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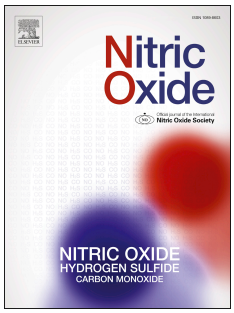

Sprint and endurance training in relation to redox and inflammatory status and biomarkers of aging in master athletes

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

Thiago Santos Rosa[1✉], Rodrigo Vanerson Passos Neves[1], Lysleine Alves Deus[1✉], Caio Victor Sousa[1], Samuel da Silva Aguiar[1], Michel Kendy de Souza[2], Milton Rocha Moraes[1], Érica Carine Campos Caldas Rosa[3], Rosangela Vieira Andrade[4], Marko T. Korhonen[5] Herbert Gustavo Simões[1✉]

[1] Graduate Program in Physical Education, Catholic University of Brasília, Brasília, Brazil.

[2] Department of Nephrology, Federal University of São Paulo, São Paulo, Brazil

[3] Graduate Program in Health Sciences, University of Brasilia, Brasília, Brazil

[4] Program in Genomic Science and Biotechnology, Catholic University of Brasília, Brasília, Brazil

[5] Faculty of Sport and Health Sciences, University of Jyvaskyla, Finland

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Corresponding author (✉)

Name: Thiago S. Rosa / Lysleine A. Deus / Herbert G. Simões

ORCID iD: 0000-0003-0418-0945 / 0000-0001-5594-3717 / 0000-0002-2378-4026

Mailling address: Universidade Católica de Brasília – UCB, Brasília DF, Brazil. EPTC, QS07, LT1 s/n. Bloco G Sala 119. CEP 72030-170, Águas Claras, Taguatinga/DF-Brazil.

Telephone: +55 61 3356 9350

E-mail: thiagoacsdkp@yahoo.com.br / lys.deus@gmail.com / hgsimoes@gmail.com

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

Abstract

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

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

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

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

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_2max	Maximal oxygen consumption
WMA	World Masters Athletics

1. Introduction

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

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

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

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

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

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

2. Methods

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

2.1 Subjects

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

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

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

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

2.2 Redox balance

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

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

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

2.3 Inflammatory status

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

2.4 Circulating biomarkers of aging

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

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

2.5 Statistical analysis

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

3. Results

Subject and training characteristics

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

TABLE 1 AROUND HERE

Oxidative stress biomarkers

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

Related to F_2-isoprostanes, both groups of master athletes presented lower (p<0.05) values (2.1±0.2 and 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 (p<0.05), values than YC (1.7±0.4 ng/mL).

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

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

FIGURE 1 AROUND HERE

Antioxidant biomarkers

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

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

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

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

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

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

FIGURE 2 ABOUT HERE

Inflammatory markers

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 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 (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.

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 (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 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 pg/mL), and both groups of master athletes presented lower (p<0.05) values of this variable in relation to MC; Figure 3-D.

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 (11±2 pg/mL). Moreover, EA and SA (10±1 pg/mL) also presented higher values than MC (p<0.05), while SA had higher (p<0.05) values than EA; Figure 3-E.

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 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 MC; Figure 3-F.

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 ± 0.3 ratio) and SA (2.2 ± 0.3 ratio); Figure 3-G.

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

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 compared to YC (3.0±0.8 pg/mL) and EA (2.9±0.6 pg/mL); Figure 3-H.

FIGURE 3 AROUND HERE

Biomarkers of aging

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) 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 (p<0.05) than that of YC; Figure 4-A. Moreover, comparisons conducted among middle-aged participants only (excluding young controls) revealed that TL of SA was longer ($p < 0.05$) than MC.

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

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

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

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

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

FIGURE 4 ABOUT HERE

The additional analysis which we compared EA and SA showed no statistical difference for TBARS, 8-OHdG, GSH, uric acid, TNF-α, sIL-6R and TL (p>0.05). Nevertheless, statistical differences with large effect sizes were identified for F_2-isoprostanes (p<0.001; d = 1.85), protein carbonyls (p = 0.03; d = 0.81), SOD (p = 0.006; d = 0.99), NO^-_2 (p < 0.0001; d = 1.39), sTNF-RI (p = 0.011; d = 1.06), IL-6 (p = 0.014; d = 1.13), IL-15 (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, 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 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; d = 3.10), klotho (p < 0.001; d = 1.31) and klotho/FGF-23 ratio (p < 0.001; d = 1.49) were significantly lower with large effect sizes in the EA compared to SA; Table 2.

