EXERCISE AS A COUNTERMEASURE TO HUMAN AGING, VOLUME II

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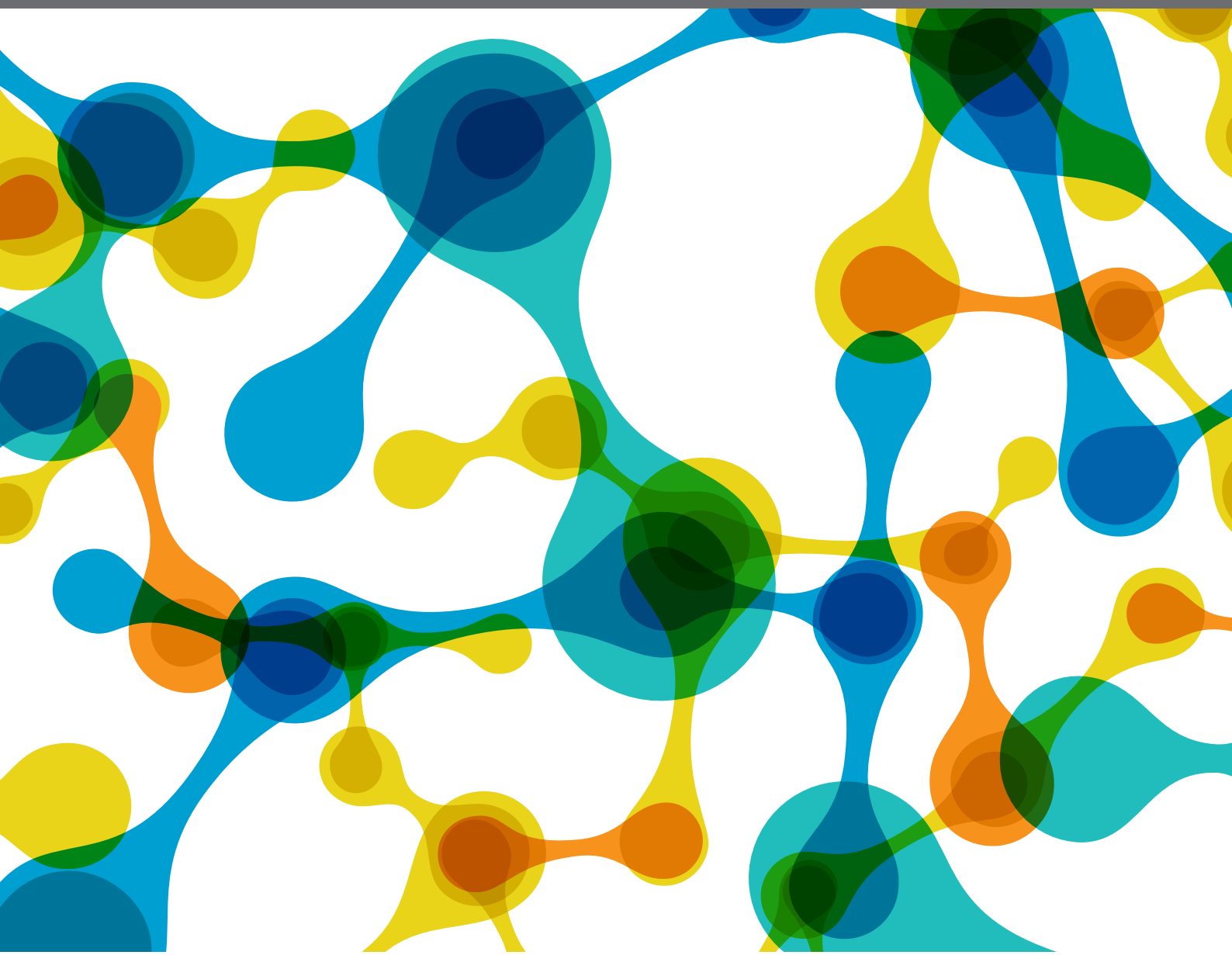

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EXERCISE AS A COUNTERMEASURE TO HUMAN AGING, VOLUME II

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EDITORIAL
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Editorial: Exercise as a Countermeasure to Human Aging, Volume II

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Keywords: ageing, exercise, sarcopenia, HIIT (high intensity interval training), editorial

Editorial on the Research Topic

Exercise as a Countermeasure to Human Aging, Volume II

Tis impossible to be sure of anything but death and taxes (Christopher Bullock in the Cobbler of Preston, 1716). We are inclined to agree with Bullock concerning the certainly of death and in this context, aging is ubiquitous amongst humans which results in a deterioration of physiological function and an inevitable march towards death. However, physical activity and exercise are known to exert positive effects on health and wider physiological function *via* multiple complex and interacting mechanisms (that have not yet been completely defined).

In volume II of this Research Topic, 10 articles covered the interplay between exercise and aging, utilizing approaches that spanned molecular, physiological, and population scale approaches, in both healthy older populations and certain disease subsets. This work builds on our previous work *Exercise as a Countermeasure to Human Aging, Volume I* and it is a pleasure to note continued progress in this field, and the range of methodological approaches authors have used.

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HIGH INTENSITY INTERVAL TRAINING

A classical exercise physiology approach of a short-term training programme over weeks-to-months in humans was performed by Hayes et al. These authors provided preliminary evidence that in a group of previously sedentary sexagenarians, moderate-intensity aerobic exercise and HIIT lowered interleukin-6 (IL-6) [and possible high sensitivity C-reactive protein (hsCRP)]. Another observation was that age-matched masters athletes had lower markers of inflammaging than the sedentary cohort through the investigation, emphasizing lifetime exercise habits are anti-inflammatory, but it is never too late to start and even after 30 years of sedentariness, inflammation was reduced after only 6 weeks of exercise. The same research group subsequently conducted a scoping review into the effects of HIIT on phenotypical characteristics of sarcopenia (Hayes et al.), with muscle function, muscle quantity, and physical performance generally improved, although there was scant literature in the oldest old (≥80 years of age), or those already sarcopenic. Therefore, more studies are needed in this population.

PERFORMANCE IN AN AGING DEMOGRAPHIC

From a population health viewpoint, increased lifelong activity, not just short-term exercise interventions, are needed. Thus, there has been much recent interest in examining highly trained

masters athletes as a physiological model of successful aging (Pollock et al., 2015; Elliott et al., 2017; Hayes et al., 2020). This Research Topic included six reports on lifelong exercising cohorts, and five examined performance of older adults in competition, with all (unfortunately!) observing poorer performance with advanced age. Firstly, Ganse et al. examined rate of performance decline in those over the age of 100 years of age between 2006 and 2019. In summary, the rate of decline was 2.53 times steeper in centenarians as is 80–96-year-olds from Sweden (previously published data from the same group). The 100 m sprint performance displayed the greatest difference between centenarians and non-centenarians, whereby centenarian performance decreased eight times steeper than in non-centenarians. The smallest differences in performance declines were in the discus and the javelin throw (1.54 and 1.27 times steeper respectively), suggesting sprint performance is most susceptible to deterioration by advanced age. Secondly, in an article from the same group Moser et al. examined pacing variation for the 200 and 400 m individual medley in age groups up to ≥75 years of age. As with the Ganse et al. article previously described, Moser and colleagues observed an increase in race time (i.e., a worsening of performance) as participant age increased. Pacing variance, using the coefficient of variance (CV) of each split exhibited greater variation in males and females in the older age groups. Pacing variation was greater in males than females, suggesting females swim a more consistent pace during competition. That said, both female and male master swimmers showed a final end spurt (i.e., the final split was the fastest) in both 200 and 400 m individual medley. Thirdly, the same research group examined historical data of the Berlin Marathon to examine whether environmental condition influenced performance in age group runners (Knechtle et al.). In a group of 869,474 valid finisher records, older age groups ran slower. Although higher daily maximum temperatures were associated with better performance in the best (and youngest) runners, increased daily maximum temperatures decreased race performance in age group marathoners from age group 35–40 years and older with no differences between the sexes. In the fourth and final paper from this research group, Reusser et al. conducted another historical analysis of the Berlin Marathon and again observed older age groups ran slower. However, an important finding was that the number of finishers increased for both women and men runners over the decades, from 236 to 8 men and women respectively in 1974, to 28,373 and 12,268 men and women respectively in 2018. This effect was manifest across all age groups, except for male athletes aged 20–49 years and athletes of both sexes above 79 years old. All these results point to an environment that is supportive of exercise participation in older age, but performance declines are inevitable. One further aspect of interest would be to examine the influence of training programme variables (i.e., volume, intensity, frequency) on the rate of performance declines. However, this would require big data approaches and continual training recording, which has only recently become possible (since cloud storage became commonplace).

FROM BIG DATA TO MICROVESICLES, MICROARCHITECTURE, AND MICRORNA

Kyriakidou et al. observed that extracellular vesicle response to muscle damaging exercise (high intensity eccentric exercise) was similar in young and old participants, suggesting a preserved means for inter-tissue crosstalk in older age following muscle damage. Using the ovariectomized mouse model of postmenopausal bone loss, Zhao et al. compared downhill running, and downhill running plus an anti-irisin receptor agent, compared to controls for bone mineral density (BMD). These authors demonstrated that downhill running improved cortical and trabecular volumetric BMD and microarchitecture compared to non-exercised ovariectomized mice. However, injection with an anti-irisin receptor agent weakened the exercise effect. These authors concluded that exercise is a potent osteogenic factor and blocking of irisin receptor signalling ameliorated this effect. Thus, irisin plays a role in regulating bone response to exercise through Akt/b-catenin pathways. Considering microRNAs (mRNAs), Garai et al. made the important observation that there was considerable overlap between exosomal mRNAs (exomiRs) of young participants on a 6 month exercise intervention, and lifelong (>25 years) exercisers. Using Kyoto Encyclopedia of Genes and Genomes pathway analysis, a large portion of these exomiRs target chronic diseases such as cancer, neurodegenerative and metabolic diseases, and viral infections. As such, exomiRs may be one of the mechanisms by which exercise prevents several chronic diseases, the emerging possibility of exomiRs as a therapeutic target is exciting.

Finally, science is, and always should be, self-correcting. We applaud Garai et al. for their corrigendum correcting an mis-acknowledgement of funding source. An error of this type would unlikely be noticed, but it was important to acknowledge correctly.

As we move towards a goal of personalised treatments and medicines, it is interesting to speculate what might fill the pages of a future *Exercise as a Countermeasure to Human Ageing, Vol III*. In the editorial accompanying our first volume, we noted the representation of sexes between published work, and in this volume it is pleasing to see that half of the human studies report both males and females and sub-group results by sex, a step towards best practice recommendations (Elliott-Sale et al., 2021). As we move towards a truly personalised future, studies that account for sex, ethnic, lifelong environmental (and indeed transgenerational (Fitz-James and Cavalli, 2022) differences and their influences on personalising exercise guidelines are called for

These results show the uses of exercise physiology to continue to probe the basic biology of aging, and further suggest emerging findings that inter-tissue cross-talk during and after physical activity is preserved with age. Exercise may truly be a countermeasure to biological human aging.

AUTHOR CONTRIBUTIONS

LH wrote the first draft. BE and MB critically reviewed, and all authors approved the final version of this editorial.

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Pacing in World-Class Age Group Swimmers in 200 and 400 m Individual Medley

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The present research investigated pacing for world-class age group swimmers competing in individual medley in 200 m and 400 m. Data on 3,242 unique finishers (1,475 women and 1,767 men) competing in four Master World Championships [XV FINA WMC held in Montreal (Canada) in 2014, the XVI FINA WMC held in Kazan (RUS) in 2015, the FINA WMC held in Budapest (HUN) in 2017, and the XVIII FINA WMC held in Gwangju (KOR] in 2019) were analyzed. Men were faster than women among all age groups in both 200 and 400 m. Additionally, differences were found between almost all adjacent age groups, with the exception ($p > 0.05$) of age groups 25–29 to 30–34, 35–39 to 40–44 years in 200 m races and 25–29 to 30–34, 30–34 to 35–39, 35–39 to 40–44, and 45–49 to 50–54 years in 400 m races. Men showed a higher pacing variation in 200 m among all male age groups and all female age groups up to 69 years. Pace-variation pairwise comparisons between men and women showed no consistencies throughout age groups, with the exception of a higher variation in men in age groups ≥55-year-old. Men were faster for all splits and strokes in both 200 and 400 m, and significant changes were identified for each split and stroke for both men and women in both 200 and 400 m. Front crawl (freestyle, 4th split) was the fastest butterfly (1st split), backstroke (2nd split), and breaststroke (3rd split). In summary, men were faster than women for all age groups in both 200 and 400 m. Men showed a higher pacing variation in 200 m in all age groups, where women had a higher variation in age groups up to 69 years. The fastest stroke for the final spurt was front crawl, followed by butterfly, backstroke, and breaststroke. Based on these findings, coaches should advise their master athletes to focus on the final spurt in both 200 and 400 m individual medley for a fast final race time.

Keywords: world class level, pacing, freestyle, backstroke, butterfly, breaststroke

INTRODUCTION

Participation in master-level swimming competitions has remarkably increased over the last 30 years (Ransdell et al., 2009; Mejias et al., 2014; Ferreira et al., 2016) and has seen an extraordinary growth in popularity since the first Fédération Internationale de Natation (FINA) World Masters Championship (WMC; Rubin, 2010) held in Tokyo in 1986. Swimming is one of the most popular sports among master athletes, attracting both casual and elite level swimmers (Ferreira et al., 2016). However, in recent years, there has been a noticeable shift from a recreational to a more elite performance while competing in WMC (Mejias et al., 2014).

The volume of pacing research has continuously grown over the last few years and is mainly dominated by endurance sports, such as running, cycling, and triathlon (McGibbon et al., 2018). However, little is known about pacing strategies in master swimmers (Nikolaidis and Knechtle, 2017; Moser et al., 2020). In contrast, most of the studies on master swimmers focused more on biomechanical and physiological determinants of performance and also how these determinants varied by age (Reaburn and Dascombe, 2008; Tanaka and Seals, 2008). Generally, pacing strategies are described as how an athlete distributes work and energy throughout an exercise task (Abbiss and Laursen, 2008). Accordingly, an athlete can select the most appropriate pacing strategy, which can include negative, all-out, positive, even, parabolic, and variable pacing (Abbiss and Laursen, 2008). Pacing in swimming is determined by plotting split times or velocity over each lap of the event (McGibbon et al., 2018).

In more recent years, research around swimming pacing has been mainly focused on freestyle swimming (Robertson et al., 2009; Mauger et al., 2012; Nikolaidis and Knechtle, 2017; Moser et al., 2020). Further, it has been noted in long-distance swimming events that the speed profile of 800 and 1,500 m international-level freestyle swimmers was generally parabolic or U-shaped (Damasceno et al., 2013). In this instance, the swimmers completed the first segment in the fastest time (assisted by the dive start), followed by a marked decrease in speed during the middle portion and then an increased speed toward the final segment of the race (Abbiss and Laursen, 2008). Further, elite 400 m freestyle swimmers have been shown to adopt a similar profile and used fast-start-even and parabolic pacing strategies (Mauger et al., 2012).

More recently, research on elite master level age group swimmers started to focus on longer swimming distance events, such as the 400 m freestyle (Lipinska and Hopkins, 2020) and the 800 m freestyle (Lipińska et al., 2016). Although previous studies have investigated performance and age progression within elite age group swimmers, little is known about age group swimmers competing at international level in the individual medley events (Saavedra et al., 2012; McGibbon et al., 2018). The individual medley is a unique event combining all four different swimming styles in the order of butterfly (1st split), backstroke (2nd split), breaststroke (3rd split), and freestyle (front crawl, 4th split) in one race (Rubin, 2010). This order might, indeed, affect pacing of the entire race (Dormehl et al., 2016). Accordingly, there is a significant variation between any given swimmer's strengths and weaknesses across all strokes (McGibbon et al., 2018). Although swimmers might produce similar performance times, their pacing strategies within the splits might be drastically different (Saavedra et al., 2012; Knechtle et al., 2016).

To date, research showed that the analysis of pacing strategies in elite freestyle swimmers (Robertson et al., 2009; Knechtle et al., 2016; Lipinska et al., 2016). Further, there is limited data available on pacing strategies in 200 and 400 m individual medley in international swimming competitions in one study (Saavedra et al., 2012). However, pacing strategies in elite master level swimmers has yet to be examined. In the only study examining international swimmers in 200 and 400 m individual medley, it was observed that butterfly (1st split) was the fastest stroke regardless of final pacing and sex (Saavedra et al., 2012). When focusing on medalists in the 200 m event, it was observed that backstroke (3rd split) was the style that most correlated with performance whereas freestyle (front crawl, 4th split) correlated most strongly with the final race time in both men and women (Thompson et al., 2003). Interestingly, in the 400 m event, it was generally observed that breaststroke (3rd split) in men and freestyle in women were the styles most strongly correlated with the final time. However, when analyzed by groups, it was found that breaststroke was not the most important style for medalists. Considering medalists in Olympic Games, World Championships, European Championships, Commonwealth Games, Pan Pacific Games, U.S. Olympic Team Trials, and Australian Olympic Trials, backstroke was the style that most determined the final race time in the 200 and 400 m race in men, whereas it was backstroke (200 m) or freestyle (400 m) in women (Saavedra et al., 2012).

The present study aimed to investigate changes in swimming times by laps (i.e., splits in 50 m pools) in age group swimmers competing in the FINA WMC in 200 and 400 m individual medley. In elite individual medley swimmers, it was shown that the men applied a positive pacing strategy in the 200 and 400 m, whereas the women applied a less positive pacing strategy (Saavedra et al., 2012). The hypothesis was that male and female master swimmers would apply a similar pacing strategy like the elite male and female individual medley swimmers in international swimming competitions.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Institutional Review Board of St. Gallen, Switzerland, with a waiver of the requirement for informed consent of the participants as the study involved the analysis of publicly available data.

Procedures

To test our hypotheses, all female and male master swimmers competing in individual medley in 200 and 400 m in four FINA World Championships were included. All women and all men were included for every 5-year age groups starting from 25 years to ≥75 years to avoid a selection bias by analyzing only a limited

sample of top athletes, such as the top 10 or top 100 of each age group. All data was sourced from the official and publicly accessible website of the FINA at www.fina.org/content/fina-masters-world-championships-results-archive. Results from 200 and 400 m individual medley were obtained from a total of four WMCs. To be eligible for FINA WMC,[1] swimmers must be older than 25 years of age and must fulfil a qualification time (see for example www.fina.org/sites/default/files/general/2018-08-30_time_standards_-_gwangju.pdf) and be registered with an official swimming club. In the XV FINA WMC held in Montreal (Canada) in 2014, in the XVI FINA WMC held in Kazan (RUS) in 2015, in the FINA WMC held in Budapest (HUN) in 2017, and in the XVIII FINA WMC held in Gwangju (KOR) in 2019, for each distance, 200 and 400 m, and each swimmer, times for each 50 m length were recorded.

A total of 3,242 age group athletes (1,475 women and 1,767 men) who competed in 200 m (n = 2,300) and 400 m (n = 942) individual medley were considered. The age groups of 25–29 to 30–34, 35–39 to 40–44 years in 200 m races and 25–29 to 30–34, 30–34 to 35–39, 35–39 to 40–44, and 45–49 to 50–54 years in 400 m races were separated by women and men and 200 and 400 m.

Statistical Analyses

All statistical procedures were carried out using the Statistical Package for the Social Sciences (SPSS version 26. IMB, Ill, USA) and GraphPad Prism (version 8.4.2. GraphPad Software LLC, CA, USA). The Shapiro-Wilk's and Levene's tests were applied for normality and homogeneity, respectively. Each participant had their mean and standard deviation (SD) calculated based on the partial race times (every 50 m) of the race. Individual mean and SD were further used to calculate an individual coefficient of variation (CV % formula: SD/mean*100) that was used a measure of pace variation. General linear models were as follows: race time (two-way ANOVA, separate for 200 and 400 m) sex × age group; pace variations (three-way ANOVA) sex × age group × distance; split/style race time (two-way ANOVA, separate for 200 and 400 m) sex × pace/style. Sex was always included as a fixed factor; all others were included as random factors. When interactions were found ($p < 0.05$), pairwise comparisons were applied to identify the differences more accurately. Partial eta squared ($_p\eta^2$) was used as estimates of effect size for the ANOVAs considering the following parameters partial eta-squared: small = 0.01; medium = 0.06; large = 0.14. The hypothesis of sphericity was verified by the Mauchly test and, when violated, the degrees of freedom are corrected by the Greenhouse-Geisser estimates. Significance level was set at 5% ($p < 0.05$).

[1]www.fina.org/discipline/masters

RESULTS

The general linear model (two-way ANOVA) for race time in 200 and 400 m showed a significant effect for sex (200 m: $F = 211.8$; $p < 0.001$; $_p\eta^2 = 0.952$; 400 m: $F = 140.7$; $p < 0.001$; $_p\eta^2 = 0.930$), age group (200 m: $F = 102.8$; $p < 0.001$; $_p\eta^2 = 0.990$; 400 m: $F = 113.1$; $p < 0.001$; $_p\eta^2 = 0.991$), and interaction sex × age group (200 m: $F = 5.1$; $p < 0.001$; $_p\eta^2 = 0.022$; 400 m: $F = 2.1$; $p < 0.001$; $_p\eta^2 = 0.022$; **Figure 1**). Pairwise comparisons showed that men had lower ($p < 0.001$) race times than women among all age groups in both 200 and 400 m. Additionally, differences were found between almost all adjacent age groups, with the exception ($p > 0.05$) of age groups 25–29 to 30–34, 35–39 to 40–44 years in 200 m races; and 25–29 to 30–34, 30–34 to 35–39, 35–39 to 40–44, and 45–49 to 50–54 years in 400 m races.

The general linear model (three-way ANOVA) for pace variation showed a significant effect for the interactions sex × age group ($F = 3.8$; $p = 0.022$; $_p\eta^2 = 0.795$) and sex × distance ($F = 11.0$; $p = 0.006$; $_p\eta^2 = 0.491$; **Figure 2**). Pairwise comparisons showed a higher pacing variation ($p < 0.05$) in 200 m races among all male age groups and all female age groups up to

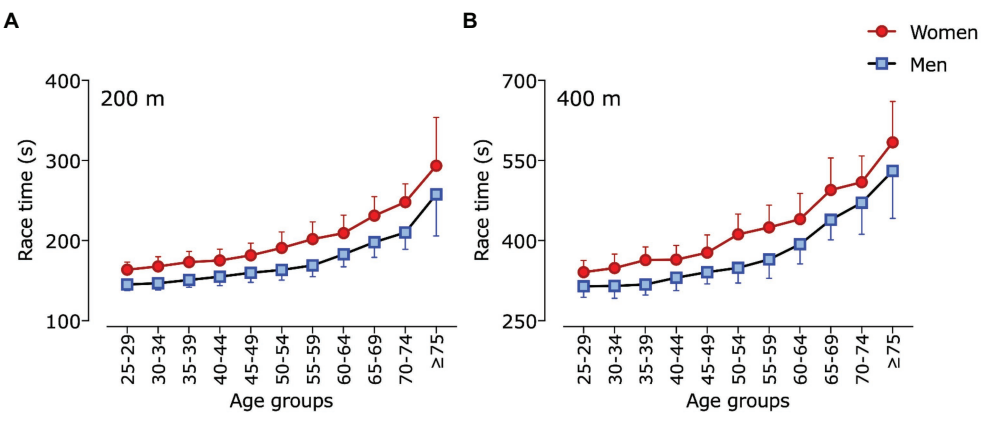

FIGURE 1 | Mean race time for each age group in **(A)** 200 and **(B)** 400 m individual medley of men and women. Data are expressed as means and standard deviations (SDs).

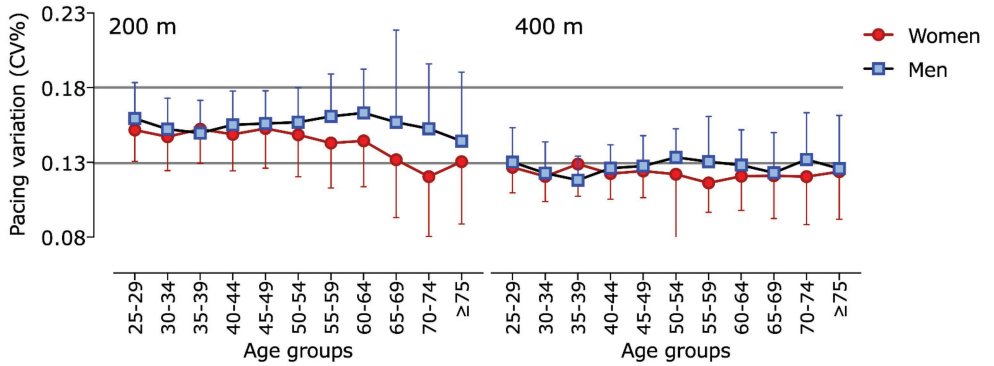

FIGURE 2 | Mean pacing variation for each age group in 200 and 400 m individual medley of men and women. Data are expressed as means and SDs.

the age of 69 years old. Pace-variation pairwise comparisons between men and women showed no consistencies throughout age groups, with the exception of higher variation in men in age groups ≥55-year-old.

The last model with pacing/style as a factor showed a significant effect for sex (200 m: $F = 256.7$; $p < 0.001$; ${}_{p}\eta^2 = 0.100$; 400 m: $F = 89.3$; $p < 0.001$; ${}_{p}\eta^2 = 0.087$), for pacing/style (200 m: $F = 10826.2$; $p < 0.001$; ${}_{p}\eta^2 = 0.825$; 400 m: $F = 11215.1$; $p < 0.001$; ${}_{p}\eta^2 = 0.923$) and for an interaction sex × pace/style (200 m: $F = 39.1$; $p < 0.001$; ${}_{p}\eta^2 = 0.017$; 400 m: $F = 22.5$; $p < 0.001$; ${}_{p}\eta^2 = 0.023$; **Figure 3**). Pairwise comparisons showed that men were faster among all splits/style in both 200 and 400 m races, and significant changes were identified among each split/style for men and women in both 200 and 400 m races, being freestyle (4th split) the fastest, followed by butterfly (1st split), backstroke (2nd split), and breaststroke (3rd split).

DISCUSSION

In this study, we intended to investigate the pacing of master swimmers in individual medley for 200 and 400 m with the hypothesis that male master swimmers would apply a positive pacing strategy in the 200 and 400 m individual medley events, whereas female master swimmers would use a negative pacing strategy. Based on our results, the main findings were: (i) freestyle was the fastest stroke, followed by butterfly, backstroke, and breaststroke, (ii) a higher pacing variation in the 200 m races compared to the 400 m races for all male age groups, and all female age groups up to 69 years old, and (iii) men were faster than women for all age groups in both the 200 and 400 m races.

A first important finding was that freestyle was the fastest stroke for both sexes followed by butterfly, backstroke, and breaststroke. This finding is in contrast to 200 and 400 m individual medley international swimming competitions, where butterfly was the fastest stroke for both sexes, and the least determinant stroke was breaststroke (Saavedra et al., 2012). Backstroke (200 and 400 m) correlated most with the final performance of men, whereas it was backstroke (200 m) and freestyle (400 m) in women (Saavedra et al., 2012). In lower classification, breaststroke was a determinant in the final performance in men, whereas it was backstroke for women (Moser et al., 2020). It might be that the competency in kinematic variables such as swimming speed, stroke rate and stroke length, and turns in freestyle stroke is higher and therefore, the age group swimmers were faster in freestyle stroke (Nikolaidis and Knechtle, 2017).

The present female and male master swimmers showed a final end spurt in both 200 and 400 m individual medley similar to female and male master freestyle swimmers competing in 200 and 400 m freestyle (Nikolaidis and Knechtle, 2017) but different to elite individual medley swimmers where elite male swimmers applied a positive pacing strategy in the 200 and 400 m individual medley events. In contrast, elite female swimmers used a negative pacing strategy (Saavedra et al., 2012). Potential explanations could be that elite swimmers adopt an all-out rather than pacing strategy (Mauger et al., 2012). Master swimmers could rely on their experience in pacing and start slow to finish fast (Nikolaidis and Knechtle, 2017), and/or master swimmers might rely on their ability for a fast end spurt compared to elite swimmers (Nikolaidis and Knechtle, 2017).

A second finding was a higher pacing variation in the 200 m races among all male age groups and all female age groups up to 69 years old. In this study, the CV increased in all males and females from the age group 65 years and above. Weir et al. (2002) noted that master level women dedicated more time to endurance and technique training than their male counterparts, who spent more time focusing on swimming speed and power training. However, younger age group swimmers could be more motivated with a stronger resilience and more stable psychological profile with a better pace control than older ones (Belinchón-deMiguel et al., 2019).

The last important finding was that men were faster than women across all age groups in 200 and 400 m. This finding confirms recent data in master butterfly swimmers where women were slower than men from 25 to 89 years (Knechtle et al., 2017) and also in master freestyle swimmers where men were faster than women for age groups 25–29 to

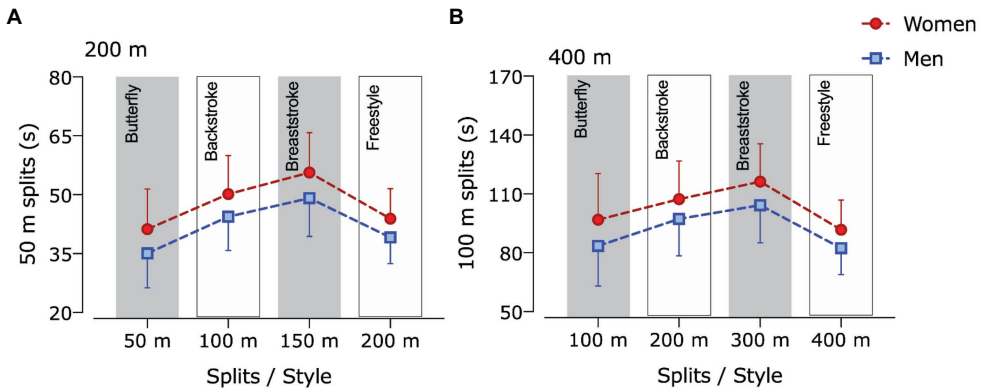

FIGURE 3 | Mean race time in each split/style in **(A)** 200 and **(B)** 400 m individual medley of men and women. Data were expressed as means and SDs.

75–79 years but not for age groups 80–84 to 85–89 years (Knechtle et al., 2016). Previously, it has been demonstrated that swimming performance decreased progressively until approximately the age of 70 years, where the decrease becomes quadratic (Donato et al., 2003). Secondly, female swimmers experienced greater performance declines in sprint events but not in endurance performance as compared to their male counterparts (Donato et al., 2003). It has been shown that men have physiological advantages such as larger body size with more skeletal muscle mass, lower body fat, and a higher maximal delivery of anaerobic and aerobic energy (Sandbakk et al., 2018). This might be the reason why men had faster race times than women among all age groups in 200 and 400 m. However, increasing age seems to have no detrimental effect on swimming times in elderly athletes.

The present study is not free of limitations. It is important to note that an essential variable could be the environmental conditions like the air and water temperature in an outdoor pool (McMurray and Horvath, 1979; Saycell et al., 2019). A further limitation is that physiological variables were not available (Donato et al., 2003; Lazarus et al., 2019).

CONCLUSION

In summary, men had lower race times than women among all age groups in 200 and 400 m. A higher pacing variation races in 200 m among all male age groups and all female age groups up to 69 years old. Men were faster among all splits/stroke in 200 and 400 m races. Freestyle (4th split) was the fastest stroke, followed by butterfly (1st split), backstroke (2nd split), and breaststroke (3rd split). The findings of the present study suggested that slower race times in both 200 and 400 m individual medley should be set as training goals for women compared to men for all age groups. In addition, when prescribing exercise for 400 m individual medley, strength and conditioning coaches should develop training programs corresponding to a relatively more even pacing. They may realize this aim by focusing on the slowest splits of 400 m individual medley (breaststroke and backstroke), i.e., a redistribution of the training load favoring these two strokes would be expected to decrease the variation of speed among strokes leading to a more even pacing.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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frontiers in Physiology

ORIGINAL RESEARCH
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Irisin Regulating Skeletal Response to Endurance Exercise in Ovariectomized Mice by Promoting Akt/β-Catenin Pathway

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Purpose: Thought irisin is recognized as a pivotal modulator for bone formation, its role in regulating skeletal response to exercise training remains unknown. Therefore, we aimed to determine the change of irisin in response to 8-week exercise training and its role in regulating the effects of exercise on bone loss in ovariectomized (Ovx) mice.

Methods: Forty 3-month old female C57BL/6 mic were randomly allocated into four groups: (1) Sham-operated (Sham); (2) ovariectomized; (3) Ovx plus 8-week downhill running exercise (Ex); (4) Ovx plus exercise and received twice weekly injection of cyclo RGDyk protein (a putative anti-irisin receptor agents) (ExRg).

Results: Ex group showed enhanced cortical and trabecular volumetric bone mineral density (vBMD) ($p < 0.05$), improved bone microarchitecture, and increased intensity of alkaline phosphatase positive (ALP^+) cells compared with Ovx group. However, cyclo RGDyk administration weakened the exercise-related improvement of vBMD, BV/TV, and ALP intensity in bone. Serum estradiol, irisin, and bone alkaline phosphatase were higher, whereas circulating tartrate-resistant acid phosphatase was lower in Ex group compared with Ovx group ($p < 0.05$). Exercise promoted mRNA expression of fibronectin type III domain-containing protein 5 (FNDC5), Akt and β-catenin, and enhanced protein levels of FNDC5, the ratio of phosphorylated Akt (p-Akt) to Akt, and β-catenin ($p < 0.05$). When irisin pathways were blocked with cyclo RGDyk, increment of Akt, p-Akt/Akt, and β-catenin in Ex mice were attenuated.

Conclusion: It is suggested that irisin plays a potential role in regulating skeletal response to exercise partly through its interaction with Akt/β-catenin pathways.

Keywords: irisin, bone loss, exercise, ovariectomy, Akt/ß-catenin

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INTRODUCTION

Osteoporosis is a common systematic skeletal disease characterized by low bone mass and impaired bone geometry and microarchitecture, presenting a major health problem in older people worldwide (Van Den Bergh et al., 2012; Compston et al., 2019). Particularly, older females after menopause are often suffered from prolonged bone loss and increased fracture risk (Black et al., 2016). Therefore, effective strategies for prevention of osteoporosis is urgently needed for improving older people' health.

Exercise is known to increase muscle mass and strength (Rogers and Evans, 1993; Roig et al., 2008), promote bone mass accumulation (Zhao et al., 2014, 2015), and correct poor balance and posture control (Howe et al., 2011a,b), etc. Compelling evidence has proved exercise to be effective in preventing bone loss and subsequent fractures (Bonaiuti et al., 2002; Zhao et al., 2017a; Sherrington et al., 2019). Zhao et al. (2017b) reported that exercise training significantly increased bone density in postmenopausal women. Another study (Bonaiuti et al., 2002) demonstrated that exercise was effective in prevention of osteoporosis in older women. A Cochrane review (Sherrington et al., 2019) also reported exercise potentially reduced falls and subsequent fractures. However, the mechanism regulating the beneficial effects of exercise on bone is still unclear.

Irisin is a recently identified myokine which is secreted by muscle, increased with exercise, and produced by the cleavage of fibronectin type III domain-containing protein 5 (FNDC5) in humans and animals (Boström et al., 2012). It mediates certain favorable effects of exercise, especially showing to have positive effects on mechanical strain-related osteoblast differentiation (Qiao et al., 2016) and then preserve bone mass in ovariectomized (Ovx) mice (Colaianni et al., 2015). Recently, the discovery of the novel αV integrin receptors in bone cells further confirmed the likelihood that irisin plays a pivotal role in modulating interactions between exercise and bone formation (Kim et al., 2018). Osteoblast differentiation is mediated by several signaling pathways, and Akt/β-catenin pathway is recognized as an important modulator in osteoblastic differentiation in response to mechanical strain (Sunters et al., 2010). And recent studies reported that irisin regulated cell differentiation mainly through Akt/β-catenin pathway (Liu et al., 2015; Shi et al., 2017). Therefore, the current evidence indicates that irisin and Akt/β-catenin pathway play an important role in regulating osteoblastic differentiation. However, whether exercise intervention affects bone formation through irisin and its interaction with Akt/β-catenin pathway remains unclear. Therefore, we conducted 8-week exercise training in Ovx mice to determine the change of irisin in response to endurance exercise and its role in regulating the effects of exercise on bone loss.

MATERIALS AND METHODS

Animals

The animals were cared for in accordance with *the principles of the Guide to the Care and Use of Experimental Animals*, and the protocol was approved by the Yangzhou University Institutional Animal Care and Use Committee (No. YZUDWLL-201905-001).

Forty 3-month old female C57BL/6 mice were purchased from Comparative Medical Center of Yangzhou University (Yangzhou, China). Four animals were housed per cage under the temperature of 23 ± 2°C and with a 12-h light-dark cycle. Food and drinking water were supplied *ad libitum*. One week after arrival, the mice were anesthetized, and sham-operated (Sham) or Ovx according to experimental protocols, and then two animals were housed for per cage. All mice were assigned randomly to 4 groups in parallel: (1) sham group; (2) Ovx group (Ovx); (3) exercise group (Ex): Ovx mice with exercise training for 8 weeks; (4) exercise and cyclo RGDyk treatment group (ExRg): Ovx mice received exercise and cyclo RGDyk [anti-irisin receptor agents (Kim et al., 2018)] interventions for 8 weeks.

Intervention Protocols

Two weeks after operation, exercise mice received 1 week of adaptive training with a protocol of daily 30-min treadmill running and the speed gradually increasing from 8 meters/min to 13 meters/min (−0° to −8° grade). After adaptive training, exercise mice were trained regularly for 5 days per week, with each training section about 45 min at a speed of 13 meters/min and with the slope of −9°. After 3 weeks of surgical operation, ExRg group mice were treated twice weekly with 2.5 mg/kg cyclo RGDyk (GLPBIO Biological Company, USA) via tail vein injection (Kim et al., 2018). At the end of interventions, all mice were anesthetized and killed within 24 h. Blood and bone tissue samples were collected. Serum was separated by centrifugation at 5000 rpm for 20 min at 4°C and then kept at −20°C. Femora and tibia bone were dissected and stored in a freezer at −80°C until PCR and Western Blot analyses.

μCT Analysis of Femur

The right femur was isolated and fixed in 4% paraformaldehyde and embedded in methyl methacrylate plastic after serial dehydration with a graded ethanol series to xylene. We used high-resolution desktop microcomputed tomography imaging (μCT40, Scanco Medical, Brüttisellen, Switzerland) for femur scanning. We assessed trabecular and cortical bone microstructure in the distal femur and femoral diaphysis. Image acquisition and analysis protocols adhered to the guidelines for the assessment of rodent bones by μCT (Bouxsein et al., 2010). In the distal femur, transverse μCT slices were evaluated in a region of interest beginning 0.2 mm superior to the distal growth plate and extending proximally 1.5 mm. The cortical and trabecular bone were identified for the following morphometric variables: bone volume fraction (BV/TV, %), volumetric bone mineral density (vBMD, g/cm^3), trabecular thickness (Tb.Th, mm), trabecular number (Tb.N, 1/mm), and trabecular separation (Tb.Sp, mm), etc.

Alkaline Phosphatase Staining and Quantitative Analysis

For ALP staining, proximal tibiae (left) were isolated and fixed in 10% paraformaldehyde fixation buffer (PFA), and decalcification performed with 10% EDTA. Eight μm-thick paraffin-embedded sections were obtained, and Improved Gomori calcium cobalt method was used to determine ALP activity after incubation with staining agents (Beijing Solarbio Science & Technology Co. Ltd, China). The slices were photographed under microscope (400 × magnification). The images were analyzed using Image-Pro Plus image analysis software version 6.0 (IPP 6.0, Media Cybernetics, USA), and the areas and integral optical density (IOD) of ALP staining were quantified.

TABLE 1 | Physical and serum parameters of mice between groups.

	Sham ($n = 10$)	Ovx ($n = 10$)	Ex ($n = 10$)	ExRg ($n = 10$)
$Weight_{pre}$ (g)	20.96 ± 1.89	21.55 ± 2.06	21.21 ± 1.43	21.5 ± 2.55
$Weight_{post}$ (g)	23.81 ± 2.48	27.99 ± 2.59*	24.13 ± 1.53†	25.29 ± 2.91
Uterus weigh (g)	0.069 ± 0.023	0.020 ± 0.012*	0.030 ± 0.017*	0.025 ± 0.018*
Serum E_2 (pg/ml)	8.09 ± 2.47	4.05 ± 1.69*	7.13 ± 1.91†	3.97 ± 1.11*§
Serum irisin (pg/ml)	5.10 ± 1.96	2.04 ± 0.64*	4.34 ± 2.01†	3.05 ± 1.09*
Serum BAP (pg/ml)	0.56 ± 0.10	0.34 ± 0.10*	0.55 ± 0.07†	0.35 ± 0.12*§
Serum TRAP (pg/ml)	4.25 ± 0.61	6.01 ± 1.20*	4.02 ± 1.2†	5.22 ± 0.89*§

*Ovx, ovariectomized; Ex, exercise; ExRg, exercise plus cRGDyk; BAP, bone alkaline phosphatase; TRAP, tartrate-resistant acid phosphatase. Data are presented as mean ± SD. *Denotes the difference between Sham group and Ovx, Ex, and ExRg groups was significant ($p < 0.05$); † indicates the difference between Ovx group and Ex and ExRg groups was significant ($p < 0.05$); § presents the difference between Ex group and ExRg group was significant ($p < 0.05$).*

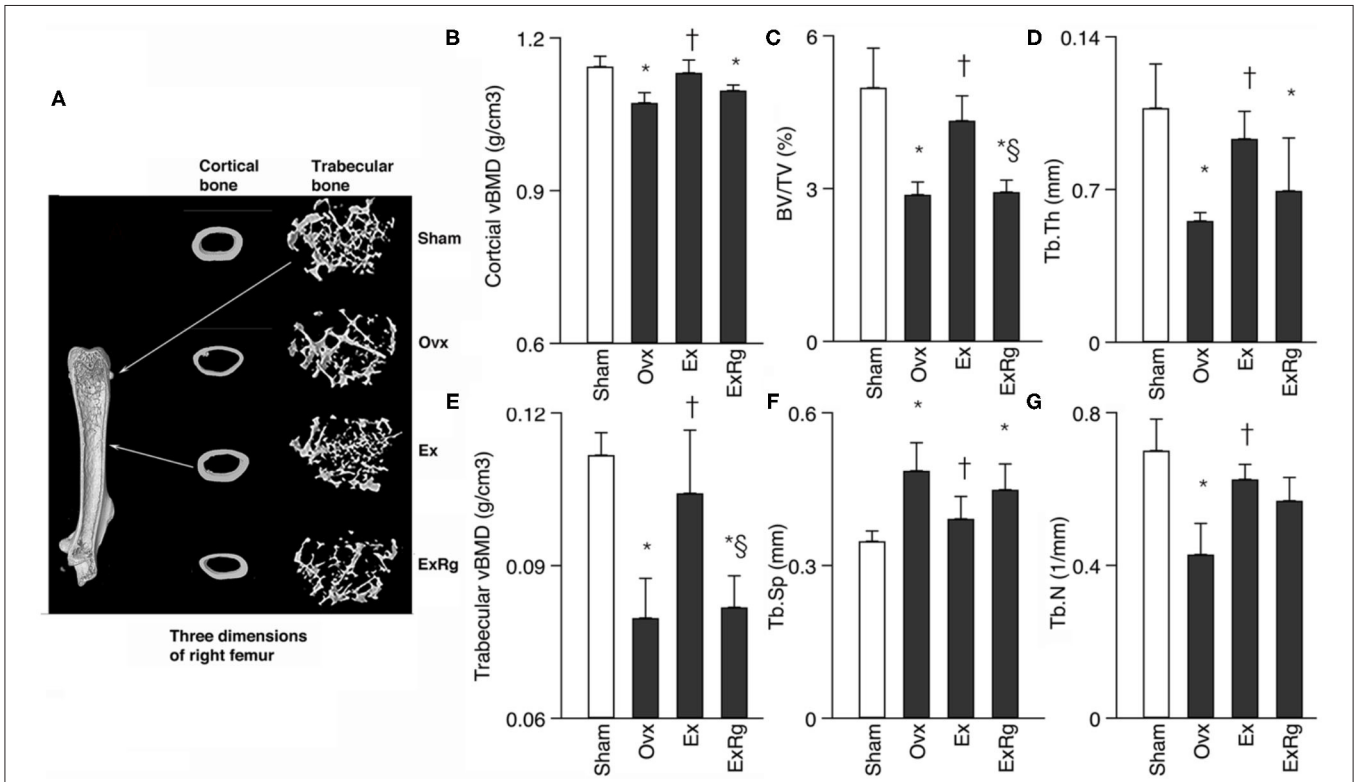

FIGURE 1 | Comparisons between groups for cortical and trabecular volumetric bone mineral density (vBMD) and trabecular bone volume fraction (BV/TV), thickness (Tb.Th), numbers (Tb.N) and separation (Tb.Sp). Micro-CT analysis was performed for sham, ovariectomized (Ovx), Ovx plus exercise (Ex), and Ovx plus exercise and cRGDyk (ExRg) groups (each group, $n = 10$). Data are presented as mean ± SD. **(A)** Three dimensions of right femur; **(B)** cortical vBMD (g/cm^3); **(C)** BV/TV (%); **(D)** Tb.Th (mm); **(E)** trabecular vBMD (g/cm^3); **(F)** Tb.Sp (mm); **(G)** Tb.N (1/mm). *Denotes the difference between Sham group and Ovx, Ex, and ExRg groups was significant ($p < 0.05$); †indicates the difference between Ovx group and Ex and ExRg groups was significant ($p < 0.05$); § presents the difference between Ex group and ExRg group was significant ($p < 0.05$).

Analysis of Serum Hormones and Biomarkers

The serum concentrations of estradiol (E_2), irisin, bone alkaline phosphatase (BAP), and tartrate-resistant acid phosphatase (TRAP) were determined with ELISA kits (Jianglai Biological Company, Shanghai, China), according to the instructions in the manufacturer's protocol.

Western Blot Analysis

Total protein (right tibia) was obtained using radioimmunoprecipitation assay (RIPA) lysis buffer (ApllyGen, Beijing, China). Protein concentration was determined using the BCA Protein Assay Kit (CWBIO, Beijing, China). Extracts were fractionated by SDS-PAGE and subsequently transferred to a polyvinylidene fluoride membrane (PVDF, IPVH00010,

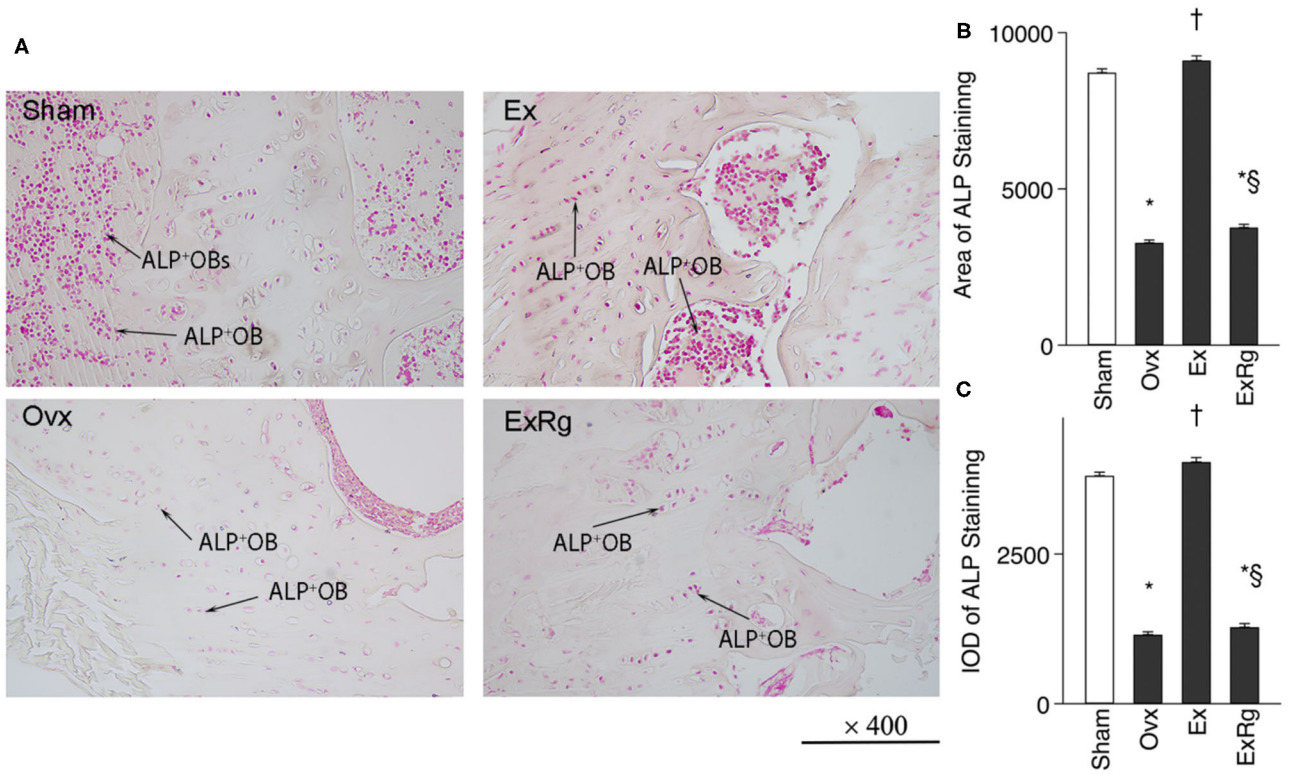

FIGURE 2 | ALP staining and quantitative analysis. **(A)** ALP (alkaline phosphatase) staining was performed for sham, ovariectomized (Ovx), Ovx plus exercise (Ex), and Ovx plus exercise and cRGDyk (ExRg) groups (each group, $n = 10$). **(B)** ALP staining area. **(C)** ALP staining IOD (integral optical density). ALP$^+$OB, ALP positive osteoblast. *The difference between Sham group and Ovx, Ex, and ExRg groups was significant ($p < 0.05$). † The difference between Ovx group and Ex and ExRg groups was significant ($p < 0.05$). §The difference between Ex group and ExRg group was significant ($p < 0.05$).

Millipore). After blocking with 3% non-fat dry milk in Tris-buffered saline (TBS), we incubated the membranes overnight at 4°C with antibody to FNDC5 (Abcam, Shanghai, China), β-catenin (Abcam, Shanghai, China), Akt (Cell Signaling Technologies, Hitchin, UK), and phospho-Akt (Ser473) (Cell Signaling Technologies, Hitchin, UK). For loading control, we used antibodies to β-actin. The secondary antibody was diluted to 1:2,000 and incubated with the membrane for 2 h at room temperature. After the last washing step, configure the luminescent liquid, soak the PVDF film with the luminescent liquid, and place it in the sample placement area of the ultra-high-sensitivity chemiluminescence imaging system (Chemi Doc^{TM} XRS+, Shanghai, China) to run the program to develop the imaging.

