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Original Research

Comparison of Muscle Damage Markers and Myokines between Adult and Middle-Aged Marathon Runners

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Abstract

This study compared markers of muscle damage and myokines between adult and middle-aged runners before and after the marathon. Seventy-four male runners: 48 adults aged 30-44 years (AA group), and 26 middle-aged individuals aged 45-59 years (MA group) participated of the study. Blood samples were collected 24 hours before, immediately after, 24 hours and 72 hours after the marathon to measure skeletal and cardiac muscle damage markers (CK, LDH, troponin, and proBNP) and myokines (IL-6, IL-15, decorin, BDNF, GDF-15, FGF-21, apelin,

musclin, myostatin, and follistatin). Before the marathon, it was observed that serum concentrations of BDNF were higher, and those of IL-15, GDF-15, apelin, and musclin were lower in the MA group. Immediately after the marathon, both groups showed higher activity of CK and LDH, as well as higher serum concentrations of ProBNP, troponin, IL-6, decorin, FGF-21, BDNF, and GDF-15. After the marathon and in the recovery period, GDF-15 concentrations remained lower and BDNF concentrations higher in the MA group compared to the AA group. In both groups, long-distance running induced muscle and cardiac damage and modulated myokines responsible for skeletal and cardiac muscle repair/adaptation. Middle-aged individuals show a reduction in the serum concentration of myokines that may contribute to muscle and cardiometabolic dysfunction in senescence. The role of higher levels of BDNF in middle-aged runners on cardiometabolic adaptation should be investigated to elucidate the molecular mechanisms of senescence.

Keywords
Exercise; BDNF; GDF-15; cardiac risk; exerkines

1. Introduction

The non-pathological aging process of senescence promotes physiological changes leading to the decline of physiological functions having its onset between 30 and 35 years of age, leading to cellular damage and dysfunctions [1, 2]. Senescence reduces protein synthesis, activation of satellite cells, and angiogenesis in muscle tissue, leading to sarcopenia and muscle dysfunction, as well as promoting immunosenescence and subclinical inflammation called inflammaging, increasing the risk for chronic diseases such as cardiovascular diseases [3].

Physical activity is the primary strategy to avoid sarcopenia and cardiovascular diseases, but it does not halt the aging process [4]. Long-distance exercise leads to muscle and cardiac damage followed by inflammatory response, muscle repair, and cardiometabolic adaptations that improve the cardiometabolic systems and maintain muscle mass [5]. Muscle contraction stimulates the secretion of cytokines, currently known as exerkines or myokines [6, 7]. Myokines have endocrine action, crosstalk between muscles and different types of organs (brain, adipose tissue, skin, bone, liver, vessels), and autocrine/paracrine function [8]. Myokines have biological effects on protein synthesis, lipid oxidation, glucose homeostasis, mitochondrial biogenesis, angiogenesis, myogenesis, muscle contractility, and inflammation, promoting the maintenance of muscle mass and muscle repair and cardiometabolic adaptations, such as improvement of glucose homeostasis and cardiac contractility, browning of adipose tissue, and angiogenesis [9].

Previous studies have shown an association between age and serum concentrations of myokines, such as interleukin 6 (IL-6), IL-15, myostatin, apelin, decorin, musclin, folistatin (FSTL), brain-derived neurotrophic factor (BDNF), fibroblast growth factor 21 (FGF-21) and growth/differentiation factor 15 (GDF-15), responsible for modulating muscle and cardiometabolic functions [10, 11]. However, few studies have been conducted on middle-aged active individuals responding to endurance exercise.

The hypothesis of the study is that the aging process may early alter the baseline myokine levels or exercise-induced myokine response in active individuals, contributing to muscle dysfunction and the non-pathological process of senescence. Thus, we compared the serum concentrations of muscle and cardiac damage markers and myokines between marathon runners, adults, and middle-aged.

2. Materials and Methods

2.1 Subjects

Participants in this study were 107 healthy male marathon finishers who had completed at least one International Marathon between 2017 (n = 54) and 2018 (n = 53), aged 30 to 59 years. The volunteers were recruited by registering in a call offered by the organizer of the International Marathon. The application was made online, and subsequently, the researchers contacted the enrollees. A complete physical examination of each participant was performed according to the rules of the *American Heart Association*. The exclusion criteria were functional alterations or pre-existing diseases, according to clinical history and physical examination. After receiving explanations of the objectives and potential benefits and risks involved in the study, the volunteers completed a free. The sample size population was calculated according to the number of runners of the International Marathon (3114, 30-59 years old), the frequency of middle-aged runners (42%, 1319 runners), 8% of margin of error and 90% of confidential levels, resulting in 100 runners (Epi Info 7.0, Center for Disease Control and Prevention, CDC).

2.2 Study Design

Clinical history, physical examination and body composition, assessed by the bioimpedance equipment (BioSeca, Germany) to determine the percentage of fat mass (% FM), fat mass (FM), fat-free mass (FFM), body mass index (BMI), were evaluated one day before the race. Blood collection from fasting runners, who had been without physical activity for at least 12 hours, was performed 24 hours before the race and 24 and 72 hours after the marathon from the antecubital vein at the Institute of Physical Activity and Sports. Blood samples were centrifuged at 4°C, 400 g, for 10 minutes to obtain serum (10 mL, vacuum-dried siliconized tube) after standing at room temperature for 30 minutes. The samples were stored at -80°C for later analysis of muscle damage markers and myokines at the University. Immediately after the race, blood samples from fed runners were kept at room temperature, placed on ice for approximately 2 hours at the International Marathon of São Paulo (competition venue near the finish line), and finally sent to the University as described by Sierra et al. (2022) [12].

