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Review

Master athletes have longer telomeres than age-matched non-athletes. A systematic review, meta-analysis and discussion of possible mechanisms

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ABSTRACT

The aim of this systematic review and meta-analysis was 1) to assess whether master athletes have longer telomeres than age-matched non-athletes and 2) discuss possible underlying mechanisms underlying telomere length preservation in master athletes. A literature search was performed in PubMed, Web of Science, Scopus and SPORTDiscus up to August 2020. Only original articles published in peer-reviewed journals that compared telomere length between master athletes and aged-matched non-athletes were included. Eleven studies fulfilled eligibility criteria and were included in the final analysis. Overall, 240 master athletes (51.9±7.5 years) and 209 age-matched non-athletes (50.1±9.1 years) were analyzed. Master athletes had been participating in high-level competitions for approximately 16.6 years. Pooled analyses revealed that master athletes had longer telomeres than aged-matched non-athletes (SMD=0.89; 95% CI=0.45 to 1.33; $p<0.001$). Master athletes showed lower pro-oxidant damage (SMD=0.59; 95% CI=0.26 to 0.91; $p<0.001$) and higher antioxidant capacity (SMD=-0.46; 95% CI=-0.89 to −0.03; $p=0.04$) than age-matched non-athletes. Further, greater telomere length in master athletes is associated with lower oxidative stress and chronic inflammation, and enhanced shelterin protein expression and telomerase activity. In conclusion, 1) master athletes have longer telomeres than age-matched non-athletes, which may be the result of 2) lower levels of oxidative stress and chronic inflammation, and elevated shelterin expression and telomerase activity.

1. Introduction

Aging is a natural and inevitable biological process that involves the progressive deterioration of bodily functions, predisposing the development of chronic diseases and ultimately resulting in death (Sousa-Victor et al., 2015). Research has shown that changes in the lifestyle, such as increased levels of physical fitness, can delay the risk of age-related diseases, such as type 2 diabetes (Francesconi et al., 2019), arterial hypertension (Diaz-Gutierrez et al., 2019), osteoporosis (Tian et al., 2017), Alzheimer (Kivipelto et al., 2018) and cancer (Vajdic et al., 2019), and increase the number of healthy life years (Blair et al., 1996; Kokkinos et al., 2010).

A well-accepted marker of biological aging is the telomere length. Telomeres are repetitive DNA sequences (TTAGGG) at the end of chromosomes (Blackburn, 1991; Blackburn et al., 2015) that are important to maintain the stability and integrity of the genome (Blackburn, 1991; Blackburn et al., 2015). Cell division leads to telomere length shortening that via cell malfunctioning in turn may in tissue impair the rate of cell division, eventually leading to loss of tissue function, characterized as "biological aging" (Blackburn et al., 2015; Sousa-Victor et al., 2015).

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Any lifestyle factor that protects telomere length is thus potentially staving off the aging process and may well lead to an increased number of healthy-life years.

Telomeres are stabilized by the shelterin complex that protect the telomeric DNA from damaging substances, such as reactive oxidative species (ROS) and pro-inflammatory cytokines (de Lange, 2018). Thus any factor other than the shelterin complex that also counteract these "pro-aging" agents, such as antioxidants and anti-inflammatory substances (Prasad et al., 2017) are likely to also protect telomere length during aging. In this context, it is promising to note that increased physical activity has indeed been associated with longer telomeres, a lower inflammatory status (Prasad et al., 2017), improved redox balance (Prasad et al., 2017) and a controlled blood glucose and lipid profile (Revesz et al., 2014; Sjogren et al., 2014).

Master athletes are people over 35 years of age (in most sports), who regularly participate in competitive events, and generally adhere to healthy lifestyle habits to preserve their competitive level, including stress management, healthful diet, and continuous, programmed and controlled practice of exercise (Korhonen et al., 2014; Kusy and Zielinski, 2015). Such habits commonly result in a more favorable metabolic profile, higher maximal oxygen uptake, and better physical fitness in favor to masters athletes in comparison to age-matched counterparts (Kusy and Zielinski, 2015). As such, master athletes have been recognized as a model of successful aging (Kusy and Zielinski, 2015).

This close relationship between master athletes' lifestyle and favorable biological aspects was in many studies associated with longer telomeres in master athletes than age-matched non-athletes (Aguiar et al., 2019; Borghini et al., 2015; Denham et al., 2013; LaRocca et al., 2010; Osthus et al., 2012; Rae et al., 2010; Simoes et al., 2017) and some even report a similar length as that seen in young adults (LaRocca et al., 2010; Rosa et al., 2020), but this is not an unequivocal observation (Laine et al., 2015; Mathur et al., 2013; Rae et al., 2010). A recent (Abrahin et al., 2019) systematic review and meta-analysis of the literature indicated that elite athletes had longer telomeres compared to sedentary controls. However, the authors did not provide the possible underlying mechanisms that might be modulated by master athletes' lifestyle to protect telomeres. This information is important and might serve as a guide for future recommendations for older adults.

Therefore, the purpose of this study was to assess 1) whether master athletes have longer telomeres than age-matched non-athletes with a meta-analysis of studies that reported telomere length in master athletes and sedentary peers and 2) discuss the possible underlying mechanisms that lead to the preservation of telomere length in master athletes.

2. Methods

This systematic review of the literature was undertaken according to the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions (version 5.1), and reported according to the Primary Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) Statement and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (Moher et al., 2015).

2.1. Eligibility criteria

The inclusion criteria of the present study were: (i) observational studies, including cross-sectional, longitudinal, and case-control studies that compared telomere length in any kind of sample (muscle; blood;

