ENDURANCE AND ULTRA-ENDURANCE SPORTS IN EXTREME CONDITIONS: PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL ISSUES

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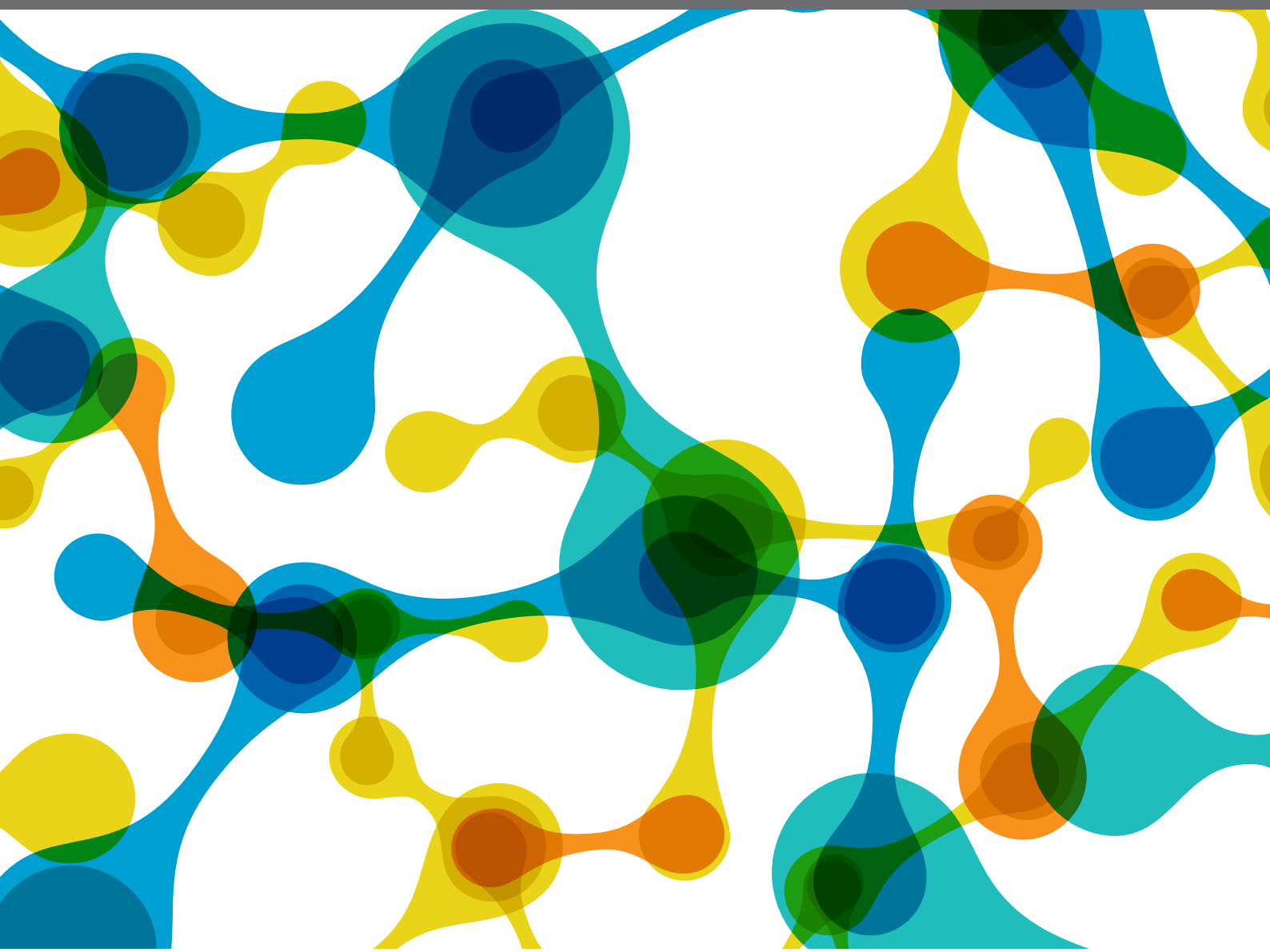

