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Endurance training alleviates MCP-1 and TERRA accumulation at old age in human skeletal muscle

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ABSTRACT

Both oxidative stress and telomere transcription are up-regulated by acute endurance exercise in human skeletal muscle. Whether and how life-long exercise training influences the antioxidant system response at transcriptional level and TERRA expression is unknown, especially during aging. Response to acute endurance exercise was investigated in muscle biopsies of 3 male subjects after 45 min of cycling. *MCP-1* and *SOD1* mRNA levels increased up to, 15-fold and 63%, respectively, after the cycling session while the mRNA levels of *SOD2* were downregulated by 25%. The effects of chronic endurance exercise and aging were tested in the blood and muscle of 34 male subjects divided into four groups: young (YU) or old (OU) untrained, young (YT) or old (OT) trained cyclists. Long-term endurance training limited the age-dependent elevation in *SOD1* (OT *vs* OU, −26%, $P = 0.03$) and the decline in *SOD2* mRNA levels (OU *vs* YU, −41%, $P = 0.04$). A high endurance training status alleviated the age-related increase in the aging biological marker MCP-1 in plasma (OU *vs* YU, +48%, $P = 0.005$). Similar results were observed for telomeric transcription as the age-associated increase in *16p TERRA* levels (OU *vs* YU, +39%, $P = 0.001$) was counteracted by a high endurance training status (OT *vs* OU, −63%, $P = 0.0005$). In conclusion, as MCP-1, we propose that the age-related TERRA accumulation might represent a novel biological marker of aging. Those aging-related increase expression might be alleviated by a high endurance training status. Whether those biological markers of aging are linked to an elevation of oxidative stress is still an open question. Therefore, whether the positive adaptations provided by endurance training indeed reduce oxidative stress, including at telomeres, and whether TERRA plays any role in this, need to be further investigated.

1. Introduction

An imbalance between the production of reactive oxygen species (ROS) and antioxidants capacities in favor of the former is associated with oxidative damage to cellular components (Jones, 2006; Powers et al., 2020). The major class of enzymatic antioxidants is the superoxide dismutase (SOD) family: SOD1, SOD2 and SOD3, localized in the cytosol, mitochondria, and extracellular space, respectively (Case, 2017). These enzymes catalyze the dismutation of $O_2^{\bullet -}$ into hydrogen peroxide (H_2O_2), a process followed by the rapid reduction of H_2O_2 into water by the catalase (CAT) and the glutathione peroxidase (GPx) (Case, 2017).

ROS are produced in an intensity-dependent way during endurance exercise bouts by skeletal muscle, a dominant source of production (Jackson et al., 2016). While serving as an important signal to stimulate, amongst others, the antioxidant system response, chronic elevated levels of ROS, combined with a low expression of antioxidant enzymes cause oxidative damage (Powers et al., 1999; Fulle et al., 2004; Powers et al., 2016). Fortunately, endurance training, *i.e.* cumulative effects from repeated bouts of exercise, seems to be associated with a decrease in ROS

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along with a concomitant increase in skeletal muscle antioxidant enzyme such as SOD2 and GPx (Vincent et al., 2000; Powers and Jackson, 2008; Gliemann et al., 2014; Wadley et al., 2016). During the aging process, the balance between ROS production and antioxidant capacities is disturbed. Indeed, antioxidant capacities of aged muscles are reduced and ROS levels thus increase, causing oxidative damages to lipids, nucleic acids, and proteins (Fulle et al., 2004; Fulle et al., 2005; Mecocci et al., 1999). This phenomenon is central to the "theory of aging" in which the age-associated functional decline of tissues is due to the accumulation of ROS-mediated damages (Beckman and Ames, 1998). Endurance training is known to prevent some of the aging-associated muscle deteriorations (McLeod et al., 2016) but how exercise may modulate the expression of antioxidant genes such as SOD1 and SOD2 in the skeletal muscle during aging awaits further investigation.

By up-regulating ROS levels, acute exercise and aging both activate the nuclear factor-kappa B (NF-κB) pathway and this induces the release of inflammatory cytokines such as the monocyte chemoattractant protein-1 (MCP-1) (Catoire et al., 2014; Inadera et al., 1999; Tantiwong et al., 2010; Powers et al., 2011). MCP-1 is a chemokine with chemoattractant activity for monocytes (Rollins, 1996) and is also known as one of the senescence-associated secretory phenotype (SASP) factors secreted by senescent cells, likely in response to NF-κB activation in these cells (Jin et al., 2016). Therefore, MCP-1 plasma levels display an age-dependent increase (Inadera et al., 1999) and are recognized as an aging marker in both mouse and human (Yousefzadeh et al., 2018).

