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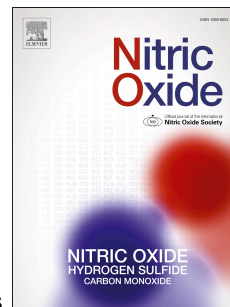
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Sprint and endurance training in relation to redox and inflammatory status and biomarkers of aging in master athletes

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SPRINT AND ENDURANCE TRAINING IN RELATION TO REDOX AND INFLAMMATORY**STATUS AND BIOMARKERS OF AGING IN MASTER ATHLETES**

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29 **SPRINT AND ENDURANCE TRAINING IN RELATION TO REDOX AND INFLAMMATORY**
30 **STATUS AND BIOMARKERS OF AGING IN MASTER ATHLETES**

31

32 **Abstract**

33 **Purpose:** Studies have shown a positive influence of intense athletic training on several biomarkers of aging, but
34 it remains unclear whether this influence is dependent of exercise-training-mode. This study compared redox
35 balance, cytokine levels and biomarkers of aging between master sprinters and endurance athletes, as well as in
36 young and middle-aged individuals as controls.

37 **Methods:** Participants were male master sprinters (SA, 50±8.9yrs; n=13) and endurance runners (EA, 53±8.2yrs;
38 n=18) with remarkable athletic experience (~25yrs of practice), besides untrained young (YC, 22.7±3.9yrs;
39 n=17) and age-matched controls (MC, 45.5±9.8yrs; n=12). Anamnesis, anthropometrics, biomarkers of aging,
40 inflammation status and oxidative stress parameters were analyzed in all participants.

41 **Results:** An increased pro-oxidant activity (elevated protein carbonyl; isoprostanes and 8-OHdG) was observed
42 for MC in comparison to remaining groups ($p<0.05$). However, SA presented a better antioxidant capacity than
43 both MC and EA, while nitrite/nitrate (NO_x) availability was higher for EA and lower for the MC ($p<0.05$). Both
44 groups of athletes presented a better anti-inflammatory status than MC (increased IL-10 and lowered IL-6, sIL-
45 6R, sTNF-RI), but worse than YC (increased TNF- α , sTNF-RI, and sIL-6R) ($p<0.05$). Telomere length was
46 shorter in MC, which also had lower levels of irisin and klotho, and elevated FGF-23 ($p<0.05$). ADMA levels
47 were higher in MC and SA, while irisin was lower in EA when compared to SA and YC ($p<0.05$).

48 **Conclusion:** Master athletes presented better redox balance and inflammatory status, with decreased biomarkers
49 of aging compared to control. Regarding exercise mode, a better NO- profile, as a marker of endothelial
50 function, was observed for EA, whereas SA had a better redox balance, cytokines profile and attenuated
51 biomarkers of aging.

52

53 **Keywords:** Master athletes; oxidative stress; cytokines; klotho-FGF-23; irisin; ADMA

54 **List of abbreviation**

ADMA	Asymmetric Dimethylarginine
BMI	Body Mass Index
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
FGF-23	Fibroblast growth factor 23
GSH	Glutathione
IL-10	Interleukin 10
IL-15	Interleukin 15
IL-6	Interleukin 6
NO _x	nitrite/nitrate
NO ₂ ⁻	Nitrite
O ₂	Oxygen
8-OHdG	8-hydroxy-2'-deoxyguanosine
sIL-6R	Soluble Interleukin-6 receptor
SOD	Superoxide dismutase
sTNF-RI	Soluble tumor necrosis factor receptor type I
TBARS	Thiobarbituric acid reactive substances
TL	Telomere length
TNF- α	Tumor necrosis factor-alpha
Trolox equivalent	Total antioxidant capacity
VO ₂ max	Maximal oxygen consumption
WMA	World Masters Athletics

55 1. Introduction

56

57 Master athletes are usually individuals over 35 years of age engaged in training routines and
58 competitions in specific modalities, composing a select portion of the middle-aged and elderly population (Kusy
59 and Zielinski 2015). Recently, the master athletes have been gaining the attention of the scientific community,
60 because they present excellent physical-functional performance (*e.g.* strength, speed, aerobic capacity), as well
61 as good clinical indexes of health (*e.g.* body composition, cholesterol, triglycerides, glycemia), becoming models
62 for studies on the health and general well-being throughout the aging process (Abrahin et al. 2019; Kusy and
63 Zielinski 2015). The healthy aging process and the good physical-functional performance of master athletes
64 compared to non-athletes may be related to the several biochemical and molecular mechanisms capable of
65 regulating cellular aging and possibly triggered, partly, by habitual physical exercise, as well as by the healthful
66 lifestyle (*e.g.* healthy nutrition, less smoking, and lower stress level) (Kusy and Zielinski 2015; Abrahin et al.
67 2019; Sousa et al. 2019; Minuzzi et al. 2019).

68 Among several factors, oxidative stress and inflammation are key points for understanding the
69 mechanisms related to the effects of chronic exercise and healthful lifestyle on aging processes (Minuzzi et al.
70 2019; Lopez-Otin et al. 2013; Koltai et al. 2018). Increased production of reactive species of oxygen and
71 proinflammatory cytokines may negatively modulate the expression of humoral factors regulating cardiovascular
72 health and mitochondrial biogenesis (Chistiakov et al. 2014), and thus induce greater telomere shortening
73 (Bernheim and Benchetrit 2011; Bode-Boger et al. 2005; Rana et al. 2014; Sahin and DePinho 2012; Chistiakov
74 et al. 2014; Nair and Gongora 2017; Ullah and Sun 2018). In general, telomere length, asymmetric
75 dimethylarginine (ADMA), klotho-FGF23 axis (fibroblast growth factor 23) and irisin can be considered good
76 aging biomarkers (Benedini et al. 2017; Amaro-Gahete et al. 2018; Kuro-o 2018; Cardoso et al. 2018; Nair and
77 Gongora 2017; Liu et al. 2018). In this case, increased levels of FGF23 and ADMA and a reduction in klotho
78 and irisin are indicators of biological aging.

79 The measurement of aging biomarkers may have practical applications in disease prevention,
80 particularly in the middle-age where the initial stages of chronic diseases such as diabetes and hypertension are
81 typically present. Moreover, these measures may contribute for studies on the molecular mechanisms of aging-
82 related to healthful lifestyle and interventions to improve human health (Lopez-Otin et al. 2013; Cardoso et al.
83 2018).

84 In recent years, science has pointed out that master athletes have good antioxidant defense system,
85 reduced levels of inflammation, increased nitrite/nitrate (NO_x) and irisin, and longer telomeres compared to
86 untrained individuals (Sousa et al. 2019; Minuzzi et al. 2019; Benedini et al. 2017). Interestingly, according to
87 some results, a former career as an elite athlete does not influence telomere length in older ages suggesting that
88 intensive exercise throughout life may be required to protect against telomere shortening (Laine et al. 2015).

89 However, to date, most studies on health-related characteristics of master athletes during the aging
90 process have focused on endurance athletes, with few studies comparing oxidative stress, inflammatory markers,
91 and biomarkers of aging among masters athletes of different modalities (Sousa et al. 2019; Minuzzi et al. 2019;
92 Benedini et al. 2017; Kusy and Zielinski 2015; Abrahin et al. 2019; Simoes et al. 2017). Because lifelong
93 exercise may exert a positive influence on the various biological factors associated with cellular aging, it is
94 critical to understand whether the protective effect is dependent on the type of exercise. We hypothesized that
95 regimens of training of master sprinters, when performed lifelong, would attenuate markers of biological aging
96 similarly to, or even in a more significant manner, in relation to master endurance athletes.