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ENDURANCE AND ULTRA-ENDURANCE SPORTS IN EXTREME CONDITIONS: PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL ISSUES

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REVIEW
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Musculoskeletal Injuries in Ultra-Endurance Running: A Scoping Review

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Ultra-endurance running (UER) has seen an important increase in participation over the last few decades. Long hours of UER can lead to excessive stress on the body, resulting in musculoskeletal injuries (MSKI). UER is not a uniform sport and events can differ considerably in distance (over 42.195 km), time (e.g., events over 6 h) and multi-day or multi-stage events on various surfaces (e.g., track, on-road, off-road). The aims of this scoping review were therefore: (1) to examine the current evidence of MSKI, providing a synthesis of the most common MSKI by anatomical region and specific diagnosis; (2) categorize MSKI by type of UER activity (competition: time-limited; multi-stage; continuous UER events and training); (3) describe knowledge gaps in the literature and provide advice on potential further research. Our electronic literature search (PubMed, SPORTDiscus, Web of Science) identified a total of 13 studies (9 in competition, and 4 in training). Anatomical site, diagnosis and rate of injuries differ between competition and training as well as between different UER types. MSKI are observed in 18% of multi-stage events (0.7–1.8 injuries/runner and 7.2 injuries/1000 h). Most MSKI involve the lower leg (35.0%), ankle (16.8%), knee (13.1%) and foot (12.6%), with main diagnosis of medial tibial stress syndrome (30.1%) and patella femoral pain syndrome (PFPS; 7.2%). Single, continuous UER events differ between a 1005 km road race with almost all of the injuries due to overuse, with the main anatomical site of the knee (31%), ankle (28%) and lower leg (14%) and main diagnosis of PFPS (15.6%), compared to a 65 km trail race, with 32.8% of MSKI, mainly on the foot [plantar fasciitis (28.6%)], ankle [sprain (28.6%)] and knee. Timed-UER events (injury rate of 2.1 injuries/athlete) observed most injuries on the ankle (36%) and knee (19%), with the main diagnosis of tendinitis of the foot dorsiflexors (30%). Injuries during training most commonly affect, the back (42%), and knee (40%) and bone stress injuries (22%). Main diagnoses include ankle sprain (18%), iliotibial band injury (16%) and Achilles tendinopathy (11%). Future considerations include examining MSKI in different UER events, environments and surfaces, and on larger study populations. Establishing risk factors, examining sex differences and using a standard reporting system of MSKI in UER are also important.

Keywords: ultra running, ultramarathon, muscle injury, injury, trail running

INTRODUCTION

Humans are well suited to running long distances, having evolved as persistence hunters, capable of covering great distances in pursuit of prey (Scheer, 2019). Ultra-endurance running (UER) tests the limits of the human body and has become increasingly popular over the last few decades, with an exponential increase that has slowed slightly since ∼2016 (Scheer, 2019). This increase is in large part due to an increase in female and master athlete (athletes ages > 35 years) participation (Eichenberger et al., 2012; Knechtle et al., 2012; Zingg et al., 2013; Scheer, 2019). In addition, there has been a similar increase in participation of youth athletes (<19 years of age), although numbers are much smaller compared to adults (Scheer and Hoffman, 2019; Scheer et al., 2020b,c). In 2019 alone, over 669,000 runners finished an UER event and there were over 7000 UER events hosted around the world (DUV, 2019). The exception of course, was 2020, with a significant reduction in UER participation and events, due to the global COVID-19 pandemic (Scheer et al., 2021).

