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RESEARCH ARTICLE

No effect of the endurance training status on senescence despite reduced inflammation in skeletal muscle of older individuals

Estelle Balan,[1] Estelle De Groote,[1] Margot Bouillon,[1] Nikenza Viceconte,[2] Manon Mahieu,[2] Damien Naslain,[1] Henri Nielens,[1] Anabelle Decottignies,[2]* and Louise Deldicque[1]*

[1]*Institute of Neuroscience, Université Catholique de Louvain, Louvain-La-Neuve, Belgium; and* [2]*De Duve Institute, Université Catholique de Louvain, Brussels, Belgium*

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Balan E, De Groote E, Bouillon M, Viceconte N, Mahieu M, Naslain D, Nielens H, Decottignies A, Deldicque L. No effect of the endurance training status on senescence despite reduced inflammation in skeletal muscle of older individuals. *Am J Physiol Endocrinol Metab* 319: E447–E454, 2020. First published July 22, 2020; doi: 10.1152/ajpendo.00149.2020.—The aim of the present study was to determine if the training status decreases inflammation, slows down senescence, and preserves telomere health in skeletal muscle in older compared with younger subjects, with a specific focus on satellite cells. Analyses were conducted on skeletal muscle and cultured satellite cells from vastus lateralis biopsies ($n = 34$) of male volunteers divided into four groups: young sedentary (YS), young trained cyclists (YT), old sedentary (OS), and old trained cyclists (OT). The senescence state and inflammatory profile were evaluated by telomere dysfunction-induced foci (TIF) quantification, senescence-associated β-galactosidase (SA-β-Gal) staining, and quantitative (q)RT-PCR. Independently of the endurance training status, TIF levels (+35%, $P < 0.001$) and the percentage of SA-β-Gal-positive cells (+30%, $P < 0.05$) were higher in cultured satellite cells of older compared with younger subjects. *p16* (4- to 5-fold) and *p21* (2-fold) mRNA levels in skeletal muscle were higher with age but unchanged by the training status. Aging induced higher *CD68* mRNA levels in human skeletal muscle (+102%, $P = 0.009$). Independently of age, both trained groups had lower *IL-8* mRNA levels (−70%, $P = 0.011$) and tended to have lower *TNF-α* mRNA levels (−40%, $P = 0.10$) compared with the sedentary subjects. All together, we found that the endurance training status did not slow down senescence in skeletal muscle and satellite cells in older compared with younger subjects despite reduced inflammation in skeletal muscle. These findings highlight that the link between senescence and inflammation can be disrupted in skeletal muscle.

age; exercise; physical activity; SASP; stem cells

INTRODUCTION

The loss of muscle functions, including degenerative loss of strength and mass, is a well-known feature of aging in human, which contributes to the progressive loss of autonomy in the elderly (25, 44). Despite the large number of studies indicating a protective role of physical activity against the sequels of aging (37), the molecular mechanisms by which exercise counteracts skeletal muscle aging need to be further investigated.

Telomeres are specialized nucleoprotein (*TTAGGG* repeats) caps at the end of the chromosomes, which protect genome stability (2). As each cell division results in telomere attrition, aging induces telomere shortening (7). Loss of telomere length impairs tissue renewal (7, 27). Adult skeletal muscle is considered as a postmitotic tissue (22). Therefore, the capacity to regenerate new myonuclei either for fiber growth or for fiber repair lies in one specific mononucleated precursor cells: the satellite cells (29). Besides the well-established loss of telomeric DNA related to the replicative history of somatic cells, telomere erosion is not constant and differs between people. These variations can be explained by environmental conditions such as oxidative stress and inflammation (38). Chronic low-grade systemic inflammation is a common manifestation of aging, with higher circulating levels of proinflammatory cytokines such as TNF-α (8). While chronic inflammation is higher with sedentary behavior (11), studies have evidenced that endurance training may reduce markers of systemic and skeletal muscle inflammation (5, 26, 46). Endurance training may have beneficial effects on telomeres. However, more research is needed to establish a consensus (3). For example, long-term high-intensity cross-country skiing provided a protective effect on muscle telomere length in older athletes compared with subjects exercising at a moderate level of activity (31), while middle age experienced runners showed shorter skeletal muscle telomeres compared with age-matching sedentary individuals (35). Moreover, the impact of endurance training on telomere integrity in a specific cell population such as satellite cells remains largely unknown.

Cellular senescence is a durable cell cycle arrest resulting notably in the accumulation of cyclin-dependent kinase inhibitor 2A (p16) and/or cyclin-dependent kinase inhibitor 1 (p21) (12, 21, 36). Telomere damage can be visualized by immunofluorescence microscopy using antibodies against DNA damage response factors, such as tumor suppressor p53-binding protein 1 (53BP1), coupled with telomere detection by fluorescent in situ hybridization (FISH) or immunofluorescence (14, 32, 41). A cell becomes senescent when multiple telomeres in the nucleus display DNA damage markers, turning them into so-called telomere dysfunction-induced foci (TIF) (41). Senescence can be induced by many stimuli (10). In addition to senescence conveyed by repeated cell divisions (i.e., replicative senescence or telomere-dependent senescence), stress, such as oxidative stress, can also induce cellular senescence in a telomere-independent manner and lead to increased p16 expression (9, 10, 43). This type of growth arrest

