

Sedentary aging increases resting and exercise-induced intramuscular free radical formation

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Bailey DM, McEneny J, Mathieu-Costello O, Henry RR, James PE, McCord JM, Pietri S, Young IS, Richardson RS. Sedentary aging increases resting and exercise-induced intramuscular free radical formation. *J Appl Physiol* 109: 449–456, 2010. First published May 27, 2010; doi:10.1152/jappphysiol.00354.2010.—Mitochondrial free radical formation has been implicated as a potential mechanism underlying degenerative senescence, although human data are lacking. Therefore, the present study was designed to examine if resting and exercise-induced intramuscular free radical-mediated lipid peroxidation is indeed increased across the spectrum of sedentary aging. Biopsies were obtained from the vastus lateralis in six young (26 ± 6 yr) and six aged (71 ± 6 yr) sedentary males at rest and after maximal knee extensor exercise. Aged tissue exhibited greater ($P < 0.05$ vs. the young group) electron paramagnetic resonance signal intensity of the mitochondrial ubisemiquinone radical both at rest ($+138 \pm 62\%$) and during exercise ($+143 \pm 40\%$), and this was further complemented by a greater increase in α -phenyl-tert-butyl nitron adducts identified as a combination of lipid-derived alkoxyl-alkyl radicals ($+295 \pm 96\%$ and $+298 \pm 120\%$). Lipid hydroperoxides were also elevated at rest (0.190 ± 0.169 vs. 0.148 ± 0.071 nmol/mg total protein) and during exercise (0.567 ± 0.259 vs. 0.320 ± 0.263 nmol/mg total protein) despite a more marked depletion of ascorbate and uptake of α/β -carotene, retinol, and lycopene ($P < 0.05$ vs. the young group). The impact of senescence was especially apparent when oxidative stress biomarkers were expressed relative to the age-related decline in mitochondrial volume density and absolute power output at maximal exercise. In conclusion, these findings confirm that intramuscular free radical-mediated lipid peroxidation is elevated at rest and during acute exercise in aged humans.

electron paramagnetic resonance spectroscopy; spin trapping; mitochondria; lipid peroxidation; Free Radical Theory of Aging

THE CENTRAL TENET of the Free Radical Theory of Aging, first conceived in 1956 by Denham Harman (23) and later refined by Jaime Miquel (30), is based on the premise that (mitochondrial) free radical formation is an underlying cause of degenerative senescence. Skeletal muscle, a highly oxidative tissue exposed to rapidly changing metabolic demands, may play an important role in this process. Indeed, evidence from animal

studies has suggested that an accumulation of oxidative damage to mitochondrial membrane phospholipids, proteins, and DNA over the animal's lifespan is a potential cause of the sarcopenia and mitochondrial bioenergetic dysfunction that distinguish senescent from youthful skeletal muscle (18).

Acute exercise has the capacity to increase oxidative stress, and Davies et al. (15) were the first to provide direct electron paramagnetic resonance (EPR) spectroscopic evidence of an exercise-induced increase in the mitochondrial ubisemiquinone radical ($UQ^{\cdot-}$) that was associated with lipid peroxidation, sarcolemmal membrane damage, and decreased mitochondrial respiratory control in rodent muscle. These findings were later confirmed in exercised human skeletal muscle, and complementary spin-trapping studies identified the additional appearance of lipid-derived oxygen-centered alkoxyl (LO^{\cdot}) and carbon-centered alkyl (LC^{\cdot}) radicals (2).

Microdialysis and intracellular fluorescence techniques have since established that exercising rodent muscle has the capacity to generate superoxide ($O_2^{\cdot-}$), hydroxyl ($\cdot OH$), hydrogen peroxide (H_2O_2), and nitric oxide (NO) radicals (24). While thermodynamically capable of causing cellular damage when in “physiological excess,” these species can also serve to optimize contractile performance and initiate long-term protective adaptations to the intermittent stress imposed by exercise training (1, 20–22, 24).

Given that aging and acute exercise exhibit independent prooxidant potential, it would therefore seem intuitively reasonable to expect a more pronounced exercise-induced oxidative stress response in senescent compared with young skeletal muscle. However, the few studies (5, 39) that have tested this hypothesis remain equivocal, were not performed in humans, and were constrained by technical limitations associated with indirect measurements and controversial sample-processing techniques. To provide further clarity, we used EPR spectroscopy, the only direct analytical technique currently available for the molecular detection and subsequent characterization of free radicals (13), to test the following hypotheses: 1) both resting and exercise-induced free radical-mediated lipid peroxidation will be greater in senescent compared with young human skeletal muscle and 2) this will be a consequence of inadequate nonenzymatic antioxidant defense in the senescent compared with young human skeletal muscle.

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EXPERIMENTAL PROCEDURES

Subjects

Six young (26 ± 6 yr) and six aged (71 ± 6 yr) males provided written informed consent after ethical approval by the University of California San Diego Human Subjects Protection Program. All subjects were normotensive, nonsmokers, free of vascular disease, and not prescribed any medication. Both groups were matched for body weight, height, and dietary status and were classified as sedentary since they had not regularly engaged in any leisure time physical activities throughout their adult lives.