97 Thus, our objective was to compare the redox balance, cytokine levels and aging biomarkers between
98 master sprinters and endurance athletes, as well as in young and middle-aged individuals as controls.

99

100 2. Methods

101 This research was approved by the local Research Ethics Committee (protocol: 1.201.316) according to
102 the declaration of Helsinki. All subjects signed a free and informed consent form with all procedures explained
103 clearly and completely. A cross-sectional design was used to analyze the oxidative stress, inflammatory status,
104 and biomarkers of aging of elite master athletes (sprinters and endurance runners) and age-matched controls.

105

106 2.1 Subjects

107 The male participants were: (i) master runners (40–65yr) from endurance (EA, 10 000m to marathon)
108 and sprint events (SA, 60-400m); (ii) untrained young (YC, 20-30yr) and middle-aged (MC, 40-65yr) controls.
109 The inclusion criteria for master athletes were: (i) have trained continuously at least 20 years; (ii) competed and
110 still participating in national and international events in their modality by the time of the data collection. Master
111 athletes were recruited from national and international athletic meetings and personal recommendations from
112 athletes to the athlete. The inclusion criteria for control groups were to be untrained and healthy.

113 The participants were submitted to anamnesis, anthropometric measures (body mass, height, and waist-
114 hip ratio), blood collection samples for biochemical and biomolecular analyzes.

115 Anamnesis was used to identify athletes' training history (training time throughout life and the current
116 volume of training per week – Table 1). The self-reported best performance by SA ranged from 84% to 97% and
117 by EA from 72% to 94% relative to the age-group World records (5-yr intervals) registered in World Masters
118 Athletics (WMA) website in 2017. Moreover, all of them reached the podium at least once in the World, national
119 and/or South American master championships.

120 Before the assessment, all participants were instructed to avoid physical exercise 24h before blood
121 donation. The participants attended the laboratory in the morning (between 7:00 - 8:00 am), after 8-hour fasting
122 to give samples for biochemical and biomolecular analyzes. Tubes for biomarkers were serum separators
123 contained EDTA. Blood samples were drawn using a butterfly needle inserted into the antecubital vein and
124 deposited in a 10 mL tube. The samples were centrifuged at 1500 x g for 15 min. After processing, the
125 specimens were aliquoted into cryovials and stored at -80° C.

126

127 **2.2 Redox balance**

128 The oxidative stress biomarkers were: lipid peroxidation, F2-isoprostanes, protein carbonyls, and 8-
129 OHdG. **Lipid peroxidation** was analyzed by thiobarbituric acid reactive substances (TBARS) as previously
130 described by Sousa et al. (Sousa et al. 2019). **F2-isoprostanes** were measured by sensitive enzyme-linked
131 immunosorbent assay (ELISA) following the manufacturer's instructions (Neogen Life Sciences, KY, USA),
132 with a minimum level of detection of 0.03 ng/mL, assay range 0.05 – 100 ng/mL. The intra- and inter-assay
133 coefficients of variation (CV) were $\leq 10\%$ and $\leq 10\%$, respectively. The determination of the **protein carbonyls**
134 was performed by colorimetric assay kit (Protein Carbonyl Content Assay Kit, Sigma-Aldrich R, CA, USA).
135 Plasma levels of **8-OHdG** were determined by ELISA, according to the manufacturer's instructions
136 (OxiSelect™ Oxidative DNA Damage ELISA Kit 8-OHdG Quantitation, Cell Biolabs, Inc., San Diego, USA).
137 The kit has an 8-OHdG detection sensitivity range of 100 pg/mL to 20 ng/mL. According to the manufacturer's
138 instructions, we made ten times plasma dilutions so that the measured concentrations were in the detection
139 sensitivity range of the test.

140 The antioxidant biomarkers included: total antioxidant capacity, activities of superoxide dismutase,
141 catalase, and NO_x, glutathione and plasma uric acid. **Total antioxidant capacity** (accessed through trolox
142 equivalent), **activities of superoxide dismutase (SOD)**; **catalase** was analyzed as presented by Sousa et al.

143 (Sousa et al. 2019). Serum levels of **glutathione** (GSH) were measured according to the manufacturer's
144 instructions using the Glutathione Assay Kit (Sigma-Aldrich R., CA, USA). **Plasma uric acid** level was
145 measured by colorimetric assay using a Labtest Uric Acid kit (Center lab Ltda, São Paulo, Brazil). Blood for
146 **nitrite/nitrate (NO_x)** analysis was collected into EDTA tubes, and plasma concentrations were determined using
147 the Griess reaction. Experiments were performed at room temperature (25°C). The 300 µL of plasma from each
148 sample were deproteinized with 20µL zinc sulfate (20%) followed by centrifugation at 10,000g. The supernatant
149 was collected and used for the following reactions. Nitrite standard solution (100 µL) was serially diluted (100 -
150 50 - 25 - 12.5 - 6.25 - 3.13 - 1.56 - 0 µM) in triplicate in a 96-well, flat-bottomed, polystyrene microtiter plate.
151 The diluting medium was used as the standard blank. After loading the plate with deproteinized samples (100
152 µL), addition 100 µL of Vanadium (III) 0.8% diluted in HCL 1M to each well was rapidly followed by addition
153 of the Griess reagents, sulfanilamide 2% diluted in HCL 5% (50 µL) and N-1-(naphthyl)ethylenediamine 0.1%
154 diluted in H₂O (50 µL) using a multichannel pipette. Sample blank values were obtained by substituting diluting
155 medium for Griess reagent. The absorbance at 540 nm was measured using a plate reader following incubation
156 (45 min in 37°C) protected from light. Linear regression of the mean values of the absorbance at 540 nm for each
157 standard set minus the blank values was utilized to determine the total NO_x concentrations in samples (Miranda
158 et al. 2001; Sousa et al. 2018).

159

160 **2.3 Inflammatory status**

161 Inflammatory markers including plasma tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6),
162 interleukin 10 (IL-10) and interleukin 15 (IL-15) levels were measured in triplicate by ELISA kits from R&D
163 Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. Levels of a soluble form of
164 interleukin-6 receptor (sIL-6R) (Invitrogen Corp., CA, USA) and soluble tumor necrosis factor receptor type I
165 (sTNF-RI) (Bender MedSystems Inc. VI, AUT) were measured in serum samples. The detectable limit for TNF-
166 α, sTNF-RI, IL-6, sIL-6R, IL-10 and IL15 were 10 pg/mL, 7,8 pg/mL, 18 pg/mL, 78 1.0 pg/mL, 0.2 pg/mL
167 respectively. The overall inter-assay CVs for inflammatory markers were respectively: 10%, 15%, 6,6%, 5%, 8%
168 and 12% for TNF-α, sTNF-RI, IL-6, sIL-6R, IL-10 and IL-15, respectively.

169

170 **2.4 Circulating biomarkers of aging**

171 The relative telomere length (TL – adjusted by a young sample subject) was measured as described
172 previously (Sousa et al. 2019). Serum ADMA concentration was determined by ELISA, the intra- and inter-

173 assay CV were $\leq 8\%$ and $\leq 12\%$, respectively (Human ADMA ELISA Kit, MyBioSource, San Diego, USA).
174 Serum irisin concentrations were measured using ELISA kits (Phoenix Pharmaceuticals, Burlingame, CA, USA)
175 following the manufacturer's instructions. The sensitivity of the assay was 0.1ng/mL and the linear range of the
176 standard was 0.1-1000ng/mL. The intra- and inter-assay CV were 4.5% and 8%, respectively. Serum levels of
177 klotho and fibroblast growth factor 23 (FGF-23) were determined using the specific human ELISA methods
178 (IBL Co., Ltd, Japan, and Immotopics Inc., USA), respectively. Both assays had intra- and inter-assay CVs less
179 than 10%.