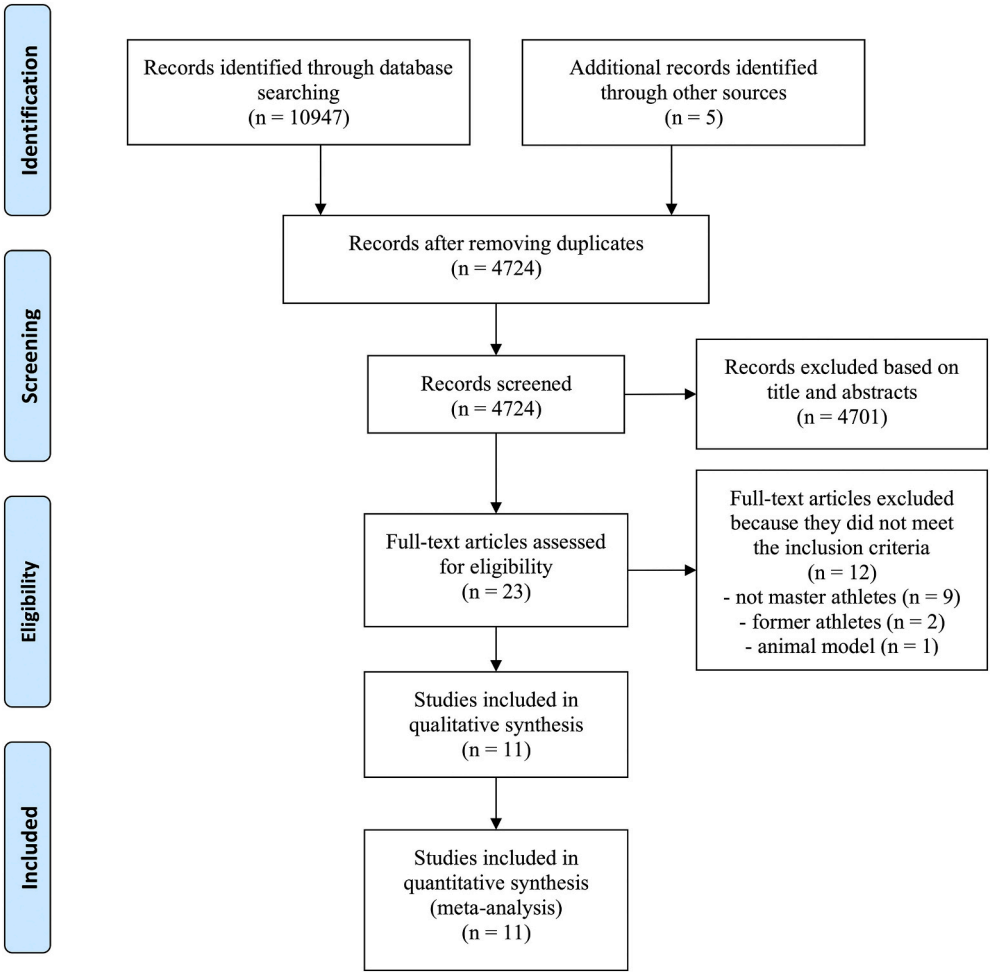

Fig. 1. Flow diagram for the strategy of searching for the studies.

saliva) between master athletes of both sexes and an age-matched control group; (ii) master athletes ≥ 35 years of age, following the recommendations of the World Masters Athletics (WMA) and > 5 years competitive experience in professional sport; (iii) high-level master athletes competing in national and international events; (iv) full reports (English language) in peer-reviewed journals. We excluded randomized clinical trials, investigations that classified master athletes according to physical activity levels, and studies using animal models (Fig. 1).

2.2. Search strategy

A comprehensive literature search was conducted up to August 2020 in the following databases: a) PubMed, b) Web of Science, c) Scopus, and d) Sports Discuss. Articles were retrieved by two independent investigators (SSA and CVS). The date of the first included article published in 2009 (Werner et al., 2009). Reference lists of reviews and retrieved articles were checked for additional studies and citation searches on key articles were performed on Google Scholar and ResearchGate for additional reports. The search strategy was based on PICOS (Population, Intervention, Comparison, Outcomes and Study design) using keywords and free text words such as telomere length and master athletes. Keywords and subject headings were exhaustively combined using Boolean operators. The complete search strategy used for PubMed is shown in Supplementary Material 1.

2.3. Data extraction and quality assessment

Two investigators (SSA and CVS) extracted coded variables using a standardized coding form. For the meta-analysis, we used the mean (± SD) of the reported telomere length. If telomere length, anti- and pro-oxidant parameters was only graphically displayed, Plot Digitalizer (SourceForge®) was used to extract the means and SD for further analysis. The methodological quality of all included articles was assessed using a modified version of the Downs and Black methodological scale. The Downs and Black scale is supported by the Cochrane Handbook as a useful tool to appraise the methodological quality of nonrandomized healthcare studies (Higgins and Green, 2011) and has been validated for use in observational studies (Downs and Black, 1998).

The number and appraisal of items from the original checklist was tailored to the scope of this systematic review (Supplementary Material 2). A total of 9 items were used, with a "1" for "yes", and "0" for "no" or "not reported" for each question. The methodological appraisal score ranged from 0 (lowest possible methodological quality) to 9 (highest possible methodological quality). To aid in interpretation, the study quality and risk of bias was based on the proportion of criteria attended: <50% = low quality, high risk of bias; 50–75% = fair quality, moderate risk of bias; 76–100% = high quality, low risk of bias.

2.4. Statistical analysis

The meta-analysis was conducted using Review Manager Software (RevMan 5.4; Cochrane Collaboration, Oxford, UK). Effect sizes were measured using mean and SD. Among all mechanisms investigated, only pro- and antioxidant markers were investigated in three or more studies. Consequently, only this mechanism was quantitatively tested. An antioxidant index, representing the total antioxidant capacity, was created using the formulas proposed by the Cochrane group (Higgins and Green, 2011).

a) Sample size = N1+N2;
b) Mean = (N1M1+N2M2)/(N1+N2);
c) SD = $\sqrt{([N1–1]\ SD12+[N2–1]SD2+N1N2/(N1+N2)\ [M12+M22—2M1M2]/N1+N2–1)}$.

Pooled effect sizes for: a) telomere length and b) antioxidant index were calculated based on standard mean difference (SMD), as different tools were used to assess oxidant status, such as thiobarbituric acid reactive substances (TBARS), catalase, superoxide dismutase and trolox equivalent. Due to the different characteristics of the included studies, a random-effect model was used to calculate the pooled effect size. Heterogeneity was assessed with the Cochran's Q test and given as tau-squared (τ^2), and inconsistency of effects (total variation across studies due to heterogeneity) was assessed using Higgins' I^2 statistic (Higgins et al., 2003). The I^2 statistic was used to indicate the percentage variance between studies with cutoff points corresponding to low (25%), moderate (50%), and high (75%) heterogeneity. The risk of publication bias was objectively assessed with Egger's test, with a cutoff of significance at an exceptional p-value of $p<0.1$, as previously suggested (Egger et al., 1997). The significance level was set at 5% ($p<0.05$) for all other analyses.

3. Results

3.1. Search results

The database search provided 10,947 references with an additional five identified through reverse search. A total of 4724 articles remained after removal of duplicates, and an additional 4701 were excluded after a review of the title/abstract indicated that the article did not meet the inclusion criteria. The remaining 23 articles were reviewed in the full-text for eligibility: nine studies were excluded as the participants were <35 years; two studies included former athletes, but not master athletes and one was an animal study. Therefore, eleven studies (n = 11) were included in the qualitative and quantitative analysis.

3.2. Study characteristics

The studies contained in total 240 master athletes and 209 age-matched non-athletes. Master athletes had a mean age of 51.9±7.5 years and had participated for 16.6±10.9 years in master athletics. Telomere length was measured in skeletal muscle (Osthus et al., 2012; Rae et al., 2010), saliva (Borghini et al., 2015) or peripheral white blood cells (Aguiar et al., 2019; Denham et al., 2013; LaRocca et al., 2010; Mathur et al., 2013; Rosa et al., 2020; Simoes et al., 2017; Werner et al., 2009). The methods used to assess telomere length were southern blotting (LaRocca et al., 2010; Rae et al., 2010), quantitative or real-time polymerase chain-reaction (Aguiar et al., 2019; Borghini et al., 2015; Denham et al., 2013; Osthus et al., 2012; Rosa et al., 2020; Simoes et al., 2017; Werner et al., 2009), or fluorescence in situ hybridization (Mathur et al., 2013). Five studies measured possible mechanisms associated with telomere length. See Table 1 for details.

3.3. Methodological quality assessment

The quality scores and risk of bias are available in Table 2. The agreement between the two reviewers was 89.9%. The disagreement was solved in a consensus meeting. Quality appraisal scores ranged from 1 (lowest quality) to 10 (highest quality) (mean score 7±3). Item 6 had the lowest score ratio with only 9.1% of the studies scoring 'yes'. Items 5 and 8 had a 100% score within all studies, whereas questions 1, 2, 3, 4, 7 and 9 had scores of 90.9%, 81.8%, 90.9%, 72.7%, 72.7% and 45.5%, respectively.