Ultra-endurance running is not a uniform sport but can be defined as a broad category with different types of running activities, such as running events by distance (e.g., any distance in excess of the standard marathon distance of 42.195 km), time (timed- UER, e.g., any events over 6 h) and multi day/multi-stage events (distance or timed events over several days or stages) (Scheer et al., 2020a). UER events can be held on various surfaces (e.g., track, on-road, off-road) (Scheer et al., 2020a) and in extreme challenging environments, putting additional strain on the human body (e.g., extreme cold, altitude, mountain, desert, heat and jungle) (Knoth et al., 2012; da Fonseca-Engelhardt et al., 2013; Gill et al., 2015; Scheer and Murray, 2015; Dawadi et al., 2020; Suter et al., 2020). The most popular race distances are those of 50 km, 100 km, and 100 miles (Cejka et al., 2014; Scheer, 2019; Knechtle et al., 2020), but can also include distances in excess of 1000 km (Fallon, 1996; Schütz et al., 2012; Scheer et al., 2020a), whereas time-limited events often include 6, 12, or 24 h events, with some lasting several days (Hutson, 1984; Bishop and Fallon, 1999; Scheer et al., 2020a). Multi day/multi-stage events are often held in extreme environments, and athletes often need to carry their provisions, resulting in additional weight while running as a further challenge (Krabak et al., 2011; Knoth et al., 2012; Dawadi et al., 2020; Scheer et al., 2020a).

Long hours of UER can lead to excessive stress on the musculoskeletal system, and potentially musculoskeletal injuries (MSKI) (Scheer and Murray, 2015). Average training loads in UER are between 66–83 km/week in adults and around 57 km/week in youth athletes (Kłapcińska et al., 2013; Scheer et al., 2018, 2020d; O'Loughlin et al., 2019). These training demands can lead to overuse injuries, especially when the load exceeds the adaptive mechanisms, affecting predominantly the lower limbs (e.g., patellofemoral pain syndrome, medial tibial stress syndrome, Achilles tendinopathy) (Khodaee and Ansari, 2012; Krabak et al., 2013, 2014; Scheer and Murray, 2015). Acute injuries are less common but may impact race performance (Khodaee and Ansari, 2012; Krabak et al., 2014; Scheer and Murray, 2015).

Musculoskeletal injuries in running have been defined as "running-related (training or competition) musculoskeletal pain in the lower limbs that causes a restriction on or stoppage of running (distance, speed, duration, or training) for at least 7 days or 3 consecutive scheduled training sessions, or that requires the runner to consult a physician or other health professional" (Yamato et al., 2015b), however, definitions of MSKI in UER vary across studies, which make comparisons difficult. Some defined MSKI as a disability resulting in a medical encounter (Krabak et al., 2011; Vernillo et al., 2016), or affecting performance (Bishop and Fallon, 1999). The severity of an injury had been described as severe, if it did not improve with rest (Hutson, 1984), major, resulting in race withdrawal, or minor, when the runner was able to continue the race (Krabak et al., 2011; Vernillo et al., 2016).

It is well established that physiological demands within this broader spectrum of UER may vary, depending on event type (e.g., distance, surface, and elevation changes) (Davies and Thompson, 1986; Bassett and Howley, 2000; Millet and Millet, 2012; Scheer et al., 2020a). For example, the fractional utilization of VO_2max in a 24 h race is between ∼40–50% (Millet et al., 2011), whereas shorter races of 6 h duration can be run at ∼70% of VO_2max (Giovanelli et al., 2017). If these higher running intensities in UER increase the risk of MSKI is currently unknown. Similarly, UER with large elevation changes, especially prolonged downhill running sections, place particular demand on the musculature with prolonged eccentric muscle action, leading to increased release of muscle enzymes (creatine kinase) and muscle damage (Dewolf et al., 2016; Giandolini et al., 2017; Vernillo et al., 2017). Such variability makes it likely that MSKI and injury rates will also be affected by the different types of UER events, like timed-events, multi-day events and continuous UER events. Similarly, injury rates and diagnosis of MSKI during competition and training may also vary, as during competitions athletes typically push themselves to the limit. Several review articles of illness and injuries exist about ultramarathon running, however, they have not specifically reviewed MSKI in different types of UER events and analyzed MSKI in competition and training (Khodaee and Ansari, 2012; Lopes et al., 2012; Krabak et al., 2013, 2014; Hoffman et al., 2014; Knechtle and Nikolaidis, 2018a,b). Proper treatment of injuries and illnesses in UER is important for avoiding long-term issues (Krabak et al., 2014) and therefore it is important to examine and summarize MSKI within these broad categories of UER during competition and training.

