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Repeated exposure to challenging environmental conditions influences telomere dynamics across adult life as predicted by changes in mortality risk

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Abstract

The effects of stress exposure are likely to vary depending on life-stage and stressor. While it has been postulated that mild stress exposure may have beneficial effects, the duration of such effects and the underlying mechanisms are unclear. While the long-term effects of early-life stress are relatively well studied, we know much less about the effects of exposure in adulthood since the early- and adult-life environments are often similar. We previously reported that repeated experimental exposure to a relatively mild stressor in female zebra finches, first experienced in young adulthood, initially had no effect on mortality risk, reduced mortality in middle age, but the apparently beneficial effects disappeared in old age. We show here that this is underpinned by differences between the control and stress-exposed group in the pattern of telomere change, with stress-exposed birds showing reduced telomere loss in middle adulthood. We thereby provide novel experimental evidence that telomere dynamics play a key role linking stress resilience and aging.

KEYWORDS

aging, glucocorticoids, stress resilience, stress vulnerability, telomere dynamics, telomere length

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLM, generalized linear model; GLMM, generalized linear mixed model; qPCR, quantitative polymerase chain reaction; S, single copy control gene; T, telomere repeat copy number; TRF, telomere restriction fragment.

1 | INTRODUCTION

Exposure to challenging environmental circumstances can accelerate the rate of aging and increase the likelihood of disease and thereby mortality risk. However, the severity of the stressor and the life stage at which this occurs is likely to influence organismal outcomes. While exposure to severe and/or chronic stress is generally detrimental, relatively milder forms of stress exposure can, in some circumstances, have positive rather than negative effects on aging and lifespan.[1-4] A substantial body of evidence across a range of taxa, including humans, shows that stress exposure during early life can alter coping abilities and have long-term phenotypic consequences, the fitness effects of which can be contingent on the adult environment.[5-7] Surprisingly, however, we know much less about variation in outcomes that results from environmentally generated stress exposure during different periods of adult life and particularly how changes in stress resistance with increasing adult age might interact with the potentially beneficial effects of mild stress exposure.[3,8]

An important pathway through which environmental conditions might alter longevity trajectories is via effects on telomeres. Telomeres comprise tandem repeats of a short DNA sequence (commonly TTAGGG), plus associated proteins found at the end of the linear chromosomes of eukaryotes. They play a key role in genome integrity and cell stability, protecting genes from the loss of coding sequences that occurs at the chromosome ends during cell division and preventing end-to-end joining of chromosomes by the DNA repair machinery.[9-11] Telomeres shorten with progressing age within somatic tissues of many species, and this shortening is a recognized hallmark of aging (recently reviewed by Ref. 12), since individuals with shorter telomeres, or with higher rates of telomere erosion, often have shorter lifespans[13-15] and show greater susceptibility to infection and disease.[16-18]

Exposure to physiological stress in early life, via direct hormonal manipulations of stress levels, changes in food availability, or social circumstances, has been shown to accelerate telomere loss with long-term consequences for health and longevity.[19-23] While stress experienced in adult life is likely to add to this early-life legacy,[24] we do not know in what way repeated or chronic stress exposure during adult life in itself affects telomere dynamics nor do we know whether effects change with advancing adult age.

In an experimental study in female zebra finches (*Taeniopygia guttata*) on the influence of persistently challenging environmental circumstances in adulthood on mortality risk, we found that effects on mortality risk varied at different stages of adult life; no initial effect of the challenging environment was observed during relatively young adulthood (5-13 months), a reduced mortality risk observed during middle age (13-36 months), and no effect in old age (36-48 months).[25,26] The differing effects of the treatment on survival during the different phases of adult life were not explained by changes or differences in individuals' breeding effort.[26] Zebra finches show telomere shortening in somatic cells during adult life.[13] Here, using stored blood samples, we examine whether the pattern of adult mortality risk that we previously reported is related to changes in telomere dynamics during these same phases of adult life. Specifically, we predicted that, if this was so, exposure to the persistently challenging conditions would have no effect on telomere shortening in early or old adulthood in comparison with control groups but would reduce telomere shortening in middle age.