TABLE 2 ABOUT HERE

4. Discussion

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

The present findings revealed that middle-aged untrained individuals have an increased concentration in markers of oxidative damage compared to the YC (F_2-Isoprostanes, carbonylated proteins and 8-OHdG). Such condition may be due to the imbalance between pro-oxidants (*e.g.* free radicals) and antioxidant defenses

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

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

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

It was identified that master athletes (both EA and SA) have lower levels of F_2-Isoprostans, higher NO_x levels, lower levels of uric acid and pro-inflammatory cytokines (sTNF-RI and IL-6), and have higher concentrations of IL-10 and IL-10 / IL-6 ratio in comparison to MC. However, the antioxidant defense and modulation adjustments of some cytokines are different between athletes. EA had higher SOD activity and

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

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

Additionally, ADMA levels were lower in the EA compared to SA, corroborating to an increased SOD and possibly eNOS activity and greater NO- bioavailability. Although the exercise effect on ADMA levels in healthy people remain controversial, in pathological situations aerobic training is effective to decrease ADMA levels, improving blood flow and O_2 supply in response to muscle work demand, which possibly leads to VO_2max maintenance throughout the aging process (Pawlak-Chaouch et al. 2019; Takada et al. 2012; Torok et al. 1995). Thus, it would be reasonable to infer that there is a possible association between ADMA, SOD, NO^- and VO_2max, which would explain the lower ADMA levels of EA in comparison to SA (Takada et al. 2012; Torok et al. 1995; Kusy and Zielinski 2015); Table 2. However, it is important to highlight that the NO metabolism is very complex and the data of the present study must be done with caution. The elevated values of NOx may be a result of the lifestyle itself, including eating habits, in addition to a possible acute residual effect of training from the days prior to collection, and not solely to the chronic effects of lifelong training (Nebl et al. 2019; Rassaf et al. 2007). On the other hand, our participants attended the laboratory after 10-hour fasting, and 48h of exercise abstinence. In addition, all studied groups underwent exactly the same research protocol, which validates the comparisons among them. We suggest, however, that future studies strictly control the eating habits of subjects, as well as the monitoring of training in relation to the intensity and duration of the week prior to data

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

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

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

Master sprinters have significantly higher levels of aerobic capacity than recreationally or sedentary trained individuals as well as a lower rate of decline in maximal heart rate and consequently absolute VO_2max values close to that in EA (Kusy and Zielinski 2015). Based on the present results, we believe that SA may have excellent mitochondrial biogenesis and oxidative function derived from a routine that mixes strength and power

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

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

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

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

5. Conclusion

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

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

5.1 Perspectives

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

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Compliance with Ethical Standards

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

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

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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.

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Figure legends

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

Data are presented as mean ± SD. TBARS (**A**), F_2-isoprostanes (**B**), Protein carbonyls (**C**) and 8-OHdG (**D**). TBARS, thiobarbituric acid reactive substances; 8-OHdG, 8-hydroxydeoxyguanosine. One-way ANOVA followed by Tukey's post hoc test was applied. [a] $p < 0.05$ *vs.* Young Control; [b] $p < 0.05$ *vs.* Middle-Aged Control; [c] $p < 0.05$ *vs.* Master Endurance. (Young Control n = 17, Middle-Aged Control n = 12, Endurance n = 18 and Sprinters n = 13).

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

Data are presented as mean ± SD. Trolox equivalent (**A**), SOD (**B**), GSH (**C**), Catalase (**D**), Uric acid (**E**) and NO_x (**F**). SOD, superoxide dismutase; GSH, glutathione; NO^-_2, nitrite. One-way ANOVA followed by the Tukey's post hoc test was applied. [a] $p < 0.05$ *vs.* Young Control; [b] $p < 0.05$ *vs.* Middle-Aged Control; [c] $p < 0.05$ *vs.* Endurance. (Young Control n = 17, Middle-Aged Control n = 12, Endurance n = 18 and Sprinters n = 13).