Out of one hundred and seven runners, twenty-two did not attend to at least one of the blood collections (before, immediately after, 24 h or 72 h after marathon) and were excluded. The other seventy-four were divided into two groups according to age: the adult age group (AA, 48 runners), aged between 30-44 years, and the middle-aged group (MA, 26 runners), aged between 45-59 years [2].

The Sao Paulo International Marathon (2017, 2018) started at 07:30 AM on April 9 and April 8, respectively. Fluid ingestion was allowed ad libitum during the race. Water was available every 2 to 3 km on the running course; sports drinks were available at 12 km, 21.7 km, 33 km, and 42 km; and

a carbohydrate source was available at 28.8 km. The weather parameters between 07:00 AM and 02:00 PM were as follows: average temperature, 19.8°C (2017) and 19.9°C (2018); and average relative humidity, 72.8% (2017) and 87.7% (2018) (National Institute of Meteorology, Ministry of Agriculture, Livestock, and Supply) as described by Sierra et al. (2022) [12].

2.3 Biochemical Measures

The lactic dehydrogenase (LDH) and creatine kinase activities were determined using enzymatic kinetics of multiple points and troponin and pro type B natriuretic peptide (ProBNP) by amplified chemiluminescence technique in the Clinical Laboratory (Associação Fundo de Incentivo à Pesquisa, AFIP). Myokines were assessed through the Milliplex technique using Luminex xMAP technology (MAP = Multiple Analyte Profiling, x = its variables) that composes several magnetic microspheres with two fluorophores (MAGPIX® System, Thermo Fisher Scientific, Boston, USA). Serum levels of IL-6, IL-15 and FGF-21 were determined using the MILLIPLEX Human Cytokine/Chemokine Magnetic Bead Panel (HCYTOMAG-60K, EMD Millipore Corporation) and serum levels of apelin, BDNF, myostatin, musclin, FSTL, IL-6, IL-15 and FGF-21 using the MILLIPLEX® human myokine magnetic sphere panel (HCYTOMAG-56K, EMD Millipore Corporation, USA) at Emergency Medicine Department, LIM-51, University of Sao Paulo. The intra-assay precision (mean coefficient variation percentage) was performed as described by the manufacturer's protocol, which was <3% for IL-6, IL-15, and <10% for apelin, irisin, BDNF, myostatin, musclin, follistatin, FGF-21. The serum concentration of decorin and GDF-15 were determined by *Enzyme-Linked Immunosorbent Assays (Duoset-ELISA, R&D Systems, USA*) according to the manufacturer's instructions (Spectra MAX Plus spectrophotometer, Molecular Devices, California, USA) at the University as described by Sierra et al. (2022) [12].

2.4 Statistical Analysis

The statistical analyses were performed using Prism 9 version 9.1.1 (GraphPad *Software*, LLC, 2021). Data normality was performed using the *Kolmogorov-Smirnov* test and was rejected (non-parametric variables). The general and training characteristics, muscle damage markers, and myokines are presented as the mean and standard deviation of the mean (SD). The association of age and myokines was performed by Spearman's nonparametric correlations. We compared the muscle damage and myokines levels using two-way ANOVA of repeated measures with Geisser-Greenhouse correction for sphericity and Holm-Sidák post-test for multiple comparisons in both intergroup (main effect) (MA and AA groups in all periods of study) and intragroup (time effect, repeated measure). The area under a curve was calculated for myokines response (immediately after, 24 h and 72 h after the race) in AA and MA groups and compared by unpaired t-test. The percentage of myokine values in the MA group relative to the AA group was calculated, with the AA group set as 100% both before and after the race. The significance level considered was $P \leq 0.05$.

2.5 Ethics Statement

Approved by the Ethics Committee of the University (CAAE: 29046919.4.0000.8084).

3. Result

Age was positively correlated with LDH activity and serum concentrations of BDNF and FSTL and negatively correlated with musclin, apelin, and IL-15 before the race (Table 1).

Table 1 Correlation of age (years) with a marker of muscle damage and myokines before the race.

Marker of muscle damage and myokines	r	p-value
LDH (U/L)	r = 0.3250	p = 0.0078
BDNF (pg/mL)	r = 0.4922	p < 0.0001
FSTL (pg/mL)	r = 0.3061	p = 0.0165
Musclin (pg/mL)	r = -0.3894	p = 0.0019
Apelin (pg/mL)	r = -0.2771	p = 0.0306
IL-15 (pg/mL)	r = -0.3389	p = 0.0076

LDH, lactate dehydrogenase; BDNF, brain-derived neurotrophic factor; FSTL, follistatin; IL-15, interleukin 15.

Seventy-four runners were divided into two groups according to age (AA and MA group), and anthropometric and training characteristics are presented in Table 2. Body mass, FFM and height were lower and age, number of marathons and training experience were higher in the MA group compared to AA group, suggesting an impairment of body composition in MA group (Table 2).

Table 2 General and training characteristics of AA and MA groups.