Four studies were classified as at moderate risk of bias (Aguiar et al., 2019; LaRocca et al., 2010; Mathur et al., 2013; Werner et al., 2009), and seven studies were classified as at low risk (Borghini et al., 2015; Denham et al., 2013; Osthus et al., 2012; Rae et al., 2010; Rosa et al., 2020; Simoes et al., 2017; Sousa et al., 2019). Only one study described the characteristics of patients lost to follow-up and/or analysis (Item 6), and five studies reported sufficient power to detect a clinically important effect where the probability value for a difference being due to chance was <5% (Item 9).

Table 1
Sample, methods and outcomes description of the studies included for the final analysis.

Study	Sample		TL assessment (DNA source; method)	Additional analyzes	Studied groups and outcomes
	Definition	Characteristics (n; age; sex; years of training; body fat; VO_2max)			
Wener et al. [28]	Young controls: <1 h/week	26; 21.8 yrs.; m/w; N/A; NR; NR	Leukocytes; fluorescence in situ hybridization (FISH)	TRF2 protein; TRF2 mRNA; Chk2 mRNA; p53; p16; Ku70; Ku80; telomerase activity	'Aged athletes' in comparison to controls: Young control: TL: ↔; TRF2 protein: ↔; TRF2 mRNA: ↑; Chk2 mRNA: ↓; p53: ↔; telomerase activity: ↑ Young athletes: TL: ↔; TRF2 protein: ↔; TRF2 mRNA: ↔; Chk2 mRNA: ↔; p53: ↔; telomerase activity: ↔ Aged control: TL: ↔; TRF2 protein: ↔; TRF2 mRNA: ↔; Chk2 mRNA: ↔; p53: ↔; p16: ↓; ku70: ↑; ku80: ↑; telomerase activity: ↔
	Young athletes: endurance athletes 13.9 h/week, 72.9 km/week	32; 20.4 yrs.; m/w; NR; NR; NR			
	Aged control: <1 h/week	21; 50.9 yrs.; m/w; N/A; NR; NR			
	Aged athletes: endurance athletes 9.6 h/week; 80.5 km/week	25; 51.1 yrs.; m/w; 35 yrs.; NR; NR			
LaRocca et al. [20]	Young sedentary: aerobic <2 days/week, <30 min/day	15; 23 yrs.; m/w; N/A; 24.6%; 43.7 ml/kg/min	Leukocytes; southern blot analysis	NR	'Older exercising' in comparison to control groups: Young sedentary: ↓ Young exercising: ↔ Older sedentary: ↑
	Young exercising: aerobic exercise ≥5 days/week, >45 min/day	10; 21 yrs.; m/w; 5 yrs.; 13.4%; 55.8 ml/kg/min			
	Older sedentary: aerobic exercise <2 days/week, <30 min/day	15; 65 yrs.; m/w; N/A; 33.7%; 25.9 ml/kg/min			
	Older exercising aerobic exercise ≥5 days/week, >45 min/day	17; 62 yrs.; m/w; 5 yrs.; 21.4%; 40.5 ml/kg/min			
Rae et al. [17]	Sedentary group: exercise <2 days/week; never participated in competitive sport	17; 38.7 yrs.; m/w; N/A; 23.7%; NR	Skeletal muscle; southern blot analysis	NR	'Athletes' in comparison to control group: Sedentary: ↔
	Athletes group: 5 days/week, 66 km/week	18; 42.4 yrs.; m/w; 15.4 yrs.; 19.7%; NR			
Østhus et al. [18]	Young non-athlete: physically active but never participated in competitive sport	5; 23.6 yrs.; m; NR; NR; 53.9 ml/kg/min	Skeletal muscle; qPCR	NR	'Older athletes' in comparison to control groups: Young non-athlete: NR Young athletes: NR Older non-athletes: ↑
	Young athlete: ski training and competing race and track running	5; 24.4 yrs.; m; NR; NR; 67.0 ml/kg/min			
	Older non-athlete: physically active but never participated in competitive sport	5; 69.8 yrs.; m; NR; NR; 39.4 ml/kg/min			
	Older athlete: training and competing cross country ski racing in previous years	5; 69.2 yrs.; m; NR; NR; 45.4 ml/kg/min			
Denhan et al. [21]	Controls walking or swimming <2 days/week	56; 42.8 yrs.; m; NR; NR; NR	Leukocytes; qPCR	CRP; IL6; Leptin; sE-selectin; sICAM-1	'Ultra-marathon runners' in comparison to control group: TL: ↑; CRP: ↓; IL6: ↔; Leptin ↓; sE-selectin ↔; sICAM-1 ↓
	Ultra-marathon runners: completed at least two ultra-marathons; 40 to 100 km/week;	67; 42.8 yrs.; m; NR; NR; NR; NR			
Mathur et al. [25]	Sedentary: physically inactive	17; 55 yrs.; m/w; N/A; NR; 33.4 ml/kg/min	Granulocytes and lymphocytes; fluorescence in situ hybridization (FISH)	CRP	'Athletes' in comparison to controls: TL: ↔; CRP: ↓
	Athletes: training and competing marathon running; 33 km/week	15; 54 yrs.; m/w; 5 yrs.; NR; 43.9 ml/kg/min			
Borghini et al. [19]	Controls: physically inactive and never participated in competitive sport	42; 45.9 yrs.; m/w; N/A; NR; NR	Saliva; qPCR	NR	'Endurance athletes' in comparison to controls: TL: ↑
	Endurance athletes: training and competing ultra-trail running; 59.4 km/week)	20; 45.4 yrs.; m/w; 13.1 yrs.; NR; NR			
Simões et al. [33]	Non-athletes: physically inactive and never participated in competitive sport	10; 45.4 yrs.; m; N/A; 26.0%; NR	Leukocytes; qPCR	NR	'Master sprinters' in comparison to controls: TL: ↑
	Master sprinters: competitive men in sprint/power athletics events	11; 50.1 yrs.; m; 10 yrs.; 12.2%; NR			
Aguiar et al. [22]	Untrained control: recreationally active but never participated in competitive sport	11; 45.4 yrs.; m; N/A; 26.0%; NR	Leukocytes; qPCR	TBARS; SOD; CAT; SOD/TBARS; CAT/TBARS	'Master athletes' in comparison to controls: TL: ↑; TBARS: ↔; SOD: ↔; CAT: ↑; SOD/TBARS: ↑; CAT/TBARS: ↑
	Master athletes: sprint/power and endurance competitive master athletes	21; 51.6 yrs.; m; 26.4 yrs.; 12.2%; NR			
Sousa et al. [34]	Young controls: recreationally active but never participated in competitive sport	11; 21.8 yrs.; m; N/A; 9.8%; NR	Leukocytes; qPCR	NO; TBARS; TEAC; SOD; CAT; SOD/TBARS; CAT/TBARS; TEAC/TBARS	'Endurance master athletes' in comparison to control groups: Young: TL: ↔; NO: ↑; TBARS: ↔; TEAC: ↔;

(*continued on next page*)

Table 1 (*continued*)

