2
Influence of investigated factors (stress - i.e., Holocaust or its consequences, PCL-C value, age, gender) on telomere length (natural log-transformed T/S ratio) based on multiple linear regression models.

Parameters	Unadjusted			adjusted[a]		
	$beta_x$	95%-CI	p-value	$beta_x$	95%-CI	p-value
Age (years), n = 285	−0.007	−0.011 to −0.003	**0.002**	−0.007	−0.010 to −0.003	< **0.001**
PCLC (number), n = 285	−0.001	−0.009 to 0.007	0.82	0.0004	−0.007 to 0.008	0.92
Gender, men vs. women, n = 285	0.560	0.398 to 0.723	< **0.001**	0.559	0.400 to 0.719	< **0.001**
G1, n = 67	0.492	0.169 to 0.815	**0.003**	0.526	0.195 to 0.856	**0.002**
G2, n = 123	0.481	0.225 to 0.738	< **0.001**	0.485	0.228 to 0.743	< **0.001**
G3, n = 95	0.665	0.393 to 0.937	< **0.001**	0.695	0.419 to 0.972	< **0.001**
Stressed group, n = 174	0.546	0.339 to 0.752	< **0.001**	0.555	0.350 to 0.761	< **0.001**
G1, n = 39	0.513	0.192 to 0.834	**0.003**	0.560	0.239 to 0.882	**0.001**
G2, n = 77	0.432	0.108 to 0.757	**0.010**	0.465	0.145 to 0.785	**0.005**
G3, n = 58	0.730	0.330 to 1.131	< **0.001**	0.747	0.336 to 1.158	< **0.001**
Control group, n = 111	0.580	0.312 to 0.849	< **0.001**	0.548	0.288 to 0.807	< **0.001**
G1, n = 28	0.486	−0.169 to 1.141	0.14	0.420	−0.266 to 1.105	0.22
G2, n = 46	0.583	0.137 to 1.030	**0.011**	0.600	0.151 to 1.048	**0.010**
G3, n = 37	0.502	0.157 to 0.846	**0.006**	0.552	0.193 to 0.911	**0.004**
Stress, yes vs. no, n = 285	−0.037	−0.212 to 0.137	0.68	−0.024	−0.197 to 0.148	0.78
G1, n = 67	0.189	−0.152 to 0.529	0.27	0.202	−0.172 to 0.576	0.29
G2, n = 123	0.025	−0.240 to 0.291	0.85	0.031	−0.235 to 0.298	0.82
G3, n = 95	−0.283	−0.585 to 0.020	0.067	−0.285	−0.593 to 0.023	0.069
Men, n = 109	−0.046	−0.320 to 0.229	0.74	0.012	−0.253 to 0.278	0.93
G1, n = 27	0.227	−0.156 to 0.609	0.23	0.023	−0.371 to 0.417	0.91
G2, n = 42	−0.124	−0.657 to 0.410	0.64	−0.055	−0.597 to 0.487	0.84
G3, n = 40	−0.061	−0.510 to 0.389	0.79	0.004	−0.436 to 0.444	0.99
Women, n = 176	−0.011	−0.214 to 0.191	0.91	−0.012	−0.213 to 0.189	0.91
G1, n = 40	0.200	−0.285 to 0.685	0.41	0.241	−0.289 to 0.770	0.36
G2, n = 81	0.027	−0.255 to 0.310	0.85	0.027	−0.257 to 0.312	0.85
G3, n = 55	−0.289	−0.650 to 0.071	0.11	−0.305	−0.677 to 0.066	0.11

Gx refers to the generation, G1 being those that lived at the time of WW2.
[a] Adjustments for age and sex (in case of all samples, otherwise sex adjustment is omitted) when comparing stress yes vs. no. In case of men vs. women comparison, adjustments for age.