	AA	MA	p-value
Age (years)	37.98 ± 3.694	49.64 ± 3.268	<0.0001
Body mass (kg)	77.47 ± 9.141	72.52 ± 10.40	0.030
Height (m)	1.74 ± 0.060	1.71 ± 0.044	0.036
BMI (kg/m^2)	25.40 ± 2.861	24.52 ± 2.875	0.435
FM (%)	21.72 ± 4.563	22.38 ± 5.498	0.923
FM (kg)	17.02 ± 5.008	16.60 ± 5.915	0.755
FFM (kg)	60.32 ± 5.850	55.93± 6.091	0.028
Marathon time (min)	256.4 ± 40.26	257.4 ± 40.70	0.970
Pace (km/min)	4.486 ± 1.049	4.706 ± 0.519	0.521
Workouts per week	4.434 ± 1.264	4.115 ± 1.143	0.599
Km per week (km)	52.60 ± 18.76	51.25 ± 16.24	0.999
Number of marathons	3.121 ± 1.673	7.263 ± 1.006	0.0007
Training Experience (years)	7.700 ± 5.495	10.35 ± 6.617	0.022

BMI, body mass index; FM, fat mass; FFM, fat-free mass. The s-values are presented as the mean, standard deviation of the mean ± of 53 runners in the adult group (AA) and 26 in the middle-aged group (MA).

The marathon race induced an increase on skeletal muscle damage markers, CK and LDH activities (time effect: F(1.6, 126) = 60.22, $p < 0.0001$; time effect: F(1.5, 114) = 27.53, $p < 0.0001$,

respectively) (Figure 1A and 1B), and on cardiac damage markers, ProBNP and troponin levels (time effect: F(1.7, 117) = 71.6 , $p < 0.0001$; time effect: F(1.4, 97) = 22.15, $p < 0.0001$, respectively), and remained elevated 24 h after the race in both groups (Figure 1C and 1D). We did not observe differences in CK (time × age effect F(3, 231) = 0.26, $p = 0.85$) , LDH (time × age effect F(2, 154) = 0.002, $p = 0.99$), ProBNP (time × age effect F(2, 132) = 1.21, $p = 0.30$) and troponin levels (time × age effect F(2, 138) = 1.66, $p = 0.20$) between AA and MA groups in all periods.

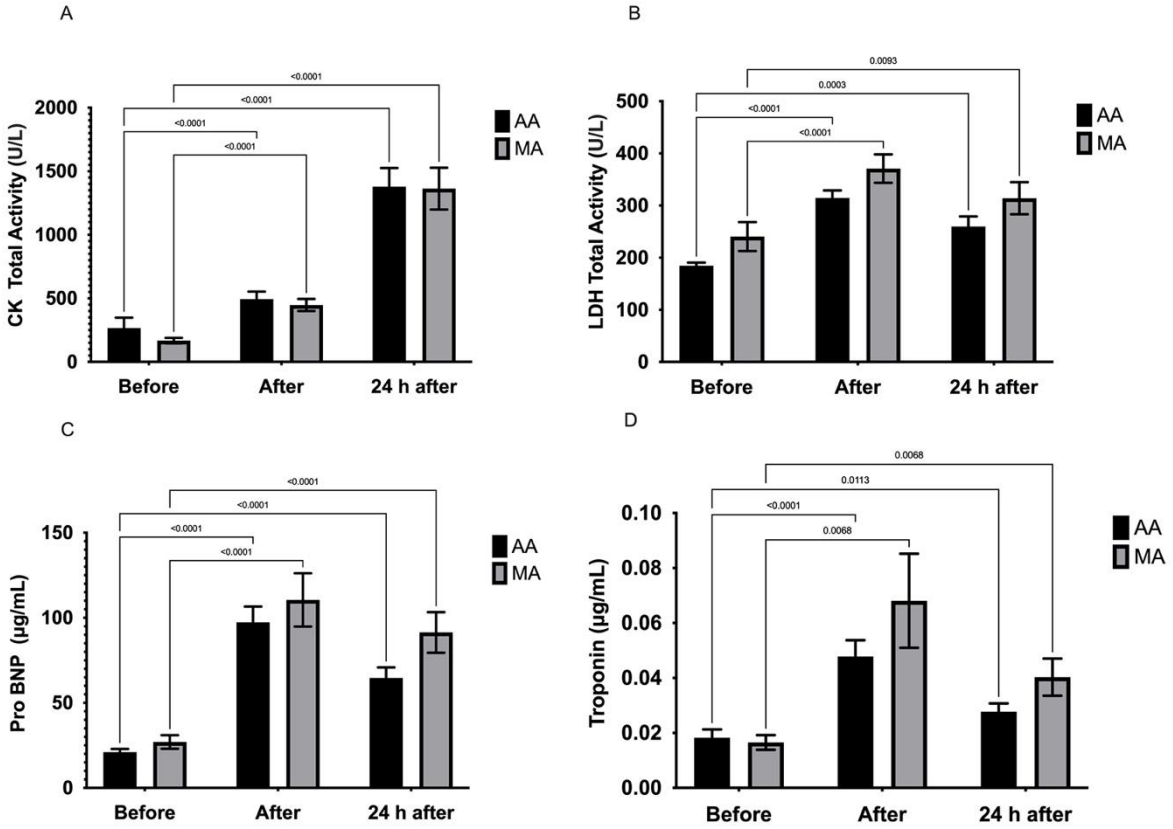

Figure 1 Skeletal and cardiac muscle damage markers before the race, immediately after, and 24 h after the race. We determined plasma creatine kinase (CK, A), lactate dehydrogenase (LDH, B), pro B-type natriuretic peptide (ProBNP, C) and troponin (D) concentration. The values are presented as mean ± standard error of the mean (SEM) of 48 runners in the adult group (AA) and 26 in the middle-aged group (MA).