Another well-established hallmark of aging is the shortening and progressive deprotection of chromosome ends, the telomeres (Palm and de Lange, 2008). Erosion-induced telomere dysfunction triggers cellular senescence, blocks cell division and interferes with tissue replenishment (Blackburn et al., 2015). For a long time, telomeres have been considered as transcriptionally silent due to their heterochromatic nature. Yet, it turned out that the C-rich strands of human telomeres are transcribed from very distal subtelomeric promoters by the RNA polymerase II, generating telomeric repeat-containing RNA (TERRA) molecules composed of subtelomeric sequences at their 5′ end followed by $(UUAGGG)_n$ repeats (Azzalin et al., 2007). TERRA molecules were detected both in cell cultures and in human tissues, including the skeletal muscle (Diman et al., 2016). By forming RNA:DNA hybrids at telomeres and through their interaction with multiple protein partners, these long non-coding RNAs play several crucial functions at telomeres such as heterochomatin formation, telomerase regulation or telomeric overhang protection (Cusanelli and Chartrand, 2015). Only few studies looked at TERRA levels in response to different cellular stresses or signals. It appears that TERRA levels increase in presence of dysfunctional/ultrashort telomeres (Cusanelli and Chartrand, 2015; Arnoult et al., 2012) and after heat shock (Schoeftner and Blasco, 2008) or oxidative stress (Galigniana et al., 2020). Our previous study revealed that acute endurance exercise up-regulates TERRA production *in vivo* in human skeletal muscle (Diman et al., 2016). Therefore, it is tempting to speculate that TERRA expression might be regulated by the oxidative stress and/or inflammation conditions induced by acute exercise (Diman et al., 2016).

The purpose of this investigation was to examine if chronic endurance training might counteract the decline in the antioxidant enzymes capacities observed in the skeletal muscle during aging and the increased inflammation measured in the blood. To identify the relevant oxidative stress mRNA markers in the skeletal muscle, we evaluated the influence of an acute endurance exercise, known to induce oxidative stress, on the transcriptional regulation of key markers of the antioxidant system and *MCP-1* mRNA levels. Moreover, in light of the proposed up-regulation of telomeric transcription by oxidative stress, we investigated the impact of both aging and chronic endurance training on TERRA levels in the skeletal muscle. We hypothesized that aging would decrease the levels of antioxidant enzymes and increase TERRA levels, which would be, at least partially, counteracted by endurance training.

2. Material and methods

All data presented here are original and unpublished. They have been acquired from left-over cDNA and plasma samples from two previous studies summarized below: one retrospective study (Balan et al., 2020) and one interventional study (Diman et al., 2016). Both protocols were approved by the local Ethical Committee of the Université catholique de Louvain and conducted in accordance with the Declaration of Helsinki.

2.1. Interventional study

The results presented here were obtained from three healthy and moderately active young Caucasian men. At least one week before the experimental session, the subjects performed a maximal incremental exercise test on a cycle ergometer (Cyclus II; RBM Electronics) to assess their maximal oxygen consumption (VO_{2max}). The starting load was 70 watts (W), incremented by 40 W every 3 min until exhaustion. On the day of the experimental session, a first biopsy (B1) was taken at rest from the *vastus lateralis* muscle under local anesthesia with 1 ml of Xylocaine 2% (AstraZeneca) using a Bergström needle. Then, the subjects cycled for 45 min at either 50 (one subject - S2) or 75% (two subjects - S13, S14) of their VO_{2max}. A second biopsy (B2) was taken immediately after exercise and a third biopsy (B3) 2.5 h post-exercise. Subjects were asked to refrain from strenuous physical activity for 2 days and from alcohol the day before the experiment.

2.2. Retrospective study

A sample size analysis has been performed to determine the optimal number of subjects to be recruited. Eight subjects per group were needed to find a difference in the mean of 50% with a SD corresponding to 35% of the means with a power of 80%. In total, 18 young (9 untrained and 9 trained) and 16 old (8 untrained and 8 trained) Caucasian men participated to the study on a voluntary basis. Any subject with smoking history was excluded from the experiment. Subjects under medication related to blood pressure or cholesterol were excluded from the study. According to their age and self-reported weekly physical activity, the subjects were divided into four groups: young untrained (YU) ($n = 9$), young trained (YT) ($n = 9$), old untrained (OU) ($n = 8$) and old trained (OT) ($n = 8$). Trained cyclist subjects reported to have been exercising for $\geq$6 h a week for at least 5 years while the untrained subjects reported no physical activity for at least 5 years before the experiment. As determined by maximal oxygen consumption (VO_{2max}) and maximal power output (W_{max}), their cardiorespiratory fitness was assessed during a maximal incremental fitness test on a cycle ergometer (Cyclus III; RBM Electronics). For the YU, YT and OT groups, the starting load was 70 W, incremented by 40 W every 3 min until exhaustion. For the OU group, the starting load was 30 W, incremented by 20 W every 3 min until exhaustion. One week after the incremental test, all subjects were asked to refrain from strenuous physical activity for 2 days and from alcohol for 1 day. A biopsy was taken in the fasted state from the *vastus lateralis* muscle using a Bergström needle under local anesthesia with 1 ml of Xylocaine 2% (AstraZeneca). A sample of about 70–100 mg was immediately frozen in liquid nitrogen for biomolecular analyses. The day before biopsy sampling, the diet of the subjects consisted in daily routine meals.

2.3. RNA extraction and quantitative real-time PCR

About 30 mg of muscle 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. For RT-qPCR analyses other than TERRA, reverse transcription was performed from 1 μg RNA using the iScript™cDNA

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 the 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 1/30 dilution. Melting curves were systematically performed to ensure the quality of the analysis. All mRNA levels were normalized to beta-2-microglobulin (β2M). For TERRA quantification, chromosome-specific subtelomeric sequences were used and qRT-PCR analyses were conducted as described in Arnoult et al. (Arnoult et al., 2012). Primers sequences are provided in Supplementary Table 1.