RT-PCR Analysis

Total RNA from right tibia was extracted using Trizol reagent (CWBIO, Beijing, China) according to the manufacturer's instructions. 200 ng of total RNA was reverse-transcribed with Ultrapure RNA extraction kit (CWBIO, Beijing, China) in a 20-μl cDNA reaction, as specified by the manufacturer. For quantitative PCR, the template cDNA was added to a 25 μl reaction with SYBR Green PCR Master Mix (Lifeint, Beijing, China) and 0.2 μM of primer. The amplification was carried out for 40 cycles under the following conditions: an initial denaturation of 95°C for 10 min, plus 40 cycles of 95°C for 10 s, then 60°C for 1 min. The fold changes were calculated relative to β-actin using the ΔΔ Ct method for FNDC5, β-catenin, and Akt mRNA analysis. The following primer sets were used: FNDC5: forward, AAGTGGTCATTGGCTTTGC; reverse, GTT GTTATTGGGCTCGTGT; β-catenin: forward, CACATCAGGA CACCCAACGG; reverse, CGTATGTTGCCACGCCTT; Akt: forward, AAGCACCGTGTGACCATGAA; reverse, TTCTCAG TAAGCGTGTGGGC; β-actin: forward, AGGGAAATCGTGC GTGAC; reverse, CATACCCAAGAAGGAAGGCT.

Statistical Analysis

Statistical analysis was performed using STATA software (*Version 15, StataCorp LP, Texas, USA*). Data were reported as mean and standard deviation (SD). A one-way analysis of variance (ANOVA) with the Bonferroni *post hoc* test was used for between-group comparisons. A level of $p < 0.05$ was accepted as significant.

RESULTS

Changes in Weights and Serum Biomarkers

The baseline weights of mice were not different between groups (**Table 1**). At the end of 8-week interventions, the weights of

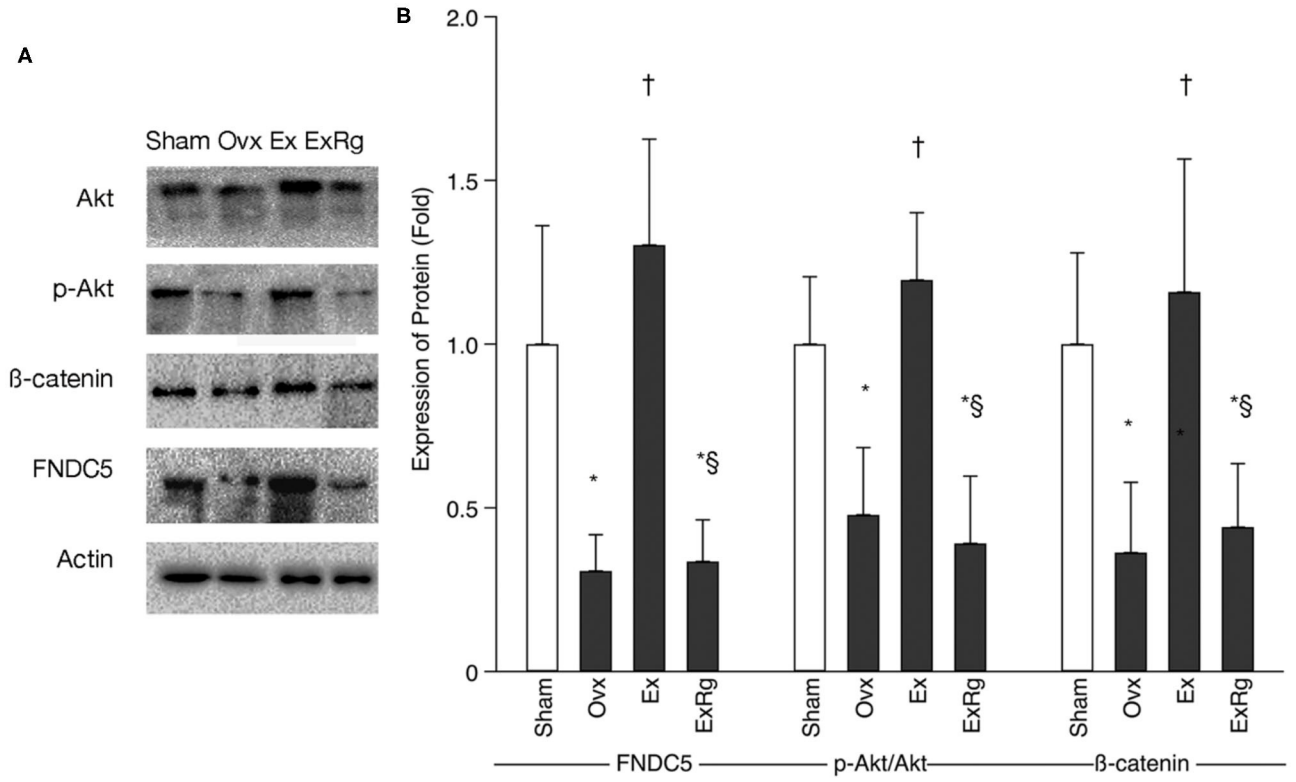

FIGURE 3 | The protein expression of FNDC5, p-Akt/Akt, and β-catenin. **(A)** Western blot analysis for fibronectin type III domain-containing protein 5 (FNDC5), phosphorylated Akt (p-Akt), Akt, and β-catenin. **(B)** Expression of proteins (fold) for sham, ovariectomized (Ovx), Ovx plus exercise (Ex), and Ovx plus exercise and cRGDyk (ExRg) groups (each group, $n = 10$). Data are presented as mean ± SD. *Denotes the difference between Sham group and Ovx, Ex, and ExRg groups was significant ($p < 0.05$); †indicates the difference between Ovx group and Ex and ExRg groups was significant ($p < 0.05$); § presents the difference between Ex group and ExRg group was significant ($p < 0.05$).

Ovx mice significantly increased compared with Sham mice, but 8-week exercise training decreased the ovariectomy-induced increment of weights. The weights of ExRg group tended to decrease compared with that in Ovx group, but the difference was not significant. Ovariectomy markedly decreased uterus weight compared with Sham operation; exercise interventions seemed to improve the reduced uterus weight, but it was not significant (**Table 1**).

Ovariectomy induced a marked reduction of serum levels of E_2, irisin, and BAP, and up-regulated serum TRAP concentrations; the ovariectomy-induced changes of serum hormones and biomarkers were reversed by exercise interventions (**Table 1**). However, the increment of serum E_2, irisin and BAP, and reduction of TRAP in Ex group were attenuated by cyclo RGDyk treatment (**Table 1**).

Changes in Volumetric Bone Density and Bone Morphometry

Data of microCT measures showed that ovariectomy caused significant reduction of cortical and trabecular vBMD, trabecular BV/TV, Tb.Th and Tb.N, and enlargement of Tb.Sp (**Figure 1**). Eight-week exercise training could significantly rescue the decreased cortical and trabecular vBMD, trabecular BV/TV, Tb.Th and Tb.N, and reduce Tb.Sp. However, cyclo RGDyk treatment weakened the exercise-related improvement of trabecular vBMD and BV/TV (**Figure 1**).

ALP Staining and Quantitative Analysis

Ovariectomy induced reduction of ALP^+ cells in tibia compared with Sham operation, which were elevated by exercise intervention (**Figure 2A**). However, cyclo RGDyk treatment seemed to decrease exercise-induced increment of ALP^+ cells. ALP staining quantitative analysis suggested that ALP intensity was lower in Ovx group compared with Sham group. Combined Ovx-exercise group had a higher ALP intensity in proximal tibia than the Ovx group, but the improvement could be diminished by cyclo RGDyk administration (**Figures 2B,C**).

Western Blot and PCR Analyses

Western blot analysis indicated that FNDC5, the ratio of phosphorylated Akt (p-Akt) to Akt (p-Akt/Akt), and β-catenin levels were lower in Ovx group than Sham group (**Figure 3**). Exercise intervention up-regulated the protein expressions of FNDC5, p-Akt/Akt, and β-catenin. However, the exercise-elevated levels of FNDC5, p-Akt/Akt, and β-catenin decreased with cyclo RGDyk intervention (**Figure 3**).

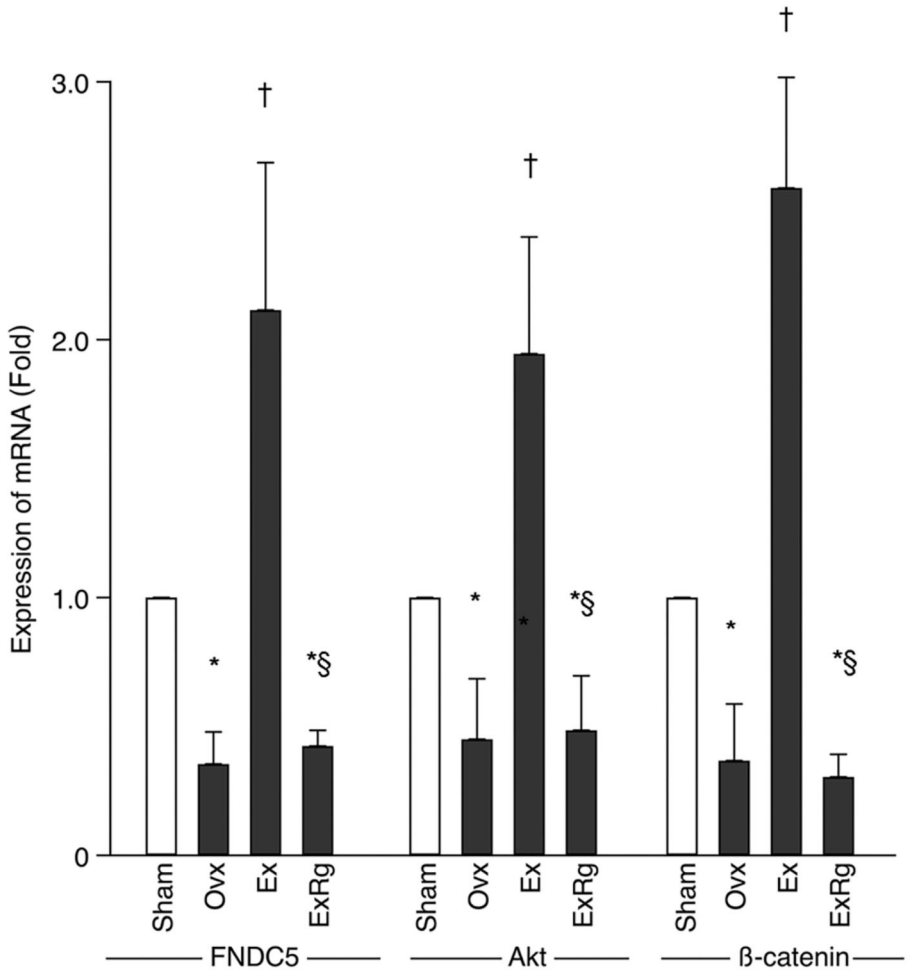

FIGURE 4 | The mRNA levels of FNDC5, Akt, and β-catenin. PCR analysis was performed for sham, ovariectomized (Ovx), Ovx plus exercise (Ex), and Ovx plus exercise and cRGDyk (ExRg) groups (each group, $n = 10$). Data are presented as mean ± SD. FNDC5, fibronectin type III domain-containing protein 5. *Denotes the difference between Sham group and Ovx, Ex, and ExRg groups was significant ($p < 0.05$); †indicates the difference between Ovx group and Ex and ExRg groups was significant ($p < 0.05$); § presents the difference between Ex group and ExRg group was significant ($p < 0.05$).

PCR analysis showed that mRNA levels of FNDC5, Akt, and β-catenin decreased in Ovx group compared with Sham group (**Figure 4**). Eight-week exercise training significantly improved the ovariectomy-associated reduction of FNDC5, Akt, and β-catenin mRNA. However, cyclo RGDyk administration blocked the increment of FNDC5, Akt, and β-catenin mRNA levels seen in Ex group (**Figure 4**).

DISCUSSION

Our findings proved exercise effective in preventing bone wasting induced by ovariectomy. Furthermore, the exercise-related improvement of bone mass could be attenuated by blocking of irisin receptor signalings, and Akt/β-catenin pathways might participate in this regulation process.

Isaksson and colleagues (Isaksson et al., 2009) reported that long-term exercise could generate beneficial outcomes on cortical and trabecular bone. Our findings were in agreement with previous reports. Recently, Luo and colleagues (Luo et al., 2020) found that recombinant irisin intervention could ameliorate bone loss in Ovx mice. Another study (Colaianni et al., 2017) also confirmed that irisin administration effectively prevented and restored bone loss in hind-limb suspended mice. Given the close correlation between exercise and irisin, it is suggested that irisin likely play an important role in regulating the effects of exercise on bone and blocking irisin pathways might affect skeletal response to exercise.

Kim and colleagues (Kim et al., 2018) first reported that by blocking irisin receptor (αV/β5), cyclo RGDyk could reduce irisin-induced signalings. Our findings indicated that cyclo RGDyk treatment decreased the exercise-related beneficial effects on cortical bone mass and trabecular BV/TV. Furthermore, exercise-elevated ALP staining positive osteoblasts were also affected by cyclo RGDyk. Colaianni and colleagues (Colaianni et al., 2015) reported that irisin treatment could promote

osteoblast differentiation; and when irisin pathways were blocked, some osteoblastogenic genes were decreased (Kim et al., 2018), which might contribute to the cyclo RGDyk-induced reduction of osteogenic differentiation. Therefore, exercise-induced production of irisin helped to promote bone formation.

Several studies suggested that both endurance training (Miyamoto-Mikami et al., 2015; Korkmaz et al., 2019) and acute exercise (Nygaard et al., 2015; Kabak et al., 2018) could significantly rise circulating irisin concentrations. Our finding was in agreement with previous reports. Additionally, circulating BAP concentrations were up-regulated, whereas TRAP levels were down-regulated with exercise intervention, indicating that exercise had positive effects on bone metabolism. Given the important role of irisin in regulating osteoblastic differentiation (Qiao et al., 2016; Kim et al., 2018), the exercise-elevated irisin levels might increase its concentrations in bone tissue and then promote bone formation.

It is known that exercise is a strong stimulator for PGC-1α expression which in turn promotes FNDC5 expression in osteoblasts (Wrann et al., 2013) and subsequently regulates osteoblastic proliferation and differentiation (Qiao et al., 2016; Kim et al., 2018). Our studies agreed with previous findings, showing that exercise group had higher FNDC5, Akt and β-catenin mRNA levels in bone tissue, whereas the expression of Akt and β-catenin were down-regulated by blocking irisin signalings. Previous studies (Liu et al., 2015; Shi et al., 2017) reported that irisin regulated cell differentiations through promoting Akt/β-catenin pathway. Therefore, it was suggested that FNDC5/irisin signaling pathways affected the process of skeletal response to mechanical loading partly through its interaction with Akt/β-catenin pathway. Akt/β-catenin signaling pathway has been recognized as an important modulator in regulating the effects of mechanical strain on osteoblast differentiation (Sunters et al., 2010). The increased levels of Akt and β-catenin in osteoblasts can stimulate lymphoid-enhancing factor/T cell factor-mediated transcription (TCF/LEF) transcriptional activity of the osteopontin promoter, and then promote osteoblast differentiation (Armstrong et al., 2007; Sunters et al., 2010). However, because the interaction between irisin and Akt pathway is complicated and different results have been reported in previous studies (Liu et al., 2015; Shi et al., 2017; Zhang et al., 2019; Vadala et al., 2020), the findings should be interpreted with caution.

One limitation of this study is that since cyclo RGDyk is not a specific agent for blocking irisin signaling pathway, it might generate "non-specific" effects, for example, cyclo RGDyk also affects αvβ3 integrin signaling pathway (Yu et al., 2014) which possibly has effects on skeletal response to mechanical loading (Rubin et al., 2006). Several studies (Moghadasi and Siavashpour, 2013; Ketabipoor and Koushkie Jahromi, 2015) also reported that increased levels of estrogen were found after exercise intervention. The elevated circulating estrogen levels in exercise group implied that other pathways regulating skeletal response to exercise might exist. Armstrong and colleagues (Armstrong et al., 2007) reported that mechanical strain up-regulated estrogen receptor signaling pathways and then promoted osteoblast differentiation. This study did not conduct sham-operated exercise and sham-exercise plus cyclo RGDyk intervention groups which could help to detect the role of estrogen in affecting response of bone to exercise. It was another limitation. Given the fact that multiple molecular pathways involve in skeletal response to mechanical loading (Thompson et al., 2012), interactions between signaling pathways may exist. Therefore, to discern the specific effects of irisin interacting with other signaling pathways or the effects of cyclo RGDyk on irisin pathway, further studies are needed, e.g., adding sham-operated exercise and sham-exercise plus cyclo RGDyk intervention groups, and performing *in vitro* molecular experiment upon mechanical loading.

CONCLUSION

Our findings have provided experimental evidence on the crosstalk between exercise and irisin in regulating skeletal response to endurance training. Irisin plays a potential role in regulating the beneficial effects of exercise on bone formation, partially through up-regulating Akt/β-catenin pathways. Future study should determine the molecular mechanism that irisin regulates osteoblast differentiation in response to mechanical strain.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by The Animal Ethics Committee at University of Yangzhou.

AUTHOR CONTRIBUTIONS

RZ and WB designed the study and wrote the first draft of the manuscript. RZ, YZ, JLi, JLin, WC, YP, and WB performed the material preparation, data collection, and analysis. All authors read and approved the final manuscript.

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frontiers in Physiology

ORIGINAL RESEARCH
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Trends in Weather Conditions and Performance by Age Groups Over the History of the Berlin Marathon

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The effect of different environmental conditions such as temperature, wind, barometric pressure, and precipitation has been well investigated in elite marathoners, but not by age categories (i.e., age group marathoners). The aim of the study was to investigate the potential influence of environmental conditions such as temperature, precipitation, and atmospheric pressure on marathon performance in age group marathoners competing in the 'Berlin Marathon' from 1974 to 2019. A total of 869,474 valid finisher records were available for analysis, of which 711,136 correspond to males and 158,338 to females. The influence of temperature, atmospheric pressure, and precipitation on marathon race times was investigated in age group marathoners grouped in 5-year-intervals. Within the 46 years of Berlin marathons under investigation, there was some level of precipitation for 18 years, and 28 years without any rain. Sunshine was predominant in 25 of the events, whilst in the other 21 years, cloud cover was predominant. Marathon race times were significantly and positively correlated with age (i.e., older runners were slower than younger runners) where the correlation was higher for males than for females. Marathon race times were significantly and positively correlated with both the hours of sunshine and the daily maximum temperature. The fastest marathon runners (meaning the minimum times) achieved the fastest race times on race days with higher maximum temperatures (i.e., 15–30°C). Daily maximum temperatures showed an influence on age group marathoners from age group 35–40 years and older. Higher precipitation levels impaired performance across most age groups. In summary, higher daily maximum temperatures (i.e., >15°C) and higher precipitation levels impaired performance of master marathoners (i.e., 35–40 years and older) competing in the 'Berlin Marathon' in the last 45 years. Master marathoners should start in marathon races with temperatures < 15°C and no precipitation in order to achieve a fast marathon race time.

Keywords: running, heat, cold, rain, performance

INTRODUCTION

Marathon running is of high popularity with a documented increase in participants in recent decades mainly in large city marathons such as the 'New York City Marathon' (Jokl et al., 2004; Vitti et al., 2020). The increase in marathoners in large city marathons is mainly due to an increase in both female (Vitti et al., 2020) and age group marathoners (i.e., master marathoners older than 35 years) (Jokl et al., 2004) and in particular of female master marathoners who increased their participation to a greater extent compared to male master marathoners (Lepers and Cattagni, 2012). In addition to the increase in participation, master marathoners of higher ages (i.e., 75 years and older) competed in these races and they also improved their performances in recent decades (Ahmadyar et al., 2015, 2016).

Regarding marathon performance, it is well-known that environmental conditions such as ambient temperature (Cheuvront and Haymes, 2001; Nikolaidis et al., 2019), wind (El Helou et al., 2012), cloud cover (Trapasso and Cooper, 1989; Ely et al., 2007a), barometric pressure (Knechtle et al., 2019; Nikolaidis et al., 2019), and precipitation (Trapasso and Cooper, 1989; Knechtle et al., 2019; Nikolaidis et al., 2019) have a considerable effect on marathon running performance. An analysis investigating marathon races times of the World Marathon Major races for Boston Marathon, London Marathon, Berlin Marathon, Chicago Marathon, and New York City Marathon showed that weather rather than course had an effect on marathon race times (Maffetone et al., 2017).

Among all the weather variables, ambient temperature seemed to have the highest influence on marathon race times (Zhang et al., 1992; Ely et al., 2007b). There is evidence that performance in a marathon race is impaired with increasing temperature (Trapasso and Cooper, 1989; Ely et al., 2007b; González-Alonso, 2007; El Helou et al., 2012). The optimum temperature for a fast marathon race time is generally at ∼10–12°C (Ely et al., 2007a; Maughan, 2010) or even lower at ∼8°C (Trapasso and Cooper, 1989).

The influence of temperature on marathon performance seemed, however, to depend on the performance level of a runner (Ely et al., 2008; El Helou et al., 2012) where the optimum temperature for a fast marathon race time may be lower for faster runners than for slower runners (Maughan, 2010). In some investigations, higher temperatures seemed to slow down faster runners compared to slower runners (Ely et al., 2008) whereas in other circumstances, slower runners suffered a greater performance decline in higher temperatures than faster runners (Montain et al., 2007; Vihma, 2010).

Based on these findings, we have knowledge that environmental conditions such as temperature, barometric pressure, cloud cover, and rain have a remarkable influence on marathon running performance regarding performance level (i.e., slower and faster runners), but we have no knowledge about the influence of environmental conditions on performance in age group marathoners (i.e., master marathoners). What we know from scientific literature is the fact that middle-aged and older adults have an impairment in performance in the heat (Larose et al., 2013; Stapleton et al., 2015; McGinn et al., 2017; Balmain et al., 2018) which is most probably due to a deterioration of the thermoregulatory response with advancing age (Bongers et al., 2014).

The aim of the present study was, therefore, to investigate the influence of different environmental conditions such as temperature (i.e., mean temperature and daily highest temperature on race day), sunshine duration, precipitation, barometric pressure on marathon race times in age group marathoners (i.e., master marathoners) competing in all editions of the 'Berlin Marathon' since its first edition in 1974 until 2019. Based upon the existing knowledge of the influence of heat on athletic performance in middle-aged and older adults, we assumed that higher ambient temperatures would impair marathon performance in master marathoners. However, also other variables such as sunshine duration, cloud cover, barometric pressure, and precipitation might have a minor influence on master marathoners.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Institutional Review Board of Kanton St. Gallen, Switzerland, with a waiver of the requirement for informed consent of the participants as the study involved the analysis of publicly available data.

Data Set and Data Preparation

The 'Berlin Marathon' was chosen due to the fact that 'Berlin Marathon' is the fastest marathon race course in the world[1] and weather data from all editions since the first edition in 1974 was available. The athlete data with name, surname, year of birth, sex, and nationality was obtained directly from the website of the 'Berlin Marathon'[2]. We were able to download the entire dataset for each year in JSON format and then convert it to an Excel file using a custom Python script (Python 2016, Python Software Foundation, United States). The weather data on the race day was downloaded from the website of 'Deutscher Wetterdienst'[3] with temperature (maximum, average in °C), sunshine (duration in hours), precipitation (mm), cloud cover (duration in hours), and atmospheric pressure (mbar) and filtered by the respective race dates. We chose the data from the weather station Berlin Dahlem because of its proximity to the 'Berlin Marathon' route.

Data Processing

Two data files have been used in this study: A register of Berlin marathon runner's finishing times between 1974 and 2019 (with the exception of 1978 and 1980 for which no data was available), including the runner's finish time in the format HH:MM:SS, along with their sex and age, and the year of the marathon. The following age groups were defined: 18 (less than 20 years

[1]www.runnersworld.com/races-places/a20823734/these-are-the-worlds-fastest-marathoners-and-marathon-courses/

[2]www.bmw-berlin-marathon.com/impressionen/statistik-und-geschichte/ergebnisarchiv/

[3]www.dwd.de/DE/leistungen/klimadatendeutschland/klarchivtagmonat.html?nn=16102

of age), 20 (20–29 years), 30 (30–34 years), 35 (35–39 years), 40 (40–44 years), 45 (45–49 years), 50 (50–54 years), 55 (55–59 years), 60 (60–64 years), 65 (65–69 years), 70 (70–74 years), 75 (75–79 years), and 80 (80 years of age or older). For each gender, race times in each age group were further filtered by each of the four originally continuous weather variables under consideration, converted into categories (ranges), as follows: Temperatures (°Celsius) grouped in three ranges: 0–8, 8–15, 15–30°C, atmospheric pressure values (mbar) in two ranges: 900–1013, 1013–1030 mbar, and precipitation values (mm) in three ranges: 0–10, 10–20, 20–50 mm. These groups are selected based on existing results from the Boston Marathon (Knechtle et al., 2019; Nikolaidis et al., 2019). A second register of the weather conditions on each marathon day between 1974 and 2019, including temperature values (average and maximum), and average atmospheric pressure and precipitation, along with sunshine and cloud cover hours. Sunshine is highly correlated with temperature, and cloud cover with sunshine, so only temperature is used in the analysis. These files were visually inspected on an Excel spreadsheet first, where minor changes were made (renaming of header columns and removing of unused columns) and then uploaded into a Google Colab[4] notebook, where Python (Python Software Foundation[5]) was used to conduct the statistical processing and to create the results tables and charts. Given the main goal of performing descriptive statistics on the available data, the decision was made not to establish cut-off finish times on either end of the range.

Statistical Analysis

Descriptive statistical analysis has been performed by age group, gender, and environmental conditions (multi-variable analysis). The resulting values of the marathon race times are presented in terms of their average value (mean) and standard deviation (std), along with maximum (max) and minimum (min) values for each category. The column named as "n" represents the number of samples in each specific category. The Kolmogorov–Smirnov two-sample test was applied to the male/female sub-populations to validate the assumption of the statistical significance of the resulting finishing times by gender. ANOVA two-way tests were run to explore the statistical significance of the differences observed in the finish times by age group and weather conditions. Statistical significance was set at 5% ($p < 0.05$) in all cases. All analyses were carried out using the Python programming language (Python Software Foundation, see footnote 5), on a Google Colab notebook (see footnote 4), and the Statistical Software for the Social Sciences (IBM SPSS v26. Chicago, Ill, United States).

[4]https://colab.research.google.com/

[5]www.python.org

RESULTS

After cleaning up and processing the data, a total of n = 869,474 valid finisher records were available for the analysis, of which 711,136 correspond to males and 158,338 to females.

The mean finish time for the full sample was 04:25:52 ± 00:40:29 h:min:s for females and 03:56:38 ± 00:41:07 h:min:s for males. 95% confidence intervals were (04:25:40 h:min:s, 04:26:04 h:min:s) for females and (03:56:32 h:min:s, 03:56:44 h:min:s) for males (see **Table 1** for a full description including mean, std, max, min and 25, 50, and 75% percentiles). **Figure 1** shows the distribution of marathon race times in h:min:s for each sex. Mean race time was faster in men compared to women. **Figure 2** presents the distribution of marathon race times by age group and sex. Beyond the unavoidable growing trend, the boxes tend to be bigger (taller) at

TABLE 1 | Basic values of the full sample for race times in h:min:s.

Full sample finish times	*n*	Mean	std	Min	25%	50%	75%	Max
Females	158,338	04:25:52	00:40:29	02:18:11	03:57:26	04:22:52	04:50:40	09:49:41
Males	711,136	03:56:38	00:41:07	02:01:39	03:28:01	03:53:00	04:21:23	08:47:19

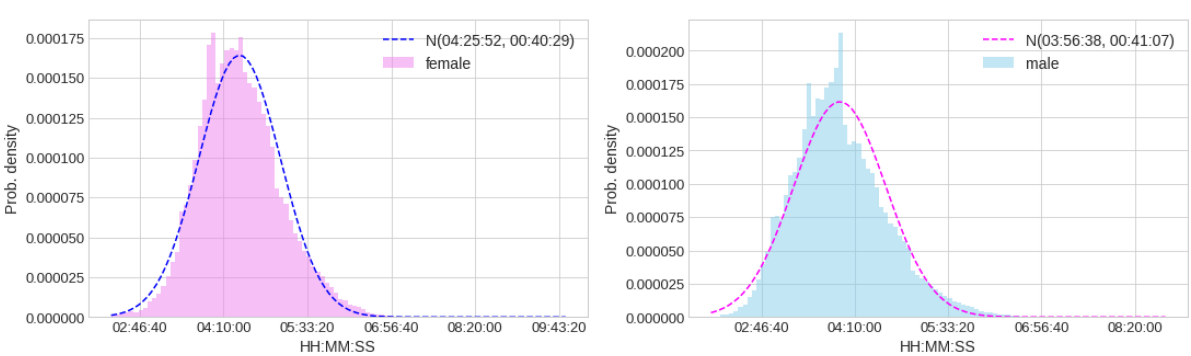

FIGURE 1 | Marathon race time histograms and probability distributions for women and men.

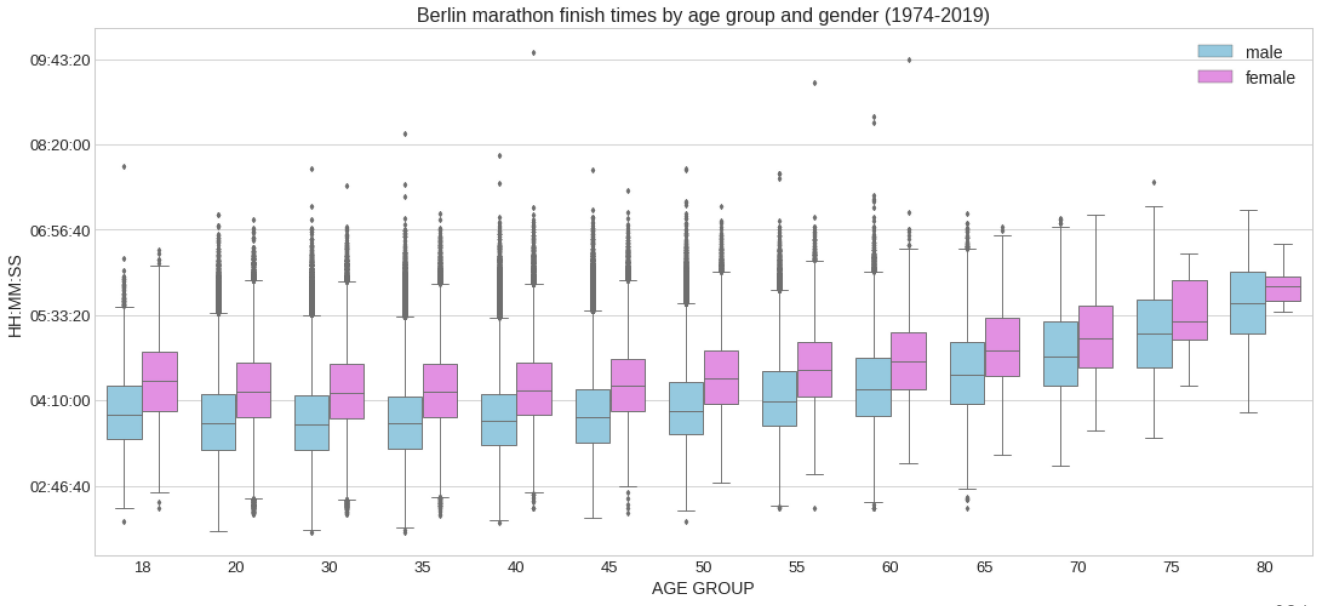

FIGURE 2 | Distribution of marathon race times by age group and sex. The chart shows five key data values: minimum, first quartile (Q1), median (~mean), third quartile (Q3), and maximum.

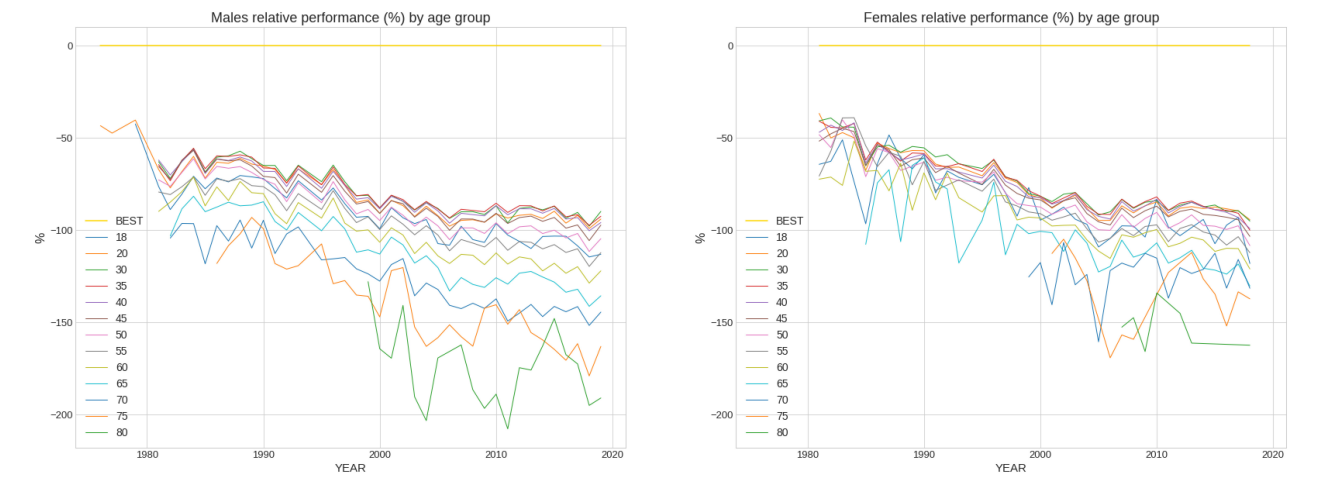

FIGURE 3 | Relative male and female performance by sex and age expressed in % of the annual best time.

both ends of the chart, appearing slightly smaller in the central age groups (40–50 years), indicating less data dispersion in the latter. **Figure 3** shows the relative male and female performance by sex and age expressed in % of the annual best time. It is interesting to see how the curves for age groups 20–40 years stay consistently close together through the years, and how the age group 18 years is behind them. **Figure 4** presents the time profiles of the measured weather variables between 1974 and 2019 and **Table 2** shows the details of the weather variables with mean, standard deviation, minimum and maximum values.

Within the 46 years of the 'Berlin Marathon,' there was some minor precipitation in 18 years with 28 years without any rain; sunshine was predominant in 25 of the events, whilst in the other 21 years, cloud cover was predominant. There was no trend with time in any of the weather variables (e.g., no increase in temperature over the years). **Figure 5** presents the correlation matrix of the finish times with the descriptors (age and weather variables). Marathon races times show a statistically weak ($r = 0.17$, $p < 0.05$) but positive correlation with age group, clearly shown in the earlier boxplot chart. The correlation of marathon races times with age is, however, higher for males ($r = 0.2$, $p < 0.05$) than for females ($r = 0.148$, $p < 0.05$) suggesting a larger impact of age on male performance. Other noticeable (but weaker) correlation coefficients of marathon races times are sunshine duration ($r = 0.11$, $p < 0.05$) and maximum temperature ($r = 0.12$, $p < 0.05$) where these variables were strongly correlated

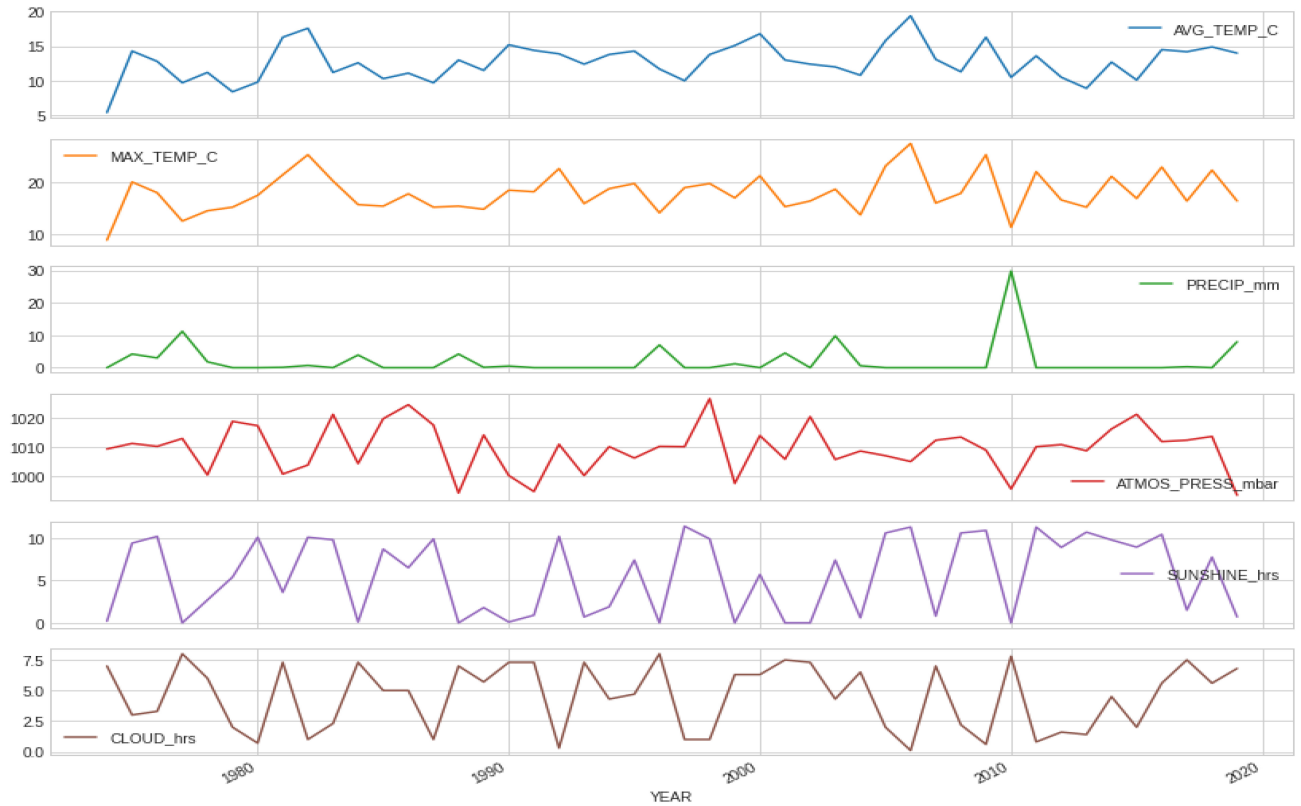

FIGURE 4 | Time profiles of measured weather variables from 1974 to 2019. PRECIP, precipitation in mm; SUNSHINE duration in hours; CLOUD cover in hours; ATMOS_PRESS, atmospheric pressure in mbar; AVG_TEMP, average temperature in °Celsius; MAX_TEMP, maximum temperature in °Celsius; MN_TEMP, minimum temperature in °Celsius.

TABLE 2 | Details of the weather variables with mean, standard deviation, minimum, and maximum values.

	Precipitation (mm)	Sunshine (hrs.)	Cloud cover (hrs.)	Atmospheric pressure (mbar)	Average temperature (°C)	Maximum temperature (°C)
Mean	2.0	5.6	4.5	1009.8	12.7	18.0
std	5.0	4.5	2.7	8.0	2.7	3.8
Min	0.0	0.0	0.1	993.6	5.4	8.8
25%	0.0	0.7	2.0	1005.4	10.9	15.4
50%	0.0	7.0	5.0	1010.3	12.8	17.6
75%	1.1	10.0	7.0	1014.0	14.3	20.2
Max	29.8	11.4	8.0	1026.8	19.4	27.6

Mean, average value; std, standard deviation; Min, minimum value; Max, maximum value.

among them ($r = 0.66$, $p < 0.05$). **Table 3** presents the analysis by maximum temperature and **Figure 6** the corresponding charts of marathon race times (in seconds) by age group and sex for maximum temperatures on race day. Charts A and D (average race times) show the curves of the higher temperatures over the curves of the lower temperature indicating that marathon races times increased when temperatures increased ($p < 0.05$ for females and $p = 0.39$ for males). Charts C and F on the right side present the minimum (fastest) race times. The darker curves (higher temperatures) are below the light-colored curves for all age ranges and sex showing that those runners who achieved the fastest race times competed on race days with higher maximum temperatures ($p < 0.05$ for both males and females). This is in contrast to average race times which are more influenced by runners of all performance tiers. Also, in charts C and F (minimum race times), there seems to be a growing gap between the curves from age group 35–40 years, so the temperature difference does not seem to have an influence on younger age groups in the same way. **Table 4** presents the analysis by average temperature and **Figure 7** the corresponding charts of marathon race times by age group and sex for average temperatures on race day. Charts C and F show again a widening gap between the curves from age group 35 years. **Table 5** presents the analysis by atmospheric pressure range and **Figure 8** the charts of marathon

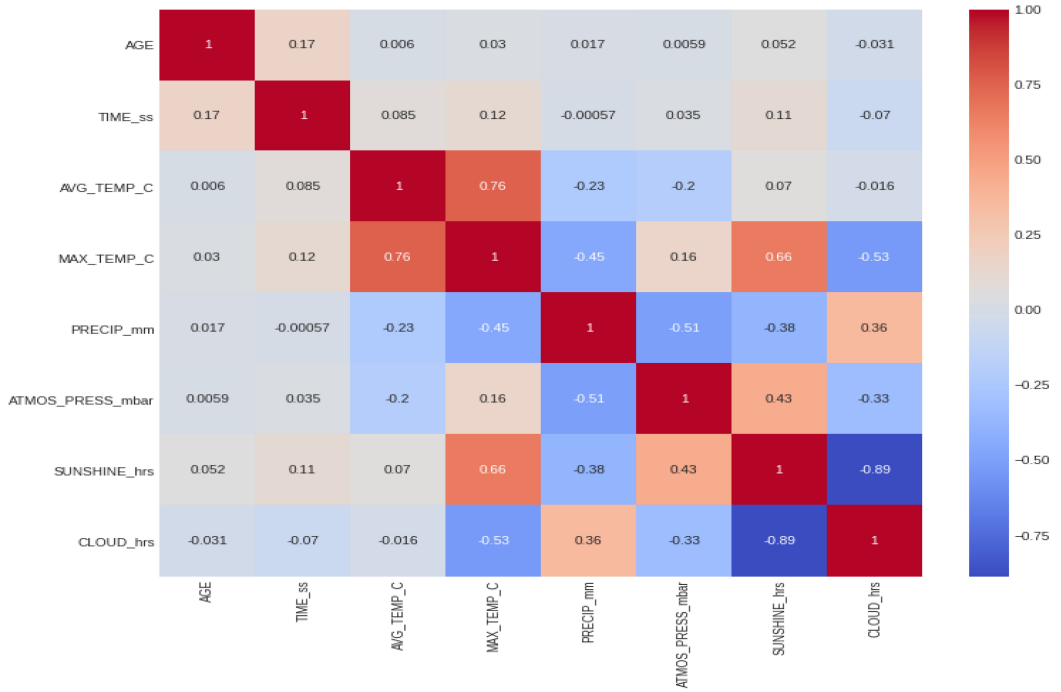

FIGURE 5 | Correlation matrix of marathon race times with the descriptors (age and weather variables).

race times by age group and sex for atmospheric pressure range on race days. **Table 6** presents the analysis by precipitation and **Figure 9** the charts of marathon race times by age group and sex for precipitation on race days. In the context of the negligible correlation of finish times with the precipitation, there are still some insights in the latter charts: curves on charts A and D (average finish times) stay pretty close together, so the levels of rain do not seem to affect the average finish times ($p = 0.49$ for females, $p < 0.05$ for males). Curves on C and F (minimum – or best – finish times) show a widening gap from age 20 years for males and age 30 years for females, indicating that higher precipitation levels were matched by a generalized worse performance across most age levels ($p < 0.05$ for both genders).

DISCUSSION

This study investigated the influence of environmental conditions such as temperature, sunshine duration, precipitation, barometric pressure on marathon race times in age group marathoners with the assumption that higher ambient temperatures would impair marathon performance in athletes in master marathoners.

The main findings were (*i*) marathon race times were significantly and positively related with age where the correlation was higher for males than for females, (*ii*) marathon race times were significantly and positively correlated with both sunshine duration and daily maximum temperature with no differences between the sexes, (*iii*) the fastest marathon runners achieved the fastest race times on race days with higher maximum temperatures with no differences between the sexes, (*iv*) increased daily maximum temperatures decreased race performance in age group marathoners from age group 35–40 years and older with no differences between the sexes, and (*v*) higher precipitation levels impaired top performance across most age groups with no differences between the sexes.

We found different influences of temperature on different performance levels (i.e., younger and age group runners) with no differences between the sexes. Marathon race times of the whole field of runners were significantly and positively related with both sunshine duration and daily maximum temperature, where sunshine duration and daily maximum temperature were highly correlated. The fastest marathon runners, however, achieved the fastest race times on race days with higher maximum temperatures. In contrast, regarding age group marathoners, the daily maximum temperatures showed an influence on age group marathoners from age group 35–40 years and older with no differences between the sexes.

It is well-known that marathon race times are impaired with increasing temperatures (Trapasso and Cooper, 1989; Cheuvront and Haymes, 2001; Ely et al., 2007b; González-Alonso, 2007; Maughan, 2010; El Helou et al., 2012; Nikolaidis et al., 2019). The temperature on race day has, however, a different influence on slower and faster runners (Montain et al., 2007; Ely et al., 2008; El Helou et al., 2012) where the optimum temperature for a fast marathon race time may be lower for faster runners than for slower runners (Maughan, 2010).

Since elite marathoners are generally faster than master marathoners (Lepers and Cattagni, 2012), higher ambient temperatures might have a higher impact on master marathoners

TABLE 3 | Analysis by maximum temperature.

Age groups	Maximum temperature range (°C)	Marathon race time (Females)					Marathon race time (Males)				
		n	Mean	std	Min	Max	*n*	Mean	std	Min	Max
18	8–15	109	04:23:06	00:37:54	02:39:39	06:27:09	564	03:52:30	00:35:50	02:33:00	06:04:08
	15–30	647	04:31:59	00:41:59	02:25:00	06:36:24	4261	03:59:52	00:39:05	02:12:00	07:58:43
20	8–15	2623	04:16:44	00:39:49	02:19:41	06:28:57	10995	03:45:15	00:38:56	02:05:08	06:35:43
	15–30	21875	04:21:48	00:41:41	02:18:34	07:07:05	84717	03:50:47	00:41:29	02:02:48	07:11:36
30	8–15	2323	04:16:00	00:40:39	02:26:21	06:30:11	10403	03:44:37	00:38:04	02:06:49	07:55:55
	15–30	21306	04:20:55	00:41:13	02:18:55	07:40:08	85825	03:50:17	00:40:52	02:01:39	07:19:28
35	8–15	2674	04:16:21	00:36:38	02:27:41	06:34:03	12971	03:45:53	00:37:22	02:09:08	07:41:00
	15–30	23423	04:21:59	00:40:13	02:18:11	07:12:28	105567	03:51:02	00:39:55	02:01:41	08:30:02
40	8–15	3072	04:19:20	00:36:00	02:47:16	06:40:50	14330	03:48:32	00:36:05	02:17:10	06:30:36
	15–30	27113	04:23:57	00:39:05	02:24:54	09:49:41	116916	03:53:40	00:39:12	02:10:24	08:09:19
45	8–15	2628	04:23:48	00:35:57	02:57:00	06:27:20	11894	03:52:35	00:35:38	02:25:36	06:42:56
	15–30	23397	04:28:10	00:38:27	02:20:32	07:34:33	102378	03:57:46	00:39:18	02:15:00	07:55:39
50	8–15	1492	04:29:28	00:36:23	03:00:04	06:39:52	7719	03:58:32	00:36:05	02:28:15	07:07:24
	15–30	14860	04:35:24	00:38:46	02:49:55	07:19:21	72120	04:05:11	00:39:24	02:12:08	07:55:55
55	8–15	594	04:37:54	00:38:01	03:09:35	07:09:24	3858	04:06:38	00:37:11	02:33:23	06:40:51
	15–30	6294	04:43:02	00:40:18	02:25:00	09:19:54	36797	04:14:47	00:39:54	02:25:04	07:52:04
60	8–15	224	04:44:57	00:36:51	03:23:37	06:21:50	1854	04:18:07	00:38:32	02:48:00	06:49:34
	15–30	2498	04:51:16	00:40:27	03:08:10	09:43:23	17539	04:26:09	00:41:35	02:25:00	08:47:19
65	8–15	79	04:51:54	00:35:05	03:45:41	06:05:05	689	04:32:26	00:41:16	02:43:27	06:40:46
	15–30	825	05:04:25	00:39:54	03:17:10	07:00:00	6726	04:39:39	00:44:07	02:25:00	07:12:17
70	8–15	20	05:10:24	00:32:39	04:23:29	06:25:41	220	04:53:16	00:40:09	03:26:09	06:59:28
	15–30	202	05:13:29	00:43:09	03:40:34	07:11:15	2122	04:57:05	00:45:09	03:06:18	07:07:46
75	8–15	1	05:17:36	00:00:00	05:17:36	05:17:36	34	05:02:19	00:41:23	04:00:00	06:20:42
	15–30	48	05:31:45	00:35:52	04:24:55	06:33:20	538	05:17:26	00:46:21	03:32:53	07:43:32
80	8–15	1	05:36:59	00:00:00	05:36:59	05:36:59	4	06:13:00	00:43:57	05:15:46	06:47:37
	15–30	10	06:04:40	00:17:36	05:44:22	06:42:36	95	05:42:59	00:43:05	03:58:23	07:15:28

Mean, average value; std, standard deviation; Min, minimum value; Max, maximum value.

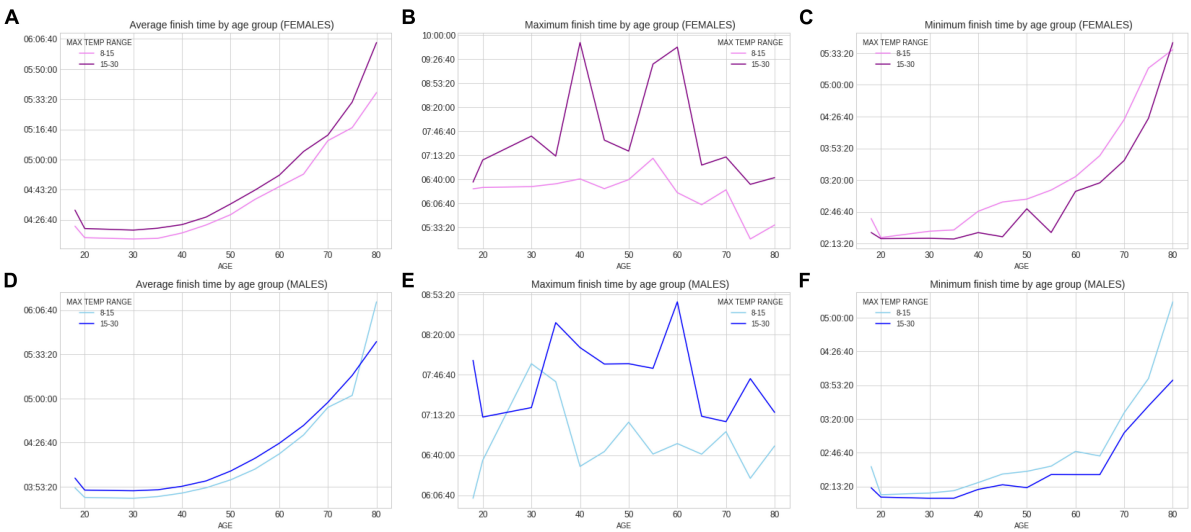

FIGURE 6 | Charts of marathon race times by age group and sex for maximum temperatures on race day.

compared to elite marathoners. However, higher temperatures seemed to slow down faster runners compared to slower runners. A study investigating the influence of air temperature on female marathoners of different performance levels showed that increasing air temperatures slowed pace more in faster runners (winner, 25th place) than slower runners (50th place,

TABLE 4 | Analysis by average temperature range.