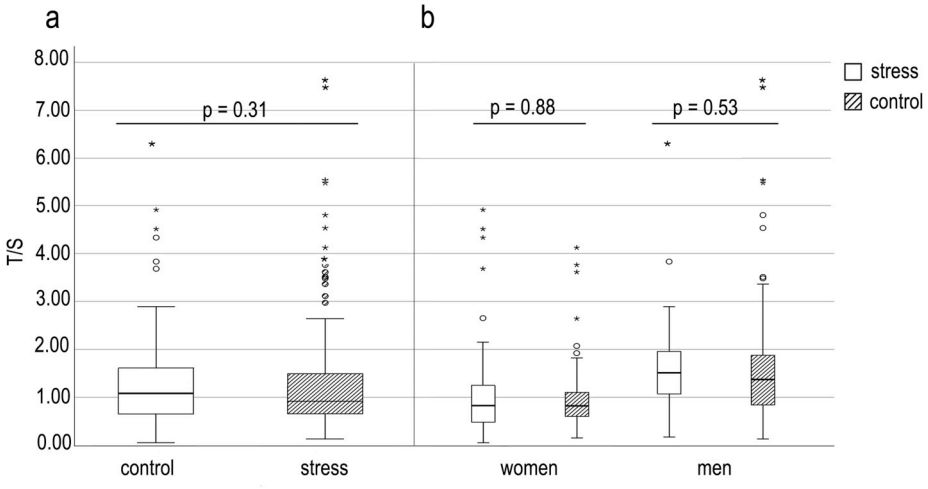

Fig. 1. Relative lengths of telomeres in Holocaust survivors and their progenies and controls of comparable age (a) and within these groups with respect to gender (b). The median of telomere lengths is represented by the horizontal line; 25% and 75% quartiles are represented by the lower and the upper borders of the rectangle, respectively; non-outlier range represented by abscissas; outlying values by dots (outlier values) and asterisks (extreme values). Relative lengths of telomeres were expressed as T/S ratios; T represents relative telomere signal; S represents the relative signal of the reference gene. In total, 174 Holocaust survivors and their progenies (109 women and 65 men) and 111 controls (67 women and 44 men) were evaluated. Statistically significant difference in telomere lengths between stressed and control groups was not detected, either when all respondents were compared (p = 0.31) or when gender was considered (women, p = 0.88; men, p = 0.53); statistical evaluation was done using Mann-Whitney test.

2. Materials and methods

2.1. Participants and nomenclature of samples

In total, 285 persons were investigated, 109 men and 176 women. Blood for analysis of relative telomere length was taken from 174 persons who survived the Holocaust and from two generations of their progenies (stress-G1: 39 persons, 15 men and 24 women; stress-G2: 77 persons, 29 men and 48 women; and stress-G3: 58 persons, 21 men and 37 women). G1 Holocaust survivors suffered from the extreme and long term stress during the World War II, e.g., long term humiliation, deprivation of basic human rights, life threating internment in prisons, death camps, hiding, false identities, fighting as partisans and for all of them assassination of family members; mostly, Holocaust survivors were exposed to combination of these stress factors. In the offspring, details of the relationship to previous generation(s), i.e., mother, father or both were exposed to the stress/were progenies of the person(s) exposed to the stress, were not considered. As control groups, 111 people of comparable ages as Holocaust survivors and their progenies who were not exposed to extreme stress were investigated (control-G1: 28 persons, 12 men and 16 women; control-G2: 46 persons, 13 men and 33 women; and control-G3: 37 persons, 19 men and 18 women). Contrast to Holocaust groups, control people were not of the Jewish ethnicity, because in the Czech Republic, there is actually no Jewish person without the Holocaust history. All people were volunteers having responded positively to a public appeal presented by Masaryk