Laboratory Testing

Subjects arrived at the laboratory after a 12-h overnight fast. During *visit 1*, subjects performed a two-legged cycling test for the determination of peak O_2 consumption ($\dot{V}\text{O}_{2\text{max}}$), which was defined using established criteria, namely, respiratory exchange ratio of >1.15 , whole blood lactate of >8 – 9 mM, and, ultimately, failure to maintain the required cadence (a consistent fall of >10 rpm below the target of 70 rpm) despite verbal encouragement (4). After two familiarization bouts (*visits 2* and *3*) using the single-leg dynamic knee extension apparatus, they performed an incremental test to exhaustion to determine the maximal work rate (WR_{max}) with either the right leg or left leg in a randomized balanced design (*visit 4*). After a 7-day rest, subjects performed an acute bout of exercise consisting of 2 min at 50% WR_{max} followed by 3 min at 100% WR_{max} with the original exercise-tested limb used during the familiarization bouts (*visit 5*).

Tissue Sampling

Muscle biopsies (Bergstrom technique) were obtained from the vastus lateralis muscle of the resting or exercised contralateral leg in a randomized balanced design. Thus, in six of the subjects (3 young and 3 old), a resting biopsy was obtained before the subsequent exercise biopsy, with an intersampling time difference of exactly 15 min. In the six remaining subjects, the resting biopsy was obtained 15 min after the completion of the exercise challenge and immediate retrieval of the biopsy from the exercised contralateral leg.

Sample Organization

Samples were immersed in liquid N_2 , dissected with a glass rod, and distributed among five vials. We specifically chose not to stabilize samples with either bathocuproinedisulfonic acid or 2,2'-dipyridyl (27) *ex vivo* since exercise and/or aging may have influenced the metabolism of redox-reactive transition metal ions. Vials were stored at -196°C (model CP100, Cryopak Series) for the following analyses: *vials 1–3* were used for the EPR spectroscopic detection of free radicals in frozen tissue ($g = 2.004$ signal), thawed tissue/homogenate was used for α -phenyl-*tert*-butylnitron (PBN) adducts and DMSO-stabilized ascorbate radical (DMSO/ $\text{A}^{\cdot-}$), *vial 4* was used for lipid hydroperoxides (LOOH) and lipid-soluble antioxidants (LSA), and *vial 5* was used for tissue morphology and histology. A second tissue block assigned for structural analyses (rest sample only) was immersion fixed at 21°C in glutaraldehyde fixative (6.25% glutaraldehyde solution in 0.1 M sodium cacodylate buffer, 1,100 mosM, pH 7.4) for low-power electron microscopy.

Oxidative Stress Biomarkers

$g = 2.004$ signal. Frozen tissue was pulverized under liquid N_2 and added to a quartz finger dewar. Samples were analyzed under liquid N_2 using an X-band EPR spectrometer (Varian E-104). The intra- and interassay coefficients of variation (CVs) were both $\sim 5\%$.

PBN adducts. A section of this frozen tissue was subsequently transferred to a glass vial that contained 1 ml of a 190 mM solution of PBN dissolved in 0.9% NaCl and allowed to incubate at 21°C for exactly 60 min in the dark. The perfusate (800 μl) was added to an

equal volume of HPLC-grade toluene, vortexed for 30 s, and centrifuged at 4,000 rpm for 10 min. Adducts (200 μl) were pipetted into a precision-bore quartz EPR sample tube and vacuum degassed before analysis at X-band (Bruker EMX) at 21°C . The intra- and interassay CVs were both $<15\%$.

Residual $\text{A}^{\cdot-}$ (proxy for tissue ascorbate). A final section of frozen tissue was weighed, pulverized under liquid N_2 , and vortexed for 60 s with 2 ml of DMSO to enhance the EPR detection of $\text{A}^{\cdot-}$ (34). The homogenate was centrifuged at 4,000 rpm for 5 min, and 1 ml of the supernatant was injected into a high-sensitivity multiple-bore sample cell (AquaX, Bruker Instruments). Samples were also analyzed by X-band (Bruker EMX) at 21°C . The intra- and inter-assay CVs were both $<5\%$.

EPR settings. The following parameters were used: microwave power = 10 mW ($g = 2.004$ and $\text{A}^{\cdot-}$) or 20 mW (PBN); modulation frequency = 100 kHz; modulation amplitude = 4.0 G ($g = 2.004$), 0.5 G (PBN), or 0.65 G ($\text{A}^{\cdot-}$); gain = 8×10^3 ($g = 2.004$), 1×10^5 (PBN), or 2×10^5 ($\text{A}^{\cdot-}$); magnetic field center = 3,258 G ($g = 2.004$), 3,465 G (PBN), or 3,477 G ($\text{A}^{\cdot-}$); scan width = ± 400 G ($g = 2.004$) or ± 50 G (PBN and $\text{A}^{\cdot-}$); scan time = 240 s ($g = 2.004$) or 84 ms (PBN and $\text{A}^{\cdot-}$); and time constant = 128 ms for 1 sweep ($g = 2.004$), 82 ms for 10 incremental sweeps (PBN), or 41 ms for 3 incremental sweeps ($\text{A}^{\cdot-}$). All EPR data were analyzed using commercially available software (version 2.11, Bruker Win EPR System) and filtered identically. The average spectral peak-to-trough line height was considered as a measure of the (relative) radical concentration.