2.4. Plasma cytokine levels

Plasma levels of IL-1ra, IL-8, TNFα, IL-6, IL-1ß and MCP-1 were measured by ELISA using a personalized MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel – Immunology Multiplex Assay (Merck) according to manufacturer instructions.

2.5. Statistics

Results are presented as means ± standard error of the mean (SEM) excepted for Fig. 1 (individual values). All data are reported to the YU group for the retrospective study and to B1 of each subject for the interventional study, which were arbitrarily set to 1. For each variable, any potential outlier was identified and excluded from the calculations (Hoaglin and Iglewicz, 1987). All RT-qPCR data were normalized to β2M. Gene expression of the housekeeping gene β2M is reported in Supplemental Fig. 1. Statistical analyses were performed using GraphPad Prism 7.0. After ensuring that the variable satisfied the D'Agostino & Pearson normality test and Bartlett's test for homogeneity of variance, a two-way ANOVA was performed. When a main effect was found, Fisher's LSD tests were performed. Only plasma levels of IL-8, IL-6, IL-1β and IL-1ra did not satisfy the normality and/or the homogeneity of variance tests. Therefore, a nonparametric Kruskal-Wallis test was performed. Correlations were determined by Pearson correlation coefficient (r). Statistical significance was set at $P \leq 0.05$.

3. Results

3.1. Subjects characteristics

For the interventional study, the three subjects were 19 y old. The VO_{2max} and BMI values were 51 ml·min^{-1}·kg^{-1} and 18.9 kg·m^{-2} for subject 2, 45 ml·min^{-1}·kg^{-1} and 23.3 kg·m^{-2} for subject 13 and 59 ml·min^{-1}·kg^{-1} and 19.3 kg·m^{-2} for subject 14. For the retrospective study, the mean age was 22 ± 1 y in both YU and YT, 67 ± 1 y in OU and 68 ± 1 y in OT, with no difference between the untrained and trained groups BMI was higher in OU (26.4 ± 1.4 kg·m^{-2}) than in YU (23.0 ± 1.1 kg·m^{-2}, $P = 0.039$) and YT (23.1 ± 1.0 kg·m^{-2}, $P = 0.049$) but not different from OT (24.6 ± 0.8 kg·m^{-2}). VO_{2max} was about 25% higher in YT compared to YU (61 ± 3 *vs* 48 ± 4 ml·min^{-1}·kg^{-1}, $P = 0.035$) and about 50% higher in OT compared to OU (44 ± 4 *vs* 30 ± 2 ml·min^{-1}·kg^{-1}, $P = 0.029$). W_{max} was 75% higher in OT compared to OU (3.3 ± 0.2 *vs* 1.9 ± 0.2 W·kg^{-1}, $P < 0.001$) and 40% higher in YT compared to YS (4.3 ± 0.3 *vs* 3.0 ± 0.2 W·kg^{-1}, $P < 0.001$).

3.2. MCP-1 and SOD1 mRNA levels in skeletal muscle biopsies seem to increase after a single cycling session

We found an increase in *MCP-1* mRNA levels of up to 15-fold immediately after exercise (S2, B2) and of up to 10-fold 2.5 h post-exercise (S14, B3) compared to pre-exercise (B1) (Fig. 1A). The magnitude of the increase observed post-exercise in *MCP-1* mRNA levels seemed to be different considering the intensity of the exercise. As shown in Fig. 1B, we also detected higher post-exercise *SOD1* mRNA levels in B2 (~46%) and B3 (~63%) compared to pre-exercise (B1). Conversely, the *SOD2* mRNA levels appeared to be slightly down-regulated by the acute exercise bout (Fig. 1B).

3.3. Aging tended to increase MCP-1 mRNA levels in the skeletal muscle that was partly counteracted by training

MCP-1 mRNA levels displayed a tendency towards increase with age in the skeletal muscle (main effect of aging, $P = 0.08$; Fig. 2A), that was possibly alleviated by endurance training (YT *vs* YU, −22%, $P = 0.13$; YT *vs* OU, −27%, $P = 0.07$; Fig. 2A). Similarly, *SOD1* mRNA levels tended to increase with age and this was compensated by a high training status (main effect of training status, $P = 0.037$; Fig. 2B). Post-hoc analyses confirmed that OT and YT had lower *SOD1* mRNA levels than OU (−26%, $P = 0.03$; −25%, $P = 0.031$, respectively; Fig. 2B). Old untrained exhibited lower *SOD2* mRNA levels compared to younger ones (main effect of aging, $P = 0.08$; post-hoc, OU *vs* YU, −41%, $P = 0.04$; Fig. 2C). This decrease was no longer observed when comparing OT with YT subjects ($P = 0.40$, Fig. 2C).

A high endurance training status might alleviate the induction of MCP-1 caused by aging.