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

Data are presented as mean ± SD. TNF-α (**A**), sTNF-RI (**B**), IL-6 (**C**), sIL-6R (**D**), IL-10 (**E**), IL-10/TNF-α ratio (**F**), IL-10/IL-6 ratio (**G**) and IL-15 (**H**). TNF-α, tumor necrosis factor alpha; sTNF-RI, soluble tumor necrosis factor receptor type I; IL-6, interleukin 6; sIL-6R, soluble form of interleukin-6 receptor; IL-10, interleukin 10; IL-10/TNF-α ratio, interleunkin 10/tumor necrosis factor alpha ratio; IL-10/IL-6 ratio, interleunkin 10/interleukin 6 ratio; IL-15, interleukin 15. One-way ANOVA followed by the Tukey's post hoc test was applied. [a] $p < 0.05$ *vs.* Young Control; [b] $p < 0.05$ *vs.* Middle-Aged Control; [c] $p < 0.05$ *vs.* Master Endurance. (Young Control n = 17, Middle-Aged Control n = 12, Endurance n = 18 and Sprinters n = 13).

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

588 Data are presented as mean ± 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).

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

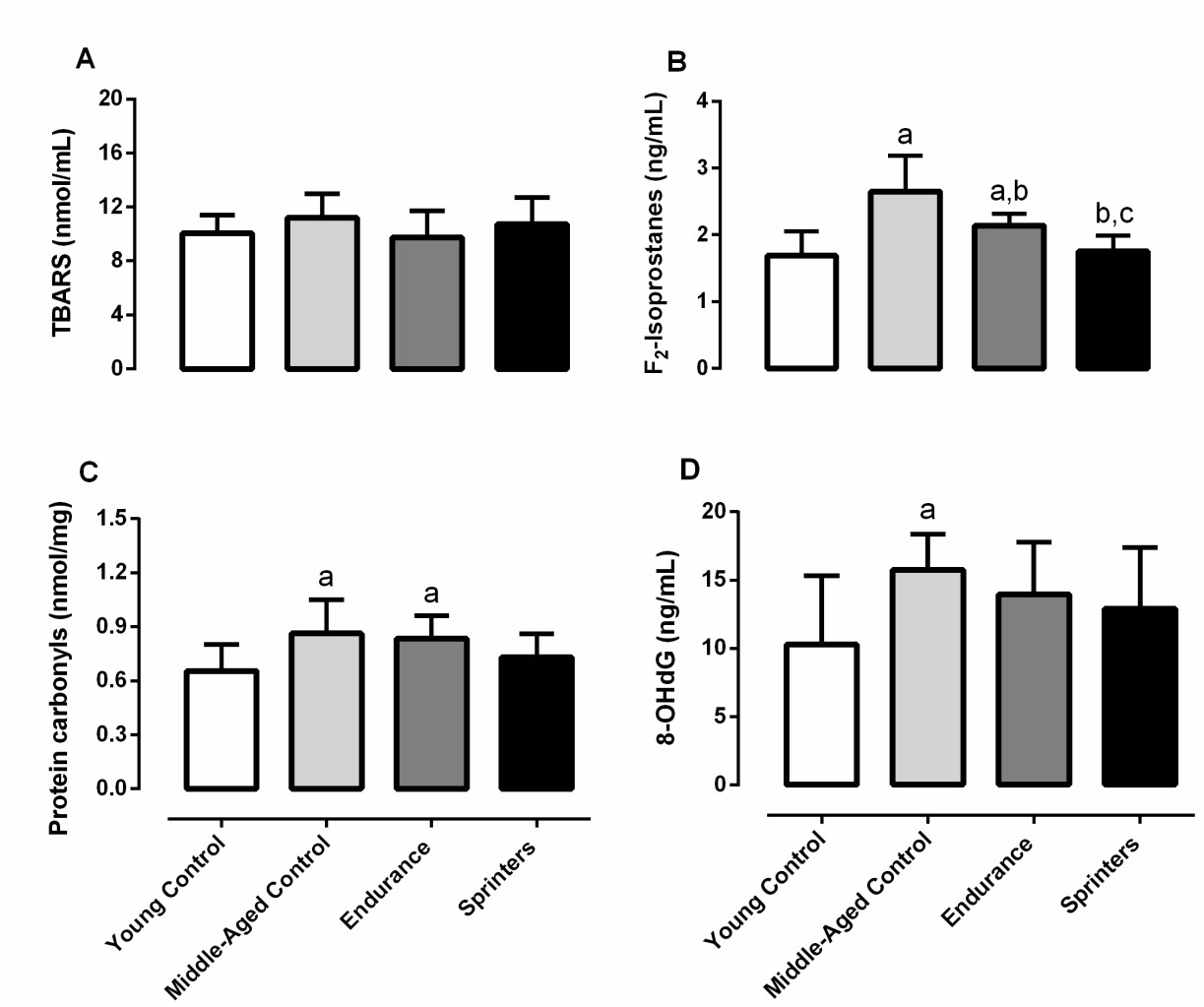
Age and anthropometrics	**Young control (n = 17)**	**Middle-aged control (n = 12)**	**Master endurance (n=18)**	**Master sprinters (n=13)**
Age (years)	22.7 ± 3.9	***45.5 ± 9.8*** [a]	***53 ± 8.2*** [a]	***50 ± 8.9*** [a]
Body mass (kg)	78.8 ± 11.2	91.3 ± 16.4	68.3 ± 7.0	68.6 ± 6.8
Height (cm)	176 ± 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]

