J Appl Physiol 129: 873–879, 2020.
First published September 3, 2020; doi:10.1152/japplphysiol.00570.2020.

RESEARCH ARTICLE

The effect of long-term ultra-endurance exercise and *SOD2* genotype on telomere shortening with age

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Submitted 7 July 2020; accepted in final form 2 September 2020

Hernando B, Gil-Barrachina M, Tomás-Bort E, Martinez-Navarro I, Collado-Boira E, Hernando C. The effect of long-term ultra-endurance exercise and *SOD2* genotype on telomere shortening with age. *J Appl Physiol* 129: 873–879, 2020. First published September 3, 2020; doi:10.1152/japplphysiol.00570.2020.—Telomere shortening, a well-known biomarker of aging, is a complex process influenced by several intrinsic and lifestyle factors. Although habitual exercise may promote telomere length maintenance, extreme endurance exercise has been also associated with increased oxidative stress—presumed to be the major cause of telomere shortening. Therefore, the pace of telomere shortening with age may also depend on antioxidant system efficiency, which is, in part, genetically determined. In this study, we aimed to evaluate the impact of ultra-endurance exercise and oxidative stress susceptibility (determined by the rs4880 polymorphism in the superoxide dismutase 2 (*SOD2*) gene) on telomere length. Genomic DNA was obtained from 53 sedentary individuals (34 females, 19–67 yr) and 96 ultra-trail runners (31 females, 23–58 yr). Indeed, blood samples before and after finishing a 107-km-trail race were collected from 69 runners to measure c-reactive protein (CRP) levels and, thus, analyze whether acute inflammation response is modulated by the *SOD2* rs4880 polymorphism. Our results revealed that telomere length was better preserved in ultra-trail runners compared with controls, especially in elderly runners who have been regularly training for many years. Carrying the *SOD2* rs4880*A allele was significantly associated with having shorter telomeres, as well as with having increased CRP levels after the ultra-trail race. In conclusion, habitual ultra-endurance exercise had a beneficial effect on telomere length maintenance, especially at older ages. This study also suggested that the *SOD2* rs4880 polymorphism may also have an impact on acute and chronic oxidative-related damage (inflammatory response and telomere length) after an ultra-trail race.

NEW & NOTEWORTHY Habitual ultra-endurance exercise seems to promote telomere length maintenance, especially at older ages. In addition, the beneficial effect of ultra-endurance training on biological aging is higher in ultra-trail runners who have been engaged to ultra-endurance training during many years. Finally, and for the first time, this study shows that the *SOD2* rs4880 polymorphism has a significant impact on telomere length, as well as on acute inflammatory response to a 107-km trail race.

acute inflammatory response; oxidative stress; polymorphism; telomere; ultra-endurance training

INTRODUCTION

Telomeres are repetitive DNA sequences found at distal ends of each chromosome that function to maintain genome integrity. Telomeres shorten with each cell division as a normal cellular process, and telomere length is, therefore, a well-known biomarker of cellular replicative history and biological age (7). Telomere length is a dynamic feature, and its regulation is a complex process influenced by several lifestyle factors, including body weight, smoking, diet, physiological stress, and physical activity (24, 37). The extent of telomere shortening with age may, thus, depend on the oxidative stress experienced by an individual after interacting with its environment. Oxidative stress, caused by an imbalance between the production of reactive oxygen species (ROS) and the intrinsic antioxidant capacity (5), preferentially damages telomeric regions due to their high content of guanines and inhibits telomerase activity (19, 29, 31).

On the other hand, upregulation of shelterin-telomerase complex may prevent or postpone the continued telomere attrition with age. A beneficial effect of long-term endurance exercise on telomere length has been recently reported (8, 13, 21, 33), which may be related to the increased telomerase activity and greater expression of shelterin-associated genes observed in nonsedentary individuals (14, 22, 32). However, extreme exposure to ultra-endurance exercise (such as an ultra-trail race, which has a total distance longer than a marathon) has also been associated with increased ROS generation and oxidative stress (38), and with telomere shortening (8). The increased ROS production may additionally promote the well-described increase of acute inflammation biomarkers following ultra-endurance exercise (1, 20). Although habitual exercise seems to increase the plasma antioxidant capacity (10), genetic variants in genes involved in the antioxidant defense system may lead to reduced protection against oxidative stress (3).