The aims of this scoping review were therefore: (1) to examine the current evidence of MSKI, providing a synthesis of the most common MSKI by anatomical region and specific diagnosis; (2) categorize MSKI by type of UER activity (competition: time-limited; multi-stage; continuous UER events) and training); (3) describe knowledge gaps in the literature and provide advice on potential further research.

MATERIALS AND METHODS

This review is based on the recommendations for scoping reviews, with the purpose of identifying and mapping the

available evidence and identifying knowledge gaps (Armstrong et al., 2011). As such we used a broad research question, for example: what are the musculoskeletal injuries in UER? What are the different anatomical distributions and incidence/prevalence of MSKI in UER competition and training? Our aim was to review the existing literature, summarize those findings, identify knowledge gaps, as done in previous scoping reviews (Armstrong et al., 2011; Granacher et al., 2016). The review considered scientific papers that investigated MSKI in UER. UER is defined as a broad category with different types of running activities, such as running events by distance in excess of 42.195 km (standard marathon distance), timed-events over 6 h durations, and multi-day or multi stage running events, on all surfaces (road, off-road, track) and terrains (Scheer et al., 2020a). All studies that examined MSKI in UER were included irrespective of participants age and/or sex, however, detailed information of MSKI according to sex or age was not available in the majority of studies, therefore a comprehensive breakdown of MSKI according to these parameters was not possible. However, those studies that did provide specific results on age and/or sex aspects were highlighted in the respective sections in the discussion (Micklesfield et al., 2007; Scheer et al., 2020d).

An electronic literature search was performed using different databases (PubMed, SPORTDiscus, Web of Science) from January 1st, 1984- September 30th, 2020. The following search terms were used: 'ultra endurance running' or 'ultra running' or 'ultramarathon' or 'trail running' or 'ultra trail running' and 'injury' or 'musculoskeletal injury' or 'muscle injury.' We identified a total of 771 studies meeting our initial search criteria. **Figure 1** gives an overview of article selection process in accordance with PRISMA (Moher et al., 2009). After removal of duplicates, the abstracts of the remaining 229 studies were reviewed. Only studies written in the English language were considered. Studies that did not examine MSKI in UER, did not provide any data on general or specific MSKI, provided data on mixed population (e.g., different running populations including UER and non-UER) were excluded. Similarly, studies that only examined muscle damage from biochemical markers or muscle cramps, as well as case reports were not included in this review. Studies on blisters, dermatological or other illnesses and injuries were excluded. A total of 35 full texts were assessed for eligibility and after review, a total of 13 studies met our inclusion criteria and were therefore included in the review. All abstracts and manuscript were reviewed by the two authors.

The definitions of MSKI varied across the different studies, with some providing no specific definitions (Scheer and Murray, 2011; Graham et al., 2012; McGowan and Hoffman, 2015; Dawadi et al., 2020), whereas other defined MSKI as a disability resulting in a medical encounter (Krabak et al., 2011; Vernillo et al., 2016), or affecting performance (Bishop and Fallon, 1999). All studies reporting MSKI during a race were either documented by the attending medical team (through self-referral/self- reporting system via medical encounters) or by a routine daily medical assessment questionnaire (Hutson, 1984; Fallon, 1996; Bishop and Fallon, 1999; Krabak et al., 2011; Scheer and Murray, 2011; Graham et al., 2012; McGowan and Hoffman, 2015; Vernillo et al., 2016; Dawadi et al., 2020). Studies examining MSKI in training studies defined injuries as either an athlete reporting the diagnosis based on a health care provider encounter or through athlete self-assessment (Micklesfield et al., 2007; Hoffman and Krishnan, 2014; Malliaropoulos et al., 2015; Scheer et al., 2020d).