Age groups	Average temperature range (°C)	Marathon race time (Females)					Marathon race time (Males)				
		n	Mean	std	Min	Max	*n*	Mean	Std	Min	Max
18	8–15	599	04:28:27	00:41:09	02:25:00	06:36:24	3800	03:56:39	00:37:53	02:12:00	06:28:50
	15–30	157	04:39:17	00:41:52	03:17:03	06:27:03	1025	04:07:44	00:40:51	02:33:00	07:58:43
20	8–15	20213	04:20:18	00:41:32	02:18:34	07:07:05	77380	03:48:38	00:41:06	02:02:48	07:11:36
	15–30	4285	04:25:47	00:41:11	02:19:12	06:48:36	18332	03:56:33	00:41:15	02:06:44	06:484
30	8–15	19538	04:19:53	00:41:31	02:18:55	07:40:08	77456	03:48:31	00:40:41	02:01:39	07:55:55
	15–30	4091	04:23:03	00:39:28	02:21:34	06:44:00	18772	03:54:29	00:39:59	02:05:56	06:58:45
35	8–15	21236	04:20:56	00:40:28	02:18:11	07:12:28	94191	03:49:14	00:39:45	02:01:41	08:30:02
	15–30	4861	04:23:24	00:37:17	02:26:24	06:41:48	24347	03:55:18	00:39:04	02:06:08	06:53:43
40	8–15	24475	04:22:43	00:39:07	02:24:54	09:49:41	104255	03:51:54	00:38:54	02:10:24	08:09:19
	15–30	5710	04:26:46	00:37:18	02:40:05	07:00:54	26991	03:57:45	00:38:33	02:23:56	07:42:28
45	8–15	21682	04:27:12	00:38:33	02:20:32	07:34:33	92967	03:56:15	00:39:02	02:15:00	07:55:39
	15–30	4343	04:30:24	00:36:28	02:56:00	06:49:53	21305	04:01:31	00:38:24	02:28:00	07:00:52
50	8–15	13973	04:34:43	00:38:53	02:49:55	07:19:21	65807	04:03:59	00:39:16	02:12:08	07:55:06
	15–30	2379	04:35:43	00:36:51	02:55:37	06:38:54	14032	04:07:11	00:38:29	02:28:00	07:55:55
55	8–15	5974	04:42:33	00:40:30	02:25:00	09:19:54	33928	04:13:39	00:39:54	02:25:04	07:52:04
	15–30	914	04:42:51	00:37:37	03:00:52	06:55:14	6727	04:15:51	00:38:44	02:39:00	06:53:43
60	8–15	2377	04:50:42	00:40:34	03:08:10	09:43:23	16081	04:25:00	00:41:27	02:25:00	08:47:19
	15–30	345	04:51:02	00:37:37	03:27:22	06:34:23	3312	04:27:16	00:40:58	02:41:00	06:59:23
65	8–15	762	05:03:07	00:40:29	03:17:10	07:00:00	6090	04:38:03	00:44:01	02:25:00	07:12:17
	15–30	142	05:04:23	00:34:59	03:45:00	06:29:41	1325	04:43:15	00:43:09	02:57:45	07:03:21
70	8–15	203	05:12:51	00:43:09	03:40:34	07:11:15	2021	04:56:25	00:44:55	03:06:18	07:07:46
	15–30	19	05:17:08	00:31:54	04:19:23	06:07:10	321	04:58:40	00:43:20	03:21:53	06:40:36
75	8–15	48	05:30:25	00:35:10	04:24:55	06:33:20	515	05:17:04	00:46:07	03:32:53	07:43:32
	15–30	1	06:21:10	00:00:00	06:21:10	06:21:10	57	05:11:38	00:46:50	04:01:33	07:07:44
80	8–15	9	05:57:05	00:14:07	05:36:59	06:17:46	88	05:43:29	00:42:16	03:58:23	07:15:28
	15–30	2	06:24:54	00:25:01	06:07:13	06:42:36	11	05:49:50	00:52:47	04:26:42	06:55:52

Mean, average value; std, standard deviation; Min, minimum value; Max, maximum value.

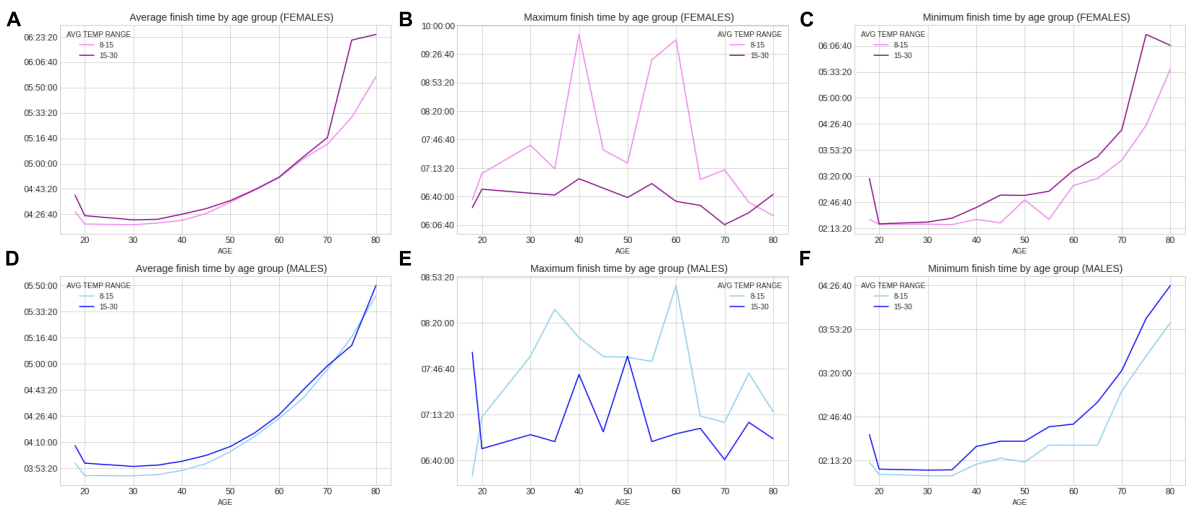

FIGURE 7 | Charts of marathon race times by age group and sex for average temperatures on race day.

100th place) (Ely et al., 2008). The disparate findings compared to our findings that the fastest marathon runners achieved the fastest race times on race days with higher maximum temperatures might be explained by the different statistical approach and the fact that Ely et al. (2008) investigated only female marathoners, whereas we investigated both female and

TABLE 5 | Analysis by atmospheric pressure range.

Age group	Atmospheric pressure range (mbar)	Marathon race time (Females)					Marathon race time (Males)				
		n	Mean	std	Min	Max	*n*	Mean	Std	Min	Max
18	900–1013	524	04:29:56	00:42:28	02:25:00	06:33:14	3101	04:00:19	00:39:02	02:12:00	07:58:43
	1013–1030	232	04:32:25	00:39:18	03:02:00	06:36:24	1724	03:56:39	00:38:15	02:25:00	06:16:30
20	900–1013	16563	04:21:51	00:40:54	02:19:12	07:07:05	64548	03:51:09	00:41:07	02:02:48	07:10:51
	1013–1030	7935	04:20:02	00:42:45	02:18:34	06:57:20	31164	03:48:03	00:41:25	02:05:21	07:11:36
30	900–1013	15959	04:20:30	00:40:46	02:20:23	06:58:53	67079	03:50:02	00:40:17	02:03:03	07:55:55
	1013–1030	7670	04:20:17	00:42:03	02:18:55	07:40:08	29149	03:48:51	00:41:20	02:01:39	06:55:02
35	900–1013	17822	04:20:54	00:38:49	02:22:18	06:57:37	83401	03:51:08	00:39:18	02:01:41	07:41:00
	1013–1030	8275	04:22:28	00:42:07	02:18:11	07:12:28	35137	03:48:54	00:40:33	02:03:59	08:30:02
40	900–1013	20874	04:23:29	00:38:03	02:24:54	07:18:23	93566	03:53:40	00:38:40	02:10:24	07:42:28
	1013–1030	9311	04:23:29	00:40:27	02:30:47	09:49:41	37680	03:51:43	00:39:27	02:12:45	08:09:19
45	900–1013	17898	04:27:29	00:37:09	02:25:00	06:54:45	80309	03:57:57	00:38:37	02:15:00	07:55:39
	1013–1030	8127	04:28:17	00:40:31	02:20:32	07:34:33	33963	03:55:33	00:39:46	02:24:00	07:15:57
50	900–1013	11134	04:33:46	00:37:37	02:49:55	07:04:09	56512	04:04:31	00:38:40	02:22:23	07:55:55
	1013–1030	5218	04:37:11	00:40:30	02:51:47	07:19:21	23327	04:04:35	00:40:17	02:12:08	07:21:15
55	900–1013	4545	04:42:01	00:39:11	02:25:00	07:09:24	28746	04:13:36	00:39:15	02:25:04	07:52:04
	1013–1030	2343	04:43:41	00:41:54	03:04:31	09:19:54	11909	04:15:00	00:40:48	02:38:52	07:51:18
60	900–1013	1793	04:49:30	00:38:49	03:14:10	06:57:25	13498	04:25:14	00:41:10	02:25:00	08:47:19
	1013–1030	929	04:53:08	00:42:40	03:08:10	09:43:23	5895	04:25:43	00:41:50	02:39:42	07:30:15
65	900–1013	624	05:01:33	00:38:27	03:17:10	06:41:28	5301	04:39:06	00:43:41	02:25:00	07:12:17
	1013–1030	280	05:07:15	00:42:01	03:25:12	07:00:00	2114	04:38:40	00:44:27	02:54:35	06:58:40
70	900–1013	149	05:15:47	00:42:42	03:40:34	06:55:36	1651	04:57:08	00:44:17	03:06:18	07:06:50
	1013–1030	73	05:07:57	00:41:09	03:45:14	07:11:15	691	04:55:43	00:45:43	03:13:45	07:07:46
75	900–1013	29	05:34:58	00:37:27	04:24:55	06:29:33	381	05:13:17	00:46:44	03:39:39	07:43:32
	1013–1030	20	05:26:21	00:32:52	04:37:51	06:33:20	191	05:23:00	00:44:28	03:32:53	07:18:33
80	900–1013	7	06:04:26	00:21:19	05:36:59	06:42:36	69	05:46:55	00:44:10	04:26:42	07:15:28
	1013–1030	4	05:58:09	00:14:49	05:44:47	06:17:46	30	05:37:56	00:41:16	03:58:23	06:52:16

Mean, average value; std, standard deviation; Min, minimum value; Max, maximum value.

male marathoners. Furthermore, Ely et al. (2008) analyzed national championship marathon runners which were all very fast comparing to our age group runners. And their slower runners were actually quite fast comparing to the recreational runners (Ely et al., 2008). We investigated both female and male marathoners with no differences between the sexes. Future studies might investigate other large city marathons regarding this aspect.

In contrast, it has also been shown that slower runners suffered a greater performance decline in higher temperatures than faster runners. An analysis regarding the effects of air temperature on performance in marathoners competing in the 'Stockholm Marathon' between 1980 and 2008 showed that slower runners were more affected by unfavorable weather conditions than faster runners (Vihma, 2010). Air temperature was the single weather parameter with the highest correlation with finishing time anomaly (i.e., deviation of the annual finishing time from the linear trend of the finishing time) with the highest values for slowest runners (Vihma, 2010). Regarding the sexes, the effects of warm weather were less evident for female than for male runners (Vihma, 2010). The finding of Vihma (Vihma, 2010) is in line with our finding that race times of the whole field of runners (i.e., the general mass) was related with both sunshine duration and daily maximum temperature (i.e., daily maximum temperature increased with increasing sunshine duration).

Apart the influence from temperature, we also found an influence of precipitations on marathon race times. Higher precipitation levels impaired performance across most older age groups (i.e., master marathoners older than 35–40 years and older) with no differences between the sexes. The influence of precipitation has been investigated in the 'Stockholm Marathon' (Vihma, 2010) and in the 'Boston Marathon' (Knechtle et al., 2019; Nikolaidis et al., 2019) for marathoners of different performance levels, but not for age group marathoners. Vihma investigated the effects of different variables (i.e., air temperature, relative and specific humidity, wind speed, solar shortwave radiation, thermal longwave radiation, and rain) on the performance of female and male participants in the annual 'Stockholm Marathon' from 1980 to 2008 (Vihma, 2010). The occurrence of rain was related to finishing time anomaly, expressed as deviation of the annual finishing time from the linear trend of the finishing time. However, the effects of rain only arose from the negative correlation the air temperature Vihma (Vihma, 2010). A study examining the relationship of weather conditions with running performance in the Boston Marathon from 1972 to 2018 showed that increasing

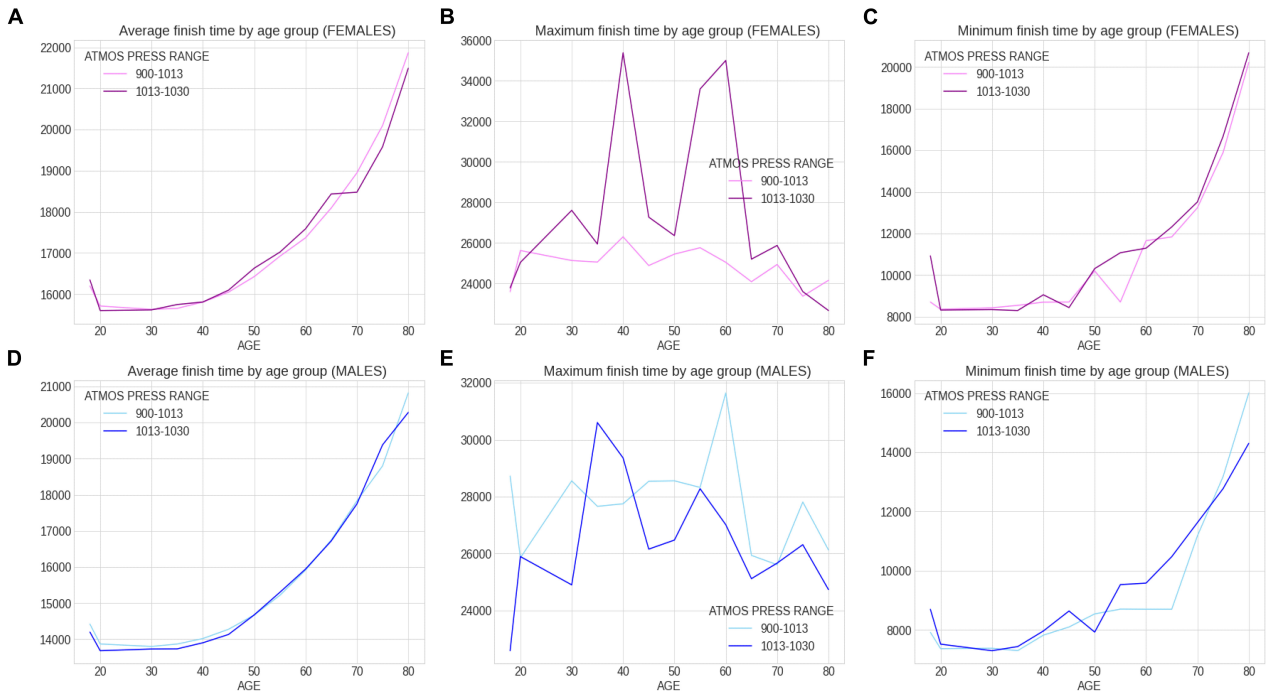

FIGURE 8 | Charts of marathon race times by age group and sex for atmospheric pressure range on race day.

precipitation was significantly related to slower performances in runners of all performance levels, except the annual winners (Knechtle et al., 2019). Also, when male performances in the 'Boston Marathon' from 1897 to 2018 were investigated, increasing precipitations worsened performances of both the top 100 and the top 10 finishers (Nikolaidis et al., 2019). Obviously, precipitation has only an influence on slower runners, but not on faster runners, regarding our findings from the 'Berlin Marathon' and the findings from 'Boston Marathon' (Knechtle et al., 2019).

The impact of precipitation on marathon performance might be attributed to the role of thermoregulation and particularly, to the association of higher humidity with reduced capacity to lose heat using evaporation (Wilmore and Costill, 1999). Humidity has been suggested as a factor of heat dissipation, and consequently, an increase of humidity would affect negatively the balance between heat production ad dissipation (Bouscaren et al., 2019). In addition, the thermoregulation has been well-documented to be impaired with aging reducing sport performance for master athletes especially in hot environmental conditions (Kenney et al., 2020). These age-related differences in thermoregulation might be attributed to differences in performance related characteristics (e.g., physical fitness and body composition) (Kenney and Munce, 2003).

We also found that marathon race times of the whole field of runners were significantly and positively related with age where the correlation was higher for male than for female marathoners suggesting a higher impact of age on male than female performance. An effect of age on marathon performance has already been reported (Nikolaidis and Knechtle, 2017; Stones, 2019). Stones investigated in his analysis the top 100 age group performances in master marathoners and found higher performance times for women than for men where the performance decline was greater at older ages and in women than men (Stones, 2019). The disparate findings that we found a higher impact on male performance with age and Stones a greater decline in performance at higher ages in women might be explained by the different samples that were investigated (i.e., the whole field of marathon race over decades compared to the top 100 age group performances). When the performance was expressed in percent of the annual fastest, we found that performance of female and male athletes in age groups 20–40 years remained very close over years and an increasing drop in performance with increasing age over calendar years. The gradual drop in relative performance through the years is most likely explained by the increasingly popular character of the marathon where every year more and more recreational runners join it. This is very obvious in the very old age groups (i.e., 75 years and older).

Limitations

Since the first edition of 'Berlin Marathon', several changes occurred such as moving the race course from outside Berlin into the city of Berlin, changing the start procedure from mass start in the beginning to start in blocks by performance levels, moving race start from afternoon to morning, aid stations with food and drinks, and including professional runners. Most of these changes cannot be included in this kind of performance analysis. Regarding temperature, historic weather data had only

TABLE 6 | Analysis by precipitation.

Age group	Precipitation range (mm)	Marathon race time (Females)					Marathon race time (Males)				
		n	Mean	std	Min	Max	*n*	Mean	Std	Min	Max
18	0–10	723	04:31:01	00:41:51	02:25:00	06:36:24	4727	03:58:52	00:38:51	02:12:00	07:58:43
	20–50	33	04:23:49	00:33:06	02:41:41	05:19:39	98	04:05:44	00:35:08	02:48:00	05:39:52
20	0–10	23475	04:21:04	00:41:35	02:18:34	07:07:05	92975	03:49:56	00:41:14	02:02:48	07:11:36
	10–20	–	–	–	–	–	126	03:20:52	00:32:05	02:16:20	05:17:56
	20–50	1023	04:25:54	00:39:36	02:23:58	06:28:57	2611	03:59:15	00:40:38	02:05:08	06:35:43
30	0–10	22633	04:20:14	00:41:12	02:18:55	07:40:08	93181	03:49:33	00:40:40	02:01:39	07:55:55
	20–50	996	04:24:50	00:40:29	02:26:21	06:30:11	3047	03:53:37	00:38:44	02:13:46	06:25:35
35	0–10	24996	04:21:23	00:40:00	02:18:11	07:12:28	114432	03:50:25	00:39:46	02:01:41	08:30:02
	20–50	1101	04:21:56	00:37:31	02:39:29	06:34:03	4106	03:51:58	00:37:32	02:19:55	06:34:45
40	0–10	28741	04:23:28	00:38:54	02:24:54	09:49:41	125719	03:53:05	00:39:01	02:10:24	08:09:19
	20–50	1444	04:23:43	00:36:44	02:52:49	06:40:50	5527	03:53:38	00:36:13	02:27:58	06:30:36
45	0–10	24635	04:27:39	00:38:18	02:20:32	07:34:33	109305	03:57:10	00:39:07	02:15:00	07:55:39
	20–50	1390	04:29:09	00:36:56	02:58:51	06:27:20	4967	03:58:47	00:35:39	02:25:36	06:42:56
50	0–10	15485	04:34:54	00:38:43	02:49:55	07:19:21	76519	04:04:31	00:39:15	02:12:08	07:55:55
	20–50	867	04:34:04	00:36:32	03:06:53	06:39:52	3320	04:05:13	00:36:42	02:37:03	07:07:24
55	0–10	6528	04:42:32	00:40:11	02:25:00	09:19:54	39042	04:13:58	00:39:49	02:25:04	07:52:04
	20–50	360	04:43:44	00:39:19	03:09:35	07:09:24	1613	04:15:12	00:37:09	02:49:17	06:40:51
60	0–10	2602	04:50:54	00:40:20	03:08:10	09:43:23	18589	04:25:22	00:41:32	02:25:00	08:47:19
	20–50	120	04:47:24	00:37:08	03:23:37	06:21:50	804	04:25:42	00:37:33	02:50:53	06:49:34
65	0–10	858	05:03:37	00:39:54	03:17:10	07:00:00	7100	04:38:49	00:44:04	02:25:00	07:12:17
	20–50	46	04:57:51	00:34:39	03:45:41	06:05:05	315	04:42:32	00:39:45	03:16:07	06:40:46
70	0–10	206	05:13:29	00:42:53	03:40:34	07:11:15	2191	04:56:43	00:45:06	03:06:18	07:07:46
	20–50	16	05:09:46	00:34:14	04:23:29	06:25:41	151	04:56:53	00:38:36	03:42:27	06:29:17
75	0–10	49	05:31:27	00:35:33	04:24:55	06:33:20	554	05:17:02	00:46:22	03:32:53	07:43:32
	20–50	–	–	–	–	–	18	05:00:53	00:37:26	04:07:38	06:14:19
80	0–10	10	06:04:40	00:17:36	05:44:22	06:42:36	97	05:43:50	00:43:10	03:58:23	07:15:28
	20–50	1	05:36:59	00:00:00	05:36:59	05:36:59	2	06:01:36	01:04:49	05:15:46	06:47:26

Mean, average value; std, standard deviation; min, minimum value; max, maximum value.

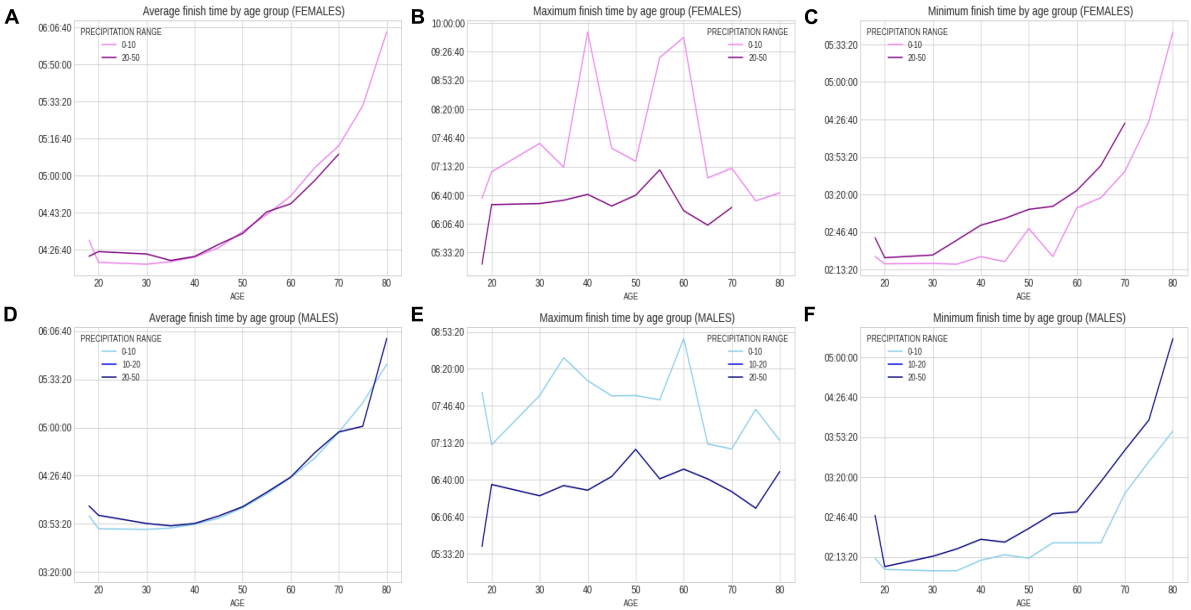

FIGURE 9 | Charts of marathon race times by age group and sex for precipitation on race day.

average, minimum (morning) and maximum (afternoon) values for a day. There might also have been a change of measurement in weather data during the decades, which also may have an impact on the results. Future studies might investigate the influence of temperature considering temperatures at hourly intervals during race time. A further limitation is the introduction of electronic time measurement in recent years. Before this area, slower runners had to wait and walk for the start line until the large mass of runners has started. This difference between 'gun time' and 'chip time' leads to partially massive differences especially in the race time of slower and older runners. Race data from 1978 and 1980 are missing in the race results from 'Berlin Marathon' which could also have an influence on our analysis.

CONCLUSION

In summary, higher daily maximum temperatures and higher precipitation levels impaired performance of master marathoners (35–40 years and older) competing in the 'Berlin Marathon' in the last 45 years. For master marathoners, temperatures below 15 °C and no precipitation would be beneficial for a fast marathon race time. The findings of an impaired performance in older age groups might also be due to the continuous decrease in performance in older age groups across calendar years.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

BK and VS drafted the manuscript. DV performed the statistical analyses. EV collected the all data. JA-C, PN, IC, and TR helped in drafting the final version of the manuscript. All the authors contributed to the article and approved the submitted version.

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Increased Participation and Decreased Performance in Recreational Master Athletes in "Berlin Marathon" 1974–2019

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The aspect of participation and performance trends in marathon running has been investigated mainly in marathons held in the United States of America (e.g., "New York City Marathon," "Boston Marathon"), but not for the fastest course in the world, the "Berlin Marathon" held in Berlin, Germany. This study aimed to examine trends in participation and performance in the "Berlin Marathon" on all its previous 46 editions from 1974 to 2019, the largest dataset ever studied in this event with 696,225 finishers (after data cleaning). Athletes in all age groups increased their participation, except for male athletes aged 20–49 years and athletes of both sexes above 79 years of age. This overall increase in participation was more pronounced in women, but still, there are more men than women participating in "Berlin Marathon" nowadays. All age group athletes decreased their performance across years overall, whereas the top ten recreational athletes improved their performance over the years. Our findings improved the knowledge about the evolution of male and female marathoners across calendar years, especially for the fastest marathon race in the world, the "Berlin Marathon."

Keywords: marathon, running, participation, performance, age of peak performance, performance decline, sex differences in endurance

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INTRODUCTION

The inaugural modern marathon event was first held during the 1896 Summer Olympic games and was refined to the official distance of 42.195 km for the 1908 Olympic Games in London (Burfoot, 2007; Wilcock, 2008). However, these races were only for men. The women's marathon event was added to the official program nearly 99 years later, during the 1984 Los Angeles Olympic Games (Burfoot, 2007). In the 1970s and 80s – in line with the upcoming fitness trend – a skyrocketing boom of these marathon events occurred (Valentine, 1982; Maffetone et al., 2017; Knechtle et al., 2018) and marathon races have become more and more popular all over the world (Vitti et al., 2020). The largest participation numbers so far were reached in 2016, with approximately nine million runners crossing

finish lines all over the world (The State of Running, 2019). A total of 12% of those finishers were marathoners (The State of Running, 2019), and most of them were age group athletes (Rüst et al., 2013; Lara et al., 2014).

With such participation numbers and demographic diversity, marathon running provides scientifically interesting samples for research in endurance sport (Stones and Baker, 2020). Further analyses of these data can contribute to, e.g., a better understanding of aging processes and consecutively age-related performance declines (Leyk et al., 2007; Reaburn and Dascombe, 2008; Tayrose et al., 2015), differences between the sexes regarding physiology and performance (Rüst et al., 2013; Knechtle and Nikolaidis, 2018; Nikolaidis et al., 2018, 2019c), the influence of lifestyle (Leyk et al., 2009, 2010), environmental (Nielsen, 1996; El Helou et al., 2012; Maffetone et al., 2017; Knechtle et al., 2019) and demographic factors (Knechtle et al., 2017; Maffetone et al., 2017; Nikolaidis et al., 2017; Knechtle and Nikolaidis, 2018) on performance and a better understanding of motivational factors for running of different cohorts and decades (Krouse et al., 2011; Nikolaidis et al., 2019b).

The "Berlin-Marathon," for the first time in 2019, marked the final race of the "World Marathon Majors," a series of six of the largest and most renowned marathons in the world and popularly known as the "Marathon's Champion's League" (World Marathon Majors, 2021). This may be a deserved position as today, the "Berlin Marathon" ranks third in the world regarding the size of the runner field and is the fastest course among the city marathons worldwide in men's racing and third fastest in women's (Berlin Marathon, 2021). Seven of the ten fastest men's marathon times and numerous world records were set during the "Berlin-Marathon" (Berlin Marathon, 2021). Among those records is the current men's world record, achieved in 2018 by the Kenyan Eliud Kipchoge in a time of 2:01:39 (World Athletic Records, 2021). The current course record for women in "Berlin-Marathon" was set by Kipchoge's countrywomen Gladys Cherono in 2018 (2:18:11 h) (BMW Berlin Marathon, 0000). This running time is close to the current world record for "women only marathon" (as is also "Berlin Marathon" since 2011) set by Mary Jepkosgei in 2017 in the "London Marathon" with a time of 2:17:01[1].

More recently, research has tended to focus primarily on marathon races held in the United States, with the "New York City Marathon, 2021" – the largest marathon race nowadays – being one of the most investigated (Jokl et al., 2004; Santos-Lozano et al., 2015; Zavorsky et al., 2017; Nikolaidis et al., 2018). Alongside the "New York City Marathon, 2021" is the "Boston Marathon, 2021" generating the next most scientific interest (Maffetone et al., 2017; Knechtle et al., 2018, 2020). Most studies conducted on those two marathon events (the "New York City Marathon, 2021" and the "Boston Marathon, 2021") have demonstrated increasing participation rates over the last two decades (more pronounced in women than in men) and a concomitant increase in mean race times (i.e., decreased performance) across calendar years (Jokl et al., 2004; Mathews et al., 2012; Nikolaidis et al., 2018; Knechtle et al., 2020). Even though the "Berlin-Marathon" is one of the fastest and most popular marathon events in the world, there has not been any complete analysis of participation and performance trends on all its 46 previous editions. Therefore, it is interesting to see whether the above-mentioned consensus about the development of participation rates and running times found in American marathon events would also be applicable for European Marathon races such as the "Berlin Marathon."

Much of the current research on participation and performance in marathon racing also was done with regard only at a short period of time or limited participation group. For example, Ahmadyar et al. (2015, 2016) studied elderly marathoners (>75 years of age) in the four largest marathon events nowadays in the time period from 1990 to 2014 and 2004 to 2011. Knechtle et al. (2018) analyzed participation and performance for all editions of "Boston Marathon" from 1879 to 2017, but only in male runners. Jokl et al. (2004) included runners of all age groups and both sexes in their analysis of the "New York City Marathon," but only analyzed the time period between 1983 and 1999 (Jokl et al., 2004). Mathews et al. (2012) studied the mortality among marathon runners in the United States, and thereby analyzed a variety of events throughout the United States but could only include data from 2000 to 2009 in their study. More recently, an interesting perspective that investigated the motivation for running in the "Athens Classic Marathon" was done by Nikolaidis et al. but only focused on the 2017 edition (Nikolaidis et al., 2019b). Therefore, by analyzing the full data of the "Berlin Marathon" since its inaugural event in 1974, we hope to provide valuable new information to the above-mentioned ongoing research.

Furthermore, the current study aims to contribute to the growing literature on women's participation in endurance sports. Previously, women were barred from participating in sporting events primarily based on Victorian area myths about endurance exercise and the fragility of the female body (Wrynn, 2014; Hill, 2017). It has taken several decades for women to be permitted the same sporting opportunities as their male counterparts, and even still, equity has not been achieved (Capranica et al., 2013). The first time women were permitted to officially run a marathon race occurred during the 1972 "Boston Marathon" (Thibault et al., 2010; Knechtle et al., 2020). In 2018, for the first time in history, equal participation between men and women in running events was achieved with women representing 50.24% of runners at events all around the world (The State of Running, 2019). Race organizers at the "Berlin-Marathon" in its 46th edition (2019) set its focus for the first time officially on the women's race (Berlin Marathon, 2021).

Although progress toward equal opportunity has been consistent for female athletes, sports science research focusing on women is still sorely lacking. Only 4% of the present research in sport sciences is conducted exclusively on female athletes, whereas 27% of those studies are conducted exclusively on male athletes (Women in Sports are Often Underrepresented in Science, 2016; Costello et al., 2014). Further, an analysis of 1,382 articles published from 2011 to 2013 showed that female participation rate per article was around 36 percent (added up

[1]www.worldathletics.org/records/by-discipline/road-running/marathon/outdoor/women

in this analysis was a total of more than six million participants) (Women in Sports are Often Underrepresented in Science, 2016). As physiology and biomechanics properties differ between the sexes, it is not applicable to transfer study results found in a predominantly male population to a female population, which – as mentioned – accounts for more than half of the athletic population currently competing in running (Women in Sports are Often Underrepresented in Science, 2016; Lewis et al., 1986). To optimize female performances and health in sport, we need to include women in our analyses in order to better understand the peculiarities that may exist in physiology. Therefore, we are happy to enrich the existing pool of knowledge with more data on female participation and performance in marathon racing.

Taken together, our understanding of the characteristics of participation and performance are well known for only a handful of important marathon events (Jokl et al., 2004; Mathews et al., 2012; Santos-Lozano et al., 2015; Maffetone et al., 2017; Zavorsky et al., 2017; Nikolaidis et al., 2018, 2019c; Knechtle et al., 2020) and/or limited periods or participation groups (Jokl et al., 2004; Mathews et al., 2012; Ahmadyar et al., 2015, 2016; Nikolaidis et al., 2019b). Drawing general conclusions out of these limited data, which can be important for the above-mentioned research fields in sport, epidemiological and medical sciences, should be done cautiously.

The aims of the present study were, therefore, (i) to analyze the changes in participation and performance trends of age group marathon runners in the "Berlin-Marathon" for all its previous editions, (ii) to compare the sex differences in performance as a function of age across the years, and (iii) by this to provide one more complete analysis on participation and performance of female athletes in the history of a significant event in order to allow best possible future findings of particularities in female sports physiology. Based upon existing evidence, we hypothesized that for "Berlin-Marathon" between 1974 and 2019, the participation of all age groups would grow, with more substantial growth in female participation and, therefore, a narrowing sex gap in participation. Further, we hypothesized the performance of top age group athletes would improve over calendar years, whereas the performance of the average age groupers would decrease.

MATERIALS AND METHODS

Ethics Approval

The institutional review board of St Gallen, Switzerland, approved this study (EKSG 01/06/2010). Since the study involved the analysis of publicly available data, the requirement for informed consent was waived.

Participants

To test our hypothesis, data (i.e., first and last name, sex, age, calendar year, and running time) on all successful female and male finishers in the "Berlin-Marathon" since 1974, the inauguration year of the "Berliner Volksmarathon," was obtained from the official race website (BMW Berlin Marathon, 0000). To compete in the "Berlin-Marathon," athletes must be 18 years old or older but must not meet specific time standards (BMW Berlin Marathon, 0000). Starting places are limited and assigned via raffle. Initially, 884,927 finishers were considered in our analysis.

The Race

The "Berlin-Marathon" takes place from mid to end of September, depending on several logistical factors. The course builds one large loop through the historic city of Berlin, with the finish line lying almost under the "Brandenburger Tor." Berlin lies 34 meters above sea-level, the average temperature in September is about 14.9°Celsius and average humidity about 75% (Klimatabelle, 2021) and the total elevation of the course is only 50 meters (Berlin Marathon, 2021).

Data Analysis

First and last name, sex, age, calendar year, and running time on all successful female and male finishers in the "Berlin-Marathon" from 1974 to 2019 were obtained from the official race website (BMW Berlin Marathon, 0000). We cleaned the dataset removing runners with missing or questionable (unreliable) information on race time, i.e., race time under 2 h or over 6 h. With respect to age stratification, finishers were classified in 10-year age groups (e.g., 20–29 years to 70–79 years) to analyze performance and participation. We compared top ten age group runners to the age group average in order to highlight differences in performance over calendar years between those groups and both sexes. Further, we compared top five age group runners of each age-category to average age group runners of the same age category to examine performance declines during aging.

Statistical Analysis

All statistical procedures were carried out using the Statistical Package for the Social Sciences (SPSS version 26. IMB, IL, United States) and GraphPad Prism (version 8.4.2. GraphPad Software LLC, CA, United States). The Shapiro-Wilk and Levene's tests were applied for normality and homogeneity, respectively. Six General Linear Models (two-way ANOVA) were used as follows: model 1 – all participants by sex and calendar year; model 2 – top ten athletes in each race by sex and calendar year; model 3 – all participants by age group and sex; model 4 – top five athletes in each age group in each race by age group and sex; model 5 – all men participants by age group and calendar year; model 6 – all women participants by age group and calendar year. When interactions were found ($p < 0.05$), pairwise comparisons were applied to identify the differences more accurately. Calendar years without sex or age information were removed from the analysis. For performance analysis, age groups, or calendar years with less than five participants were removed. Applying those criteria, the calendar years 1976 to 1984, 1994 to 1998, and 2019 had to be removed from the performance × years for men and women analysis, the calendar years 1976 to 1978, 1980 to 1981, 1994 to 1998 and 2019 had to be removed from the performance × years × age group analysis for men and the calendar years 1974 to 1984, 1994 to 1998 and 2019 had to be removed from the performance × years × age group analysis for women. For the participation × year analysis for both sexes (total participants)

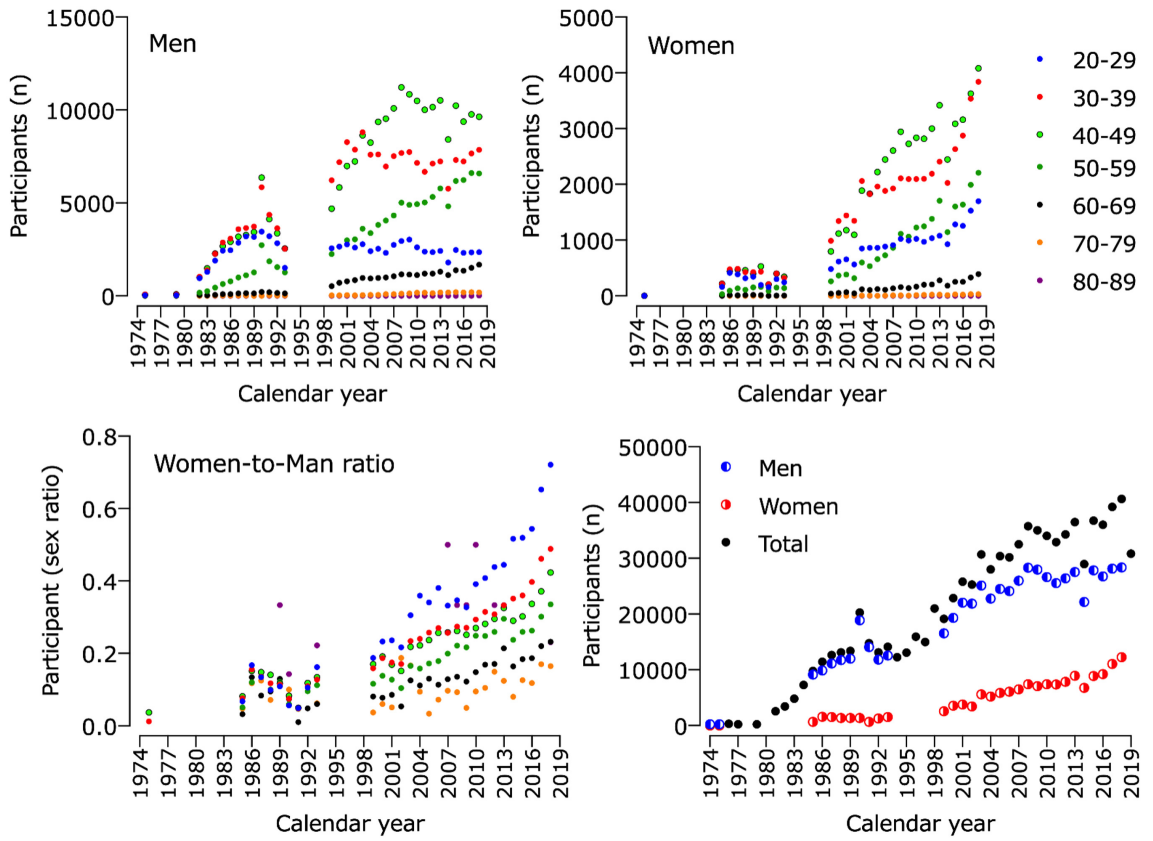

FIGURE 1 | Number of participants in "Berlin Marathon" from 1974 to 2019 by sex and age groups.

we had to remove the years 1976 to 1984, 1994 to 1998 and 2019, for the participation × sex × age analysis, we had to remove the years 1974, 1976 to 1978, 1980 to 1981, 1994 to 1998 and 2019 for men and the years 1974, 1976 to 1984, 1994 to 1998 and 2019 for women. The level of significance utilized was $p \leq 0.05$.

RESULTS

The total number of athletes ever registered in the 46 editions of the "Berlin-Marathon" between 1974 and 2019 was 884,927 finishers. After filtering out invalid data regarding our criteria, 696,225 finishers were included for the final analysis. The number of athletes participating in the "Berlin-Marathon" increased from only 236 men and 8 women in 1974 to 28,373 men and 12,268 women in 2018 (**Figure 1**). Moreover, all age groups increased their participation, except for male athletes aged 20–49 years and athletes of both sexes above 79 years old (**Figure 1**).

The first two ANOVA models (model 1 and 2) to analyze performance showed a significant effect on sex, year, and interaction sex × year (**Table 1**). However, pairwise comparisons and the linear trend show that model 1 tends to increase race time across calendar years. In contrast, in model 2, which only included the top ten athletes in each race, race time has a decreasing trend across calendar years (**Figure 2**).

Models 3 and 4 showed significant effects on sex, age group, and interaction sex × age group (**Table 1**). Both models showed similar performance trends across age groups, with the lowest race times between 20 and 39 years old, and slight increases across each next age group (**Figure 3**).

Models 5 and 6 showed significant effects for age group, year, and an interaction age group × year (**Table 1**). Both men and women showed similar performance trends among all age groups, with increasing race time across calendar years (**Figure 4**).

DISCUSSION

The main aims of this study were (i) to analyze the changes in participation and performance trends of age group marathon runners in the "Berlin-Marathon" for all its previous editions, (ii) to compare the sex differences in performance as a function of age across the years, and (iii) by this to provide one more complete analysis on participation and performance of female athletes in the history of a significant event in order to allow best possible future findings of particularities in female sports physiology. The main findings were that (i) the number of finishers increased for both women and men

TABLE 1 | ANOVA results of performance analysis in "Berlin marathon" in different models.

	Factor	*F*	*p*-value
Model 1	Sex	294.5	<0.001
	Year	29.0	<0.001
	Sex × Calendar year	85.4	<0.001
Model 2 (Top ten athletes)	Sex	91.6	<0.001
	Year	3.4	0.001
	Sex × Calendar year	12.8	<0.001
Model 3	Sex	280.4	<0.001
	Age group	155.3	<0.001
	Sex × Age group	19.9	<0.001
Model 4 (Top five athletes in each age group)	Sex	29.5	0.001
	Age group	38.7	<0.001
	Sex × Age group	13.1	<0.001
Model 5 (Men)	Age group	208.3	<0.001
	Calendar year	80.0	<0.001
	Age group × Calendar year	7.6	<0.001
Model 6 (Women)	Age group	100.4	<0.001
	Calendar year	39.2	<0.001
	Age group × Calendar year	1.8	<0.001

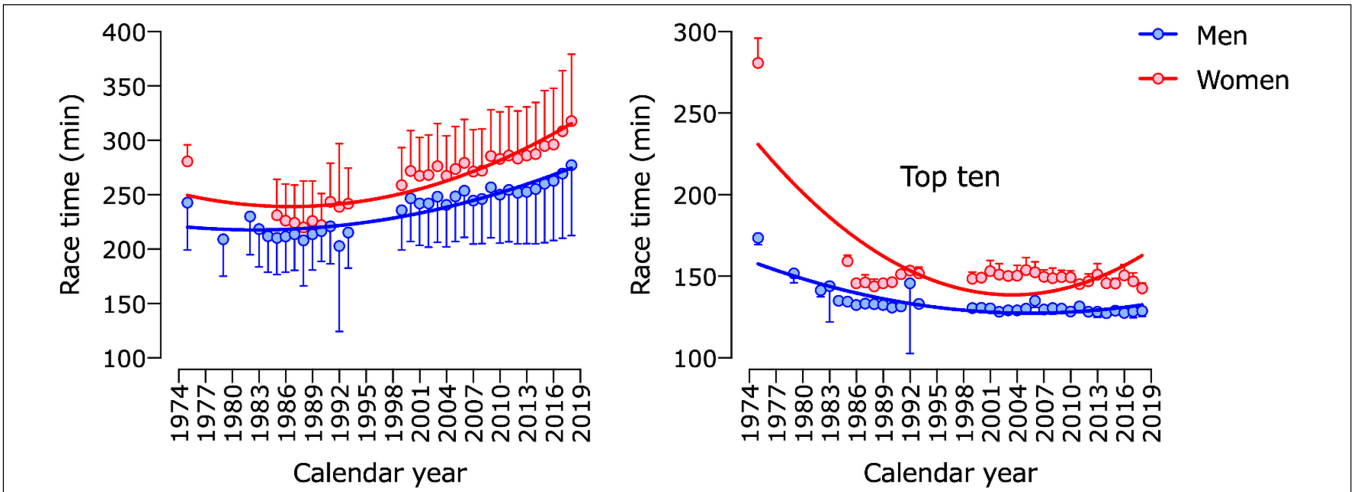

FIGURE 2 | Race time of men and women in "Berlin Marathon" across calendar years.

runners over the decades, (ii) this increase in participation was more pronounced in women than in men, and (iii) all age group athletes decreased their overall performance, whereas the top ten recreational athletes improved their performance over the years.

Participation Trends

The first important finding was that the number of finishers increased for both women and men runners over the decades, which supports our first hypothesis. In the first edition of the "Berliner Volksmarathon" in 1974, only 236 men and 8 women took part, and by 2018 there were 28,373 men and 12,268 women participating. The increase in participation in recent years was more pronounced in women, whereas men of the age groups 20 – 49 years showed a slight decrease in participation numbers in the last two decades. This narrowing of the gender gap in participation has been previously reported in well-studied city marathons such as the "New York City Marathon" (Hunter and Stevens, 2013; Nikolaidis et al., 2018) and the "Boston Marathon" (Knechtle et al., 2020). Some researchers even predicted a closing of the gender gap in participation in the marathon; however, it has not appeared yet for the large city marathons such as the "New York City Marathon" (Jokl et al., 2004; Knechtle et al., 2020; Vitti et al., 2020) and after analysis of the present study, neither in the "Berlin-Marathon" so far.

Still, an extensive but not officially validated research of Andersen et al. showed that in 2018 – "for the first time in history" – there were more female than male runners competing worldwide (50.24% women) (The State of Running, 2019).

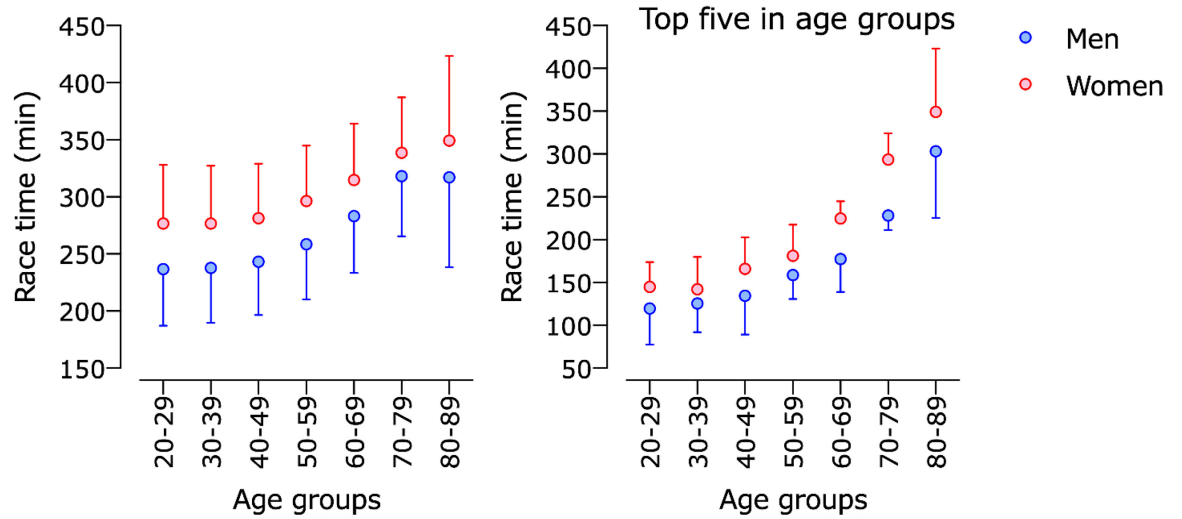

FIGURE 3 | Race time of men and women in "Berlin Marathon" across age groups.

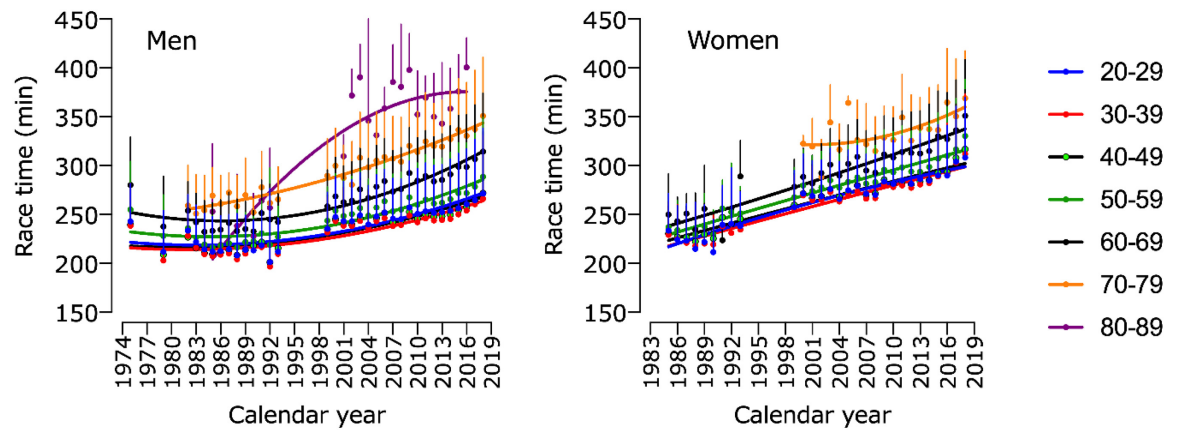

FIGURE 4 | Race time of men and women in "Berlin Marathon" in age groups across calendar years.