Taking everything in mind, recent studies have suggested a potential relationship between exercise, oxidative stress, inflammation response, and telomere length (28, 39). However, little is known about the role of individual's susceptibility to oxidative damage in this scenario. Increasing our knowledge on the molecular pathways responsible for exercise-modulated telomere maintenance may be of vital value for both scientists and practitioners.

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In this study, we compared telomere length, as well as the pace of telomere shortening with age, between 53 sedentary individuals and 96 ultra-trail runners. The large size of our cohort, compared with analogous works, allowed us to explore the effect of several risk factors on telomere length. First, variability of telomere length across ultra-trail runners was analyzed, taking into account the individual's age and years of training. Furthermore, for the first time, this study assessed the impact of the rs4880 polymorphism in the superoxide dismutase 2 (*SOD2*) gene, which encodes one of the components of key enzymes of the antioxidant defense system, on telomere length and acute inflammation response. We focused on analyzing this genetic variant because a potential role of *SOD2* rs4880 on oxidative stress response after performing an intense physical activity has been previously described (9).

MATERIALS AND METHODS

Study subjects and data collection. A total of 149 nonsmoking, healthy individuals (53 sedentary individuals and 96 ultra-trail runners), aged from 19 to 67 yr, were included in this case-control study. (A supplemental data set is available at https://doi.org/10.6084/m9.figshare.12618725.) From all participants, sixty-five were females (43.62%). Selected ultra-trail runners were participants of the Penyagolosa Trails CSP race in 2019 and were required to have previously completed at least one ultra-marathon (>60 km). Sedentary individuals were randomly recruited among the students and staff of the Jaume I University of Castellon. All individuals were Europeans of Spanish origin with no apparent pathology and did not take any medications. Written informed consent was provided by all participants, and the study was approved by the Ethics Committee of the Jaume I University of Castellon (Spain), with the code number CD/007/2019. This study is enrolled in the ClinicalTrails.gov database, with the code number NCT03990259 (https://www.clinicaltrials.gov/).

A standardized questionnaire was used to collect information on sex, age, average training distance per week, and years of endurance training (Supplemental Material S1, https://doi.org/10.6084/m9.figshare.12781817). To avoid misclassification, each individual completed the questionnaire under the supervision of a professional. A buccal swab was used to collect a saliva sample from each participant. Genomic DNA was isolated from saliva samples using the QIAamp DNA mini kit (Qiagen, Hilden, Germany), following the manufacturer's recommendations.

Additionally, 43 ultra-trail runners voluntarily donated a blood sample the day before the Penyagolosa Trails CSP race and after crossing the finish line. From all of them, 32 runners finished the 107-km trail race and were, therefore, analyzed. With the aim of increasing sample size, blood samples from 37 finishers of the Penyagolosa Trails CSP 2015 were also analyzed. Blood samples were collected and processed as previously described (25).

Analysis of telomere length. A real-time PCR assay was applied for estimating the relative telomere length of each sample (11), which reflects the ratio (T/S) of telomere repeat copies (T) to copy numbers of the 36B4 single-copy gene (S). The quantitative real-time PCR was performed on a StepOnePlus Real-Time PCR System. For quality control, we included a negative and positive control in each 96-well plate. Each analysis was performed in triplicate. All intra-assay standard errors were less than 5% of the respective sample mean.

Genotyping assays. The *SOD2* rs4880 (c.47G>A, p.Ala16Val) polymorphism was selected to be genotyped to assess the potential relationship between the susceptibility to oxidative stress and telomere length. Genotyping of the single nucleotide polymorphism (SNP) was conducted by the Spanish National Genotyping Centre (CeGen-PRB2, Santiago de Compostela, Spain) as a contract service, as previously described (17). Individuals were then classified according to their genotype.