* A. Decottignies and L. Deldicque contributed equally to this study.
Correspondence: L. Deldicque (louise.deldicque@uclouvain.be).

is called stress or aberrant signaling-induced senescence (16). Higher lysosomal β-galactosidase activity is often reported in senescent cells, leading to measurable senescence-associated β-galactosidase (SA-β-Gal) activity (21). Moreover, senescent cells induce a cellular program called senescence-associated secretory phenotype (SASP), resulting in the secretion of cytokines with potent proinflammatory effects (20, 21). Indeed, SASP involves the production of factors that reinforce the senescence arrest, alter the microenvironment, and trigger immune surveillance, notably by recruiting macrophages (20, 21). SASP cytokine production depends largely on stress-induced nuclear factor-κB (NF-κB) signaling, which promotes the transcription of tumor necrosis factor-α (TNF-α) and interleukin-8 (IL-8) coding genes (12).

The aim of the present study was to determine if a better endurance training status decreases inflammation, slows down senescence, and preserves telomere health in skeletal muscle of older compared with younger subjects, with a specific focus on satellite cells.

MATERIALS AND METHODS

Human study. The present work has been conducted in parallel with another one looking at the effects of aging and the training status on mitophagy on the same muscle samples (4). Briefly, 34 healthy men were recruited, and their cardiorespiratory fitness was assessed during a maximal incremental fitness test on a cycle ergometer (Cyclus III; RBM Electronics). Based on their age and physical level, the subjects were randomly divided into four groups: young sedentary (YS) (n = 9), young trained (YT) (n = 9), old sedentary (OS) (n = 8), and old trained (OT) (n = 8). None of the sedentary subjects were engaged in any weekly physical activity session for at least 5 yr whereas physically active subjects were all experienced cyclists and reported ≥6 h training a week for at least 5 yr. None of the volunteers participated in resistance training before their enrollment in our study. One week later, a skeletal muscle biopsy was taken in the fasted state from the vastus lateralis muscle under local anesthesia with 1 ml of Xylocaine 2% (AstraZeneca) using a Bergström needle. A biopsy of ~70–100 mg of skeletal muscle was immediately frozen in liquid nitrogen for biomolecular analyses and satellite cell isolation while ~20 mg were embedded in optimum cutting temperature compound and immediately frozen in cooled isopentane so immunofluorescence could be performed on muscle sections. Before muscle biopsy, all subjects were asked to refrain from strenuous physical activity for 2 days and from alcohol for 1 day. The protocol was approved by the local Ethical Committee of the Université Catholique de Louvain and conducted in accordance with the Declaration of Helsinki. All participants provided their written consent after being fully informed about the experimental procedure.

RNA extraction and quantitative real-time PCR. About 30 mg of muscle biopsy were homogenized using a Polytron (Kinematica) in 1 ml Trizol reagent (Invitrogen). RNA isolation was achieved according to the manufacturer's instructions. RNA quality and quantity were assessed by Nanodrop (Thermo Fisher Scientific) spectrophotometry. Reverse transcription was performed from 1 μg RNA using the iScriptcDNA Synthesis Kit (Bio-Rad), following the manufacturer's instructions. PCR analyzes were conducted using the following conditions: 3 min at 95°C, followed by 40 cycles of 15 s at 95°C and 30 s at 60°C using CFX Connect Real-Time System (Bio-Rad Laboratories). All samples were run in duplicate. Each reaction was processed in a 10-μl volume containing 4.8 μl SsoAdvanced Universal SYBR Green SuperMix (Bio-Rad), 0.1 μl of each primer (100 nM final), and 5 μl cDNA at the appropriate dilution. Melting curves were systematically performed to ensure the quality of the analysis. All mRNA levels were normalized to β2-microglobulin (*β2M*). Primers sequences are provided in Table 1.

Immunofluorescence on cryosections. Cryosections (6 μm) were air dried and directly blocked with PBS/20% normal goat serum for 30 min at room temperature. Slides were then incubated for 1 h at 37°C with the following primary antibodies: anti-laminin (1/200, NB600-883, Novus) and anti-CD68 (1/200, no. 76437, Cell Signaling). After several washes with PBS, slides were incubated for 1 h at room temperature with the following secondary antibodies: Alexa Fluor 568 anti-mouse (A11031) and Alexa Fluor 488 anti-rabbit (A25034) (1/500, Thermo Fisher Scientific). Samples were mounted with Vectashield containing DAPI (Vector Laboratories). Images were acquired with a Vert.A1 (Zeiss) microscope using a ×40 objective.

Satellite cell isolation. Satellite cell isolation was performed on four biopsies per group. About 20 mg of muscle were scissor minced, and the tissue fragments were plated onto collagen-coated dishes. The explants were maintained in the dish with a thin layer of Matrigel (Corning Life Sciences) and incubated in DMEM (Dutscher) supplemented with 10% FBS (VWR) and 1% Ultroser G (Pall Biosepra) at 37°C in humidified air with 5% CO_2. After 6–8 days, cells migrated out of the explants and were enzymatically harvested using dispase (Merck). Satellite cells were purified using an immunomagnetic bead-based sorting system with high gradient magnetic cell separation (MACS) microbeads (Miltenyi Biotec) directly linked to an antibody against CD56 (BD559044, BD Biosciences).