Serum concentrations of IL-6 and decorin, which participate in the muscle repair process, increased in both groups after the marathon (time effect: F(1.5, 114) = 97.34 , $p < 0.0001$; time effect: F(1.9, 109) = 19.68, $p < 0.0001$, respectively) (Figure 2A and 2B). AA group presented an elevation in FSTL levels after the race, followed by a reduction of IL6, FSTL, and IL-15 levels in the recovery period (time effect: F(2.1, 151) = 23.9, $p < 0.0001$) (Figure 2A, 2C, and 2D). Before the race, IL-15 levels were lower in the MA group compared to the AA group (time × age effect F(3, 216) = 7.32, $p = 0.0001$) (Figure 2D). IL-6, decorin and FSTL were not different between AA and MA groups (time × age effect F(3, 216) = 1.76, $p = 0.16$, time × age effect F(3, 168) = 1.85, $p = 0.14$, time × age effect F(3, 216) = 0.31, $p = 0.82$).

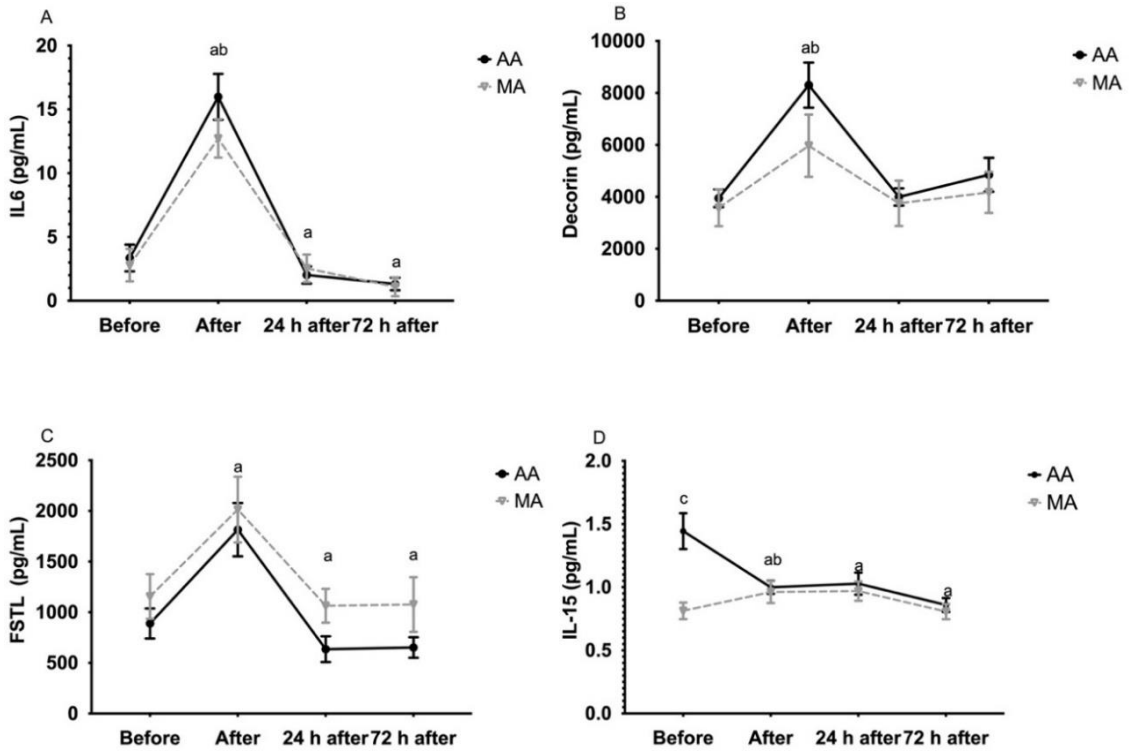

Figure 2 Serum concentration of IL-6 (A) decorin (B), FSTL (C) and IL-15 (D) before the race, immediately after, 24 h and 72 h after the race. IL-15, interleukin 15; FSTL, folistatin; IL-6, interleukin 6; AA adult group; MA middle-aged group. The values are presented as mean ± SEM of 48 runners in the AA and 26 in MA. [a] $p \leq 0.05$ vs before race in AA group; [b] $p \leq 0.05$ vs before race in MA group and [c] $p \leq 0.05$ AA vs MA group.

Before the marathon, myostatin levels were lower in the MA group than in the AA group, although this difference was insignificant (time × age effect: $F(3, 216) = 1.46$, $p = 0.22$). The marathon induced a reduction in myostatin concentrations immediately after the race and during the recovery period in the AA group (time effect: $F(2.3, 170.2) = 6.143$, $p = 0.0015$), which may contribute to muscle repair (Figure 3).

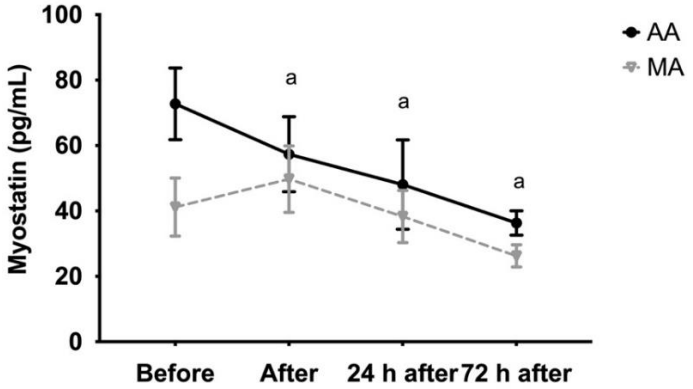

Figure 3 Serum concentration of myostatin before the race, immediately after, 24 h and 72 h after the race. AA adult group; MA middle-aged group.The values are presented as mean ± SEM 48 runners in AA and 26 in MA. [a] $p \leq 0.05$ vs. before the race in the AA group.