Assessment of acute inflammation response. Serum levels of C-reactive protein (CRP) were measured to evaluate the acute inflammation response to achieving the 107-km trail race. For each runner, we calculated the fold change of CRP values from the day before (B) to the finish line (F), as follows:

$$\Delta\text{CRP} = \frac{CRP_F - CRP_B}{CRP_B}$$

Statistical analysis. Statistical analyses were performed using the R software (http://www.R-project.org). A significance level of 0.05 was considered for rejection of the null hypothesis. All analyses were two-sided. Standardized measures of effect size were additionally estimated for determining the meaningfulness of outcomes (16). Unknown values were excluded at each specific analysis.

A linear model was applied to correlate the relative telomere length (T/S ratio) determined per sample with individual's age. A Kruskal-Wallis test was used for testing differences in telomere length among groups, since T/S ratio was not normally distributed according to the Shapiro-Wilk test.

Fisher's exact test was used both to check for deviations from Hardy-Weinberg equilibrium (HWE) of rs4880 genotype frequencies and to compare differences in allele counts between cases and controls. Association between the *SOD2* rs4880 genotype and telomere length was assessed according to the additive model of inheritance via linear regression, which was adjusted by sex and individual's subgroup (control or runner) or training years to minimize confounding effects. The relative telomere length of each sample was normalized by an individual's age for genetic association analysis performed with all individuals pooled, in order to account for interindividual variability related to age effects. The mean difference per alternative allele carried and its corresponding 95% confidence interval with respect to the reference-allele homozygous genotype, as well as the associated β and P value obtained from an overall allele effect, were estimated.

A Kruskal-Wallis test was used for comparing the percentage of ΔCRP according to individual's genotype in the *SOD2* rs4880 polymorphism. Individuals were classified as noncarriers and carriers of the alternative A allele (GG versus AG/AA).

Except for comparison of ΔCRP levels, according to individual's genotype, all statistical analyses were additionally performed splitting volunteers as young (with less than 40 yr of age, the cohort median age) and elderly individuals ($\geq$ 40 yr old). The cohort median age was used as cut-off to have equivalent sample sizes in both age groups (70 young and 73 elderly individuals).

RESULTS

In this study, we aimed to evaluate the effects of ultra-endurance exercise on cellular aging by measuring the telomere length in 96 ultra-trail runners (34 females, 35.42%) and 53 sedentary controls (31 females, 58.49%). The ratio of female runners included in this study was significantly higher than the percentage of females participating in the Penyagolosa Trails CSP in 2019 (8%). However, the sex ratio of our ultra-trail runners' cohort was representative to the current population of ultra-endurance athletes (20, 30), and the trend of increasing female participation (18). No differences were observed between the mean age of sedentary individuals (39.96 yr, range 19–67) and ultra-trail runners (39.38 yr, range 23–58).

As expected, telomere length decreased with age for the entire sample independently of sex and subgroup (P value = 0.022, $\beta = -0.19$). However, telomere shortening with age was observed for the control group (P value = 6.93×10^{-3},

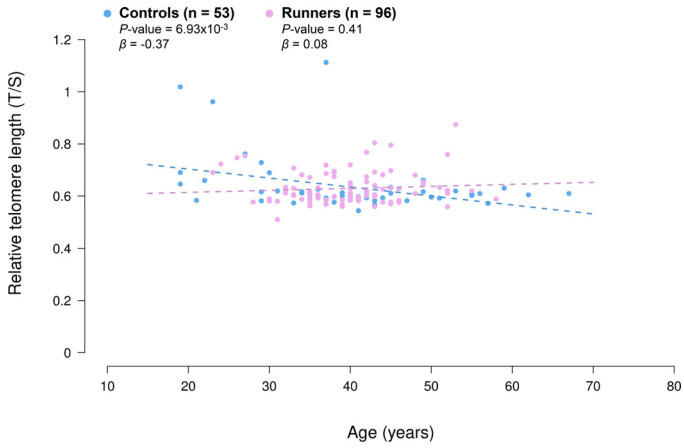

Fig. 1. Pace of telomere shortening with age in sedentary individuals and ultra-trail runners. Each individual is represented by a specific point. A linear model was applied to correlate the relative telomere length (T/S ratio) determined per sample with individual's age.