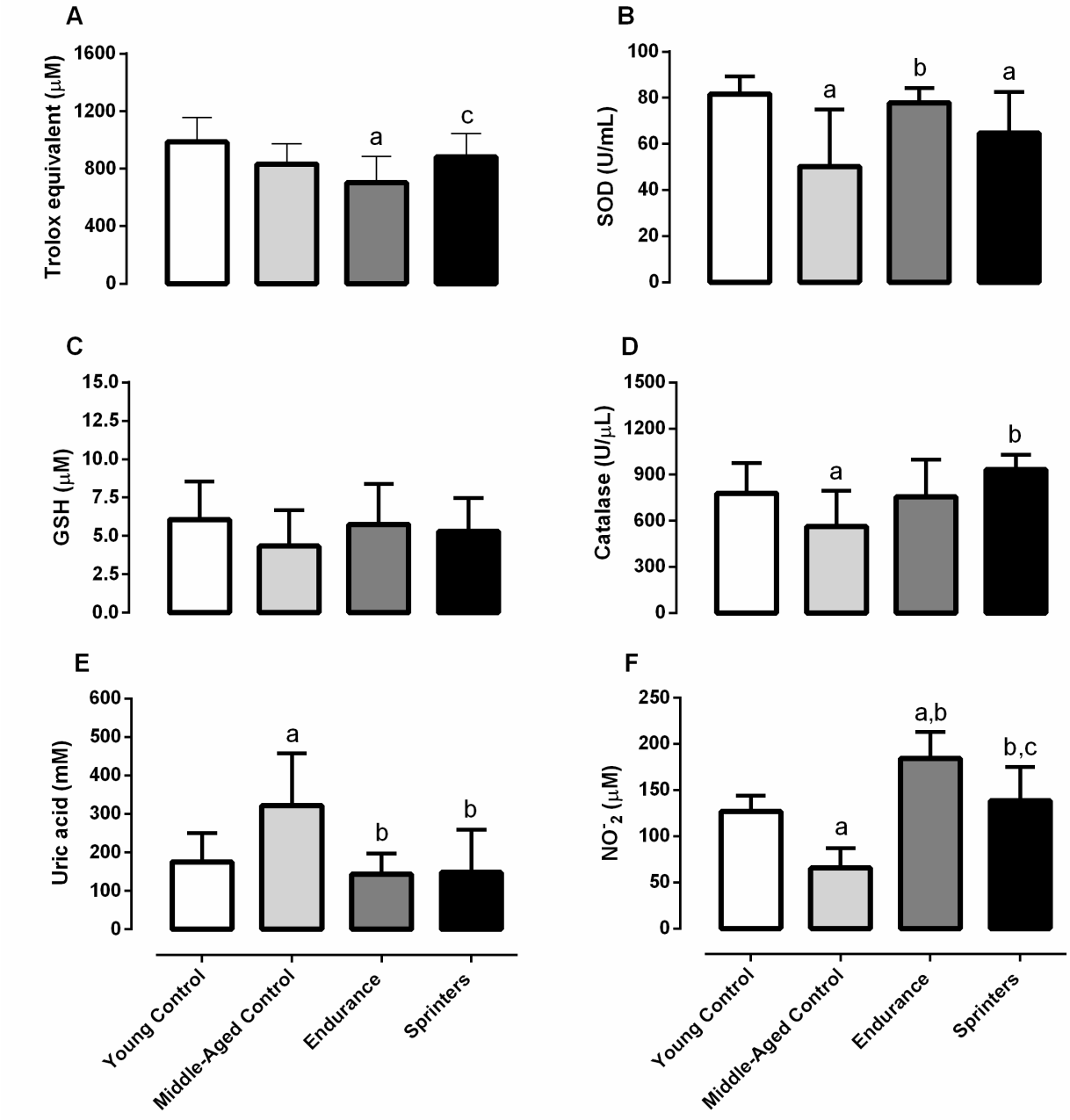
Data expressed as mean ± standard deviation. BMI: body mass index. [a] $p < 0.05$ in comparison to the young control group. [b] $p < 0.05$ in comparison to the middle-aged control group. [c] $p < 0.05$ in comparison to the master endurance athletes group.