The analysis by Andersen and colleagues covered 96% of US-based running race results, 91% of the race results from the EU, Canada, and Australia and a "big portion" from Asian, Africa, and South America. Andersen et al. analyzed not only marathon races, but all types of running events. This may be the crucial point: Women seemed to be more engaged in shorter running events. Moreover, it was noted that the longer events were, women were less likely to participate showing a decreased number of starters in relation to men. However, further investigation in worldwide participation in running events of different distances considering demographical conditions of the participants is required. However, also an analysis of running races from one single country would be of interest.

A significant observation described previously by Nikolaidis et al. (2018) found that women, in general, start and stop racing at a younger age than men. That men-to-women-ratio in the older age groups compared to their younger counterparts could be explained through the existence of historical and social barriers (Vitti et al., 2020). Pointing in the same direction are the findings of Andersen et al., who – by comparing sex participation rates for running events between different countries – found a clear correlation between "general gender equality" and equality in participation rates (The State of Running, 2019). When taking into account the historical perspective of women's competitive sports, female athletes have been subjected to a variety of discriminatory practices and gender-based social barriers, many of which are still ongoing (Costello et al., 2014). The 2012 Summer Olympic Games were an important milestone, whereby every participating country's delegation included at least one female competitor (Costello et al., 2014), albeit a positive step, but far from what is required for a true closing of the gender gap.

However, it must be noted that some countries have been successful in improving women's participation rates and some have even tipped the scales completely. Higher participation rates in running events for women than for men have been

observed with Iceland on the top of the board (59%), followed by the United States (58%) and Canada (57%) (The State of Running, 2019). Switzerland (16%) and Italy (19%) are among the countries with the least female participation in running events (The State of Running, 2019). In the case of Switzerland, this participation rate seems to contradict to the fact that 49% of the regularly running population in Switzerland (which is about one-third of the Swiss population) are women (Sport Schweiz 2020, 2020). This finding may be quite surprising, given the mentioned country's apparent progressiveness, but not so, if one considers the repeated international criticism on Switzerland's gender policy (Human Rights Switzerland, 2021). When examining sports such as road cycling, the gender gap in Switzerland seems even more prominent (in 2019, only 14% of the licensed road cyclists in Switzerland were women) (*personal and unpublished written communication with Stefania Ratano, the responsible for members and licenses at the Swiss national cycling federation "Swiss Cycling," mid of June 2020*). This possible correlation between actual and effective "general gender equality" and participation rates in endurance events could be an interesting subject for future research.

Performance Trends

As expected, performance in all age group athletes decreased, whereas the top ten age group athletes improved their finishing times across calendar years. This tendency was found for the "Boston Marathon" already (Maffetone et al., 2017; Knechtle et al., 2019, 2020). Vitti et al. (2020) analyzed 1.2 Million runs "during half a century" in the "New York City Marathon" and described the phenomenon of "the faster get faster and the slower get slower." They stated that nowadays, more women, more recreational and more elderly runners participate in most of the marathons worldwide, while in the 1970s, participation was limited to mainly elite male runners (Vitti et al., 2020). Knechtle et al. (2018) who analyzed men's participation and performance in the Boston Marathon from 1897 to 2017 commentated the same way: There is more variability on performance introduced by the increased number of age group runners in marathon running. We also see these changes in the marathoners' community over the years as an important factor that explains why "the slower get slower."

The factors that influence the improvement in running times of the already fast marathoners seem to be more complex and multi-factorial. Historically, marathon running training, pre-race preparation, nutrition, fluids and equipment were significantly different than what is available today (Ineos 1:59 Running Challenge, 2019; Joyner et al., 2020). Special attention should be given to recent advancements in running shoe technology and as a result, improved running times by professional runners, who primarily wear them (Carbon Fiber Racing Shoe Battle, 2020). In 2017 "Nike" released the first carbon fiber shoe, triggering a technology advancement race between commercial shoe companies (Carbon Fiber Racing Shoe Battle, 2020). Independent tests showed significantly lower oxygen uptake by runners at higher running speeds wearing carbon sole "Nike" shoes (Carbon Fiber Racing Shoe Battle, 2020; Hoogkamer et al., 2018; Hunter et al., 2019).

Therefore, improvements in "running economy" seem to be crucial for the constantly dropping running times. This is also seen in analyses of East-African runners, who comprise most of today's elite in big-city-marathons (World Athletic Records, 2021). Those runners, who originate from specific regions in Kenya and Ethiopia (Scott et al., 2003; Onywera, 2009), show a profile that allows them to run with an exceptional high running economy (Weston et al., 2000; Lucia et al., 2006; Kong and De Heer, 2008; Mooses and Hackney, 2017). This outstanding running economy is seen as one of the important factors for the dominance of East-African runners (Weston et al., 2000; Kong and De Heer, 2008; Mooses and Hackney, 2017). Still, even if the reasons for Kenyan and Ethiopian apparent dominance in endurance running races have been deeply analyzed, there remains no clear consensus on what contributes to their dominance (Hamilton, 2000). So, one part of the explanation why the "faster" become faster, could be a growing participation rate of mentioned African ethnicities in the big city marathons during the last decades (Vitti et al., 2020). Still, extensive research about worldwide participation rates in marathon running for the last decades split up in ethnicities or countries of origins are missing.

Not all studies conducted on a large population of endurance athletes find improving race times of the "fast athletes" and slowing race times of recreational athletes in the past though. Research conducted on elite and master Ironman triathletes e.g., showed improving race times for both these groups of athletes during the last decades while also the average age of the athletes augmented (Lepers et al., 2013; Gallmann et al., 2014). Regarding at these results, the importance of interaction of age of a certain study population and age of peak performance in endurance sport has to be underlined. Future studies need to take into consideration the current ideas about age of peak performance (see below) in a certain discipline and changing age of participations over decades of analysis.

Athletes in the age groups 20–29 years and 30–39 years showed the fastest race times in the "Berlin-Marathon" for both sexes, as well as the top five were the fastest in those two age groups. For the following older age groups, we constated slight increases in race times. Above the age of 60 years, the increase in average race time was more pronounced, with a greater decline of performance for top five age group athletes than for the average age group. All those findings account for both sexes, with only minor differences in-between.

There is no consensus about the precise age of peak performance and the dynamics of the age-related performance decline in endurance sport in the current scientific literature (Lara et al., 2014; Zavorsky et al., 2017; Nikolaidis et al., 2018, 2019a; Jäckel et al., 2020). Depending on the discipline ("locomotion models") (Jäckel et al., 2020), the study population (recreational athletes versus top age group athletes (Lepers and Cattagni, 2012; Zavorsky et al., 2017) versus top professional athletes (Knechtle et al., 2018, 2019) and other factors like research period (Leyk et al., 2007; Lara et al., 2014; Knechtle et al., 2018), the outcomes are different. For example, Jäckel et al. (2020) stated a progressive running performance decline for recreational half-ironman triathletes after the age of 50 years. The same was

constated for age group marathoners by Zavorsky et al. (2017) who examined data from the New York City, Boston and Chicago marathons, in addition to Leyk et al. (2007) who examined 69 marathons and 65 half-marathons performed between 2003 and 2005 in Germany. At the same time, Käch et al. (2018), who investigated recreational Ironman triathletes, reported a much earlier decline in running performance starting at about 30–34 years in women and 35–39 years in men. Reaburn et al. summarized the same for recreational runners (Reaburn and Dascombe, 2008). Their review reported that the declines in performance are curvilinear from age 35 years until approximately the age of 60–70 years (Reaburn and Dascombe, 2008). Additionally, Lepers et al. (2010) showed that elite triathletes maintained their performance up to the fourth or fifth decade of life, i.e., a curvilinear decline from 50 years onward in Olympic Triathlon World Championships and from 45 years onward in the "Ironman Hawaii."

Also, there is the question of sex and age of peak performance, which is important for athletes and coaches to plan a career (Allen and Hopkins, 2015). In contrast to existing findings reporting a higher age of peak marathon performance in women compared to men (Nikolaidis et al., 2018), it was found that women achieved their best marathon race time ∼5 years earlier in life than men by analyzing all finishers of the "New York City Marathon" between 2006 and 2016 (Nikolaidis et al., 2018). More data from big events over large periods of time is needed to discuss those questions and find consensus about the age of peak performance and the dynamics of the age-related performance decline in endurance sport.

Limitations

Several limitations of this study should be noted. First, the data obtained for the "Berlin Marathon" database only included finishing times, gender and age of the participants. Other factors, such as training volume and intensity, previous experience, ethnicity and physiological variables (VO_2max, lactate threshold, and running economy) were not recorded. Still, those other factors are known to affect endurance running performance, and therefore some part of the variance in endurance performance explained by age may actually be related to those (Lara et al., 2014). For this reason, the outcomes found in this investigation should be reinforced by collecting experimental data. Second, there are limited participation places for the "Berlin Marathon," which are allocated by raffle. Information about the year of installment of the raffle and places distributed per year and sex since then are not available[2]. If there had been a distribution key respecting the sex of participants, it consecutively must have had an influence on sex ratios found in finisher reports and thereby on our results. At the same time, drawing lots shouldn't influence mean running times because the sample sizes in "Berlin Marathon" are large enough to ensure an evenly distributed composition of the runners. In the present study, we only considered finishing numbers but neither registration nor starting numbers, which are not accessible to public "(see text footnote 2)". By this, we cannot exclude to actually report more on the ability of participants to make it to the finish line within the limit of 6 h, than on the actual trend to participate and register for the "Berlin Marathon." We do not expect the latter to differ by much, still those analyses would be needed to confirm our findings. Third, sex and age data are unavailable for the years 1976 to 1984, 1994 to 1998 and 2019. For those years, the analyses are missing between sexes, age groups, and sex ratio. Therefore, our analysis on that subject is not fully complete. Nevertheless, the available data allows us to see trends and make statistically reliable statements on sex ratios. Further, the way the platform of "Berlin Marathon" displays the data about participants has changed throughout the years, so as how they store data (BMW Berlin Marathon, 0000). For some years, we suspect that they calculated the age of each participant as "current year – year of birth." This can generate a problem with participants with missing data because they would enter as 90 + years old, and we excluded this age group from our analysis. Finally, in order to make statements about age of peak performance and the dynamics of age-related performance declines in marathon running, data should be split up into rather small age group fragments. The present work analyzed age groups in 10-year intervals, which doesn't allow us to draw conclusions regarding this issue.

[2]www.bmw-berlin-marathon.com/en/your-registration/registration-information/general/

CONCLUSION

This study tested the hypothesis that for "Berlin-Marathon" over all its previous 46 editions (1974–2019), participation of age group athletes would increase, the over-all performance of age group athletes would decrease and top age-group performances would improve over calendar years. Participation for female and male runners increased, with a stronger increase in female participation and thereby narrowing sex gap in participation, the fastest age group women and men became faster across years and average age group performance decreased. This is the largest dataset ever studied in this event and provides valuable information in the ongoing research about characteristics in participation and performance in large city marathons. Future studies might investigate the influence of other parameters such as country of origin, training volume, training years and motivation of athletes to understand how training and life of athletes can be planned best to achieve maximal performance.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MR and BK designed the study. EV collected the data. CS performed the statistical analyses. JA, LH, TR, and PN contributed by writing and editing a part of the manuscript. All authors read and approved the final manuscript.

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Physical Activity as a Preventive Lifestyle Intervention Acts Through Specific Exosomal miRNA Species—Evidence From Human Short- and Long-Term Pilot Studies

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Exercise initiates systemic adaptation to promote health and prevent various lifestyle-related chronic diseases. Emerging evidence suggests that circulating exosomes mediate some of the beneficial effects of exercise via the transfer of microRNAs between tissues. Yet to date, a comprehensive profile of the exosomal miRNA (exomiR) content released following short-term (0.5 year in this study) and long-term (25 + years in this study) regular bouts of exercise is still lacking. However, a better understanding of these miRNA species would assist in clarifying the role of regular exercise at the molecular level in the prevention of chronic diseases. In the present pilot studies we analyzed serum exomiR expression in healthy young, sedentary participants (n = 14; age: 23 ± 2 years) at baseline and following a half year-long moderate-intensity regular exercise training. We also analyzed serum exomiR expression in older, healthy trained participants (seniors, n = 11; age: 62 ± 6 years) who engaged in endurance activities for at least 25 years. Following the isolation and enrichment of serum exosomes using Total Exosome Isolation Reagent (TEI) their exomiR levels were determined using the amplification-free Nanostring platform. Hierarchical cluster analysis revealed that the majority of exomiRs overlap for short-term (0.5 year in this study) and long-term (25 + years in this study) regular bouts of exercise. The top 12 significantly altered exomiRs (let-7a-5p; let-7g-5p; miR-130a-3p; miR-142-3p; miR-150-5p; miR-15a-5p; miR-15b-5p; miR-199a-3p; miR-199b-3p; miR-223-3p; miR-23a-3p, and miR-451a-3p) were used for further evaluation. According to KEGG pathway analysis a large portion of the exomiRs target chronic diseases including cancer, neurodegenerative and metabolic diseases, and viral infections. Our results provide evidence that exosomal miRNA modulation is the molecular mechanism through which regular exercise prevents various chronic diseases. The possibility of using such exomiRs to target diseases is of great interest. While further validation is needed, our comprehensive exomiR study presents, for the first time, the disease-preventive molecular pattern of both short and long-term regular exercise.

Keywords: regular exercise, exosome, miRNA, chronic disease, prevention

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INTRODUCTION

Regular exercise has been known as a major intervention tool not only to attenuate the risk of a multitude of disorders from metabolic disease and neurodegenerative disorders to cancer, but also to delay the occurrence of numerous age-related diseases (Brahmer et al., 2019). While most molecular mechanisms mediating the long-term beneficial effects of exercise remain unexplored, growing evidence suggests the involvement of tissue crosstalk via the release of exosomes following exercise (Frühbeis et al., 2015; Estébanez et al., 2021). Exosomes are small extracellular vesicles (sEVs) (30–150 nm) that are secreted by fusion of multivesicular bodies to the plasma membrane (Brennan et al., 2020). These vesicles transport a large variety of cargo molecules including miRNAs, DNA and proteins that may be taken up by distant cell types and alter the phenotype of these recipients (Kowal et al., 2014). Since miRNA species are well recognized for playing important roles in many physiological and pathological processes, they could also be involved in exercise-related benefits of disease prevention. Deciphering the contribution of miRNAs present in exercise-derived exosomes and their downstream targets is crucial for the better comprehension of how preventive lifestyle actually acts at the molecular level. According to a study, the expression of certain circulating miRNA species increases with age in plasma microvesicles (Rani et al., 2017). Of notable example, hsa-miR-223-3p, hsa-miR-23a-3p, hsa-let-7g-5p, hsa-miR-199a-5p, hsa-miR-15a-5p, and has-miR-142-3p show positive correlation with age and the development of specific chronic diseases (Rani et al., 2017). Recently it has been shown that healthy aging is also reflected by the profile of circulating exosomes, and exercise−induced beneficial effects may be related with the modulation of these exosomes (Bertoldi et al., 2018). There are reports indicating the changes of various miRNA species in exosomes following an acute of exercise (D'souza et al., 2018; Yin et al., 2019), however only a small number of studies examine exosomes in response to long-term training (Nederveen et al., 2021). Of note, in a mammalian study the levels of miR−19b, miR−148a, miR−150, miR−221, miR−361, and miR−486 were up-regulated during the first month of exercise, but returned to baseline by completion of a 4−month study period (Muroya et al., 2015). Regarding long-term human experiments, a significant increase in exosome release was shown after a single bout of flywheel exercise (Annibalini et al., 2019), whereas no change was found after a full year of rowing training (Hou et al., 2019). These conflicting results could potentially be attributed to the adaptation process that occurs with time. Additional research is crucial with various training modalities and durations to further understand the role of exosomes and their miRNA content in the prevention of chronic diseases induced by long-term exercise. In the present study first we investigated the effect of a 0.5 year-long, moderate intensity, personal trainer-supervised, concurrent resistance and aerobic training program on the overall circulating exomiRs expression profile of healthy, young, previously sedentary individuals. We also assessed whether exomiRs differentially expressed after a 0.5 year regular exercise in young adults were similarly present in healthy senior trained participants who have engaged in regular exercise activities for at least 25 years. The effect of short- and long-term regular exercise on the miRNA profile was determined by comparing baseline vs. 0.5 year, and baseline vs. 25 + years miRNA levels. As anticipated we found that the levels of the exomiRs are fairly consistent in comparison of the 0.5 year (short-term adaptation) and the 25 + years (long-term adaptation) active groups. Twelve exomiRs showed overlap for both study periods (baseline vs. 0.5 year and baseline vs. 25 + years). Of note, all of them were significantly down-regulated. Bioinformatics analysis was used to evaluate the interplay between biological signaling pathways offering insight into mechanisms linking exercise and chronic disease prevention. Our results prove that full miRNome analysis might be a useful tool to identify exomiRs acting on particular pathways that prevent the development of specific chronic diseases.

MATERIALS AND METHODS

Participants and Applied Training Protocol

Healthy young, sedentary (n = 14; age: 23 ± 2 years) and senior trained (n = 11; age: 62 ± 6 years) individuals were recruited. Participants were in good general health, defined as having no chronic diseases (e.g., metabolic disorders, cardiovascular disease, cancer, etc.). Main characteristics of the subjects are summarized in **Table 1** (see **Supplementary Material** for further details). Healthy, young sedentary individuals completed moderate-intensity, concurrent resistance and aerobic exercises regular exercise training three times a week for half a year (Garai et al., 2019). Our exercise bouts consisted of four parts: warm-up, resistance training, aerobic exercises and cool-down with stretching. The heart rate of the participants was measured continuously during exercise with a heart rate monitor (Polar Team System, Polar Electro, Finland). Age-predicted maximum heart rates were estimated with the following calculation: 220—age (years). Every trainings began with standardized, active warm-up protocol applying mobility and stability exercises, gymnastic exercises and moderate stretching. After warming-up resistance training was performed. During this part the heart rate of the subjects was allowed to reach 85% of individual heart rate maximum. Aerobic exercises included walking and jogging, if the subject's heart rate was lower than 65% of the individual heart rate maximum. The cool-down protocol included 2 min of slow walking and 8 min of static stretching exercises of all major muscle groups. Participants were asked to keep their diet and daily activity level unchanged during the 6 month-long lifestyle program. Training diary was prepared during the 6 months and compliance was calculated accordingly.

Senior trained subjects were engaged in regular exercise activities for at least 25 years. The exercise behavior of senior participants was assessed with the use of a general lifestyle questionnaire as well as with the International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003). We obtained information on smoking-, alcohol consumption status and physical activity (frequency, type, duration). Senior trained

participants also performed both types of exercise (endurance and resistance training), including running, swimming, weightlifting, cycling, skating, adrenaline sports, walking, hiking and spinning. Over half (54%) of the senior participants performed physical activity on a daily basis, while the rest performed physical activity at least twice a week. For details please check the **Supplementary Material Section**. Each participant gave written informed consent before completing any data collection. The study was conducted according to the Declaration of Helsinki principles and approved by the Regional and Local Ethics Committee of Clinical Center, University of Pecs (ref. no.: 6439/2016 and 7755/2019).

Collection and Preparation of Human Serum Samples

Human blood samples were collected around 7:00 a.m. after a 12 h fasting in blood collection tubes (BD Vacutainer, SST: BD SST Tubes with Silica Clot Activator and Polymer Gel, Franklin Lakes, NJ, United States) at two time-points: at baseline and after the 0.5 year long training program from the young individuals and at one time-point from seniors. Participants were asked to avoid excessive exercise the day before each testing condition. Blood samples were clotted for approximately 30 min at room temperature. Samples were then centrifuged at 1,500 g for 10 min at room temperature. Serum samples were stored at −80°C until further analysis. The same procedure was carried out with the samples of seniors.

Exosome Isolation

In order for their samples to be processed participants had to show min. 85% compliance with regular exercise mandated by the program. Before exosome isolation, equal volumes of serum (100 μl each) from 14 healthy young participants and 11 seniors were pooled, separately (**Figure 1**). Prior to pooling we have carefully evaluated the participants for potential outliers based on the assessment of physiological and blood parameters. Only those participants' samples were pooled who constituted a homogenous population for the evaluated physiological and blood parameters (baseline, 0.5 year, 25 + years). Then, exosomes were isolated from the three pooled serum samples (baseline; n = 1, 0.5 year; n = 1 and 25 + years; n = 1 pooled samples) using TEI (from serum) (Invitrogen, Thermo Fisher Scientific, Waltham, MA, United States) following the manufacturer's protocol. TEI reagents contain volume-excluding polymers (e.g., polyethylene glycol, dextrans, or polyvinyls). According to Andreu et al. (2016) and Banfai et al. (2019), the use of precipitation reagents provide good reproducibility and are suitable for an easy and cost-efficient enrichment of serum exosomes for miRNA analyses. As a result TEI was chosen for studying exosomal miRNA content in our study.

NTA Measurement With Nanosight NS300

NTA Protocol

Exosome-enriched preparations were measured and quantified using Nanosight NS300 instrument (Malvern Panalytical Ltd., Malvern, United Kingdom). The camera level for each sample was manually adjusted to achieve optimal visualization of particles following the manufacturer's instructions. Samples were injected with a syringe pump (infuse = 50). Detection threshold was set for maximum sensitivity with a minimum of background noise. All measurements were performed in five replicates for each sample, collecting 60-s videos. Following capture, the videos were analyzed by the in-built NTA v3.2 software (Gardiner et al., 2013).

Particle Size and Concentration Analysis

The samples of 3 individuals were randomly chosen from each group (baseline, n = 3; 0.5 year, n = 3; and 25 + years, n = 3). All samples were diluted in PBS. Ideal measurement concentrations were achieved by pre-testing the ideal particle per frame value (40–100 particles/frame).

Single EV Direct Immunolabeling and NTA Evaluation

The following monoclonal antibodies were used for immune-labeling: anti-human-CD63-FITC (MEM-259) (Thermo Fisher Scientific) anti-human-CD81-PE/Cy7 (TAPA-1) (Sony Biotechnology). Particle concentrations were established for unlabeled EV sample prior to immune-labeling. The

TABLE 1 | Subject characteristics.

	Baseline	0.5 years	p	25 + years
Age (years)	23 ± 2	23.5 ± 2		62 ± 6
BMI	21.64 ± 1.57	21.46 ± 1.44	0.382	27.92 ± 2.95
Body weight (kg)	60.39 ± 5.42	59.55 ± 5.74	0.166	75.16 ± 7.18
Body fat percentage (%)	31.79 ± 3.39	31.49 ± 3.47	0.61	21.23 ± 6.03
VO_2 max (ml/kg/min)	36.41 ± 6.67	39.81 ± 6.20*	0.047	32.9 ± 6.99
LDL (mmol/L)	2.35 ± 0.9	2.44 ± 0.83	0.481	3.63 ± 1.19
HDL (mmol/L)	1.81 ± 0.55	2.13 ± 0.61*	0.002	1.68 ± 0.53
Glucose (mmol/L)	4.94 ± 0.39	4.63 ± 0.31 **	<0.001	5.67 ± 0.45
Systolic BP (Hgmm)	114.5 ± 14.18	108.07 ± 8.69	0.55	131.2 ± 19.42
Diastolic BP (Hgmm)	76.07 ± 9.19	72.93 ± 7.92	0.223	87.00 ± 9.25

Values are expressed as mean ± SD. Paired t-test ($p < 0.05$; **$p < 0.001$); p-values were calculated for baseline vs. 0.5 year as applicable; VO_2 max, maximal oxygen uptake (cardiorespiratory fitness); LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; BP, Blood Pressure.*

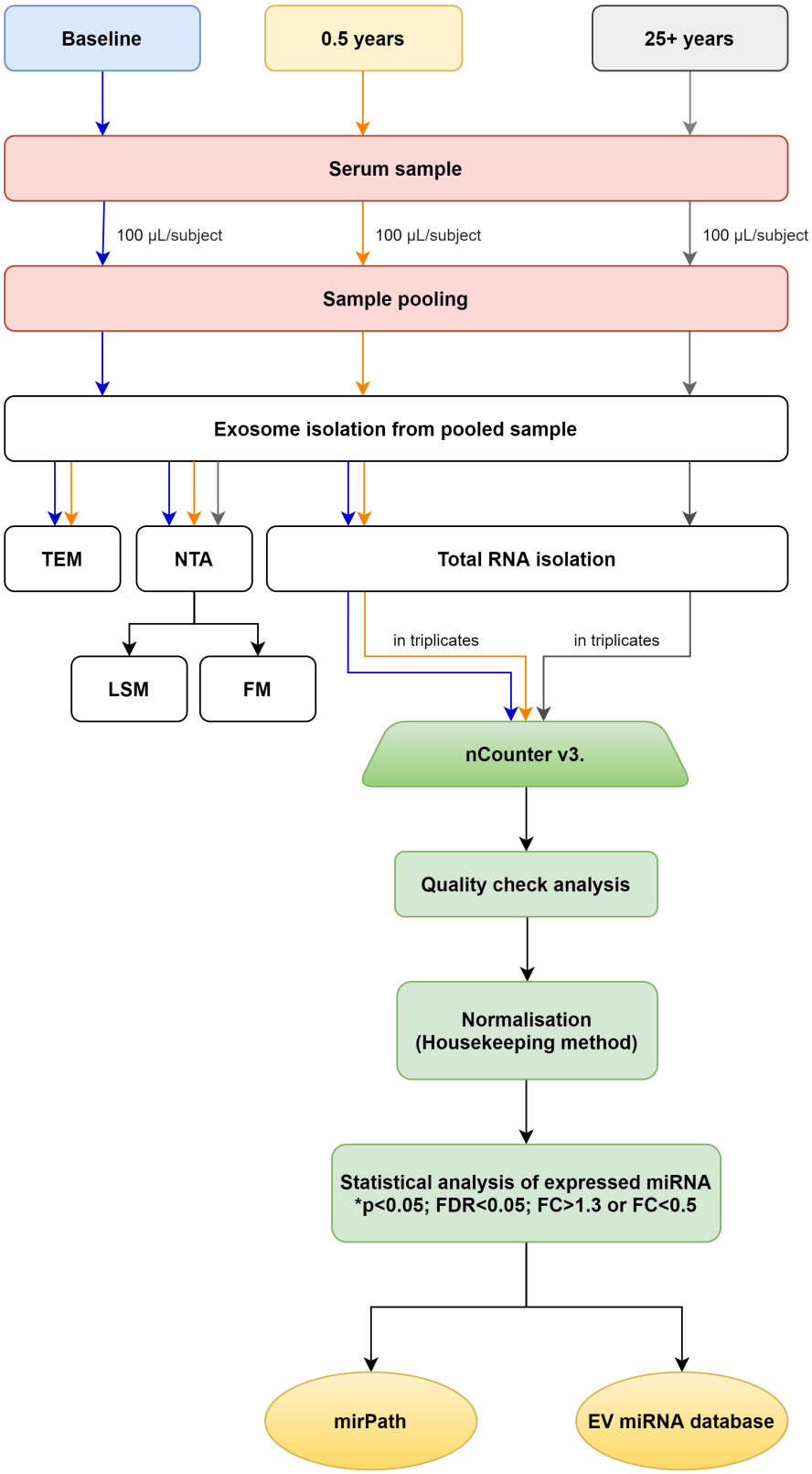

FIGURE 1 | Workflow. Flowchart representing the entire workflow.

concentration of the exosome stock solution was 3.17×10^{10} particles/ml (based on NTA). Sample aliquots were prepared to ensure equal dilution effects for each arm of the experiment. Varying concentration anti-CD63 and anti-CD81 antibodies was added to the 50 μl exosome stock solution to yield a volume of 100 μl and to determine an optimized antibody to exosome ratio for single-vesicle labeling. The samples (anti-CD63 labeled; $n = 1$ and anti-CD81 labeled; $n = 1$) were incubated in the dark for 1 h at room temperature. In order to minimize photobleaching during fluorescence mode (FM), all immune-labeled samples were evaluated first in FM, followed immediately by evaluation in light scatter mode (LSM). Then, the FM/LSM percentage was calculated (Thane et al., 2019).

Transmission Electron Microscopy (TEM)

Exosomes were visualized by transmission electron microscopy. Sample volume of 2.5 μl was placed onto a 300 mesh grid. The grid was left to air dry overnight. Then 5% uranyl-acetate and later 3% sodium-citrate were added to the grid. After 5 min incubation, the grid was allowed to air dry. Twenty four hours later the grid was analyzed using JEOL TEM 1,200 EX. The average diameter of the isolated exosomes was determined using three independent TEM preparations and ImageJ software.

Exosomal Total RNA Purification and Complete miRNome Profiling

Total RNA from exosomes was extracted using Total Exosome RNA and Protein Isolation Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, United States) according to the manufacturer's protocol. ExomiR level profiling was performed using the Nanostring platform (NanoString Technologies, Seattle, WA) according to the manufacturer's instructions to analyze 800 human miRNAs. Since the extraction of exosomal miRNA yields low amounts of RNA, but amplification-free methods require high amounts, we adopted the standard method of using pooled samples to yield reproducible reads. Three technical replicates were run per sample (baseline; 0.5 year and 25 + years groups). Quality check confirmed the reliability of the run and also the validity and reproducibility of the miRNA screening protocol. nSolver software was used for data analysis and normalization. Normalization was performed using the Housekeeping method according to nCounter miRNA expression analysis in plasma and serum samples technote instructions. Briefly, NormFinder was used to identify putative housekeeping miRNAs. First, raw data (RCC files) were imported into nSolver and any sample which failed QC was removed. An experiment was built and background subtraction was set to the Mean + 1 SD of the NEG control probes. Of note, we kept normalization options turned off during this process. Data from the completed experiment were exported into an excel file derived from the normalized dataset. Using NormFinder background subtracted data were sorted by average counts across all samples, and all miRNAs expressed below 50 mean counts were deleted when averaged across all samples. NormFinder created a worksheet listing all the genes and a stability value for each of them. With the aid of NormFinder the potential housekeepers with the most stringent stability values were identified. After that we have applied normalization using the geometric mean of five stably expressed miRNAs (hsa-miR-495-3p; hsa-miR-302d-3p; hsa-miR-3144-3p; hsa-miR-612; hsa-miR-548ar-5p) (Andersen et al., 2004). Quality Control fulfilled all the requirements set by the manufacturer.

Statistical Analysis

All statistical analyses were performed with R (R Core Team, 2019). Paired *t*-tests (baseline vs. 0.5 year) and *t*-tests (baseline vs. 25 + years; 0.5 year vs. 25 + years) were used. We adjusted the *P*-values due to the multiple comparisons therefore False Discovery Rate (FDR) correction was also applied. Heatmap was created in R with the help of "heatmap.2" function from g-plots package (Bolker et al., 2020).

miRNA Target Prediction and Pathway Analysis

After identifying a dozen similarly expressed exomiRs in the active young and senior groups, miRNA—mRNA signaling pathway interaction analysis was performed. Briefly, online available software mirPath v.3 was used for this purpose (Vlachos et al., 2015). Human database of the mirPath v.3 and the TarBase v7.0 were used for mRNA target prediction. *P*-value and MicroT thresholds were kept as default, 0.05 and 0.8, respectively. False discovery rate (FDR) correction was applied.

ExomiRs as Biomarkers of Chronic Disease

The exomiR biomarkers related to certain types of chronic diseases were screened through the EVmiRNA database[1] (Liu et al., 2019). Studies were included if they were original research and evaluated the exomiR levels in a specific disease.

RESULTS

Anthropometric and Physiological Parameters

The study comprised 14 healthy, young, previously inactive and 11 senior trained participants. Healthy, young sedentary individuals completed moderate-intensity regular exercise training three times a week for half a year. Senior subjects have done regular exercise for at least 25 years. Participant parameters are listed in **Table 1**. After half a year of regular exercise, the previously inactive young individuals showed significant improvement in cardiorespiratory fitness (VO_2max), glucose and lipid metabolism. All physiological parameters of senior trained participants were within a normal range. For them, the VO_2max values were far better than the age-matched reference range (**Supplementary Material**).

Validation of Isolated Exosomes

Exosomes were isolated from blood serum samples and obtained from study participants, as described in section "Materials and

[1] http://bioinfo.life.hust.edu.cn/EVmiRNA

Methods." The purified exosomes were characterized using TEM, a gold-standard technique for nanoparticle validation (Kestens et al., 2017). Our TEM analysis showed typical exosomal round morphology (**Figure 2A**). Nanoparticle Tracking Analysis (NTA) allowed us to obtain the size distribution of EVs and estimate particle concentration. The mean size of particles (n = 9) was 143.2 ± 16.43 nm, which falls into the size range of exosomes (Brennan et al., 2020), confirming that the purified EVs contained exosomes (**Figure 2B**; see **Supplementary Material** for further details). Exosome concentrations in our preparations (n = 9) ranged from 1.97×10^{10} to 3.75×10^{10} particles/ml. For details please see the **Supplementary Material section**. Immune-labeled EV sample was evaluated using NTA in FM and LSM modes. The FM:LSM percentage was 83.87% for of CD63. With the CD81-labeled sample, the FM:LSM percentage was 76.95% (**Figure 2C**; please also refer **Supplementary Videos**).

The Expression Patterns of exomiRs After 0.5 Year of Regular Exercise

In order to study regular exercise-related changes in serum exomiR expression, we used amplification-free Nanostring technology. The effect of regular exercise on circulating exomiRs was assessed by comparing baseline (inactive status) and active status (after 0.5 year of regular exercise) expression levels. After analyzing and normalizing raw data, we identified 54 exomiRs (**Figure 3**). Then, we applied filtering criteria to differentiate baseline vs. 0.5 year results ($^*p < 0.05$; #FDR < 0.05; FC > 1.3 or FC < 0.5). Through this analysis, we have observed significant differences in exomiR abundance for several exomiRs (let-7a-5p, $p < 0.05$; let-7g-5p, $p < 0.05$; miR-130a-3p, FDR < 0.05; miR-142-3p, $p < 0.05$; miR-150-5p, $p < 0.05$; miR-15a-5p, $p < 0.05$; miR-15b-5p, FDR < 0.05; miR-199a-3p, FDR < 0.05; miR-199b-3p, FDR < 0.05; miR-223-3p, FDR < 0.05; miR-23a-3p, FDR < 0.05; miR-451a-3p, FDR < 0.05; miR-126-3p, $p < 0.05$; miR-199a-5p, $p < 0.05$; miR-21-5p, FDR < 0.05; miR-25-3p, $p < 0.05$; miR-374a-5p, $p < 0.05$) (for further details please refer to **Supplementary Material**) (ArrayExpress accession number: **E-MTAB-10067**).

ExomiR Overlap of the 0.5 Year- and the 25 + Years of Exercise Groups

Going further we then wished to assess whether exomiRs differentially expressed after 0.5 year of regular exercise were similarly expressed in healthy senior trained participants who engaged in endurance activities for at least 25 years. Therefore, using Nanostring technology we examined the miRNA copy numbers in 11 trained senior individuals focusing on the levels of serum exomiRs. Then, we utilized a hierarchical clustering method to compare circulating exomiR profiles at baseline, after 0.5 year and 25 + years of exercise. As shown by **Figure 3**, the 0.5 year and 25 + years group share an exomiR expression profile that is completely different from that of the sedentary group. In contrast, the 0.5 year and 25 + years active groups showed a highly similar exomiR expression pattern. In addition, 12 exomiRs (let-7a-5p; let-7g-5p; miR-130a-3p; miR-142-3p; miR-150-5p; miR-15a-5p; miR-15b-5p; miR-199a-3p; miR-199b-3p; miR-223-3p; miR-23a-3p, and miR-451a-3p) showed overlap between the two tested signatures (baseline vs. 0.5 year and baseline vs. 25 + years) (**Supplementary Material**). Notably, all 12 exomiRs were significantly down-regulated both in the 0.5 year and the 25 + years trained groups as compared to the sedentary group. Having performed a detailed comparison of the 0.5 year vs. 25 + years trained group profiles, miR-411-5p ($p < 0.05$; FC = 0.879) and miR-144-3p (FC = 1.322)

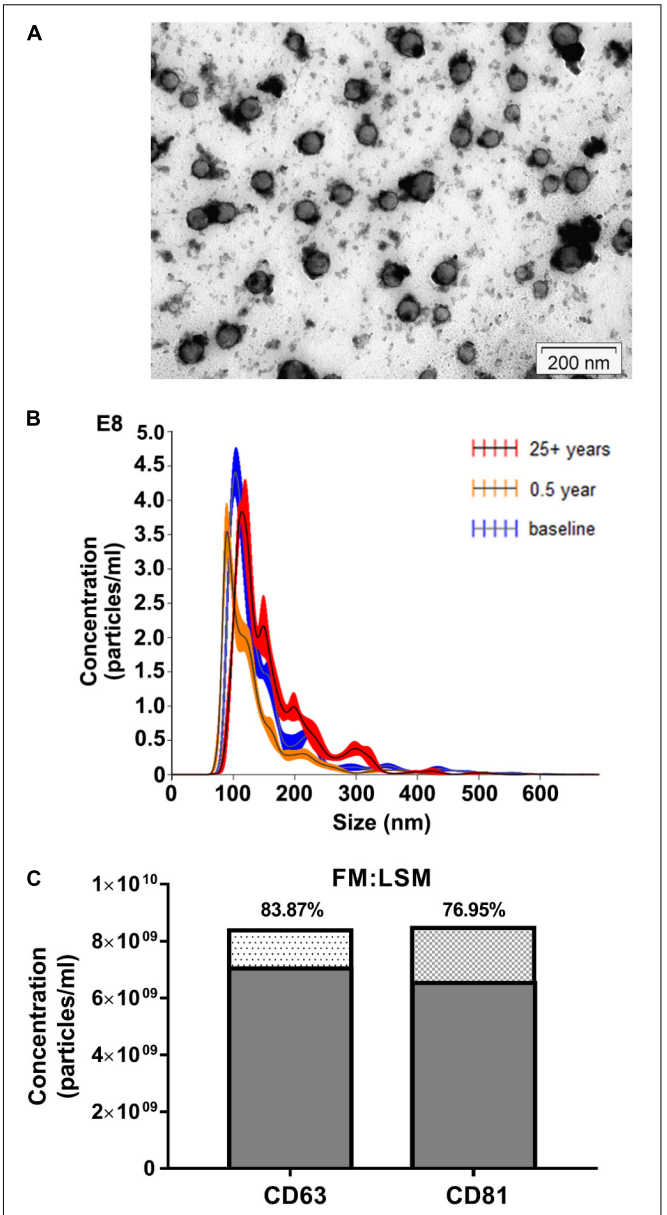

FIGURE 2 | Characterization of exosome-enriched preparations isolated from human serum. **(A)** Transmission electron microscope (TEM) image of exosomes purified from human serum samples (Scale bar: 200 nm). **(B)** Analysis of EV size distribution and concentration in exosome-enriched preparations using a NanoSight NS300 instrument. **(C)** Evaluation of CD63 and CD81 surface expression abundance in exosome-enriched preparations using a NanoSight NS300 instrument.

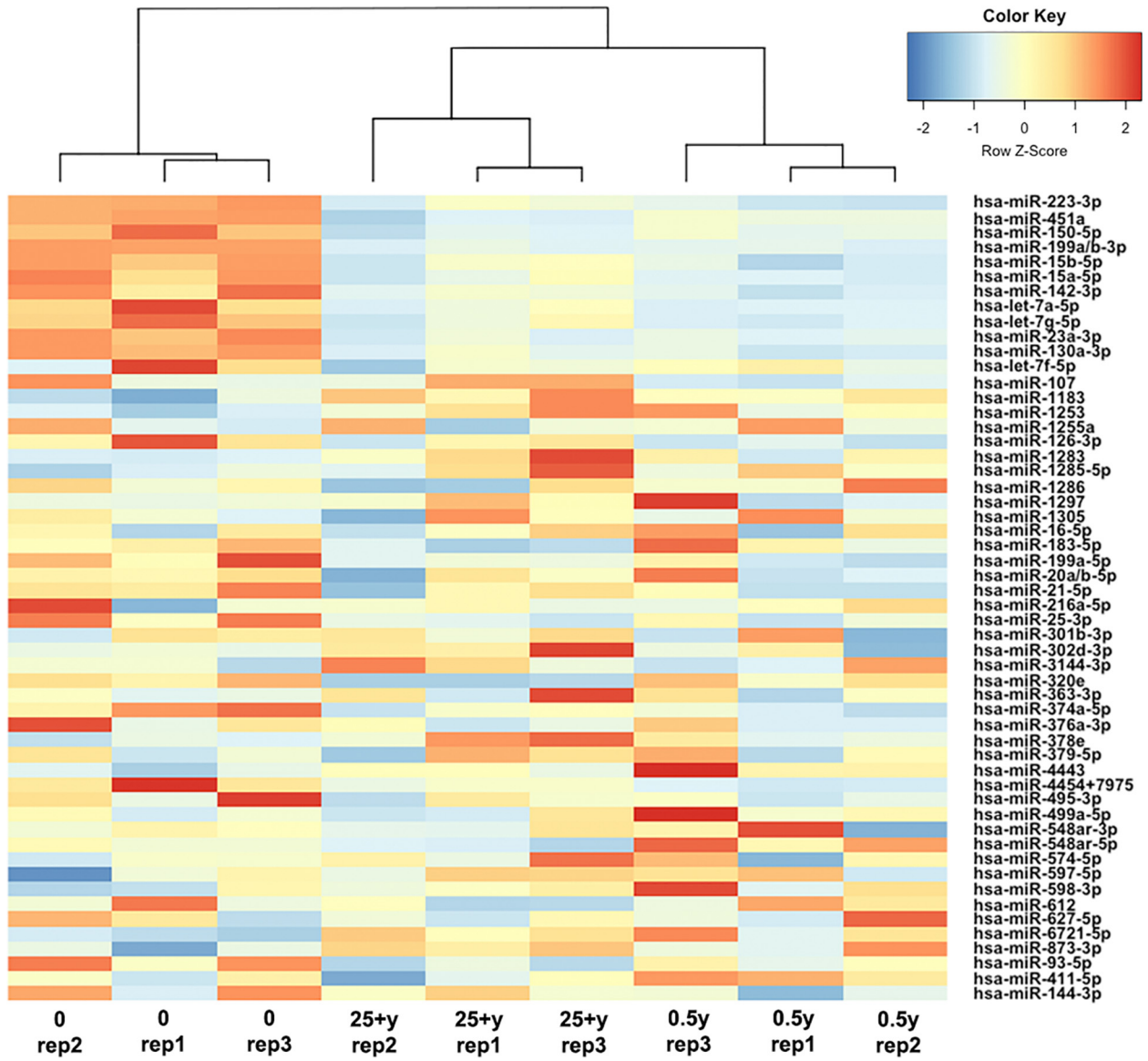

FIGURE 3 | Differential expression of circulating exomiRs. Heatmap with dendrogram shows clustering results for 54 exomiRs at baseline (0), after 0.5 year and 25 + years of regular exercise. Colors represent the level of exomiR expression; red: high expression; blue: low expression.

showed remarkably different expression. Specifically, miR-411-5p was significantly down-regulated, while miR-144-3p was up-regulated in the 25 + years trained group.

Pathway Analysis

To better understand how these exomiRs may contribute to the health benefits of exercise, we examined the mRNA targets of the 12 similarly expressed exomiRs of the 0.5 year and 25 + year trained groups. Then, to reveal the top targeted pathways associated with each exomiRs, KEGG database analysis was used. We found that 38 KEGG signaling pathways were significantly affected by the 12 selected exomiRs (**Figure 4B**). Of these Pathways in cancer (hsa05200) had the largest number of targeted mRNAs (148 genes) (**Figure 4A**). The 148 genes were targeted by four differentially expressed exomiRs (let-7a-5p; let-7g-5p; miR-15b-5p; miR-23a-3p). These findings are consistent with the fact that regular exercise is associated with reduced risk of cancer development. Going further, most exomiRs targeted proteoglycans in cancer pathways (nine exomiRs) and let-7g-5p appeared to affect the most pathways (26 pathways) (for details see **Supplementary Material 2**).

ExomiRs as Biomarkers of Chronic Diseases

exomiR biomarkers related to specific diseases were evaluated using an EVmiRNA database. It is the first concise database

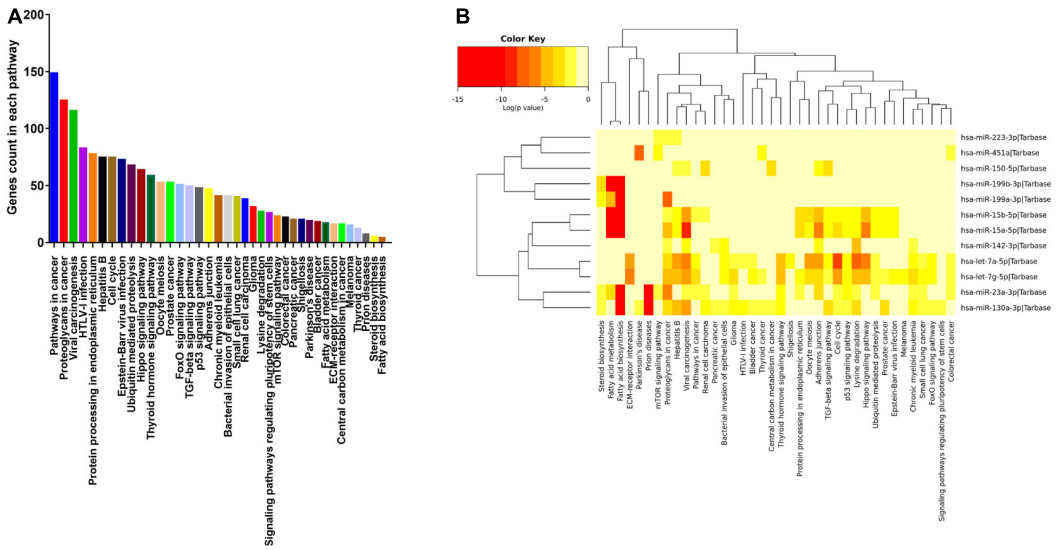

FIGURE 4 | Pathways targeted by the 12 similarly expressed exomiRs in the active young and active senior group. **(A)** Number of genes targeted within each pathways ranked based on high to low. **(B)** The 12 similarly expressed exomiRs in the active young and active senior group regulate 38 signaling pathways in total.

focusing on miRNA expression profiles in EVs (Liu et al., 2019). Several studies have reported the aberrant expression of the 12 identified exomiRs in various chronic diseases (as summarized by **Table 2**).

DISCUSSION

Regular exercise has a beneficial role in preventing a number of chronic diseases. This is primarily due to the fact that regular exercise acts at a systemic level (Anderson and Durstine, 2019). However, a gap remains between identifying in detail the molecular mechanisms induced by exercise and the observed potential benefits in health (Sanford et al., 2020). A better understanding of these biological processes and pathways could allow for the development of targeted exercise intervention and also provide basis for developing exercise-mimetic molecular level interventions (Sanford et al., 2020).

Therefore, in the present study we examined, for the first time, the effect of short-term (0.5 year in this study) and long-term (25 + years in this study) regular exercise on global circulating exomiR profile. To the best of our knowledge, this is the first study to use an amplification-free platform (Nanostring) to determine the miRNA expression profile of exercise-derived exosomes as most studies of the field evaluate specific miRNA species by amplification-based RT-qPCR (Estébanez et al., 2021). The technology applied in the current study is not only amplification-free, but also a sensitive, robust and reproducible state-of-the-art method (Hong et al., 2021). Exosomal miRNA analysis showed a significant number of differentially expressed exosomal miRNAs in all group comparisons. Comparing the miRNAs enriched or depleted in both groups (0.5 year and 25 + years), we have identified 12 similarly regulated exomiRs in the young and senior trained groups as compared to the sedentary group as shown by **Figure 3** (for details please refer to **Supplementary Material**). The KEGG pathway analysis of similarly expressed serum-derived exomiRs confirmed their involvement in pathways related to cancer development affecting TGF-beta, p53 and mTOR signaling. In support of our observations, physical activity has been shown to be associated with lower cancer risks (Li et al., 2020). Moreover, the overall cancer incidence is lower in athletes than in the general population (Sormunen et al., 2014). Recently, a number of studies have indicated that certain exosomal miRNA species (**Table 2**), can be used as biomarkers of cancer and other chronic diseases (references of the studies are listed in **Table 2**).

An elevated expression level of miR-23a has been identified in the serum of various types of human cancer, including breast, gastric, pancreatic, and esophageal squamous cell carcinoma (Wang et al., 2019). Further analysis showed that miR-23a travels as exosomal cargo, and circulating exosomal miR-23a is up-regulated in the serum of early stage colorectal cancer patients (Yong et al., 2013). As a robust cellular regulator of gene expression, miR-23a targets a broad range of mRNA species in cancer cells by directly binding to their three prime untranslated regions (3'-UTR), which in turn suppresses gene expression (Wang et al., 2019). For example, the up-regulation of miR-23a in gastric cancer promotes cell proliferation and inhibits apoptosis (Hua et al., 2018). Zhu et al. (2010) suggested that miR-23a can target IL6R in gastric adenocarcinoma thus encouraging the proliferation of tumor cells. Based on literature data, the inhibition of miR-23a by antisense oligonucleotide inhibits proliferation and promotes the apoptosis of gastric adenocarcinoma cells (Liu et al., 2014). Its biological functions encompass drug resistance, metastasis formation and cancer

TABLE 2 | Summary of exomiR biomarkers related to certain diseases according to EVmiRNA database.

miRNA species	Affected age-related chronic disease, autoimmune condition or infection	References
hsa-let-7a-5p	Colorectal-, renal-, prostate-, ovarian-, breast-, lung-, pancreas-, gastric-, esophageal-, thyriod cancer, Ewing's and Kaposi's sarcoma, glioblastoma, AML and MML; metastasis formation; cell cycle control; inflammation; diabetes; cardiovascular disease; hepatitis B infection	Iliopoulos et al., 2009; Trang et al., 2010; Lee et al., 2011
hsa-let-7g-5p	Breast-, esophageal-, lung cancer, glioblastoma, AML and CML; graft-vs.-host disease; inflammation; autoimmune thyroid disease; cell cycle control; diabetes; cardiovascular disease; metabolic syndrome; hepatitis B and influenza A infection	Arora et al., 2011; Wang et al., 2013; Biamonte et al., 2019
hsa-miR-130a-3p	Lung-, liver-, prostate-, ovarian-, breast-, cervical-, nasopharyngeal-, prostate cancer, myeloma, CML and glioblastoma; cardiovascular disease; fibrosis; inflammation; autophagy; diabetes; Crohn's disease; hepatitis C infection; cardiac arrhythmia; renal GBM disease; UV damage	Osbourne et al., 2014; Huang et al., 2015; Eichelmann et al., 2018
hsa-miR-142-3p	Liver-, lung-, colorectal-, breast-, cervical-, esophageal cancer, osteosarcoma, prolactinoma, ALL, AML, CLL and MALT lymphoma; graft rejection; Hashimoto's thyroiditis; multiple sclerosis; cardiovascular disease; inflammation; rotavirus infection; Alzheimer's disease; fibrosis	Ma et al., 2016; Sukma Dewi et al., 2017; Wang et al., 2017
hsa-miR-150-5p	Colorectal-, lung-, liver-, prostate-, cervical-, pancreas-, breast-, ovarian-, esophageal cancer, osteosarcoma, glioblastoma, melanoma; Burkitt lymphoma, ALL and MML; inflammation; cardiovascular disease; fibrosis; irritable bowel syndrome; myasthenia; diabetes; SLE; psoriasis	Roderburg et al., 2013; Qu et al., 2014; Yu et al., 2015
hsa-miR-15a-5p	Gastric-, colorectal-, lung-, breast-, liver-, ovarian-, prostate cancer, melanoma, osteosarcoma, neuroblastoma, pheochromocytoma, AML, CLL and multiple myeloma; inflammation; cell cycle control; apoptosis induction; autophagy; multiple sclerosis; hepatitis B infection; fibrosis; diabetes	Xia et al., 2008; Bandi et al., 2009; Sun et al., 2013
hsa-miR-15b-5p	Liver-, gastric-, lung-, liver-, pancreas-, ovarian-, squamous cell cancer, glioblastoma, melanoma, CLL and thymoma; apoptosis induction; metastasis formation; angiogenesis; fibrosis; bipolar disorder; insulin-resistance; skin photoaging; multiple sclerosis; diabetes	Zhang et al., 2015; Li et al., 2016; MacLean et al., 2016
hsa-miR-199a/b-3p	Liver-, gastric-, lung-, renal cell-, ovarian-, pancreas-, colorectal, liver-, breast-, testicular germ cell-, thyroid-, colorectal cancer, endometriosis, glioblastoma, CLL, melanoma, chondrosarcoma and osteosarcoma; osteoarthritis; COPD; autophagy; angiogenesis; HCV infection; inflammation	Li et al., 2015
hsa-miR-223-3p	Ovarian-, gastric-, colorectal-, prostate-, pancreas-, lung-, liver cancer, CLL, AML, ALL, glioblastoma and osteosarcoma; metastasis formation graft rejection; inflammation; osteoarthritis; lipid metabolism; obesity; rheumatoid arthritis; psoriasis; cardiovascular disease; diabetes; COPD; Alzheimer's disease	Wong et al., 2008; Filková et al., 2014; Lunavat et al., 2015
hsa-miR-23a-3p	Gastric-, colorectal-, esophageal-, liver-, renal-, breast-, prostate-, pancreas-, lung-, laryngeal-, lung cancer, CML, AML, Burkitt lymhoma, melanoma, osteosarcoma and endometriosis; retinal degeneration; UV damage; apoptosis induction; autophagy; progeria; osteoarthritis; obesity	Wang et al., 2014; Yang et al., 2014; Zheng et al., 2014
hsa-miR-451a	Lung-, colorectal-, breast-, skin-, bladder-, gastric-, renal-, esophageal-, thyroid-, liver cancer, T-ALL, AML, CML, multiple myeloma, endometriosis, prolactinoma, osteosarcoma and glioblastoma; drug transporters; cell cycle; metastasis formation; angiogenesis; rheumatoid arthritis; cardiomyopathy	Lopotová et al., 2011; Song et al., 2014; Riquelme et al., 2016

progression, suggesting its potential role as an emerging targetable entity in cancer treatment (Wang et al., 2019). Of note, miR23a shows natural correlation with age, partly explaining the correlation of the above cancers with senior age (Rani et al., 2017).