LOOH. LOOH were determined spectrophotometrically using the ferrous iron-xylene orange technique (42). The intra- and interassay CVs were both $<5\%$.

LSA. The homogenate was assayed for α -tocopherol (α -TOH), α -carotene, β -carotene, retinol, and lycopene using an HPLC method (16, 17). The intra- and interassay CVs were both $<5\%$.

Tissue Morphology and Histology

An additional biopsy was obtained from the resting leg and separated into two smaller blocks (*vials 5* and *6*). The first tissue block was frozen in liquid N_2 , and 8- μm -thick transverse sections were cut at -24°C with a cryostat (Jung-Reichert Cryocut 1800) and kept at -20°C . After 5 min of fixation in Guth and Samaha fixative, sections were incubated at 37°C for 1 h in Pb-ATPase staining medium to stain for fiber types I and II (29). The relative cross-sectional area and numbers of type I and type II fibers were estimated by point counting using eyepiece square grid test A100 on histochemical sections examined at a magnification of $\times 250$ with a light microscope (29). The second tissue block was prepared for ultrastructural morphometric analyses (mitochondrial volume, myofibrillar volume, and intramuscular fat) by low-power electron microscopy according to established techniques (29). The intra- and interassay CVs were both $<5\%$.

Normalization Procedures

Biomarkers were normalized relative to dry tissue weight (DMSO/ $\text{A}^{\cdot-}$ and PBN adducts) or total protein concentration ($g = 2.004$ signal, LOOH, and LSA). For total protein concentrations, tissue samples were homogenized with 1 part butylated hydroxytoluene (200 μM dissolved in methanol) to 99 parts PBS (10 mM) and adjusted to pH 7.4. The mixture was added at a concentration of 1 ml/100 mg tissue, and the supernatant was analyzed spectrophotometrically using the Bradford assay. After this initial normalization, oxidative stress biomarkers ($\text{UQ}^{\cdot-}$, PBN adducts, and LOOH) were expressed relative to mitochondrial volume density (in %) and maximal absolute power output (in W).

Table 1. Subject characteristics

Group	Young Group	Aged Group
Anthropometrics		
Age, yr	26 ± 6	71 ± 6†
Weight, kg	76.3 ± 7.5	79.1 ± 4.7
Height, m	1.75 ± 0.04	1.72 ± 0.03
Quadriceps muscle mass, kg	2.45 ± 0.11	2.00 ± 0.22†
Tissue morphology/histology		
Type I fiber content, %	37 ± 15	53 ± 12†
Type II fiber content, %	63 ± 15	47 ± 12†
Capillary-to-fiber ratio	1.82 ± 0.79	1.51 ± 0.39
Mitochondrial volume density, %	4.3 ± 0.7	3.4 ± 0.5†
Myofibrillar volume, %	82.2 ± 1.4	85.1 ± 1.8†
Intramuscular fat, %	0.5 ± 0.4	0.5 ± 0.3
Exercise performance		
Two-legged maximal O ₂ uptake, ml·kg ⁻¹ ·min ⁻¹	33 ± 2	22 ± 3†
Single-leg maximal work rate, W	28 ± 8	24 ± 7†

Values are means ± SD; *n* = 6 subjects/group. †Significantly different (*P* < 0.05) between groups.

Statistical Analysis

After confirmation of distribution normality using Shapiro-Wilk *W*-tests, data were analyzed using a two-factor mixed ANOVA (group: young vs. aged × condition: rest vs. exercise). A combination of Bonferroni-corrected paired and independent sample *t*-tests were used to make post hoc comparisons. Relationships were identified using Pearson product moment correlations. Significance was established at *P* < 0.05, and data are presented as means ± SD.

RESULTS

Subject Characteristics

The needle biopsy technique yielded adequate muscle samples both at rest and exercise in the young (rest: 187 ± 40 mg and exercise: 132 ± 43 mg) and old (rest: 172 ± 26 mg and exercise: 121 ± 30 mg) subjects. Aged subjects demonstrated lower values for quadriceps muscle mass, type II fiber content, mitochondrial volume density, (two-legged) $\dot{V}O_{2\max}$, and (single-leg) WR_{\max} and higher values for type I fiber content and myofibrillar volume (*P* < 0.05 vs. young subjects; Table 1).

Antioxidants

Exercise generally increased (*P* < 0.05 vs. rest) the tissue concentration of all LSA (Table 2), and the reduction in DMSO/A^{•-} (*a*_{H4}; ≈1.76 G) indicated that ascorbate content had decreased (Fig. 1A). An inverse relationship was observed between the reduction in ascorbate and increase in α-TOH