180

181 2.5 Statistical analysis

182 Total sample size used in this study ($n = 60$) conferred a statistical power of 86 % ($\beta = 0.86$) with a
183 significance level of 5% ($\alpha = 0.05$) and moderate effect size ($d = 0.6$). Initially, normality and homoscedasticity
184 were calculated using the Shapiro-Wilk and Levene tests, respectively. Data are presented as mean \pm standard
185 deviation. One-way ANOVA followed by Tukey's post-test was applied to verify possible differences among all
186 studied groups, as well as among middle-aged groups only (master sprinters, endurance runners, and middle-
187 aged controls) to minimize type II error in the comparisons. In this sense, comparisons between athletes only
188 (master endurance and sprinters) were conducted by using the *Student's t-test* followed by effect size (Cohen's d
189 – Small 0.2; Medium 0.5; Large > 0.8). Statistical significance was accepted with $p < 0.05$. All procedures were
190 performed using Graph Pad Prism version 6.0 (Graph Pad Software, San Diego – California), Gpower® version
191 3.1 (Franz Faul, Germany) and Statistical Package for the Social Sciences – SPSS Statistics® version 21 (IBM
192 Corporation, New York – United State).

193

194 3. Results

195 *Subject and training characteristics*

196 The anthropometric indexes (*e.g.* BMI and waist-hip ratio) master athletes had lower values than the
197 MC ($p < 0.05$). There were no differences in these variables of master athletes when compared to YC ($p > 0.05$).
198 See Table 1 for details.

199

TABLE 1 AROUND HERE

200 *Oxidative stress biomarkers*

201 There was no difference between groups for TBARS ($p > 0.05$).

202 Related to F₂-isoprostanes, both groups of master athletes presented lower ($p<0.05$) values (2.1 ± 0.2 and
203 1.8 ± 0.2 ng/mL, for EA and SA, respectively) than MC (2.7 ± 0.5 ng/mL). Also, MC and EA presented higher
204 ($p<0.05$), values than YC (1.7 ± 0.4 ng/mL).

205 The results of protein carbonyls revealed the MC (0.9 ± 0.2 nmol/mg) and EA (0.8 ± 0.1 nmol/mg) groups
206 to have higher ($p<0.05$) values than the YC (0.7 ± 0.1 nmol/mg), with no statistical differences ($p>0.05$) between
207 SA (0.7 ± 0.1 nmol/mg) neither YC; Figure 1-C.

208 Regarding 8-OHdG, MC (16 ± 3 ng/mL) presented higher ($p<0.05$) values than YC (10 ± 5 ng/mL) and no
209 differences ($p>0.05$) were identified between YC and master athletes from SA (14 ± 4 ng/mL) or SA (13 ± 4
210 ng/mL) events; Figure 1-D.

211 **FIGURE 1 AROUND HERE**

212 *Antioxidant biomarkers*

213 The total antioxidant capacity (trolox equivalent) of SA (703 ± 184 μ M) was lower ($p< 0.05$) in
214 comparison to YC (985 ± 171 μ M) and SA, with no differences ($p>0.05$) between the MC (833 ± 139 μ M), YC and
215 SA (882 ± 164 μ M); Figure 2-A.

216 SOD values were lower ($p<0.05$) for both MC (50 ± 25 U/mL) and SA (65 ± 18 U/mL) when compared to
217 YC (78 ± 13 U/mL), whereas EA (75 ± 18 U/mL) had higher ($p<0.05$) values than MC; Figure 2-B.

218 No differences among groups ($p>0.05$) were identified for GSH; Figure 2-C.

219 The MC (563 ± 234 U/mL) presented lower values ($p<0.05$) of catalase than YC (780 ± 196 U/mL).
220 Moreover, the results of catalase for the SA (935 ± 93 U/mL) were higher ($p<0.05$) than MC. Catalase values of
221 EA (756.92 ± 241.00 U/mL) and SA (934.56 ± 93.33 U/mL) athletes did not differ from YC; Figure 2-D.

222 Regarding uric acid, MC (322 ± 136 mM) presented higher values ($p<0.05$) compared to the other
223 groups: YC (176 ± 75 mM), SA (144 ± 53 mM) and SA (148 ± 111 mM); Figure 2-E.

224 Additionally, NO_x results for YC (127 ± 17 μ M), MC (66 ± 21 μ M), EA (184 ± 29 μ M) and SA (138 ± 36
225 μ M) revealed that MC and EA presented, respectively, the lowest and the highest level among studied groups (p
226 < 0.05); Figure 2-F.

227 **FIGURE 2 ABOUT HERE**

228 *Inflammatory markers*

229 TNF- α values were higher ($p<0.05$) in MC (14.9 ± 1.7 pg/mL), EA (14.7 ± 1.7 pg/mL) and SA (15.1 ± 1.9
230 pg/mL) in comparison to YC (4.7 ± 0.8 pg/mL); Figure 3-A. YC presented higher values ($p<0.05$) of sTNF-RI
231 (0.7 ± 0.3 ng/mL) than MC (3.4 ± 0.8 ng/mL), EA (1.7 ± 0.3 ng/mL) and SA (1.4 ± 0.3 ng/mL); Figure 3-B.

232 IL-6 was higher ($p<0.05$) in MC (10.1 ± 1.3 pg/mL) and EA (5.1 ± 0.7 pg/mL) in comparison to YC
233 (3.7 ± 0.7 pg/mL). Both EA and SA (4.4 ± 0.4 pg/mL) had lower ($p<0.05$) IL-6 than MC; Figure 3-C. MC (137 ± 11
234 pg/mL), EA (122 ± 7 pg/mL) and SA (119 ± 7 pg/mL) had higher ($p<0.05$) sIL-6R values than YC (84 ± 22
235 pg/mL), and both groups of master athletes presented lower ($p<0.05$) values of this variable in relation to MC;
236 Figure 3-D.

237 The IL-10 levels were lower ($p<0.05$) for the MC (6 ± 1 pg/mL) and EA (8 ± 1 pg/mL) compared to YC
238 (11 ± 2 pg/mL). Moreover, EA and SA (10 ± 1 pg/mL) also presented higher values than MC ($p<0.05$), while SA
239 had higher ($p<0.05$) values than EA; Figure 3-E.

240 The IL-10/TNF- α ratio was higher ($p<0.05$) in YC (2.1 ± 0.5 ratio) than all other groups: MC (0.4 ± 0.1
241 ratio), EA (0.5 ± 0.1 ratio) and SA (0.7 ± 0.1 ratio). Additionally, SA had higher ($p<0.05$) values in comparison to
242 MC; Figure 3-F.

243 The IL-10/IL-6 ratio of YC (2.8 ± 0.3 ratio) was higher ($p < 0.05$) than MC (0.6 ± 0.2 ratio), EA ($1.5 \pm$
244 0.3 ratio) and SA (2.2 ± 0.3 ratio); Figure 3-G.

245 On the other hand, the IL-10/IL-6 ratio MC was also significantly lower ($p<0.05$) than all other groups
246 and the ratio for the SA group was significantly higher ($p<0.05$) than EA.

247 Moreover, the IL-15, MC (1.5 ± 0.5 pg/mL) and SA (2.0 ± 0.6 pg/mL) had lower ($p<0.05$) values
248 compared to YC (3.0 ± 0.8 pg/mL) and EA (2.9 ± 0.6 pg/mL); Figure 3-H.