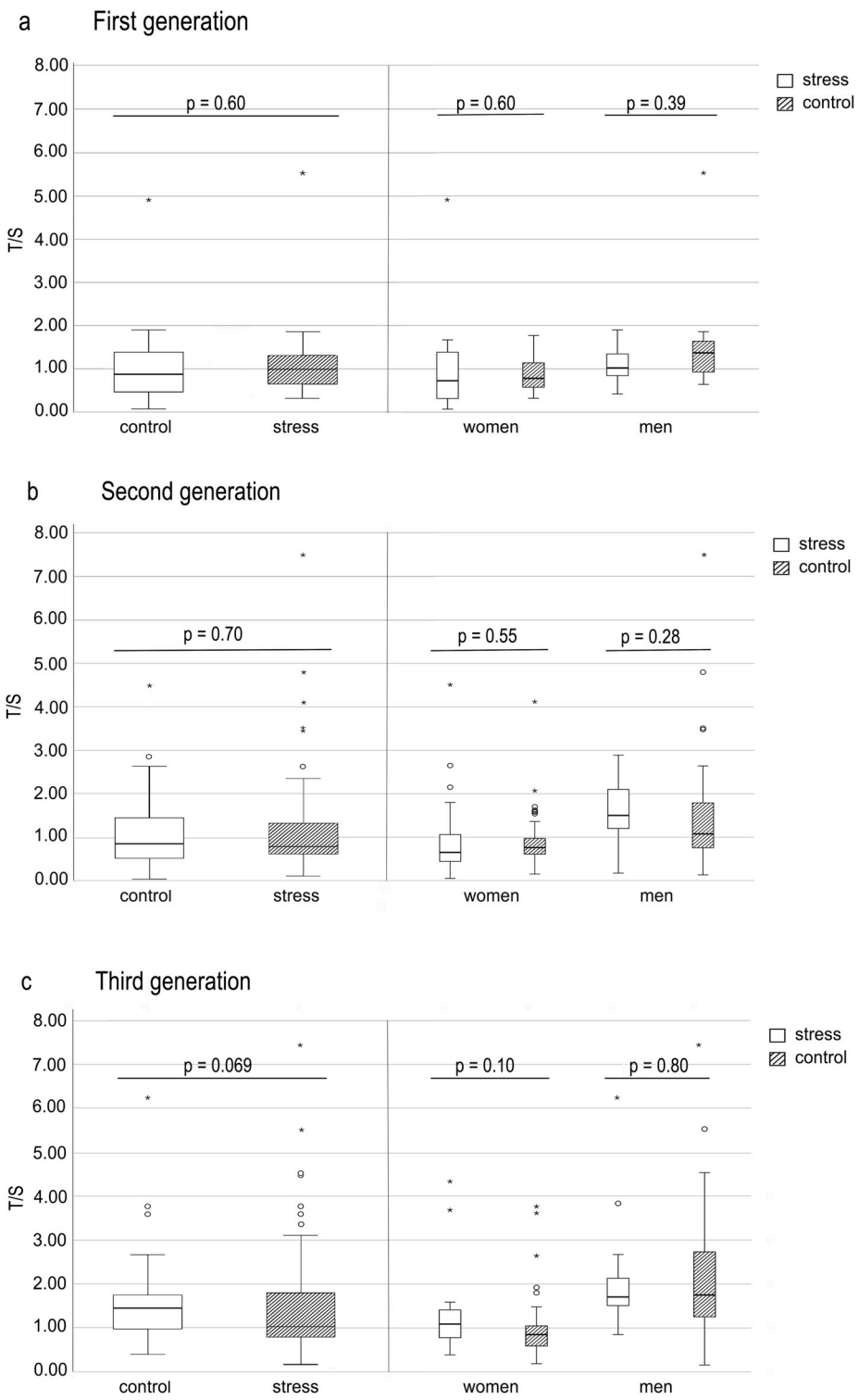

Fig. 2. Relative lengths of telomeres in Holocaust survivors (a, G1), in two generations of their progenies (b, G2; c, G3), and in relevant control groups. In G1 Holocaust survivors, 39 people (24 women and 15 men) were compared to 28 controls (16 women and 12 men); in G2 Holocaust survivors' progenies 77 people (48 women and 29 men) were compared to 46 controls (33 women and 13 men); and in G3 Holocaust survivors' progenies, 58 people (37 women and 21 men) were compared to 37 controls (18 women and 19 men). No statistically significant differences in telomere lengths between stressed and control groups were found, neither when gender was considered (right panels). For details, see Fig. 1 legend and Table 1.