The marathon induced an increase in serum level of FGF-21 immediately after the marathon and returned to basal levels after 24 h in both groups (time effect: F(1.9, 136.8) = 17.95, $p < 0.0001$) (Figure 4A). Serum level of apelin was higher in the AA group before the race compared to the MA group (time × age effect: F(3, 216) = 4.947, $p < 0.0024$) and decreased immediately after the race and in the recovery period in AA group (time effect: F(2.6, 187) = 3.358, $p < 0.0001$) (Figure 4B).

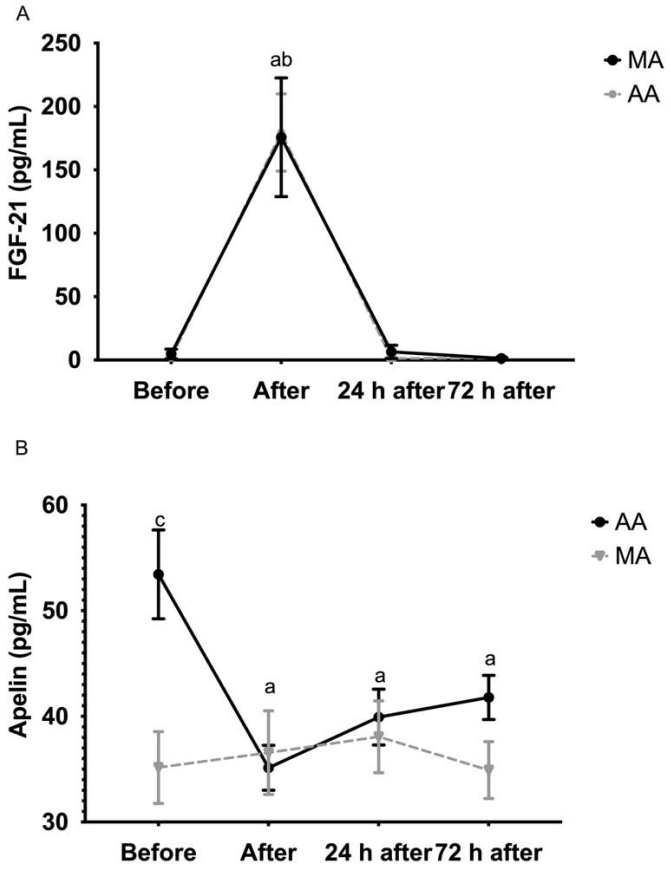

Figure 4 Serum concentration of FGF-21 (A) and apelin (B) before, immediately after, 24 h and 72 h after the marathon. FGF-21, fibroblast growth factor 21; AA adult group; MA middle-aged group. The values are presented as mean ± SEM of 48 runners in AA and 26 in MA. [a] $p \leq 0.05$ vs before race in AA group; [b] $p \leq 0.05$ vs before race in MA group and [c] $p \leq 0.05$ AA vs MA group.

We observed higher serum concentration of BDNF in all periods and AUC BDNF in the MA group compared to AA group (age effect: F(1, 72) = 27.02, $p < 0.0001$) (Figure 5A and 5B), as well as, lower serum concentration of GDF-15 in all periods and AUC GDF-15 in the MA group compared to AA group (age effect: F(1, 47) = 16.45, $p = 0.0002$) (Figure 5C and 5D). Marathon induced an increase in serum level of BDNF (time effect: F(1.9, 135) = 52.36, $p < 0.0001$) and GDF-15 (time effect: F(2.139, 100.5) = 28.61, $p < 0.0001$) in both groups (Figure 5A and 5C). Musclin concentration and musclin AUC decreased in the AA group (time effect: F(2.901, 208.9) = 7.285, $p < 0.0001$), as the musclin concentration was already reduced before the marathon in the MA group (age effect: F(1, 72) = 7.8, $p = 0.0066$) (Figure 5E and 5F).