Study	Sample		TL assessment (DNA source; method)	Additional analyzes	Studied groups and outcomes
	Definition	Characteristics (n; age; sex; years of training; body fat; VO_2max)			
	Age-matched controls: recreationally active but never participated in competitive sport	17; 46.6 yrs.; m; N/A; 24.4%; NR			SOD: ↔; CAT: ↔; SOD/TBARS: ↔; CAT/TBARS: ↔; TEAC/TBARS: ↓ Age-matched: TL: ↑; NO: ↑; TBARS: ↔; TEAC: ↔; SOD: ↔; CAT: ↔; SOD/TBARS: ↔; CAT/TBARS: ↑; TEAC/TBARS: ↔
	Endurance master athletes: competitive and experienced master athletes in endurance events (10 km to marathon)	10; 51.6 yrs.; m; 28.4 yrs.; 12.7%; NR			
Rosa et al. [20]	Young controls: untrained and health	17; 22.7 yrs.; m; N/A; NR; NR	Leukocytes; qPCR	F_2-Isoprostanes; Protein carbonyls; TBARS; 8-OHdG; TEAC; SOD; CAT; NO^-_2; GSH; Uric acid; TNF-α; sTNF-RI; sIL-6R; IL-6; IL-10; IL-15; IL-10/TNF-α; IL-10/IL-6; ADMA; Irisin; Klotho; FGF-23; Klotho/FGF-23	'Endurance athletes' in comparison to control groups: Young: TL: ↔; F_2-Isoprostanes: ↑; Protein carbonyls: ↑; TBARS: ↔; 8-OHdG: ↔; TEAC: ↓; SOD: ↔; CAT: ↔; NO^-_2: ↑; GSH: ↔; Uric acid: ↔; TNF-α: ↑; sTNF-RI: ↑; sIL-6R: ↑; IL-6: ↑; IL-10: ↓; IL-15: ↔; IL-10/TNF-α: ↓; IL-10/IL-6: ↓; ADMA: ↔; Irisin: ↓; Klotho: ↓; FGF-23: ↔; Klotho/FGF-23: ↓ Middle-aged: TL: ↔; F_2-Isoprostanes: ↓; Protein carbonyls: ↔; TBARS: ↔; 8-OHdG: ↔; TEAC: ↔; SOD: ↑; CAT: ↔; NO^-_2: ↑; GSH: ↔; Uric acid: ↓; TNF-α: ↔; sTNF-RI: ↓; sIL-6R: ↓; IL-6: ↓; IL-10: ↔; IL-15: ↑; IL-10/TNF-α: ↔; IL-10/IL-6: ↑; ADMA: ↓; Irisin: ↔; Klotho: ↔; FGF-23: ↓; Klotho/FGF-23: ↔ 'Sprint athletes' in comparison to control groups: Young: TL: ↔; F_2-Isoprostanes: ↔; Protein carbonyls: ↔; TBARS: ↔; 8-OHdG: ↔; TEAC: ↔; SOD: ↓; CAT: ↔; NO^-_2: ↔; GSH: ↔; Uric acid: ↔; TNF-α: ↑; sTNF-RI: ↑; sIL-6R: ↑; IL-6: ↔; IL-10: ↔; IL-15: ↓; IL-10/TNF-α: ↓; IL-10/IL-6: ↓; ADMA: ↑; Irisin: ↔; Klotho: ↔; FGF-23: ↔; Klotho/FGF-23: ↔ Middle-aged: TL: ↑; F_2-Isoprostanes: ↓; Protein carbonyls: ↔; TBARS: ↔; 8-OHdG: ↔; TEAC: ↔; SOD: ↔; CAT: ↑; NO^-_2: ↑; GSH: ↔; Uric acid: ↓; TNF-α: ↔; sTNF-RI: ↓; sIL-6R: ↓; IL-6: ↓; IL-10: ↑; IL-15: ↔; IL-10/TNF-α: ↑; IL-10/IL-6: ↑; ADMA: ↔; Irisin: ↑; Klotho: ↑; FGF-23: ↓; Klotho/FGF-23: ↑ 'Endurance athletes' in comparison to 'Sprint athletes': TL: ↔; F_2-Isoprostanes: ↑; Protein carbonyls: ↑; TBARS: ↔; 8-OHdG: ↔; TEAC: ↓; SOD: ↑; CAT: ↓; NO^-_2: ↑; GSH: ↔; Uric acid: ↔; TNF-α: ↔; sTNF-RI: ↑; sIL-6R: ↔; IL-6: ↑; IL-10: ↓; IL-15: ↑; IL-10/TNF-α: ↓; IL-10/IL-6: ↓; ADMA: ↓; Irisin: ↓; Klotho: ↓; FGF-23: ↑; Klotho/FGF-23: ↓
	Middle-aged controls: recreationally active but never participated in competitive sport	12; 45.5 yrs.; m; N/A; NR; NR			
	Endurance athletes: competitive and experienced master athletes in endurance events (10 km to marathon)	18; 53.0 yrs.; m; 25.3 yrs.; NR; NR			
	Sprint athletes: competitive and experienced master athletes in sprint/power events (60 m to 400 m)	13; 50.0 yrs.; m; 25.3 yrs.; NR; NR			

TL: telomere length; m: men; w: women; VO_2max: maximal oxygen uptake; N/A: non-applicable; NR: non-reported; qPCR: quantitative polymerase chain-reaction; CRP: C-reactive protein; IL6: interleukin 6; SOD: superoxide dismutase; CAT: catalase: TEAC: Trolox-equivalent antioxidant capacity; TBARS: thiobarbituric acid-reactive substances; NO: nitric oxide; NO^-2: nitrite; ADMA: asymmetric dimethylarginine; 8-OHdG: 8-hydroxydeoxyguanosine; GSH: glutathione; FGF-23: fibroblast growth factor 23.

3.4. Meta-analysis

A total of 11 studies were included in the pooled analysis and results are shown in Fig. 2. Master athletes had longer telomeres than aged-matched controls (SMD=0.89, 95% CI=0.45 to 1.33, $p<0.001$, Z=3.96, $p<0.001$). There was a high 75% of heterogeneity (Q=44.50, df 11, $p<0.001$, τ^2=0.41) in the pooled analysis. The Egger's test (Egger et al., 1997) showed an objective asymmetry (p=0.137). Fig. 3 shows the analysis of telomere length in blood and buccal cells and skeletal muscle separately. Master athletes had longer blood and buccal cells telomeres than the control group (SMD=0.99, 95% CI=0.50 to 1.49, $p<0.001$, Z=3.93, $p<0.001$). There was a high 78% of heterogeneity (Q=40.38, df 9, $p<0.001$, τ^2=0.46). No differences were found between groups for telomeres measured in skeletal muscle (SMD=0.43, 95% CI=-0.51 to 1.37, Z=0.90, p=0.37). In addition, master athletes had lower values for pro-oxidant parameters (SMD=0.59; 95% CI=0.26 to 0.91, $p<0.001$; Z=3.48, $p<0.001$) (Fig. 4) and a higher antioxidant defense (SMD=-0.46; 95% CI=-0.89 to −0.03, p=0.04, Z=2.10, p=0.04) compared with age-matched non-athletes (Fig. 5). Sub-group analysis with young sedentary controls vs. middle-aged adults were not performed since only a small number of articles included any of these. Besides, due to the small number of articles to measure anti- and pro-inflammatory cytokines (2 articles), and shelterin protein expression (1 article), quantitative analyzes were not performed on these parameters, but they were used to explain their potential influence on telomere length.