University and Czech national media (online press release, local broadcasts; for details in Czech see Supplementary File 1), with the cooperation of the Jewish community of Brno and Prague. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Masaryk University, Brno, Czech Republic (project NV18-04-00559).

2.2. Evaluation of post-traumatic stress disorder (PTSD) markers using the PTSD CheckList – civilian version (PCL-C)

Markers of the PTSD were evaluated using a PCL-C questionnaire. The PCL-C questionnaire is available at http://www.istss.org/assessing-trauma/posttraumatic-stress-disorder-checklist.aspx. We chose the PCL-C questionnaire as it is used in the diagnostic assessment of PTSD in civilian subjects.

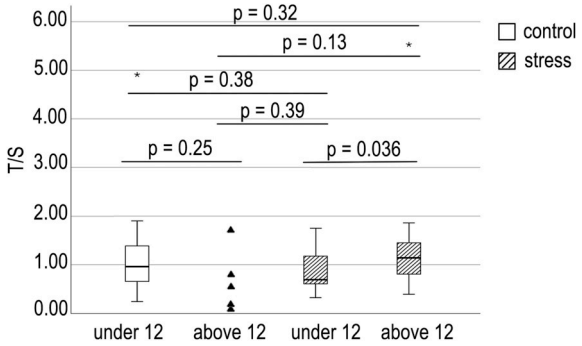

Fig. 3. Relative lengths of telomeres in G1 Holocaust survivors compared to relevant control groups with respect to the age of people in the period of extreme stress exposure; as a cut-off, the age of 12 years in 1945 was selected. From 28 control people, 23 were of age under 12, and 5 were of age above 12; from 39 G1 Holocaust survivors, 19 were of age under 12 years in 1945, 20 were of age above 12. Significantly shorter telomeres were detected in the group of Holocaust survivors of the age under 12 compared to Holocaust survivors of the age above 12. For details, see Fig. 1 legend.

2.3. Isolation of peripheral blood mononuclear cells (PBMC) and DNA

PBMC were isolated from whole blood using ficoll (Histopaque, Sigma) density gradient centrifugation (Ulmer et al., 1984). Samples were collected within years 2016–2019 and were processed using the same protocols. Genomic DNA was purified by proteinase K (Roth) treatment, chloroform extraction, and isopropanol precipitation; for step-by-step protocol of DNA isolation see Supplementary File 2. Quality and concentration of DNA were analyzed by agarose electrophoresis and spectrophotometrically using Nanodrop 2000 (Thermo Fisher Scientific), (Supplementary Fig. 1).

Table 3
Age-related changes of telomere length (natural log-transformed T/S ratio) based on Pearson's correlation coefficient calculation.

Parameter	Correlation coefficient	p-value
All participants, n = 285	−0.184	**0.002**
Men, n = 109	−0.300	**0.002**
Women, n = 176	−0.136	0.072
Stressed group, n = 174	−0.107	0.16
Men, n = 65	−0.235	0.060
Women, n = 109	−0.0709	0.46
Control group, n = 111	−0.295	**0.002**
Men, n = 44	−0.430	**0.004**
Women, n = 67	−0.221	0.072

2.4. Quantitative PCR

Analysis of relative telomere length was performed by quantitative PCR (qPCR). DNA (10 ng) was analyzed using EliZyme Green MIX AddROX (EliZyme) and a Rotorgene Q cycler (72-well rotor) and software (Qiagen). Telomeres were analyzed by TelC.

(5′- TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA - 3′) and TelG.