Senescence-associated galactosidase staining in cultured satellite cells. Cultured satellite cells were fixed with 3.7% paraformaldehyde (PFA) (Merck) for 5 min at room temperature before incubation at 37°C in freshly prepared SA-β-Gal staining solution [1 mg/ml 5-bromo-4-chloro-3-indoly β-D-galactoside (X-Gal) (Merck), 40 mM citric acid/sodium phosphate (Merck), pH 5.5, 5 mM potassium ferrocyanide (Merck), 5 mM potassium ferricyanide, 150 mM NaCl (Merck), and 2 mM $MgCl_2$ (Merck)]. Blue cells were counted as senescent cells. Two hundred cells were counted for each subject.

TIF detection and telomere length analysis in satellite cells. As previously reported (15), TIF detection was performed to evaluate telomere integrity. After fixation with 3.7% PFA (Merck) for 15 min and permeabilization [20 mM Tris·HCl (Merck), pH 8.0, 50 mM NaCl (Merck), 3 mM $MgCl_2$ (Merck), 300 mM sucrose (Merck), and 0.5%

Table 1. *Primers sequences*

	Forward	Reverse
p16	GGAAGGTCCCTCAGACATCC	TACGAAAGCGGGGTGGGTTG
p21	GCAGACCAGCATGACAGATTT	GGATTAGGGCTTCCTCTTGGA
CD68	TCCAGGGAAGCTGTGAGGGT	AGCCGAGAATGTCCACTGTGC
IL-8	GACATACTCCAAACCTTTCCAC	GCTTTACAATAATTTCTGTGTTGGC
TNF-α	GCTGCACTTTGGAGTGAT	GGTTCGAGAAGATGATCTGAC
β2M	ATGAGTATGCCTGCCGTGTGA	GGCATCTTCAAACCTCCATG

p16, cyclin-dependent kinase inhibitor 2A; p21, cyclin-dependent kinase inhibitor 1; CD68, cluster of differentiation; IL-8, interleukin 8; TNF-α, tumor necrosis factor-α; β2M, β2-microglobulin.

Triton X-100 (Merck)], the antibody 53BP1 (NB100-304, Novus) was used to visualized DNA damage by immunofluorescence microscopy while telomeres were marked by fluorescent in situ hybridization (FISH). Colocalization events between 53BP1 and telomeric DNA are counted as TIF. Briefly, after immunofluorescence against 53BP1, satellite cells were fixed again with 3.7% PFA for 2 min and treated with ribonuclease (100 ug/ml) for 1 h at 37°C. After a second incubation with permeabilization buffer (20 mM Tris·HCl, pH 8.0, 50 mM NaCl, 3 mM $MgCl_2$, 300 mM sucrose, and 0.5% Triton X-100) for 10 min at room temperature and refixation for 2 min with 3.7% PFA, cells were dehydrated through successive baths of 70, 80, 90, and 100% ethanol for 2 min each. Telomeres were hybridized with 50 nM TeloG Exiqon Locked Nucleic Acid (LNA) red probe GGGTtAGGGttAGgGTTAGGGttAGGGttAGGGtTA (TAMRA) (small letters indicate LNA-modified bases) for 2 h at room temperature after denaturation for 3 min at 85°C. After several washes, samples were mounted with Vectashield containing DAPI (Vector Laboratories). Telomere length in each culture of satellite cells was normalized to the signal obtained in an immortalized mouse myoblast cell line (C2C12, ATCC) seeded on the same slide. Images were acquired with a Cell Observer spinning disk (Zeiss) confocal microscope using a ×100 objective and analyzed using ImageJ software (National Institutes of Health).

Statistics. All results are presented as means ± SE. Statistical analyses were performed using GraphPad Prism 7.0. Data were analyzed by a two-way ANOVA, and when a main effect was found, Bonferroni post hoc tests were performed. Statistical significance was set at $P \leq 0.05$.

RESULTS

Subjects characteristics. Subjects characteristics can be found in (4). Briefly, the mean age of the young subjects (YS + YT) was 22 ± 1 yr and the mean age of the old subjects (OS + OT) was 67 ± 1 yr, with no difference between the sedentary and trained groups. Body mass index (BMI) was higher in OS (26.4 ± 1.4 kg/m^2) than YS (23.0 ± 1.1 kg/m^2, $P = 0.039$) and YT (23.1 ± 1.0 kg/m^2, $P = 0.049$). $\dot{V}O_{2max}$ was ~25% higher in YT compared with YS (61 ± 3 vs. 48 ± 4 $ml \cdot min^{-1} \cdot kg^{-1}$, $P = 0.035$) and ~50% higher in OT compared with OS (44 ± 4 vs. 30 ± 2 $ml \cdot min^{-1} \cdot kg^{-1}$, $P = 0.029$). OT had similar $\dot{V}O_{2max}$ values as YS (~45 $ml \cdot min^{-1} \cdot kg^{-1}$). W_{max} was 75% higher in OT compared with OS (3.3 ± 0.2 vs. 1.9 ± 0.2 W/kg, $P < 0.001$) and 40% higher in YT compared with YS (4.3 ± 0.3 vs. 3.0 ± 0.2 W/kg, $P < 0.001$).