249 **FIGURE 3 AROUND HERE**

250 *Biomarkers of aging*

251 Relative TL (adjusted by young control subject) of EA (0.8 ± 0.5 T/S ratio) and SA (1.4 ± 1.0 T/S ratio)
252 were not different ($p>0.05$) from YC (1.9 ± 1.6 T/S ratio). TL of MC (0.5 ± 0.6 T/S ratio) was significantly shorter
253 ($p<0.05$) than that of YC; Figure 4-A. Moreover, comparisons conducted among middle-aged participants only
254 (excluding young controls) revealed that TL of SA was longer ($p < 0.05$) than MC.

255 ADMA concentrations were higher ($p<0.05$) in MC (0.73 ± 0.07 μ M) and SA (0.69 ± 0.06 μ M) in
256 comparison to YC (0.55 ± 0.07 μ M) and EA (0.59 ± 0.05 μ M); Figure 4-B.

257 Irisin levels of SA (139 ± 16 ng/mL) did not differ from YC (151 ± 17 ng/mL), and both presented higher
258 ($p<0.05$) levels than MC and EA (76 ± 13 and 90 ± 16 ng/mL respectively); Figure 4-C.

259 Results of klotho were also higher ($p<0.05$) for SA (674 ± 96 pg/mL) and YC (683 ± 101 pg/mL) in
260 comparison to MC (489 ± 59 pg/mL) and EA (522 ± 133 pg/mL); Figure 4-D.

261 Regarding to FGF-23, MC (54±9 pg/mL) showed higher values ($p < 0.05$) than YC (32±10 pg/mL), EA
262 (39±10 pg/mL) and SA (26±8 pg/mL); Figure 4-E.

263 Klotho/FGF-23 ratio was lower ($p < 0.05$) in MC (9±2) and EA (14±5) compared to YC (23±7) and SA
264 (30±14); Figure 4-F.

265 **FIGURE 4 ABOUT HERE**

266 The additional analysis which we compared EA and SA showed no statistical difference for TBARS, 8-
267 OHdG, GSH, uric acid, TNF- α , sIL-6R and TL ($p > 0.05$). Nevertheless, statistical differences with large effect
268 sizes were identified for F₂-isoprostanes ($p < 0.001$; $d = 1.85$), protein carbonyls ($p = 0.03$; $d = 0.81$), SOD ($p =$
269 0.006 ; $d = 0.99$), NO₂ ($p < 0.0001$; $d = 1.39$), sTNF-RI ($p = 0.011$; $d = 1.06$), IL-6 ($p = 0.014$; $d = 1.13$), IL-15
270 ($p < 0.001$; $d = 1.41$) and FGF-23 ($p < 0.001$; $d = 1.49$), all of them were higher for EA (Table 2). Additionally,
271 trolox equivalent ($p < 0.001$; $d = 1.03$), catalase ($p = 0.02$; $d = 0.97$), IL-10 ($p < 0.001$; $d = 1.96$), IL-10/TNF- α
272 ratio ($p < 0.001$; $d = 1.22$), IL-10/IL-6 ratio ($p < 0.001$; $d = 2.38$), ADMA ($p < 0.001$; $d = 1.92$), irisin ($p < 0.001$;
273 $d = 3.10$), klotho ($p < 0.001$; $d = 1.31$) and klotho/FGF-23 ratio ($p < 0.001$; $d = 1.49$) were significantly lower
274 with large effect sizes in the EA compared to SA; Table 2.

275 **TABLE 2 ABOUT HERE**

276 **4. Discussion**

278 Biomarkers of aging, redox balance and inflammatory status were compared between master athletes
279 from sprints, distance running and untrained controls. The main findings were that, in general, master athletes
280 presented reduced biomarkers of aging, oxidative stress and inflammation, with some of these markers at levels
281 corresponding to those of young control group. Regarding the differences between modalities, EA had a better
282 profile of markers related to the vascular system (*i.e.* NO_x as a marker of endothelial function), whereas SA had
283 a better general redox balance, cytokines and attenuated biomarkers of aging. Aging is normally related to
284 systemic functional-health decline because during the ontogenic there is cellular damage accumulation reaching
285 critical points to induce diseases (Beyret et al. 2018). Even before the onset of aging-related diseases, it is
286 possible to detect changes in the pattern of circulating molecules allowing for measures that can guide strategies
287 to prevent or attenuate the senescence (Lopez-Otin et al. 2013; Gladyshev 2014).

288 The present findings revealed that middle-aged untrained individuals have an increased concentration in
289 markers of oxidative damage compared to the YC (F₂-Isoprostanes, carbonylated proteins and 8-OHdG). Such
290 condition may be due to the imbalance between pro-oxidants (*e.g.* free radicals) and antioxidant defenses

291 (Gladyshev 2014), which was also observed in MC that presented the lower activity of antioxidant enzymes
292 (SOD and catalase), higher levels of uric acid and reduced NO_x levels compared to YC. Previous evidence
293 suggested that detection of changes in the redox balance, such as increased uric acid and a decreased NO_x, may
294 indicate the onset of hemodynamic and glyceemic disorders, and the trend for the development of chronic
295 diseases such as hypertension and diabetes (De et al. 2018; Li et al. 2019; Mohan and Gupta 2018). Oxidative
296 stress also interferes with intracellular signaling, increasing the expression of pro-inflammatory cytokines and
297 disrupting anti-inflammatory cytokines (Mohan and Gupta 2018), what in turn could explain the higher
298 circulating levels of TNF- α , sTNF-RI, IL-6, sIL6R and lower IL-10 levels for the MC compared to the YC.

299 Moderate chronic inflammation and oxidative stress triggered by aging and adiposity may uncouple the
300 endothelial enzyme nitric oxide synthase (eNOS), performing a crucial role in the synthesis of ADMA and FGF-
301 23, besides to Klotho expression inhibition, which was observed for MC compared to the YC in the present
302 study. Although the adiposity was not higher in comparison to normative values, body composition changes
303 during aging (reduced muscle mass and increased body fat) may be the reason for such outcomes (Bouras et al.
304 2013; Sydow et al. 2005; Dermaku-Sopjani et al. 2013; Bernheim and Benchetrit 2011).

305 Regarding the aging biomarkers, it is reasonable to infer that klotho action on skeletal muscle interferes
306 not only into FGF-23 but also in the irisin and IL-15 release (Kureya et al. 2016). Further, irisin improves
307 mitochondrial biogenesis and acts as a predictor of telomere length (Rana et al. 2014). Therefore, the increased
308 FGF-23 and ADMA, and a reduced klotho and irisin in MC are possible causes of a single but complex aging
309 process underlying telomere attrition. Thus, the longer TL of SA in relation to MC (comparisons with middle-
310 aged participants only) may be due to higher irisin levels, as well as higher Klotho/FGF-23 ratio, and lower
311 FGF-23 and protein carbonyl for SA in comparison to both MC and EA. Several aging biomarkers are
312 modulated by the practice of exercise and other healthy living habits (Rana et al. 2014; Beyret et al. 2018; Sousa
313 et al. 2019; Minuzzi et al. 2019; Koltai et al. 2018; Simoes et al. 2017) and master athletes become a reference of
314 training routines, adequate nutrition and stress control over decades (Kusy and Zielinski 2015). This lifestyle
315 seems to decrease oxidative stress due to the improvement of antioxidant defense (Sousa et al. 2019; Minuzzi et
316 al. 2019), corroborating with our findings.