(5′- ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT - 3′) primers (final concentration in reaction mixture 450 nM each; for design of primers, see Cawthon, 2009), in a program consisting of 94 °C for 2 min, 2 cycles of 15 s at 94 °C, 15 s at 49 °C; 25 cycles of 15 s at 94 °C, 10 s at 62 °C, and 15 s at 74 °C with signal acquisition. The reference gene *GAPDH* was amplified using GAPDG Fw (5′- GAAGGTGA AGGTCGCAGTC - 3′) and GAPDH Rev (5′- GAAGATGGTGATGGGAT

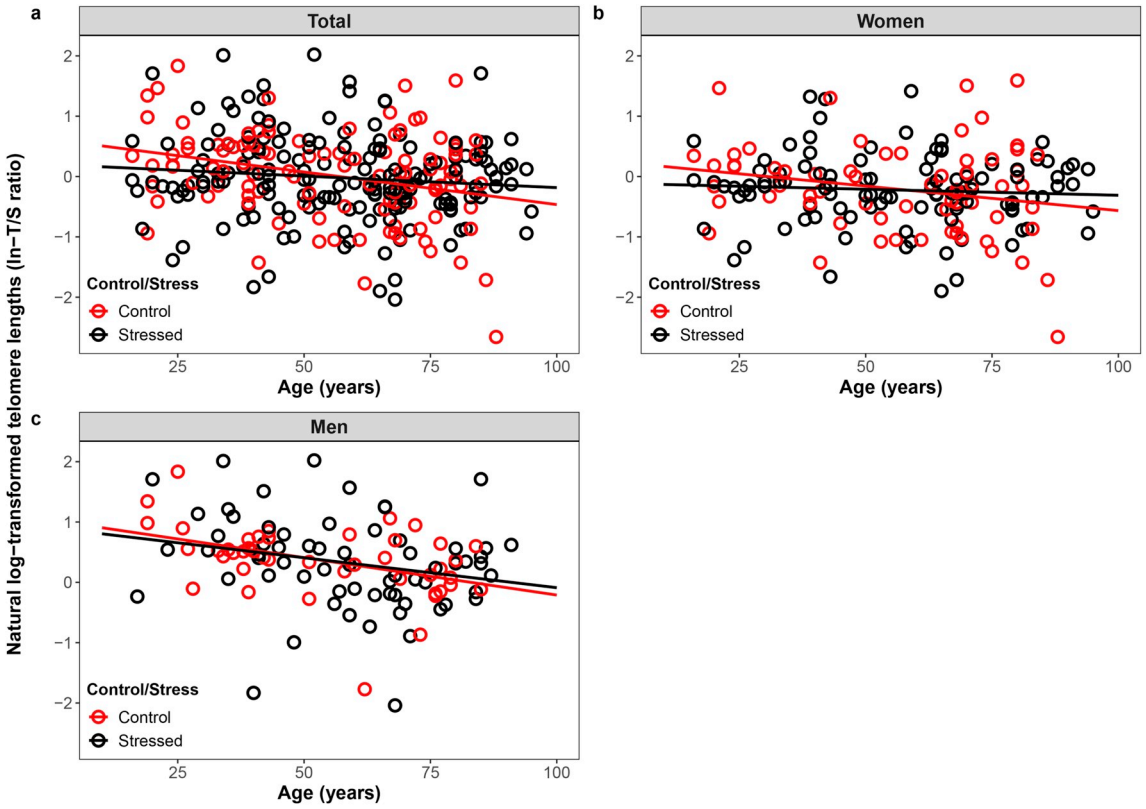

Fig. 4. The dependence of relative lengths of telomeres on the age of respondents. (a) Relative lengths of telomeres of all people (regardless of gender) within the stressed (Holocaust survivors and their progenies) and control groups; (b) relative lengths of telomeres of women in the stressed and control groups; (c) relative lengths of telomeres of men in the stressed and control groups. No statistically significant differences in age-related telomere length dynamics among stress and control groups were detected, as evaluated by ANCOVA.

TTC - 3′) primers (final concentration in reaction mixture 250 nM each) in a program consisting of 2 min at 94 °C, 30 cycles of 20 s at 94 °C, 20 s at 53 °C, 20 s at 72 °C with signal acquisition, and a final polymerization step (3 min at 72 °C). Amplification efficiencies of both reactions were calculated from slopes of standard curves which were constructed using serially diluted DNA samples (1, 1/10, 1/100, 1/1,000, 1/10,000 for *GAPDH*; 1, 1/10, 1/50, 1/100, 1/500 for telomeres). The amplification efficiency (E) and correlation coefficient (R^2) for each reaction were calculated by three experimenters independently (Table S1). As E within 90–110% and $R^2 > 0.99$ are considered as acceptable (Xiao et al., 2017), our values were clearly within these ranges.