Table 2
Methodological quality assessment scores of the included studies.

Study	Questions									Score ratio (%)	Risk of bias[a]
	1. Clear objective	2. Measure description	3. Sample description	4. Telomere assessment	5. Main findings	6. Sample loss	7. Probability values	8. Statistical tests	9. Statistical power		
Werner et al. [28]	0	1	1	1	1	0	1	1	0	66.7	Moderate
LaRocca et al. [20]	1	1	0	0	1	0	0	1	1	55.6	Moderate
Rae et al. [17]	1	1	1	1	1	1	1	1	0	88.9	Low
Østhus et al. [18]	1	1	1	1	1	0	1	1	0	77.8	Low
Denhan et al. [21]	1	1	1	0	1	0	1	1	1	77.8	Low
Mathur et al. [25]	1	0	1	0	1	0	1	1	0	55.6	Moderate
Borghini et al. [19]	1	0	1	1	1	0	1	1	1	77.8	Low
Simões et al. [33]	1	1	1	1	1	0	1	1	0	77.8	Low
Aguiar et al. [22]	1	1	1	1	1	0	0	1	0	66.7	Moderate
Sousa et al. [34]	1	1	1	1	1	0	0	1	1	77.8	Low
Rosa et al. [32]	1	1	1	1	1	0	1	1	1	88.9	Low
Score ratio (%)	90.9	81.8	90.9	72.7	100	9.1	72.7	100	45.5		

[a] To aid in interpretation, we classified study quality and risk of bias based on the proportion of criteria met:<50%=low quality, high risk of bias; 50–75%=fair quality, moderate risk of bias; 76–100%=high quality, low risk of bias.

4. Discussion

4.1. Summary of evidence

In this meta-analysis of 11 studies we found that master athletes (n=240) have longer telomeres than age-matched non-athletes (n=209). Four studies were classified as at moderate risk of bias and seven studies were classified as at low risk. Below we will discuss that lower oxidative stress and chronic inflammation, and a higher expression of shelterin proteins that protect telomeres in master athletes may contribute to their longer telomeres. We will further discuss telomere length as a marker of 'biological age' and the role of physical activity in maintaining telomere length and health.

4.2. Underlying mechanisms

4.2.1. Oxidative stress

Three of the selected studies found an association between oxidative stress parameters and telomere length in master athletes (Aguiar et al., 2019; Rosa et al., 2020). One was classified as at moderate risk of bias

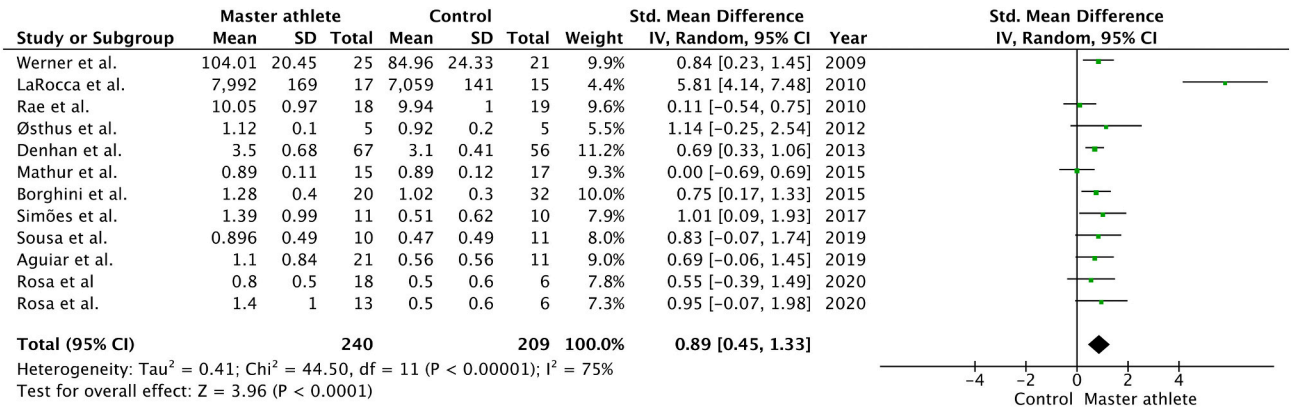

Fig. 2. Meta-analysis of all of the included articles that measured the telomere length of master athletes and age-matched non-athletes.

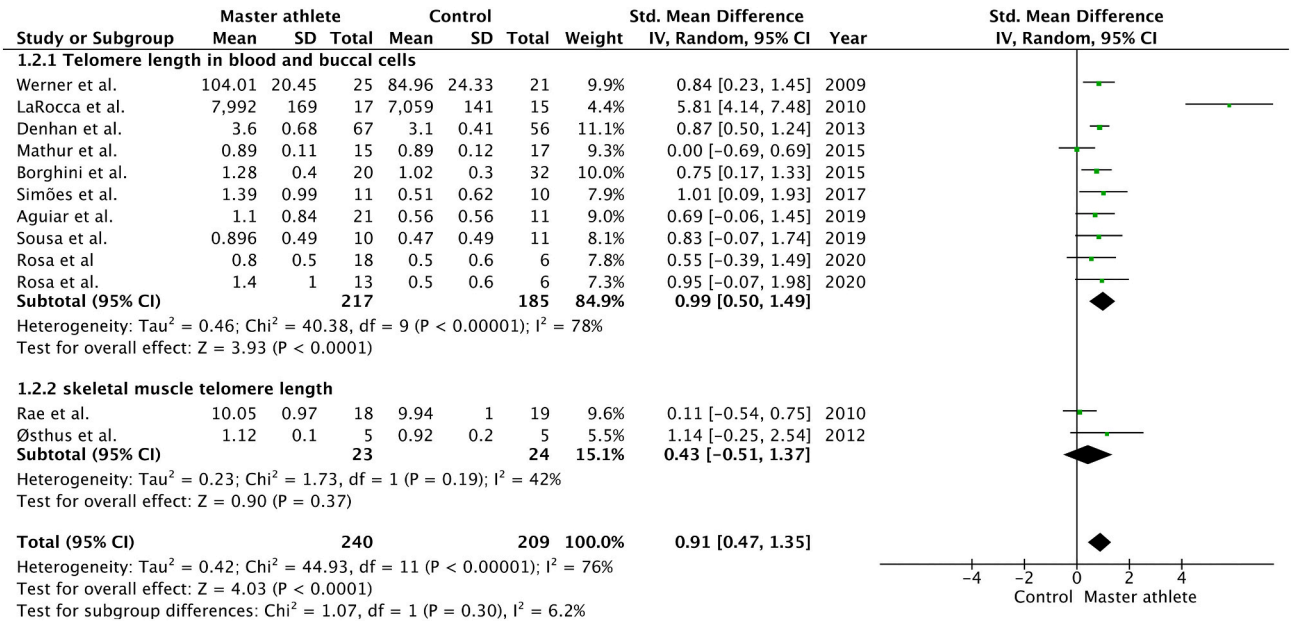

Fig. 3. Meta-analysis of articles that measured the telomere length in blood and buccal cells and skeletal muscle of master athletes and age-matched non-athletes.