Aging results in a higher number of dysfunctional telomeres in satellite cells without any effect of the training status. Neither age nor the training status appeared to modify the relative telomere length in cultured satellites cells (Fig. 1, *A* and *B*). However, the evaluation of telomere length by FISH based on microscopy data analysis is likely not sensitive enough to detect small changes in telomere length. This is why we relied on another approach. We chose to evaluate the loss of telomere integrity, through the detection of TIF. Our data indicated that the percentage of cultured satellite cells containing three or more TIF per nucleus increased with aging (main effect of aging, $P < 0.0001$; Fig. 2, *A* and *B*). Post hoc analysis showed that OS and OT had a higher number of cells containing greater than or equal to three TIF per nucleus compared with either YS (+35%, $P = 0.0007$ and +36%, $P = 0.0006$, respectively) or YT (+39%, $P = 0.0003$ and +40%, $P = 0.0003$) (Fig. 2, *A* and *B*). The training status did not modify the percentage of cells containing greater than or equal to three TIF per nucleus.

Together, these data indicate that although aging is associated with an increased frequency of damaged telomeres in satellite cells, exercise does not appear to protect from age-associated telomere uncapping.

Cellular senescence increases with age but is not influenced by the training status. In skeletal muscle biopsies, the mRNA levels of two markers of cellular senescence, *p16* and *p21* (13, 42), were higher in the older groups compared with the younger subjects, independently of the training status (both main effect of aging, $P = 0.0001$; Fig. 3, *C* and *D*). Post hoc analysis revealed that *p16* mRNA levels were fivefold higher in OS ($P = 0.0079$) and in OT ($P = 0.0044$) compared with YS and tended to be fourfold higher in OS ($P = 0.08$) and in OT ($P = 0.0507$) compared with YT. Post hoc analysis showed that *p21* mRNA levels were higher in OS (+165%, $P =$

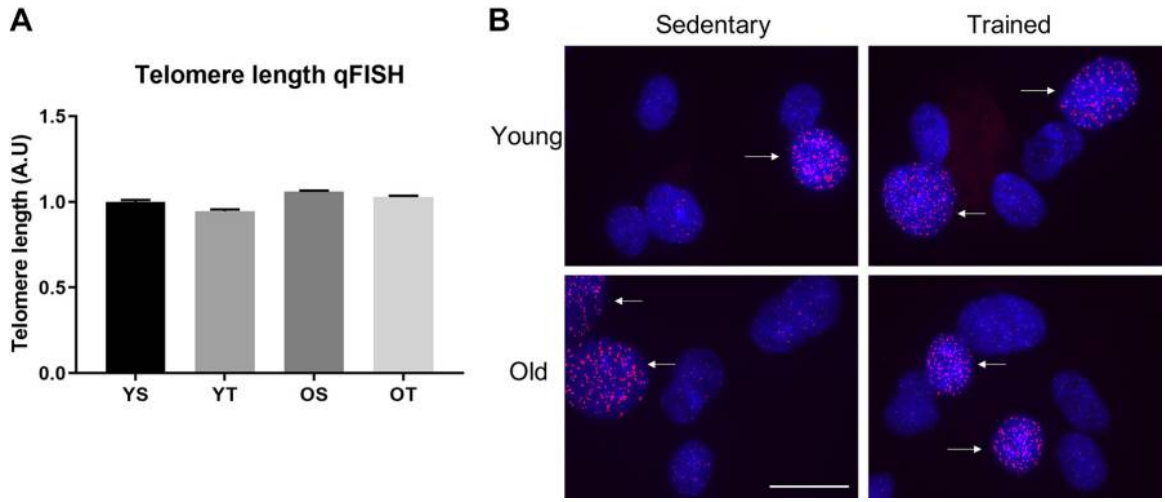

Fig. 1. Relative telomere length in cultured satellite cells. *A*: telomere length is expressed as arbitrary units. Results are normalized to C2C12 signal and reported to the young sedentary (YS) group who was arbitrarily set to a value of 1; A.U., arbitrary units. *B*: illustration of a quantitative fluorescence in situ hybridization (qFISH) signal. Nuclei are identified by DAPI in blue, and telomeres are marked by the qFISH probe in red. Human satellite cells showed a lower qFISH signal compared with the C2C12 immortalized cell line (marked by white arrows). Scale bar = 50 μm. Values are expressed as means ± SE; $n = 4$/group. YT, young trained; OS, old sedentary; OT, old trained.