A relative S value (based on the *GAPDH* signal) and a T value (based on the telomere-specific signal) were determined according to respective calibration curve, and the T/S ratio was calculated for each sample. The T/S ratio of a sample selected as a reference was arbitrarily set as 1; this sample was included in all analyses and T/S values of all other samples were related to it. Each DNA was analyzed at least twice in independent analyses, in technical triplicates with essentially the same outputs regarding the Ct values obtained for telomere-specific and *GAPDH*-specific signals, and T/S ratio (inter-assay coefficient of variability 4.8–9.3% for control samples and 3.5–10.5% for stress samples); values obtained from repeated measurements were averaged.

2.5. Statistical analysis

Continuous variables were calculated as mean ± SD in the case of a normal distribution, and for non-normally distributed data the median (range) was used. Categorical variables were presented as n (percentages). Since the T/S ratios showed a deviation from a normal distribution (D'Agostino omnibus normality test: $p < 0.001$; depicted as histogram in Supplementary Fig. 2a), data were natural log-transformed (D'Agostino omnibus normality test: $p = 0.06$; depicted as histogram in Supplementary Fig. 2b). Differences in continuous variables were tested by two-sample t-tests in the case of a normal distribution, or by the Mann-Whitney test for non-normally distributed data. The correlation between telomere length and age was evaluated by Pearson's correlation coefficient. Slopes of regression lines were compared by ANCOVA. Associations between telomere length and analyzed factors (stress, age, gender, PCL-C value) were tested by the multiple linear regression analysis. The level of statistical significance was set at $p < 0.05$ in all analyses.

All statistical calculations were carried out using version 3.5.0 of the R statistical package (cran.r-project.org).

3. Results

3.1. Relative lengths of telomeres do not differ significantly between groups of Holocaust survivors and their progenies, and control groups

In total, the relative lengths of telomeres in samples of DNA isolated from 285 persons were analyzed; 174 samples from Holocaust survivors and their progenies and 111 samples from control groups. Our results are summarized in Tables 1 and 2; the raw data are in Table S2.

When comparing the relative telomere lengths between all respondents within the stress and control groups, no significant difference was observed (Fig. 1a), neither when men and women were compared separately (Fig. 1b).

Similar results were obtained in analyses of relative lengths of telomeres in G1 respondents, survivals of the Holocaust, and control persons of comparable age; there was no statistically significant difference in telomere lengths between stressed and control groups (Fig. 2a, left panel), nor when the gender was considered (Fig. 2a, right panel).

Taking into consideration that in G1 Holocaust survivors, the age at which people were exposed to the extreme stress might be relevant for the propagation of consequences of this stress, we selected the age of 12 years in the year 1945 (the end of World War II) as a cut-off for another evaluation. Relative telomere lengths were compared for 23 persons representing controls under 12, controls above 12 (5 persons), stressed under 12 (19 persons), and stressed above 12 (20 persons). Evaluation based on gender was not performed in this case because the low number of people in the control group above 12 (control women – 3, control men – 2) would compromise the relevance of such analysis; these persons are now at the minimal age of 83. As shown in Fig. 3, significantly shorter telomeres were detected in the group of stressed people of the age under 12 in 1945 compared to Holocaust survivors of the age above 12 ($p = 0.036$).

The relative lengths of telomeres were compared in two generations of progenies of Holocaust survivors and groups of control persons. In G2, relative lengths of telomeres were comparable in stressed and control groups (Fig. 2b, left panel), and also when gender was considered, there were no statistically significant differences between stressed and control G2 women and stressed and control G2 men (Fig. 2b, right panel). Similar results were obtained for comparison of telomeres between G3 stressed and G3 control groups (Fig. 2c).