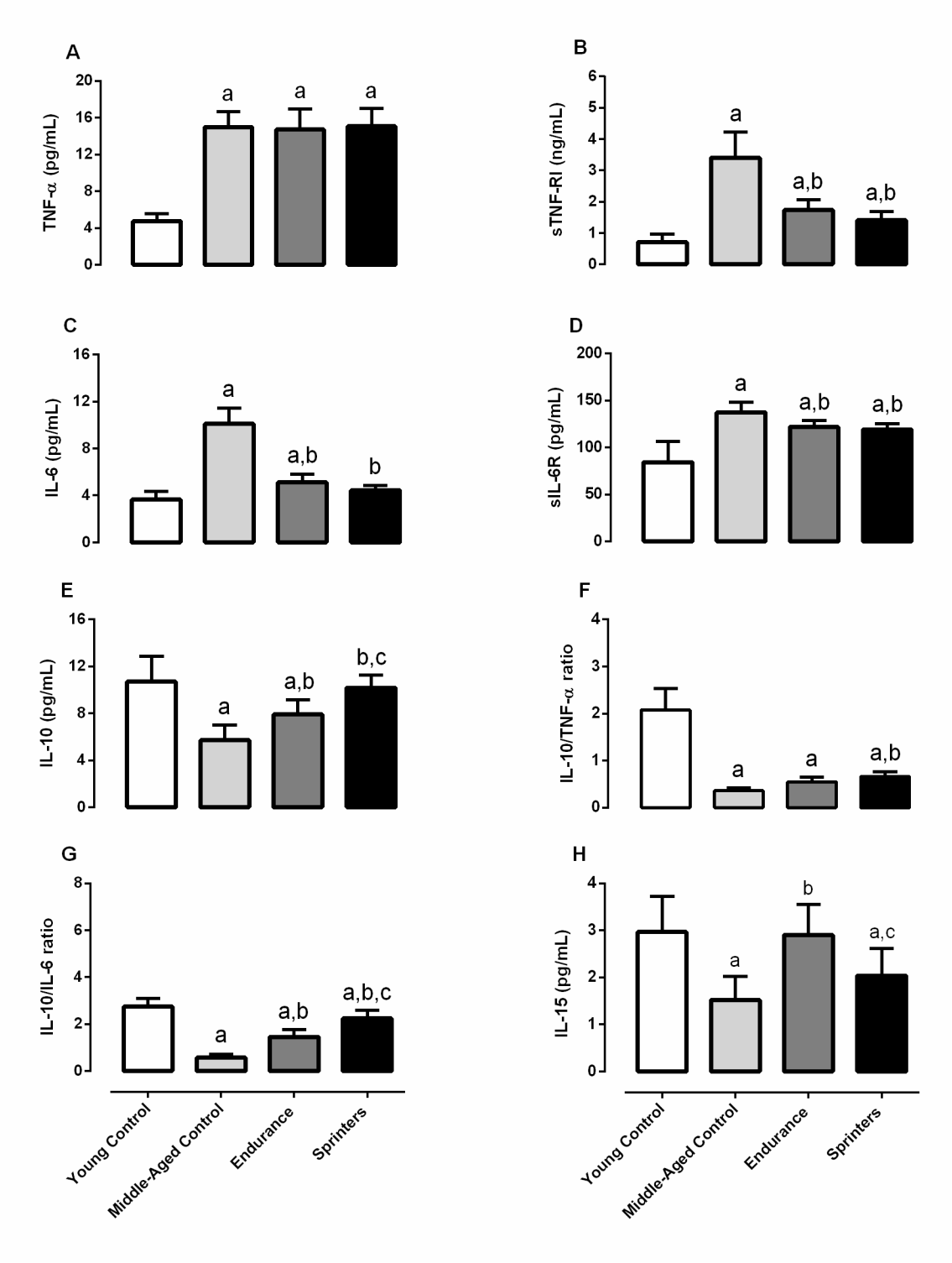
Table 2. Differences between aging markers of master endurance runners and sprinters