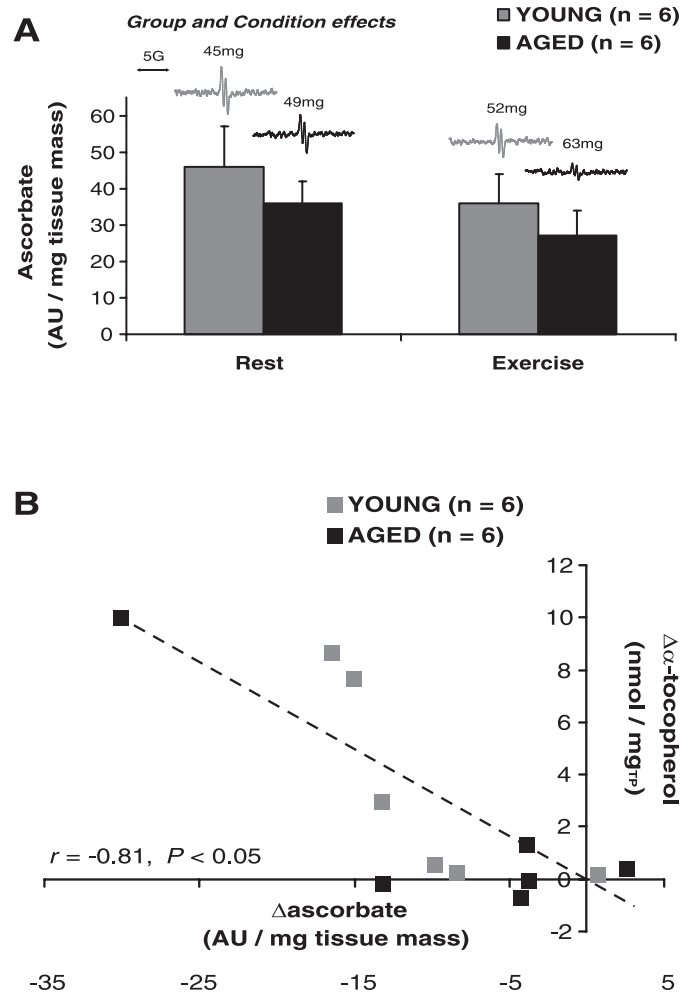


Fig. 1. A: changes in the electron paramagnetic resonance spectral intensity of the residual ascorbate free radical (A^{•-}). Typical spectra and corresponding masses are also shown for a single young and aged subject. AU, arbitrary units. B: inverse relationship (pooled data) observed between the exercise-induced changes (Δ : exercise minus rest value) in the normalized intramuscular concentration of ascorbate and α -tocopherol. TP, total protein.

(Fig. 1B). With the exception of α-TOH and retinol, all remaining LSA were generally elevated in aged subjects, whereas ascorbate (Fig. 1A) was depressed (*P* < 0.05 vs. young subjects). A linear relationship was observed between the exercised-induced increase in the cumulative concentration of LSA and LOOH (*r* = 0.69, *P* < 0.05).

Table 2. Lipid-soluble antioxidants

Group: Condition:	Young Group		Aged Group	
	Rest	Exercise	Rest	Exercise
α-Tocopherol†	2.88 ± 1.27	6.26 ± 5.02	4.20 ± 2.32	6.00 ± 3.91
α-Carotene*†	0.005 ± 0.005	0.012 ± 0.007	0.037 ± 0.048	0.066 ± 0.084
β-Carotene*	0.004 ± 0.003	0.012 ± 0.010	0.032 ± 0.061	0.062 ± 0.074
Retinol†	0.034 ± 0.012	0.122 ± 0.193	0.058 ± 0.055	0.153 ± 0.177
Lycopene*†	0.024 ± 0.021	0.077 ± 0.56	0.088 ± 0.117	0.121 ± 0.087

Values (in nmol/mg total protein) are means ± SD; *n* = 6 subjects/group. Values were normalized for total protein concentration. *Group effect, which indicates a pooled (rest + exercise) difference between young vs. aged subjects (*P* < 0.05); †condition effect, which indicates a pooled (young + aged) difference between rest vs. exercise (*P* < 0.05).