317 It was identified that master athletes (both EA and SA) have lower levels of F₂-Isoprostans, higher NO_x
318 levels, lower levels of uric acid and pro-inflammatory cytokines (sTNF-RI and IL-6), and have higher
319 concentrations of IL-10 and IL-10 / IL-6 ratio in comparison to MC. However, the antioxidant defense and
320 modulation adjustments of some cytokines are different between athletes. EA had higher SOD activity and

321 higher levels of IL-15, whereas SA had higher catalase activity and higher IL-10 / TNF ratio when compared to
322 the MC. It is possible that among multiple variables, the specificities of the modalities provide different
323 adaptations in the antioxidant defense and expression of cytokines (Kusy and Zielinski 2015; Issurin 2019;
324 Takada et al. 2012; Torok et al. 1995; Abernethy et al. 1990). Endurance training promotes the mitochondrial
325 biogenesis, while sprint training presents a high glycolytic activity (Huertas, 2019), that can influence the
326 expression of antioxidants enzymes, such as SOD which predominance is mitochondrial, and CAT which
327 predominance is cytosolic. Besides that, it was verified high NO_x levels in master endurance athletes which in
328 turn promote an inhibitory effect over CAT's activity (Nilakantan, 2005). This fact could also explain these
329 differences between modalities.

330 Furthermore, the EA presented higher SOD activity, higher NO_x levels, higher IL-15 concentration, and
331 lower ADMA level compared to SA. We would suggest that shear-stress and greater exercise-induced oxygen
332 consumption may act as a physiological trigger for the activation of SOD, eNOS and NO⁻ production, promoting
333 vasodilation and blood perfusion promoting cardiovascular adaptations that are characteristics of aerobic
334 exercise. Thus, in well-trained EA, both the NO⁻ release, and SOD activity seem to be optimized by their regular
335 training and diet (Decroix et al. 2017; Takada et al. 2012; Torok et al. 1995).

336 Additionally, ADMA levels were lower in the EA compared to SA, corroborating to an increased SOD
337 and possibly eNOS activity and greater NO⁻ bioavailability. Although the exercise effect on ADMA levels in
338 healthy people remain controversial, in pathological situations aerobic training is effective to decrease ADMA
339 levels, improving blood flow and O₂ supply in response to muscle work demand, which possibly leads to
340 VO₂max maintenance throughout the aging process (Pawlak-Chaouch et al. 2019; Takada et al. 2012; Torok et
341 al. 1995). Thus, it would be reasonable to infer that there is a possible association between ADMA, SOD, NO⁻
342 and VO₂max, which would explain the lower ADMA levels of EA in comparison to SA (Takada et al. 2012;
343 Torok et al. 1995; Kusy and Zielinski 2015); Table 2. However, it is important to highlight that the NO
344 metabolism is very complex and the data of the present study must be done with caution. The elevated values of
345 NO_x may be a result of the lifestyle itself, including eating habits, in addition to a possible acute residual effect
346 of training from the days prior to collection, and not solely to the chronic effects of lifelong training (Nebl et al.
347 2019; Rassaf et al. 2007). On the other hand, our participants attended the laboratory after 10-hour fasting, and
348 48h of exercise abstinence. In addition, all studied groups underwent exactly the same research protocol, which
349 validates the comparisons among them. We suggest, however, that future studies strictly control the eating habits
350 of subjects, as well as the monitoring of training in relation to the intensity and duration of the week prior to data

351 collection. We believe, however, that the findings regarding NO_x may be mainly due to the vascular health of
352 individuals, since the redox balance and ADMA concentrations were synergistic and / or confluent with the NO_x
353 results as previously demonstrated (Kleinbongard et al. 2003).

354 The better oxidative metabolism, characteristic of EA, may also explain the higher levels of IL-15 in
355 relation to SA, once IL-15 plays an important role in oxidative properties and skeletal muscle fatigue *in vivo*
356 (Pistilli and Quinn 2013; Minuzzi et al. 2019). Regular endurance training may play an important role in
357 reducing some markers of systemic inflammation, besides regulating metabolic and physiological parameters of
358 muscles during the aging process (Pistilli and Quinn 2013; Minuzzi et al. 2019). Literature corroborates with
359 findings of the present study when analyzing EA (Pistilli and Quinn 2013; Minuzzi et al. 2019). However, we
360 observed that in comparison to EA, SA presented even lower levels of markers of oxidative damage
361 (isoprostanes and carbonylated proteins), greater overall antioxidant defense (trolox equivalent, catalase
362 activity), lower proinflammatory cytokines (IL-6 and sTNF-RI), higher rates of anti-inflammatory indicators (IL-
363 10, IL-10 / IL-6, and IL-10 / TNF- α), and higher concentrations of irisin. Moreover, the TL of SA was longer
364 than MC; Table 2.

365 Some of these differences may be related to the higher volume of training performed by endurance
366 runners. It is known that high volumes of training may lead to immunosuppression effect (Nielsen 2013), and
367 that endurance training is known to elicit the generation of reactive oxygen species (Ismaeel et al. 2019); which
368 should interfere in the final redox balance and inflammatory adaptations. However, attention is required when
369 refers to training volume of EA, because participants of present study performed at least 25 years of training
370 lifelong, which the main feature was a greater volume of moderate-intensity continuous training. Nowadays,
371 endurance training has changed to a lower volume and higher intensity, what in turn may be the reason of
372 significant improvement in athletic performance and frequency of records being broken lately (Skovgaard et al.
373 2018; Bangsbo 2015). On the other hand, the volume of sprint training tends to be lower and intensity higher
374 than endurance training. Even though, little is known about the redox balance and inflammatory profile of SA.
375 Thus, it is feasible to assume that they presented a better redox balance and inflammatory profile than EA
376 probably due to their lower volume of training.

377 Master sprinters have significantly higher levels of aerobic capacity than recreationally or sedentary
378 trained individuals as well as a lower rate of decline in maximal heart rate and consequently absolute VO₂max
379 values close to that in EA (Kusy and Zielinski 2015). Based on the present results, we believe that SA may have
380 excellent mitochondrial biogenesis and oxidative function derived from a routine that mixes strength and power

381 training sessions with aerobic exercises (Kusy and Zielinski 2015). Usually, SA have a better body composition
382 compared to untrained subjects (Kusy and Zielinski 2015), which may be partially evidenced by the BMI data of
383 the present study. Older athletes, regardless of being SA or EA have low body fat. However, SA have a higher
384 lean body mass, indicating that exercise training aiming to increase or maintain muscle mass plays a key role in
385 attenuation of sarcopenia and functional-health decline during aging (Kusy and Zielinski 2015).

386 The number of hours dedicated to strength and power training in SA (Table 1) could explain the greater
387 muscle mass they usually exhibit (Kusy and Zielinski 2015). However, a greater oxidative function observed for
388 the SA of the present study could have been induced by aerobic components of their training routine (Issurin
389 2019). Taken together, possibly a combination of aerobic and anaerobic training could allow for a greater level
390 of irisin and better profile of pro- and anti-inflammatory cytokines throughout life, since only isolated strength
391 training does not seem to interfere with aging markers such as irisin (Tibana et al. 2017; Bonfante et al. 2017).

392 A better redox and inflammatory balance found in the group of SA compared to EA could explain the
393 higher levels of irisin and klotho and lower levels of FGF-23, which are usually modulated by the inflammatory
394 and oxidative status (Amaro-Gahete et al. 2018; Ullah and Sun 2018). Irisin and klotho are associated with
395 telomere length (Rana et al. 2014; Ullah and Sun 2018). Although not significant, it is possible to observe a trend
396 (moderate effect size; $d = 0.7$) of SA to present longer TL than EA. Moreover, only SA presented TL longer than
397 the MC. These data are interesting because our sample was composed by middle-aged individuals, and it is
398 possible that, over the years, the effects of lifestyle and training regimen of master athletes from endurance and
399 sprints could result in a progressively higher difference in telomere length, especially due to maintenance of a
400 better oxidative and inflammatory profile lifelong of the master athlete.