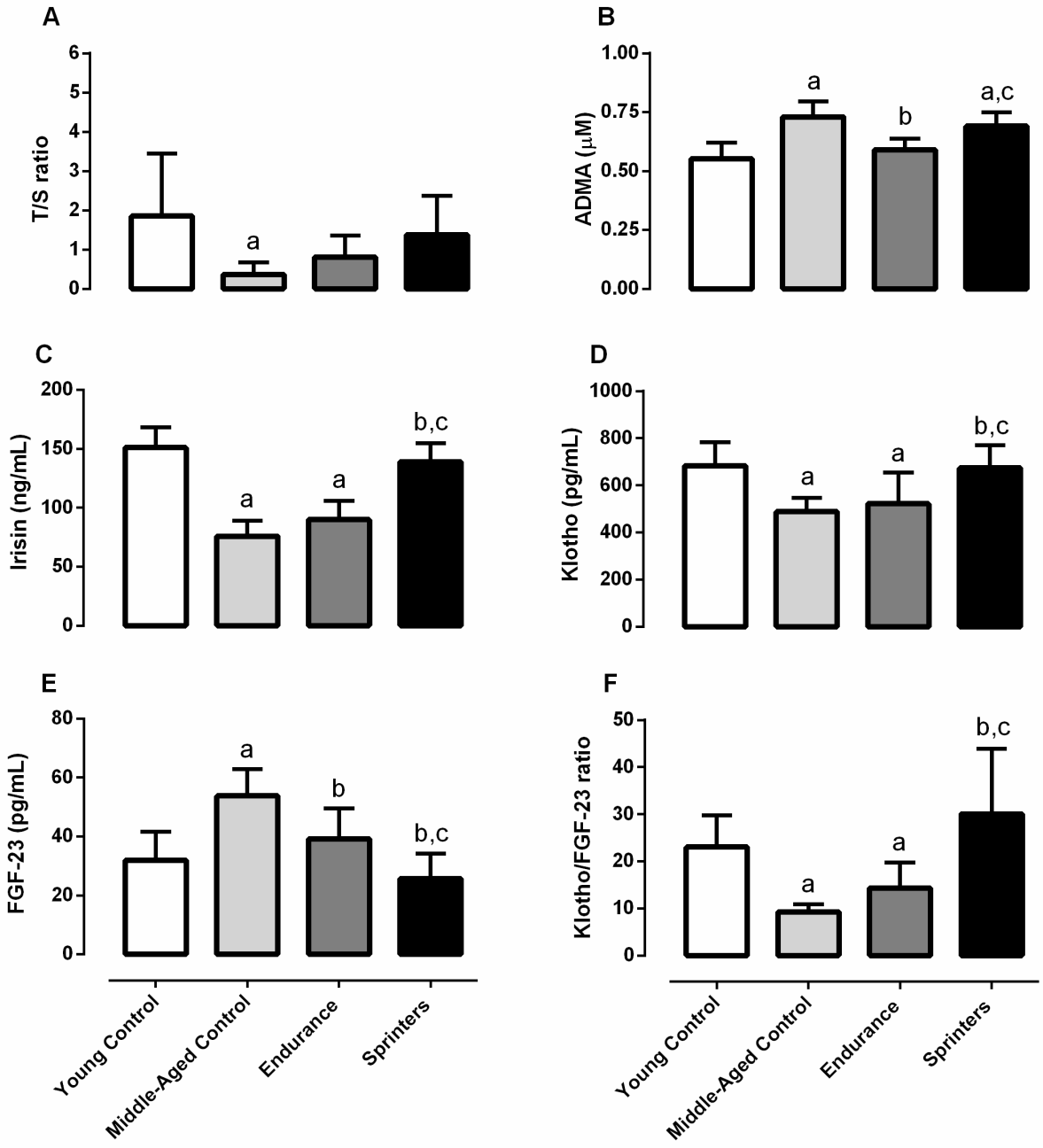
Variables	Endurance Athletes	Sprint Athletes	*p-value*	*Effect Size (Cohen's d)*
Oxidative Stress				
F_2-Isoprostanes (ng/mL)	***2.13 ± 0.17***	1.75 ± 0.23	***0.00***	***1.85***
Protein carbonyls (nmol/mg)	***0.83 ± 0.12***	0.73 ± 0.13	***0.03***	***0.81***
Antioxidants				
Trolox equivalente (µM)	702.92 ±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***
Citokynes				
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; NO^-_2, 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.