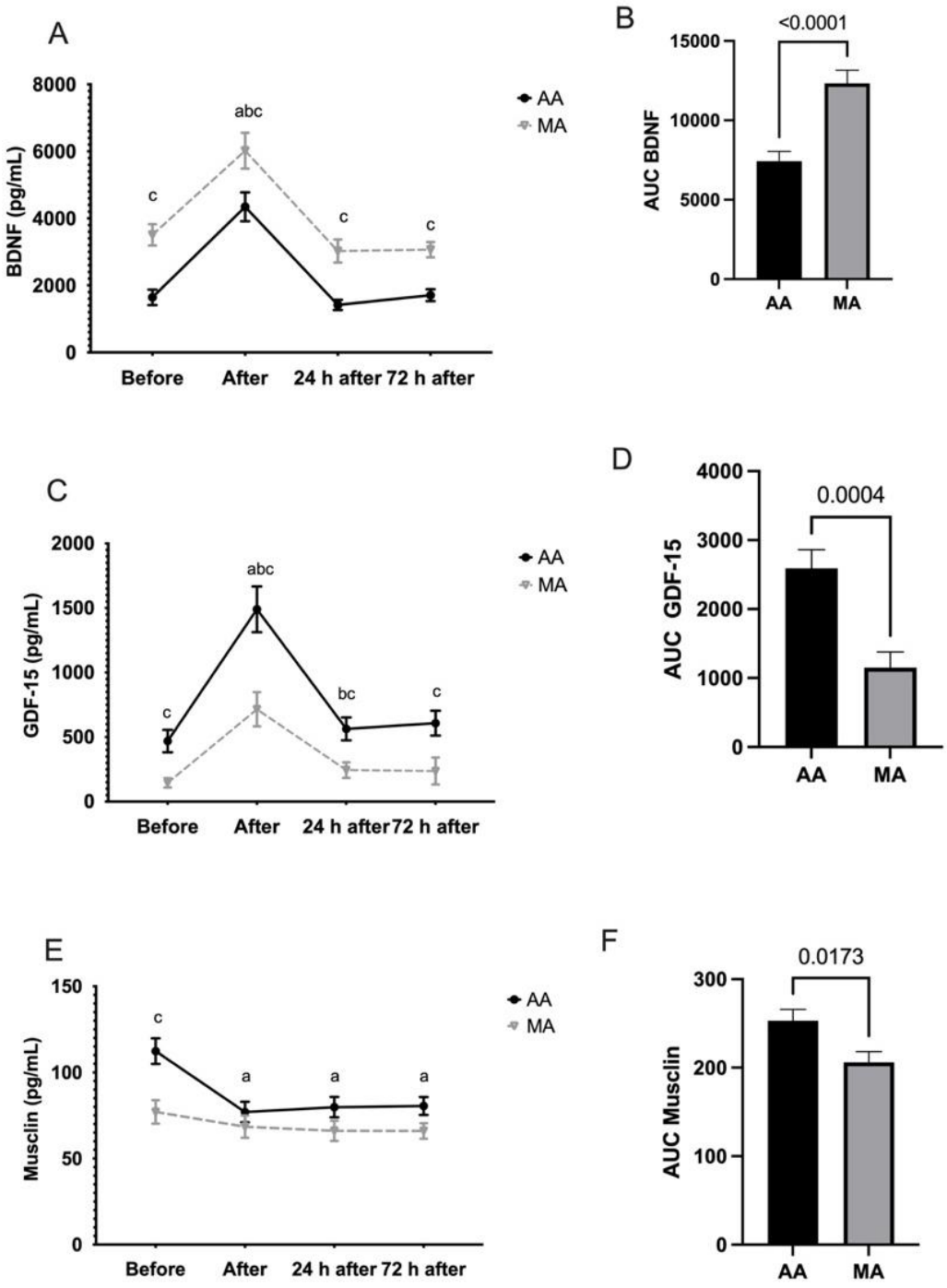

Figure 5 Serum concentration of BDNF (A), GDF-15 (C), and musclin (E) before, immediately after, 24 h and 72 h after the marathon and AUC of BDNF (B), GDF-15 (D) and musclin (F). BDNF, brain-derived neurotrophic factor; GDF-15, growth/differentiation factor 15; AUC, area under the curve; AA adult group; MA middle-aged group. The values are presented as mean ± SEM of 48 runners in the AA and 26 in MA. [a] $p \leq 0.05$ vs before race in AA group; [b] $p \leq 0.05$ vs before race in MA group and [c] $p \leq 0.05$ AA vs MA group.

Changes in BDNF levels showed positive correlations with body mass (Figure 6A), and fat-free mass (FFM) (Figure 6B) in the AA group. However, no correlation was found between BDNF changes and body composition in the MA group.

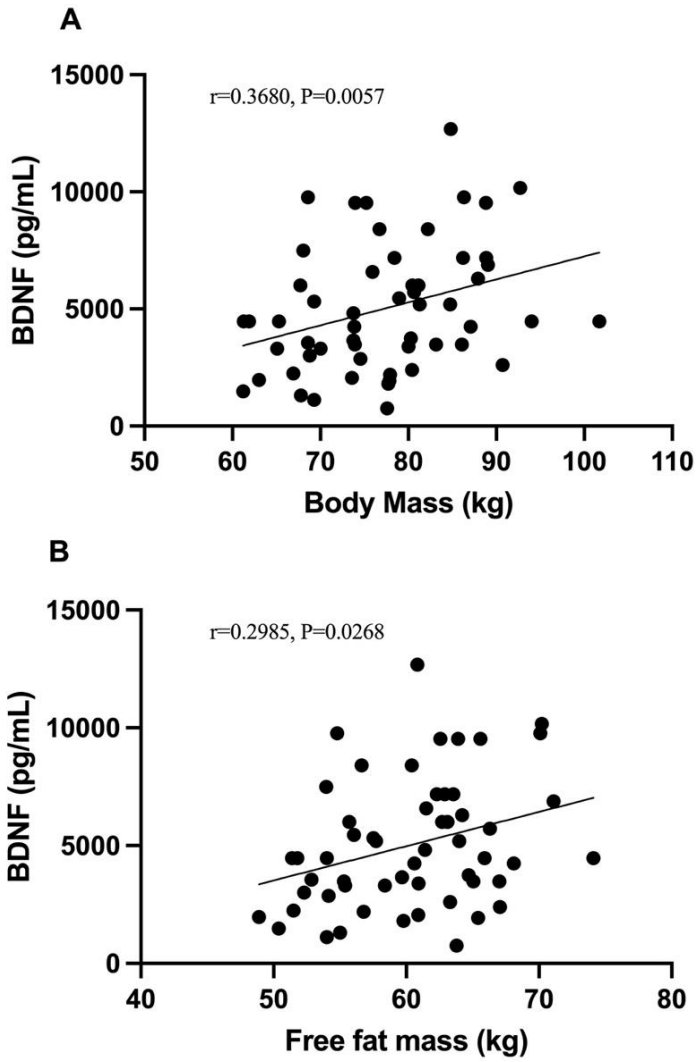

Figure 6 Correlation between changes in BDNF levels and body mass (A) and fat-free mass (B) in the AA group. BDNF, brain-derived neurotrophic factor.