401 A possible limitation of the study was the inclusion of well-trained master athletes with a large
402 experience in athletic training, which represents a very small portion from the general population preventing
403 further extrapolations for the general population. However, our study offers a range of insights into the influence
404 of vigorous training on several aging biomarkers, with new knowledge about the association of chronic exercise
405 status on the oxidative and inflammatory factors for health maintenance mitigating the effects of aging. Future
406 studies should analyze a more complete profile of hallmarks of cellular aging in a larger number of master
407 athletes of different sports and also in individuals with different levels of physical fitness.

408

409 **5. Conclusion**

410 We conclude that both EA and SA have excellent redox and inflammatory balance and better
411 biomarkers of aging in comparison to MC, with some of those biomarkers not differing from YC. Moreover, EA
412 has a better profile of markers closely related to the cardiovascular system and SA has a better general redox
413 profile and cytokines profile, and attenuated biomarkers of aging, including a trend of longer TL.

414 Finally, the presented data are circumstantial evidence once, when it comes to assessment of the effects
415 of lifelong training, there is an intrinsic complexity.

416

417 **5.1 Perspectives**

418 This study is the first to compare redox balance, inflammation profile and aging biomarkers between
419 SA and EA. In light of these promising preliminary findings, more information is needed on how lifelong
420 exercise and training type influence biomarkers of aging (Abrahin et al. 2019; Kusy and Zielinski 2015). New
421 research in this area may lead to important advances in exercise dose-response and a prescription for people
422 throughout the aging process. An important aspect of the analysis of aging biomarkers is that they may act as
423 predictors of health and general well-being towards old age. Thereby, new knowledge of the use of regular
424 exercise to counteract age-related changes in various markers of cellular aging may be important for the
425 development of new training strategies capable of promoting healthy aging.

426

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428

429 **Compliance with Ethical Standards**

430

431 **Ethical approval:** This study was performed following the ethical standards of the Declaration of Helsinki.
432 Ethics approval was obtained from the local Research Ethics Committee (protocol: 1.201.316).

433

434 **Informed consent:** Informed consent was obtained from all subjects included in this study.

435

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439 **Conflict of Interest** No conflicts of interest, financial or otherwise is declared by the authors. The results of this
440 study are presented clearly, honestly and without fabrication, falsification or inappropriate data manipulation.
441 The results and conclusions of the study do not constitute an endorsement by the American College of Sports
442 Medicine.

Journal Pre-proof

443 **6. References**

444

- 445 Abernethy PJ, Thayer R, Taylor AW (1990) Acute and chronic responses of skeletal muscle to endurance and
 446 sprint exercise. A review. *Sports medicine* 10 (6):365-389. doi:10.2165/00007256-199010060-00004
- 447 Abrahim O, Cortinhas-Alves EA, Vieira RP, Guerreiro JF (2019) Elite athletes have longer telomeres than
 448 sedentary subjects: A meta-analysis. *Exp Gerontol*. doi:10.1016/j.exger.2019.01.023
- 449 Amaro-Gahete FJ, de la OA, Jurado-Fasoli L, Ruiz JR, Castillo MJ, Gutierrez A (2018) Role of exercise on S-
 450 Klotho protein regulation: a systematic review. *Curr Aging Sci*.
 451 doi:10.2174/1874609811666180702101338
- 452 Bangsbo J (2015) Performance in sports--With specific emphasis on the effect of intensified training.
 453 *Scandinavian journal of medicine & science in sports* 25 Suppl 4:88-99. doi:10.1111/sms.12605
- 454 Benedini S, Dozio E, Invernizzi PL, Vianello E, Banfi G, Terruzzi I, Luzi L, Corsi Romanelli MM (2017) Irisin:
 455 A Potential Link between Physical Exercise and Metabolism-An Observational Study in Differently
 456 Trained Subjects, from Elite Athletes to Sedentary People. *J Diabetes Res* 2017:1039161.
 457 doi:10.1155/2017/1039161
- 458 Bernheim J, Benchetrit S (2011) The potential roles of FGF23 and Klotho in the prognosis of renal and
 459 cardiovascular diseases. *Nephrol Dial Transplant* 26 (8):2433-2438. doi:10.1093/ndt/gfr208
- 460 Beyret E, Martinez Redondo P, Platero Luengo A, Izpisua Belmonte JC (2018) Elixir of Life: Thwarting Aging
 461 With Regenerative Reprogramming. *Circulation research* 122 (1):128-141.
 462 doi:10.1161/CIRCRESAHA.117.311866
- 463 Bode-Boger SM, Scalera F, Martens-Lobenhoffer J (2005) Asymmetric dimethylarginine (ADMA) accelerates
 464 cell senescence. *Vasc Med* 10 Suppl 1:S65-71. doi:10.1177/1358836X0501000110
- 465 Bonfante IL, Chacon-Mikahil MP, Brunelli DT, Gaspari AF, Duft RG, Lopes WA, Bonganha V, Libardi CA,
 466 Cavaglieri CR (2017) Combined training, FNDC5/irisin levels and metabolic markers in obese men: A
 467 randomised controlled trial. *Eur J Sport Sci* 17 (5):629-637. doi:10.1080/17461391.2017.1296025
- 468 Bouras G, Deftereos S, Tousoulis D, Giannopoulos G, Chatzis G, Tsounis D, Cleman MW, Stefanadis C (2013)
 469 Asymmetric Dimethylarginine (ADMA): a promising biomarker for cardiovascular disease? *Curr Top*
 470 *Med Chem* 13 (2):180-200
- 471 Cardoso AL, Fernandes A, Aguilar-Pimentel JA, de Angelis MH, Guedes JR, Brito MA, Ortolano S, Pani G,
 472 Athanasopoulou S, Gonos ES, Schosserer M, Grillari J, Peterson P, Tuna BG, Dogan S, Meyer A, van
 473 Os R, Trendelenburg AU (2018) Towards frailty biomarkers: Candidates from genes and pathways
 474 regulated in aging and age-related diseases. *Ageing Res Rev* 47:214-277. doi:10.1016/j.arr.2018.07.004
- 475 Chistiakov DA, Sobenin IA, Revin VV, Orekhov AN, Bobryshev YV (2014) Mitochondrial aging and age-
 476 related dysfunction of mitochondria. *BioMed research international* 2014
- 477 De BB, Borghi C, Burnier M (2018) Uric acid and hypertension: a focused review and practical
 478 recommendations. *Journal of hypertension*
- 479 Decroix L, Tonoli C, Soares DD, Descat A, Driittij-Reijnders MJ, Weseler AR, Bast A, Stahl W, Heyman E,
 480 Meeusen R (2017) Acute cocoa Flavanols intake has minimal effects on exercise-induced oxidative
 481 stress and nitric oxide production in healthy cyclists: a randomized controlled trial. *J Int Soc Sports*
 482 *Nutr* 14:28. doi:10.1186/s12970-017-0186-7
- 483 Dermaku-Sopjani M, Kolgeci S, Abazi S, Sopjani M (2013) Significance of the anti-aging protein Klotho. *Mol*
 484 *Membr Biol* 30 (8):369-385. doi:10.3109/09687688.2013.837518
- 485 Gladyshev VN (2014) The free radical theory of aging is dead. Long live the damage theory! *Antioxidants &*
 486 *redox signaling* 20 (4):727-731
- 487 Ismaeel A, Holmes M, Papoutsis E, Panton L, Koutakis P (2019) Resistance Training, Antioxidant Status, and
 488 Antioxidant Supplementation. *International journal of sport nutrition and exercise metabolism* 29
 489 (5):539-547. doi:10.1123/ijsnem.2018-0339
- 490 Issurin VB (2019) Biological Background of Block Periodized Endurance Training: A Review. *Sports medicine*
 491 49 (1):31-39. doi:10.1007/s40279-018-1019-9
- 492 Koltai E, Bori Z, Osvath P, Ihasz F, Peter S, Toth G, Degens H, Rittweger J, Boldogh I, Radak Z (2018) Master
 493 athletes have higher miR-7, SIRT3 and SOD2 expression in skeletal muscle than age-matched
 494 sedentary controls. *Redox Biol* 19:46-51. doi:10.1016/j.redox.2018.07.022
- 495 Kureya Y, Kanazawa H, Ijiri N, Tochino Y, Watanabe T, Asai K, Hirata K (2016) Down-Regulation of Soluble
 496 alpha-Klotho is Associated with Reduction in Serum Irisin Levels in Chronic Obstructive Pulmonary
 497 Disease. *Lung* 194 (3):345-351. doi:10.1007/s00408-016-9870-7
- 498 Kusy K, Zielinski J (2015) Sprinters versus long-distance runners: how to grow old healthy. *Exercise and sport*
 499 *sciences reviews* 43 (1):57-64. doi:10.1249/JES.0000000000000033