$\beta = -0.37$), but not for the ultra-trail data subset (P value = 0.41, $\beta = 0.08$) (Fig. 1).

In fact, telomere length of ultra-trail runners tended to be positively correlated with age. This unexpected result may be explained by the long-term beneficial effects on biological aging of doing regular aerobic exercise during many years. In this regard, elderly ultra-trail runners had longer telomere length compared with elderly sedentary individuals (P value = 0.014, $d = 0.54$; Fig. 2*B*). However, telomere length was not different between ultra-trail runners and control individuals younger than 40 yr (P value = 0.18, $d = 0.21$; Fig. 2*A*).

The protective effect of long-term ultra-endurance training on telomere length was also explored by splitting ultra-trail runners, according to the number of training years. Elderly ultra-trail runners that have been training ≥11 yr (the subgroup median years of training) seemed to have longer (but not significantly), telomeres compared with elderly individuals that have been training less than 11 yr (P value = 0.069, $d = 0.46$; Fig. 2*D*). The intraindividual variability in telomere length related to the total years of ultra-endurance training was not observed in young runners (P value = 0.72, $d = 0.28$; Fig. 2*C*).

Differences in telomere length across individuals may also be explained by their susceptibility to oxidative stress, which may be, in part, determined by the antioxidant system efficiency. For that reason, we focused on exploring the effect of the *SOD2* rs4880 genotype on telomere length. The minor allele frequency (MAF) in our population was 0.47. No evi-