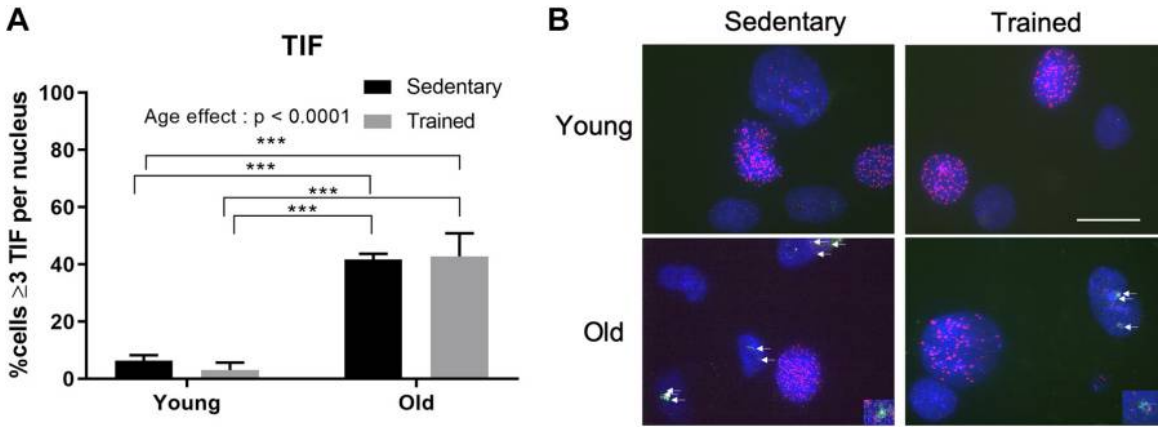

Fig. 2. Dysfunctional telomeres in cultured satellite cells. *A*: percentage of cells containing at least 3 telomere dysfunction-induced foci (TIF) per nucleus in all groups. *B*: telomeres are marked by the quantitative fluorescence in situ hybridization (qFISH) locked nucleic acid (LNA) probe in red, and DNA lesions are represented by 53BP1 foci in green and nuclei are identified by DAPI in blue. White arrows show DNA lesions at telomeric DNA sequences. Scale bar = 50 μm. Values are expressed as means ± SE; n = 4/group. ***$P < 0.001$.

0.0054) and OT (+154%, $P = 0.01$) compared with YT and tended to be higher in OS ($P = 0.06$) and OT ($P = 0.11$) compared with YS.

To check whether the increased expression of *p16/p21* mRNA detected in the tissues was associated with cellular senescence of satellite cells, we performed SA-β-Gal staining experiments on cultured satellite cells. Here, we found that the percentage of SA-β-Gal-positive cells was higher in the older compared with the young subjects (main effect of aging, $P < 0.0001$), independently of the training status. The percentage of positive cells was higher in OS and in OT compared with YS and YT (post hoc analysis; OS vs. YS, +38%, $P = 0.0009$; OT vs. YS, +29%, $P = 0.007$; OS vs. YT, +30%, $P = 0.0053$; OT vs. YT, +22%, $P = 0.0471$; Fig. 3, *A* and *B*).