The Figure 7 summarizes the differences between AA and MA groups evaluating the percentage of MA group myokines value in relation to AA group value before and after the race. Before the race, we observed a reduction in IL-15 (by 44%), musclin (by 31%), apelin (by 34%), and GDF-15 (by 69%) and an increase of BDNF by 2.1-fold in the MA group (Figure 7A). GDF-15 levels were 52% lower, and BDNF concentrations were higher (1.4-fold) in the MA group compared to the AA group after the race (Figure 7B).

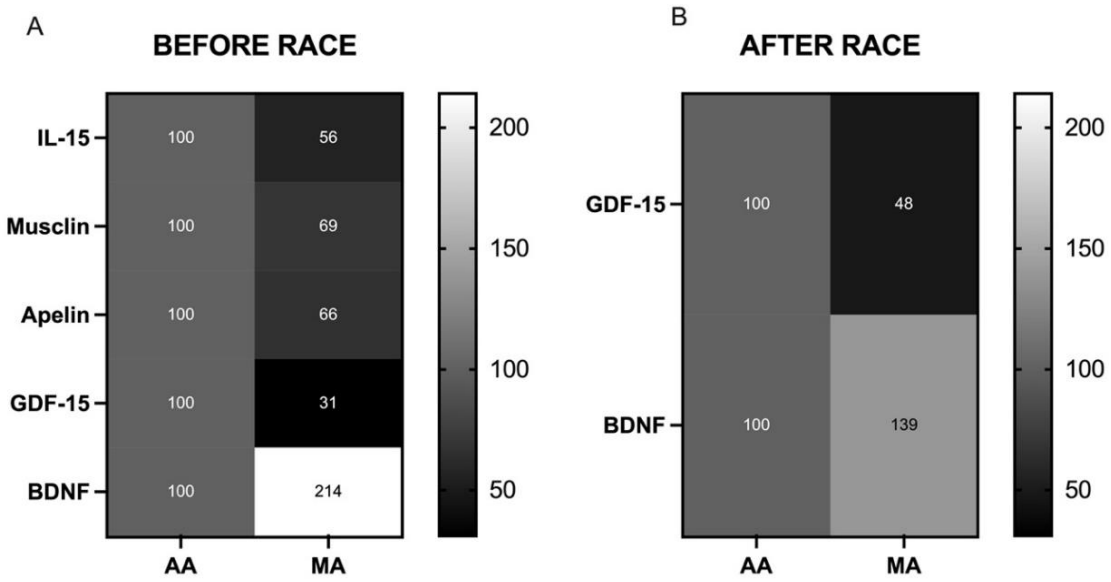

Figure 7 Percentage of MA group myokine value about AA group value before (A) and after the race (B). IL-15, interleukin 15, GDF-15, growth/differentiation factor 15; BDNF brain-derived neurotrophic factor; AA, adult group; MA, middle-aged group.

4. Discussion

Before the marathon, lower serum concentrations of IL-15, GDF-15, apelin, and musclin and higher serum concentrations of BDNF were observed in the MA group, which may affect the cardiometabolic system. The endurance exercise induced muscle and cardiac damage, as well as the release of myokines responsible for tissue repairs, such as IL-6, decorin, FGF-21, GDF-15, and BDNF, in both groups. After the marathon, serum GDF-15 concentrations remained lower and BDNF concentrations higher in the MA group compared to AA group. The reduction in myokines (myostatin, IL-15, apelin, and musclin) after the marathon was only observed in the AA group because the level of these myokines in the MA group was already at low concentrations before the marathon.

Long-distance exercise promotes muscle and myocardial damage in both adult and middle-aged individuals, with no difference between the groups, corroborating previous studies on endurance athletes [11]. Muscle repair after the marathon seems mediated by the increase in IL-6 and decorin in both groups. IL-6 has anti-inflammatory properties and modulates myogenesis and energy metabolism [13]. Decorin interferes with protein degradation pathways by inhibiting the myostatin Activin IIB receptor [14]. The decrease in decorin expression and increase in IL-6 have a direct relationship with senescence [15].

Other myokines associated with muscle repair are FSTL and IL-15, which contribute to mitochondrial biogenesis [16, 17]. FSTL is also secreted by the gonads and liver tissue in response to exercise and seems to be regulated by the glucagon-insulin ratio [18]. A previous study with different age groups showed that FSTL had a positive correlation with age and was higher in active male individuals between 61-79 years compared to individuals between 18-33 years [19]. Our study also demonstrated a positive correlation of FSTL with age, but no significant difference was observed between the AA and MA groups. An increase in serum FSTL concentrations was observed in both groups after the marathon, which was significant only in the the AA group.

Before the marathon, lower serum concentrations of IL-15 were observed in the MA group, and there was a negative correlation between IL-15 and age. Previous studies suggested a decrease in serum levels of IL-15, mainly in the quadriceps and gastrocnemius muscles in senescence [20].

The myokine that has adverse effects on repair and muscle mass is myostatin. The inhibition of myostatin expression promotes muscle hypertrophy [21]. Before the marathon, serum myostatin levels were lower in the MA group but not significantly. A study with women <30 years (young) and >30 years of age demonstrated that the chronic effect of exercise promoted the reduction of myostatin concentrations only in the group >30 years [22]. In senescence, there is an increase of type I fibers and, consequently, lower expression of myostatin [23]. After the marathon, there was a reduction of myostatin in the AA group only once the MA group already had low myostatin levels.