RESULTS

The main findings of our review were: (i) MSKI in UER are common, mostly affecting the lower limbs and are of overuse in nature; (ii) MSKI differ between competition and training, with multistage events, predominantly affecting the lower leg, foot, and knee, while timed events mainly affect the ankle, Achilles tendon, and knee; (iii) Short continuous UER events off-road have the highest incidence of MSKI, mainly affecting the foot and ankle, while long continuous UER affect the knee and ankle; (iv) During training back, knee and bone stress injuries are common. Detailed description of MSKI incidence (rate of occurrences of new cases) and/or prevalence (number of MSKI at a particular time) are provided. The manuscript is organized accordingly describing the different pertinent research sections.

Thirteen studies examined MSKI in UER and are included in this review. **Table 1** and **Table 2** provide detailed information about the study, participants (age and sex), and MSKI. Nine studies (**Table 1**) provide information from UER races/competitions (total n = 723 participants), while four studies (**Table 2**) provide information from MSKI mostly during training of UER athletes (total n = 1606 participants). Detailed information of MSKI according to sex or age was not available in the majority of studies, therefore a comprehensive breakdown of MSKI according to sex or age was not possible. One study (Micklesfield et al., 2007) specifically examined bone stress injuries in female UER, and another MSKI in youth athletes (Scheer et al., 2020d) and for those studies results by sex and age were discussed in more detail in the appropriate sections. The vast majority of MSKI involve the lower limb (Fallon, 1996; Krabak et al., 2011), and are generally minor across all types of UER (Fallon, 1996; Bishop and Fallon, 1999; Krabak et al., 2014). MSKI are predominantly overuse in nature (e.g., tendinopathies), even during competitions, with incidences in the region of between 98–100% (Fallon, 1996; Bishop and Fallon, 1999), demonstrating the exceptional demand on the musculoskeletal system during prolonged running, in contrast to 'true' acute injuries (e.g., muscle strains) that may be expected during competition in other, more explosive sports (Huxley et al., 2014; Krabak et al., 2014; Scheer and Murray, 2015).

Anatomical Regions and Specific Diagnoses

Hip

The incidence of hip injuries during multi- day UER events was around 3.8% (Krabak et al., 2011), with the iliotibial band the most commonly affected structure. Iliotibial band syndrome (ITBS) is generally an overuse injury of the connective tissue around the lateral thigh and/or knee (Taunton et al., 2002; Fredericson and Wolf, 2005; Fredericson and Weir, 2006; Strauss et al., 2011) with a prevalence of between 14.2%