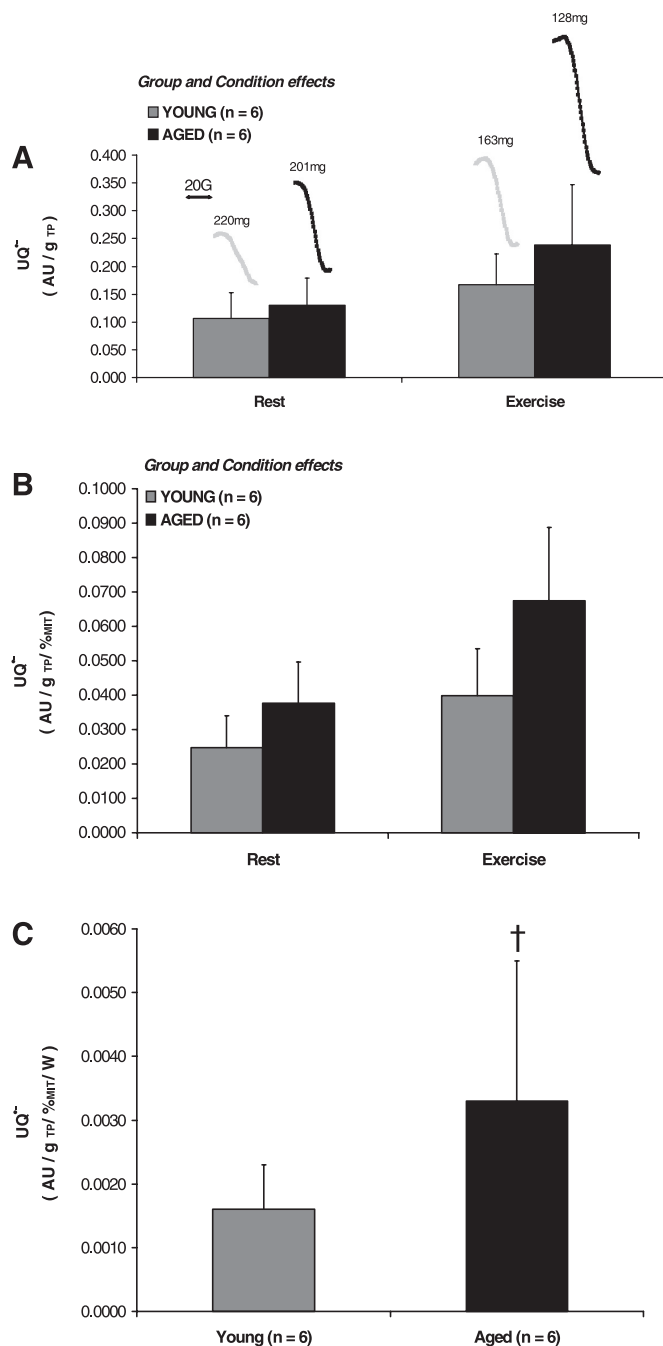


Fig. 2. A: changes in the spectral intensity of the $g = 2.004$ signal expressed relative to the TP concentration with typical spectra and corresponding masses. B: data were further normalized for mitochondrial volume density (%MIT). C: exercise values were further corrected for (single-leg) maximal absolute power output (in W). UQ•, ubisemiquinone radical. †Significantly different ($P < 0.05$) between groups.

$g = 2.004$ Signal

All frozen tissue samples exhibited an isotropic single doublet (lacking hyperfine structure) located at $g = 2.004$, as shown in Fig. 2A, consistent with a mitochondrial UQ• species (4, 5, 14, 22, 23). This signal increased more markedly with exercise in the aged group [$+0.106 \pm 0.071$ vs. $+0.063 \pm 0.051$ arbitrary units (AU)/g total protein, $P < 0.05$]. These differences were even more pronounced when biomarkers were

further normalized relative to mitochondrial volume density (Fig. 2B) and maximal (absolute) power output (Fig. 2C).

PBN Adducts

Figure 3A shows typical EPR spectra of PBN adducts extracted from thawed tissue. Computer simulation confirmed two primary species with the following hyperfine coupling constants: $a_1^N = 13.7$ G and $a_1^H = 1.9$ G ($\approx 90\%$ of total signal) and $a_2^N = 14.0$ G and $a_2^H = 4.0$ G ($\approx 10\%$ of total signal), consistent with the trapping of LO• and LC•,

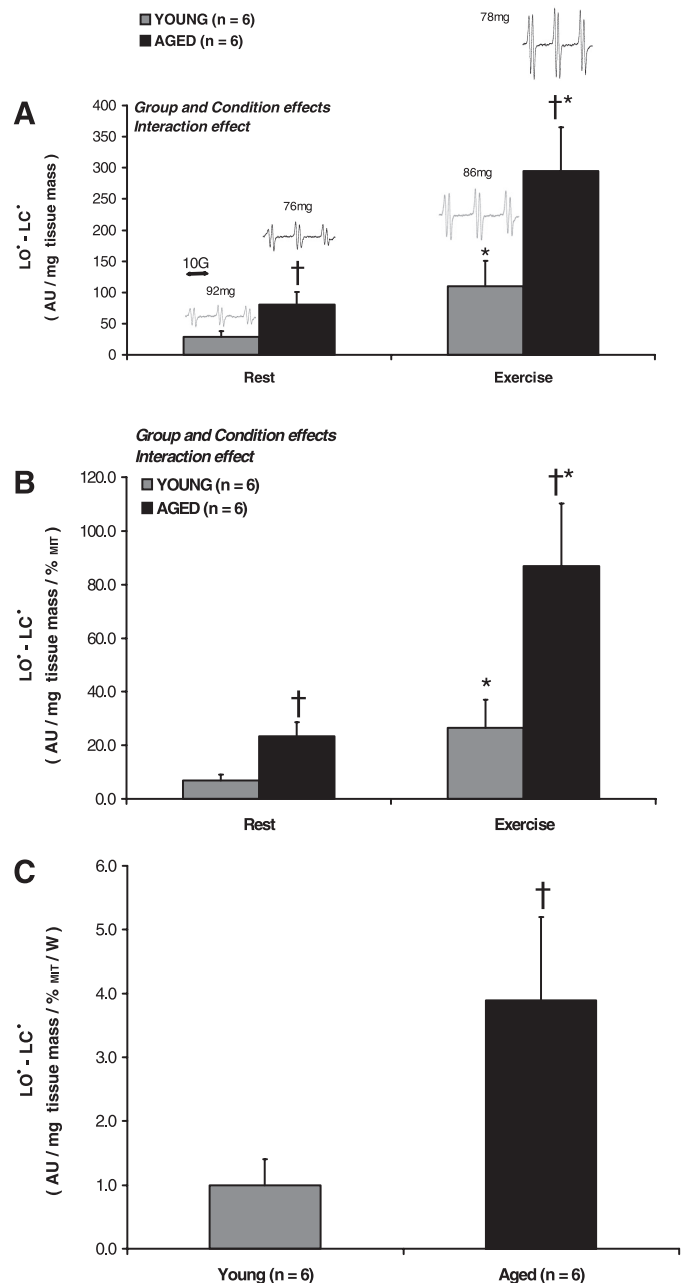


Fig. 3. A: changes in the spectral intensity of α -phenyl-*tert*-butylnitron adducts identified as a combination of lipid-derived alkoxy-alkyl radicals (LO•-LC•) expressed relative to tissue mass. B and C: data after further normalization. *Significantly different ($P < 0.05$) between conditions for a given group; †significantly different ($P < 0.05$) between groups for a given condition.