In the process of senescence, cardiometabolic changes occur that increase the risk for cardiovascular diseases [23]. Before the marathon, the serum concentration of apelin, musclin and GDF-15, which have biological effects on the cardiometabolic system, were lower in the MA group compared to AA, suggesting that these myokines may contribute to a reduction in cardiometabolic and/or cardioprotective function in senescence. The cardioprotective effect of GDF-15 induced by exercise is due to its anti-inflammatory action, ALK receptor activation (1-7) of cardiomyocytes promoting anti-apoptotic action by inhibition of the AKT and NF-kB pathway and antihypertrophic, stimulating the AKT pathway and the production of nitric oxide [24]. An increase in GDF-15 was observed in both groups; however, a negative correlation between age and GDF-15, along with the lower concentration of GDF-15 in active middle-aged individuals at all times evaluated [11] also suggests a reduction in cardiometabolic adaptations in middle-aged athletes.

Apelin and musclin also have cardioprotective properties after ischemia-reperfusion, promoting muscle repair [25, 26]. Apelin is a peptide expressed mainly in cardiomyocytes [27] and promotes vasodilation through activation of AKT, phosphorylation of eNOS, and release of nitric oxide, Ca^{2+} improving cardiac contractility [28], as well as triggering mitochondriogenesis, autophagy, and anti-inflammatory action in myofibers, contributing to muscle repair [29]. Exercise-induced musclin has been shown to regulate mitochondrial genesis and prevent the degradation of cardiac NPs, improving cardiomyocyte contractility through protein kinase and fibroblast inhibition due to protein kinase G [30]. Both myokines had a negative correlation with age, in addition to lower serum concentrations before the marathon, suggesting reduced cardiometabolic function in middle-aged individuals. Chronic exercise increases the concentrations of apelin and musclin in patients with chronic non-communicable diseases and obese [31]. However, acute resistance exercise reduces the serum concentrations of muscle, according to our study [32]. After the marathon, there was a reduction of apelin and musclin in the AA group, as the MA group already had low levels, indicating that these myokines do not participate in the cardiometabolic adaptation after acute exercise in both the AA and MA groups.

Increased FGF-21 may contribute to decreased apelin levels in response to exercise [33]. In the present study, an increase in FGF-21 concentration after the marathon was observed, in line with previous studies [34]. FGF-21 stimulates mitochondrial biogenesis, and lipid oxidation in muscle, improves glucose homeostasis, as well as induces the AMPK/FOXO3/SIRT3 signaling axis in stem-cell-derived cardiomyocytes [35, 36].

On the other hand, in our study, we observed a positive correlation between age and BDNF, as well as a higher concentration of BDNF in the MA group at all periods evaluated. BDNF is expressed in the brain and by muscle, adipose tissue, and immune cells. Aerobic exercise increases the serum

expression of BDNF chronically or acutely [37]. The muscle contraction and damage during endurance exercise increase BDNF expression in skeletal muscle, although it does not contribute to BDNF levels in circulation [38]. A recent study demonstrated that exercise promotes an increase in pro-BDNF levels in circulation (20%) and muscle (10%) [39]. BDNF increases Ca^{2+} levels in the presynaptic buds of neuromuscular junctions, resulting in improved skeletal and cardiac muscle contractility during exercise [37]; it also promotes angiogenesis due to the generation of ROS derived from NADPH oxidase by transduction of the tropomyosin kinase B receptor, activating the AKT pathway, leading to the migration of endothelial cells, in addition to having anti-apoptotic [40].

The BDNF also appears to participate in skeletal muscle regeneration, stimulating myogenesis, improving glucose transport, and enhancing fat oxidation [41]. BDNF levels have been negatively associated with cardiopulmonary function at rest in sedentary individuals and/or those with metabolic disorders [38]. However, immediately after exercise, an inverse correlation has been observed with fitness levels, highlighting the importance of BDNF in exercise adaptation [15]. Previously, our group suggested that changes in BDNF levels induced by marathon running were associated with body mass, fat-free mass, and BMI [11]. In the present study, we also showed a positive correlation between changes in BDNF levels with body mass and FFM in the AA group. In contrast, in the MA group, changes in BDNF and FFM were not correlated with body composition.

Changes in BDNF have been associated with higher walking speed, increased muscle mass, and lower body fat [42]. In older and younger trained individuals, BDNF levels decrease 1 hour after exercise, which is more pronounced in younger volunteers (48%) than older volunteers (34%). Our study is the first to demonstrate that BDNF levels in MA runners are higher than in AA runners, and we suggest that MA runners may experience less BDNF downregulation after training compared to AA runners, which could contribute to the higher BDNF levels observed in the MA group.

5. Conclusions

Although muscle and myocardial damage were similar in adults and middle-aged runners after the race, lower baseline serum concentration of several myokines in middle-aged runners (IL-15, apelin, musclin, GDF-15) suggests their involvement in the no-pathological process of senescence. Furthermore, the role of higher levels of BDNF in middle-aged runners on muscle mass and exercise-induced cardiometabolic adaptation should be investigated to elucidate the molecular mechanisms underlying senescence better.

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Author Contributions

CAZS performed data collection, analysis, and data interpretation. APRS carried out the data collection, participated in the conception of the study, and assited writing the manuscript. BFCB performed data acquisition and interpretation and helped write the manuscript. BSMG was

responsible for data collection. HVB, HPS and GHOL participated in the experimental design, data acquisition, analysis, and interpretation, as well as helping to write the manuscript. MFCB conceived the study, participated in its design and coordination, assisted with statistical analysis, and wrote the manuscript. All authors have read, revised and approved the final version of the manuscript and agree with the order of presentation of the authors.

Competing Interests

The authors declare that they have no competing interests.

Data Availability Statement

Data generated or analyzed during this study may be provided if the reviewer declares need and may be requested from the corresponding author.

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