Post-hoc analyses showed that OU, but not OT, had higher circulating MCP-1 compared to YU and YT (+48%, $P = 0.0052$ and + 33%, $P = 0.0417$, respectively; Fig. 3A). The OT group tended to have lower

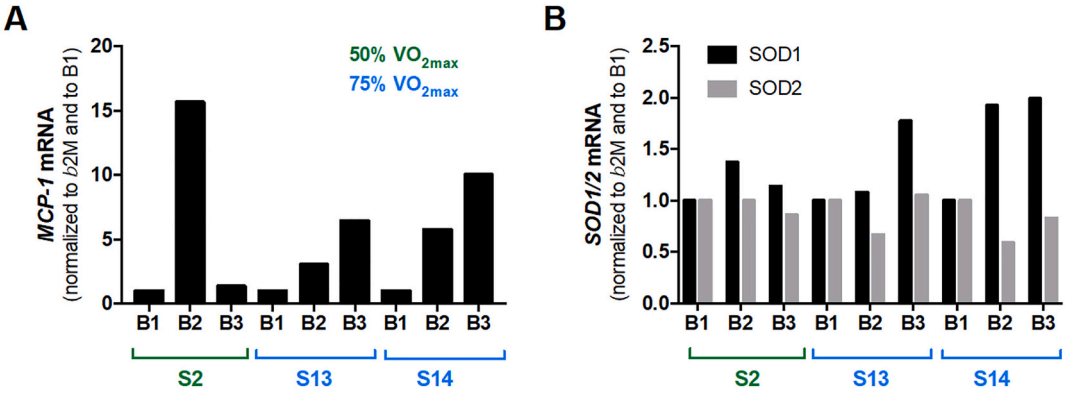

Fig. 1. Acute regulation of *MCP-1*, *SOD1* and *SOD2* mRNA levels in pre- and post-exercise young human skeletal muscle: A. *MCP-1* and B. *SOD1* - *SOD2* mRNA levels normalized to *β-2-microglobulin* (β2M) mRNA and to B1. All subjects cycled for 45 min. Subject 2 (S2) cycled at 50% VO_{2max} and subjects 13 and 14 (S13 – S14) cycled at 75% VO_{2max}. The first biopsy (B1) was taken before exercise, the second biopsy (B2) immediately after and the third one (B3) 2.5 h post-exercise. Individual values are presented. VO_{2max} = maximal oxygen consumption.

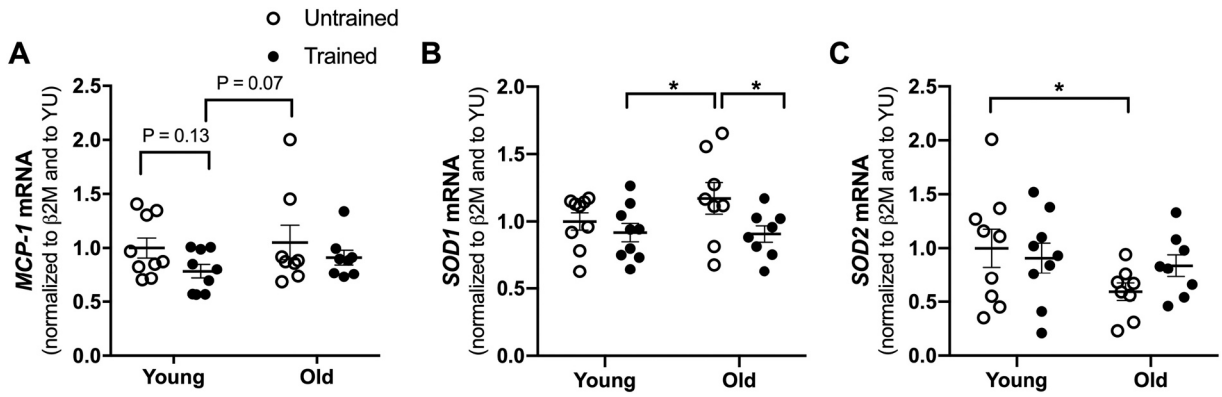

Fig. 2. Chronic regulation of *MCP-1*, *SOD1* and *SOD2* mRNA levels by aging and the endurance training status in human skeletal muscle: A. *MCP-1*, B. *SOD1* and C. *SOD2* mRNA levels normalized to *β-2-microglobulin* (β2M) mRNA and to the mean of the young untrained (YU) group. Values are expressed as means ± SEM. *$P < 0.05$.