A
TBARS (nmol/mL)
20
16
12
8
4
0
B
F_2-Isoprostanes (ng/mL)
4
3
2
1
0
a
a,b
b,c
C
Protein carbonyls (nmol/mg)
1.5
1.2
0.9
0.6
0.3
0.0
a
a
D
8-OHdG (ng/mL)
20
15
10
5
0
a
Young Control
Middle-Aged Control
Endurance
Sprinters


A
Trolox equivalent (μM)
1600
1200
800
400
0
a
c
B
SOD (U/mL)
100
80
60
40
20
0
a
b
a
C
GSH (μM)
15.0
12.5
10.0
7.5
5.0
2.5
0.0
D
Catalase (U/μL)
1500
1200
900
600
300
0
a
b
E
Uric acid (mM)
600
500
400
300
200
100
0
a
b
b
F
NO^-_2 (μM)
250
200
150
100
50
0
a
a,b
b,c
Young Control
Middle-Aged Control
Endurance
Sprinters


A
TNF-α (pg/mL)
20
16
12
8
4
0
a
a
a
B
sTNF-RI (ng/mL)
6
5
4
3
2
1
0
a
a,b
a,b
C
IL-6 (pg/mL)
16
12
8
4
0
a
a,b
b
D
sIL-6R (pg/mL)
200
150
100
50
0
a
a,b
a,b
E
IL-10 (pg/mL)
16
12
8
4
0
a
a,b
b,c
F
IL-10/TNF-α ratio
4
3
2
1
0
a
a
a,b
G
IL-10/IL-6 ratio
8
6
4
2
0
a
a,b
a,b,c
H
IL-15 (pg/mL)
4
3
2
1
0
a
b
a,c
Young Control
Middle-Aged Control
Endurance
Sprinters


A
T/S ratio
6
5
4
3
2
1
0
a
B
ADMA (μM)
1.00
0.75
0.50
0.25
0.00
a
b
a,c
C
Irisin (ng/mL)
200
150
100
50
0
a
a
b,c
D
Klotho (pg/mL)
1000
800
600
400
200
0
a
a
b,c
E
FGF-23 (pg/mL)
80
60
40
20
0
a
b
b,c
F
Klotho/FGF-23 ratio
50
40
30
20
10
0
a
a
b,c
Young Control
Middle-Aged Control
Endurance
Sprinters