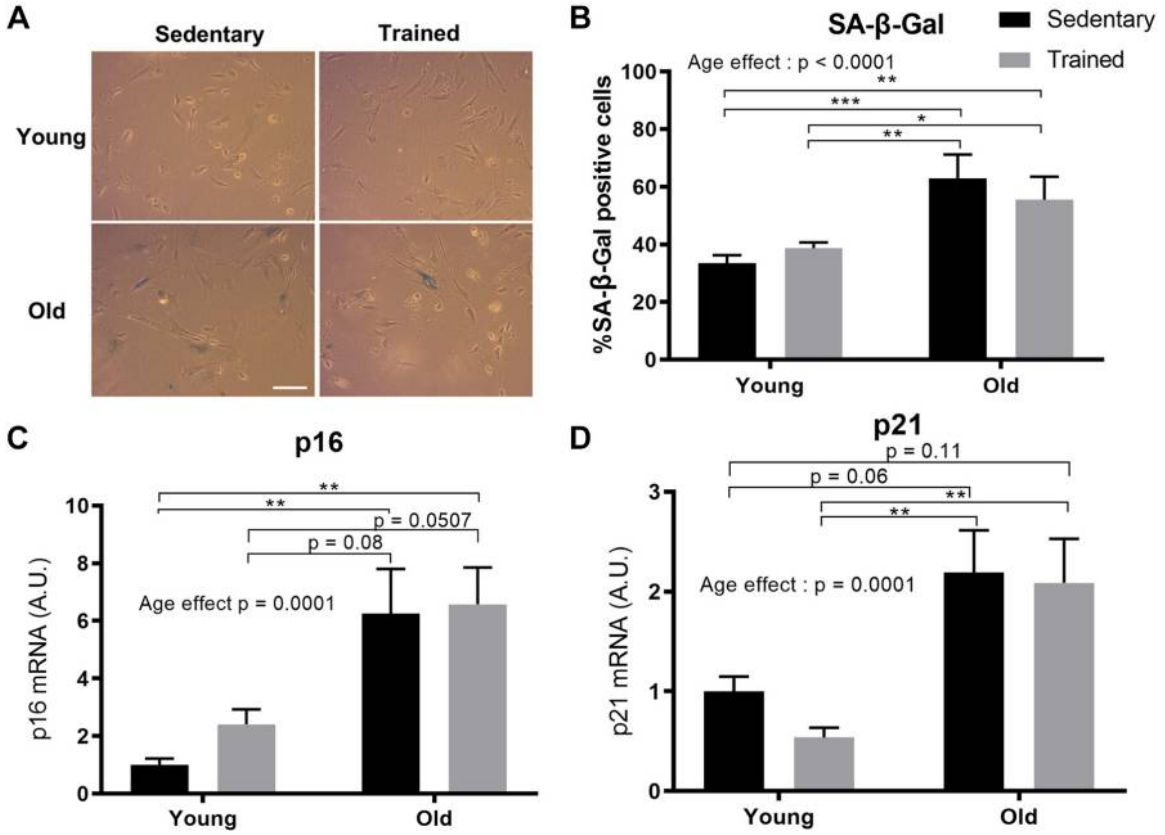

Fig. 3. Senescence in cultured satellite cells and human skeletal muscle. *A*: senescence-associated β-galactosidase (SA-β-Gal) staining in cultured satellite cells. Blue cells are senescent cells; n = 4/group. Scale bar = 50 μm. *B*: percentage of positive cells after SA-β-gal staining. *C* and *D*: relative p16 mRNA levels (*C*) and relative p21 mRNA levels (*D*) normalized to β-2-microglobulin in human skeletal muscle; A.U., arbitrary units. Young sedentary (YS) and young trained (YT), n = 9/group; old sedentary (OS) and old trained (OT), n = 8/group. Values are expressed as means ± SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

The SA-β-Gal analysis performed in cultured satellite cells thus revealed that, in line with the TIF data, cellular senescence increases with age but is not counteracted by endurance exercise.

A good training status alleviates chronic inflammation and seems to lower the macrophage tissue infiltration in old subjects. To evaluate tissue inflammation on muscle sections, macrophages were marked by CD68 staining (23). Interestingly, numerous $CD68^+$ macrophages were found in OS compared with both young groups and OT (Fig. 4*B*), suggesting that a good training status might have a protective role on the inflammation profile of older subjects. Aging also induced higher *CD68* mRNA levels in human skeletal muscle of sedentary subjects (main effect of aging, $P = 0.009$; post hoc, OS vs. YS, +99%, $P = 0.032$; OS vs. YT, +105%, $P = 0.0215$), but post hoc analysis did not show any difference between OT and YS nor between OT and YT (Fig. 4*A*). As SASP cytokine production largely depends on stress-induced NF-κB signaling (12), we also measured *IL-8* and *TNF-α* mRNA levels in skeletal muscle. Our data did not reveal any upregulation of *IL-8* and *TNF-α* mRNA with age (Fig. 4*C*). However, we found that both trained groups had lower *IL-8* mRNA levels (~70%, $P = 0.011$; Fig. 4*C*) and tended to have lower *TNF-α* mRNA levels (~40%, $P = 0.10$; Fig. 4*D*) compared with the sedentary subjects.

Together, these data highlight distinct impacts of aging and the training status on $CD68^+$ macrophages, on the one hand, and *IL-8* and *TNF-α* mRNA levels, on the other hand. Exercise appears to reduce macrophage infiltration in older subjects but not in younger ones. However, in both groups, it reduces *IL-8* and *TNF-α* transcripts.

DISCUSSION

Our study revealed that a good endurance training status does not counteract the age-dependent upregulation of *p16*/*p21* mRNA senescence markers in skeletal muscle tissues, despite reducing inflammation, as measured by a lower number of $CD68^+$ macrophages in tissues of older trained individuals and lower *IL-8* and *TNF-α* mRNA levels in trained versus sedentary subjects. These observations suggest that the links between cellular senescence and inflammation, through the senescence-associated secretory phenotype, or SASP, may be masked by additional effects of endurance training on inflammation in the skeletal muscle (Fig. 5).

Even though skeletal muscles are mostly postmitotic tissues, recent reports have suggested the development of a senescent phenotype in postmitotic cells (19). In line with our observations, higher expression levels of p21 were previously detected in skeletal muscle tissues of older compared with younger women (45). Similarly, *p16* mRNA levels were twofold higher