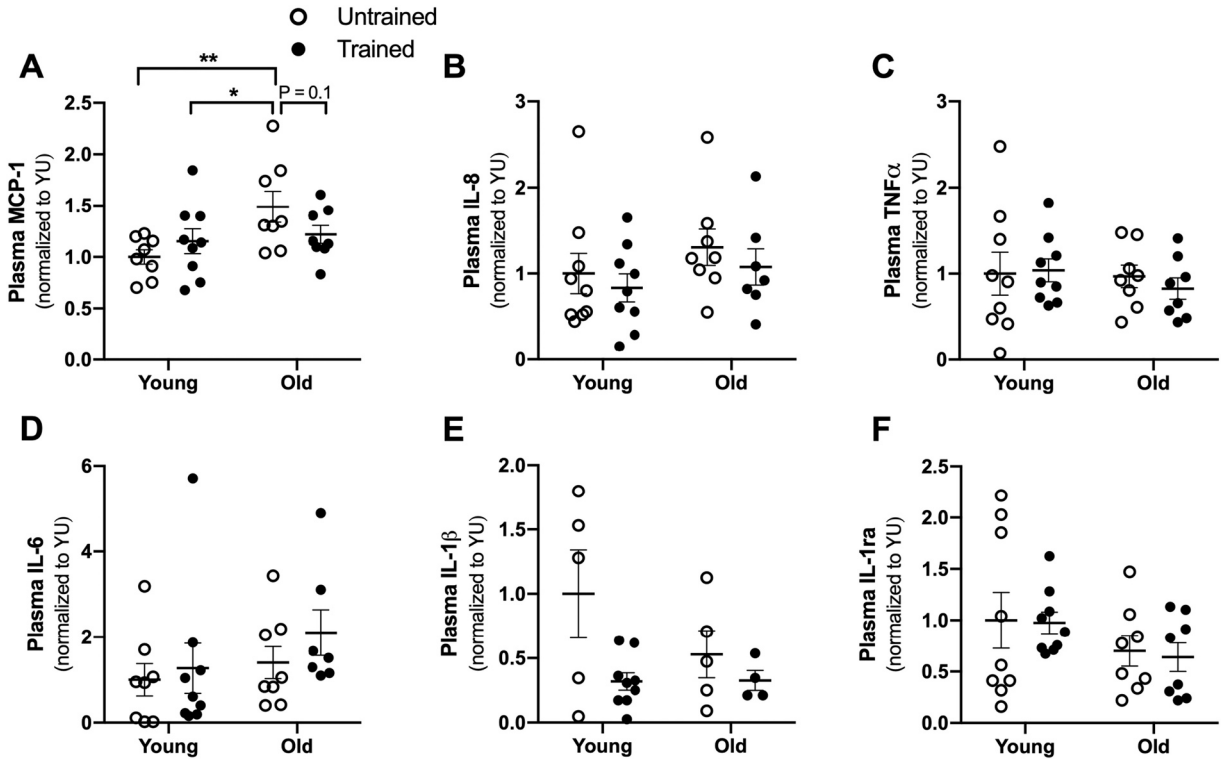

Fig. 3. Chronic regulation of systemic inflammation by aging and the endurance training status in human plasma: Relative plasma cytokines concentration normalized to the mean of the young untrained (YU) group: A. MCP-1, B. IL-8, C. TNFα, D. IL-6, *E. IL*-1β and F. IL-1ra. Values are expressed as means ± SEM. *P < 0.05, **$P < 0.01$.

levels of circulating MCP-1 levels compared to OU (−27%, $P = 0.1$; Fig. 3A).

Conversely, neither age nor the training status appeared to influence the systemic inflammation measured by the plasma levels of the cytokines IL-8, TNFα, IL-6, IL-1β and IL-1ra (Fig. 3B, C, D, E and F, respectively).

3.4. A high endurance training status counteracts the higher age-dependent TERRA levels

Post-hoc analyses revealed higher *16p* TERRA levels in OU *vs* YU (+39%, $P = 0.001$), suggesting that age could induce higher TERRA levels in untrained subjects (Fig. 4A). Moreover, we found that, whether in young or in old subjects, training was associated with reduced *16p* TERRA levels in the muscle (main effect of training status, $P = 0.0013$; Fig. 4A). As *16p* TERRA levels were reduced by 63% in old trained subjects compared to the untrained ones ($P = 0.0005$; Fig. 4A), training appeared to counteract the age-dependent increase of TERRA observed in skeletal muscle of old subjects. Although not significant, *1q-2q-4q-10q-13q-22q* TERRA levels tended to be higher in OU *vs* YT (+27%, $P = 0.06$, Fig. 4B). Similarly to the situation observed for *16p*, we found an impact of training on *1q-2q-4q-10q-13q-22q* TERRA levels in both groups (main effect of training status, $P = 0.023$, Fig. 4B). Post-hoc analysis showed that OT had lower *1q-2q-4q-10q-13q-22q* TERRA levels compared to OU (−33%, $P = 0.04$; Fig. 4B), confirming the high training status-dependent downregulation of telomeric transcription at old age.