2 | MATERIALS AND METHODS

2.1 | Study subjects and housing conditions

All females used in this study were produced from the breeding stock at the University of Glasgow. All the birds had the same, benign early-life environment with ad lib high-quality food available (*ad lib* supply of mixed seeds—common millet, yellow millet and canary seed in a ratio of 3:1:1 (Johnson and Jeff, UK)—oyster shell grit, cuttlefish, weekly protein supplement, and *ad lib* water used for stock birds in our facilities). We conducted two replicates of the experiment; replicate 1 females were produced from our breeding stock in April-June 2011, and replicate 2 females were produced in August-September 2011. For each replicate, the environmental manipulations started when the study females were fully grown, sexually mature, young adults (~5 months old; mean ± SE: 152 ± 1 days). Birds were housed in treatment-specific groups (n = 7-10 per 120 × 50 × 50-cm cage). When possible, females that hatched in the same nest (part of the same brood) were counterbalanced between the two treatment groups and family of origin was taken into account in the analyses. The photoperiod was always maintained at 14 hours:10 hours light:dark cycle and the temperature was between 20℃ and 24℃. All procedures were carried out under UK Home Office Project Licence 60/4109.

2.2 | Environmental manipulation

When the females were ~5 months of age, they were randomly allocated to one of the two environmental treatment groups: a challenging (replicate 1, n = 49; replicate 2, n = 66) or control environment (replicate 1, n = 49; replicate 2, n = 64). In the challenging environment, food was made unavailable for a continuous period of ~one third of the daylight period (4.9 hours), 4 days/week on a random time schedule. For the remaining two thirds of the day, and on the remaining 3 days/week, challenged females received

ad lib food. We found no substantial effect of the challenging environment on annual measures of body mass up to 3 years of age.[25] At 4 years the challenged birds were on average slightly lighter (by 7%) than the controls (control: 18.16 ± 0.38 g, challenged: 16.87 ± 0.27 g; full statistics in Table S1). We did not closely monitor changes in body mass between days in which the food was temporally limited and days in which it was *ad lib* to avoid high levels of disturbance. Measurements of body mass at the time of blood sampling were always included in all initial statistical models of telomere change or length as clarified in the "Data Analysis" paragraph. Challenged females always experienced this food regime except during breeding when they were given *ad lib* access to food until they completed the breeding event (~2 months for each breeding event). Control females were always provided with *ad lib* food and experienced exactly the same breeding scheduling as the challenged birds. As previously shown, the challenging environmental conditions led to increases in corticosterone secretion, the primary avian glucocorticoid. At the end of each food withdrawal exposure, challenged females had higher corticosterone than controls (on average 1.6-fold increase and within the baseline range of variation for our study species—Ref. 25). This moderate physiological increase in glucocorticoids remained consistent throughout the course of the experiment with no sign of habituation to the environmental manipulation.[26] Thus, the level of increase in glucocorticoids is consistent with relatively mild stress exposure, related to the unpredictable availability of food.

2.3 | Adult female breeding timeline

The study females from both treatment groups were given the opportunity to breed four times during the study period (full detail provided in Ref. 26). Briefly, females were always paired with an unrelated, relatively young male (6 months to 1.8 years of age). The first breeding event occurred when the control and challenged females were on average 6 months old (188 ± 0.89 days of age). The females were paired again at 1.1 years (408 + 0.82 days of age), 1.8 years (653 + 0.78 days of age), and finally when they were 3.5 years old (1270 + 0.92 days of age; mean + SEM for all). Number of eggs laid and number of young reared were recorded for each individual female, giving a measure of breeding effort.