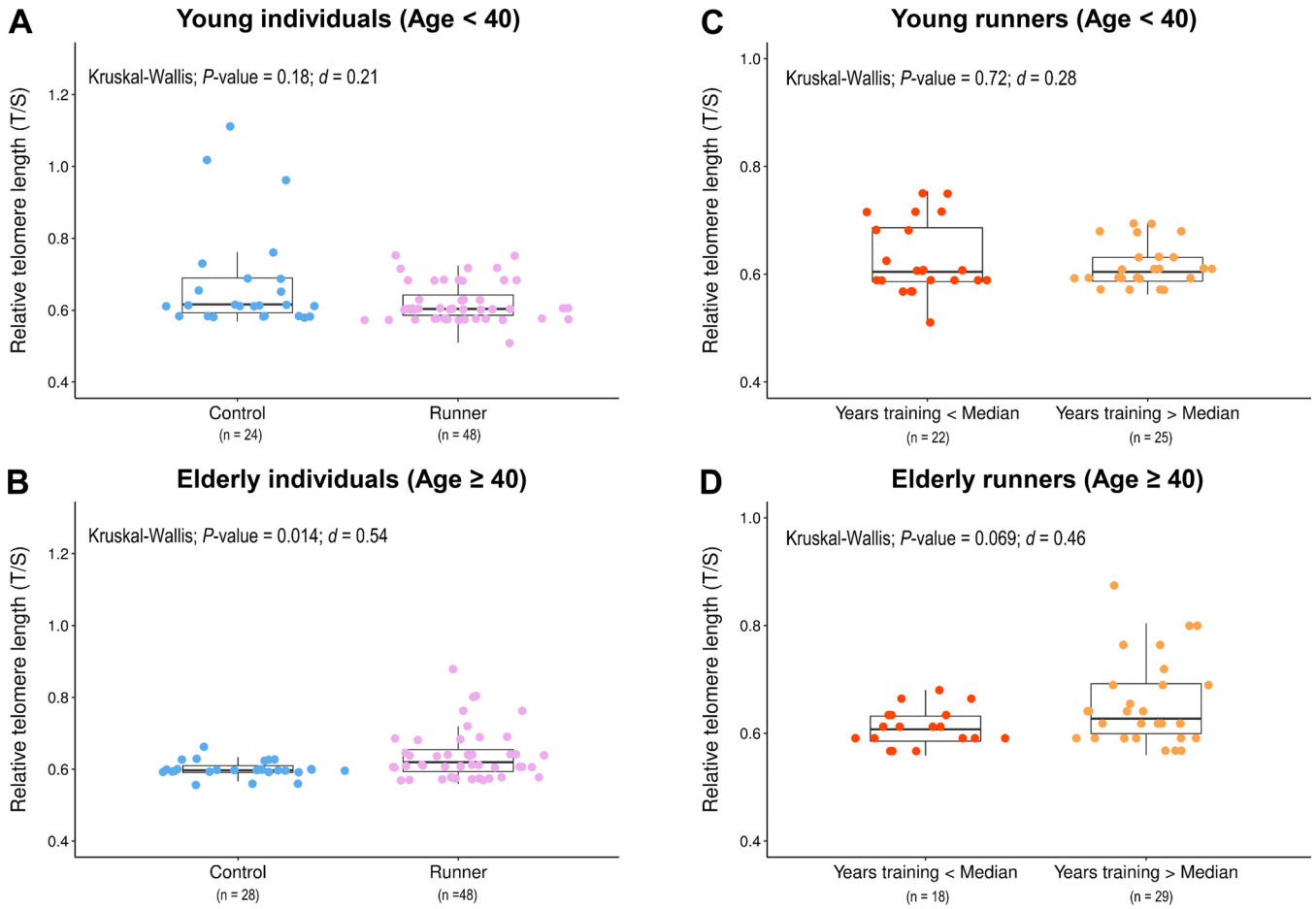

Fig. 2. Boxplots showing the beneficial effect of long-term ultra-endurance training on telomere length. *A* and *B*: telomere length comparison between sedentary individuals and ultra-trail runners. Comparison was performed by splitting the cohort in young individuals (*A*) and elderly individuals (*B*). Telomere length comparison between ultra-trail runners, according to the total years of training. Comparison was performed by splitting the cohort in young individuals (*C*) and elderly individuals (*D*). A Kruskal-Wallis test was applied to perform comparisons among groups. In all boxplots, each individual is represented by a specific point.

dence of departure from HWE for the *SOD2* rs4880 polymorphism was found (P value = 1.00). No differences in the distribution of genotype frequencies between runners and controls were observed (P value = 0.89, $d = -0.02$).

The alternative allele (A) in the *SOD2* rs4880 polymorphism was correlated with having a shorter telomere length, independently of sex and individual's subgroup (P value = 0.011, $\beta = -0.21$; Fig. 3*A*). Interestingly, the association of this genetic variant with telomere length was statistically significant for young ultra-trail runners (P value = 0.046, $\beta = -0.30$; Fig. 3*B*), but not for elderly individuals (P value = 0.125, $\beta = -0.22$; Fig. 3*C*). This result suggests that environmental influences (as performing regular aerobic exercise during many years) may have a key long-term impact on biological aging that masks genetic effects.