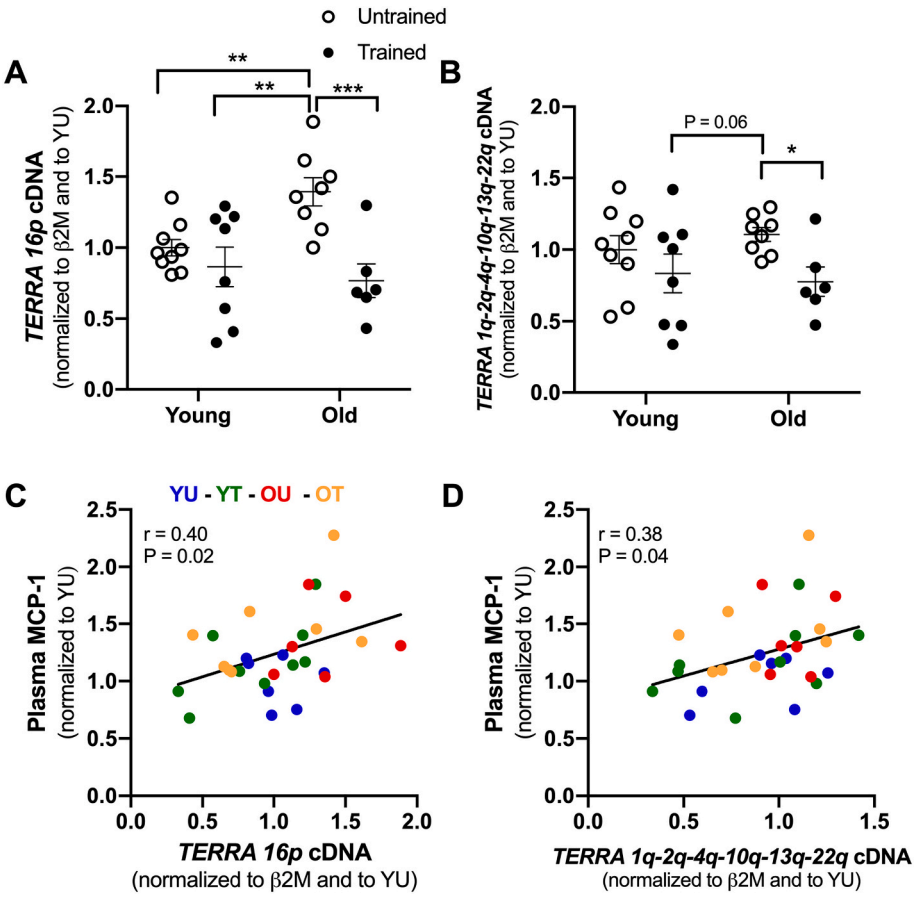

Fig. 4. Chronic regulation of Telomeric Repeat-containing RNA (TERRA) expression by aging and endurance training status in human skeletal muscle: A. *16p TERRA* levels and B. *1q-2q-4q-10q-13q-22q TERRA* levels normalized to *β-2-microglobulin* (β2M) mRNA and to the mean of the young untrained (YU group). C. Correlation between relative MCP-1 plasma concentration and relative *TERRA 16p* levels. **D.** Correlation between relative MCP-1 plasma concentration and relative *TERRA 1q-2q-4q-10q-13q-22q* levels. Young untrained (YU) in blue, young trained (YT) in green, old untrained (OU) in red and old trained (OT) in orange. Values are expressed as means ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.5. Plasma MCP-1 levels are positively correlated to telomeric transcription

Having shown that both MCP-1 serum levels and telomeric transcription increase with age and that chronic endurance exercise may counteract this upregulation, we next tested whether plasma MCP-1 levels were correlated to TERRA expression in the skeletal muscle. Accordingly, we found that plasma MCP-1 levels were positively correlated with either *16p* ($r = 0.4$, $P = 0.02$; Fig. 4C) or *1q-2q-4q-10q-13q-22q* ($r = 0.38$, $P = 0.04$, Fig. 4D) TERRA levels in the skeletal muscle. Conversely, *MCP-1, SOD1* or *SOD2* mRNA levels measured in the skeletal muscle were not correlated to either *16p* or *1q-2q-4q-10q-13q-22q* TERRA levels (data not shown).

4. Discussion

Endurance training induces numerous health benefits (Ruegsegger and Booth, 2018) and improves the quality of life of elderly people (Ozaki et al., 2007). In the skeletal muscle, the transient ROS production that occurs with an acute bout of endurance exercise is one of the mechanisms by which exercise may provide adaptive changes (Powers et al., 2016). However, how the antioxidant system reacts to counteract ROS elevation and avoid oxidative stress damage needs to be further investigated, especially in humans. Here, we had the unique opportunity to study the acute and chronic effects of endurance exercise as well as the influence of age on the transcriptional regulation of key markers of the antioxidant system, namely *SOD1* and *SOD2* genes. Indeed, the recruitment of human subjects matched for age and training status allow us to distinguish the effect of age and endurance training on the results. Moreover, to our knowledge, this study is the first to investigate how endurance training influences telomeric transcription during aging and linking TERRA expression to pro-inflammatory cytokines such as MCP-1.

4.1. An acute bout of endurance exercise seems to induce an opposite transcriptional regulation of SOD1 and SOD2

Acute aerobic exercise, through the production of ROS, induces a physiological oxidative stress (Powers et al., 2016). The impact of an acute aerobic exercise on the transcriptional response of the antioxidant system is, however, poorly characterized. Therefore, we measured the mRNA levels of *SOD1* and *SOD2* in skeletal muscle biopsies obtained pre-exercise, immediately post-exercise and after 2.5 h of recovery during our former interventional study (Diman et al., 2016). A single cycling session increased *SOD1* while decreasing *SOD2* mRNA levels in the skeletal muscle of young subjects, revealing a possible opposite effect of endurance exercise on the transcriptional regulation of these two main antioxidant enzymes. In mouse and rat skeletal muscle, several studies reported an increase of *SOD1* and/or *SOD2* mRNA post-exercise (Merry and Ristow, 2016; Hollander et al., 2001). Therefore, the increase observed here in *SOD1* mRNA levels might be part of the antioxidant reaction to protect human skeletal muscle cells against excessive ROS production. While it does not fit with its known role as a first-line antioxidant defense against $O_2^{\bullet-}$ superoxide radical anions, the decrease in *SOD2* mRNA levels could rather be related to its more recently discovered role as a peroxidase, *i.e.* utilization of H_2O_2 and oxidation of other molecules that result in mitochondrial damage (Ansenberger-Fricano et al., 2013; Ganini et al., 2018). However, the decrease observed here is moderate and the prooxidant role has been previously evidenced when SOD2 was overexpressed. Therefore, the physiological significance of a decrease in *SOD2* gene expression in the human skeletal muscle after a single exercise session remains to be

determined.