Exosomal miR-451a was highly expressed in non-small-cell lung carcinoma patients (NSCLC) compared to healthy individuals. This miRNA was strongly associated with tumor progression, recurrence, and poor prognosis in NSCLC patients. According to literature data, it may serve as a potential predictive biomarker for NSCLC (Kanaoka et al., 2018). Zhu et al. (2014) also found that miR-451 levels were consistently elevated in the plasma of patients with gastric cancer providing high diagnostic accuracy for early stage gastric adenocarcinoma. To date, numerous genes have been confirmed as actual targets of miR-451, covering multiple biological signaling pathways including apoptosis, cell invasion and migration, cell proliferation and angiogenesis. Taken together, an accumulating body of evidence indicates that miR-451 is a potential biomarker for cancer diagnosis and prognosis, possibly a treatment target in combination with established drugs (Bai and Wu, 2019).

Exosomal miR-223-3p level in the serum of patients with breast cancer was significantly higher in comparison with healthy controls (Yoshikawa et al., 2018). Its expression was tightly associated with the malignancy of breast cancer, suggesting that exosomal miR-223-3p might be a useful biomarker for the early detection of invasive breast cancer. Of further note, miR223-3p also shows correlation with the advance of age, and these cancers are known to emerge at senior age (Rani et al., 2017).

Elevated expression of miR-150-5p has been shown in breast cancer (BC), described as a good prognostic biomarker for patients with HER2-positive BC (Ozawa et al., 2020).

Exercise was shown to modulate the expression of several miRNA species that in turn are protective against cancer (Li et al., 2020; Pulliero et al., 2020). In our report we demonstrate this modulation observed after both short-term (0.5 year) and long-term (25 + years) regular exercise since our miR-23a, 451a, 223-3p, and miR-150-5p were all suppressed emphasizing the role of exercise in the prevention of several cancer entities. Nevertheless, data on exercise-derived exosomal miRNA species in modulating cancer prevention is still in its infancy (Pulliero et al., 2020). Therefore, elaborate research effort

is required to reveal the role of exosomal miRNA species in this particular field.

The deregulation of miRNA species described in conjunction with other chronic diseases has also been observed in our study. According to our results, regular exercise altered the levels of miR-15a and miR-142 in the opposite direction as observed in patients with diabetes and neurodegenerative disease, supporting that regular exercise (either short- or long-term) reduces the risk of developing such chronic diseases. In more detail, miR-15a was shown to be elevated in the plasma of diabetic patients also showing correlation with disease severity (Kamalden et al., 2017). Xiong et al. (2020) demonstrated that miR-15a-3p is up-regulated in exosomes of diabetic patients, and impairs wound healing. When miR-15a-3p was knocked down and such exosomes were utilized later on, their negative effects on the metabolism and wound healing in particular were partially reversed both *in vitro* and *in vivo* (Xiong et al., 2020).

Barbagallo et al. (2020) isolated exosomal miRNA from the serum of 30 Parkinson disease (PD) patients and compared it with that of 30 healthy controls. The expression levels of ex-miR-23a; ex-miR-142-3p were significantly elevated in the serum of PD patients, unlike in our study where miR-142 showed a decrease in expression compared to healthy, but sedentary state (Barbagallo et al., 2020). Previous studies have also reported the benefits of physical exercise in improving the symptoms in individuals with PD (Da Silva et al., 2021). Taken together these reports suggest the protective role of miR-142-3p in PD, though further studies are required.

Taken together, the miRNAs that we have observed to be modulated by both short and long-term exercise are mostly involved in cancer prevention mechanisms including tumor suppression (miR-223-3p; miR-451a; miR-15a/b-5p; let-7a/7g-5p) (Wang et al., 2016; Gao et al., 2011), aging (miR-223-3p; miR-451a; miR-15a/b-5p; miR-23a-3p) (Mercken et al., 2013; Teteloshvili et al., 2015), induction of apoptosis (miR-150-5p; miR-15a/b-5p; miR-130a-3p) (Xu et al., 2014; Wang et al., 2015) and reduction of inflammation (miR-199a/b-3p; miR-142-3p) (Cai et al., 2012). In addition, the inverse deregulation of miR-15a characteristic to diabetes, and miR-142 featured in Parkinson's disease has also been recorded in our study. Potential applications target these miRNA species to prevent the development of cancer, diabetes and neurodegenerative disease or to be used as adjuvant therapy in established diseases. However, to date no such experiments exist supporting that exercise-derived exomiRs could prevent or treat chronic diseases.

So, beyond the utility of serum-derived exomiRs as potential biomarkers of physical fitness or chronic diseases, our work suggests their key role in essential pathways, potentially preventing the development of multiple chronic diseases. In the future the evaluation of physical activity level may be used to predict the risk of developing various chronic diseases. Furthermore, this study is important as a starting point to understand the global pattern of regular exercise-related exomiRs and their target pathways in health and disease. However, the present study must be seen as an exploratory study. Our current pilot-study is limited by the number of biological replicates. Therefore, further studies are required with larger sample size to comprehensively examine the effect of regular exercise on circulating exomiR profile.

CONCLUSION

Both short- (0.5 year) and long-term (25 + years) regular exercise significantly alters the serum miRNA profile in healthy individuals, potentially reducing the risk of a number of malignant, metabolic and neurodegenerative diseases. Combining an amplification-free miRNome profiling platform and bioinformatics analysis, our study revealed that numerous disease-associated exomiRs show differential expression toward a more beneficial pattern. Physiological relevance is also supported by the large number of genes targeted by these miRNAs. Future work lies ahead in determining the exact mechanism of action and the potential use of exomiRs as therapeutic tools to efficiently prevent or successfully treat age-related diseases.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: EBI ArrayExpress, accession no: E-MTAB-10067.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Regional and Local Ethics Committee of Clinical Centre, University of Pecs (ref. no.: 6439/2016 and 7755/2019). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KG, KK, and MW designed the study. KG, ZA, KB, and AdG recruited participants, collected samples and performed the experiments. RH and AtG analyzed the data. KG and ZA interpreted data and drafted the manuscript. JP, MW, and KK critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.658218/full#supplementary-material

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Corrigendum: Physical Activity as a Preventive Lifestyle Intervention Acts Through Specific Exosomal miRNA Species—Evidence From Human Short- and Long-Term Pilot Studies

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A Corrigendum on

Physical Activity as a Preventive Lifestyle Intervention Acts Through Specific Exosomal miRNA Species—Evidence From Human Short- and Long-Term Pilot Studies

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Short-Term and Lifelong Exercise Training Lowers Inflammatory Mediators in Older Men

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Increased basal low-grade inflammation is observed with advancing age, which is augmented by physical inactivity. However, data regarding the influence of lifelong exercise training and particularly high-intensity interval training (HIIT) on inflammatory mediators in older men are scarce. Therefore, we examined effects of 6 weeks of aerobic preconditioning followed by 6 weeks of HIIT on inflammatory mediators [interleukin (IL)-6, homocysteine, and high-sensitivity C-reactive protein (hsCRP)] in previously sedentary older men (SED) and masters athletes (LEX). Further, we investigated whether SED exhibited greater basal inflammatory biomarkers compared to LEX. Twenty-two men (aged 62 ± 2 years) participated in the SED group, while 17 age-matched LEX men (aged 60 ± 5 years) also participated as a positive comparison group. In SED, preconditioning ($P = 0.030$, $d = 0.34$) and HIIT ($P = 0.030$, $d = 0.48$) caused a reduction in IL-6 compared to enrollment. SED homocysteine did not change throughout ($P > 0.57$; $d < 0.26$), while the decrease in hsCRP after preconditioning ($P = 0.486$, $d = 0.25$) and after HIIT ($P = 0.781$, $d = 0.23$) compared to enrollment was small. HIIT did not influence IL-6 or hsCRP in LEX (all $P > 0.42$; $d < 0.3$). Homocysteine increased from enrollment to post-HIIT in LEX ($P = 0.144$, $d = 0.83$), but all other perturbations were trivial. IL-6 and hsCRP were greater in SED than LEX throughout the investigation (all $P < 0.029$; $d > 0.72$), but homocysteine was not different (all $P > 0.131$; $d < 0.41$). Results of this study suggest moderate-intensity aerobic exercise and HIIT lowers IL-6 (and possible hsCRP) in previously sedentary older men. Moreover, lifelong exercise is associated with reduced concentrations of some inflammatory biomarkers in older males, and therefore, physical activity, rather than age *per se*, is implicated in chronic low-grade inflammation. Moreover, physical inactivity-induced inflammation may be partly salvaged by short-term exercise training.

Keywords: Aging, C-reactive protein, high-intensity interval training, homocysteine, interleukin-6, inflammaging, inflammation

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INTRODUCTION

Increased basal inflammation is generally considered a side effect of poor health and an unhealthy lifestyle (Beavers et al., 2010; Nimmo et al., 2013; Papadopoulos and Santa Mina, 2018). Moreover, the increased concentrations of pro-inflammatory cytokines are associated with several noncommunicable diseases, such as cardiovascular disease (Ramage and Guy, 2004), diabetes

(Qu et al., 2014), cancer (Papadopoulos and Santa Mina, 2018), and obesity (Monzillo et al., 2003; Accattato et al., 2017). The significance of chronic low-grade inflammation in disease risk is compelling. For example, there is evidence for high-sensitivity C-reactive protein (hsCRP; whose level rises in response to inflammation) as a strong independent predictor of cardiovascular disease (Ridker et al., 1997, 1998; Pai et al., 2004; Ridker, 2004). Likewise, the Rancho Bernardo Study of Healthy Aging demonstrated that for each standard deviation (SD) increase in interleukin (IL)-6, lifespan was 1 year less, underlining the importance of inflammatory cytokines in longevity (Wassel et al., 2010; Lee et al., 2012).

Systemic inflammation typically increases with age (termed *inflammaging*; Franceschi et al., 2000), and circulating cytokine dysregulation is a well-recognized result of biological aging (Álvarez-Rodríguez et al., 2012). The inflammaging hypothesis proposes aging increases reactive oxygen species and leads to a more pro-inflammatory basal state (Franceschi et al., 2007). For example, tumor necrosis factor alpha (TNF-α) has been reported to be increased in a stepwise manner, with centenarians displaying greater concentrations than 80-year-olds, who in turn display greater concentrations than younger individuals (Franceschi et al., 2007). Similarly, homocysteine is greater in those aged over 50 years of age compared to younger individuals and is associated with unhealthy lifestyle habits, such as smoking (Xu et al., 2020). Moreover, IL-6 is greater in advanced age (Bruunsgaard et al., 1999; Baylis et al., 2013), while intracellular pro-inflammatory cytokines, such as interferon gamma (IFN-γ) and TNF-α, are greater in older participants' T cells than younger counterparts' T cells (Zanni et al., 2003).

This increased pro-inflammatory state is ameliorated by physical activity (Nimmo et al., 2013; Hennigar et al., 2017; Cabanas-Sánchez et al., 2018). It is thought that exercise exerts a direct anti-inflammatory effect, but also alleviation of adipose-induced inflammation (Beavers et al., 2010). In support of this, Taaffe et al. (2000) reported that IL-6 and CRP were inversely related with hours per year of moderate and strenuous physical activity. Moreover, physical function measured by walking speed and grip strength was inversely correlated with IL-6 and CRP in septuagenarians (Taaffe et al., 2000). Based on the available literature, aerobic exercise training appears more likely to reduce inflammation than resistance training (Nicklas et al., 2008; Beavers et al., 2010). Moreover, both greater frequency and greater intensity of aerobic training enhance anti-inflammatory effects (Hsu et al., 2009; Hawkins et al., 2012). In a recent systematic review covering 2016–2020, consistent anti-inflammatory effects of exercise included lowering of CRP, IL-6, and TNF-α (Bautmans et al., 2021), mirroring the findings of an earlier review by the same authors (Liberman et al., 2017). One striking outcome of these systematic reviews, however, was that resistance exercise was the most examined exercise type, followed by aerobic conditioning, then a combination of the two, and finally "other" which consisted of tai chi, Pilates, and balance training. The reason this observation is of note is that despite interventions incorporating resistance training being most prevalent, this is not reflected in the number of people adhering to the public health guidelines regarding muscle strengthening exercise (Strain et al., 2016). In fact, in those >70 years of age, fewer than 98% of people complete twice weekly muscle strengthening exercises (Strain et al., 2016). In similar fashion, none of the studies included within these meta-analyses utilized a high-intensity interval training (HIIT) intervention.

Given that higher exercise intensity is reportedly more efficacious in reducing inflammatory mediators, it would be prudent to consider HIIT as a viable alternative to moderate-intensity continuous exercise and/or resistance exercise to reduce chronic low-grade inflammation. HIIT is considered healthogenic across several physiological systems (Gillen and Gibala, 2014; Weston et al., 2014; Grace et al., 2015; Knowles et al., 2015; Ramos et al., 2015), yet there are scarce data concerning HIIT-induced alterations to inflammatory cytokines and mediators in older adults. Taken together, the aims of this study were: (a) to examine effects of HIIT preceded by preconditioning on IL-6, homocysteine, and hsCRP in previously sedentary older men (SED); (b) to examine the impact of HIIT (and simultaneous reduction of other habitual training) on IL-6, homocysteine, and hsCRP in lifelong exercising masters athletes (LEX); and (c) to test whether LEX exhibited lower concentrations of inflammatory mediators than age-matched SED men. Our *a priori* hypotheses were as follows: (a) Six weeks of HIIT following preconditioning exercise would reduce IL-6, homocysteine, and hsCRP in SED men; (b) LEX would exhibit no alteration to these inflammatory mediators following HIIT; and (c) IL-6, homocysteine, and hsCRP would be greater in SED than LEX.

MATERIALS AND METHODS

Participants

Following ethical approval by the Institutional Ethics Committee and written informed consent, 22 volunteered and were included in the SED group [62 ± 2 years of age, with a mass of 91 ± 16 kg, stature of 175 ± 6 cm, and peak oxygen uptake (VO_{2peak}) of 28 ± 6 ml kg min^{-1}] and 17 men participated as LEX (60 ± 5 years of age, with a mass of 78 ± 12 kg, stature of 173 ± 6 cm, and VO_{2peak} of 39 ± 6 ml kg min^{-1}). The SED group had not participated in exercise programs for over 30 years. Conversely, LEX participants had all been training for >30 years and competed in masters events, such as triathlon, water-polo, road cycling, track cycling, and distance running. One of the authors (PH) questioned participants in advance of the study, and the majority of LEX included some infrequent, generally isolated high-intensity efforts which were not in an organized manner (i.e., cannot constitute interval training *per se*). Therefore, despite being well trained, LEX participants were considered HIIT-naïve at enrollment. On testing days, participants reported to the laboratory between 07:00 and 09:00 h after an overnight fast and had not exercised for >48 h. Similarly, participants had not drunk alcohol or caffeine for a minimum of 36 h. As a condition to study enrollment, general medical practitioners

(GPs) for each potential participant were contacted and provided with a copy of the study design, protocols, and intended exercise programs. GPs were invited to contact the investigators with any query relating to the study and were further required to provide a written letter of approval for their patient to enroll to the study. Participants were withdrawn if, in the opinion of their GP, risks to their health were present. This could include a history of myocardial infarction, angina, stroke, and chronic pulmonary disease. Consequently, three of the original 47 participants withdrew under GP advice (**Figure 1**). Four of the SED group were on antihypertensive medication according to their physical activity readiness questionnaire, and all remaining participants indicated "nil" to the question concerning medication.

Blood Collection and Analysis

Venous blood samples were collected from an antecubital vein at each phase between 07:00 and 09:00 h, 48–72 h following the last exercise session, by the same phlebotomist, as previously described (Hayes et al., 2017, 2020; Herbert et al., 2017a). Serum IL-6, homocysteine, and hsCRP were determined by electrochemiluminescent immunoassay (E601 module of the Roche Cobas 6000, Burgess Hill, West Sussex, United Kingdom) in the Clinical Biochemistry Laboratory at Royal Glamorgan Hospital (Wales, United Kingdom). Coefficients of variations (CV) over 6 months were all <5%.

Body Composition and Physical Fitness

Bioelectrical impedance analysis (Tanita MC-180MA Body Composition Analyzer, Tanita UK Ltd.) as used to determine body composition (i.e., lean body mass, fat mass, and body fat percentage). VO_{2peak} was measured using breath-by-breath gas analysis (Cortex II Metalyser 3B-R2, Cortex, Biophysik, Leipzig, Germany) using a modified Storer Test (Storer et al., 1990), as reported previously (Knowles et al., 2015). The 6 s Herbert test (Herbert et al., 2015) was used to determine peak power output (PPO) on a cycle ergometer (Wattbike Pro, Wattbike Ltd., Nottingham, United Kingdom). Order of measurement was blood draws, body composition, PPO, and VO_{2peak}. All details have been previously described (Grace et al., 2015).

Exercise Training

Two six-week training blocks separated the three testing phases (phase A, B, and C). The full protocol has been previously reported by Grace et al. (2015), so training is detailed here briefly to avoid replication. During training block 1 (between testing phases A and B), SED underwent the physical activity guidelines of moderate to vigorous aerobic exercise for 150 min wk^{-1} (Riebe et al., 2015) of which was recorded through heart rate telemetry (Polar FT1, Polar, Kempele, Finland). During this time, LEX continued their habitual training which we monitored by heart rate telemetry. During training block 2 (between testing phases B and C), both groups underwent a HIIT program. Sessions consisted of efforts at 40% PPO for 30 s with 3 min recovery between each interval. Frequency of training was once every five days (i.e., nine HIIT sessions in total).

Statistical Analysis

Data analysis was completed with Jamovi version 1.6.23.0. Following confirmation of normal distribution (Shapiro-Wilk test) and homogeneity of variance (Levene's test), 2 × 3 [group (SED, LEX) × time (phase A, B, C)] mixed factorial ANOVAs were conducted to test for differences in concentrations of IL-6, homocysteine, and hsCRP between groups and time points. Subsequently, one-way ANOVA with *post-hoc* paired *T*-tests with Bonferroni corrections were performed to locate differences between phases A, B, and C in each group. As the Bonferroni correction can be simplified to $\frac{\alpha}{n}$; where α is the set probability threshold and n is the number of comparisons, we report P values from each *T*-test multiplied by the number of comparisons [three for within-group between-phase comparisons (i.e., SED A vs. SED B vs. SED C)]. Moreover, *posteriori* independent samples *T*-tests were performed to locate differences between LEX and SED at a particular test phase, without correcting for the number of comparisons [as there was only one (i.e., SED A vs. LEX A)]. As adiposity is known to influence inflammatory biomarkers, analyses of covariance (ANCOVAs) were performed on inflammatory biomarkers at each phase to locate differences between SED and LEX with body fat percentage as the covariate. Correlations between variables were examined with Pearson's correlation coefficient. Alpha levels are reported as exact P values, and we do not defined P values as "significant" or "non-significant" as advised by the American Statistical Association (Hurlbert et al., 2019). Effect sizes are reported using Cohen's *d* and classified using thresholds specific to gerontology (Brydges, 2019) which are 0.15 ≥ small, 0.40 ≥ moderate, and 0.75 ≥ large. Figures are shown as grouped dot plots with mean and SD as recommended by Drummond and Vowler (2012), but also dot plots with connected lines to show individual responses to the intervention. Data are presented as mean ± SD [95% confidence intervals (CI)].

RESULTS

Adherence to the intervention was 100%.

Interleukin-6

The mixed factorial ANOVA concerning IL-6 resulted in a main effect of time ($P = 0.017$), group ($P = 0.008$), and interaction between time and group ($P = 0.017$). SED IL-6 was 3.0 ± 1.7 (95% CI 2.4–3.6), 2.5 ± 1.2 (95% CI 2.0–2.9), and 2.3 ± 1.2 (95% CI 1.9–2.8) pg ml^{-1} at phase A, B, and C, respectively (A vs. B: $P = 0.030$, $d = 0.34$, B vs. C: $P = 1.000$, $d = 0.17$, A vs. C: $P = 0.030$, $d = 0.48$). LEX IL-6 was 1.6 ± 0.8 (95% CI 1.0–2.3), 1.7 ± 0.7 (95% CI 1.2–2.2), and 1.6 ± 0.6 (95% CI 1.1–2.1) pg ml^{-1} at phases A, B, and C, respectively (A vs. B: $P = 1.000$, $d = 0.13$, B vs. C: $P = 0.520$, $d = 0.15$, A vs. C: $P = 1.000$, $d = 0.02$). Thus, IL-6 concentrations were greater in SED than LEX at phase A ($P = 0.004$, $d = 1.05$), B ($P = 0.026$, $d = 0.81$), and C ($P = 0.028$,

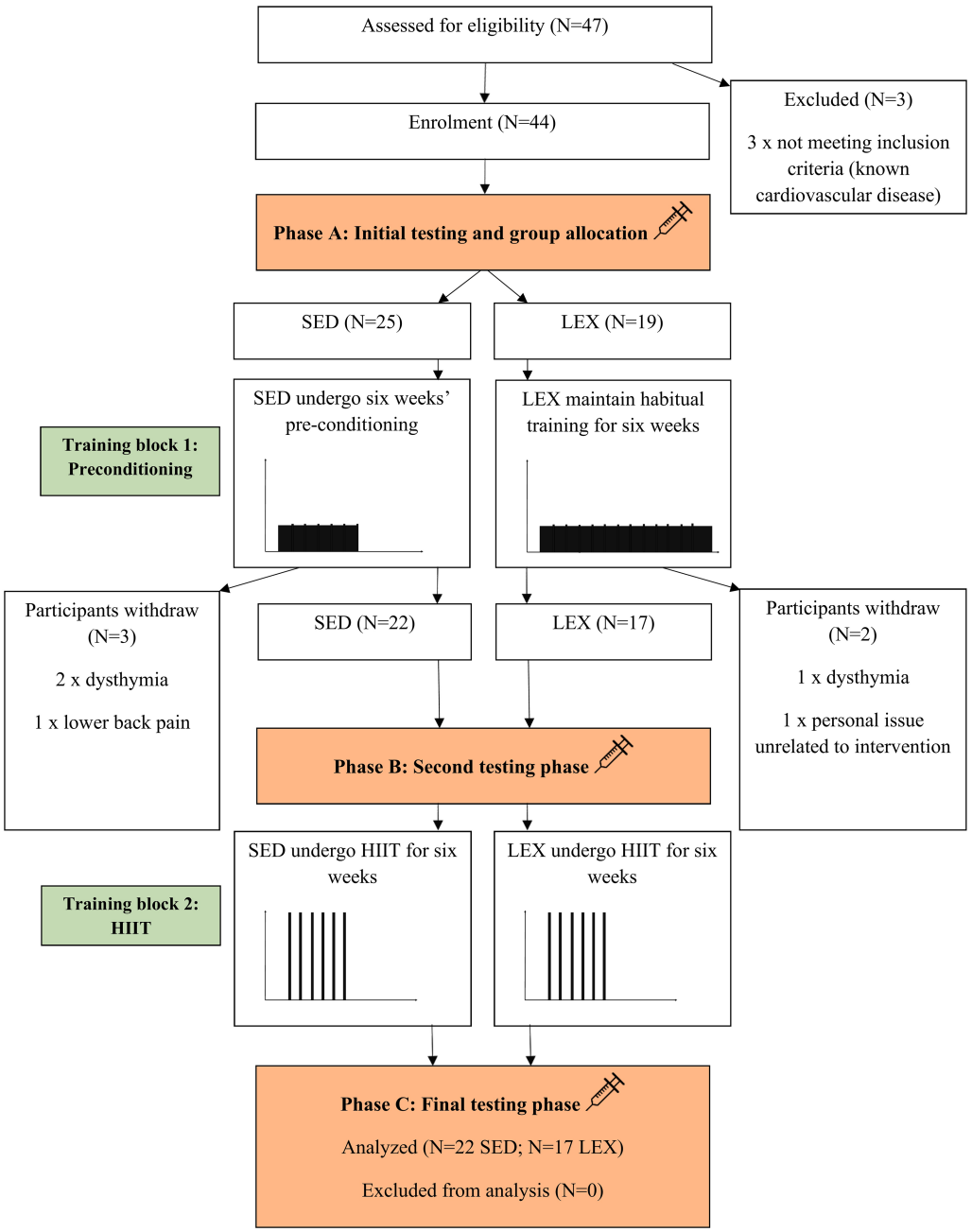

FIGURE 1 | The Consolidated Standards of Reporting Trials (CONSORT) flow diagram detailing the experimental process in which lifelong exercising (LEX) and lifelong sedentary (SED) men completed the intervention. HIIT = high-intensity interval training.

$d = 0.74$; **Figures 2–4**). Controlling for body fat percentage with ANCOVA did not have a large effect on between group P or d values (phase A: $P = 0.039$, $d = 0.75$, phase B: $P = 0.041$, $d = 0.73$, Phase C: $P = 0.038$, $d = 0.75$).

Homocysteine

The mixed factorial ANOVA concerning homocysteine resulted in time effect of $P = 0.171$, group effect of $P = 0.816$, and interaction effect of $P = 0.339$. SED homocysteine was 16.6 ± 3.6 (95% CI 15.0–18.2), 17.7 ± 4.8 (95% CI 14.8–20.5), and 16.7 ± 3.9 (95% CI 15.0–18.5) μmol L^{-1} at phases A, B, and C, respectively (A vs. B: $P = 0.806$, $d = 0.26$, B vs. C: $P = 0.573$, $d = 0.23$, A vs. C: $P = 1.000$, $d = 0.26$). LEX homocysteine was 15.3 ± 2.8 (95% CI 13.6–17.0), 17.1 ± 6.8 (95% CI 14.0–20.1), and 17.8 ± 3.2 (95% CI 15.9–19.7) μmol L^{-1} at phases A, B, and C, respectively (A vs. B: $P = 0.680$, $d = 0.35$, B vs. C: $P = 1.000$, $d = 0.13$, A vs. C: $P = 0.144$, $d = 0.83$). Homocysteine between LEX and SED was similar throughout the study (phase A: $P = 0.132$, $d = 0.40$,

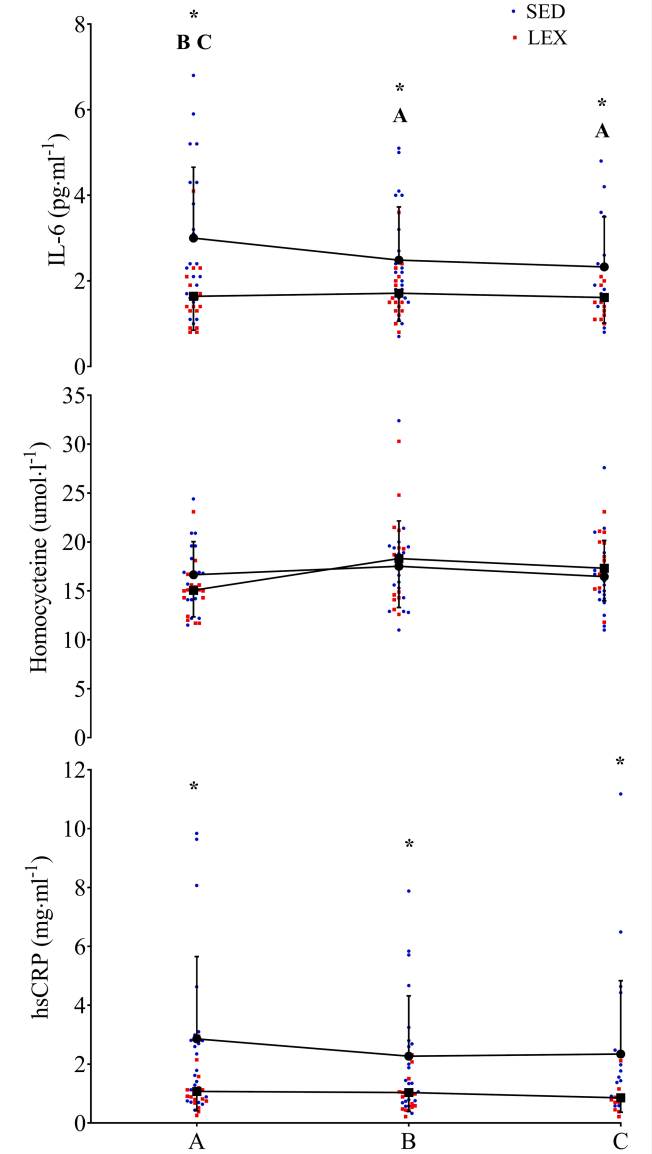

FIGURE 2 | IL-6 (top), homocysteine (middle), and high-sensitivity C-reactive protein (hsCRP; bottom) in a group of previously sedentary (SED) older men and LEX older men. Data are presented as mean ± SD, plus individual data points. *Group differences at this experimental phase at the $P < 0.05$ level. *A* = SED group difference from phase A at the $P < 0.05$ level. *B* = SED group difference from phase B at the $P < 0.05$ level. *C* = SED group difference from phase C at the $P < 0.05$ level.

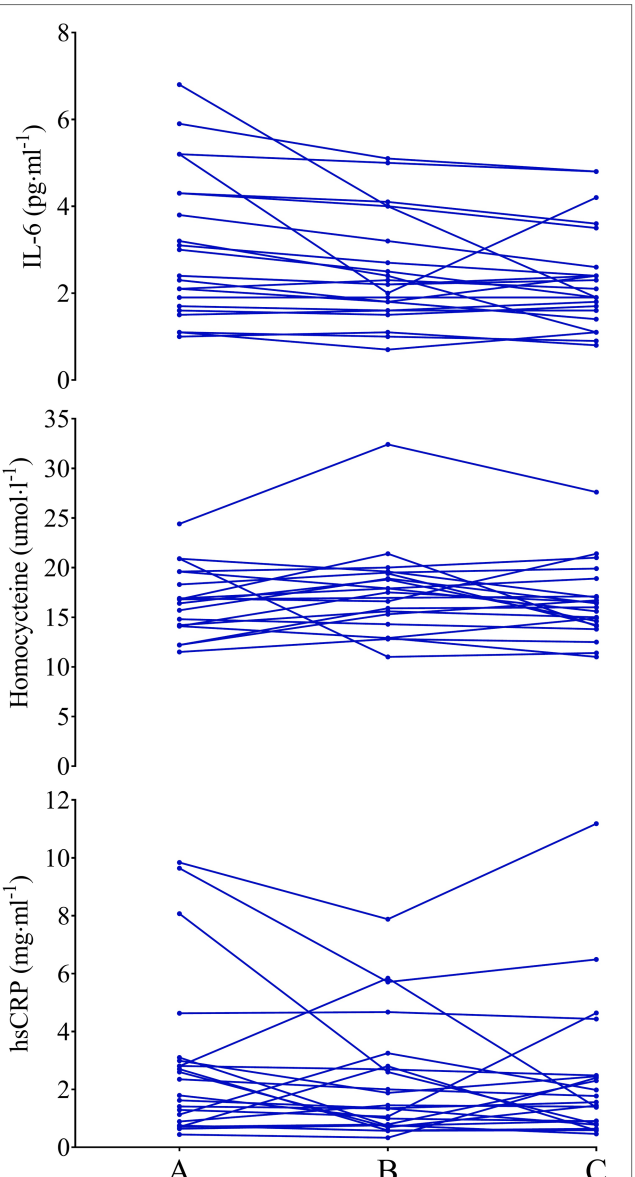

FIGURE 3 | IL-6 (top), homocysteine (middle), and hsCRP (bottom) in previously sedentary older men. Data are presented as individual data points to visualize individual responses.

phase B: $P = 0.830$, $d = 0.10$, phase C: $P = 0.457$, $d = 0.31$). Applying ANCOVA with body fat percentage as the covariate did not have a large effect on between group P or *d* values (phase A: $P = 0.243$, $d = 0.44$, phase B: $P = 0.960$, $d = 0.02$, phase C: $P = 0.181$, $d = 0.47$).

High-Sensitivity C-reactive Protein

The mixed factorial ANOVA resulted in a time effect of $P = 0.267$, a group effect of $P = 0.009$, and an interaction effect of $P = 0.526$ for hsCRP. In SED, hsCRP was 2.9 ± 2.8 (95% CI 1.9–3.8), 2.3 ± 2.0 (95% CI 1.6–3.0), and 2.3 ± 2.5 (95% CI 1.5–3.2) mg·ml^{-1} at phases A, B, and C, respectively (A vs. B: $P = 0.486$, $d = 0.25$, B vs. C: $P = 1.000$, $d = 0.01$, A vs. C: $P = 0.781$, $d = 0.23$). LEX hsCRP was 1.1 ± 0.7 (95% CI 0.02–2.1), 1.0 ± 0.6 (95% CI 0.2–1.8), and 0.9 ± 0.5 (95% CI 0.0–1.8) mg ml^{-1} at phases A, B, and C, respectively (A vs. B: $P = 1.000$, $d = 0.15$, B vs. C: $P = 0.720$, $d = 0.18$, A vs. C: $P = 0.425$, $d = 0.33$). Thus, hsCRP was lower in LEX than SED at phases A ($P = 0.014$, $d = 0.88$), B ($P = 0.022$, $d = 0.88$), and C ($P = 0.021$, $d = 0.79$). Controlling for body fat percentage using ANCOVA increased the P value and reduced effect size for differences between LEX and SED at phases A ($P = 0.268$, $d = 0.40$), B ($P = 0.205$, $d = 0.44$), and C ($P = 0.303$, $d = 0.36$).

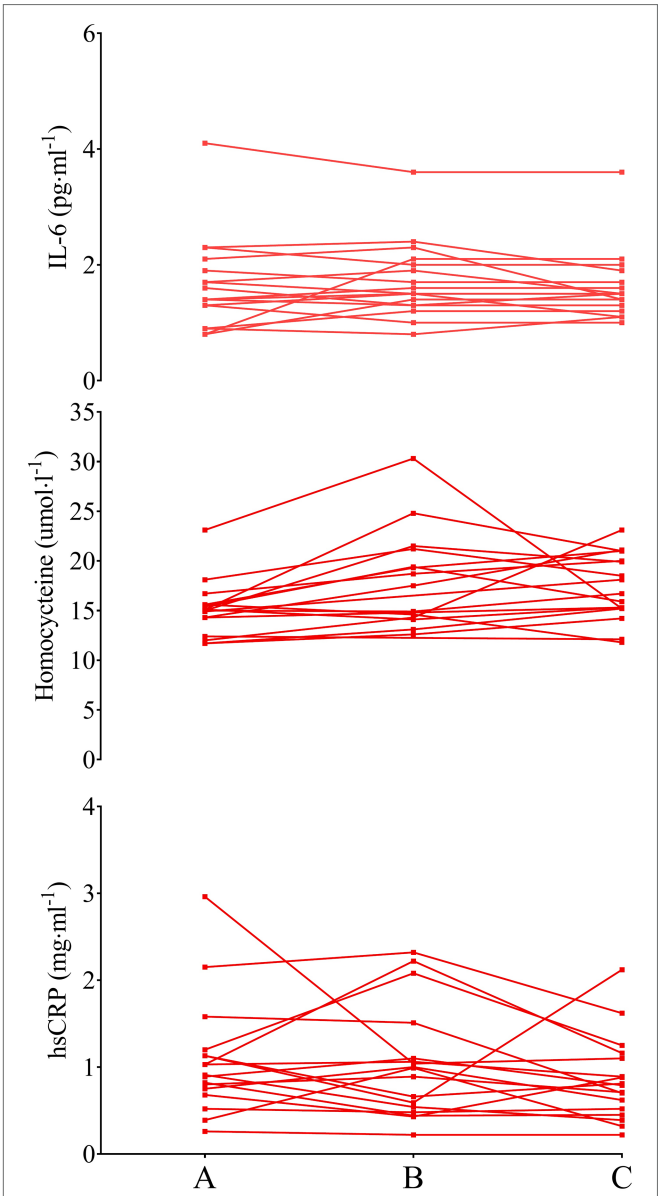

FIGURE 4 | IL-6 (top), homocysteine (middle), and hsCRP (bottom) in LEX older men. Data are presented as individual data points to visualize individual responses. Note, the y-axis for IL-6 and hsCRP has been compressed compared to **Figures 2, 3**.

Correlative Analysis

IL-6 was correlated with hsCRP at phases A, B, and C (all $P < 0.009$; $r = 0.413–0.835$). Moreover, IL-6 concentrations were correlated with fat mass and body fat percentage at phase A ($P < 0.001$; $r = 0.531$ and $P < 0.001$; $r = 0.456$, respectively). However, this relationship did not exist at phase B or C ($P > 0.224$; $r < 0.199$). IL-6 was correlated with VO_{2peak} at phase A ($P = 0.006$; $r = -0.430$), but not B ($P = 0.260$; $r = -0.185$) or C ($P = 0.445$; $r = -0.126$). hsCRP concentrations were associated with total fat mass and body fat percentage at phases A ($P < 0.001$; $r = 0.744$ and $P < 0.001$; $r = 0.665$, respectively), B ($P < 0.001$; $r = 0.632$ and $P < 0.001$; $r = 0.606$, respectively), and C ($P < 0.001$; $r = 0.732$ and $P < 0.001$; $r = 0.678$, respectively). hsCRP was correlated with VO_{2peak} at phase A ($P = 0.002$; $r = -0.488$), phase B ($P = 0.003$; $r = -0.459$), and phase C ($P < 0.001$; $r = -0.570$). Homocysteine was not related to any body composition or fitness parameter at any phase.

The change in (Δ) IL-6 (i.e., phase C IL-6 – phase A IL-6) was not associated with ΔhsCRP ($P = 0.074$; $r = 0.289$), Δhomocysteine ($P = 0.789$; $r = 0.044$), Δbody fat percentage ($P = 0.608$; $r = -0.085$), or ΔVO_{2peak} ($P = 0.309$; $r = -0.167$). ΔIL-6 was most strongly correlated with IL-6 at phase A ($P < 0.001$; $r = -0.742$). ΔhsCRP was not associated with Δhomocysteine ($P = 0.725$; $r = 0.061$), Δbody fat percentage ($P = 0.441$; $r = -0.127$), or ΔVO_{2peak} ($P = 0.412$; $r = -0.135$). ΔhsCRP was most strongly correlated with hsCRP at phase A ($P < 0.001$; $r = -0.513$). Δhomocysteine was not associated with Δbody fat percentage ($P = 0.133$; $r = 0.255$), or ΔVO_{2peak} ($P = 0.712$; $r = 0.064$). Δhomocysteine was strongly correlated with homocysteine at phase A ($P < 0.001$; $r = -0.533$) and homocysteine at phase C ($P < 0.001$; $r = 0.648$).

DISCUSSION

The primary finding from the present investigation was that a period of preconditioning produced a moderate reduction in IL-6 and a small reduction in hsCRP, without change to homocysteine in SED. Moreover, HIIT maintained lower concentrations of IL-6 and hsCRP, despite the reduced training volume in SED. No alterations to these inflammatory mediators were seen in LEX, likely due to their low initial concentrations at baseline (the ceiling effect). Finally, LEX had lower concentrations of IL-6 and hsCRP, but not homocysteine, than SED throughout the investigation. However, when controlling for body fat percentage, the difference in hsCRP was attenuated. These data suggest lifetime exercise training habits are anti-inflammatory, in part through reduced adiposity, and a short period of HIIT after aerobic conditioning can produce moderate reductions in IL-6 and small reductions in hsCRP in previously sedentary older men. These data may also suggest homocysteine is not a viable target for exercise interventions.

Here, we report aerobic conditioning reduced IL-6 in lifelong sedentary older adults, which was maintained following the transition to HIIT despite the very low training volume. Interestingly, ΔIL-6 was not related to change in body composition, suggesting exercise exerted a direct effect on IL-6 rather than an indirect effect mediated by reduced adiposity. As ΔIL-6 was mostly strongly related to IL-6 at phase A (in both groups, and combined), this suggests individuals with the highest concentrations at enrollment experienced the greatest benefit, which could be interpreted as those who had the greatest IL-6 at enrollment had the most "potential" to improve. This corroborates several investigations which show those with the poorest values at baseline have the most "potential" for improvement across several physiological parameters (Little et al., 2011; Herbert et al., 2017b; Hayes et al., 2020). Our finding of improved IL-6 in SED post-training is in line with other intervention studies in older adults following adoption

of exercise or physical activity (Kohut et al., 2006; Nicklas et al., 2008). For example, Nicklas and colleagues observed a decrease in IL-6 of $0.53 \pm 4.19\,pg\,ml^{-1}$ in older adults following a 12-month physical activity intervention. In the present investigation, we report a larger magnitude of change following our 12-week exercise intervention [Cohen's $d = 0.48$ for phase A vs. C in the present study; Cohen's $d = 0.15$ for baseline vs. 12 months in Nicklas et al. (2008)]. An elucidation of greater effect size seen in the present investigation could be the exercise was of a greater intensity than used in Nicklas et al. (2008) investigation, which focused on walking with a targeted perceived exertion of 1,116 out of 20. Conversely, HIIT by definition is high intensity and muscle glycogen is depleted in an intensity-dependent manner, and IL-6 is dependent on muscle glycogen availability (Keller et al., 2001; Steensberg et al., 2001; Chan et al., 2004).

In reporting lower concentrations of inflammatory mediators in a trained group of older adults (Cohen's $d > 0.75$; large effect for IL-6 and hsCRP), our findings support those of Aguiar et al. (2020), who reported middle-age masters athletes had lower IL-6 and TNF-α than an age-matched control group. These findings were observed in participants ~46–52 year of age, and the present study extends these findings into sexagenarians (termed the "young-old"), supporting the contention that exercise habits modify inflammation, potentially through indirect adipose tissue reduction. In terms of inflammation and physical function correlates, Santos Morais Junior et al. (2020) observed IL-6 was correlated with frailty in very old adults which adds weight to the notion increased inflammatory mediators are consistently associated with frailty and mortality in the very old (Michaud et al., 2013; Chen et al., 2014). This investigation extends these findings to the young old, suggesting inflammatory mediators are associated with physical function, as evidenced by the correlation between IL-6 and VO_{2peak} at baseline, and hsCRP and VO_{2peak} throughout the investigation. The differences between SED and LEX at baseline also suggest lifelong exercise may be protective against inflammatory markers known to associate with frailty, in addition to the direct anti-frailty effect (Theou et al., 2011; Viña et al., 2016; da Silva et al., 2019; Oliveira et al., 2020).

Data in this study, primarily the relationships between IL-6 and hsCRP with fat mass and body fat percentage, suggest adiposity influenced initial concentrations of inflammatory mediators. Interestingly, the changes in fitness and fatness were not related to changes in inflammatory mediators in the present investigation. We suggest this may due to different temporal adaptations in these systems (i.e., cardiorespiratory vs. immune), and a longitudinal study may be required to confirm these relationships over a longer duration.

Data from this study are supportive of a recent article which suggested inflammation is physical inactivity driven, rather than a factor of age *per se* (Yasar et al., 2021). These authors examined multiple pro- and anti-inflammatory cytokines in aerobically trained older and younger males. Interestingly, of the 12 cytokines studied [epidermal growth factor (EGF), IL-1a, -1b, -2, -4, -6, -8, and -10, IFN-γ, monocyte chemoattractant protein-1, TNFα, and vascular endothelial growth factor], only EGF was different between the young and old cohort. With reference to the biomarkers measured in this study, Yasar et al. (2021) noted IL-6 was similar between young and old trained groups. We believe this is supportive of data from the present investigation as we observed differences in IL-6 between men of the same age, but divergent lifetime exercise habits, suggesting exercise rather than age influence IL-6. Likewise, Yasar et al. (2021) noted similar IL-6 in participants with an age difference of ~40 years, but similar exercise habits. This leads us to agree with several authors' conclusions that *inflammaging* is partly inactivity-driven, and not exclusively a consequence of chronological aging (Minuzzi et al., 2017, 2019; Yasar et al., 2021). Interestingly, however, Minuzzi et al. (2019) did not observe differences in IL-6 or TNFα between masters athletes and age-matched healthy middle-aged controls. This could suggest the physical inactivity-induced pro-inflammatory environment may only manifest later in life (i.e., in the old, rather than middle aged). This contention is supported by the same authors observing no difference between the middle-aged groups and a young cohort in basal IL-6 or TNFα. However, on further examination of the complete data set of Minuzzi et al. (2019), these authors did observe differences in IL-1ra, IL-1β, IL-4, and IL-8 between masters athletes and age-matched controls and the young group, causing these authors to suggest lifelong training improves the anti-inflammatory environment, which also supports their previous work (Minuzzi et al., 2017). Taken together, future research could consider both pro- and anti-inflammatory biomarkers to examine the effects of age and exercise on inflammation more comprehensively, although this would require significant resource commitment.

The present investigation is not without limitations, which we accept. Firstly, it is impossible to ascribe positive adaptations in SED to HIIT alone, but a combination of training block 1 (preconditioning) and training block 2 (HIIT) as a result of our study design. We made the decision to structure the exercise intervention this way considering the ACSM guidelines for exercise for older adults (Riebe et al., 2015) and considered it safety-conscious to precede HIIT with preconditioning. Secondly, it would have been interesting to include a trained and sedentary younger cohort to examine the age and training interplay more comprehensively. Moreover, a non-exercise control arm would have added clarity to this interaction. However, this would have necessitated greater resource commitments which were outside the scope of this project. Penultimately, the cross-sectional comparison of SED and LEX as baseline limits the conclusion that training habits rather than age drive inflammation. While LEX and SED exercise habits during adulthood were known, inflammatory biomarkers were only quantified at one age. Thus, we were unable to determine whether LEX inflammatory biomarkers were unchanged throughout adulthood or whether a gradual increase occurred irrespective of LEX/SED status, but LEX to a lesser extent than SED. Although the data from Yasar et al. (2021) suggested young (~28 years of age) and old (~68 years of age) trained individuals' inflammatory biomarkers were similar and thus support our supposition, these data were also a comparison made at one time point. Therefore, serial sampling throughout adulthood (i.e., a longitudinal cohort study) would be required to determine whether training habits rather than age are entirely

responsible for levels of inflammation. The greatest limitation however was that inflammatory mediators measured in this study were secondary outcomes of a previous study (Knowles et al., 2015), which used VO_{2peak} for sample size calculations. In this context, an *a posteriori* power analysis with SED IL-6 at phases A and C, and an alpha level of 0.05 resulted in statistical power of 0.52 for a one-sided test. With an alpha level of 0.1, proposed as a suitable compromise between risk of type I and type II error (Nio et al., 2017), observed power was 0.66. For homocysteine and hsCRP, using the highest and lowest concentrations observed (thus, generating the greatest statistical power), observed power was even lower.

Despite the limitations of this study, there are numerous strengths which we feel obliged to emphasize. Firstly, our use of ECLA, rather than cytokine array, ensured suitable sensitivity for the inflammatory markers investigated. This was crucial as LEX exhibited low concentrations of pro-inflammatory cytokines, which would not be detected by biochip assays used to detect clinically relevant concentrations of these cytokines. Secondly, incorporating a SED and a LEX group allowed us to compare effect of lifelong exercise habits vs. sedentarism (LEX vs. SED), and the effects of HIIT in sedentary and lifelong exercising (but HIIT naïve) participants. In this context, results presented here are encouraging, as they provide novel data supporting aerobic preconditioning preceding HIIT as an IL-6 (and possibly hsCRP) lowering intervention in previously sedentary older men. The change in IL-6 and hsCP in SED was largest from A to B (i.e., during preconditioning), but 6 weeks of HIIT maintained this reduction despite reduced time commitments. High-intensity aerobic training and high-intensity resistance training have been shown to ameliorate inflammaging; however, the intensity and volume of these exercise modes are rarely met by the general public. Thus, HIIT may provide an alternative option to the current physical activity guidelines to attenuate inflammaging. HIIT has been perceived as more enjoyable than typically time-consuming aerobic conditioning (Thum et al., 2017), and previous investigations from our group have demonstrated HIIT increases perceptions of health-related quality of life, exercise motives, and VO_{2peak} in older men (Knowles et al., 2015; Herbert et al., 2021). Moreover, using the training intervention within this investigation, we have previously reported a shift in the hormonal milieu toward a more "youthful" profile (Hayes et al., 2017, 2020; Herbert et al., 2017a,b). However, 6 weeks aerobic conditioning followed by 6 weeks of HIIT cannot be considered to reverse inflammatory changes in sedentary older males since the difference between LEX and SED in IL-6 and hsCRP (Cohen's $d \geq 0.74$) was much greater than the change in these biomarkers induced by 12 weeks of exercise training (Cohen's $d \leq 0.48$). In this context, LEX possess a preferential biochemical profile than SED (Hayes et al., 2015, 2020; Elliott et al., 2017) and this investigation extends these data by reporting lower concentrations of some inflammatory mediators due to LEX habits, which were not reduced further by HIIT.