Next, all participants were investigated for signs of PTSD using the PCL-C questionnaire (raw data are summarized in Table S2). Data were tested using multiple linear regression analysis. Based on whole model, only age and gender have proven to have a significant effect on the telomere length; estimate ± standard error and p-value for analyzed factors were as follows: Age: −0.007 ± 0.002, 0.001; Gender: 0.560 ± 0.082, < 0.001; Stress: −0.016 ± 0.085, 0.85; PCL-C: 0.0006 ± 0.004, 0.88. Thus, these two parameters were later used for an adjustment. No association between telomere length and PCL-C value was found, even when the adjustment for other factors (age, gender) was taken into account (Table 2).

3.2. Dynamics of age-related telomere length changes are comparable for Holocaust survivors and their progenies vs. controls

The gradual age-related telomere shortening is a consequence of the inability of DNA polymerase to complete replication of 5′ ends of lagging DNA strands which is not compensated by telomerase activity in terminally differentiated human tissues (reviewed in Blackburn, 1991). We compared the dynamics of age-related telomere length changes between the Holocaust survivors and their progenies vs. control group (Fig. 4a) and within the same groups with respect to gender (Fig. 4b and c). As expected, a relationship was found between the age of respondents and the relative length of their telomeres (Table 3; Pearson's correlation coefficient −0.184; $p = 0.002$). Interestingly, distinct relationship with respect to age-related decrease of relative length of telomeres was detected for the sub-group of control men, but not for other sub-groups (Table 3). Comparison of the slopes of regression lines based on ANCOVA between men and women did not show any significant difference ($p = 0.10$), which is also the case when the samples were separated by their affiliation to the stress group ($p = 0.14$ for stressed group, $p = 0.51$ for control group). Similarly, comparison of the slopes based on stress also did not show any significant difference ($p = 0.10$), even when separated by gender ($p = 0.21$ for women, $p = 0.72$ for men).

4. Discussion

Faster telomere shortening has been reported repeatedly as a consequence of a strong and/or long-term stress exposure. As stress conditions result in increased production of reactive oxygen species, G-rich telomere repeats are preferential targets for oxidative damage which contributes substantially to their degradation. Even an inhibition of telomerase by oxidative stress products was recently reported (for review, see Ahmed and Lingner, 2018). Very relevant to our study was the finding that telomeres of survivors of the famine during the Leningrad

siege were significantly shorter, with the extent of shortening in correlation with the famine period (Rotar et al., 2015).

In our study, relative telomere lengths of Holocaust survivors and two generations of their progenies were comparable with telomeres measured in control groups (Fig. 2) and also the dynamics of age-related telomere length changes were not influenced by the strong stress experience and its consequences (Fig. 4). According to a recent comprehensive review summarizing data from 105 studies dealing with the impact of various socioeconomic stressors and stressful life events, including those in early life, on telomere lengths (Willis et al., 2018), more than half of the studies of adults having suffered from early stress reported an association with shorter telomere lengths. Although the authors found the correlations between early life stress and telomere shortening persisting across the life course as significant, they pointed out that this relationship may be influenced by other factors, including race and ethnicity. In this respect, two aspects of our study are to be stressed. First, we compared survivors of the Holocaust and their progenies with control groups represented by non-Jewish people. The reason is that there was nobody of the Jewish origin in the Czechoslovakia during World War II who was spared Nazis' "final solution" plan. The second important point is that the G1 survivors represent specific, highly selected group of persons. They were able to cope with unprecedented conditions during the Holocaust and even following its end, when they learned and had to accept that many of their relatives and friends had not survived. Moreover, despite this they were able to survive to a better-than-average age; the average age of the G1 Holocaust survivors was 84 years. Thus, and as concluded in other studies (Sagi-Schwartz et al., 2008; Zerach and Solomon, 2016), this may reflect a distinct resilience which may even have a genetic background. This may be correlated also with positive patterning, when the miserable experience from the Holocaust was presented as a factor making them and their progenies stronger and able to cope with common – not always positive – life conditions. In support of this hypothesis, genetic selection during the Holocaust and/or posttraumatic growth associated with protective factors were mentioned as possible reasons of longer life expectancy in the group of Holocaust survivors compared to Jewish people having immigrated to Israel before the year 1939 (Sagi-Schwartz et al., 2013). Next, physical separation of children from parents has been associated with accelerated biological ageing, including telomere shortening, only in adult participants who self-reported traumatic experience during their lifespan, while separation only (or self-reported traumatic experience in the control group) was not reflected in telomere length (Savolainen et al., 2014).