respectively, in identical solvents (10). Exercise increased LO \cdot -LC \cdot more markedly in the aged group ($+215 \pm 67$ vs. $+81 \pm 39$ AU/mg tissue mass, $P < 0.05$), with the differences becoming more apparent upon further normalization (Fig. 3, *B* and *C*).

LOOH

Likewise, exercise increased LOOH (Fig. 4*A*) more markedly in the aged group ($+0.377 \pm 0.342$ vs. $+0.173 \pm 0.234$ nmol/mg total protein, $P < 0.05$), with the differences

becoming more apparent after normalization (Fig. 4, *B* and *C*).

DISCUSSION

The findings of this study are consistent with an accumulating body of cellular and animal-based literature and confirm that intramuscular free radical-mediated lipid peroxidation is elevated in aged humans. Even under resting conditions, the concentration of free radicals in the form of mitochondrial UQ \cdot^- was greater relative to that observed in young tissue, and the corresponding rise in the secondary reactants, LOOH and LO \cdot -LC \cdot , confirmed that lipid peroxidation was also elevated. This was further compounded by acute exercise despite a more pronounced intramuscular mobilization of LSA and depletion of ascorbate. The impact of senescence was especially apparent when exercise-induced oxidative stress biomarkers were expressed relative to mitochondrial volume density and absolute power output, acknowledged prooxidant sources that decline with sedentary aging subsequent to sarcopenia.

Antioxidant Defense

The present findings extend what we have previously documented in young humans (2) to the elderly population and reveal an even more pronounced resting and exercise-induced increase in the LSA content of senescent skeletal muscle. The latter may be explained by a blood flow-mediated increase in the delivery of triglyceride-rich lipoproteins since they are the major, if not the only, carriers of blood-borne LSA (33). However, a previous thermodilution study (28) revealed that sedentary aged subjects, similar to those in the current study, exhibit $\sim 20\%$ lower quadriceps muscle blood flow per unit muscle mass during exercise (28), thus arguing against the (increased) "perfusion concept."

While we do not currently have a satisfactory answer to explain this unique phenomenon, we remain confident that it is indeed a genuine observation and not an artifact caused by blood contamination of biopsy specimens with lipoproteins since identical protocols were applied in all subjects. The intramuscular mobilization of LSA may represent an adaptive countermeasure (akin to the acute-phase response), the magnitude of which correlates in direct proportion to the increase in oxidative stress. The linear relationship observed between the exercised-induced increase in the cumulative concentration of LSA and LOOH provides tentative support to this contention.

The intramuscular enrichment of α -TOH typically concentrated in the inner mitochondrial membrane was found to be present at a concentration approximately two to three orders of magnitude higher than the other LSA measured, which would have been expected to increase the ability of senescent tissue to "chain break" lipid peroxidation. This is due to the fact that the phenolic OH group located in its chromanol ring scavenges lipid-derived peroxy radicals faster than the equivalent reaction with lipid side chains:

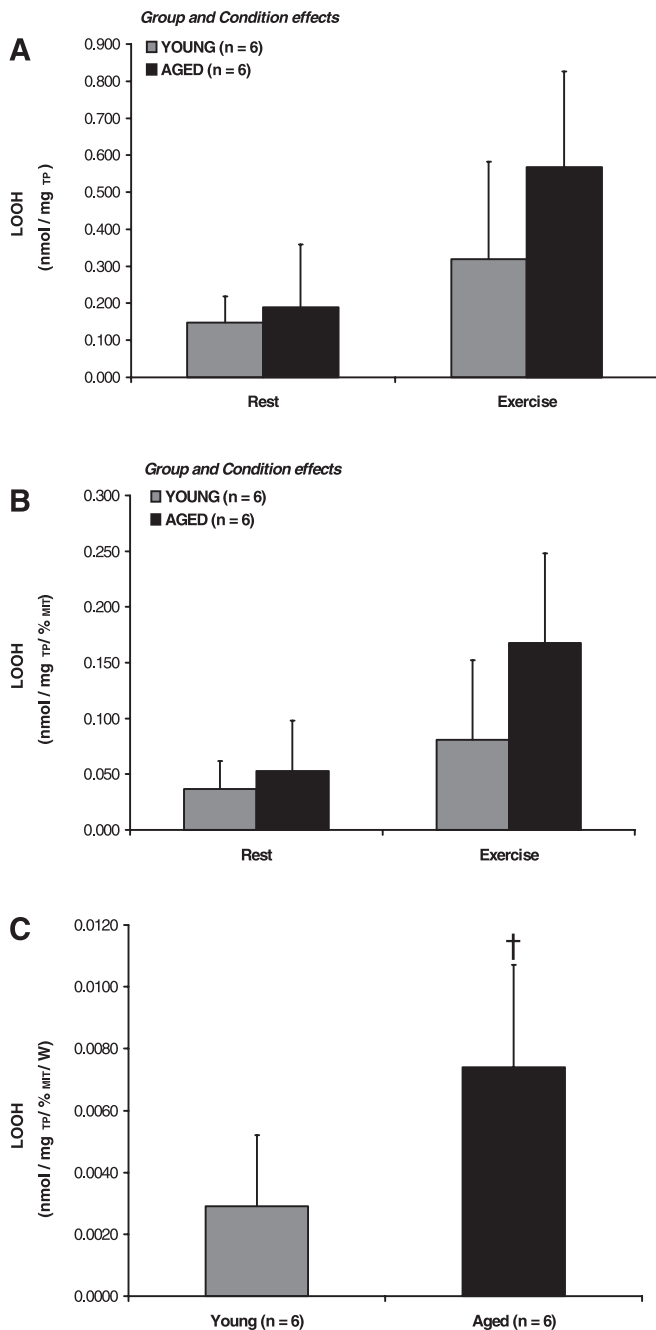
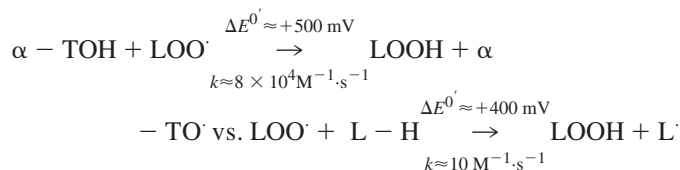


Fig. 4. *A*: changes in the concentration of lipid hydroperoxides (LOOH) expressed relative to TP concentration. *B* and *C*: data after further normalization. †Significantly different ($P < 0.05$) between groups for a given condition.



where LOO^\cdot is the lipid peroxy radical, $\alpha\text{-TO}^\cdot$ is the α -tocopheroxy radical, E^0 is the reduction potential, k is a constant, L-H represents a polyunsaturated lipid, and L^\cdot represents a carbon-centered lipid radical. Likewise, the enrichment of carotenoids and retinol would have been expected to increase the quenching capacity of tissue against singlet O_2 and LOO^\cdot formation, with higher rate constants observed at low(er) ambient partial pressures of O_2 (11), typically encountered in exercising muscle in the order of $\sim 2\text{--}5$ mmHg (37).

The intramuscular content of ascorbate was determined using an enhanced EPR technique, taking advantage of DMSO to promote the quantitative oxidation of ascorbate (AH^-) to yield the resonance-stabilized tricarbonyl species $\text{A}^{\cdot-}$ (34). In stark contrast to the LSA data, exercise and aging were both shown to deplete tissue of ascorbate, as indicated by the persistent reduction in the spectral intensity of $\text{DMSO/A}^{\cdot-}$. In light of the low E^0 value associated with the $\text{A}^{\cdot-}/\text{AH}^-$ couple ($E^0 = 282$ mV) (41), ascorbate is considered the most effective water-soluble chain-breaking antioxidant (19), and its disappearance likely reflects the ongoing consumption by tissue during targeted “repair” of $\text{O}_2^{\cdot-}$, OH^\cdot , LOO^\cdot , LO^\cdot , and LC^\cdot . Furthermore, ascorbate can recycle $\alpha\text{-TO}^\cdot$ at the aqueous-lipid interface to regenerate the fully functional $\alpha\text{-TOH}$ isomer (9, 38), which may account for the inverse relationship observed between (exercise-induced) ascorbate depletion and $\alpha\text{-TOH}$ enrichment. Constrained by the available tissue mass, it was not possible to assess glutathione, although this would have been of interest given its role as a substrate for glutathione peroxidase, thus targeting H_2O_2 and LOOH and with the potential to reduce $\text{A}^{\cdot-}$ and $\alpha\text{-TO}^\cdot$ (36). Additionally, complementary assays of catalase and superoxide dismutase would have provided useful information regarding age-related changes in muscle antioxidant capacity and the potential impact endogenous enzymatic antioxidants could have had on the present results.

Free Radical-Mediated Lipid Peroxidation

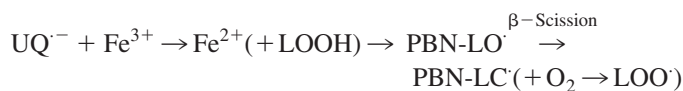
The reactive biomarkers measured in the present study suggest that the antioxidant defense response likely constrained but was clearly unable to prevent some degree of oxidative stress. EPR spectroscopy revealed a persistent exercise- and age-induced increase in the signal centered at $g = 2.004$. This has previously been detected in contracting rodent skeletal muscle (15, 25), in the rodent diaphragm (7), and, more recently, in human skeletal muscle (2), but to our knowledge, this is the first time free radicals have been documented not only directly in the muscle of elderly humans but also in greater concentration than in young subjects both at rest and as a consequence of exercise. This radical has been identified as a quasistable $\text{UQ}^{\cdot-}$ species generated within the Q_0 and Q_i sites of the electron transport chain (14) and complements a previous report (24) of other mitochondrial ROS/RNS, albeit in rodent tissue, notably, OH^\cdot , $\text{O}_2^{\cdot-}$, H_2O_2 , and NO (24).