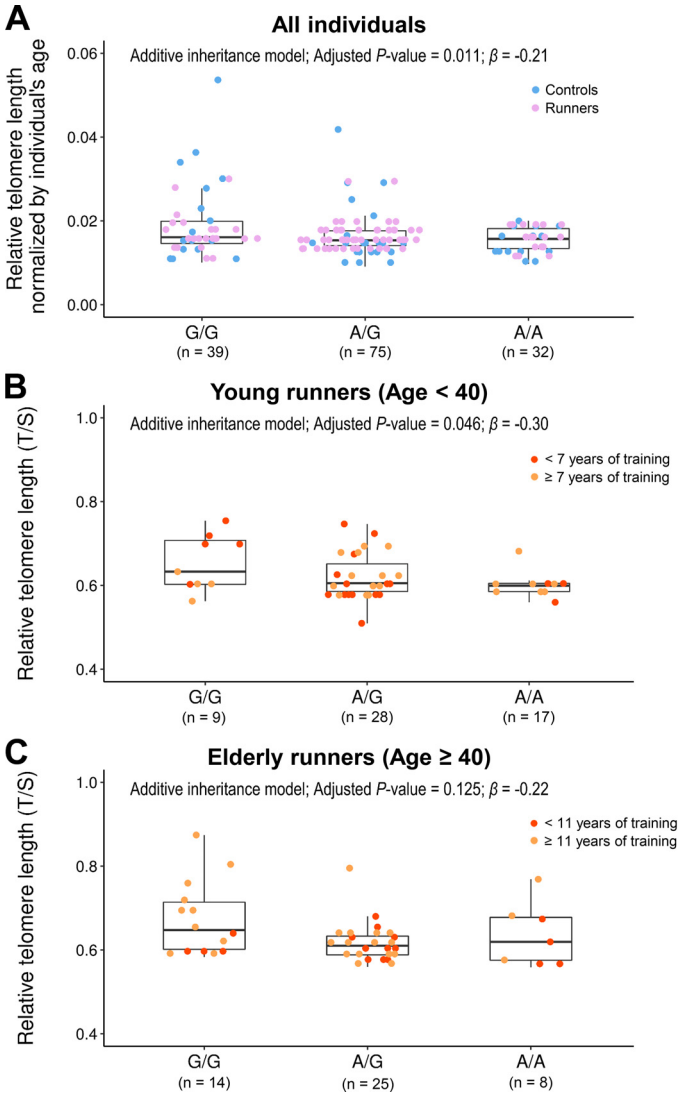

Fig. 3. Association of the *SOD2* rs48800 polymorphism with telomere length. Genetic association was assessed according to the additive model of inheritance via logistic regression for all individuals pooled (*A*), for young ultra-trail runners (*B*), and for elderly ultra-trail runners (*C*). Telomere length was normalized by individual's age when all individuals were assessed together. Association analyses were adjusted by sex and individual's subgroup (control or runner, in *A*) or years of training (in *B* and *C*). In all boxplots, each individual is represented by a specific point.

Given the close relationship between oxidative stress and inflammatory response, we also analyzed weather *SOD2* genotype is associated with the levels of acute inflammatory biomarkers after running the Penyagolosa Trails CSP race. Our results show that carriers of the alternative allele in the *SOD2* rs4880 polymorphism (A/−) increased the levels of CRP, an acute inflammatory protein, more than homozygotes for the reference G allele (P value = 0.023, $d = 0.44$). Note that CRP was only measured before and after the race in a total of 32 ultra-trail runners from the Penyagolosa Trails CSP 2019 (only 32 out of 43 ultra-trail runners who voluntarily donated blood samples finished the race) and 37 ultra-trail runners from the Penyagolosa Trails CSP 2015.

DISCUSSION

Because of the characteristics of ultra-trail races, training for and racing an ultra-trail normally result in a huge variety of injuries and acute physiological disorders (20), which are normally transient and tend to be recovered within a few days (6, 25, 46). However, although ultra-endurance athletes regularly perform such exhaustive physical effort, several studies have demonstrated greater longevity of individuals who frequently perform vigorous aerobic activities compared with the general population (23, 35, 36).

Apart from reducing risk for cardiovascular and metabolic diseases, habitual endurance exercise may have a long-term beneficial effect on telomere length maintenance and, therefore, on biological aging (13, 21, 45). In this regard, we have not only verified that elderly ultra-trail runners exhibit longer telomeres compared with age-matched sedentary individuals, but we have also shown that the pace of telomere shortening with age is significantly reduced in ultra-trail runners in comparison to nonactive controls.