2.4 | Blood sampling and telomere length analysis

We measured telomere length in blood samples taken from the females prior to the start of the experiment at approximately 5 months (mean ± SE: 152 ± 1 days, range: 117-168 days) at approximately 13 months (mean ± SE: 380 ± 1 day, range: 352-412 days), at approximately 36 months (mean ± SE: 1101 ± 1 day, range: 1081-1183 days), and approximately 48 months (mean ± SE: 1460 ± 1 day, range: 1445-1483 days). This provided us with information on the pattern of telomere change in early adulthood, middle age, and old age. DNA from red blood cells was extracted using commercial kits and following the manufacturer's protocol (Macherey-Nagel, USA). Relative telomere length (RTL) was quantified in the red blood cell DNA by using qPCR as described elsewhere[27]; this correlates well with measurements using the TRF method.[27] Briefly, the RTL of each sample was measured by determining the ratio (T:S) of telomere repeat copy number (T) to a single copy control gene (S), relative to the same DNA reference sample run on each plate. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the single copy control gene. The telomere and GAPDH reactions were carried out on separate plates, and in both reactions the number of PCR cycles (Ct) required for the products to accumulate enough fluorescent signal to cross a threshold was determined. Reaction efficiencies were always within the acceptable range (ie, 100% ± 10%). All samples fell within the bounds of the standard curve run on every plate (6 standard dilutions, from 40 to 1.25 ng of DNA). All telomere assays were run at the end of the animal experiment (between October 2015 and February 2016). Samples were randomly spread with respect to treatment, sampling age, replicate, and individual across the different qPCR plates; each plate contained a standard curve, and all standards and samples were always run in triplicate. The intraplate coefficients of variation for the telomere and GAPDH assays for the raw Ct values were 0.65% and 0.97%, respectively; the interplate coefficients of variation calculated using the standard dilutions that were run across each plate for both the telomere and GAPDH assays were 1.63% and 1.96%, respectively.

The raw qPCR data were analyzed using the software qBase+.[28] Mean Ct values were used to calculate a relative measure of telomere length as a T:S ratio of telomere repeat copy number to a control, single copy gene number (GAPDH). The qBase+ software provides the advantage of adjusting for differences in amplification efficiencies among plates (as described in Ref. 28) and correcting for further interrun variation by including three interrun calibrators (ie, the reference sample and two points from the standard curve—10 ng and 5 ng of DNA). For each sample, the software produced a calibrated normalized relative telomere measurement, which is similar to the T:S ratio described by Ref. 29 but offers a greater control of inter-plate stochastic variation. The interassay coefficient of variation for the calibrated normalized T:S ratios calculated using the standard dilutions run across each plate was 15.25%.

2.5 | Survival

We monitored the survival of the birds until they were 4 years old (48 months), by which point 52% had died. Experimental birds were inspected daily. Where birds showed clear signs that death was imminent and their welfare severely compromised (not able to fly and/or feed independently, and our veterinarian confirmed that death was imminent— about 2 days), they were culled for welfare reasons under the advice of our veterinarian in line with UK Home Office legislation (16 control and 20 challenged of 105 females that died). Generally, deaths were unpredictable, with the majority of the birds being found dead on the cage floor without having shown prior health symptoms. A few birds (6 control and 8 challenged birds) died from accidental injury and were not included in the survival analyses.

2.6 | Data analysis

Analyses were performed in R (version 3.6.2; R core team, 2014) using generalized linear models (GLMs)/generalized linear mixed model (GLMMs) (package "lme4",[30] "lmerTest"[31]). First, we checked whether telomere length prior to the start of the experiment (5 months of age) differed between the treatment groups and replicates; telomere length data were ln-transformed to improve normality of model residuals. One individual female sampled at 5 months of age with a telomere length value of 4.61 was excluded from this model because this value was an extreme statistical outlier as shown by inspection of model residuals and because it exceeds the upper quartile by more than three times the interquartile range.[32] We then assessed potential treatment differences in telomere change patterns during the same periods used in our mortality risk assessment early (5-13 months of age), middle (13-36 months of age), and old adulthood (36-48 months of age) in three separate GLMs or GLMMs. These ages correspond to different phases of adult life with respect to when increases in mortality and declines in reproductive performance are observed.[26] Telomere change between sampling points was calculated as the standardized measure of change (D) suggested by Ref. 33, an index that corrects for regression to the mean; following this adjustment, there was no relationship between initial telomere length and telomere change over any of the three time periods. We expressed D so that a negative value indicates telomere loss and a positive value indicates increase. Each of the three standardized measures of telomere change (ie, during early, middle, and old adulthood) was correlated with the simple difference in telomere length between the same two time points ($P < .0001$ for all, Pearson's r: $0.68 \leq r \geq 0.71$). In initial models of telomere change during each of the three adult stages, we also included the individuals' change in body mass over the time period involved as well as their cumulative reproductive effort during that time (ie, number of eggs laid or number of chicks raised, up to 13, 36, or 48 months as appropriate) as continuous covariates; neither body mass change nor reproductive effort was significant at any adult stage. Replicate (1 or 2) was always included in the final models as a main factor, and we also initially entered the interaction treatment x replicate to check for potential differences in the effect of the treatment between the two replicates; this interaction was never significant demonstrating that even if there were differences in actual telomere length between birds in the two replicates, the differences due to our experimental treatments were consistent across the two replicates of the experiment and the replicates are combined in the figures of telomere change to show the best overall representation of the data. These nonsignificant variables/interactions ($P > .3$) were removed from the final models. We also performed a complementary analysis of telomere length across the three age-time points, using the full dataset and using only the females that survived up to 48 months of age to exclude potential bias in the results due to selective mortality (supporting information). All models met the assumptions of normality and homogeneity, which was assessed via graphical diagnostics of the residuals as recommended in Ref. 34. Family identity was included as random factors to account for nonindependence of females from the same brood. We then tested using GLMMs with a binomial error structure and logit function whether telomere measurements at either 36 or 48 months predicted the probability of survival during middle adulthood or old adulthood,