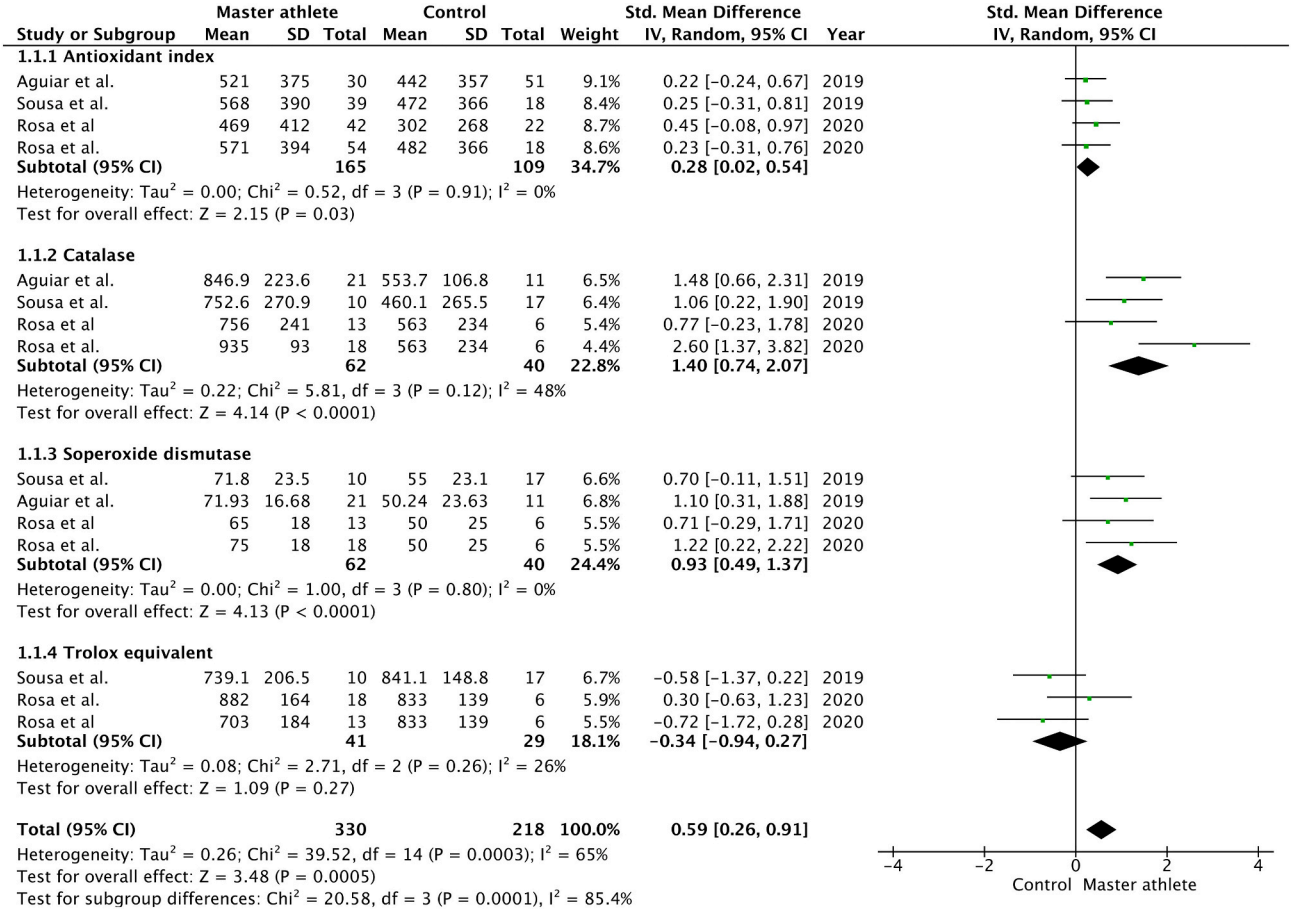

Fig. 4. Meta-analysis of the antioxidant index, catalase, superoxide dismutase and trolox equivalent of master athletes and age-matched non-athletes.

(Aguiar et al., 2019) and two as at low risk of bias (Rosa et al., 2020; Sousa et al., 2019). Aguiar et al. (2019) demonstrated that master athletes (100 m to marathon) have longer telomeres and lower levels of TBARS, and higher antioxidant defenses, as reflected by higher catalase and *superoxide dismutase/TBARS ratio* (SOD/TBARS), when compared to untrained age-matched individuals. Similarly, reported that master endurance runners not only have a better oxidative balance and longer telomeres than age-matched non-athletes, but these were even better

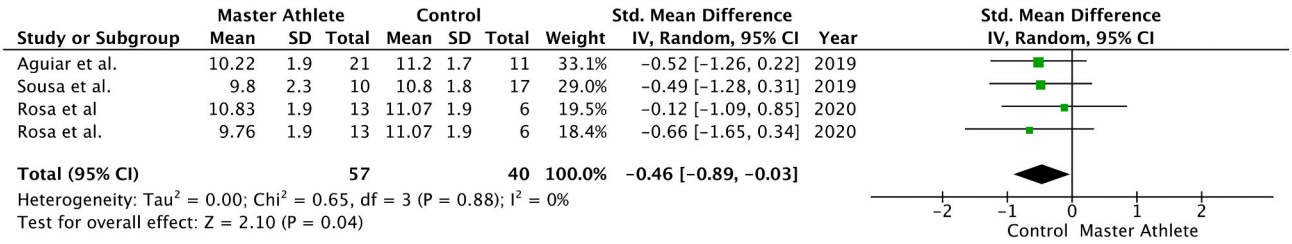

Fig. 5. Meta-analysis of the thiobarbituric acid reactive substances (pro-oxidant) of master athletes and age-matched non-athletes.

than that in young untrained individuals. These observations applied to both sprint and endurance master athletes (2020), but endurance athletes had lower levels of Trolox equivalent, catalase, F_2-isoprostanes and Protein carbonyls, and higher levels of superoxide dismutase and NO^-_2 than sprint athletes. The training sessions-induced oxidative stress may well contribute to increase the efficiency of antioxidant defense systems, leading to a greater cytosolic and mitochondrial capacity to eliminate free radicals (de Sousa et al., 2017) and a reduction in the production of reactive oxygen species (Bouzid et al., 2015; Daussin et al., 2012). Therefore, lifelong training seems to be a key point to improve the oxidative balance of master athletes (Barranco-Ruiz et al., 2017a; Barranco-Ruiz et al., 2017b), mitigating biological aging and the onset of chronic diseases, providing a healthy and functional trajectory of life.