4.2. Endurance training counteracts the age-associated regulation of SOD1 and SOD2 in an opposite way

While numerous studies are trying to understand how an acute exercise influence the oxidative stress response (Powers et al., 2016; Catoire et al., 2014; Tantiwong et al., 2010), few are looking at the effect of long-term training on the basal levels of this cellular stress especially at old age. Therefore, we next looked at the long-term effects of regular endurance exercise as well as the effects of aging on *SOD1/2* mRNA levels. While other studies did not find any difference in SOD1 protein expression (Cobley et al., 2014) or mRNA levels (Johnson et al., 2014) at rest between young and old participants, we found that aging tended to increase *SOD1* mRNA levels and this was attenuated by a high endurance training status. At first, the elevation in *SOD1* mRNA levels with aging might be a response to the well-known age-associated ROS increase (Fulle et al., 2004). Secondly, it is possible that the reported down-regulation of ROS accumulation after repeated endurance exercise contributes to the mitigation of aging-induced increase in *SOD1* expression. Contrary to *SOD1*, aging was associated with decreased *SOD2* mRNA levels in our study, which was partially reversed by regular endurance exercise as no difference was detected between old and young trained participants. These results confirm the previously reported age-dependent decline in skeletal muscle *SOD2* mRNA levels (Johnson et al., 2014). In the latter study, however, cycling 3 times a week for 8 weeks did not modify skeletal muscle *SOD1* and *SOD2* mRNA or protein expression at old age (Johnson et al., 2014), indicating that 2 months may not be enough to modulate the age-related changes in *SOD1 and SOD2* expression. Two other studies found that either lifelong exercise training (Cobley et al., 2014) or training for at least 16 years (Koltai et al., 2018) increased SOD2 protein expression in master athletes compared to age-matched sedentary controls. It seems therefore likely that several years are necessary to influence the transcriptional and post-transcriptional regulation of *SOD1* and *SOD2* genes. Altogether, regular endurance exercise modulates the age-associated regulation of SOD1 and SOD2, which seem to be differentially, if not oppositely, regulated by age. Further studies are warranted to better understand whether the modifications induced by regular endurance exercise on their expression effectively translate into a better handling of ROS.

4.3. Endurance training limits the accumulation of the biological marker MCP-1 at old age

In addition to activate the antioxidant system, the up-regulation of ROS by endurance exercise and aging activates the NF-κB pathway and induces the transcription of pro-inflammatory cytokines such as MCP-1 (Catoire et al., 2014; Inadera et al., 1999; Tantiwong et al., 2010). Despite the fact that MCP-1 is mainly expressed and secreted by the adipose tissue (Arner et al., 2012), previous studies reported that cycling exercise can activate *MCP-1* transcription in the human skeletal muscle (Catoire et al., 2014; Tantiwong et al., 2010). Here, we confirmed that an acute bout of endurance exercise increased *MCP-1* mRNA levels by up to 15-fold in the skeletal muscle. Aging, as well, tended to increase *MCP-1* gene expression in the skeletal muscle with a possible protective effect of regular endurance training. Beyond its role as pro-inflammatory cytokine, MCP-1 is also known as a chemokine as it attracts macrophages during the inflammatory response (Rollins, 1996; Deshmane et al., 2009). We have previously shown that a good endurance training status reduced the age-associated macrophages tissue infiltration, assessed by cluster of differentiation 68 (*CD68*) mRNA levels and immunofluorescence staining in the same muscle samples as those used here (Balan et al., 2020). Therefore, we propose that MCP-1 could be a master regulator of aging-related macrophage infiltration in the skeletal muscle and that this might be alleviated by endurance training.

4.4. The age-related accumulation of the plasma biomarker of aging and inflammation MCP-1 is alleviated by endurance training

As plasma levels of MCP-1 were reported to increase with age, likely as a consequence of increased oxidative stress (Inadera et al., 1999), we next checked whether *i*) we could confirm this observation and, *ii*) whether endurance exercise may be able to counteract this increase. In accordance with a previous study (Inadera et al., 1999), we confirmed the age-dependent increase in MCP-1 plasma levels. Regarding the possible influence of endurance training on this variable, while a 12-week resistance training was previously reported to keep MCP-1 plasma levels unchanged in old subjects (Della Gatta et al., 2014), our lifelong data suggest a protective influence of the endurance training status by limiting the amount of MCP-1 at old ages at both plasma and skeletal muscle mRNA levels. We hypothesize that the increase observed in MCP-1 expression at old ages might be triggered by the age-related ROS accumulation and that endurance training could limit the amount of ROS and thereby lower MCP-1 expression. Being one of the SASP factors, MCP-1 is recognized as a hallmark of biological aging in mouse and human (Yousefzadeh et al., 2018) and elevated circulating MCP-1 levels reflect a greater burden of senescent cells which promote aging and age-related diseases (Jin et al., 2016). Altogether, our results confirm that MCP-1 plasma levels increase with age and we found that endurance training might exert a protection influence on this increase. One interesting candidate to be investigated in relation to aging, exercise, oxidative stress and inflammation is the tumor protein p53. Indeed, ROS can directly activate p53 as well as indirectly *via* NF-κB activation (Schneider et al., 2010). Depending on the levels and duration of oxidative stress, p53 can either exacerbate the stress (high levels) or favor the expression of antioxidant enzymes (low levels) such as SOD2, CAT and GPx (Liu and Xu, 2011; Beyfuss and Hood, 2018). The molecular mechanisms behind this dual role of p53 remains unclear and deserve further investigation. Interestingly, p53 has also been reported to up-regulate TERRA production through non-canonical p53-binding sites within human subtelomeres (Tutton et al., 2016).