TABLE 1 GLMM modeling with Gaussian distribution to test for potential treatment differences in telomere length upon the start of the challenging exposure at 5 months of age between the two treatment groups and replicates

Parameter	Estimate	SE	*df*	*t*	*P*
Family identity (*r*)	0.028				
Residual	0.182				
Intercept	−0.391	0.060	142.438	−6.493	<.0001
Treatment (challenged)	−0.034	0.060	156.739	−0.559	.577
Replicate (2)	−0.043	0.068	95.247	−0.633	.528

Note: Female identity was added as random factor, *r* indicates the associated estimated variance, and fixed factor estimates are indicated in parenthesis. Number of females: 214.

Abbreviation: GLMM, generalized linear mixed model.

respectively. In preliminary survival models we entered the interaction between treatment and replicate, which was never significant ($P \geq .5$) and consequently removed from the final models. Model fit of the survival binomial models was evaluated via graphical check of the binned plot residuals versus fitted values as recommended in Ref. 34. Unless otherwise specified descriptive statistics are provided as mean ± SEM.

3 | RESULTS

3.1 | Effects of age and environmental circumstances on telomere dynamics

Prior to the start of the experiment (5 months of age), there were, as expected, no differences in telomere length between those individuals randomly allocated to either the control or challenged group within each replicate (full statistics in Table 1; see also Figure S1). We then examined the pattern of telomere change in the same young-, middle-, and old-age periods as in our previous study of mortality patterns. We found no difference in telomere change between the control and the environmentally challenged groups during early adulthood, that is between 5 and 13 months of age (Figure 1A and Table 2a). During middle adulthood (between 13 and 36 months of age), the magnitude of telomere change significantly differed between the two groups in the direction predicted by the mortality data. Telomere loss in middle age was much higher in the high mortality group—that is, the control group (averaging 20%) than in the challenged group (averaging 7%—Figure 1B and Table 2b). In old adulthood (between 36 and 48 months of age), however, the pattern of telomere change appeared reversed (Figure 1C and Table 2c) due to a slight increase in telomere length in the control females (averaging 10%) and a slight decrease in the challenged group (averaging 9%). The complementary analysis of telomere length, as opposed to telomere change, across the three adult stages (performed using both the full dataset, and using only those birds that survived to 48 months of age) yielded similar statistical differences between the two treatment groups, suggesting that the higher telomere loss in the middle-aged controls compared to the middle-aged challenged birds occurred within individuals and was not due to selective mortality (Table S2 and Figure S1).