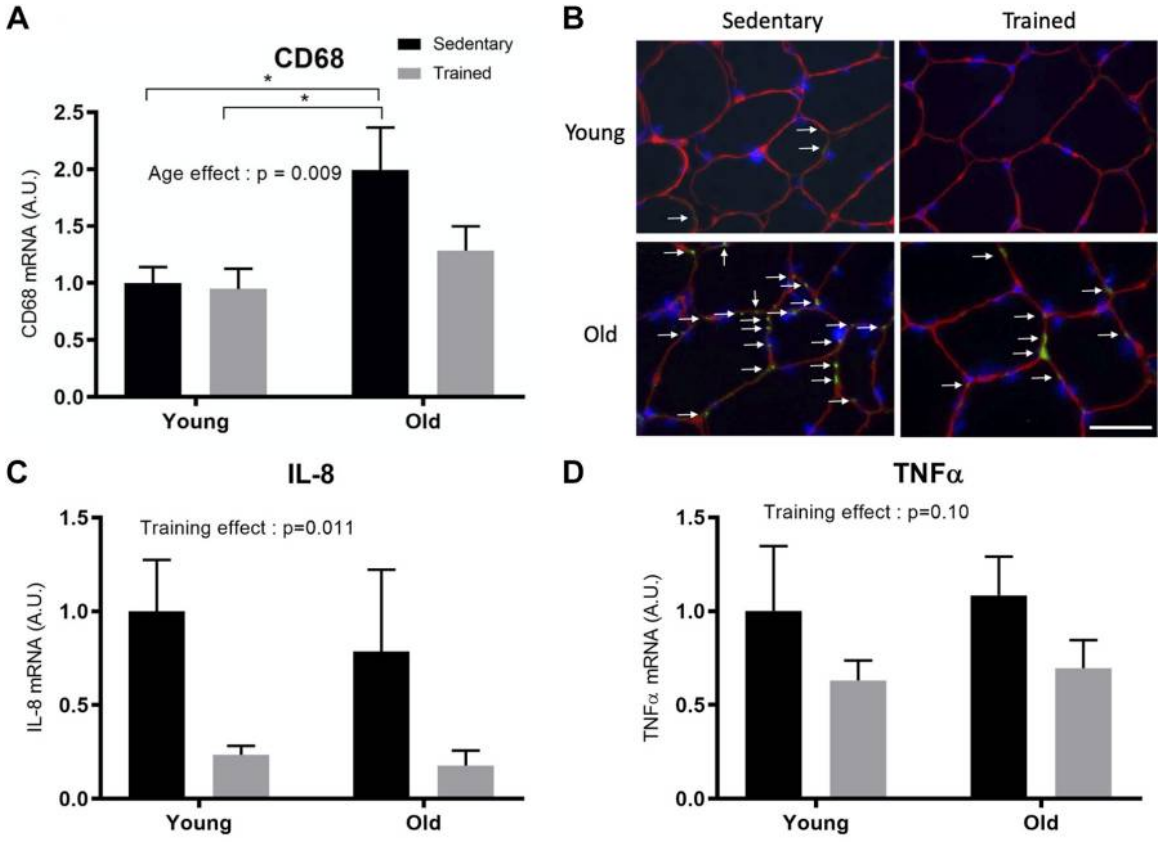

Fig. 4. Chronic inflammation in human skeletal muscle. *A*: relative CD68 mRNA levels normalized to β-2-microglobulin; A.U., arbitrary units. *B*: immunofluorescence staining on representative skeletal muscle cross sections representing $CD68^+$ macrophage infiltration: CD68 (green), shown by white arrows, identified proinflammatory macrophages; laminin (red) outlined the muscle fiber basement membrane; and DAPI (blue) represented the nuclei. Scale bar = 50 μm. *C* and *D*: relative IL-8 mRNA levels (*C*) and relative TNF-α mRNA levels (*D*) normalized to β2-microglobulin in skeletal muscle. Young sedentary (YS) and young trained (YT), $n = 9$/group; old sedentary (OS) and old trained (OT), $n = 8$/group. Values are expressed as means ± SE. $^*P < 0.05$.

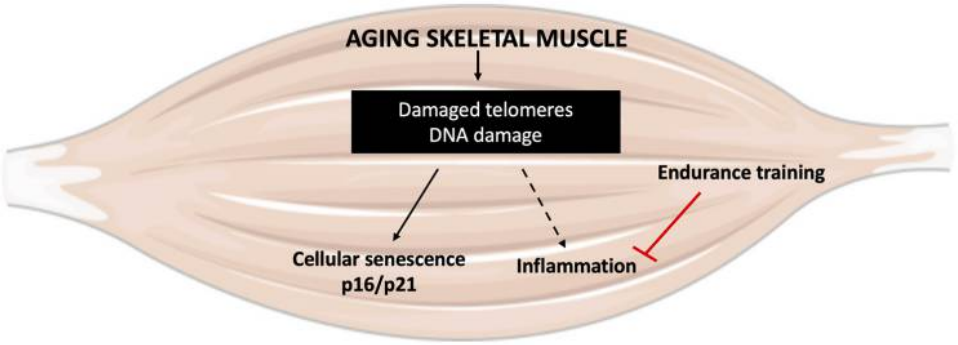

Fig. 5. Proposed model for the effect of endurance training on skeletal muscle aging with a specific focus on telomeres and senescence-associated secretory phenotype. In skeletal muscle, aging leads to DNA damage including telomere sequences. Damaged telomeres increase cellular senescence notably by activating p16/p21 signaling. Dotted line indicates that further studies are necessary to better understand whether aging influences the inflammatory profile of skeletal muscle. Despite reducing inflammation, endurance training does not counteract the age-dependent upregulation of senescence. We suggest that the links between cellular senescence and inflammation, through the senescence-associated secretory phenotype (SASP), may be masked by additional effects of endurance training on inflammation.

in differentiating myoblasts from older versus young individuals (6). One factor that may induce the expression of p16/p21 senescence markers in muscle biopsies of older subjects is the age-dependent phenomenon of telomere shortening and/or stress-induced telomere dysfunction. Our quantitative (q)FISH analysis of telomere length in satellite cells did not highlight any influence of age or the training status. As healthy skeletal muscle is a slow-turnover tissue (40), meaning that satellite cells undergo a limited number of cell divisions, qFISH might not be sensitive enough to detect small changes in telomere length. The flow-FISH technique, which combines FISH with flow cytometry, might be more suitable but requires more cells, thus limiting its feasibility and applicability for human skeletal muscle studies. We therefore relied on the evaluation of telomeric integrity loss through TIF measurement, as telomeric damages are considered as hallmark of cellular senescence (41). To our knowledge, we were the first to quantify TIF in primary cultures of human satellite cells. In addition, we performed SA-β-Gal staining to evaluate cellular senescence in cultured satellite cells. In accordance with the increase of *p21*/*p16* mRNA levels in muscle biopsies from older subjects, we found that both damaged telomeres and SA-β-Gal activity were increased in satellite cells isolated from both older groups compared with younger groups. TIF detection provides a way to investigate whether the senescence observed through SA-β-Gal staining and p21/p16 induction may be linked to telomere dysfunction. These observations match the well-known decline in skeletal muscle regenerative capacity observed with aging (17, 24).