4.5. Telomeric transcription might be a novel biological marker of aging

As TERRA expression appears to increase with different cellular stresses, including oxidative stress (Cusanelli and Chartrand, 2015; Arnoult et al., 2012; Schoeftner and Blasco, 2008), and, as we showed previously, in muscle biopsies after an acute bout of endurance exercise (Diman et al., 2016), we anticipated that old untrained subjects, characterized by an up-regulated oxidative stress response (Mecocci et al., 1999), could possibly display higher TERRA levels and that this might be alleviated by training. As expected, the untrained subjects of the retrospective study displayed an age-dependent increase in TERRA levels in the skeletal muscle. Although not tested here, the age-dependent increase in ROS could damage/shorten telomeres and this, in turn, would be associated with an increased production of TERRA as shown previously (Sedelnikova et al., 2004; Aguado et al., 2020). Alternatively, the up-regulated transcriptional activity at telomeres of older untrained subjects may result from an increased activation of the NRF1 transcription factor activity that, as we showed previously, regulates TERRA production in human muscle cells (Diman et al., 2016).

Supporting a possible relationship between the MCP-1 marker of biological aging and TERRA, we found a moderate correlation between skeletal muscle TERRA transcript abundance and MCP-1 plasma levels. To our knowledge, our study is the first to quantify TERRA transcripts in the old human skeletal muscle and we propose that the age-dependent TERRA accumulation might represent a novel biological marker of aging. Previously, aberrant telomere transcriptional activity has been reported as a main driver of premature aging disorders (Yehezkel et al., 2013; Sagie et al., 2017). Noteworthy, the age-dependent increase in TERRA expression was alleviated in the old trained participants. As an acute cycling exercise increases telomeric transcription (Diman et al.,

2016), our observation that chronic endurance training, known to be associated with lower oxidative stress levels (Vincent et al., 2000), has the opposite effect and counteracts the age-dependent increase of TERRA, further supports that telomeric transcription may be part of the cellular anti-oxidant response. Consisting of G-rich telomeric repeats prone to oxidation, being closely associated with telomeres and known for their protective roles at telomeres (Chu et al., 2017), it is tempting to speculate that TERRA molecules may possibly protect the guanine residues of telomeric DNA repeats from ROS-induced oxidative lesions. We therefore hypothesize that the excessive amounts of ROS observed during aging might, by a telomere damage-dependent or independent pathway, trigger telomeric transcription and that this would be alleviated by endurance training. More research is needed to establish the molecular mechanisms by which endurance training alleviate the aging-related increase in TERRA expression.

4.6. Limitations

One of the main limitations of the study is that we could not confirm whether the positive adaptations provided by endurance training indeed reduced oxidative stress as we only have indirect markers of the anti-oxidant response. Analyzing oxidative markers directly is quite challenging and requires a specific handling of the samples at the time of collection, that we did not apply. In addition, the amount of biological material available was very limited. We thus focused on the transcriptional response to oxidative stress, not on the protein levels or activity of SOD1 and SOD2.

Another limitation is the small sample size regarding the acute study. With only 3 subjects committed to different exercise intensities, no statistics were performed and interpretation of the results should be made with caution. In addition to the different exercise intensity, it is known that a high variability exists between individuals regarding the oxidative stress response following an acute exercise (Mullins et al., 2013). This said, the purpose of the acute interventional study was to get hints into the transcriptional regulation of the two main antioxidant enzyme-encoding genes, *SOD1* and *SOD2*, and of *MCP-1*, to be further analyzed in the chronic retrospective study, not to draw conclusions about the regulation of those genes upon acute exercise in the skeletal muscle.

5. Conclusion

The age-dependent increase in TERRA expression might represent a novel biological marker of aging. At old age, a high endurance training status might be a key point to alleviate the MCP-1 and TERRA aging-related accumulation. Whether those biological markers of aging are linked to an elevation of oxidative stress at old age is still an open question. Therefore, whether the positive adaptations provided by endurance training indeed reduce oxidative stress, including at telomeres, and whether TERRA plays any role in this, need to be further investigated.

Author contributions

LD and A Decottignies designed the studies; HN took skeletal muscle biopsies and blood samples; EB, A Diman and AE acquired the data, EB, LD and A Decottignies analyzed the data; EB, LD and A Decottignies wrote the manuscript. All authors revised and approved the last version of the manuscript.

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Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Appendix A. Supplementary data

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