In conclusion, short-term exercise training can reduce some (IL-6 and possibly hsCRP), but not all (homocysteine) inflammatory mediators in SED men, but not LEX men. Moreover, lifelong exercise appears partly anti-inflammatory in sexagenarian men. Taken together, we propose that exercise habits, rather than age *per se*, is more causative of IL-6 (and possibly hsCRP) concentrations in older men. One area for future research could be to extend these data into the oldest old to determine whether lifelong exercise attenuates inflammaging into the eight decade of life and beyond.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of the West of Scotland. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PH, NS, and FG: conceptualization and methodology. LH, PH, NS, and FG: formal analysis, investigation, resources, writing – original draft preparation, writing – review and editing, project administration, and funding acquisition. LH: visualization. NS and FG: supervision. All authors contributed to the article and approved the submitted version.

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High Intensity Interval Training (HIIT) as a Potential Countermeasure for Phenotypic Characteristics of Sarcopenia: A Scoping Review

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Background: Sarcopenia is defined as a progressive and generalized loss of skeletal muscle quantity and function associated predominantly with aging. Physical activity appears the most promising intervention to attenuate sarcopenia, yet physical activity guidelines are rarely met. In recent years high intensity interval training (HIIT) has garnered interested in athletic populations, clinical populations, and general population alike. There is emerging evidence of the efficacy of HIIT in the young old (i.e. seventh decade of life), yet data concerning the oldest old (i.e., ninth decade of life onwards), and those diagnosed with sarcopenic are sparse.

Objectives: In this scoping review of the literature, we aggregated information regarding HIIT as a potential intervention to attenuate phenotypic characteristics of sarcopenia.

Eligibility Criteria: Original investigations concerning the impact of HIIT on muscle function, muscle quantity or quality, and physical performance in older individuals (mean age ≥60 years of age) were considered.

Sources of Evidence: Five electronic databases (Medline, EMBASE, Web of Science, Scopus, and the Cochrane Central Register of Controlled Trials [CENTRAL]) were searched.

Methods: A scoping review was conducted using the Arksey and O'Malley methodological framework (2005). Review selection and characterization were performed by two independent reviewers using pretested forms.

Results: Authors reviewed 1,063 titles and abstracts for inclusion with 74 selected for full text review. Thirty-two studies were analyzed. Twenty-seven studies had a mean participant age in the 60s, two in the 70s, and three in the 80s. There were 20 studies which examined the effect of HIIT on muscle function, 22 which examined muscle quantity, and 12 which examined physical performance. HIIT was generally effective

in Improving muscle function and physical performance compared to non-exercised controls, moderate intensity continuous training, or pre-HIIT (study design-dependent), with more ambiguity concerning muscle quantity.

Conclusions: Most studies presented herein utilized outcome measures defined by the European Working Group on Sarcopenia in Older People (EWGSOP). However, there are too few studies investigating any form of HIIT in the oldest old (i.e., ≥80 years of age), or those already sarcopenic. Therefore, more intervention studies are needed in this population.

Keywords: aging, exercise, HIIT, high intensity, power, sarcopenia, sprint, strength

KEY POINTS

- A variety of intensity prescriptions were utilized in previous experiments, which included "all-out" effort, percentage of maximal heart rate, perceived a percentage of peak oxygen uptake, percentage of intensity at termination of a ramped exercise test, percentage of peak instantaneous power, rating of perceived exertion, and percentage of maximum gait speed.
- Twenty-seven studies had a mean participant age in the 60s, two in the 70s, and three in the 80s. There were 20 studies which examined the effect of HIIT on muscle function, 22 studies which examined the effect of HIIT on muscle quantity, and 12 studies which examined the effect of HIIT on physical function (which are the outcomes used to diagnose sarcopenia).
- No previous investigation had considered HIIT in a sarcopenic or pre-sarcopenic population, and only three studies were in the oldest old humans.

INTRODUCTION

Rationale

Sarcopenia is a progressive skeletal muscle disorder, characterized by reduced skeletal muscle quantity and function which is associated with a range of negative health outcomes including frailty, falls, reduced quality of life, and mortality (Cruz-Jentoft and Sayer, 2019; Cruz-Jentoft et al., 2019). In addition to these individual health impacts, sarcopenia places a considerable economic burden on healthcare systems with the associated costs in the UK estimated at £2.5 billion per year (Pinedo-Villanueva et al., 2019). Taken together, these effects highlight the need to develop treatment strategies to counteract the deleterious consequences of sarcopenia.

Factors including chronic inflammation, mitochondrial dysfunction, and reduced satellite cell function contribute to the onset and progression of sarcopenia (Ziaaldini et al., 2017). Exercise training has the potential to counteract these cellular, molecular, and neural alterations (Marzetti et al., 2017; Seo and Hwang, 2020) with aerobic and resistance exercise capable of inducing differential adaptations (Hawley et al., 2014). Previous work has demonstrated that resistance exercise has multisystem effects, acting at both the physiological [e.g., improvements in mitochondrial function (Melov et al., 2007) and reduced inflammation (Beyer et al., 2012)] and the functional level [e.g., improvements in muscle strength and physical performance (Peterson et al., 2010; Steib et al., 2010)]. To date there remains no pharmacological treatment approved for the treatment of sarcopenia and resistance exercise training is recommended as its primary treatment (Dent et al., 2018). Given the multi-factorial nature of sarcopenia, exercise programmes for older adults living with sarcopenia often involve a combination of exercise modes (Witham et al., 2020) with the aim of simultaneously improving muscular and cardiorespiratory function (Hurst et al., 2019a). Offering a range of alternative exercise training approaches which can simultaneously improve multiple outcomes (e.g., muscle strength, physical performance, and cardiorespiratory fitness) could help to maximize the potential of exercise as a therapeutic strategy for older people living with sarcopenia.

High intensity interval training (HIIT) has previously been shown to exert substantial cardio-protective effects, across a range of population groups (Knowles et al., 2015; Hwang et al., 2016; Batacan et al., 2017; Füzéki and Banzer, 2018; Hannan et al., 2018; Hayes et al., 2020; Herbert et al., 2021). In the clinical context, HIIT has been shown to be a safe, feasible and effective therapeutic strategy in patients living with diabetes (Little et al., 2011), heart failure (Angadi et al., 2015) and coronary artery disease (Warburton et al., 2005). From a pragmatic perspective, HIIT can be embedded within the clinical pathway (Way et al., 2020) and can be delivered using a range of exercise modes (e.g., stair climbing, stepping, cycling, walking).

Despite this, much less is known about how HIIT could improve elements of muscular structure and function. A recent narrative review (Callahan et al., 2021) outlined several mechanistic explanations as to why HIIT *might* be anabolic in nature. These authors called for further investigation of HIIT in populations of different age groups and training status to explore this phenomenon further. Moreover, they proposed HIIT may be beneficial in middle and older age where physical conditioning (i.e., aerobic fitness) and increased muscle quantity were simultaneously desired. Whether HIIT could provide the necessary improvements in muscle quantity, quality, and strength, in addition to cardioprotective effects however, remain unclear (Hurst et al., 2019b). The potential for HIIT to simultaneously induce improvements in cardiometabolic health and muscular health is an appealing strategy. However,

until now there has not been a comprehensive review of HIIT within older adults pertaining to phenotypic characteristics of sarcopenia using a systematic search strategy.

Given that exercise programmes delivered to older people with sarcopenia in clinical practice are varied and often poorly prescribed (Witham et al., 2020), delivering effective and engaging exercise programmes to older people is of prime concern (Dismore et al., 2020; Collado-Mateo et al., 2021). HIIT is reportedly enjoyable (Thum et al., 2017), can be completed without gym equipment (Blackwell et al., 2017; Dunford et al., 2021; Yasar et al., 2021), and deliver self-perceived health and fitness improvements (Knowles et al., 2015). However, before HIIT can be proposed as a viable countermeasure to phenotypic characteristics of sarcopenia, it is important to consider the existing literature in terms of methodologies, quality of research and heterogeneity, to determine whether a systematic review and meta-analysis is possible, and if not to identify the areas in which the current literature is deficient. A comprehensive review of HIIT and its effect on phenotypic characteristics of sarcopenia is important for clinicians and exercise practitioners to ensure they are equipped to support community-dwelling older adults and their families/caregivers. Therefore, it seemed prudent to conduct a scoping review in this area to map the existing literature in terms of the volume, nature, and characteristics of the primary research (Arksey and O'Malley, 2005). We used a scoping review rather than systematic review and meta-analysis because our aim was not to ask a precise question and were more interested in the characteristics of investigations conducted (Munn et al., 2018). Moreover, the topic has not yet been extensively reviewed and may have been complex or heterogeneous in nature. If existing research was heterogeneous, a systematic review and meta-analysis would not have been possible, and therefore we opted to scope the area in this manuscript (Mays et al., 2001).

Objectives

We aimed to provide an overview of existing literature relating to phenotypic characteristics of sarcopenia pre- and post-HIIT in older adults. The four specific objectives of this scoping review were to (1) conduct a systematic search of the published literature for the effect of HIIT on muscle strength, muscle quantity or quality, and physical performance [aligned to the 2018 operational definition of sarcopenia (Cruz-Jentoft et al., 2019)] in older adults, (2) map characteristics and methodologies used and classified as "HIIT" within the interventions, (3) outline the range and characteristics of outcome variables used, and (4) provide recommendations for the advancement of the investigative area.

METHODS

Protocol and Registration

The review was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for scoping reviews (PRISMA-ScR) guidelines (Tricco et al., 2018) and the five-stage framework outlined in Arksey and O'Malley (Arksey and O'Malley, 2005). A review protocol was not published.

Eligibility Criteria

Studies that met the following criteria were included: (1) involvement of human participants with a mean age of $\geq$ 60 years [considered the start of old age (United Nations, 2020)]; (2) not a review; (3) an intervention which included bouts of high intensity exercise interspersed with periods of recovery, including exercise defined as HIIT or sprint interval training (SIT). We defined high intensity as exercise >85% peak oxygen uptake (VO_{2peak}) or 85% maximal heart rate (HR_{max}) or equivalent perception-based approaches (e.g., Borg 6–20 scale or similar); (4) employing an intervention design and include an exercise training period of >2 weeks; (5) including HIIT in isolation or performed in combination with another form of exercise; (6) including outcome measures related to either (i) muscle function (either strength or power), (ii) muscle quantity, or (iii) physical performance.

Search Strategy

The search strategy consisted of a combination of free-text and MeSH terms relating to "high-intensity interval training," "sarcopenia," and "older adults" which were developed through examination of previously published original and review articles (e.g., screening of titles, abstracts, keywords). Filters were applied to ensure that only records published in English language involving human participants were included in the search results. Full search terms and the complete search strategy can be found in the online **Supplementary Material** associated with this article (**Supplementary Material 1**).

Information Sources

Five electronic databases (Medline, EMBASE, Web of Science, Scopus, and the Cochrane Central Register of Controlled Trials [CENTRAL]) were searched to identify original research articles published from the earliest available up until 12th March 2020. Reference lists from included studies and previously published review articles were examined for potentially eligible papers.

Study Selection

Data were extracted by two reviewers (LH & CH) independently and compared in an unblinded and standardized manner. Once each database search was completed and manuscripts were sourced, all studies were downloaded into a single reference list with duplicates removed. Titles and abstracts were then screened for eligibility and full texts were only retrieved for studies with HIIT incorporated. Two reviewers then read and coded all the included articles using the PEDro scale (de Morton, 2009). Full texts were then thoroughly assessed using the complete eligibility criteria with first (LH) and last (CH) authors confirming inclusion and exclusion. Following this quality assessment, the same reviewers read and coded each of the studies and assessed the following moderators: design method (randomized control trial; RCT, controlled trial; CT or uncontrolled trial; UCT), combined or HIIT in isolation, and outcome variable. Furthermore, participant descriptions and training programme variables were extracted with as much detail provided by the authors. Any disagreement between reviewers

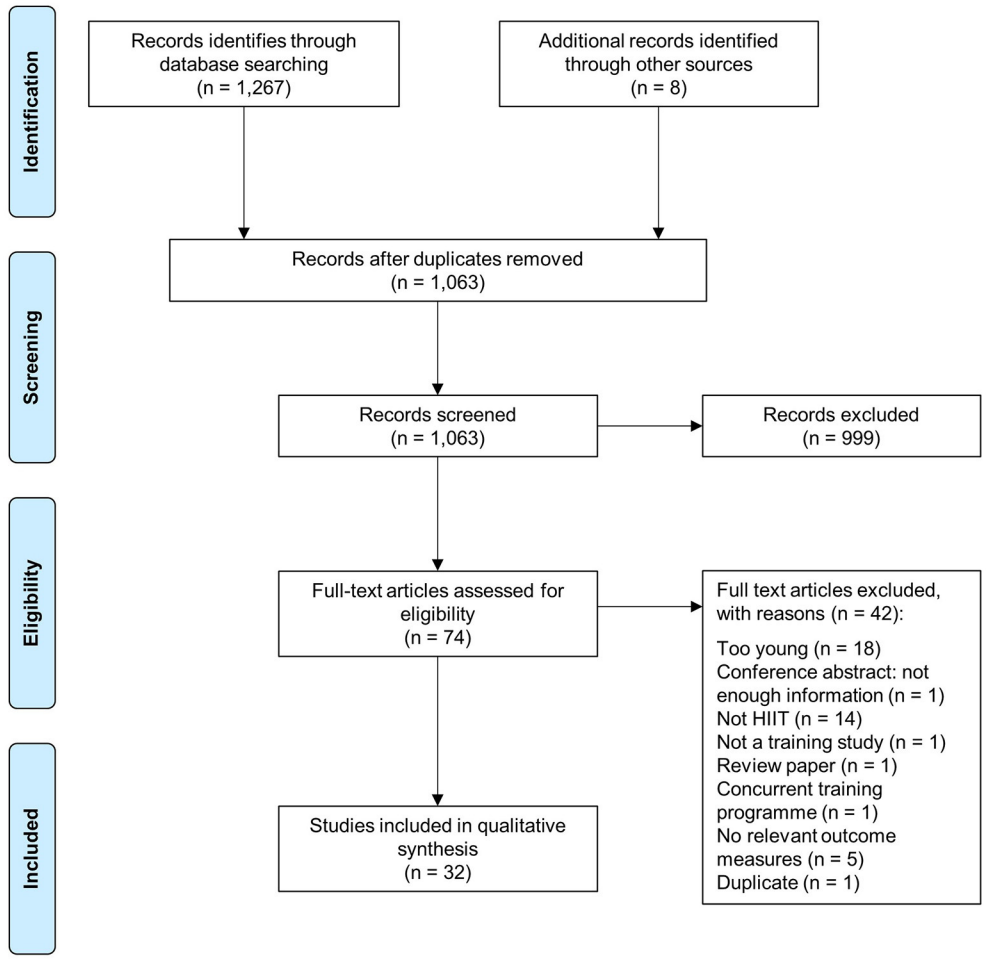

FIGURE 1 | Schematic flow diagram describing exclusions of potential studies and final number of studies.

was discussed in a consensus meeting, and unresolved items were addressed by a third reviewer.

Data Items

Data extracted from each study included sample size, group descriptions, study design, analysis method, and outcome data. Methodological quality was assessed using the modified 0–10 PEDro scale (de Morton, 2009). The primary outcome variables were defined as muscle strength or power, muscle quantity or quality, and physical performance, pre- and post-intervention. There was heterogeneity in study inclusion criteria, interventions, assessment tools, and outcomes, thus a pooled analysis was not appropriate.

RESULTS

Study Selection

Following the initial database search, 1,267 records were identified (**Figure 1**). Once duplicates were removed, 1,063 titles and abstracts remained, and were screened for inclusion, resulting in 74 full-text articles being screened. Of these, 42 were excluded and 32 remained.

Study Characteristics

Of the 32 studies included, 14 were RCTs (Adamson et al., 2014, 2020; Hwang et al., 2016; Coetsee and Terblanche, 2017; Sculthorpe et al., 2017; Aboarrage Junior et al., 2018; Malin et al., 2018; Martins et al., 2018; Ballesta-García et al., 2019; Hurst et al., 2019c; Jiménez-García et al., 2019; Nunes et al., 2019; Taylor et al., 2019; Coswig et al., 2020), one was a quasi-experimental, non-randomized, single-blinded controlled study (Losa-Reyna et al., 2019), 16 were observational cohort studies (Bruseghini et al., 2015, 2019; Boereboom et al., 2016; Guadalupe-Grau et al., 2017; Hayes et al., 2017; Herbert et al., 2017a,b; Robinson et al., 2017; Wyckelsma et al., 2017; Andonian et al., 2018; Bartlett et al., 2018; Buckinx et al., 2018, 2019; Søgaard et al., 2018, 2019; Snijders et al., 2019), and one was a pilot study (although randomized; (Beetham et al., 2019) (**Table 1**). Where a study had multiple outcome measures, they were examined separately. Three out of 32 (9%) included HIIT in

TABLE 1 | General study information of investigations concerning HIIT and phenotypic characteristics of sarcopenia.

Reference	Population	Study design	Study protocol available/ Preregistered	Intervention characteristics						Outcome(s)	PEDro score
				Duration (weeks)	Total sessions	Exercise protocol	Exercise intensity	Adherence/ Compliance/ Attendance	Adverse events		
Aboarrage Junior et al. (2018)	25 untrained females total, 15 in training group (aged 65 ± 7 years); normal body mass; disease free.	RCT	No	24 weeks	72	5 min warm-up preceded jump-based SIT (20 min of 20 repetitions of 30 s work, 30 s rest); 5 min cool-down on a cycle ergometer.	All-out	>90% inclusion criteria	"No participants in either group left the study or Presented any injuries as result of the exercise program"	Muscle quantity Physical performance	5
Adamson et al. (2014)	12 untrained older adults in total, 6 in training group (aged 65 ± 4 years); normal body mass; disease free.	RCT	No	6 weeks	12	6–10 6 s sprints on a cycle ergometer against ∼7% body mass,∼ 60 s rest.	All-out	-	Not reported	Physical performance	5
Adamson et al. (2020)	34 untrained older adults, 11 in once per week training, and 11 in twice per week training group (aged 65 ± 3 years); disease free.	RCT	No	8 weeks	8 for the once per week training group 16 for the twice per week training group	6–10 6 s sprints on a cycle ergometer against ∼7% body mass,∼ 60 s rest.	All-out	-	Not reported	Physical performance	5
Andonian et al. (2018)	21 untrained, sedentary older adults with rheumatoid arthritis ($n = 12$; 64 ± 7 years) or prediabetes ($n = 9$; 71 ± 5 years), free of CVD or diabetes, able-bodied.	Observational cohort study	The study was registered with ClinicalTrials.gov	10 weeks	30	5 min warm-up preceded 90 s work, 90 s rest); 5 minute cool-down on a treadmill.	80-90% HRR	-	Not reported	Muscle quantity	2
Ballesta-García et al. (2019)	54 individuals ($n = 18$, 66 ± 5 years in the HIIT group, $n = 18$, 70 ± 9 years in the MICT group, and, $n = 18$, 67 ±69 years in the control group group), without hypertension or a disease that would interfere with exercise.	RCT with MICT and non-exercise control	The study was registered prospectively with ClinicalTrials.gov	18 weeks	36	1–1.5 min work, 2–2.5 min rest). 6–12 intervals. The programme was progressed over the 18 weeks. "Movements of the lower limbs, combined with the movements of the upper limbs with or without external load."	14–18 on the Borg scale	>80% inclusion criteria. There were registered adverse events in MICT and control groups. Four women in the MICT group and one in control were lost to follow-up due to eye surgery, foot surgery, clavicle fracture, and two hip fractures after a fall. These adverse events did not occur during exercise classes.	Not reported	Physical performance Muscle function	6
Bartlett et al. (2018)	12 untrained, sedentary older adults with rheumatoid arthritis (64 ± 7 years), free of CVD or diabetes, able-bodied.	Observational cohort study	The study was registered with ClinicalTrials.gov	10 weeks	30	5 min warm-up preceded 60–90 s work, 60–90 s rest; 5 min cool-down on a treadmill. Time per session was matched at 30 min.	80–90% VO_2 reserve targeted. 85 ± 5% achieved.	99% adherence.	Not reported	Physical performance	2
Beetham et al. (2019)	21 individuals with stage 3–4 kidney disease ($n = 9$, 61 ± 6 years in the HIIT group and $n = 5$, 63 ± 11 years in the MICT group), overweight and varied diabetic status.	Randomized pilot trial vs MICT	The study was registered at the Australian and New Zealand Clinical Trials Registry	12 weeks	36	5 min warm-up preceded 4 × 4 min intervals with 3 min rest on a treadmill. The programme was progressed over the 12 weeks.	80–95% peak heart rate.	33/36 for HIIT, 34/36 for MICT.	None attributed to the intervention.	Muscle quantity	8

(Continued)

TABLE 1 | Continued

Reference	Population	Study design	Study protocol available/ Preregistered	Intervention characteristics						Outcome(s)	PEDro score
				Duration (weeks)	Total sessions	Exercise protocol	Exercise intensity	Adherence/ Compliance/ Attendance	Adverse events		
Boereboom et al. (2016)	21 individuals (aged ~ 67 years)	Observational cohort study	The study was registered with ClinicalTrials.gov	31 days	12	2 min warm-up preceded 5 × 60 s intervals with 90 s rest on a cycle ergometer.	100–110% power achieved during a ramped CPET protocol to failure.	12 (full compliance)	Not reported	Muscle quantity	2
Bruseghini et al. (2015)	12 healthy older adults (aged 68 ± 4 years).	Proof-of-concept observational cohort study	No	8 weeks	24	10 min warm-up preceded 7 × 2 min intervals with 2 min rest on a cycle ergometer.	85–95% VO_{2peak}	Not reported	Not reported	Muscle function Muscle quantity	2
Bruseghini et al. (2019)	12 moderately active healthy men (aged 69 ± 4 years), normal body mass, disease free.	Observational cohort study	No	8 weeks	24	10 min warm-up preceded 7 × 2 min intervals with 2 min rest on a cycle ergometer. The programme was progressed every 2 weeks.	85–95% VO_{2peak}	Not reported	None attributed to the intervention.	Muscle function	2
Buckinx et al. (2019)	33 untrained adults (aged 69 ± 4 years), non-smoking, low alcohol consuming, postmenopausal (if female), without counter-indication to exercise.	Observational cohort dataset	No	12 weeks	36	5 min warm-up preceded 10 × 30 s intervals with 90 s rest on an elliptical device. The programme was progressed.	80–85% peak heart rate or >17 on the Borg scale	>80% inclusion criteria	Not reported	Physical performance Muscle function Muscle quantity	3
Buckinx et al. (2018)	30 untrained adults (aged 69 ± 4 years), non-smoking, low alcohol consuming, postmenopausal (if female), without counter-indication to exercise.	Observational cohort dataset	No	12 weeks	36	5 min warm-up preceded 10 × 30 s intervals with 90 s rest on an elliptical device. The programme was progressed.	80–85% peak heart rate or >17 on the Borg scale	>80% inclusion criteria	Not reported	Physical performance	3
Coetsee and Terblanche (2017)	67 inactive individuals ($n = 13$, 65 ± 6 years in the HIIT group and $n = 129$, 63 ± 6 years in the control group), normal BMI, no cognitive impairment, and no comorbidities.	RCT	No	16 weeks	48	4 × 4 min intervals with 3 min rest on a treadmill. The programme was progressed	90–95% peak heart rate.	Not reported	Not reported	Physical performance	5
Coswig et al. (2020)	46 untrained female nursing home residents (aged 81 ± 5 years), $n = 15$ in HIIT group. Comorbidities that did not preclude involvement.	RCT with MICT as a positive control group	No	8 weeks	16	5 min warm-up preceded 4 × 4 min intervals with 4 min rest on a treadmill. The programme was progressed	85–95% peak heart rate.	>80% inclusion criteria.	Not reported	Physical performance Muscle quantity	5
Guadalupe-Grau et al. (2017)	9 males (aged 84 ± 3 years) with low to severe COPD. Participants were overweight according to BMI, and 4/9 were sarcopenic.	Observational cohort study	No	9 weeks	18	Strength training plus HIIT. HIIT commenced from the third week: 5 min warm-up preceded 4 × 15 s, progressing to 5 × 25 s intervals with 60 s rest on a cycle ergometer.	"Sprints" at 80–90% HRR	>80% inclusion criteria. 14 started. 9 completed.	Not Not reported	Physical performance Muscle function	2
Hayes et al. (2017)	22 sedentary but otherwise healthy, males (62 ± 2 years)	Observational cohort study with MICT phase	No	6 weeks HIIT preceded by 6 weeks MICT	9 HIIT sessions	6 × 30 s intervals with 3 min rest on a cycle ergometer.	40% PPO or ~141% power achieved during a ramped CPET protocol to failure.	100% adherence	Not reported	Muscle quantity	2

(Continued)

TABLE 1 | Continued

Reference	Population	Study design	Study protocol available/ Preregistered	Intervention characteristics						Outcome(s)	PEDro score
				Duration (weeks)	Total sessions	Exercise protocol	Exercise intensity	Adherence/ Compliance/ Attendance	Adverse events		
Herbert et al. (2017a)	22 sedentary but otherwise healthy, males (62 ± 2 years) 17 male masters athletes (60 ± 5 years)	Observational cohort study with MICT phase	No	6 weeks HIIT preceded by 6 weeks MICT	9 HIIT sessions	6 × 30 s intervals with 3 min rest on a cycle ergometer.	40% PPO or ∼141% power achieved during a ramped CPET protocol to failure.	100% adherence	Not reported	Muscle quantity	2
Herbert et al. (2017b)	17 male masters athletes (60 ± 5 years)	Observational cohort study	No	6 weeks HIIT preceded by 6 weeks MICT	9 HIIT sessions	6 × 30 s intervals with 3 min rest on a cycle ergometer.	40% PPO or ∼141% power achieved during a ramped CPET protocol to failure.	100% adherence	Not reported	Muscle function	2
Hurst et al. (2019c)	36 untrained older adults, who were disease free ($n = 18$ HIIT; aged ∼62 years, $n = 18$ control; aged ∼63 years).	RCT	The study was registered with ClinicalTrials.gov	12 weeks	24	6 min warm-up preceded 4 sets of 4 resistance exercises. The programme was progressed	>90% peak heart rate was targeted. 89% peak heart rate achieved. Mean heart rate was 82% maximum.	>90% inclusion criteria. 99% achieved.	None attributed to the intervention.	Muscle function	7
Hwang et al. (2016)	51 untrained older adults, who were disease free ($n = 15$ completed HIIT; aged 65 ± 1 years, $n = 15$ completed control; aged 64 ± 2 years).	RCT	No	8 weeks	32	10 min warm-up preceded 4 × 4 min intervals with 3 min rest of synchronous arm and leg exercise on a non-weight bearing all-extremity air-braked ergometer. The programme was progressed.	>90% peak heart rate.	84% completed the study. Of those who completed the study, 89% attendance was achieved for HIIT.	None attributed to the intervention.	Muscle quantity	6
Jiménez-García et al. (2019)	82 healthy older adults 68 ± 5 years of age ($n = 26$ in HIIT)	RCT	The study was registered with ClinicalTrials.gov	12 weeks	24	10 min warm-up preceded 4 × 4 min suspension squats with 3 min rest.	90–95% peak heart rate.	>80% attendance as inclusion criteria.	None attributed to the intervention.	Physical performance	8
Losa-Reyna et al. (2019)	20 pre-frail or frail patients without multiple comorbidities, 84 ± 5 years of age ($n = 11$ in HIIT)	Quasi-experimental, non-randomized, single-blinded controlled study	No	6 weeks	12	Resistance training plus HIIT. 5 min warm-up preceded resistance exercise, and then 6–10 × 10–30 s with 40–100 s rest on a treadmill. The programme was progressed	90% maximal gait speed	16 started, 11 finished.	Not reported	Physical performance Muscle function	5
Malin et al. (2018)	Sedentary obese subjects (61±3 years)	RCT with MICT as control	No	2 weeks	12	10 × 3 min intervals with 4 min rest on a cycle ergometer. The programme was progressed	90% peak heart rate	Not reported	Not reported	Muscle quantity	5
Martins et al. (2018)	16 postmenopausal sedentary women at high risk of type II diabetes ($n = 8$ HIIT; aged 64 ± 7 years, $n = 8$ combined training; aged 65 ± 6 years).	RCT with combined training (resistance and aerobic) as control	The study was registered with ClinicalTrials.gov	12 weeks	36	5 min warm-up preceded 10 × 60 s with 60 s rest bodyweight squats and steps. The programme was progressed	>85% peak heart rate	14 started, 8 finished.	Not reported	Muscle quantity Physical performance Muscle function	5

(Continued)

TABLE 1 | Continued

Reference	Population	Study design	Study protocol available/ Preregistered	Intervention characteristics						Outcome(s)	PEDro score
				Duration (weeks)	Total sessions	Exercise protocol	Exercise intensity	Adherence/ Compliance/ Attendance	Adverse events		
Nunes et al. (2019)	24 postmenopausal obese sedentary women ($n = 12$ HIIT; aged ~63 years, $n = 12$ combined training; aged ~63 years).	RCT with combined training (resistance and aerobic) as control	The study was registered with ClinicalTrials.gov	12 weeks	36	5 min warm-up preceded 10 × 60 s with 60 s rest bodyweight squats and steps. The programme was progressed	>85% peak heart rate	13 started, 12 finished. 91% adherence	Not reported	Muscle quantity Physical performance Muscle function	5
Robinson et al. (2017)	8 untrained older adults (71 ± 6 years), disease free, non-smokers.	Observational cohort study with sedentary control phase, followed by randomization into HIIT, combined training (resistance and aerobic), or resistance only training.	The study was registered with ClinicalTrials.gov	12 weeks	36	4 × 4 min with 3 min rest on a cycle ergometer.	>90% VO_{2peak}	27 started, 23 finished.	Not reported	Muscle quantity Muscle function	3
Sculthorpe et al. (2017)	22 sedentary older males (62 ± 4 years), disease free.	RCT	No	12 weeks, of which 6 weeks was HIIT	9	5 min warm-up preceded 6 × 60 s with 3 min rest on a cycle ergometer.	40% PPO for the first 3 sessions, then 50% PPO for the remaining 6 sessions.	100% adherence.	None attributed to the intervention.	Muscle quantity Muscle function	5
Snijders et al. (2019)	14 sedentary men (74 ± 8 years), disease free, non-smokers.	Observational cohort study	The study was registered with ClinicalTrials.gov	12 weeks	36	Resistance training plus HIIT 3 min warm-up preceded 10 x 60 s with 60 s rest on a cycle ergometer.	~90% peak heart rate	Not reported	Not reported	Muscle function Muscle quantity	2
Søgaard et al. (2018)	22 sedentary older adults (aged 63 ± 1 years), disease free, non-smokers.	Observational cohort study	No	6 weeks	18	2 min warm-up preceded 5 × 60 s with 90 s rest on a cycle ergometer.	>85% power achieved during a ramped protocol to failure (individualized so participants could maintain intensity for 60 s).	28 started, 22 finished.	Not reported	Muscle quantity	2
Søgaard et al. (2019)	22 sedentary older adults (aged 63 ± 1 years), disease free, non-smokers.	Observational cohort study	No	6 weeks	18	2 min warm-up preceded 5 × 60 s with 90 s rest on a cycle ergometer.	>85% power achieved during a ramped protocol to failure (individualized so participants could maintain intensity for 60 s).	Not reported	Not reported	Muscle quantity	2
Taylor et al. (2019)	29 older adults (aged 64 ± 8 years) split into HIIT and MICT.	RCT with MICT as control	No	12 weeks	36	Not reported	Not reported	Not reported	Not reported	Muscle quantity	4
Wyckelsma et al. (2017)	15 older adults (aged 69 ± 4 years) disease free.	Observational cohort study	No	12 weeks	36	3 min warm-up preceded 4 × 4 min with 4 min rest on a cycle ergometer.	90–95% peak heart rate.	Not reported	Not reported	Muscle quantity	2

RCT, randomized control trial; MICT, moderate intensity continuous training; SIT, sprint interval training; HIIT, high intensity interval training.

a multicomponent intervention (Guadalupe-Grau et al., 2017; Losa-Reyna et al., 2019; Snijders et al., 2019). Sixteen studies included HIIT on a cycle ergometer, six included HIIT on a treadmill, seven included resistance exercise HIIT (including bodyweight exercises), two included HIIT on an elliptical trainer, and one study did not detail the intervention. Three studies used an "all-out" intensity, 15 used a percentage of HR_{max} or heart rate reserve (HRR) to prescribe intensity, four used a percentage of VO_{2peak} to prescribe intensity, three used a percentage of intensity at termination of a ramped incremental exercise protocol to prescribe intensity, four used percentage of peak power output to prescribe intensity, one used the Borg scale to prescribe intensity, one study used a percentage of maximum gait speed to prescribe intensity, and one study did not detail the intervention. Twenty-seven studies had a mean age in the 60s, two in the 70s, and three in the 80s. One study considered frail participants. There were 20 studies which examined the effect of HIIT on muscle function, 22 studies which examined the effect of HIIT on muscle quantity, and 12 studies which examined the effect of HIIT on physical function (**Figure 2**). Several studies investigated more than one parameter, thus why the sum of the studies above is greater than the number of included studies.

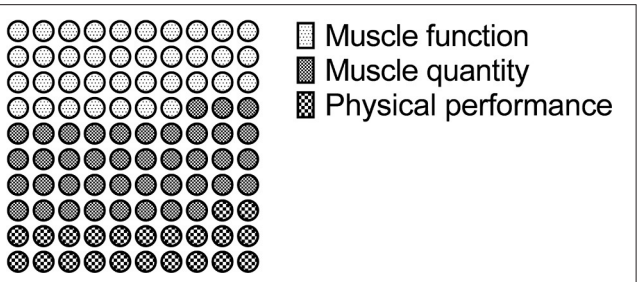

FIGURE 2 | Schematic representation of frequency of outcome examined ($n = 54$) within the 32 included studies concerning HIIT and phenotypic characteristics of sarcopenia.

HIIT and Muscle Function

There were 20 studies which examined the effect of HIIT on muscle function using one or more of the criteria outline by EWGSOP (Cruz-Jentoft et al., 2019) (**Table 2**). Of these, 18 measured muscle strength, and five measured muscle power (some studies measured both, thus why this total is not 20). Of those reporting strength, six used the handgrip test, one used a 30 s arm curl test, five used a 30 s chair stand test, four used the 5 repetitions chair stand test, one used a 10 repetition chair stand test, two used knee extensor isokinetic dynamometry, one used a strain gauge for the knee extensors, four used a leg press, two used a chest press, three used a knee extension machine (which was not a dynamometer), and one used *latissimus dorsi* pull-down, horizontal row, and shoulder press. Of the 20 studies examining strength outcomes, 15 reported ≥1 strength parameter having been improved by HIIT compared to pre-training or compared to a moderate intensity continuous training (MICT) or non-exercise control. Of the remaining three (Robinson et al., 2017; Martins et al., 2018; Nunes et al., 2019), they all reported strength had improved more in a combined aerobic and resistance training group than a HIIT group.

There were five studies which examined the effect of HIIT on muscle power, with two studies examining peak power output during cycle ergometry, and the remaining three determined power during a resistance training exercise (leg extension or leg-press). One investigation examined power during a 5 repetition chair stand test (Losa-Reyna et al., 2019). Of these studies, all reported improved power output post-HIIT. There was no evident association between change in muscle function (either 5 rep chair stand, 30 s chair stand, or grip strength) and number of bouts completed (**Supplementary Figures 1a–c**).

HIIT and Muscle Quantity or Quality

There were 22 studies which examined the effect of HIIT on muscle quantity or a surrogate (fat free mass, lean mass, thigh volume; **Table 3**). Of these, 13 measured whole body lean mass by dual-energy X-ray absorptiometry (DEXA), nine measured leg lean mass by DEXA (of these, all nine also reported whole body lean mass), one measured whole body lean mass by air plethysmography, one measured *M. vastus lateralis* muscle thickness by ultrasonography, two measured quadriceps muscle volume by magnetic resonance imaging (MRI), two measured quadriceps cross-sectional area (CSA) or anatomical CSA (ACSA) by MRI, one measured whole body lean mass by MRI, one measured thigh muscle area by peripheral quantitative computed tomography (pQCT), and six measured whole body lean mass by bioelectrical impedance analysis (BIA). Of the 22 studies examining muscle quantity or quality outcomes, 11 reported ≥1 muscle quantity parameter was improved by HIIT, 14 reported no difference in ≥1 measure from pre-intervention or vs. a no exercise control, two reported inferior adaptation following HIIT compared to a group undertaking resistance training in ≥1 measure, one study reported lean mass was lost post-HIIT to a similar extent as a non-exercise control, and one did not report post-intervention lean mass (some studies measured several outcomes, thus why this total is not 22). There was no evident relationship between change in muscle quantity (as measured by lean mass) and number of bouts completed (**Supplementary Figure 1d**).

HIIT and Physical Performance

There were 12 studies which examined the effect of HIIT on physical function (**Table 4**). One used the short physical performance battery (SPPB), eight used gait speed or the 6 min walk test (6MWT), nine used the timed up and go (TUG) test, and one used the 400 m walk test (some studies utilized more than one outcome). Of the 12 studies examining physical performance, all reported ≥1 parameter was improved by HIIT. The only study examining SPPB reported HIIT improved SPPB performance.

There was no evident relationship between change in muscle performance (as measured by TUG and 6MWT) and number of bouts completed (**Supplementary Figures 1e,f**).

TABLE 2 | Summary of study details concerning HIIT and muscle function.

Reference	Method of outcome measurement	Summary of results
MUSCLE STRENGTH		
Aboarrage Junior et al. (2018)	30 s chair stand test	**↗ vs. pre-HIIT, ↗ vs. control** HIIT group 30 s chair stand test was 16 ± 4 repetitions and 19 ± 5 repetitions pre- and post-intervention, respectively. Control group 30 s chair stand test was 20 ± 2 repetitions and 19 ± 2 repetitions pre- and post-intervention, respectively.
Adamson et al. (2014)	5 rep chair stand test	**↗ vs. pre-HIIT, ↗ vs. control** HIIT group 5 rep chair stand test was 10.5 ± 2.2 s and 9.0 ± 1.6 s pre- and post-intervention, respectively. Control group 5 rep chair stand test was 12.1 ± 4.9 s and 11.9 ± 4.0 s pre- and post-intervention, respectively.
Adamson et al. (2020)	5 rep chair stand test	**↗ vs. pre-HIIT, ↗ vs. control** HIIT once weekly group 5 rep chair stand test was 11.9 ± 1.8 and 10.6 ± 2.1 s pre- and post-intervention, respectively. HIIT twice weekly group 5 rep chair stand test was 12.0 ± 2.1 s and 9.3 ± 1.1 s pre- and post-intervention, respectively. Control group 5 rep chair stand test was 12.1 ± 4.3 and 12.3 ± 4.2 s pre- and post-intervention, respectively.
Ballesta-García et al. (2019)	30 s arm curl test 30 s chair stand test	**↗ vs. pre-HIIT, ↗ vs. control, ↗ vs. MICT** HIIT group 30 s arm curl test was 28.9 ± 5.2 repetitions and 31.7 ± 5.5 repetitions pre- and post-intervention, respectively. Control group 30 s arm curl test was 20.6 ± 3.0 repetitions and 22.4 ± 2.9 repetitions pre- and post-intervention, respectively. MICT group 30 s arm curl test was 25.6 ± 5.2 repetitions and 25.1 ± 4.1 repetitions pre- and post-intervention, respectively. HIIT group 30 s chair stand test was 15.1 ± 2.7 repetitions and 20.7 ± 3.2 repetitions pre- and post-intervention, respectively. Control group 30 s chair stand test was 16.8 ± 2.9 repetitions and 14.9 ± 2.9 repetitions pre- and post-intervention, respectively. MICT group 30 s chair stand test was 13.7 ± 3.4 repetitions and 17.5 ± 4.9 repetitions pre- and post-intervention, respectively.
Bartlett et al. (2018)	30 s chair stand test Handgrip strength	**↗ vs. pre-HIIT** 30 s chair stand test was 14 ± 4 repetitions and 17 ± 5 repetitions pre- and post-HIIT, respectively. **→ vs. pre-HIIT** Handgrip strength was 18.3 ± 7.2 and 19.0 ± 8.1 kg pre- and post-HIIT, respectively.
Bruseghini et al. (2015)	Knee extensor isokinetic dynamometry.	**↗ vs. pre-HIIT, ↘ vs. resistance training** HIIT group isometric knee extensor torque at 60° knee flexion was 200 ± 21 Nm and 215 ± 32 Nm pre- and post-intervention, respectively. Resistance training group isometric knee extensor torque at 60° knee flexion was 202 ± 23 Nm and 223 ± 39 Nm pre- and post-intervention, respectively. **→ vs. pre-HIIT, ↘ vs. resistance training** HIIT group isometric knee extensor torque at 90° knee flexion was 169 ± 34 and 165 ± 31 Nm pre- and post-intervention, respectively. Resistance training group isometric knee extensor torque at 90° knee flexion was 166 ± 38 and 177 ± 42 Nm pre- and post-intervention, respectively. HIIT group concentric knee extensor torque at 60° · s^{-1} was 160 ± 24 and 163 ± 22 pre- and post-intervention, respectively. Resistance training group concentric knee extensor torque at 60° · s^{-1} was 164 ± 26 and 179 ± 31 Nm pre- and post-intervention, respectively. HIIT group concentric knee extensor torque at 120° · s^{-1} was 130 ± 23 and 133 ± 24 pre- and post-intervention, respectively. Resistance training group concentric knee extensor torque at 120° · s^{-1} was 132 ± 23 and 139 ± 23 Nm pre- and post-intervention, respectively.
Bruseghini et al. (2019)	Knee extensor isokinetic dynamometry.	**→ vs. pre-HIIT, ↘ vs. resistance training** Knee extensor isokinetic dynamometry results at 90° knee flexion and 120° · s^{-1} are identical to Bruseghini et al. (2015).
Buckinx et al. (2018)	10 rep chair stand test	**↗ vs. pre-HIIT** 10 rep chair stand test was 18.8 ± 3.7 and 15.6 ± 3.7 s pre- and post-HIIT, respectively.
Buckinx et al. (2019)	Handgrip strength Knee extensor isometric strength using a chain-mounted strain gauge.	**↗ vs. pre-HIIT** Relative handgrip strength was 0.41 ± 0.11 and 0.43 ± 0.12 kg·kg^{-1} pre- and post-HIIT respectively, in a low protein group. Relative handgrip strength was 0.40 ± 0.09 and 0.41 ± 0.08 kg·kg^{-1} pre- and post-HIIT respectively, in a high protein group. **→ vs. pre-HIIT** Relative knee extensor isometric strength was 9.8 ± 2.5 and 10.1 ± 1.9 N·kg^{-1} pre- and post-HIIT, respectively, in a low protein group. Relative knee extensor isometric strength was 10.2 ± 1.6 and 10.4 ± 1.6 N·kg^{-1} pre- and post-HIIT, respectively, in a high protein group.
Coswig et al. (2020)	30 s chair stand test	**↗ vs. pre-HIIT, ↗ vs. MICT** HIIT group 30 s chair stand test was 8.4 ± 1.4 repetitions and 11.8 ± 2.1 repetitions pre- and post-intervention, respectively. MICT group 30 s chair stand test was 8.5 ± 1.1 repetitions and 11.0 ± 1.6 repetitions pre- and post-intervention, respectively.

(Continued)

TABLE 2 | Continued

Reference	Method of outcome measurement	Summary of results
Guadalupe-Grau et al. (2017)	30 s chair stand test Upper- and lower-limb isometric strength using a hydraulic hand dynamometer. 3 RM leg press and chest press. Handgrip strength	**↗ vs. pre-HIIT** 30 s chair stand test was 11.9 ± 4.2 repetitions and 17.0 ± 3.8 repetitions pre- and post-HIIT, respectively. Shoulder abduction strength was 10.9 ± 3.8 and 15.8 ± 4.3 kg pre- and post-HIIT, respectively. Hip flexion strength was 14.8 ± 3.7 and 21.1 ± 4.7 kg pre- and post-HIIT, respectively. Leg extension strength was 11.9 ± 2.1 and 18.2 ± 2.8 kg pre- and post-HIIT, respectively. 1-RM leg press strength was ∼90 ± 20 and 145 ± 10 kg pre- and post-HIIT, respectively. 1-RM chest press strength was ∼22 ± 8 and 40 ± 10 kg pre- and post-HIIT, respectively. **→ vs. pre-HIIT** Handgrip strength was 28.4 ± 5.0 and 30.3 ± 5.2 kg pre- and post-HIIT, respectively.
Hurst et al. (2019c)	Handgrip strength	**→ vs. pre-HIIT, → vs. control,** HIIT group handgrip strength was 36.2 ± 10.9 and ∼38.1 kg pre- and post-intervention, respectively. Control group handgrip strength was 33.9 ± 11.0 and ∼ 33.4 kg pre- and post-intervention, respectively.
Jiménez-García et al. (2019)	Handgrip strength	**↗ vs. pre-HIIT, ↗ vs. control, ↗ vs. MICT** HIIT group handgrip strength was ∼25 ± 1 and ∼28 ± 2 kg pre- and post-intervention, respectively. Control group handgrip strength was ∼27 ± 2 and ∼27 ± 2 kg pre- and post-intervention, respectively. HIIT group handgrip strength was ∼25 ± 2 and ∼26 ± 2 kg pre- and post-intervention, respectively.
Losa-Reyna et al. (2019)	5 rep chair stand test Leg-press force-velocity testing and 1-RM Handgrip strength	**↗ vs. pre-HIIT, ↗ vs. control** HIIT group 5 rep chair stand test was 15.6 ± 2.7 and 10.8 ± 2.5 s pre- and post-intervention, respectively. Control group 5 rep chair stand test was 15.7 ± 3.0 and 14.8 ± 4.0 s pre- and post-intervention, respectively. HIIT group handgrip strength was 16.3 ± 3.6 and 18.3 ± 2.3 kg pre- and post-intervention, respectively. Control group handgrip strength was 20.8 ± 6.0 and 20.1 ± 5.7 kg pre- and post-intervention, respectively. **↗ vs. pre-HIIT** 1-RM leg-press strength was 49.2 ± 19.0 and 62.4 ± 23.2 kg pre- and post-HIIT, respectively. Load at peak power leg-press was 36.3 ± 18.1 and 42.3 ± 17.4 kg pre- and post-HIIT, respectively.
Martins et al. (2018)	1-RM unilateral knee extension.	**↗ vs. pre-HIIT, ↘ vs. combined training** HIIT group unilateral knee extension strength was 56.2 ± 17.7 and 56.8 ± 21.9 kg pre- and post-intervention, respectively. Combined training group unilateral knee extension strength was 47.8 ± 8.5 and 64.0 ± 64.0 kg pre- and post-intervention, respectively.
Nunes et al. (2019)	5 rep chair stand test 1-RM unilateral knee extension.	**↗ vs. pre-HIIT, → vs. combined training** HIIT group 5 rep chair stand test was 12.3 (10.2–14.5) s and 9.3 (7.5–11.1) s pre- and post-intervention, respectively. Combined training group 5 rep chair stand test was 11.0 (9.7–12.4) s and 7.8 (6.8–8.8) s pre- and post-intervention, respectively. **↗ vs. pre-HIIT, ↘ vs. combined training** HIIT group unilateral knee extension strength was 57.9 (47.6–68.1) and 61.5 (45.7–77.2) kg pre- and post-intervention, respectively. Combined training group unilateral knee extension strength was 50.7 (41.1–60.3) and 65.4 (54.8–75.9) kg pre- and post-intervention, respectively.
Robinson et al. (2017)	1-RM leg press.	**↗ vs. pre-HIIT, ↘ vs. combined training, ↘ vs. resistance training** HIIT group increased 1-RM leg press ∼1.0 kg·FFM_{leg}^{-1} from pre- to post-intervention. Combine training group increased 1-RM leg press ∼3.5 kg·FFM_{leg}^{-1} from pre- to post-intervention. Resistance training group increased 1-RM leg press ∼4.3 kg·FFM_{leg}^{-1} from pre- to post-intervention.
Snijders et al. (2019)	1-RM leg press, chest press, *latissimus dorsi* pull-down, horizontal row, shoulder press, and knee extension.	**↗ vs. pre-HIIT** 1-RM leg press was 72 ± 25 and 92 ± 35 kg pre- and post-HIIT, respectively. 1-RM chest press was 21 ± 6 and 24 ± 7 kg pre- and post-HIIT, respectively. 1-RM *latissimus dorsi* pull-down was 26 ± 4 and 30 ± 5 kg pre- and post-HIIT, respectively. 1-RM knee extension was 27 ± 8 and 35 ± 9 kg pre- and post-HIIT, respectively. **→ vs. pre-HIIT** 1-RM shoulder press was 24 ± 7 and 27 ± 8 kg pre- and post-HIIT, respectively. 1-RM horizontal row was 28 ± 9 and 29 ± 5 kg pre- and post-HIIT, respectively.
MUSCLE POWER		
Buckinx et al. (2018)	Leg extensor power.	**↗ vs. pre-HIIT** Leg extensor power was 155 ± 70 and 186 ± 69 W pre- and post-HIIT, respectively.
Herbert et al. (2017a)	Peak power output, determined by a 6 s sprint on a cycle ergometer.	**↗ vs. pre-HIIT** Peak power output was 766 ± 163 and 856 ± 211 W pre- and post-HIIT, respectively.

(Continued)

TABLE 2 | Continued

Reference	Method of outcome measurement	Summary of results
Hurst et al. (2019c)	Leg extensor power.	**↗ vs. pre-HIIT, ↗ vs. control,** HIIT group leg extensor power was 159 ± 65 W and ∼165 W pre- and post-intervention, respectively. Control group leg extensor power was 162 ± 63 and ∼162 W pre- and post-intervention, respectively.
Losa-Reyna et al. (2019)	Leg-press force-velocity testing. 5 rep chair stand test power	**↗ vs. pre-HIIT, ↗ vs. control** HIIT group 5 rep chair stand power was 104 ± 32 and 156 ± 50 W pre- and post-intervention, respectively. Control group 5 rep chair stand test was 123 ± 23 and 134 ± 35 W pre- and post-intervention, respectively. **↗ vs. pre-HIIT** HIIT group 5 leg-press peak power was 113 ± 62 and 153 ± 96 W pre- and post-intervention, respectively.
Sculthorpe et al. (2017)	Peak power output, determined by a 6 s sprint on a cycle ergometer.	**↗ vs. pre-HIIT, ↗ vs. control** HIIT group peak power output was 699 ± 180 and 831 ± 171 W pre- and post-intervention, respectively. Control group peak power output was 655 ± 130 and 657 ± 133 W pre- and post-intervention, respectively.

1-RM, One repetition maximum; MICT, Moderate intensity continuous training; MIIT, Moderate intensity interval training ↗, superior to; ↘, worse than; →, equal to (according to statistical interpretation of original authors). Data are presented as mean ± standard deviation or mean (95% confidence intervals).

DISCUSSION

This scoping review provided an overview of existing literature pertaining to HIIT and phenotypic characteristics of sarcopenia. We examined outcomes according to the revised EWGSOP definition (Cruz-Jentoft et al., 2019) to facilitate translation of research findings into clinical practice. Firstly, the earliest article cited was Adamson et al. (2014) published in 2014, which speaks to this rapidly emerging area of research. This review catalogs existing literature, with a view to facilitating discussion of research opportunities and issues that need to be addressed in future studies.