These findings point toward the mitochondrion as a potential, although not exclusive, subcellular source of oxidative stress. In support of the present data, a functional deficiency in the Q_0 binding site within cytochrome b of complex III has been identified as the primary cause for increased electron “leakage” and subsequent $\text{UQ}^{\cdot-}$ formation in aged mitochondria (31). Precisely why exercise should promote further $\text{UQ}^{\cdot-}$ formation, especially in senescent tissue, is not entirely clear. Mitochondrial $\text{O}_2^{\cdot-}$ formation in vitro has been shown to be greatest during state 4 respiration and decreases markedly during state 3 (8) (akin to the rest \rightarrow exercise transition in vivo). Thus, the traditional belief that ROS formation would be expected to increase in exercising humans in direct proportion to (muscle) O_2 consumption when most muscle mitochondria will be in state 3 remains a contradiction. In support, we would have anticipated $\sim 30\%$ lower maximal quadriceps muscle O_2 consumption in aged subjects (17), yet exercise-induced $\text{UQ}^{\cdot-}$ formation remained clearly elevated, confirming that mitochondrial O_2 “flux” per se is not the primary mechanism responsible for exercise-induced ROS formation in vivo (2, 3).

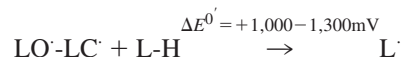
An alternative explanation may relate to age-related differences in the (extra)mitochondrial PO_2 response to exercise. Since maximal quadriceps muscle O_2 delivery (decreased quadriceps muscle blood flow) and extraction (decreased mitochondrial volume density) decline with sedentary aging (17), we would have expected a more marked exercise-induced fall in intracellular PO_2 and thus, by consequence, mitochondrial PO_2 . In vitro findings have suggested that this may alter the lipid-protein structure within the mitochondrial membrane, thereby increasing the “lifetime” of $\text{UQ}^{\cdot-}$ by slowing its rate of oxidation by cytochrome b (22).

Furthermore, despite not being especially reactive ($E^0 = 200$ mV) (16), $\text{UQ}^{\cdot-}$ is the primary source of mitochondrial $\text{O}_2^{\cdot-}$ ($\text{UQ}^{\cdot-} + \text{O}_2 \rightarrow \text{UQ} + \text{H}^+ + \text{O}_2^{\cdot-}$) (35), the stoichiometric precursor to H_2O_2 , which can freely diffuse to the cytoplasm and initiate lipid peroxidation. The observed rise in LOOH and spin-trapped $\text{LO}^\cdot\text{-LC}^\cdot$ in the present study, which was further evident upon normalization, provides convincing evidence for an exercise- and aging-induced increase in free radical-mediated lipid peroxidation.

The secondary formation of extramitochondrial $\text{LO}^\cdot\text{-LC}^\cdot$ detected may have evolved during the metal-catalyzed reductive decomposition of LOOH after attack by an initiating radical (e.g., $\text{UQ}^{\cdot-}$):



These species are thermodynamically capable of initiating additional chain reactions (9):



In support of this concept, exercise and aging are both associated with an increase in both the intramuscular content and circulating vascular bioavailability of catalytic iron (26), which may prove the unifying mechanism responsible for the increased oxidative stress observed in senescent tissue.

Although these findings demonstrate that acute exercise and aging are independent variables that compound free radical-mediated lipid peroxidation, they do not suggest that

aged individuals should refrain from engaging in long-term physical activity. To the contrary, emerging evidence suggests that while thermodynamically capable of causing cellular damage when in "physiological excess," these species also serve as integral components of the signal transduction sequence capable of regulating antioxidant gene expression and initiating protective adaptations to the intermittent stress of exercise training for the long-term maintenance of homeostasis (1, 20–22, 24). Thus, the controlled formation of ROS may contribute, at least in part, to the observed benefits of physical activity in the elderly (32).

Experimental Limitations

While the free radicals detected in muscle are known to be relatively stable (12), there was an unavoidable time delay between the recovery of the biopsy sample after the exercise challenge and freezing (~60 s). Thus, it is conceivable that the radicals detected probably underestimate the real-time concentration in vivo. Furthermore, it is important to recognize the limitations of spin trapping, which ultimately relies on the ex vivo detection of second-generation radicals that are formed downstream of the primary reaction pathway that we interpret to reflect dynamic events in vivo (6). Thus, we cannot exclude potential artifacts generated by ex vivo chemistry during the incubation phase of tissue with PBN. However, fact that all samples were treated identically suggests that the exercise and aging responses observed are indeed authentic.

Conclusions

In support of our original hypotheses, these findings are consistent with the Free Radical Theory of Aging and demonstrate that both resting and exercise-induced free radical-mediated lipid peroxidation is more pronounced in senescent compared with young human skeletal muscle. Despite an increased mobilization of LSA, aged muscle was identified as a more active source of mitochondrial $UQ^{\cdot-}$ that likely contributed to the extramitochondrial formation of the secondary reactants $LO^{\cdot-}$ - $LC^{\cdot-}$ and LOOH, which have the capacity to initiate further membrane peroxidation.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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