- 500 Laine MK, Eriksson JG, Kujala UM, Raj R, Kaprio J, Backmand HM, Peltonen M, Sarna S (2015) Effect of
501 intensive exercise in early adult life on telomere length in later life in men. *Journal of sports science &*
502 *medicine* 14 (2):239-245
- 503 Li WF, Huang YQ, Feng YQ (2019) Serum uric acid concentration is associated with ascending aortic dilatation
504 in newly diagnosed nondiabetic hypertensive patients. *Clinical and experimental hypertension*:1-6.
505 doi:10.1080/10641963.2019.1571602
- 506 Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. *Cell* 153
507 (6):1194-1217. doi:10.1016/j.cell.2013.05.039
- 508 Minuzzi LG, Chupel MU, Rama L, Rosado F, Munoz VR, Gaspar RC, Kuga GK, Furtado GE, Pauli JR, Teixeira
509 AM (2019) Lifelong exercise practice and immunosenescence: Master athletes cytokine response to
510 acute exercise. *Cytokine* 115:1-7. doi:10.1016/j.cyto.2018.12.006
- 511 Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous
512 detection of nitrate and nitrite. *Nitric oxide : biology and chemistry* 5 (1):62-71.
513 doi:10.1006/niox.2000.0319
- 514 Mohan S, Gupta D (2018) Crosstalk of toll-like receptors signaling and Nrf2 pathway for regulation of
515 inflammation. *Biomed Pharmacother* 108:1866-1878. doi:10.1016/j.biopha.2018.10.019
- 516 Nair N, Gongora E (2017) Oxidative Stress and Cardiovascular Aging: Interaction Between NRF-2 and ADMA.
517 *Curr Cardiol Rev* 13 (3):183-188. doi:10.2174/1573403X13666170216150955
- 518 Nielsen HG (2013) Exercise and immunity. *Current Issues in Sports and Exercise Medicine*:121-140
- 519 Pawlak-Chaouch M, Boissiere J, Munyaneza D, Tagougui S, Gamelin FX, Cuvelier G, Heyman E, Goossens JF,
520 Descat A, Berthoin S, Aucouturier J (2019) Plasma asymmetric dimethylarginine concentrations are not
521 related to differences in maximal oxygen uptake in endurance trained and untrained men. *Exp Physiol*
522 104 (2):254-263. doi:10.1113/EP087398
- 523 Pistilli EE, Quinn LS (2013) From anabolic to oxidative: reconsidering the roles of IL-15 and IL-15Ralpha in
524 skeletal muscle. *Exercise and sport sciences reviews* 41 (2):100-106.
525 doi:10.1097/JES.0b013e318275d230
- 526 Rana KS, Arif M, Hill EJ, Aldred S, Nagel DA, Nevill A, Randeva HS, Bailey CJ, Bellary S, Brown JE (2014)
527 Plasma irisin levels predict telomere length in healthy adults. *Age (Dordr)* 36 (2):995-1001.
528 doi:10.1007/s11357-014-9620-9
- 529 Sahin E, DePinho RA (2012) Axis of ageing: telomeres, p53 and mitochondria. *Nat Rev Mol Cell Biol* 13
530 (6):397-404. doi:10.1038/nrm3352
- 531 Simoes HG, Sousa CV, Dos Santos Rosa T, da Silva Aguiar S, Deus LA, Rosa E, Amato AA, Andrade RV
532 (2017) Longer Telomere Length in Elite Master Sprinters: Relationship to Performance and Body
533 Composition. *Int J Sports Med* 38 (14):1111-1116. doi:10.1055/s-0043-120345
- 534 Skovgaard C, Christiansen D, Christensen PM, Almquist NW, Thomassen M, Bangsbo J (2018) Effect of speed
535 endurance training and reduced training volume on running economy and single muscle fiber
536 adaptations in trained runners. *Physiol Rep* 6 (3). doi:10.14814/phy2.13601
- 537 Sousa CV, Aguiar SS, Santos PA, Barbosa LP, Knechtle B, Nikolaidis PT, Deus LA, Sales MM, Rosa E, Rosa
538 TS, Lewis JE, Andrade RV, Simoes HG (2018) Telomere length and redox balance in master endurance
539 runners: The role of nitric oxide. *Exp Gerontol*. doi:10.1016/j.exger.2018.11.018
- 540 Sousa CV, Aguiar SS, Santos PA, Barbosa LP, Knechtle B, Nikolaidis PT, Deus LA, Sales MM, Rosa E, Rosa
541 TS, Lewis JE, Andrade RV, Simoes HG (2019) Telomere length and redox balance in master endurance
542 runners: The role of nitric oxide. *Exp Gerontol* 117:113-118. doi:10.1016/j.exger.2018.11.018
- 543 Sydow K, Mondon CE, Cooke JP (2005) Insulin resistance: potential role of the endogenous nitric oxide
544 synthase inhibitor ADMA. *Vasc Med* 10 Suppl 1:S35-43. doi:10.1177/1358836X0501000106
- 545 Takada S, Okita K, Suga T, Omokawa M, Morita N, Horiuchi M, Kadoguchi T, Takahashi M, Hirabayashi K,
546 Yokota T, Kinugawa S, Tsutsui H (2012) Blood flow restriction exercise in sprinters and endurance
547 runners. *Medicine and science in sports and exercise* 44 (3):413-419.
548 doi:10.1249/MSS.0b013e31822f39b3
- 549 Tibana RA, da Cunha Nascimento D, Frade de Souza NM, de Souza VC, de Sousa Neto IV, Voltarelli FA,
550 Pereira GB, Navalta JW, Prestes J (2017) Irisin Levels Are not Associated to Resistance Training-
551 Induced Alterations in Body Mass Composition in Older Untrained Women with and without Obesity. *J*
552 *Nutr Health Aging* 21 (3):241-246. doi:10.1007/s12603-016-0748-4
- 553 Torok DJ, Duey WJ, Bassett DR, Jr., Howley ET, Mancuso P (1995) Cardiovascular responses to exercise in
554 sprinters and distance runners. *Medicine and science in sports and exercise* 27 (7):1050-1056
- 555 Ullah M, Sun Z (2018) Klotho deficiency accelerates stem cells aging by impairing telomerase activity. *J*
556 *Gerontol A Biol Sci Med Sci*. doi:10.1093/gerona/gly261

558 Figure legends

559

560 Figure 1. Pro-oxidant status of young control, middle-aged control, and master endurance and sprinter athletes.

561

562 Data are presented as mean \pm SD. TBARS (**A**), F₂-isoprostanes (**B**), Protein carbonyls (**C**) and 8-OHdG (**D**).