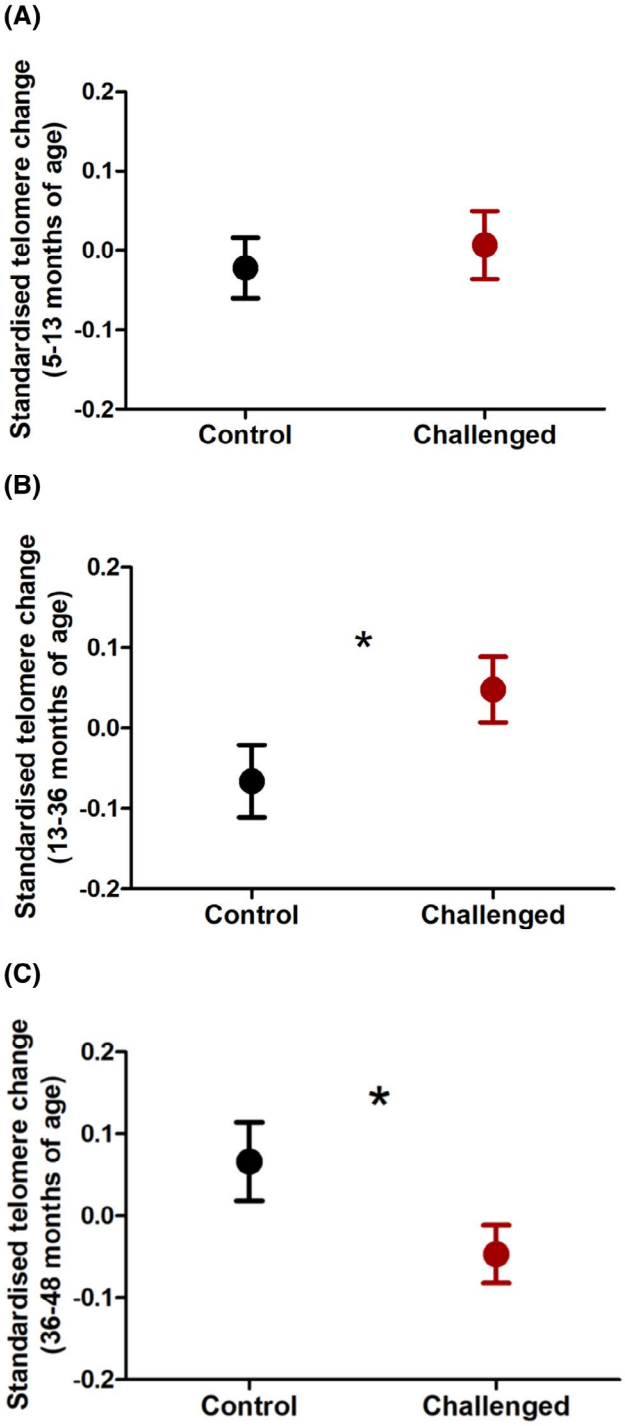

FIGURE 1 Standardized average telomere change (±SEM) corrected for regression to the mean using[33] in relation to the treatment group during (A) early adulthood (5-13 months of age), (B) middle adulthood (13-36 months of age), and (C) old adulthood (36-48 months of age) in female zebra finches. Sample sizes in (A) 101 controls and 102 challenged, in (B) 65 controls and 81 challenged, and in (C) 44 controls and 51 challenged

3.2 | Telomere length, environmental conditions, and probability of survival

We further examined the link between survival, stress exposure, and telomere length at the individual level. During the young adult phase, as would be expected, very few birds died in either treatment group (4 out of 107 controls and 2 out of 107 challenged birds). For the two older adult phases, we examined the extent to which individuals' telomere length at the start of each phase was predictive of the probability of

survival to the end of that phase. While the mortality risk was lower in the challenged group during middle age (ie, 13 to 36 months of age when 35 out of 103 controls died and 22 out of 105 challenged birds died; Table 3a), this was not linked to differences in individual telomere length. On the other hand, the probability of death during the old age period (36-48 months, when 17 out of 68 control birds and 25 out of 83 challenged birds died) was related to telomere length at 36 months (Table 3b); regardless of the environmental circumstances, those birds which died during this old-aged phase had shorter telomere lengths at 36 months than those that survived (Figure 2).

4 | DISCUSSION

The design of this study was such that we manipulated stress exposure only in adulthood, with all the study animals having had the same, nonchallenging early-life conditions. Our environmental manipulation produced relatively moderate increases in corticosterone (ie, within the baseline rather than acute range of variation of the stress response[25,26]). We performed two replicates of the experiment, and the results showed the same patterns in telomere change in both the replicates (we never found an interaction effect between treatment and replicate in our analyses). We previously reported that, during middle age, the risk of death was reduced in the birds kept in the more challenging conditions in comparison with the control birds, but mortality risk in early and late adulthood was unaffected.[26] This demonstrates that the stress exposure itself is not compromising survival. We found that the persistent and repeated exposure to mildly challenging environmental conditions during adult life influenced telomere dynamics during adulthood in a manner that was consistent with our predictions based on the observed mortality patterns. There was no effect of the treatment on the change in telomere length during early adulthood (5-13 months of age). During middle adulthood, (13-36 months of age), telomere length decreased in birds in both treatments, but females exposed to challenging conditions experienced substantially less telomere loss than controls, which is in line with their higher survival during this time. In old adulthood