4.2.2. *Chronic inflammation*

Three studies addressed the effects of lifelong exercise on pro-inflammatory cytokines and telomere length in master athletes. One was classified as moderate risk of bias (Mathur et al., 2013) and two as low risk of bias (Denham et al., 2013; Rosa et al., 2020). Mathur et al. (2013) found no differences in telomere length between master marathon runners and sedentary controls, but they did have lower levels of c-reactive protein (CRP). While comparing telomere length and cardiovascular risk markers (including interleukin-6 and CRP) in master ultra-endurance runners and age-matched controls, Denham et al. (2013) also observed lower c-reactive protein levels but, in contrast to Mathur et al. (2013), they verified that master athletes had longer telomeres. Moreover, Rosa et al. (2020) demonstrated that master athletes have longer telomeres, lower levels of inflammatory cytokines (sTNF-RI, IL-6, sIL6R) and higher levels of anti-inflammatory cytokines (IL-10, IL-10/TNF-α ratio, and IL-10/IL-6 ratio) than middle-aged controls. In this study, endurance athletes had higher levels of sTNF-RI, IL-6 and IL-15 than sprint athletes, while sprint athletes had higher levels of IL-10, IL- 10/TNF-α ratio and IL-10/IL-6 ratio than endurance athletes. These observations corroborate with the study of Sousa et al. (2020). This last reported a negative correlation between markers of chronic inflammation and telomere length, and a positive association between telomere length and the performance level of master athletes.

Like the benefits of exercise for the oxidative status, the evidence also suggest that exercise is beneficial to reduce or attenuate the inflammatory status in older age. The anti-inflammatory effects of long-term physical training may be mediated by a low body fat content, reducing the release of pro-inflammatory cytokines from adipose tissue, and simultaneously increasing the release of anti-inflammatory cytokines through skeletal muscle contractions (Aguiar et al., 2020; Mikkelsen et al., 2013; Minuzzi et al., 2019). This may include an (i) increased production and release of interleukin-10 (IL-10), and other anti-inflammatory myokines from working skeletal muscle; (ii) reduced expression of Toll-like receptors (TLRs) in monocytes and macrophages and consequently inhibition of pro-inflammatory cytokine production; (iii) inhibition of adipose tissue infiltration by monocytes and macrophages; (iv) reduction in the circulating number of pro-inflammatory monocytes; (v) and an increase in the circulating number of regulatory T (Treg) cells (Gjevestad et al., 2015; Gleeson et al., 2011). In practical terms, the literature indicates that lifelong training of master athletes has a beneficial effect on the balance of pro and anti-inflammatory cytokines, and thereby attenuate both inflammaging, immunosenescence and thus the risk of age-related chronic diseases.

4.2.3. *Shelterin proteins*

The enzyme telomerase help to maintain telomeres by adding TTAGGG sequences at the end of the telomeres, using RNA as a template. In addition to telomerase, a group of proteins called shelterin proteins (Shay and Wright, 2019) plays a key role in telomere maintenance. The shelterin complex is composed of six proteins: telomere-related factors 1 (TRF1) and 2 (TRF2), TRF1-interacting protein 2 (TIN2), protection of telomeres 1 (Pot-1), the Pot-1- and Tin2-organizing protein repressor/activator protein 1 (RAP1), and tripeptidyl peptidase 1 (TPP1) (Laye et al., 2012; Martinez and Blasco, 2010). These proteins protect telomeric termini from triggering an inappropriate DNA damage response and are thought to contribute to the formation of T-loop structures that protect the end of the telomere (Morrish et al., 2013).

Only one study (Werner et al., 2009), classified as moderate risk of bias, investigated telomere length and its regulatory proteins in master athletes. These authors evaluated telomere length, TRF2 protein, TRF2 mRNA, Chk2 mRNA, p53, p16, ku70, ku80 and telomerase activity in healthy young, healthy middle-aged individuals, young endurance athletes, and master endurance athletes. They reported that athletes (young and aged-athletes) had similar telomere length and p53 expression compared to controls (young and aged-control), but young and aged athletes had higher expression of TRF2 mRNA, greater telomerase activity and lower expression of Chk2 mRNA than young controls. Finally, the aged athletes showed downregulation of p16 and greater expression of ku70 and 80 mRNA. These data suggest that higher physical fitness has beneficial effects on shelterin complex proteins and telomerase activity.

4.2.4. *An integrative perspective*

It thus appears that high physical fitness effectively preserves telomere length (LaRocca et al., 2010; Osthus et al., 2012) . Although the current literature does not provide experimental evidence to fully establish all mechanisms that underlie the preservation of telomere length in master athletes, there are several potential mechanisms: lowered oxidative stress (increased antioxidant defenses), reduced chronic inflammation, a higher activity of telomerase and of proteins related to telomere integrity (Aguiar et al., 2019; Arsenis et al., 2017; Sousa et al., 2019 #491; Rosa et al., 2020) (Barranco-Ruiz et al., 2017a; Barranco-Ruiz et al., 2017b; Minuzzi et al., 2019; Werner et al., 2009) (Fig. 6).

As discussed above, high physical fitness is associated with less oxidative damage probably consequent to a higher activity of antioxidant enzymes (Aguiar et al., 2020; Barranco-Ruiz et al., 2017b). For example, the greater activity of SOD dismutates the superoxide (O_2^-) into the less harmful hydrogen peroxide (H_2O_2). The H_2O_2, can be neutralized in two ways: (i) through catalase, forming H_2O and O_2; and (ii) by glutathione (GSH) forming 2 H_2O (Silva and Coutinho, 2010). This mechanism is fundamental to reduce the formation of peroxynitrite ($ONOO^-$) what in turn would decrease the availability of nitric oxide (NO^-) (Forstermann et al., 2017).