Actually, telomere length seemed to be elongated with age in ultra-trail runners. We are aware that telomere lengthening in cross-sectional studies may be an artefact since measurement is made at only one time point. However, previous longitudinal studies show that following a program of moderate physical exercise significantly increases telomere length (27, 32). Lifestyle of participants may also be a confounding factor in cross-sectional studies by increasing variability across cohorts. For this reason, we applied a selection criteria focused on having comparable participants—except for years of training—regarding their nonsmoking and health status.

The long-term beneficial effect of endurance training on biological aging was also supported by the lack of significant differences in telomere length between young ultra-trail runners and age-matched sedentary individuals. Accordingly, previous studies also stated that ultra-endurance exercise is associated with preserved telomeres in elderly trained athletes, but not in young athletes, in comparison with their sedentary counterparts (8, 33). The nonsignificant positive correlation between ultra-endurance exercise and telomere length in young runners may be explained by the lower number of years engaged in regular aerobic training. In this regard, our data revealed that elderly ultratrail runners who had been training for many years tended to have longer telomeres than those reporting fewer years of training, corroborating the long-term protection effect of ultra-endurance exercise on telomere length. However, we cannot ignore the possible confounding

effects of other unmeasured variables, such as antioxidant intake and vitamin supplementation.

It is well documented that the elevation of oxygen uptake by exhaustive exercise results in greater production of superoxide radicals and other ROS (2, 15, 26, 40), and finally in increased oxidative stress—presumed to be the major cause of telomere shortening (19). On the other hand, numerous studies confirmed that regular training causes adaptation to acute effects of high-intensity exercise by, among others, upregulating antioxidant enzymes (28, 34, 39). The better redox balance status (according to antioxidant/prooxidant ratios) displayed by endurance runners may lead to less telomere attrition (39). Since antioxidant enzyme capacity to scavenge oxygen radical is partially explained by individual's genotype (3), we speculated that genetic variants in antioxidant-related genes may impact on intraindividual variability of telomere length—especially on inactive and less-trained individuals given that the high-trained runners may be adapted to acute damage of exhaustive exercise by multiple mechanisms.

With the purpose of exploring this hypothesis, we focused on analyzing the rs4880 polymorphism in the *SOD2* gene, a G-to-A transition at nucleotide 47 (c.47G>A), that results in a change from alanine to valine at codon 16 (p.Ala16Val). The alternative A allele has been shown both to decrease mRNA stability and to disrupt the structure of SOD2, thus, limiting its import into mitochondria. As a result, the mutant protein presents significantly lower activity than the wild-type form (41). This genetic variant has been previously associated with several chronic, metabolic, and cardiovascular diseases (4, 43, 44), as well as with biological aging profile (higher senescence markers and DNA damage) (42). Our results supported that the alternative A allele may have a negative impact on telomere length (a well-known aging biomarker), presumably by not hampering the oxidative DNA damage. However, because no association between *SOD2* genotype and telomere length was found in elderly runners, its adverse effect may be, in turn, mitigated by long-term regular ultra-endurance exercise adaptations. Actually, elderly runners carrying the alternative A allele (associated with having shorter telomeres) with relative longer telomeres were those that had been performing regular ultra-endurance training for more than a decade (tan/beige dots in Fig. 3*C*).

Finally, we also explored the potential effect of the *SOD2* rs4880 polymorphism on the acute inflammatory response after running a 107-km-trail race (Fig. 4). Running an ultra-endurance race has been shown to increase serum inflammation biomarkers such as c-reactive protein (20), which may be, in part, promoted by increased ROS production (1, 12). Therefore, it was reasonable to speculate that runners with decreased antioxidant capability (carriers of the *SOD2* A allele) may present an exacerbate inflammatory response after running an ultra-trail race, compared with runners who are homozygous for the reference G allele. Since blood samples before and after the race were available only from 69 ultra-trail runners, this interesting result should be verified by increasing sample size.