TABLE 2 GLMM modeling to assess the effect of the treatment on standardized telomere change during (a) early adulthood, (b) middle adulthood, or (c) old adulthood (measure corrected for the regression to the mean following,[33] full details in data analyses)

Parameter	Estimate	SE	*df*	*t*	*P*
(a) Standardized telomere change during early adulthood (5-13 months of age), n = 203					
Family id (r)	0.002				
Residual	0.148				
Intercept	−0.202	0.073	174.219	−2.786	.006
Treatment (challenged)	0.030	0.054	169.829	0.545	.586
Replicate (2)	**0.276**	**0.056**	**100.102**	**4.977**	**<.0001**
Telomere length at 5 months of age	0.029	0.065	197.884	0.446	.656
(b) Standardized telomere change during middle adulthood (13-36 months of age), n = 146					
Family id (*r*)	0.008				
Residual	0.125				
Intercept	−0.073	0.084	136.810	−0.878	.381
Treatment (challenged)	**0.127**	**0.060**	**120.542**	**2.098**	**.038**
Replicate (2)	0.093	0.067	85.273	1.389	.168
Telomere length at 13 months of age	−0.056	0.079	141.369	−0.713	.477
(c) Standardized telomere change during old adulthood (36-48 months of age), n = 95					
Family id (r)	0.011				
Residual	0.062				
Intercept	0.112	0.076	89.026	1.480	.142
Treatment (challenged)	**−0.123**	**0.055**	**79.353**	**−2.246**	**.027**
Replicate (2)	**−0.190**	**0.059**	**67.488**	**−3.240**	**.002**
Telomere length at 36 months of age	0.070	0.085	90.732	0.830	.409

Note:: Family id was added as random factor, *r* indicates its estimated variance; fixed factors estimates are indicated in parenthesis, in bold significant factors ($P < .05$), n indicates sample size for each age period.

Abbreviation: GLMM, generalized linear mixed model.

(36-48 months of age) the pattern of telomere change appeared reversed, with telomere loss, albeit modest in both groups, now being somewhat greater in the birds in the challenging conditions. We also found that, at the individual level, the relationship between telomere length and survival differed depending on treatment and life stage. Mortality rate increased in middle and old age as would be expected. However, risk of dying during middle age was not predicted by earlier telomere length at 13 months of age. Obviously, stress exposure has multiple effects on physiology, and our data support the proposal that telomere dynamics might represent an integrative measure of the impact of an individual's experience on its overall biological state.[35-37] However, it was only in old age that telomere length was predictive of mortality risk, with telomere length reached by 36 months predicting the probability of dying within the following year. This suggests that there could be a critical telomere length below which the expected association with mortality risk emerges and above which it disappears. This might be because the cause of death prior to 36 months is less likely to be due to age-related degeneration.

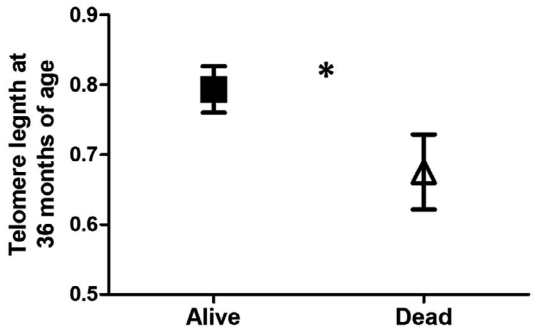

FIGURE 2 Relative telomere length (calibrated normalized T:S ratios) at 36 months of age in zebra finch females alive (square) or dead (triangle) by 48 months of age (data right-censored at 1455 days of age) in female zebra finches. Data are shown as average ± SEM