In relation to our first objective, which was to search the literature for the effect of HIIT on phenotypic characteristics of sarcopenia in older adults, we observed most studies reported at least one positive change in characteristics when compared to vs. pre-HIIT, vs. non-exercise control, or vs. MICT. In this context, 19 of 20 studies reported an improvement to ≥1 muscle function outcome for ≥1 comparisons examined (vs. pre-HIIT, vs. non-exercise control, or vs. MICT) (Bruseghini et al., 2019). Similarly, twelve of 22 reported an improvement to ≥1 muscle quantity outcome for ≥1 comparison examined, and 11 of 12 reported an improvement to for ≥1 physical performance outcome for ≥1 comparison examined.

In relation to our second objective, training programmes ranged in duration from 2 to 24 weeks (median = 9.5 weeks), incorporated resistance training based HIIT, running/walking HIIT, cycling HIIT, and HIIT combined with other exercise modes (i.e., resistance training). Populations studied were commonly in the 7th decade of life, and mostly living independently. In relation to our third objective, muscle quantity, or quality was most frequently studied in the included literature. DEXA was the most utilized measurement method, which is in line with the EWGSOP algorithm for sarcopenia case findings in clinical practice (Cruz-Jentoft et al., 2019). However, these are only routinely found in research facilities and hospitals and would likely require a referral from primary care before an individual received a DEXA scan. Importantly, none of the included studies involved participants who had been diagnosed with sarcopenia using a formalized definition. This limits the clinical significance of the included literature and clearly highlights a need for further work in this population.

HIIT and Muscle Function

According to the revised EWGSOP definition of sarcopenia (Cruz-Jentoft et al., 2019), muscle function is primarily considered as muscle strength. Yet, the chair stand test (or its variations) is named as a parameter that measures muscle strength. However, as the chair stand test relies on the ability to generate force over a short period of time, this could be considered a test of muscle power, rather than a measure of maximal force. The term *dynapenia* [i.e., the age-associated reduction in muscle strength and power (Clark and Manini, 2012; Manini and Clark, 2012)] was originally used to differentiate itself from sarcopenia (Clark and Manini, 2008), which has its roots in age-related reduced muscle mass [Greek translation = "poverty of flesh" (Kim and Choi, 2013)]. However, more recent definitions and diagnoses of sarcopenia have broadened to include muscle function. In this context, when one measures muscle strength using non-isometric movements (i.e., when work occurs), force, distance, and time can be extracted, which is quantification of power. Thus, we believed it pertinent to include studies which concerned muscle power within this review. In fact, muscle power associates more strongly with physical performance and independence than muscle quantity (Clark and Manini, 2010; Trombetti et al., 2016), which may explain why the chair stand test is at the forefront of the revised EWGSOP algorithm for diagnosing and quantifying sarcopenia (Cruz-Jentoft et al., 2019). Moreover, as this is a scoping review, our *a priori* aim was to outline the range and characteristics of outcome variables examined.

In this review, only six studies used grip strength as an outcome measure (Guadalupe-Grau et al., 2017; Buckinx et al., 2019; Hurst et al., 2019c; Jiménez-García et al., 2019). This is interesting to note as EWGSOP propose grip strength as the primary measurement of muscle strength in clinical practice

TABLE 3 | Summary of study details concerning HIIT and muscle quantity or quality.

Reference	Method of outcome measurement	Summary of results
Aboarrage Junior et al. (2018)	Whole body lean mass by DEXA.	**→ vs. control** HIIT group lean mass was 40 ± 6 and 41 ± 6 kg pre- and post-intervention, respectively. Control group lean mass was 40 ± 9 and 41 ± 10 kg pre- and post-intervention, respectively.
Andonian et al. (2018)	Whole body lean mass by air displacement plethysmography.	**→ vs. pre-HIIT** Rheumatoid arthritis group lean mass was 44.9 ± 8.9 and 44.7 ± 7.8 kg pre- and post-HIIT, respectively. Prediabetes group lean mass was 50.1 ± 12.2 and 50.1 ± 12.0 kg pre- and post-HIIT, respectively.
Beetham et al. (2019)	Whole body and lower limb lean mass by DEXA.	**→ vs. pre-HIIT, → vs. MICT** HIIT group low limb lean mass was 17.6 ± 6.6 and ∼17.2 kg pre- and post-intervention, respectively. MICT group lower limb lean mass was 17.0 ± 2.8 and ∼17.3 kg pre- and post-intervention, respectively.
Boereboom et al. (2016)	Whole body and leg lean mass by DEXA. *M. vastus lateralis* muscle thickness determined by ultrasonography.	**→ vs. pre-HIIT** Lean mass was 45.2 ± 11.1 and 45.3 ± 11.1 kg pre- and post-HIIT, respectively. **↗ vs. pre-HIIT** Leg lean mass was 4.1 ± 1.3 and 4.2 ± 1.2 kg pre- and post-HIIT, respectively. *m. vastus lateralis* thickness was 2.04 ± 0.27 and 2.17 ± 0.28 cm pre- and post-HIIT, respectively.
Bruseghini et al. (2015)	Whole body and lower limb lean mass by DEXA. CSA and volume of the quadriceps by MRI.	**→ vs. pre-HIIT, ↘ vs. resistance training** HIIT group lean mass was 56.9 ± 6.2 and 57.7 ± 5.3 kg pre- and post-intervention, respectively. Resistance training group lean mass was 57.3 ± 5.9 and 57.6 ± 5.8 kg pre- and post-intervention, respectively. **↗ vs. pre-HIIT, → vs. resistance training** HIIT group total quadriceps CSA was 60.3 ± 10.6 and 62.9 ± 10.5 cm^2 pre- and post-intervention, respectively. Resistance training group total quadriceps CSA was 59.5 ± 9.3 and 62.0 ± 9.3 cm^2 pre- and post-intervention, respectively. HIIT group total quadriceps volume was 820 ± 198 and 865 ± 199 cm^3 pre- and post-intervention, respectively. Resistance training group total quadriceps volume was 812 ± 184 and 852 ± 188 cm^3 pre- and post-intervention, respectively.
Bruseghini et al. (2019)	Volume and ACSA of the quadriceps by MRI.	**↗ vs. pre-HIIT, → vs. resistance training** Total quadriceps volume results are identical to Bruseghini et al. (2015). HIIT group total quadriceps ACSA increased 3.09 ± 1.38, 2.27 ± 252, and 2.65 ± 3.04 cm^2 at 25, 50, and 75% femur length, respectively, compared to pre-intervention. Resistance training group total quadriceps ACSA increased 3.19 ± 1.24, 3.03 ± 3.04, and 3.40 ± 3.21 cm^2 at 25, 50, and 75% femur length, respectively compared to pre-intervention. **→ vs. pre-HIIT, ↘ vs. resistance training** HIIT group PCSA at 50% femur length was unchanged post-intervention. Resistance training group PCSA at 50% femur length increased post-intervention.
Buckinx et al. (2019)	Whole body and leg lean mass by DEXA. Thigh muscle area by pQCT.	**→ vs. pre-HIIT** Lean mass was 51.8 ± 7.3 and 53.0 ± 7.9 kg pre- and post-HIIT, respectively, in a low protein group. Lean mass was 43.1 ± 9.3 and 43.4 ± 9.5 kg pre- and post-HIIT, respectively, in a high protein group. Leg lean mass was 18.4 ± 3.0 and 18.8 ± 3.3 kg pre- and post-HIIT, respectively, in a low protein group. Leg lean mass was 15.4 ± 3.5 and 15.7 ± 3.5 kg pre- and post-HIIT, respectively, in a high protein group. Thigh muscle area was 91.8 ± 11.9 and 94.4 ± 15.6 cm^2 pre- and post-HIIT, respectively, in a low protein group. Thigh muscle area was 99.3 ± 21.7 cm^2 and 95.7 ± 21.8 cm^2 pre- and post-HIIT, respectively, in a high protein group.
Coswig et al. (2020)	Whole body lean mass by BIA.	**→ vs. pre-HIIT, → vs. MICT** HIIT group lean mass was 29.4 ± 2.8 and 29.6 ± 2.7 kg pre- and post-intervention, respectively. MICT group lean mass was 30.1 ± 3.5 and 29.9 ± 3.6 kg pre- and post-intervention, respectively.
Hayes et al. (2017)	Whole body lean mass by BIA.	**↗ vs. pre-HIIT** Lean mass was 66.7 ± 7.1 and 69.1 ± 8.3 kg pre- and post-HIIT, respectively.
Herbert et al. (2017b)	Whole body lean mass by BIA.	**↗ vs. pre-HIIT** Sedentary group lean mass was 66.7 ± 7.1 and 69.1 ± 8.3 kg pre- and post-HIIT, respectively. Masters athlete group lean mass was 65.2 ± 6.4 and 67.9 ± 5.1 kg pre- and post-HIIT, respectively.
Hwang et al. (2016)	Whole body lean mass by DEXA.	**→ vs. pre-HIIT, → vs. control, → vs. MICT** HIIT group lean mass was 44.6 ± 2.6 and 45.0 ± 2.4 kg pre- and post-intervention, respectively. MICT group lean mass was 47.8 ± 2.1 and 47.7 ± 1.9 kg pre- and post-intervention, respectively. Control group lean mass was 48.3 ± 2.9 and 48.4 ± 2.9 kg pre- and post-intervention, respectively.
Jiménez-García et al. (2019)	Whole body lean mass by BIA.	**→ vs. pre-HIIT, → vs. control, → vs. MIIT** HIIT group lean mass was 24.9 ± 5.7 and 25.7 ± 6.7 kg pre- and post-intervention, respectively. MIIT group lean mass was 25.6 ± 6.6 and 24.5 ± 6.3 kg pre- and post-intervention, respectively. Control group lean mass was 24.6 ± 4.8 and 23.8 ± 4.5 kg pre- and post-intervention, respectively.

(Continued)

TABLE 3 | Continued

Reference	Method of outcome measurement	Summary of results
Malin et al. (2018)	Whole body lean mass by BIA.	**↘ vs. pre-HIIT, → vs. control** HIIT group lean mass decreased 0.4 ± 0.1 kg from pre- to post-intervention. Control group lean mass decreased 0.4 ± 0.1 kg from pre- to post-intervention
Martins et al. (2018)	Whole body lean mass by DEXA, expressed as muscle mass index.	**↗ vs. pre-HIIT, → vs. combined training** HIIT group muscle mass index was 6.6 ± 0.7 and 6.8 ± 0.9 kg·m^2 pre- and post-intervention, respectively. Combined training group muscle mass index was 6.6 ± 1.1 kg and 6.8 ± 1.3 kg·m^2 pre- and post-intervention, respectively.
Nunes et al. (2019)	Whole body and leg lean mass by DEXA.	**→ vs. pre-HIIT, → vs. combined training** HIIT group lean mass was 37.5 (33.9–41.1) kg and 37.5 (33.8–41.2) kg pre- and post-intervention, respectively. Combined training group lean mass was 36.0 (32.7–39.2) kg and 36.3 (32.8–39.8) kg pre- and post-intervention, respectively. **↗ vs. pre-HIIT, → vs. combined training** HIIT group leg lean mass was 12.7 (11.1–14.2) kg and 12.9 (11.3–14.6) kg pre- and post-intervention, respectively. Combined training group leg lean mass was 12.3 (10.8–13.8) kg and 12.7 (11.1–14.4) kg pre- and post-intervention, respectively.
Robinson et al. (2017)	Whole body lean mass by DEXA.	**↗ vs. pre-HIIT, → vs. combined training, → vs. resistance training** HIIT group increased fat free mass ∼0.9 kg from pre- to post-intervention. Combine training group increased fat free mass ∼1.0 kg from pre- to post-intervention. Resistance training group increased fat free mass ∼1.2 kg from pre- to post-intervention.
Sculthorpe et al. (2017)	Whole body lean mass by BIA.	**↗ vs. pre-HIIT, ↗ vs. control** HIIT group lean mass was 65.9 ± 6.7 and 68.1 ± 7.5 kg pre- and post-intervention, respectively. Control group lean mass was 63.4 ± 6.9 and 63.6 ± 7.3 kg pre- and post-intervention, respectively.
Snijders et al. (2019)	Whole body and leg lean mass by DEXA.	**→ vs. pre-HIIT** Lean mass was 55.0 ± 7.8 kg and 55.3 ± 7.7 kg pre- and post-HIIT, respectively. Leg lean mass was 19.3 ± 3.6 kg and 19.5 ± 3.4 kg pre- and post-HIIT, respectively.
Søgaard et al. (2018)	Whole body and leg lean mass by DEXA.	**→ vs. pre-HIIT** Female lean mass was 43.3 ± 1.0 and 43.7 ± 1.0 kg pre- and post-HIIT, respectively. Male lean mass was 59.6 ± 2.0 and 60.0 ± 2.0 kg pre- and post-HIIT, respectively. Female leg lean mass was 15.5 ± 0.4 kg and 15.5 ± 0.5 kg pre- and post-HIIT, respectively. Male leg lean mass was 21.0 ± 0.7 and 21.2 ± 0.7 kg pre- and post-HIIT, respectively.
Søgaard et al. (2019)	Whole body and leg lean mass by DEXA.	**↗ vs. pre-HIIT** Lean mass was 51.5 ± 2.1 and 51.8 ± 2.1 kg pre- and post-HIIT, respectively.
Taylor et al. (2019)	Whole body lean mass by MRI.	**↗ vs. pre-HIIT, → vs. MICT** HIIT group increased fat free mass 0.3 ± 0.9 kg from pre- to post-intervention. MICT group increased fat free mass 0.9 ± 1.5 kg from pre- to post-intervention.
Wyckelsma et al. (2017)	Whole body and leg lean mass by DEXA.	Data not reported post-intervention

DEXA, Dual-energy X-ray absorptiometry; MRI, Magnetic resonance imaging; CSA, Cross sectional area; ACSA, Anatomical cross-sectional area; pQCT, peripheral quantitative computed tomography; BIA, bioelectrical impedance analysis; MICT, Moderate intensity continuous training; MIIT, Moderate intensity interval training; ↗, superior to; ↘, worse than; →, equal to (according to statistical interpretation of original authors). Data are presented as mean ± standard deviation or mean (95% confidence intervals).

and research studies (Cruz-Jentoft et al., 2019). However, of these six investigations, two were published before the revised EWGSOP guidelines, and four were published the same year, so data collection may have been pre-update. Wiśniowska-Szurlej et al. (2019) examined handgrip strength and other mobility parameters including gait speed, balance, and chair stand and observed weak correlations between handgrip strength and mobility in older adults under long-term care facilities. Yee et al. (2021) corroborated this finding reporting weak correlations between chair stand test and handgrip strength in community-dwelling older adults. Similarly, changes in handgrip strength do correlate with changes in leg muscle strength of physical performance during an exercise intervention program in frail older people (Tieland et al., 2015), suggesting it is not a good surrogate of mobility, muscle function, or change in muscle function of muscle other than those involved in gripping. If the two proposed measures of muscle strength to diagnose sarcopenia are not in agreement, then an alternative method for measuring muscle strength is necessary in this population. This may explain why most studies in this review have not measured handgrip and instead opted for isokinetic dynamometry, considered the gold standard for assessing muscle strength but not commonly used in a clinical setting. When considering the body of studies examining muscle function, the majority report increased strength (70% of studies) or power (100% of studies) following HIIT.

Considering reduced muscle function is at the forefront of the recent update on the definition and treatment of sarcopenia (Cruz-Jentoft et al., 2019), any intervention targeting the prevention or reversal of phenotypic characteristics of sarcopenia must be capable of enhancing muscle strength. To our knowledge, Losa-Reyna et al. (2019) is the only investigation

TABLE 4 | Summary of study details concerning HIIT and physical performance.

Reference	Method of outcome measurement	Summary of results
Aboarrage Junior et al. (2018)	TUG	**↗ vs. pre-HIIT, ↗ vs. control** HIIT group TUG was 6.86 ± 1.24 and 6.22 ± 1.13 s pre- and post-intervention, respectively. Control group TUG was 5 ± 1 and 6 ± 1 s pre- and post-intervention, respectively.
Adamson et al. (2014)	TUG	**↗ vs. pre-HIIT, ↗ vs. control** HIIT group TUG was 6.5 ± 0.8 and 5.8 ± 0.6 s pre- and post-intervention, respectively. Control group TUG was 6.9 ± 1.0 and 6.7 ± 1.0 s pre- and post-intervention, respectively.
Adamson et al. (2020)	TUG	**↗ vs. pre-HIIT, ↗ vs. control** HIIT once weekly group TUG was 6.7 ± 0.9 and 6.2 ± 0.7 s pre- and post-intervention, respectively. HIIT twice weekly TUG was 7.0 ± 1.2 and 5.9 ± 0.5 s pre- and post-intervention, respectively. Control group TUG was 7.0 ± 1.1 and 6.7 ± 1.1 s pre- and post-intervention, respectively.
Ballesta-García et al. (2019)	TUG 6MWT	**↗ vs. pre-HIIT, ↗ vs. control** HIIT group TUG was 6.08 ± 1.31 and 5.30 ± 0.80 s pre- and post-intervention, respectively. MICT group TUG was 6.40 ± 1.23 and 5.53 ± 1.28 s pre- and post-intervention, respectively. Control group TUG was 5.89 ± 0.74 and 6.25 ± 0.89 s pre- and post-intervention, respectively. HIIT group 6MWT was 564 ± 41.0 and 600 ± 74.9 m pre- and post-intervention, respectively. MICT group 6MWT was 502 ± 72.3 and 545 ± 72.6 m pre- and post-intervention, respectively. Control group 6MWT was 510 ± 59.0 and 494 ± 49.5 m pre- and post-intervention, respectively.
Bartlett et al. (2018)	TUG 400 m walk	**→ vs. pre-HIIT** TUG was 8.8 ± 1.8 and 8.4 ± 1.9 s pre- and post-intervention, respectively. **↗ vs. pre-HIIT** 400 m walk was 251 ± 62 and 233 ± 51 s pre- and post-intervention, respectively.
Buckinx et al. (2018)	TUG 6MWT	**↗ vs. pre-HIIT** HIIT group TUG was 7.5 ± 1.1 and 6.6 ± 0.9 s pre- and post-intervention, respectively. HIT group 6MWT was 550 ± 85 and 618 ± 91 m pre- and post-intervention, respectively.
Coetsee and Terblanche (2017)	TUG	**→ vs. pre-HIIT, → vs. control, → vs. MICT, → vs. resistance training** HIIT group TUG was 5.6 ± 0.7 and 5.3 ± 0.7 s pre- and post-intervention, respectively. Control group TUG was 5.5 ± 1.1 and 5.7 ± 0.8 s pre- and post-intervention, respectively. MICT group TUG was 5.6 ± 0.7 and 5.4 ± 0.8 s pre- and post-intervention, respectively. RT group TUG was 5.4 ± 0.9 and 5.1 ± 0.8 s pre- and post-intervention, respectively.
Coswig et al. (2020)	Gait speed (10 m) 6MWT	**→ vs. pre-HIIT, → vs. MIIT, → vs. MICT** HIIT group gait velocity was 1.3 ± 0.1 and 1.3 ± 0.1 $m \cdot s^{-1}$ pre- and post-intervention, respectively. MIIT group gait velocity was 1.3 ± 0.1 and 1.2 ± 0.1 $m \cdot s^{-1}$ pre- and post-intervention, respectively. MICT group gait velocity was 1.3 ± 0.1 and 1.3 ± 0.1 $m \cdot s^{-1}$ pre- and post-intervention, respectively. **↗ vs. pre-HIIT, → vs. MIIT, → vs. MICT** HIIT group 6MWT was 406 ± 74 and 454 ± 72 m pre- and post-intervention, respectively. MIIT group 6MWT was 403 ± 83 and 451 ± 84 m pre- and post-intervention, respectively. MICT group 6MWT was 413 ± 58 and 427 ± 68 m pre- and post-intervention, respectively.
Guadalupe-Grau et al. (2017)	TUG 6MWT	**↗ vs. pre-HIIT** TUG was 9.1 ± 1.6 and 7.0 ± 0.9 s pre- and post-intervention, respectively. 6MWT was 286.1 ± 107.2 and 396.2 ± 106.5 m pre- and post-intervention, respectively.
Jiménez-García et al. (2019)	Gait speed (via TUG test)	**↗ vs. pre-HIIT, ↗ vs. MIIT, ↗ vs. control** HIIT group gait speed was 0.73 and 0.89 $m \cdot s^{-1}$ pre- and post-intervention, respectively. MIIT group gait speed was 0.75 and 0.75 $m \cdot s^{-1}$ pre- and post-intervention, respectively. Control group gait sped was 0.75 and 0.75 $m \cdot s^{-1}$ pre- and post-intervention, respectively.
Losa-Reyna et al. (2019)	SPPB 6MWT	**↗ vs. pre-HIIT, ↗ vs. control** HIIT group SPPB was 6.8 ± 1.5 points and 9.8 ± 1.5 points pre- and post-intervention, respectively. Control group SPPB was 7.4 ± 2.0 points and 6.9 ± 2.7 points pre- and post-intervention, respectively. **→ vs. pre-HIIT** 6MWT was 257 ± 62 and 302 ± 72 m pre- and post-intervention, respectively. 6MWT was not performed in the control group.
Martins et al. (2018)	6MWT	**↗ vs. pre-HIIT, → vs. combined training** HIIT group 6MWT was 577 ± 83 and 600 ± 92 m pre- and post-intervention, respectively. Combined training group 6MWT was 614 ± 89 and 669 ± 105 m pre- and post-intervention, respectively.

TUG, timed up and go; 6MWT, 6-min walk test; SPPB, short physical performance battery; HIIT, high intensity interval training; MICT, moderate intensity continuous training; MIIT, Moderate intensity interval training; ↗, superior to; ↘, worse than; →, equal to (according to statistical interpretation of original authors). Data are presented as mean ± standard deviation or mean (95% confidence intervals).

to examine an exercise intervention containing HIIT in frail older adults. These authors examined the influence of a 6-week multicomponent exercise intervention (including walking-based HIIT) focused on enhancing muscle power in ∼84-year olds (range 77–96 years; 75% females; 35% pre-frail and 65% frail). Post-intervention, leg press strength had improved by 34%, and muscle power improved by 47%. Moreover, load at peak power on the force-velocity curve increased by 23%, which suggests this type of intervention may improve muscle strength and power in frail and pre-frail elderly.

HIIT and Muscle Quantity or Quality

In this review, 20/21 (95%) of studies report appendicular skeletal muscle mass measured by DEXA, BIA, or MRI, or cross-sectional area of the thigh by MRI or pQCT scan, which are the primary measurement of muscle quantity proposed by EWGSOP in clinical practice and research (Cruz-Jentoft et al., 2019). The remaining investigation used air plethysmography to determine whole body lean mass (Andonian et al., 2018). When considering the body of studies examining total body lean mass, several reported no increase from pre-HIIT (Bruseghini et al., 2015; Boereboom et al., 2016; Hwang et al., 2016; Andonian et al., 2018; Malin et al., 2018; Søgaard et al., 2018; Beetham et al., 2019; Buckinx et al., 2019; Jiménez-García et al., 2019; Nunes et al., 2019; Snijders et al., 2019; Coswig et al., 2020), whereas some reported an increase post-HIIT compared to pre-HIIT (Hayes et al., 2017; Herbert et al., 2017a; Sculthorpe et al., 2017). To add further uncertainty, two studies which observed no increase in whole body lean quantity observed increased thigh lean mass (Boereboom et al., 2016; Bruseghini et al., 2019). Taken together, it is unclear whether HIIT can significantly increase muscle quantity or quality, and the result may be determined by measurement technique of muscle quantity.

There are no data concerning the effect of HIIT on skeletal muscle quantity or its surrogates (e.g., fat free mass [FFM], lean body mass) in adults diagnosed with sarcopenia, or oldest old humans, despite emerging evidence in the rodent model (Seldeen et al., 2018). Thus, data from the middle old and young old must be extrapolated until these studies exist. In this context, and despite no changes in muscle strength, Robinson et al. (Robinson et al., 2017) observed a ∼1 kg increase in FFM in sedentary ∼71 year olds following 3 days/week cycling HIIT and 2 days/week of treadmill walking. This increase was greater in a resistance training only group, however. Interestingly, FFM was also increased to the same extend in a young (∼25 years old) sedentary cohort, suggesting HIIT can increase FFM in the young and old to equal magnitude. This can be interpreted in two ways: 1) sedentary older adults maintain muscle plasticity and sensitivity to HIIT into older age, and 2) HIIT can increase FFM quantity in young sedentary adults who have not experienced muscle wastage. However, as all participants were untrained, increased FFM could be attributed to both young and old participants being HIIT-naïve.

It would have been a reasonable *a priori* hypothesis to predict HIIT performed at the greatest relative intensity (i.e., all-out or SIT) would result in the greatest increases in muscle quantity, as intensities closer to maximal voluntary contraction are known to induce muscle hypertrophy (Schoenfeld, 2010; Krzysztofik et al., 2019). However, this was not observed as Aboarrage Junior et al. (2018) utilized an all-out protocol, with no reported increases in lean mass. Likewise, it may have been expected untrained participants would exhibit the greatest increase in muscle quantity. However, Herbert et al. (2017a) examined the body composition changes in a group of previously sedentary older males and masters athletes, and reported FFM increased ∼3% (from ∼67 to ∼69 kg) and ∼4% (from ∼65 to ∼68 kg), respectively. This suggests HIIT may be efficacious at increasing FFM in highly active older males and previously sedentary older male, if they are HIIT-naïve. Yet, these data are not ubiquitous through the included literature of this review. Adequate intake of dietary protein is also an important consideration for older adults and any potential exercise induced increases in muscle mass are likely to be influenced by this (Beaudart et al., 2019).

HIIT and Physical Performance

In this review, all of the studies assessing physical performance reported gait speed (part of the SPPB), the SPPB, or the TUG test as an outcome, which are the primary measurements of physical performance proposed by EWGSOP in clinical practice and research (Cruz-Jentoft et al., 2019). Four investigations also reported the 5 repetitions chair stand test separately (Adamson et al., 2014, 2020; Losa-Reyna et al., 2019; Nunes et al., 2019). However, this is one element of the SPPB, so those reporting SPPB values will have conducted this test. When considering the body of literature examining physical performance, all studies reported improvements post-HIIT. When considering studies examining physical performance, all studies report increased physical performance of ≥1 parameter following HIIT. In some instances HIIT did not improve performance more than another training method, where investigations had a parallel arm (Martins et al., 2018; Ballesta-García et al., 2019; Nunes et al., 2019). Physical performance represents a multidimensional construct involving a range of physiological systems across the whole-body (Beaudart et al., 2019) and is a key component in the definition of severe sarcopenia (Cruz-Jentoft et al., 2019).

Losa-Reyna et al. (2019) observed that a 6-week multicomponent exercise intervention (including walking-based HIIT) focused on enhancing muscle power improved the frailty phenotype by 1.6 points, muscle strength by 34%, and muscle power by 47%, suggesting this type of intervention is feasible in frail and pre-frail elderly. As this intervention was multicomponent, it is not possible to quantify the contribution of HIIT to the overall improvement, and therefore it is difficult to ascertain whether adaptations would have occurred were HIIT examined in isolation, rather than simultaneously with a resistance training programme.

Strengths and Limitations

In cataloging the research concerning HIIT and phenotypic characteristics of sarcopenia, several issues and considerations came to light, all of which have important implications for the interpretation of this body of literature, and improvement of future investigations. Firstly, the use of exercise terminology requires clarity. In this context, we mean the definition of "HIIT." HIIT has previously been described as periods of work

>85% VO_{2peak} or 85% HR_{max} or equivalent perception-based approaches, interspersed by recovery periods (Gibala et al., 2012). Only articles matching this description were included in this article. Several articles were returned from our database searching which termed the exercise intervention HIIT, but often these did not reach this threshold of intensity. Similarly, when exercise is described as "all-out," this should be termed SIT, which although a subcategory of HIIT, is unique in its prescription (Weston et al., 2014). It is imperative to classify protocols based on the nature of exercise prescription as different interval exercise classifications will alter experience and potentially subsequent adaptation to the exercise (Biddle and Batterham, 2015). Penultimately, the majority of studies considered small samples sized, which limits interpretation. Finally, the major limitation of the present scoping review is the lack of studies in older adults diagnosed with sarcopenia. Whilst the literature assessment was comprehensive, it is possible that studies may have been missed from the analysis, but as three databases were searched, it is unlikely enough were missed to create a large void in the included literature.

One questions that cannot be answered in the current scoping review is the effect of age on adaptations in physical performance, muscle function, or muscle quantity with HIIT. Whilst we attempted to examine results by decade (60–69, 70–79, and ≥80 years of age), it was noted that most published results were performed in "younger old" participants between 60 and 70 years of age. Further meta-analytical subgroup analysis or meta-regression may thus be required to examine differing responses by age group. In a similar manner, another limitation noted is the inability to examine potential sex differences in responses to HIIT for any outcome. Whilst most studies utilized both male and female participants, groups were typically mixed and thus no insight into sex difference of HIIT responses is attempted here. With a need to better describe and report female physiology in exercise physiology literature (Elliott-Sale et al., 2021), more work in this area may this be called for.

It is also important to acknowledge that the studies included in this review were delivered across a range of settings and involved a diverse range of older adults of varying health and fitness status. While this makes generalizing findings difficult, it does suggest that HIIT may be feasible across a broad range of settings with a wide range of older people. However, it is important to make clear that HIIT may not be suitable for all older people and all exercise programmes should be individually prescribed based on the characteristics of the individual.

Recommendations for Advancement of the Investigative Area

In relation to our fourth objective (provide recommendations for the advancement of the investigative area), this review revealed a dearth of studies considering participants diagnosed with sarcopenia. Therefore, our primary recommendation for advancement of the research area is to increase studies that recruit participants or patients with sarcopenia, or those who are at risk from sarcopenia (i.e., the oldest old). These studies could be feasibility trials, as there is little information as to whether HIIT is a feasible exercise approach in older people. Secondly, given the issue regarding terminology and exercise intensity discussed above, authors are encouraged to be consistent in the use of exercise terminology by adhering to the consensus on exercise reporting template [CERT; (Slade et al., 2016)] in future investigations, which would permit assessment of intervention heterogeneity. Thirdly, studies included within this review had a sample size ranging from 8 to 82 participants, possibly due to resource commitments associated with having large sample sizes and/or rigorous research design. We suggest multicentre RCTs to improve (a) statistical power, and (b) the quality of available evidence, as only 17/32 studies achieved ≥5 on the PEDro scale. Finally, although this review focused directly on phenotypic characteristics of sarcopenia (i.e., quantitative assessment), qualitative investigations on the perceptions of adults with phenotypic characteristics of sarcopenia on this type of exercise and how it could be delivered to this population with minimizing any barriers will be beneficial for the field of gerontology.

CONCLUSIONS AND PRACTICAL RECOMMENDATIONS

In conclusion, most studies presented herein utilized outcome measures defined by the revised EWGSOP guidelines. There was divergence observed in exercise interventions, with HIIT interventions involving a range of exercise modes delivered in a range of settings. Currently, there is some evidence suggesting HIIT may improve phenotypic characteristics of sarcopenia. However, there are few studies investigating any form of HIIT in the very old, or those diagnosed with sarcopenia. Therefore, more intervention studies are needed in this population to confirm this phenomenon and confidently quantify the effectiveness of HIIT. In addition, we need to understand if this is a safe and feasible training approach in this population. In a practical context, combined interventions involving HIIT and resistance training are a worthy avenue for investigation as resistance training is the most potent stimulus to increase muscle quantity and studies herein showed divergent results concerning HIIT and muscle quantity. Finally, HIIT or SIT that is easy to apply (i.e., without equipment needs, travel, specialist training, and intensity monitoring such as heart rate or power output) or can be supported virtually is likely needed to promote the transition of HIIT from the laboratory to the real world.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

LH and CH: conceptualization, methodology, investigation, and project administration. LH and NS-H: formal analysis and investigation. LH, BE, ZY, TB, NS, NS-H, and

CH: writing—original draft preparation. LH, BE, TB, NS, NS-H, and CH: writing—review and editing. LH and BE: visualization. LH and CH: funding acquisition. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.715044/full#supplementary-material

Supplementary Figure 1 | Percent change (Δ%) in outcome measures for muscle function **(a)** 5 s chair stand, **(b)** 30 s chair stand, **(c)** grip strength, muss quality **(d)** lean mass, or muscle performance **(e)** timed up and go (TUG), and **(f)** 6 min walk test (6MWT), all as a function of the number of bouts completed. Dashed lines indicate 95% confidence intervals.

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Preliminary Investigations Into the Effect of Exercise-Induced Muscle Damage on Systemic Extracellular Vesicle Release in Trained Younger and Older Men

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Background: Exercise-induced muscle damage (EIMD) results in transient muscle inflammation, strength loss, and muscle soreness and may cause subsequent exercise avoidance. Research has recently proven that skeletal muscle can also release extracellular vesicles (EVs) into the circulation following a bout of exercise. However, EV's potential role, including as a biomarker, in the response to eccentric resistance exercise stimulus remains unclear.

Methods: Twelve (younger, $n = 7$, 27.0 ± 1.5 years and older, $n = 5$, 63.0 ± 1.0 years) healthy, physically active males, undertaking moderate, regular physical activity (3–5 times per week) performed a unilateral high intensity eccentric exercise protocol. Venous plasma was collected for assessment of EVs and creatine kinase (CK) prior to EIMD, immediately after EIMD, and 1–72 h post-EIMD, and maximal voluntary isometric contraction (MVIC) and delayed onset muscle soreness (DOMS) were assessed at all time points, except 1 and 2 h post-EIMD.

Results: A significant effect of both time ($p = 0.005$) and group ($p < 0.001$) was noted for MVIC, with younger participants' MVIC being higher throughout. Whilst a significant increase was observed in DOMS in the younger group ($p = 0.014$) and in the older group ($p = 0.034$) following EIMD, no significant differences were observed between groups. CK was not different between age groups but was altered following the EIMD (main effect of time $p = 0.026$), with increased CK seen immediately post-, at 1 and 2 h post-EIMD. EV count tended to be lower in older participants at rest, relative to younger participants ($p = 0.056$), whilst EV modal size did not differ between younger and older participants pre-EIMD. EIMD did not substantially alter EV modal size or EV count in younger or older participants; however, the alteration in EV concentration (ΔCount) and EV modal size (ΔMode) between post-EIMD and pre-EIMD negatively associated with CK activity.

No significant associations were noted between MVIC or DOMS and either ΔCount or ΔMode of EVs at any time point.

Conclusion: These findings suggest that profile of EV release, immediately following exercise, may predict later CK release and play a role in the EIMD response. Exercise-induced EV release profiles may therefore serve as an indicator for subsequent muscle damage.

Keywords: eccentric exercise, muscle damage, inflammation, extracellular vesicles, ageing, strength, delayed onset muscle soreness, recovery

INTRODUCTION

Resistance exercise has a myriad of beneficial effects that can offset negative physiological effects associated with ageing and is highly recommended as a strategy to minimise loss in muscle mass and function across the lifespan, and to improve quality of life (Little and Phillips, 2009; Bei et al., 2017; Bagheri et al., 2019). In older adults, eccentric resistance exercise interventions have been suggested due to high load and potentially greater anabolic response at a low energy cost (Gault and Willems, 2013; Lim, 2016; Franchi et al., 2017). It has also been proposed that eccentric exercise-induced muscle damage (EIMD) may be used to develop safer and more effective personalised training and recovery protocols (Givli, 2015). However, unaccustomed exercise, especially high load eccentric muscle contractions, is associated with temporary muscle damage, muscle pain, reductions in muscle force output, an avoidance of repeated loading and transient muscle inflammation (Jouris et al., 2011; Hyldahl and Hubal, 2014; Owens et al., 2019).

Whilst the characteristics of this eccentric type of EIMD have been well defined in healthy young participants (Nosaka et al., 2002; Damas et al., 2016; Kyriakidou et al., 2021), less research has been conducted in older individuals. More specifically, ageing involves a reduction in function of most physiological systems, including muscle mass and function, and is coupled with increased inflammatory signalling (Franceschi and Campisi, 2014). Ageing has been also associated with decreased bone density, which in turn negatively affects physical performance (Reid et al., 2016). Better understanding of any mechanistic ageing-associated differences in muscle damage, inflammation, and pain responses may thus aid both our understanding of physiological differences in older individuals, and also ultimately aid personalised exercise prescription in this population.

Extracellular vesicles (EVs) are lipid-bilayer membrane vesicles, released from the cell of origin, are found in most body fluids and participate in cellular communication *via* transfer of cargo proteins and genetic material systemically between cells (Inal et al., 2013; Colombo et al., 2014; Lange et al., 2017; Turchinovich et al., 2019; Vagner et al., 2019). As EV cargo is comprised of a large range of proteins, enzymes, and genetic material, circulating EVs and their amount, composition, and profile reflect the physiological and pathophysiological condition. Therefore, EV profiles can be useful biomarkers and are easily isolated and quantified from a range of body fluids, including sera and plasma (Hessvik and Llorente, 2018; Ramirez et al., 2018).

Research into EV profiles has largely focussed on human pathologies, including cancer and autoimmune diseases (Withrow et al., 2016; Garcia-Contreras et al., 2017; Lange et al., 2017; Dolcetti et al., 2020; Urabe et al., 2020; Zhao et al., 2020), and is linked to crucial roles in the pathophysiology of inflammation-associated disorders, particularly in relation to larger sized EVs (Słomka et al., 2018). In comparison, explorations of the roles for EVs in normal physiology are fewer, with one plausible mechanism of action being adaptation and recovery from exercise stimuli. For instance, following treadmill running an increase in circulating EVs was seen in mice (Bei et al., 2017), whilst EV associated proteins were elevated in humans following 90 min exhaustive aerobic exercise (Fruhbeis et al., 2015). Different intensities of aerobic treadmill exercise equally increased circulating EV concentrations, whilst increases in modal size were only seen with moderate intensity, not low or high intensity exercise (Oliveira et al., 2018). However, exercise modalities outside of endurance exercise have hitherto not been examined. Importantly, EVs have been shown to be involved in acute responses to injury and inflammatory stimuli in non-exercise models (Middel et al., 2016; Słomka et al., 2018). Whilst it is known that eccentric exercise induces the greatest magnitude of EIMD (Clarkson and Hubal, 2002; Herzog, 2014; Owens et al., 2019), it is likely that EVs will be involved in acute aspects of this response.

To our knowledge, no studies to date have examined the effect of eccentric exercise or the effect of ageing on circulating EV profiles following exercise. Therefore, this study aimed at isolating, quantifying, and size profiling EVs from the plasma of exercised human participants, to investigate whether there is any interplay between acute EIMD-induced changes in EV release profiles in younger and older participants, and whether such EV-related changes would correlate with other biological and muscle functional markers of EIMD, such as creatine kinase (CK) activity, strength, and muscle soreness.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was obtained by the College of Liberal of Arts and Sciences Research Ethics Committee, University of

Westminster, United Kingdom (ETH1819-0328). All work herein conforms to the standards set by the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their participation.

Participants

Twelve (younger $n = 7$, 27.0 ± 1.5 years and older $n = 5$, 63.0 ± 1.0 years) healthy, physically active males, undertaking moderate, regular physical activity (3–5 times per week) volunteered to participate in this experimental study to perform a unilateral eccentric exercise protocol [seven sets of 10 repetitions at one repetition maximum (1RM), leg press machine]. The physical characteristics of the participants are presented in **Table 1**.

Exclusion criteria included smoking, sex, taking any medication (e.g., non-steroidal anti-inflammatory drugs), and/or consuming anti-inflammatory (e.g., fish oil) supplements <6 months prior to commencing the study and the presence of any known immune, cardiovascular or metabolic disease. To further confirm participants were free from upper respiratory tract infections, they completed an illness-specific questionnaire (WURSS-21; Barrett et al., 2009). Additionally, participants were free from any pain or injury as determined by the physical activity readiness questionnaire (PAR-Q) pre-exercise participation screening. Participants were also excluded if they regularly undertook downhill running or eccentric exercise (e.g., resistance exercise, squats, and lunges) as part of their normal training <6 months prior to commencing the study. Participants were required to refrain from any exercise for 24 h prior to baseline visit and 48 h prior to EIMD visit, and from alcohol and caffeine 24 h before baseline and EIMD visit. Further, they were asked to refrain from exercise during the recovery phase (for the subsequent 72 h following the muscle-damaging exercise bout).

Experimental Design

All participants were required to attend the human performance laboratory at the University of Westminster, London, United Kingdom, at the same time of day (±1 h) in the morning on five occasions over a 2-week period. During visit 1 (baseline), in an overnight fasted-state, participants performed baseline measurements to ensure familiarisation of testing equipment and 5RM was determined. The baseline visit included anthropometric measurements, a venous blood sample, perceived muscle soreness, and maximal voluntary isometric contraction (MVIC) on the leg, described fully below.

On visit 2 (7 days later), participants reported to the laboratory at 07:00 am having fasted overnight to complete the EIMD exercise protocol. All above measurements were repeated prior to (pre-EIMD) and immediately post (post-EIMD) the EIMD trial, and an additional blood sample was collected at 1 and 2 h post-EIMD. Identical follow-up assessments were repeated at visits 3, 4, and 5 (24, 48, and 72 h post-EIMD). An overview of the study design is presented in **Figure 1**.

TABLE 1 | Characteristics of participants at baseline.

	Total ($n = 12$)	Younger (18–35; $n = 7$)	Older (≥60; $n = 5$)	p
Age (years)	42.00 (±5.32)	27.00 (±1.34)	63.00 (±0.93)	0.001*
Weight (kg)	74.02 (±2.77)	73.83 (±3.16)	74.30 (±5.46)	0.939
Height (cm)	180.50 (±1.45)	181.14 (±2.06)	179.60 (±2.16)	0.624
BMI (kg/m^2)	22.73 (±0.77)	22.56 (±0.98)	22.98 (±1.35)	0.800
Body fat (%)	19.07 (±1.99)	16.90 (±2.60)	22.10 (±2.83)	0.212
Muscle mass (kg)	28.70 (±0.81)	29.53 (±1.04)	27.54 (±1.22)	0.243
1RM leg press (kg)	145.29 (±6.69)	152.16 (±7.66)	135.67 (±11.52)	0.241

*Independent sample t-test comparison between younger (18–35 years of age) and older (≥60 years of age). Values are expressed as mean ± SEM. BMI, body mass index; RM, repetition maximum. *$p < 0.05$.*

Anthropometric Measurements

Height (to nearest 0.1 cm) was measured using a wall-mounted Holtain Harpenden Stadiometer (Holtain Ltd., Crymych, Wales, United Kingdom), and body weight (to nearest 0.1 kg), BMI and body fat % (to nearest 0.1%) were measured using Seca® (mBCA 514 Medical Body Composition Analyzer, Gmbh&Co. KG, Hamburg, Germany) with participants being fasted, with an empty bladder and with standardised exercise clothing.

Participants' Determination of 1RM

A 5RM protocol was employed at baseline visit after anthropometric measurements, blood sampling and functional assessments to avoid residual EIMD from baseline affecting experimental measures. 5RM test was performed for the prediction of 1RM to minimise myofibrillar damage to the contractile proteins of the knee extensors, as well as to avoid adaptations to muscle damage and potential repeated bout effect for the EIMD trial. Each participant performed six concentric repetitions of incremental weight until failure, with 3 min rest between sets. 5RM leg press predictive equation (Reynolds et al., 2006) was then applied to determine 1RM for each participant. The predicted 1RM weight lifted concentrically was then used to calculate 120% of the weight to be performed eccentrically at EIMD visit.

Eccentric Leg Press Exercise Protocol

Participants performed a muscle-damaging exercise protocol known to successfully induce delayed onset muscle soreness (DOMS) in younger individuals (Vaile et al., 2008). The protocol comprised of seven sets of 10 eccentric single-leg press repetitions on a leg press machine (Body-Solid G9S Multi-Station Home Gym, Taiwan), with the first five sets of 10 repetitions at 120% of 1RM and final two sets of 10 repetitions at 100% of 1RM. A timed rest period of 3 min took place between each set. The protocol was performed unilaterally on each participant's dominant leg. Before performing each eccentric contraction, participants raised the weight using both legs, concentrically. Each eccentric contraction lasted 3–5 s, during which participants resisted the load with the dominant leg from full knee extension to 90 degrees angle of knee flexion (Vaile et al., 2007, 2008). All participants completed all seven sets. Water was provided *ad libitum* every 15 min.

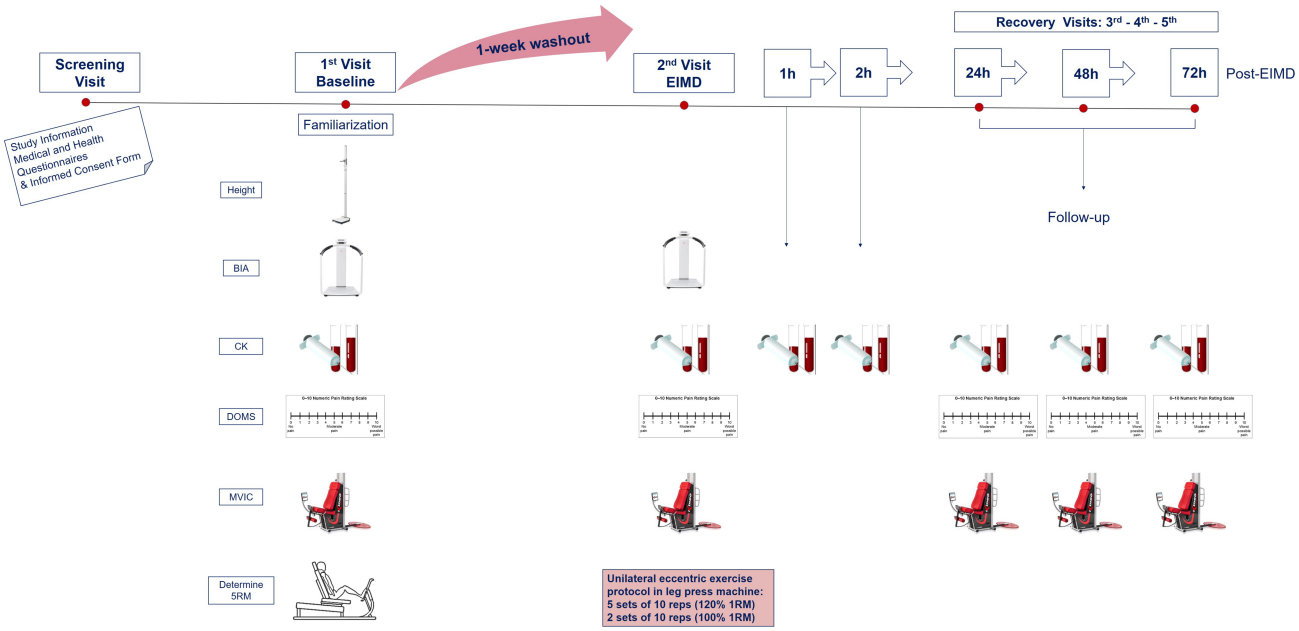

FIGURE 1 | Schematic of experimental procedures. BIA, body impedance analysis; CK, creatine kinase; MVIC, maximal voluntary isometric contraction for peak force; DOMS, delayed onset muscle soreness *via* visual analogue scale; RM, repetition maximum; and EIMD, exercise-induced muscle damage. Second visit combines both pre- and post-measurements, immediately prior and following EIMD stimulus, respectively.

Assessment of Muscle Function

Muscle Soreness

Magnitude of DOMS was quantified using a visual analogue scale (VAS), and it was self-rated by participants on a 10-point-validated VAS indicating on a horizontal line with anchor points from 0 (no pain) to 10 (extreme pain; Carlsson, 1983; McCormack et al., 1988). Participants were seated with both legs in passive 90 degrees of flexion during a wall squat. Participants then placed a mark at the point on the VAS corresponding to their perception of soreness on the quadriceps muscle. Participants were blinded to the scores they had previously reported.

Maximal Voluntary Isometric Contraction

Maximal voluntary isometric contraction leg strength of the quadriceps was assessed on KINEO dynamometer (Globus Kineo 7000, Italy). Participants were seated upright and strapped into the dynamometer to limit excess motion. The chair was adjusted so that the leg pad was placed on the lower part of the tibialis anterior, and the pivot was located on the lateral epicondyle of the dominant leg. Maximal force was measured at an angle of 60 degrees leg extension. The protocol consisted of three maximal isometric contractions with 120 s recovery between each repetition. Following a 2-min rest period, participants employed maximal isometric force against the leg pad. Peak force was determined by the average of three maximal isometric contractions lasting 3–5 s. From pilot data ($n = 6$ healthy younger participants) the within-day coefficient of variation (CV) for leg extension MVIC was calculated as 6.2% and the day-to-day CV was calculated as 8.7%. Verbal encouragement was given throughout each repetition.

Venous Plasma

A 6 ml vacutainer tube of venous blood was collected at each time point (lithium-heparin; BD, Oxford, United Kingdom). Whole blood was spun (Hettich Universal 320 R, Germany) at 3,857 g for 10 min at 4°C, with plasma aliquoted and frozen at −80°C.

Circulating CK activity was measured using a clinical chemistry analyser (Werfen ILab Aries, Italy). CK activity was determined using kinetic spectrophotometry at 340 nm with a minimum detection limit of 3 U/L, an undiluted linearity up to 900 U/L. CV for CK was within run <1.2%, total <2.5%. All samples and standards were analysed in duplicate.

EV Isolation and Characterisation From Human Plasma

Isolation of Plasma-EVs

Plasma EVs were prepared from the individual plasma (thawed on ice) aliquots (100 µl per individual) from each participant, under the different conditions, using sequential centrifugation and ultracentrifugation according to previously standardised and described protocols and procedures (Kosgodage et al., 2018; Criscitiello et al., 2019; Pamenter et al., 2019), also following the recommendations of The International Society for Extracellular Vesicles (MISEV2018; Thery et al., 2018). For each individual plasma-EV preparation, 100 µl of plasma was diluted 1:5 in Dulbecco's PBS (DPBS, ultrafiltered using a 0.22 µm filter, before use). This was then centrifuged for 20 min at 3,000 *g* at 4°C, to remove apoptotic bodies and aggregates. Supernatants were then collected and ultra-centrifuged at 100,000 *g* at 4°C for 1 h. This resulted in EV-enriched pellets, which were resuspended each in 500 µl DPBS and thereafter

ultra-centrifuged again for 1 h at 100,000 *g*, at 4°C. The final resulting EV pellets were resuspended each in 100 µl of DPBS. The EV pellets were kept frozen at −80°C until used for nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM) in the procedures described below (all assessments were performed with EV preparations that had not been frozen for longer than 1 week).

Nanoparticle Tracking Analysis

Plasma-EV quantification and size distribution profiles were established by NTA, based on Brownian motion of particles in suspension, using the NanoSight NS300 system (Malvern, United Kingdom). For NTA, the EV samples were diluted 1/100 in DPBS (10 µl of EV preparation diluted in 990 µl of DPBS). The diluted EV samples were applied to the NanoSight NS300 (Malvern Panalytical, United Kingdom), recording five repetitive reads, 60 s each. Particle numbers per frame were 40–60, camera settings were at level 10 for recording and for post-analysis the detection threshold was set at 5. Replicate histograms were generated from these videos using the NanoSight software 3.0 (Malvern), representing mean and ±SEM of the five recordings for each sample.