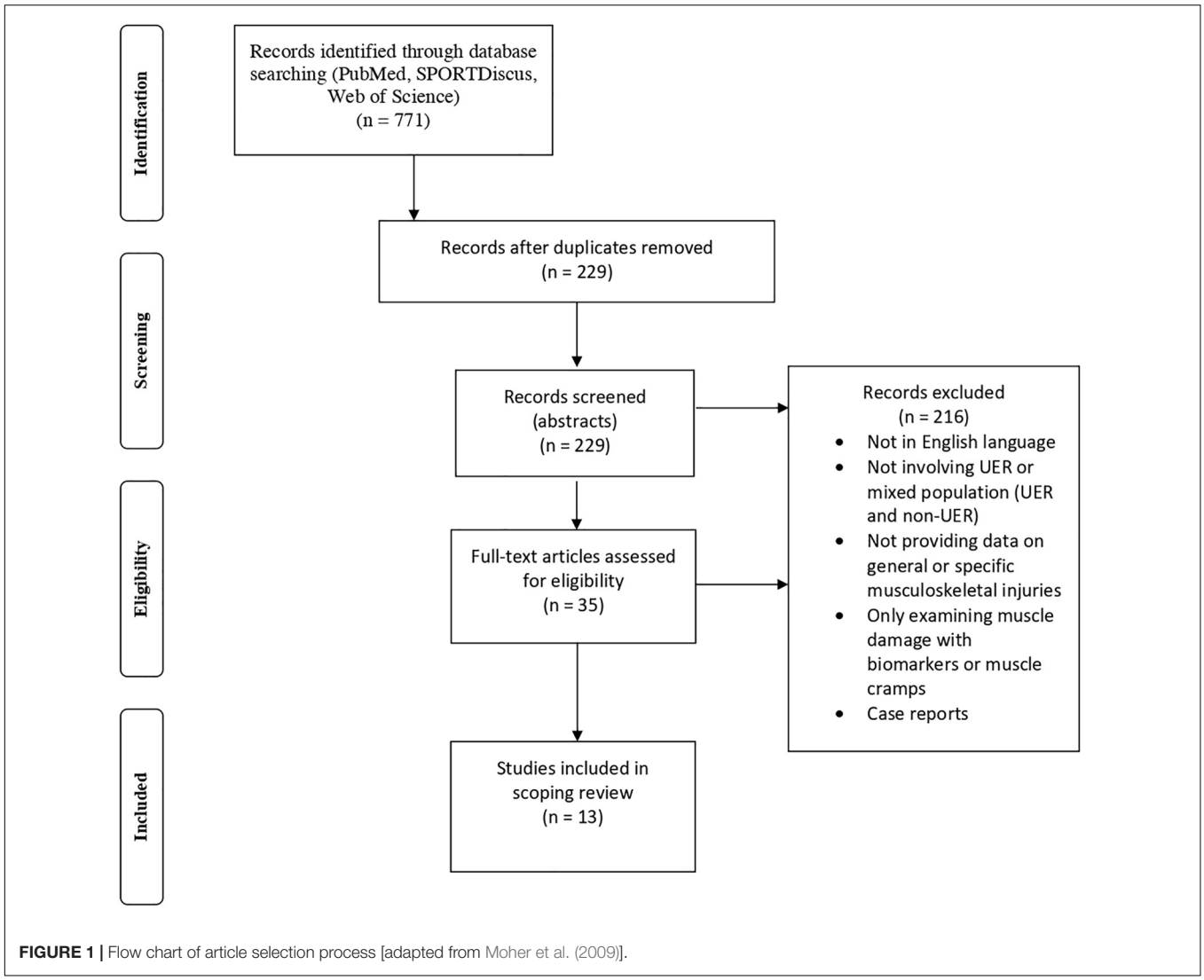

FIGURE 1 | Flow chart of article selection process [adapted from Moher et al. (2009)].

(Malliaropoulos et al., 2015) and 15.8% in adult UER (Hoffman and Krishnan, 2014), and 14.2% in youth UER (Scheer et al., 2020d). The incidence on race day during a 1005 km continuous road race was lower with an incidence of approximately 4.7% (Fallon, 1996). Hip injuries encountered during race day were typically secondary to a bursitis [psoas bursitis 11.1% (Hutson, 1984), greater trochanteric bursitis (1.4%–.1% (Fallon, 1996; Scheer and Murray, 2011)], while injuries during training often included hip flexor strains (3.6–8.7%) (Scheer and Murray, 2011; Hoffman and Krishnan, 2014) and adductor tendinopathy (2%) (Malliaropoulos et al., 2015).

Upper Leg

Injuries to the structures of the upper leg were mostly encountered during competition, and include injuries such as quadriceps muscle pain (1.4%) (Scheer and Murray, 2011), quadriceps muscle strain or tear [ranging from 4.7% (Fallon, 1996) to 14.3% (Vernillo et al., 2016)], and quadriceps tendinitis [7.8% (Fallon, 1996)]. During training hamstring muscle strains have been described in 3.8% of youth athletes (Scheer et al., 2020d) and 11.8% in adult UER (Hoffman and Krishnan, 2014).

Knee

The knee is one of the regions most frequently injured, with incidences during competition ranging between 13.1% (Krabak et al., 2011) to 31.3% (Fallon, 1996). Diagnosis included patellar tendinitis/tendinopathy (18.5%) (Hutson, 1984), knee sprains (14.3%) (Vernillo et al., 2016) or other non-specific knee pains (3.1%) (Fallon, 1996). Patella femoral joint disorders or patella femoral pain syndrome (PFPS) were frequent diagnoses and the incidence ranged between 7.2–15.6% (Fallon, 1996; Scheer and Murray, 2011). During training the prevalence of knee injuries was between 21.4% in youth athletes (Scheer et al., 2020d) to 24% in adult UER (Hoffman and Krishnan, 2014). Meniscal injuries were also prevalent (12%) (Malliaropoulos et al., 2015).