Our data support the idea that relatively mild physiological stress experienced only in adulthood can slow the onset of senescence. This is further supported by our previous finding that the challenged females showed delayed age-specific declines in reproductive performance.[26] It is unlikely that such an effect reflects age-specific changes in trade-offs between reproduction and self-maintenance processes. In fact, reproductive effort was not related to either telomere length or change, nor did we previously find an effect of lifetime reproductive effort on probability of survival.[26] A more plausible explanation is that the repeated mild stress exposure during adult life altered physiological capacities by promoting stress resilience. The observed mortality patterns are in line with a hormetic effect of exposure to mild dose stressors, as observed across various species.[38,39] Our environmental stressor was not designed to induce caloric restriction as the birds had over two thirds of daylight hours in which to replenish their daily energy requirements. The slight decline in body mass observed at 4 years of age (by 7%) in the challenged birds compared to the controls is not on the scale expected under caloric restriction protocols, which usually involve reduction in body mass higher than 10-15%.[40,41] The treatment is more akin to intermittent energy restriction paradigms and in particular, time restricted feeding in which energy intake is limited to specific time windows of the day.[42] Intermittent fasting regimes have been associated with hormesis-based processes[43] and are thought to induce some stress resilience.[44] Low to moderate stimulation of the physiological stress system could be an important mechanism here as it can give rise to

TABLE 3 Model outcomes of GLMMs (using a binomial distribution) to test if variation in telomere length measured at (a) 13 months of age or (b) at 36 months of age predicted subsequent survival

Parameter	Estimate	SE	*z*-Value	*P*
(a) Telomere length at 13 months and probability of survival at 36 months, n = 201				
Family id (r)	0.036			
Intercept	−0.672	0.435	−1.543	0.123
Treatment (challenged)	**−0.759**	**0.335**	**−2.267**	**.023**
Replicate (2)	−0.019	0.355	−0.054	.957
Telomere length at 13 months of age	0.046	0.434	0.106	.915
(b) Telomere length at 36 months and probability of survival at 48 months, n = 142				
Family id (r)		0.645		
Intercept	−0.653	0.596	−1.096	.273
Treatment (challenged)	0.411	0.447	0.918	.359
Replicate (2)	0.869	0.479	1.814	.070
Telomere length at 36 months of age	**−1.648**	**0.768**	**−2.145**	**.032**

Note: Fixed factors estimates are indicated in parenthesis, in bold significant factors ($P < .05$); *r* indicates random factor with its estimated variance; n indicates sample size in each model. Data were right-censored at 1096 days of age (analysis of survival up to 36 months of age) or 1455 days of age (analysis of survival up to 48 months of age).

adaptive neuroendocrine responses that can promote stress resilience, restorative processes, and repair capabilities.[3,45] Acute and short-term exposure to environmentally generated stress in both humans and laboratory rats can lead to rapid upregulation of telomerase activity.[46,47] In our study, it is thus possible that the increased resilience of the better surviving challenged birds during middle age mitigated telomere loss possibly by stimulating telomerase activity.

Overall, our experiment shows that repeated stress exposure in adult life can alter telomere dynamics in a way that is consistent with observed mortality patterns. Our data provide important experimental evidence that changes in telomere dynamics are likely to be a fundamental component of the link between stress resilience and aging rate. Our study design, which examined the effect of repeated stress exposure, does not allow us to separate the effects of age and the duration of the challenging exposure as the two are inextricably interlinked, as it would happen in nature. However, we did not find any evidence to suggest that the response to the stress exposure in terms of corticosterone exposure changed across the experimental period[25,26] and the fact the effects wax and wane across the life course suggest that duration of exposure is not the key factor. Nonetheless, it would be interesting to experimentally manipulate the duration of stress exposure in such a way to at least partially uncouple this from age, for example, by starting the exposure at different ages and fixing its duration, though this would inevitably mean that the stress exposure would be relatively short-term.

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AUTHOR CONTRIBUTIONS

V. Marasco, W. Boner, B. Heidinger, and P. Monaghan designed the experiment; V. Marasco and P. Monaghan analyzed the data and wrote the manuscript; all authors carried out the animal experimental procedures; W. Boner and V. Marasco carried out the laboratory telomere analyses; all authors commented on previous drafts of the manuscript.

Data accessibility. For requests of the data used in this study, please contact the corresponding authors.

DATA AVAILABILITY STATEMENT

Data will be made publicly available upon publication.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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