In addition to a better defense against reactive oxidative species, the

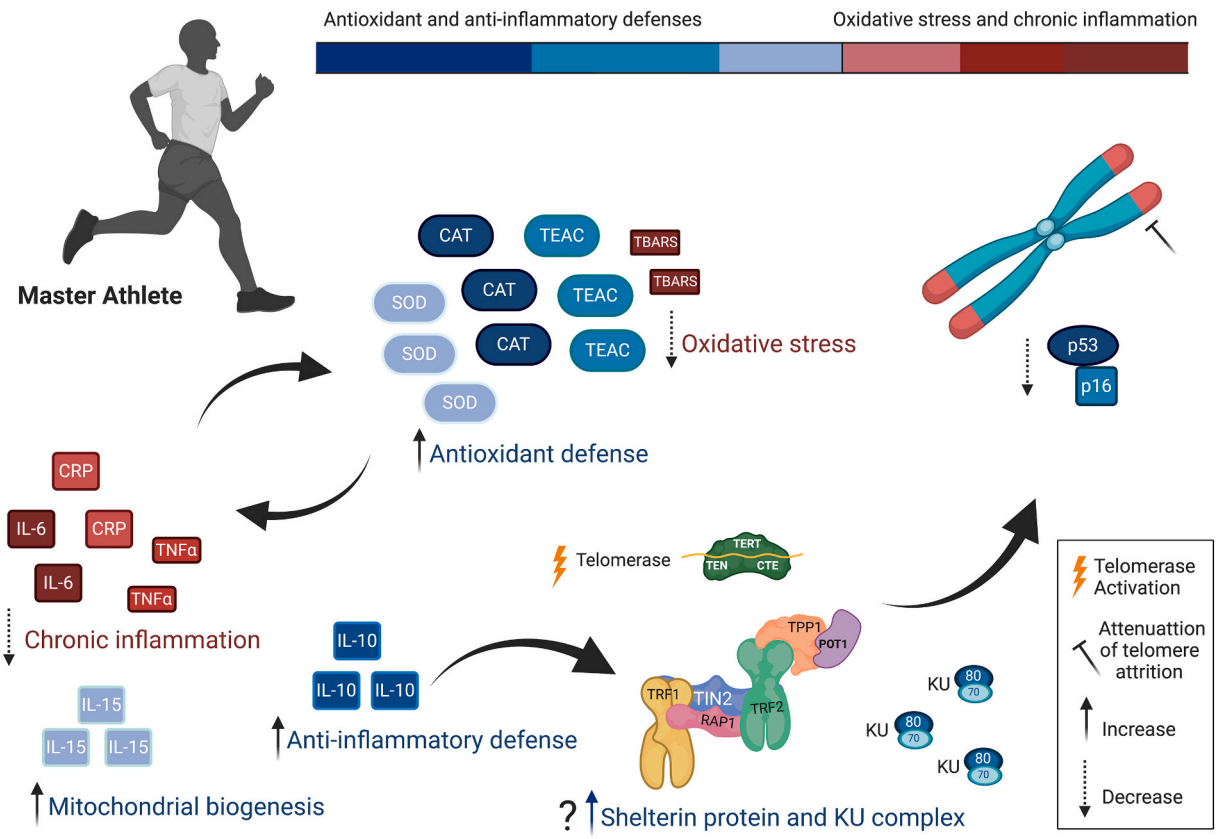

Fig. 6. Schematic representation of the possible physiological mechanisms associated with the protection of telomeres in master athletes. For details see the "*An integrative perspective*" section.

production of reactive oxidative species production is also less in master athletes than age-matched non-athletes. For instance, the cascade reaction (mainly via NF-κB and MCP-1) is attenuated, decreasing the production of several pro-inflammatory cytokines, such as TNF-α, CRP, IL-1β, and IL-6 (Aguiar et al., 2020; Mikkelsen et al., 2013; Minuzzi et al., 2019), that in turn minimizes the activation of specific ROS-generating enzymes, and thus, break the vicious cycle between the production of oxidative stress and pro-inflammatory cytokines (Aguiar et al., 2020). Moreover, the training history of master athletes leads to a greater expression of anti-inflammatory cytokines IL-4, IL-15 (Aguiar et al., 2020; Mikkelsen et al., 2013; Minuzzi et al., 2019; Rosa et al., 2020) that contributes to IL-10 production (Mitchell et al., 2017), which plays a key role in the inhibition of pro-inflammatory responses of both innate and adaptive cells of immune system, enhancing survival, proliferation, differentiation, and antibody production (Wang and Karin, 2015).

As a result of lower oxidative stress and inflammation, the telomerase enzyme suffers less damage and downregulation (Arsenis et al., 2017; Khan et al., 2012). This may be further enhanced by regular exercise via an increase in TERT, TRF1, TRF2 and TPP1 mRNA (Laye et al., 2012; Werner et al., 2009), preserving telomere length. Furthermore, master athletes have a higher expression of ku70/80 mRNA (Werner et al., 2009). Although these proteins are not inside the shelterin complex, they interact with it, helping to protect DNA from damage (Laye et al., 2012; Werner et al., 2009). As shortened telomeres will ultimately cause cell senescence, it is encouraging to see that the attenuated age-related shortening of telomeres in master athletes is a accompanied with lower levels of markers of cellular senescence and apoptosis, such as p16, Chk2, and p53 (Khan et al., 2012; Werner et al., 2009). There is thus a substantial amount of, at least circumstantial, evidence that the longer telomeres in master athletes than age-matched non-athletes is a result of these protective mechanisms, reduced inflammation and oxidative stress (Korhonen et al., 2014; Kusy and Zielinski, 2015), but it can not be entirely excluded that master athletes have longer telomeres due to being born with longer telomeres.

Other markers follow a similar pattern, such as higher levels of Klotho (an anti-aging protein) and lower levels of FGF23 (a marker of renal endothelial dysfunction and cardiovascular risk factor) in master athletes than age-matched controls, and even reaching similar levels as those seen in young adults (2020).

The better antioxidant and anti-inflammatory defense, greater telomerase activity, upregulation of shelterin proteins, lower apoptotic signaling and associated longer telomeres undoubtedly contribute to the above-average general health and physical capacity for their chronological age, indicating they are biologically younger. Therefore, regular physical exercise (including high intensity stimulus, at proper dosage and frequency), besides a controlled diet, a reasonable stress management, and adequate body composition should be targeted by non-athletes willing to attenuate biological aging (Korhonen et al., 2014; Kusy and Zielinski, 2015).

4.2.5. *Dose-response of exercise in master athletes*

Although the master athlete is widely recognized as a model of successful aging (Korhonen et al., 2014; Kusy and Zielinski, 2015), a small number of athletes may experience muscle damage and chronic exercise-related fatigue, a condition known as "fatigued myopathic athlete syndrome" (FAMS) (Collins et al., 2003; Lambert et al., 2000). This condition may be due to endurance training with very high volumes and intensity and participation in an excessive number of competitions throughout the season (Collins et al., 2003; Rae et al., 2010). Collins et al. (2003) demonstrated that endurance master athletes diagnosed with FAMS have shorter telomere length in skeletal muscle compared to athletes without FAMS symptoms. This shortening of telomeres may be attributable to oxidative damage, as telomeres area significant target of

reactive oxygen species (Arsenis et al., 2017; Blackburn et al., 2015) and during high-volume exercise, there may be an increase in the production of reactive oxygen species by oxidative metabolism, in such a way that it exceeds the antioxidant capacity. It is important to note, however, that only a small proportion of master athletes suffer from FAMS, and except during over training, the training-induced increase in oxidant defenses appear sufficient to scavenge ROS and prevent any oxidative stress-induced telomere shortening.

4.3. Perspectives and limitations

Some limitations of the present study have to be addressed. High heterogeneity was identified in the present meta-analysis, possibly due to differences in the methods to assess telomere length (FISH, southern blot, and qPCR), the tissue (muscle, blood, and saliva), and the sample size. Moreover, comprehensive experimental studies that thoroughly investigated telomere preservation mechanisms in master athletes are still scarce, making it difficult to explain the phenomenon precisely. We suggest that future research should focus on explaining the telomere length preservation mechanisms in the master athletes to assess a more comprehensive net of age-related markers, such as oxidative stress, inflammatory markers, hormones, sheltering proteins, and other aging markers.

4.4. Conclusions

In conclusion, master athletes have longer telomeres than their non-athletes peers, possibly due to reduced oxidative stress and chronic inflammation, and an up regulation of both shelterin proteins and telomerase activity. These findings maybe not be a result of regular physical training only, but a combination of lifestyle factors including stress management, proper resting, balanced nutrition and better psychological traits. Original research on athletes that suddenly stop regular exercise could provide important information whether the benefits of an athletic training could be retained or not.

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CRediT authorship contribution statement

SSA and CVS conceived the idea for the review. SSA and CVS conducted the study selection and data extraction. SSA and CVS conducted the quality assessment. JG conducted the statistical analyses. SSA drafted the initial manuscript. PAS, LPB, LAM, HJCJ, DMS, TSR, HD, and HGS contributed to writing the manuscript. All authors approved the final version of the manuscript.

Declaration of competing interest

Samuel S Aguiar, Caio V Sousa, Patrick A Santos, Lucas P Barbosa, Larissa Alves Maciel, Hélio J Coelho-Júnior, Daisy Motta-Santos, Thiago Santos Rosa, Hans Degens and Herbert G Simões have no conflicts of interest that are directly relevant to the content of this article.

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