The only category, in which significant difference in relative lengths of telomeres was observed, was comparison of Holocaust survivors of the age under 12 in the year 1945 and those of the age above 12, with significantly shorter telomeres in the first group (Fig. 3). No significant changes in telomere lengths were detected among groups of Holocaust survivors and groups of controls, which may be related to the relatively low number of participants (e.g., 5 persons in the group of controls above 12 were investigated). Based on this result, the impact of the extreme stress during the very early childhood seems to leave behind the strongest consequences with respect to telomere maintenance.

Regarding the biological point of view, many factors cooperate to modulate the length of telomeres. For telomere maintenance the activity of telomerase in stem cells is crucial, and telomerase is influenced by other proteins which modulate its enzymatic activity or the structure and thus accessibility of chromosome ends. Except for genetic factors determining the telomere length at birth, many life circumstances guide telomere developmental shortening. Evaluation and unambiguous interpretation of results of telomere lengths in population studies are thus complicated by high variability of telomere lengths among individuals of comparable age (reviewed in Ishikawa et al., 2016). This is a probable reason for the broad distribution of relative lengths of telomeres of persons that were evaluated in this study (Fig. 4).

For analysis of telomere lengths, PBMC were isolated with lymphocytes making up the majority of their population. The life span of lymphocytes is significantly longer compared to that of other blood cells, which is crucial for proper functioning of the immune system. Unlike most human somatic cells, lymphocytes are able to reactivate telomerase for a limited time period, which is part of the process of lymphocyte activation during the immune response (reviewed in Barsov, 2011). Although telomerase reactivation in lymphocytes or telomerase activity in stem cells from which lymphocytes are differentiated is not supposed to fully compensate for age-related telomere shortening, it is possible that except for the genetic predispositions mentioned above (including longer telomeres or their slower age- and/or stress-related shortening), telomerase activity can, at least partially, contribute to the compensation of Holocaust stress-related telomere erosion.

Although this was not a goal of our study, significantly longer telomeres in men compared to women were detected within each group (Table S3). This was obviously not related to the Holocaust stress. According to the general view, telomeres in women are considered longer, although results from published studies are sometimes contradictory and largely dependent on the method used. In meta-analyses, telomeres in women were shown to be longer than those in men, but this difference was significant only when a Southern blot-based method was used for analysis and not in qPCR or flow-FISH data. Gardner and colleagues (2014) considered technology-specific impact of some polymorphisms. Except of polymorphisms in subtelomeric regions which may influence Southern blot data, interstitial telomeric sequences (ITSs) are considered. ITSs are internally located telomere repeats within the human genome which are highly polymorphic (Samassekou and Yan, 2011) and, importantly, display high inter-individual variability (Foote et al., 2013). Thus, although a strong correlation between the mean telomere length measured by the Southern blot method and qPCR telomere-specific signal was presented (Cawthon, 2009), a method-specific bias may exist and further analyses will be needed to solve the problem of sex-related differences in telomere lengths.

Altogether, according to our data no difference in relative lengths of telomeres or in age-related telomere length dynamics were observed between survivors of the Holocaust during World War II and two generations of their progenies, and non-Jewish control groups of comparable age which were not exposed to this extreme and long-term stress. This analysis represents, to the best of our knowledge, the first systematic study of telomere dynamics in Holocaust survivors. Unfortunately, due to the advanced age of the Holocaust survivors it could also be the last chance to make such studies.

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Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2019.06.018.

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