Much work remains to be done before a full understanding of the impact extent of *SOD2* rs4880 polymorphism on the intraindividual variability on telomere length, as well as on acute inflammatory response to an ultratrail race. Genetic association analyses should always be interpreted with caution and cannot be extrapolated to other populations due to the differences on genetic background. Thus, results should be verified in an independent population of similar ethnic origin. In addition, further research should also be done to correlate the *SOD2* rs4880 genotype with the plasma redox status (by measuring antioxidant/pro-oxidant activity and oxidative damage levels), as well as with the ability to recover of exercise-induced damage.

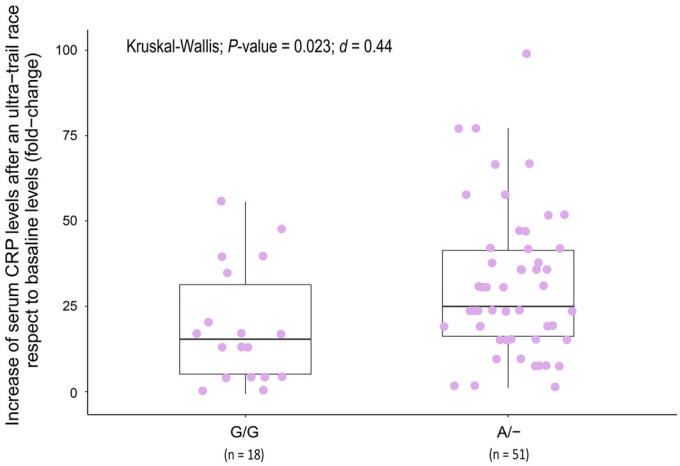

Fig. 4. Impact of the *SOD2* rs4880 polymorphism on acute inflammatory response after running a 107-km-trail race. Increase of c-reactive protein (CRP) levels respect to baseline were compared according to individual's *SOD2* genotype. A Kruskal-Wallis test was applied to perform comparisons between carriers and noncarriers of the alternative A allele. In all boxplots, each individual is represented by a specific point.

In summary, our study suggested that habitual ultra-endurance exercise have a beneficial effect on telomere length maintenance, especially at older ages. We also observed a significant impact of the *SOD2* rs4880 polymorphism on telomere length, which seems to be mitigated by the total years of ultra-endurance training, as well as on acute inflammatory response to running an ultra-trail race. Therefore, knowing the *SOD2* rs4880 genotype of an ultra-trail runner may help coaches and practitioners establish individualized training and nutrition routines that prevent carriers of *SOD2* A allele from telomere shortening and having severe inflammation levels, as consequence of their increased susceptibility to oxidative stress.

ACKNOWLEDGMENTS

This research has been performed thanks to the collaboration of Vithas-Nisa Hospitals group, Penyagolosa Trails, and Catedra Endavant Villarreal CF de l'Esport. The authors are also grateful to all the staff involved in the organization of the Penyagolosa Trail CSP 2019 and all volunteers participating in this study. We would like to thank Maria Torres for her expert technical support with genotyping.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

B.H. and C.H. conceived and designed research; B.H., M.G.-B., and E.T.-B. performed experiments; B.H., M.G.-B., and E.T.-B. analyzed data; B.H., M.G.-B., E.T.-B., and C.H. interpreted results of experiments; B.H. prepared figures; B.H. drafted manuscript; B.H., I.M.-N., E.C.-B., and C.H. edited and revised manuscript; B.H., I.M.-N., E.C.-B., and C.H. approved final version of manuscript.

ENDNOTE

At the request of the authors, readers are herein alerted to the fact that additional materials related to this manuscript may be found at https://doi.org/10.6084/m9.figshare.12618725.v1. These materials are not a part of this manuscript and have not undergone peer review by the American Physiological Society (APS). APS and the journal editors take no responsibility for these materials, for the website address, or for any links to or from it.

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