Transmission Electron Microscopy

Plasma EVs were further assessed by morphological analysis using TEM. EVs were resuspended in 100 mM sodium cacodylate buffer (pH 7.4). One drop (~3–5 µl) of the EV suspension was placed onto a grid, which held a carbon support film which had been previously glow discharged. Following partial drying of the EV suspension, the sample was fixed for 1 min at room temperature (RT) by placing the grid onto a drop of a fixative solution (2.5% glutaraldehyde) in 100 mM sodium cacodylate buffer (pH 7.4). The grid was applied to the surface of three drops of distilled water for washing of the EV sample, removing excess water using a filter paper. The EVs were then stained for 1 min with 2% aqueous uranyl acetate (Sigma-Aldrich), removing excess stain with a filter paper and air drying the grid. TEM imaging of EVs was carried out with a JEOL JEM 1400 transmission electron microscope (JEOL, Tokyo, Japan), which was operated at 80 kV, using a magnification of 30,000x to 60,000x. Recording of digital images was performed with an AMT XR60 CCD camera (Deben, United Kingdom).

Western Blot Analysis

Extracellular vesicles were assessed for the EV-specific markers CD63 and Flotillin-1 (Flot-1), using western blotting. EV samples were diluted 1:1 in denaturing 2×Laemmli sample buffer (containing 5% beta-mercaptoethanol, BioRad, United Kingdom) and heated for 5 min at 100°C. Protein separation was carried out at 165 V using 4–20% gradient TGX gels (BioRad United Kingdom), followed by western blotting at 15 V for 1 h using a Trans-Blot® SD semi-dry transfer cell (BioRad, United Kingdom). Membranes were blocked with 5% bovine serum albumin (BSA, Sigma, United Kingdom) in Tris buffered saline (TBS) containing 0.1% Tween20 (BioRad, United Kingdom; TBS-T) for 1 h at RT and primary antibody incubation was carried out overnight at 4°C using the EV-marker CD63 (ab216130, Abcam, United Kingdom) and Flot-1 (ab41927, Abcam); diluted 1/1,000 in TBS-T. The membranes were then washed at RT in TBS-T for 3×10 min and thereafter incubated with HRP-conjugated anti-rabbit IgG secondary antibodies (BioRad), diluted 1/3,000 in TBS-T, for 1 h at RT. The membranes were then washed for 4×10 min TBS-T, and visualised, using enhanced chemiluminescence (ECL, Amersham, United Kingdom) in conjunction with the UVP BioDoc-ITTM System (Thermo Fisher Scientific, United Kingdom).

Statistical Analysis

Normal distribution of data was examined by QQ plot visual inspection. Following Levene's test of equality of variance, baseline characteristics were compared between groups using a two-tailed independent samples *t*-test. Exercise-induced changes in EV profiles, CK, and MVIC were analysed using a mixed model ANOVA with repeated measures [group (younger, older) × time (pre-, post-, at 1, 2, 24, 48, 72 h post-EIMD)]. Tukey's correction was used for *post hoc* analysis to perform pairwise comparisons. As an ordinal measure, Mann-Whitney U test was used to determine between group differences in DOMS. The EIMD effects on DOMS within-group were determined across time using Freidman ANOVA, and the Wilcoxon matched pairs signed ranks test was performed for *post hoc* analysis to test differences in this variable. The relationship between EV profiles, and CK, MVIC, and DOMS were performed with Pearson correlation. Partial eta-squared (η^2_p) values were calculated as measures of effect size for mixed model ANOVA when necessary, and were considered small (0.01), medium (0.06), or large (>0.14) effect, and for Wilcoxon matched pairs tests, effect size (*r*) was considered small (0.10), medium (0.30), or large (0.50) by the formula $z/\sqrt{n}$; where n = the number of observations over the two time points (Pallant, 2016); all were calculated using methods proposed by Cohen (1988). Values were considered statistically significant if $p < 0.05$. Values were expressed as mean ± SEM for data from parametric tests, and as median and interquartile range for data from non-parametric tests. All figures were generated in, and statistical analysis performed in GraphPad Prism (Version 9.1.1, GraphPad, United States), except generation of NTA curves which was carried out using the Nanosight 3.0 software (Malvern, United Kingdom). Subsequent power calculations on data presented within was calculated using G*Power (3.1.9.7).

RESULTS

Participant characteristics are presented in **Table 1**. Besides age, participants were reasonably homogenous, with no differences noted between body fat [younger 16.90 (±2.60) % vs. older 22.10 (±2.83) %, $p = 0.212$], or muscle mass [younger 29.53 (±1.04) kg vs. older 27.54 (±1.22) kg, $p = 0.243$].

Extracellular vesicle profile (both modal size and particle concentration) was quantified by NTA (representative sample shown in **Figure 2A**) and were characterised by Western blotting for EV surface markers (CD63 and Flot-1; **Figure 2B**) and TEM

for morphology (**Figure 2C**). Pre-exercise circulating blood samples suggested that EV modal size did not differ between younger and older participants [younger 109.33 (±7.64) vs. older 115.68 (±6.37) nm, $p = 0.538$, **Figure 2D**], whilst EV count showed a trend of being lower in older participants at rest, relative to younger participants [younger 1.15×10^{10} ($\pm 3.72 \times 10^{9}$) vs. older 2.75×10^{10} ($\pm 3.08 \times 10^{9}$), $p = 0.056$, **Figure 2E**].

Repeated measures ANOVA showed no interaction between age group and time on leg MVIC ($p = 0.064$). However, following the EIMD protocol, a main effect of both time ($p = 0.005$, $\eta^2_p = 0.894$) and age group ($p < 0.001$, $\eta^2_p = 0.437$) was noted for MVIC with a large effect size, suggesting that younger participants' MVIC was higher throughout, and the EIMD protocol successfully reduced muscle force in both groups (**Figure 3A**). *Post hoc*

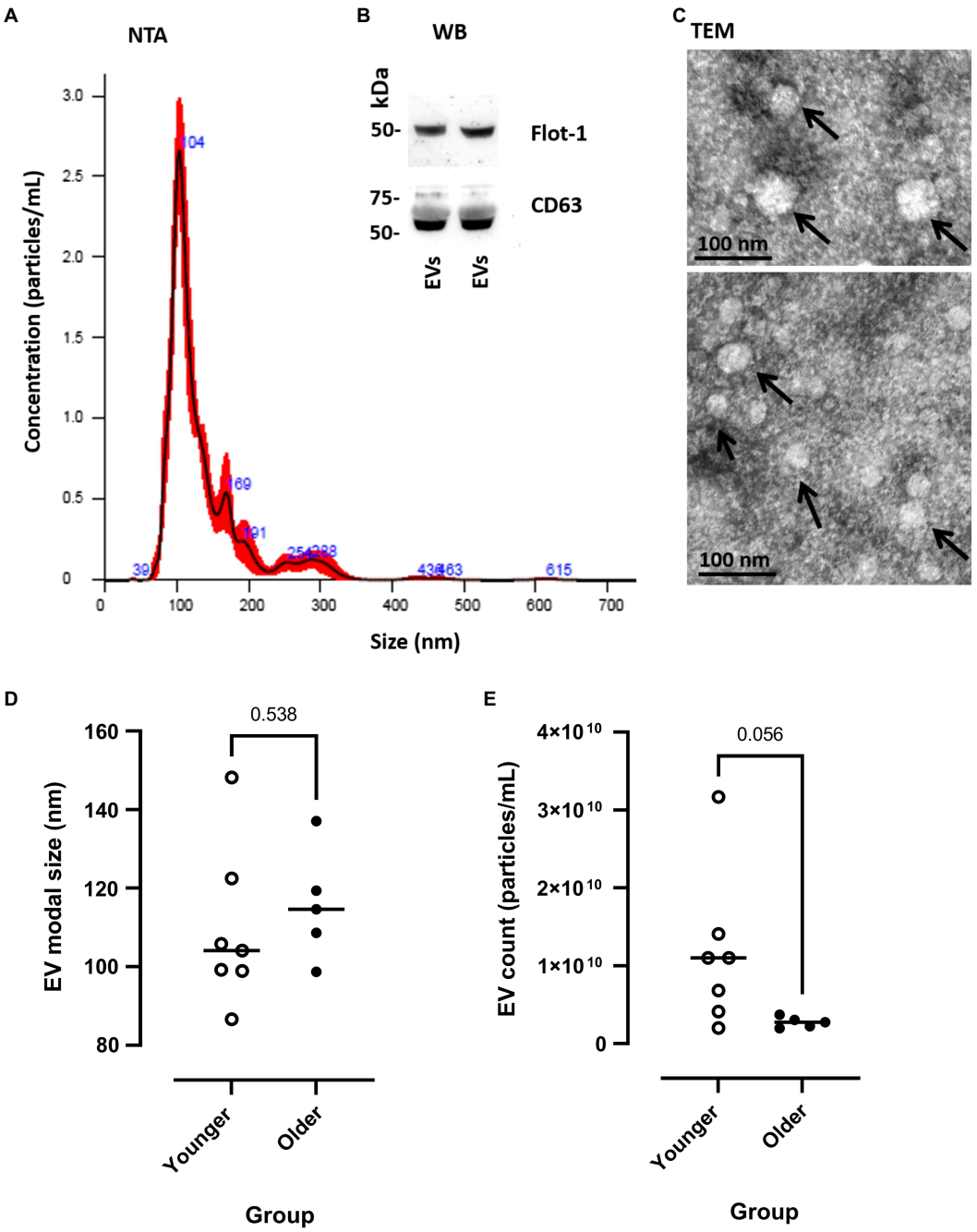

FIGURE 2 | Measurement of EV modal size and count in younger and older participants. **(A)** Representative example of nanoparticle tracking analysis (NTA), SEM shown in red and mean in black line. **(B)** Western blotting of human plasma extracellular vesicles (EVs) showing positive for Flot-1 and CD63. **(C)** Transmission electron microscopy (TEM) images of human plasma-EVs, showing EV morphology; scale bar indicates 100 nm. **(D)** EV modal size (nm) and **(E)** EV count (particles/ml) at pre-EIMD in younger (open circles) and older (closed circles) participants. Horizontal line indicates group means.

testing suggests force significantly decreased immediately post-EIMD [pooled pre-MVIC, 17.21 (±1.40) kg to pooled post-MVIC, 14.15 (±0.99) kg, $p=0.006$], and then started to return in a linear recovery at 24 h post-EIMD [pooled 24 h MVIC, 16.07 (±1.22) kg, $p=0.280$] and at 48 h post-EIMD [pooled 48 h MVIC, 16.62 (±1.42) kg, $p=0.845$], but was not fully restored by 72 h post-EIMD [pooled 72 h MVIC, 16.05 (±1.32) kg, $p=0.334$].

Mann-Whitney U test showed no significant difference in DOMS between groups at any timepoint. However, following the EIMD protocol, Freidman test suggests a significant increase in DOMS across time in the younger group ($p=0.014$) and in the older group ($p=0.034$). Nevertheless, DOMS returned to pre-EIMD values by 72 h post-EIMD in the younger group, but not in the older group (**Figure 3B**). *Post hoc* pairwise comparisons showed that both younger and older group had significantly elevated DOMS immediately post- [younger, Md = 4.00 (3.00), $p=0.042$, r = 0.54; older, Md = 2.00 (3.75), $p=0.039$, $r=0.65$] and at 24 h post-EIMD [younger, Md = 5.00 (2.00), $p=0.034$, $r=0.57$; older, Md = 2.00 (3.25), $p=0.042$, $r=0.64$] relative to pre-EIMD [younger, Md = 1.50 (3.00) and older, Md = 0.00 (2.75)], indicating a large effect size for both time points.

Exercise-induced muscle damage showed no group by time interaction on CK activity ($p=0.398$). However, CK was significantly altered following the EIMD (main effect of time $p=0.026$, $\eta^2_p=0.519$, suggesting a large effect size), with increased CK seen at immediately post-EIMD [pooled pre-CK, 170.18 (±27.26) vs. pooled post-CK 198.36 (±33.01) U/L; $p=0.041$], at 1 h post- [pooled 1 h CK, 208.26 (±33.78) U/L; $p=0.034$], and at 2 h post- [pooled 2 h CK, 216.13 (±35.40) U/L; $p=0.035$] EIMD completion. Circulating CK was not different between age group (**Figure 3C**, main effect of age group $p=0.121$).

Whilst the EIMD protocol visually appeared to induce increased expression and greater variability in circulating plasma-EV modal size in the younger group (**Figure 4A**), repeated measures ANOVA suggested EIMD had no significant effect on group by time interaction ($p=0.898$), nor a main effect of either group (younger or older, $p=0.377$), or time ($p=0.309$; **Figure 4A**). In a similar manner, the EIMD protocol did not substantially alter plasma-EV count, with no group by time interaction ($p=0.416$), nor a main effect of group (younger or older, $p=0.227$) or time ($p=0.074$; **Figure 4B**). These results are maintained if participants are examined independent of age ($n=12$), with one-way ANOVA suggesting no effect of time on EV modal size ($p=0.269$; **Figure 4C**) or count ($p=0.134$; **Figure 4D**). As a preliminary study into changes in EV profile with EIMD in younger and older participants, required sample size for future studies using a condition × time model with seven time points (as presented in **Figure 4**) was calculated as $n=398$ per group for EV modal size, and $n=57$ per group for EV count ($\alpha=0.05$, power $(1-\beta=0.8)$, effect size of 0.035 for EV modal and 0.093 for EV count).

To explore a correlation between EV release profiles as a putative biomarker of muscle damage, the numerical difference in EV modal size (ΔMode) and EV count (ΔCount) between post-EIMD and pre-EIMD was examined relative to CK (U/L), MVIC (kg), or DOMS at each time point measured. A significant association between ΔMode and circulating CK was seen at

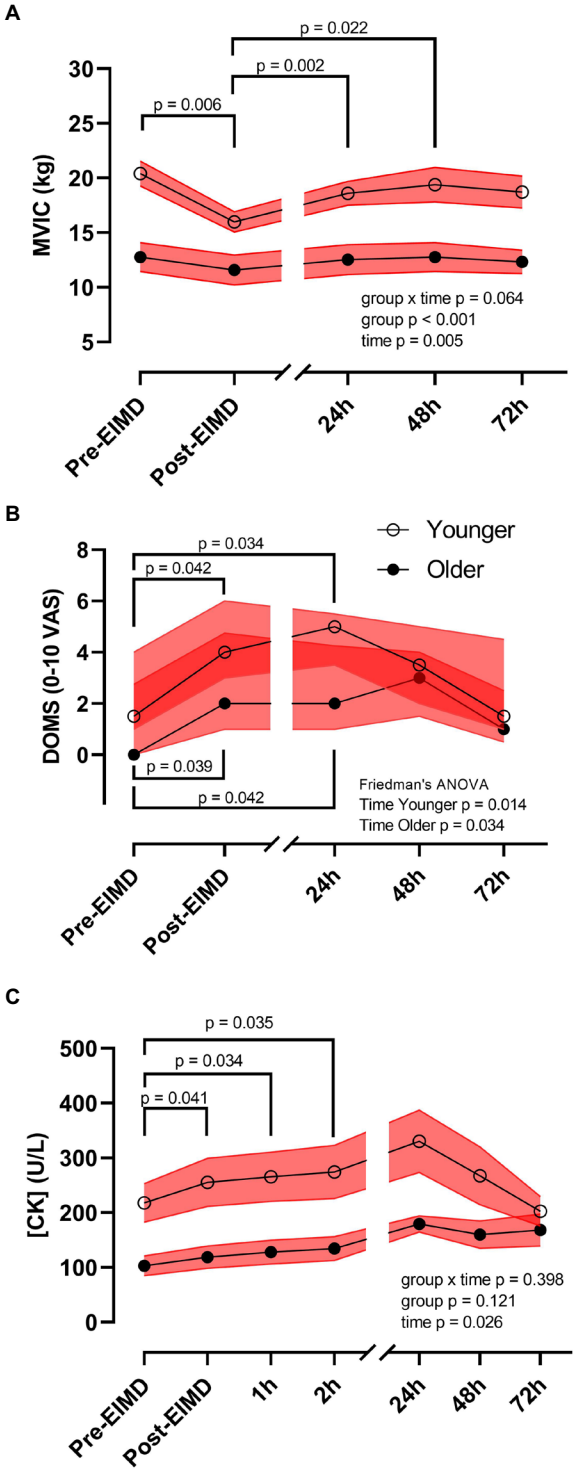

FIGURE 3 | Exercise-induced muscle damage reduces muscle force and increases circulating creatine kinase (CK) concentrations independent of age. **(A)** MVIC (kg), **(B)** DOMS [0–10 visual analogue scale (VAS)], and **(C)** plasma CK (U/L) of younger (open circles) and older (closed circles) participants. Red shaded zones indicate SEM in **(A,C)**, and interquartile range in **(B)**, and black connected line indicates group means in **(A,C)**, and medians in **(B)**, values of *p* between timepoints as indicated. The scale brake indicates from hourly testing to 24-h intervals.

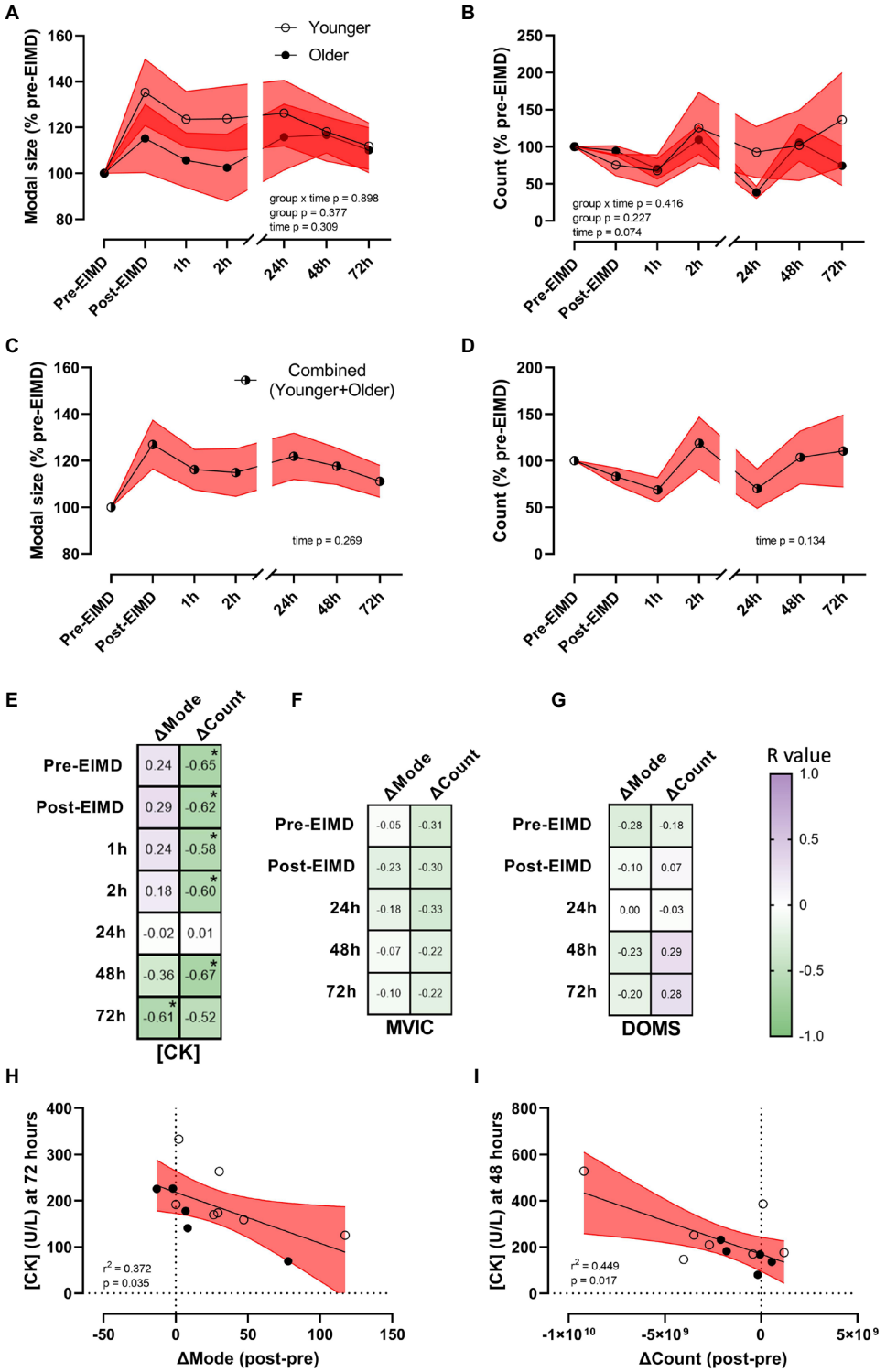

FIGURE 4 | Alterations in EV modal size and count with exercise, and EV correlations with muscle damage markers. **(A)** EV modal size (% pre-EIMD) and **(B)** EV count (% pre-EIMD) as a function of timepoint between younger and older groups, **(C)** EV modal size (% pre-EIMD) and **(D)** EV count (% pre-EIMD) as a function of timepoint combined younger and older participants ($n = 12$). Red shaded zones indicate SEM and black connected line indicates group means. The scale brake indicates from hourly testing to 24-h intervals. **(E)** Correlation matrix between change in EV modal size (ΔMode) or in EV count (ΔCount) as a function of CK (U/L), **(F)** as a function of MVIC (kg) and **(G)** as a function of DOMS (0–10 VAS) at each timepoint measured, with r values as shown. *Indicates significant association between variables (each $p < 0.05$). Colour intensity for r values (purple indicates positive r value, green negative, white = 0) as indicated. **(H)** CK (U/L) at 72 h as a function of ΔMode (post-EIMD – pre-EIMD and **I**) CK (U/L) at 48 h as a function of ΔCount (post-EIMD – pre-EIMD). Red shaded zone indicates 95% CIs. Open circles indicate younger, closed indicate older.

72 h only, post-EIMD (**Figure 4E**; $r^2 = 0.372$, $p = 0.035$ visualised in **Figure 4H**). Circulating CK was shown to significantly associate negatively with ΔCount at every time point measured, except 24 h post-EIMD (**Figure 4E**; largest $r^2 = 0.449$ at 48 h visualised in **Figure 4I**, $p = 0.017$). No significant associations were noted between MVIC and either ΔMode or ΔCount (**Figure 4F**), or DOMS and either ΔMode or ΔCount at any time point measured (**Figure 4G**).

DISCUSSION

Exercise is associated with a number of immediate physiological responses. Circulating EVs can act as plasma-based biomarkers, reflecting physiological and pathophysiological conditions of the body (Withrow et al., 2016; Zhao et al., 2020). Thus, this study analysed EVs in blood plasma isolated during the acute phase of EIMD and during a recovery period of 72 h in younger and older healthy, physically active male adults. In this study, we show that a single bout of EIMD triggers apparent changes to EV concentration and size distribution profiles, but in trained older men there is no clear differences in this EV signature from that of younger men. However, unlike prior studies on the effects of acute endurance exercise on EV release profiles, acute eccentric resistance exercise does not appear to predictably alter EV modal size or EV concentration. Furthermore, immediate changes in EV profiles as observed here may associate with later changes in biological markers of muscle damage, such as CK, as found in the current study.

No significant effect on EV profiles was observed in relation to age at pre-exercise values, with younger and older participants showing relatively homogeneous EV profile responses. Nonetheless, older participants had lesser magnitude of CK response than their younger counterparts. Whilst the younger group showed a greater signal in CK response and returned to pre-exercise values by the end of the experimental period, suggesting a better resolution in recovery, the older group did not attain absolute values by the end of the recovery period. Unexpectedly, both groups had similar recovery in leg strength changes following EIMD. Likewise, a previous study has reported no age differences in muscle function after muscle-damaging exercise (Heckel et al., 2019). However, others concluded that younger individuals were able to recover and adapt quicker in functionality following EIMD, confirming that muscle function declines through the ageing process (Tieland et al., 2018; Fernandes et al., 2019). In the current study, muscle soreness significantly peaked immediately post- and at 24 h post-EIMD for both groups, but the younger group consistently scored higher on perception of pain than the older group during the experimental period. This may have been attributed to a higher muscle damage, as indicated by the increased CK activity for the younger men, or hypothetically due to the larger ratio of type II fibres typically seen in younger individuals, which have been suggested to be more susceptible to injury (Byrne et al., 2004; Verdijk et al., 2014). However, muscle biopsies would be required to confirm the fibre type shift. Similarly, Lavender and Nosaka (2006) reported older males experienced lower muscle soreness than younger males following EIMD. A review by Gibson and Helme (2001) also reported that pain perception is decreased with ageing. This may explain the lower DOMS score of the older group compared with the younger group in the present study. Nevertheless, no significant differences were observed between groups following EIMD. Similarly, Nikolaidis (2017) and Heckel et al. (2019) demonstrated no differences between age groups after lower-body resistance exercise. However, Lavender and Nosaka (2008) found opposite findings after eccentric exercise. The contrast in research findings was attributed to the magnitude of muscle damage induced by the exercise protocol used (bilateral vs. unilateral) or due to the different muscle group (arm vs. leg) involved in the studies. Overall, the current study showed that EIMD recovery took a similar course in both muscle function and DOMS for physically active younger and older individuals. Therefore, the data presented here suggests that when younger and older individuals are matched for activity status, ageing does not appear to impair recovery from voluntary eccentric exercise.

Endurance exercise has been shown to alter EV profiles (Oliveira et al., 2020; Soares et al., 2021). Chronic exercise in murine models (3 weeks swim training) was, for example, shown to significantly increase serum EV count (Bei et al., 2017), whilst the modal size of EVs was unchanged. Both EV count and modal EV size were elevated in race horses following a single bout sustained (160 km) endurance exercise (Oliveira et al., 2020), which may correlate with previous observations of larger EVs being associated with inflammation (Słomka et al., 2018). Alternatively, in humans Fruhbeis et al. (2015) reported a significant increase in EV concentration immediately after an incremental cycling exercise to failure (typically 12–20 min), but EVs were found to be cleared from the circulation during the early recovery period (90 min after exercise). However, the concentration of plasma-EVs remained elevated after exhaustive running. In murine models, Oliveira et al. (2018) showed that 40 min of moderate intensity endurance exercise immediately increased EV modal size, but neither low, nor high intensity exercise had any effect on modal size. It is therefore of interest that in the current study we did observe a shift in EV modal size towards larger EVs, at 48 h post-EIMD (**Supplementary Figure 1**), albeit this trend was not statistically significant. Our findings are also in line with previous work of Lovett et al. (2018) who reported no significant change in EVs size or number over time after an acute muscle-damaging exercise (combination of plyometric jumping and downhill running). Thus, it may be that exercise duration, intensity, and modality, in addition to differential species responses may yield variable results, and this warrants further exploration to fully understand effects of an acute exercise bout on circulating EVs. Indeed, as already noted, great individual variability is observed in human responses to various exercise modalities, and thus differing EV profile response may in part underlie differing adaptation to these modalities (Trovato et al., 2019). Alternatively, in lieu of changes to the number and morphology of circulating EVs, their transported cargo may be more relevant to the exercise response, and thus future studies may choose to examine this variable.

Unlike research on endurance models, the current literature is lacking in resistance training investigations, and specifically eccentric muscle-damaging protocols, such as those used in the current study. Whilst Cui et al. (2017) assessed three different types of resistance exercise, they reported only changes in circulating microRNAs (miRNAs), not in EV profile states. Our study provides evidence that early changes in EV profile following EIMD significantly correlate with subsequent changes in CK, a known biomarker of muscle damage, and thus acute changes in EV profile post-exercise may indicate subsequent magnitude of muscle damage. Both processes may result from the mechanical EIMD stimulus (e.g., the mechanical "stretching" of the muscle cell membrane may promote both CK and EV release). Alternatively, it is tempting to speculate that the EV response may be causative of subsequent changes in muscle damage markers, such as CK; however, such causality is not possible to ascribe with the data collected here. Outside of EIMD, other types of muscle damage, such as laser membrane ablation and cellular hypoxia, have been reported to induce rapid increases in EV release; however, these have hitherto been performed on either zebrafish (Middel et al., 2016) or mouse models (Scheffer et al., 2015), muscle tissue *ex vivo*, and thus the results presented here are the first to extend these findings into human models of muscle damage.

Whilst associations have previously been observed between EV release profiles in response to inflammatory disorders (Hosseinkhani et al., 2018) and older individuals are noted to have elevated basal systemic inflammatory cytokines concentrations (Franceschi and Campisi, 2014), circulating miRNAs (Jung and Suh, 2014) and increased EV release is seen from senescent cells (Hitomi et al., 2020; Riquelme et al., 2020). Therefore, we were interested in examining any putative age differences in EV release profiles between older and younger individuals following a bout of EIMD. Whilst our results presented here suggest no major differences in EV modal size or EV plasma concentration in younger vs. older individuals, following a single bout of EIMD, some caution should be taken in the interpretation of these results due to the small sample size assessed and volunteer selection. The findings presented here are with recreationally active younger and older participants, all participants habitually engaged in structured physical activity, and thus are not representative of wider physically inactive Western populations (Farrell et al., 2013; Lindsay et al., 2019). Importantly also, in the ageing population, reduced physical activity and increase in sedentary time are typically observed (Lindsay et al., 2019). Furthermore, no difference in muscle mass or fat mass was seen in our study population, unlike that witnessed in wider society (Volpi et al., 2004; Barrios-Silva et al., 2018). By studying highly active ageing cohorts, we can separate physiological differences of ageing from inactivity induced changes (Harridge and Lazarus, 2017). Our results, therefore, should be interpreted in light of the relatively physical trained cohort presented here. Any potential differences suggested by the results presented here between age groups may be enhanced when expanding this study to exercise naive younger and older individuals; however, this may reflect effects of long-term inactivity, not ageing *per se*.

Whilst this pilot study on EIMD has presented some interesting results in relation to EVs as putative biomarkers for muscle damage, these findings will need further validation in larger cohorts that can be guided in sample size collection by the results presented here. Future investigations should also conduct in depth analysis of EV cargo composition will be of considerable interest for the identification of EV-related biomarkers in EIMD. Therefore, it will be of great interest to perform full EV profiling analysis using RNA sequencing, proteomics and metabolomics to reveal the EV cargo profiles in response to EIMD, also in different age populations. Whilst EV cargo biomarkers have been implicated in the pathophysiology of inflammation-associated disorders, research regarding their role in EIMD and ageing remains limited. This study therefore provides the first insights into the potential of EV-profiling in association with muscle-damaging exercise and ageing and paves the way for future studies, aiming to extend current knowledge on their roles as mediators of health-promoting effects, and as biomarkers, associated with physical activity.

In conclusion, here we show that physical responses to eccentric exercise induces plasma-EV changes that correlate with CK release post exercise, a biological marker of muscle damage. EV profiles did not appear to change significantly in relation to age groups assessed (active younger vs. older), which importantly may make them a reliable biomarker to assess effects of exercise interventions across age groups. As EV release has previously been associated with small animal models of muscle damage, our study further supports that EV release profiles immediately following exercise may also play a role in the EIMD response in humans. If the post-exercise EV response does indeed reflect physiological injury recovery responses, the magnitude and content of EV profile changes could be of interest for strategies to reduce the impairing effects of EIMD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by College of Liberal of Arts and Sciences Research Ethics Committee, University of Westminster, United Kingdom. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YK and BE: conceptualisation, methodology, project administration, and writing – original draft preparation. YK, IK, and SL: formal analysis and investigation. YK: human fitness testing. YK, IC, IK, SL, and BE: data curation. YK, IK, SL, and BE: visualisation. BE: supervising and funding acquisition.

YK, IC, SL, and BE: writing – review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.723931/full#supplementary-material

Supplementary Figure 1 | Representative example of EV profiles responses of a younger and older participant, showing shifts of EV modal size to larger EVs in response to EIMD during the post-exercise recovery period.

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GLOSSARY

Term	Definitions
ANOVA	Analysis of variance
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BSA	Bovine serum albumin
CK	Creatine kinase
CV	Coefficient of variation
DOMS	Delayed-onset muscle soreness
ECL	Enhanced chemiluminescence
EIMD	Exercise-induced muscle damage
EVs	Extracellular vesicles
Flot-1	Flotillin-1
miRNAs	microRNAs
MVIC	Maximal voluntary isometric contraction
NTA	Nanoparticle tracking analysis
PAR-Q	Physical activity readiness questionnaire
Post-EIMD	Following exercise-induced muscle damage
Pre-EIMD	Before exercise-induced muscle damage
RM	Repetition maximum
RT	Room temperature
SEM	Standard error mean
TBS	Tris buffered saline
TEM	Transmission electron microscopy
VAS	Visual analogue scale
WURSS	Wisconsin upper respiratory symptom survey
ΔCount	Difference between EV concentration at post-EIMD and pre-EIMD time points
ΔMode	Difference between in EV modal size at post-EIMD and pre-EIMD time points

frontiers in Physiology

BRIEF RESEARCH REPORT
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Acceleration of Longitudinal Track and Field Performance Declines in Athletes Who Still Compete at the Age of 100 Years

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While physical performance decline rates accelerate after around the age of 70 years, longitudinal athletic performance trends in athletes older than 95 years are unknown. We hypothesized a further accelerated decline in human performance in athletes who still perform at the age of 100 years. To investigate this, longitudinal data of all athletes with results at or over the age of 100 years were collected from the "World Master Rankings" data base spanning 2006–2019 (138 results from 42 athletes; 5 women, 37 men; maximum 105 years) and compared to previously published longitudinal data from 80- to 96-year-old athletes from Sweden (1,134 results from 374 athletes). Regression statistics were used to compare performance decline rates between disciplines and age groups. On average, the individual decline rate of the centenarian group was 2.53 times as steep (100 m: 8.22x; long jump: 0.82x; shot put: 1.61x; discus throw: 1.04x; javelin throw: 0.98x) as that seen in non-centenarians. The steepest increase in decline was found in the 100-m sprint (*t*-test: $p < 0.05$, no sign. difference in the other disciplines). The pooled regression statistics of the centenarians are: 100 m: $R = 0.57$, $p = 0.004$; long jump: $R = 0.90$, $p < 0.001$; shot put: $R = 0.65$, $p < 0.001$; discus throw: $R = 0.73$, $p < 0.001$; javelin throw: $R = 0.68$, $p < 0.001$. This first longitudinal dataset of performance decline rates of athletes who still compete at 100 years and older in five athletics disciplines shows that there is no performance plateau after the age of 90, but rather a further acceleration of the performance decline.

Keywords: aging, master athletics, physical activity, longevity, oldest-old, centenarian, javelin throw, long jump

INTRODUCTION

Human longevity, limits of the human life span and physical performance in old age are of great interest due to the increasing proportion of older people in western societies. It has been speculated that the human mortality rate reaches a "plateau" after the age of 105 (Barbi et al., 2018) but it is unknown whether a plateau, or attenuated decline, also exists for physical performance. Frailty that is associated with mobility limitations and loss of autonomy is a major cause of a poor quality of life of the oldest old (Portegijs et al., 2016; Ding et al., 2017). Indeed, for the oldest old, key to a good quality of life is physical independence (Arai et al., 2014). Data of the few extraordinary individuals who are still able to compete in sports at an age where most people are unable to move and care for themselves are therefore of particular interest for the older people themselves, healthcare providers and insurers, as they may not only inspire other older people, but also show the limits of the physiologically achievable in the oldest old.

In the master athlete population, performance decline rates based on results from annual rankings are currently known up until the age of 95, but not beyond (Ganse et al., 2020a). Analyses of such databases have revealed that the rate of decline of physical performance is initially almost linear, and then accelerates from around the age of 70 years (Da Silva Aguiar et al., 2020). This acceleration has not only been shown in cross-sectional (Young and Starkes, 2005; Ganse et al., 2018), but also in longitudinal data (Lazarus and Harridge, 2017; Ganse et al., 2020b). The decline rate accelerates even further beyond the age of 80 (Ganse et al., 2020b). However, while in longitudinal data most individuals show an acceleration, some athletes have a slower or faster performance decline than others (Donato et al., 2003; Rubin et al., 2013; Ganse et al., 2020b; Hoog Antink et al., 2021). Only few cross-sectional data of centenarian athletes are available (Lepers et al., 2016), but there are no longitudinal data on athletes around 100 years, despite the need for more knowledge in the field of frailty and aging-research (Arai et al., 2014; Portegijs et al., 2016; Ding et al., 2017). Increased participation of centenarians in competitive sports now allows us to assess the rate of performance decline also in a population of oldest-old athletes.

Based on the observation that world records up to the age of 105 years suggest a progressive age-related acceleration of performance decline in many disciplines (Baker and Tang, 2010), we hypothesized a further acceleration of the decline rate in performance of master athletes that are around 100 years of age. To determine whether the rate of performance decline in master athletes is accelerated after the age of 90 years, we combined the data from 80+-year-old athletes in 5 disciplines from a longitudinal data set we published recently (Ganse et al., 2020b) with new longitudinal data of athletes who still competed at age 100 years or older from the database "World Master Rankings." This represents the first master athletics performance data set ever published of this age group.

MATERIALS AND METHODS

Ethical approval was obtained from the IRB of Saarland Medical Board (Ärztekammer des Saarlandes, application number 135/21).

Generation of Data Set

Performance data of all athletes with results at age 100 years and older were collected from the publicly available "World Master Rankings" data base (www.mastersrankings.com). As the data base reached back to the year 2006, and since restrictions due to the COVID-19 pandemic led to the absence of data in the year 2020, results of both sexes from 2006 to 2019 were considered. For all athletes who had a result at an age of 100 years and older, the data base was searched for additional results from previous ages going back to as early as the age of 90 years to obtain a longitudinal trajectory of the individual.

The Swedish Data Set

The new data were compared to the data of all 80+-year-old athletes in a data set from Sweden that was published previously (Ganse et al., 2020b). Briefly, this data set comprises all data from the Swedish master athletics rankings from the years 1901 to 2019 and is the largest longitudinal master athletics data set published to date.

Implements

Changes in weights of javelins, discusses, and shots with age did not affect the results in the present analysis, as implement weights in the throwing disciplines stay constant for athletes 80 years and older. The following implements are used by athletes 80 years and older: shot put: men 3 kg, women 2 kg; discus throw: men 1 kg, women 750 g; javelin throw: men and women 400 g.

Statistical Analysis

All statistical tests were executed with IBM SPSS Statistics version 25. Regression statistics were employed to compare performance decline rates between disciplines and age groups. Regression coefficients and their corresponding p-values were calculated (significance level 0.05). Disciplines were included in the study if the following inclusion criteria were met: at least three athletes with at least two results in the data set and at least one result at an age of 100 years or more. Linear regression decline rates were computed for the younger (the data from Sweden) and the older (the data of the athletes who still compete at 100 years) data set separately to allow direct comparison of declines, even though other regression types would have delivered higher R^2-values. Two separate analyses were conducted: linear regression on all pooled data points of each group, and linear regression on the individual regression lines of each athlete, combined with t-tests. We chose linear regression, as the optimal type of regression function differed between disciplines, and to be able to compare decline slopes between the centenarians and the non-centenarian old athletes.

TABLE 1 | Numbers of athletes and data points per discipline in the present centenarian (100+-years group) and Swedish (80+ group) data sets, separated by sex.

	Number of centenarian athletes			**Number of centenarian data points**		
Discipline	**Men**	**Women**	**Total**	**Men**	**Women**	**Total**
100 m	7	3	10	18	6	24
Long jump	3	0	3	10	0	10
Shot put	12	1	13	36	2	38
Discus throw	9	0	9	39	0	39
Javelin throw	6	1	7	23	4	27
Sum	**37**	**5**	**42**	**126**	**12**	**138**
	Number of athletes 80+ years (Sweden)			Number of data points 80+ years (Sweden)		
Discipline	Men	Women	Total	Men	Women	Total
100 m	51	4	55	94	11	105
Long jump	31	5	36	83	10	93
Shot put	81	16	97	290	35	325
Discus throw	91	18	109	307	41	348
Javelin throw	68	9	77	248	15	263
Sum	**322**	**52**	**374**	**1,022**	**112**	**1,134**

RESULTS

Characterization of the Data Sets

We collected 138 results from 42 athletes (5 women and 37 men) of whom results at the age of 100 years or more up to the age of 105 were present in the data base (**Table 1**). The most data in this age group were available from discus throw and shot put, followed by javelin throw, 100-m sprint and long jump. **Table 1** also shows the numbers of athletes and results in the disciplines in the other longitudinal data set of athletes between 80 and 96 years of age with 1,134 results from 374 athletes.

Decline Rates

Figures 1A–E and **Table 2** show that in all five disciplines the average decline rate was higher in the older compared to the younger athletes. The steepest increase in performance decline was found in the 100-m sprint (t-test, $p < 0.05$), where the decline rate was 8x as steep in the older compared to the younger group (**Table 2**). The least steep increase in performance decline was found in the javelin throw. The increases in decline were not significant in long jump and in the throws (t-test, **Table 2**). On average, the decline rate of centenarians was 2.53x as steep as compared to that between 80 and 96 years. **Figure 1F** shows a direct comparison of the disciplines.

Sex Differences

Only 13.5% (5 out of 42 athletes) among the centenarians were women, which is in line with the 13.9% (52 out of 374 athletes) in the Swedish data set. Three women had results in the 100-m sprint, one in the shot put and one in the javelin throw. Due to the low number of women in the data-set, an analysis of sex differences in decline rates was not possible.

DISCUSSION

In the present study, we analyzed performance declines in five athletic disciplines of 42 master athletes who still performed at 100 years of age and older, and compared the data to the 80+-year-old athletes from a longitudinal data set we have published previously (Ganse et al., 2020b). The main finding of the study was that performance decline rates of centenarian athletes were on average 2.53-times as steep as the decline rate between 80 and 96 years of age. The fastest drop occurred in the 100-m sprint, while the javelin throw showed the least steep increase in the slope of decline. Of the centenarians, 13.5% were women, which is in line with the 13.9% in the younger collective.

Performance Trends in Aging

It has been reported that the rate of performance decline was more than three times as fast in athletes older than 80 years compared to 30- to 69-year-old athletes (Ganse et al., 2020a,b). Accelerated decline rates after the age of 70 have also been shown in other disciplines and sports (Young and Starkes, 2005; Berthelot et al., 2012; Rubin et al., 2013; Dahl et al., 2020). The "plateau" in mortality rate in the oldest old (Barbi et al., 2018) may suggest a stabilization of the age-related rate of decrease in physical decline. Our data, however, do not show evidence for such a plateau in physical performance, but rather that the rate of decline is accelerated even further in 90+-year-old athletes beyond that seen in 80+-year-old athletes. Ongoing increases in decline rates were also previously reported from master world records (Baker and Tang, 2010; Akkari et al., 2015). At the same time, our data suggest that there is variation in the decline rate between individuals, that was also shown in the Swedish data set (Hoog Antink et al., 2021). Understanding the underlying factors for these differences could help to develop and improve interventions to slow down performance decline rates.

Differences in decline rates between track and field disciplines may be explained by specific skills required in each discipline,

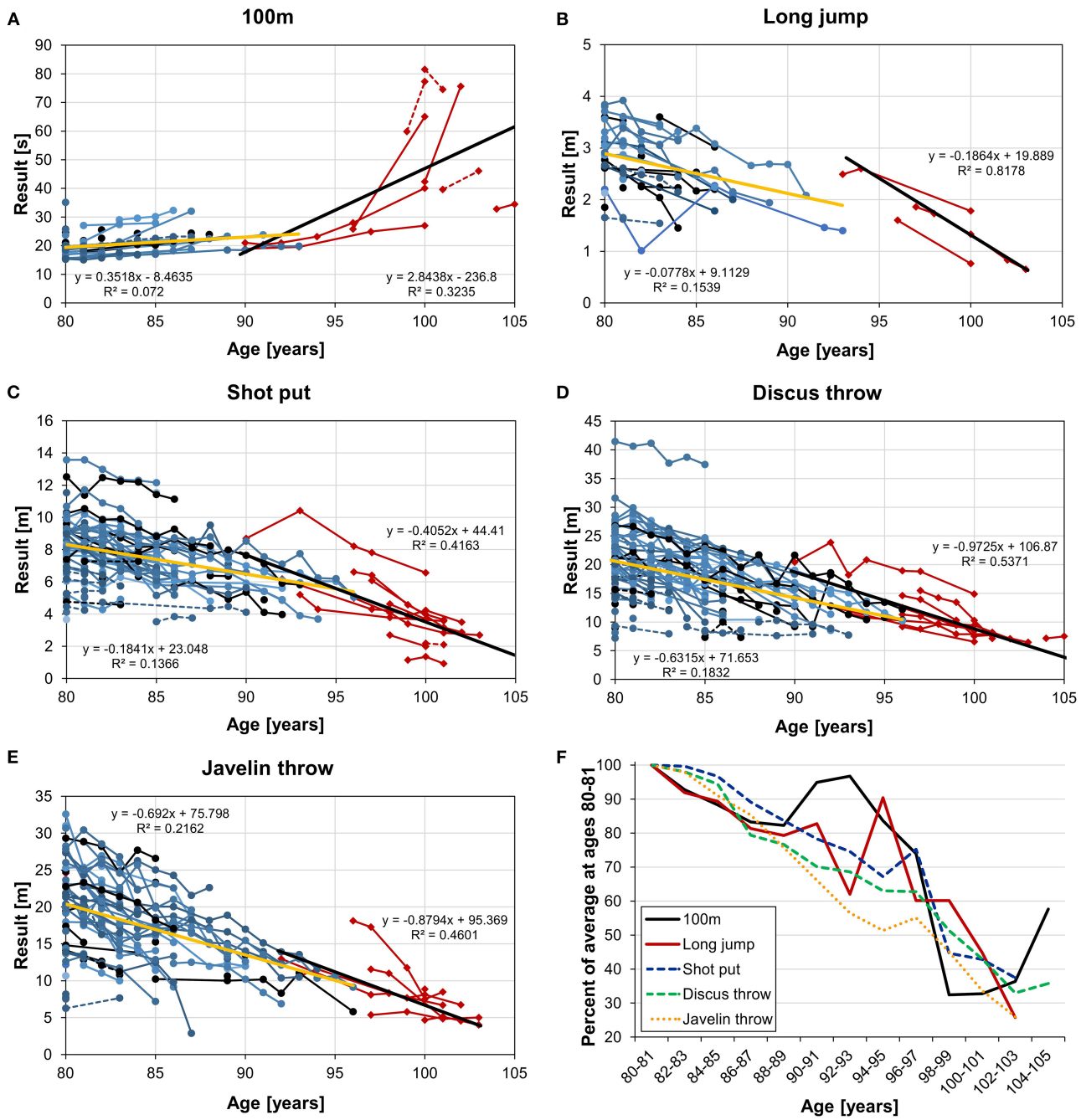

FIGURE 1 | Longitudinal decline trajectories of individual athletes for 100-m sprint **(A)**, long jump **(B)**, shot put **(C)**, discus throw **(D)**, and javelin throw **(E)**. Dashed lines: women; solid lines: men. Blue/black lines and circles: data from the "Swedish Veteran Athletics" data base (Ganse et al., 2020b); red lines and rhombi: data of athletes with data at age 100 and/or older. Linear regression lines and functions are shown to allow comparison with the literature. Yellow line: regression line of data from "Swedish Veteran Athletics" (80+ years); black line: regression line of the new data from the "World Master Rankings" (100+ years). **(F)** Direct comparison of the performance declines normalized to ages 80–81 in the five disciplines, shown in pooled 2-year steps.

such as power, speed, agility, coordination or endurance, that may show different rates of age-related decline. For instance, the fastest performance decline was observed in the 100-m sprint that requires mainly speed and power (Dahl et al., 2019), while the javelin throw showed the least steep decline rate and depends besides power, on agility and coordination (Ganse and Degens, 2018). This needs to be studied in more detail, however, as previously we have observed that there was no significant difference in the rate of decline in aerobic and anaerobic power in master athletes (Bagley et al., 2019). It is

TABLE 2 | Comparison of decline rates between the 80+-years-old and centenarian group in seconds or meters per year and the ratio.

	Linear regression, all data points pooled (R)				Decline rates all data points pooled (slopes)			Average individual decline rates (slopes)			
Discipline	**R 80+**	**R Centenarians**	***p* 80+**	***p* Centenarians**	**80+ group**	**Centenarians**	**Ratio**	**80+ group**	**Centenarians**	**Ratio**	***P*-value (*t*-test)**
100 m	0.27	0.57	0.006	0.004	0.35	2.84	8.08	0.68 ± 0.86	5.59 ± 8.42	8.22	<0.05
Long jump	0.39	0.90	<0.001	<0.001	0.08	0.19	2.40	0.22 ± 0.45	0.18 ± 0.06	0.82	0.43
Shot put	0.38	0.65	<0.001	<0.001	0.18	0.51	2.74	0.23 ± 0.26	0.37 ± 0.21	1.61	0.06
Discus throw	0.43	0.73	<0.001	<0.001	0.63	0.97	1.54	0.79 ± 1.14	0.82 ± 0.70	1.04	0.47
Javelin throw	0.47	0.68	<0.001	<0.001	0.69	0.88	1.27	0.94 ± 0.67	0.92 ± 0.88	0.98	0.48
Average							**3.21**			**2.53**	

The R and slope values were calculated on all data points pooled. "Average individual decline rates" were derived as the average of the individual regression slopes.

clear that different physiological systems contribute to the success in different disciplines, where for instance power events are particularly dependent on muscle power, and endurance events are most limited by the cardiovascular system. It is therefore of interest to assess to what extent performance declines in different disciplines are attributable to proportional decrements in the muscle, respiratory (Degens et al., 2013), or cardiovascular function (Ganse and Degens, 2021).

Centenarian Disciplines

Data from the oldest-old athletes were only available in 1 of the 10 individual running events, 1 of the 4 jumping events, but 3 of the 5 throwing events contested at the International Masters Games[1]. Interestingly, the event selection of athletes changes in the oldest old compared to earlier ages. Perhaps these disciplines were chosen, as the oldest-old athletes are physically unable to compete in other events. Such a limitation may already occur before the age of 100 years, as in another data set none of the 80–94-year-old athletes participated in hurdles or in other jumps (Ganse et al., 2020a). Possible factors that may contribute to the absence of oldest-old competitors in theses disciplines may be lack of sufficient muscle power and/or fear of injury.

Sex Differences

Despite the higher life expectancy of women[2], only 13.5% of the centenarian athletes in the present study were women. In comparison, 22% of the athletes in the largest longitudinal master athletics data set were women (Ganse et al., 2020b). Among the athletes with results at age 80 and older from the same data set, however, only 13.9% were women. The low proportion of oldest-old women may be related to a lower participation of women in sports in the past, where changes in society over the decades have stimulated a growing interest in sports and fitness in women, explaining a larger proportion of competing women at younger ages (O'Brien and Robertson, 2010).

Strengths and Weaknesses

The main strength of the present study is that it is the first to present longitudinal data on changes in performance after the age of 95 of athletes still competing at the age of 100 or over. The main weakness is that a low amount of data from women led to the inability to test for sex differences. This is particularly interesting, as differences in decline rates between men and women have been shown in previous studies (Ganse et al., 2018; Gava et al., 2020). Larger data sets may show that the increases in decline between the younger and the older group are actually significant.

Conclusions

We presented the first data set of performance decline rates after the age of 90 of athletes still competing at the age of 100 or over in five track and field disciplines. In this age group, the age-related performance decline was even faster than that previously reported in 80+-year-old athletes.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found at: www.mastersrankings.com.

ETHICS STATEMENT

Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

BG contributed the idea and worked on data analysis and interpretation, figures, tables, drafting, and manuscript submission. HD contributed to the statistical analysis and data discussion. AB, CHA, MK, and TP helped with data interpretation. All authors have contributed to manuscript drafting and revision, read, and approved the submitted version of the manuscript.

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[1] https://imga.ch/masters-sports/sports-and-disciplines/athletics/

[2] "Human Development Report", United Nations Development Program. Available at http://hdr.undp.org/en/content/2019-human-development-index-ranking.

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