Lower Leg

The lower leg was frequently injured during competition ranging from 14% (Fallon, 1996) to 35.0% for multi-day, multi-stage

TABLE 1 | Studies investigating musculoskeletal injuries (MSKI) during competition.

Study	Competition/ Race	Study design	Participants	General observations	Specific injuries	Non-finisher (MSK injury)	Definition	Additional observation
Hutson, 1984	Track; 6-day race (time-limited) (Charles Rowell Six Day Race, Nottingham, United Kingdom)	observational, prospective	21 (19 men, 2 women; average age 41)	27 MSKI, all overuse	Tendinitis of the foot dorsiflexors 29.6% (severe 18.5%), patellar tendinitis 18.5% (severe, 3.7%), Achilles tendinitis 18.5%, psoas bursitis 11.1% (severe, 3.7%), PFPS 7.4% (severe 3.7%) MTSS 10.1%, gastrocnemius strain severe 3.7%	1 (4.8%) Achilles tendinitis	Self-reported medical encounters to medical team; classification: severe, did not improve with rest	Winner covered 576 miles, 13 athletes passed 400 miles, direction of track was changed every 12 h
Fallon, 1996	Road; 1005 km (Westfield Sydney to Melbourne, Australia)	observational, prospective	32 (average age men 38, women 43; number of male/females not given)	64 MSKI (63 overuse injuries); anatomic site; knee 31.3%, ankle 28.1%, lower leg 14%, upper leg 11%, foot 6.3%, back 6.3%, upper limb 1.5%, chest wall 1.5%. Lower limb total: 90.1%	PFPS 15.6%, Achilles tendinopathy 7.8%, anterior compartment tendinitis 7.8%, extensor digitorum tendinitis 7.8%, MTSS 7.8%, Quadriceps tendinitis 7.8%, anterior compartment pain 6.3%, ITB 4.7%, quadriceps strain/tear 4.7%, back muscle strain 3.1%, peroneal tendinitis 3.1%, non-specific knee 3.1%, greater trochanteric bursitis 3.1%, extensor hallucis longus tendinitis 3.1%	None, but 72% detrimental effect on performance,	Medical team speak to runner and injury confirmed by history and examination of experienced medical team	19 completed race within cut off time of 8.5 days.

(Continued)

TABLE 1 | Continued

Study	Competition/ Race	Study design	Participants	General observations	Specific injuries	Non-finisher (MSK injury)	Definition	Additional observation
Bishop and Fallon, 1999	Track (grass); 6-day race (time-limited), (Colac, Victoria, Australia)	prospective cohort study	17 (16 men, 1 woman; average age 47)	36 MSKI in 11 runners, injury rate 2.1 injuries/person; all overuse injuries; anatomical site: ankle 36%, knee 19 %	Achilles tendinitis 19%, extensor digitorum longus tendon 14%, PFPS 14%, anterior compartment pain 11%	2 (4.3%); bilateral Achilles tendinitis and quadriceps strain	MSKI defined as affecting performance; interview every 6 h, each injury examined by two experienced physicians	Change of track direction every 2 h; Majority of MSKI presented on day 2 and 3 (75%)
Scheer and Murray, 2011	Off-road (trail); 5-day stage race, 219 km (Al Andalus Ultra Trail, Loja, Spain)	observational, prospective	69 (48 men, average age 46 years; 21 women, average age 40 years)	MSKI: 12 runners (17,4%); 1.8 injuries/competitor. Mostly knee (PFPS). Most medical encounters day 3 and 4.	PFPS 7.2%, Achilles tendinopathy 2.9%, hip (trochanteric bursitis) 1.4%, ultramarathon ankle 1.4%, ankle inversion injury 1.4%, muscle pain (quadriceps 1.4%, tibialis anterior 1.4%)	9 runners did not complete the race; 1 runner (1.4%) because of MSKI (PFPS)	Self-reported by athlete to medical team; medical encounters	Routine medical clinics held twice daily
Krabak et al., 2011	Off-road (trail); 7-day stage race, 240 km (Racing The Planet 4 Desert Series)	prospective	396 (79.2% male, 20.8% female, average age 40)	MSKI 18.2%, mostly minor, during day 3 or 4; 0.71 MSKI/runner. Major MSKI 46.2/1000 runners, 0.8/1000 h; Minor MSKI 670.0/1000 runners, 11.2 per 1000 h. Overall 7.2 injuries/1000 h	MSKI by diagnosis: tendonitis 11.3%, sprain 3.2%, bursitis 1.6%, strain 1.6%, other 4.8% Location: lower extremity 92.6%, mostly The lower leg (35%), ankle (16.8%), knee (13.1%), foot (12.6%),	1.2% race withdrawal	Self-reported medical encounter at checkpoint; MSKI: disability during the race, resulting in medical encounter, minor if able to continue, major if had to withdraw	Data from 4 races (Gobi Desert 2005 and 2006, Sahara Desert, Atacama Desert), 396 runners participated (303 unique individuals)