563 TBARS, thiobarbituric acid reactive substances; 8-OHdG, 8-hydroxydeoxyguanosine. One-way ANOVA

564 followed by Tukey's post hoc test was applied. ^a $p < 0.05$ vs. Young Control; ^b $p < 0.05$ vs. Middle-Aged565 Control; ^c $p < 0.05$ vs. Master Endurance. (Young Control n = 17, Middle-Aged Control n = 12, Endurance n =

566 18 and Sprinters n = 13).

567

568 Figure 2. Antioxidant status of young control, middle-aged control, and master endurance and sprinter athletes

569

570 Data are presented as mean \pm SD. Trolox equivalent (**A**), SOD (**B**), GSH (**C**), Catalase (**D**), Uric acid (**E**) and571 NO_x (**F**). SOD, superoxide dismutase; GSH, glutathione; NO₂, nitrite. One-way ANOVA followed by the572 Tukey's post hoc test was applied. ^a $p < 0.05$ vs. Young Control; ^b $p < 0.05$ vs. Middle-Aged Control; ^c $p < 0.05$

573 vs. Endurance. (Young Control n = 17, Middle-Aged Control n = 12, Endurance n = 18 and Sprinters n = 13).

574

575 Figure 3. Inflammatory parameters of young control, middle-aged control, and master athletes from endurance

576 and sprints.

577

578 Data are presented as mean \pm SD. TNF- α (**A**), sTNF-RI (**B**), IL-6 (**C**), sIL-6R (**D**), IL-10 (**E**), IL-10/TNF- α ratio579 (**F**), IL-10/IL-6 ratio (**G**) and IL-15 (**H**). TNF- α , tumor necrosis factor alpha; sTNF-RI, soluble tumor necrosis

580 factor receptor type I; IL-6, interleukin 6; sIL-6R, soluble form of interleukin-6 receptor; IL-10, interleukin 10;

581 IL-10/TNF- α ratio, interleukin 10/tumor necrosis factor alpha ratio; IL-10/IL-6 ratio, interleukin

582 10/interleukin 6 ratio; IL-15, interleukin 15. One-way ANOVA followed by the Tukey's post hoc test was

583 applied. ^a $p < 0.05$ vs. Young Control; ^b $p < 0.05$ vs. Middle-Aged Control; ^c $p < 0.05$ vs. Master Endurance.

584 (Young Control n = 17, Middle-Aged Control n = 12, Endurance n = 18 and Sprinters n = 13).

585

586 Figure 4. Biomarkers of aging of young control, middle-aged control, master endurance and sprinter athletes.

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588 Data are presented as mean \pm SD. T/S ratio (**A**), ADMA (**B**), irisin (**C**), klotho (**D**), FGF-23 (**E**) and klotho/FGF-
589 23 ratio (**F**). T/S ratio, leukocyte telomere length relative; ADMA, asymmetric dimethylarginine; FGF-23,
590 fibroblast growth factor 23. One-way ANOVA followed by Tukey's post hoc test was adopted to verify the
591 difference between groups. ^a $p < 0.05$ vs. Young Control; ^b $p < 0.05$ vs. Middle-Aged Control; ^c $p < 0.05$ vs.
592 Endurance. (Young Control n = 17, Middle-Aged Control n = 12, Endurance n = 18 and Sprinters n = 13).

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Table 1. General characteristics of young control, middle-aged control, and master athletes from endurance and sprinters events.

Age and anthropometrics	Young control (n = 17)	Middle-aged control (n = 12)	Master endurance (n=18)	Master sprinters (n=13)
Age (years)	22.7 ± 3.9	45.5 ± 9.8 ^a	53 ± 8.2 ^a	50 ± 8.9 ^a
Body mass (kg)	78.8 ± 11.2	91.3 ± 16.4	68.3 ± 7.0	68.6 ± 6.8
Height (cm)	176 ± 6.9	172 ± 8.5	176 ± 4.6	173 ± 4.1
BMI (kg•m ⁻²)	23.5 ± 2.2	31.2 ± 8.5 ^a	21.5 ± 5.8 ^b	23.5 ± 2.2 ^b
Waist-hip ratio	0.82 ± 0.06	0.96 ± 0.05 ^a	0.81 ± 0.12 ^b	0.84 ± 0.04 ^b
Training Characteristics				
Training time (years)	-----	-----	25.3 ± 9.2	25.3 ± 11.2
Aerobic training (hrs/wk)	-----	-----	7.8 ± 4.0	4.7 ± 2.4 ^c
Strength/Power training (hrs/wk)	-----	-----	3.0 ± 1.8	5.4 ± 1.5 ^c

Data expressed as mean ± standard deviation. BMI: body mass index. ^a $p < 0.05$ in comparison to the young control group. ^b $p < 0.05$ in comparison to the middle-aged control group. ^c $p < 0.05$ in comparison to the master endurance athletes group.

Table 2. Differences between aging markers of master endurance runners and sprinters

Variables	Endurance Athletes	Sprint Athletes	<i>p-value</i>	<i>Effect Size (Cohen's d)</i>
Oxidative Stress				
F ₂ -Isoprostanes (ng/mL)	2.13 ± 0.17	1.75 ± 0.23	0.00	1.85
Protein carbonyls (nmol/mg)	0.83 ± 0.12	0.73 ± 0.13	0.03	0.81
Antioxidants				
Trolox equivalente (μM)	702.92 ± 183.54	881.50 ± 164.05	0.00	1.03
SOD (U/mL)	77.92 ± 6.22	64 ± 17.81	0.00	0.99
Catalase (U/μL)	756.92 ± 241.00	934.56 ± 93.33	0.02	0.97
NO ₂ ⁻ (μM)	184.05 ± 28.77	138.46 ± 36.45	0.00	1.39
Citokines				
sTNF-RI (ng/mL)	1.72 ± 0.33	1.40 ± 0.27	0.01	1.06
IL-6 (pg/mL)	5.10 ± 0.70	4.44 ± 0.01	0.01	1.13
IL-10 (pg/mL)	7.92 ± 1.22	10.18 ± 1.06	0.00	1.96
IL-10/TNF-α ratio	0.54 ± 0.10	0.66 ± 0.09	0.00	1.22
IL-10/IL-6 ratio	1.45 ± 0.30	2.24 ± 0.34	0.00	2.38
IL-15 (pg/mL)	2.9 ± 0.64	2.03 ± 0.57	0.00	1.41
Biomarkers of Aging				
ADMA (μM)	0.59 ± 0.04	0.69 ± 0.05	0.00	1.92
Irisin (ng/mL)	89.83 ± 16.08	139 ± 15.64	0.00	3.10
Klotho (pg/mL)	522.16 ± 132.51	673.69 ± 96.45	0.00	1.31
FGF-23 (pg/mL)	39.22 ± 10.36	25.76 ± 8.45	0.00	1.42
Klotho/FGF-23 ratio	14.31 ± 5.46	30.06 ± 13.89	0.00	1.49

Data are presented as mean ± SD. SOD, superoxide dismutase; NO₂⁻, nitrite; sTNF-RI, soluble tumor necrosis factor receptor type I; IL-6, interleukin 6; IL-10, interleukin 10; IL-10/TNF-α ratio, interleukin 10/tumor necrosis factor-alpha ratio; IL-10/IL-6 ratio, interleukin 10/interleukin 6 ratio; IL-15, interleukin 15; ADMA, asymmetric dimethylarginine; FGF-23, fibroblast growth factor 23.