The fact that the training status was not associated with a reduction of either TIF frequency or SA-β-Gal activity in satellite cells was quite surprising as telomere integrity has been linked before to environmental conditions such as oxidative stress and inflammation (38), two factors alleviated by moderate and chronic physical activity (30). We have previously shown that acute endurance exercise induces the transcription of telomeres into TERRA noncoding RNA molecules that play multiple protective roles at telomeres (15). We showed that, upon an acute bout of endurance exercise, TERRA production was induced by a proliferator-activated receptor-γ coactivator 1α (PGC-1α)/nuclear respiratory factor 1 (NRF1)-dependent antioxidant response, suggesting that endurance exercise generates reactive oxygen species that, in turn, activate an antioxidant response at telomeres (15). Because of their high G content, telomeric DNA sequences are sensitive to oxidation. Besides, the existence of a natural antioxidant mechanism would help protecting them from 8-oxoguanine formation. Hence, in the hypothesis of a balance between pro- and antioxidant responses, endurance exercise may possibly not result in any beneficial outcome for telomeres. Our observation that the age-related increase of *p21* and *p16* mRNA in human skeletal muscle was not counteracted by a good training status contrasts with results in mice, in which 4 wk of treadmill exercise decreased p16 and p21 protein expression in skeletal muscle (47). Further studies are necessary to better understand whether endurance training and more generally, physical activity, impact on telomeres and cellular senescence in human tissues.

Although we did not detect any beneficial impact of the training status on either cellular senescence or on telomere status in muscle cells, we did observe a reduction of proinflammatory cytokines mRNA levels, like *IL-8* and *TNF-α*, along with a reduced macrophage infiltration, assessed by *CD68* mRNA levels and immunofluorescence staining in muscle tissues. Senescent cells induce a proinflammatory neighborhood environment by recruiting macrophages and by secreting proinflammatory cytokines (20, 21). This phenomenon is called SASP (21). *p16* and *p21* mRNA senescence markers were higher in older versus younger subjects, independently of the training status. Our observations suggest that the positive impact of endurance training on the age-dependent increase in macrophage infiltration occurs regardless of an impact on cellular senescence. Hence, if the age-dependent increase in macrophage infiltration is caused by muscle tissue senescence, it may be masked by a positive and independent impact of endurance exercise. Along this line, the association between physical inactivity and low-grade systemic inflammation in healthy subjects is well established. The skeletal muscle tissue is a highly and metabolically active organ, producing and secreting an array of molecules, called "myokines" in response to contraction (33). During an acute bout of endurance exercise, contracting muscle fibers produce and release the IL-6 myokine via a TNF-α-independent pathway (28, 34). The level of circulating IL-6 increases up to 100-fold in response to

endurance exercise, and declines in the postexercise period (28). IL-6 has strong anti-inflammatory effects by stimulating the appearance in the circulation of other anti-inflammatory cytokines such as IL-1 and IL-10 and inhibiting the production of proinflammatory cytokines, such as TNF-α (34). Based on those results, regular endurance training might give a protective effect against age-related proinflammation. In this context, the exact role of the proinflammatory cytokine IL-8 remains to be more clearly defined as divergent results have been found. Acute resistance exercise did not induce any change in young and old endurance-trained athletes but increased *IL-8* mRNA levels in skeletal muscle of old sedentary individuals (26) while running a marathon increased serum IL-8 levels in experienced runners (39). IL-8 levels might depend on the exercise type and intensity, the tissue, and the population studied.

If macrophage infiltration was found to increase with age, we did not detect any upregulation of *IL-8* and *TNF-α* transcripts in muscle tissues from older subjects. Along this line, a previous study showed that, 2 mo postcontusion, rat soleus muscle displayed increased p53 expression that was, however, not associated with any increase in mRNA expression levels of common SASP cytokine genes (18). Hence, we cannot exclude that senescence and SASP may be similarly disconnected in human skeletal muscle.

In conclusion, the present study demonstrates that aging is associated with increased senescence in satellite cells and skeletal muscle tissue. A good endurance training status did not reduce senescence but relieved chronic inflammation that occurred with aging in skeletal muscle. These findings highlight that under certain circumstances the link between senescence and SASP can be disrupted in human skeletal muscle.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

E.B., A.D., and L.D. conceived and designed research; E.B., E.D.G., M.B., N.V., M.M., D.N., H.N., A.D., and L.D. performed experiments; E.B., E.D.G., M.B., N.V., M.M., D.N., A.D., and L.D. analyzed data; E.B., E.D.G., M.B., N.V., M.M., D.N., A.D., and L.D. interpreted results of experiments; E.B., A.D., and L.D. prepared figures; E.B., A.D., and L.D. drafted manuscript; E.B., A.D., and L.D. edited and revised manuscript; E.B., A.D., and L.D. approved final version of manuscript.

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