(Continued)

TABLE 1 | Continued

Study	Competition/ Race	Study design	Participants	General observations	Specific injuries	Non-finisher (MSK injury)	Definition	Additional observation
Graham et al., 2012	Off-road (trail); 7-day stage race, 240 km (Gobi Desert, Mongolia)	prospective	11 (11 men, average age 33 years)	pain, lack of joint mobility (stiffness in knee), Achilles pain, shin pain (similar pattern to previous studies)	N/A	none	Medical assessment twice daily (morning/night) by physician	Abrasion and blisters 100%
McGowan and Hoffman, 2015	Off road (trail); 161 km (Western States Endurance Run, California, United States)	retrospective	63 consultations (of unique individuals), average age 42/43 years across 4 years	Consultations of MSKI 2.6% of all starters	Sprain, strain or tendinitis 0.9%, muscular pain 0.7%, contusion 0.3% (percentages are given in relation to race starters)	0.7% (muscle cramping, sprain, strain or tendinitis)	Self-reported medical encounters at aid stations, retrospective analyses; Pooled data from 4 events (consecutive years);	Total of 8.2% starters sought medical care, most for medical problems (e.g., nausea), including non-competitors (10%)
Vernillo et al., 2016	Off-road (trail); 65 km (Vigolana Trail, Trento, Italy)	prospective	77 (64 men, 13 women, average age 44 years)	MSKI 32.8%, all minor; injury rate: 614 per 1000 runners; 4285 per 1000 h,	Plantar fasciitis 28.6%, ankle sprain 28.6%, knee sprain 14.3%, thigh sprain 14.3%, Achilles tendinopathy 7.1%, neck/cervical sprain 7.1%	none	Self-reported medical encounter post-race via questionnaire; MSKI: disability during the race, resulting in medical encounter, minor if able to continue, major if had to withdrawal	No major injuries
Dawadi et al., 2020	Off-road (trail); 7-stage 212 km (Manaslu trail race, Himalaya)	retrospective	100 (60 men, 40, women)	MSKI 17%; 170 injuries/1000 athletes or 1.2 cases per 1000 km run	Most commonly ankle sprain; one case of subluxated distal phalanx of finger	none	Self-reported to medical team	Poole data from 3 consecutive events; no data on specific MSKI/incidence; competition at altitude

TABLE 2 | Studies investigating musculoskeletal injuries (MSKI) during training.

Study	Study design	Participants	General observations	Specific MSKI	Additional observation
Micklesfield et al., 2007	retrospective, questionnaire survey, at registration prior to Two Ocean Ultra (56 km), South Africa, by trained interviewer, random prior athlete selection	276 ultra runners, (all female), average age 39 years	Examined only bone stress injuries in female ultra-runners	Bone stress injury 21%	No anatomical site of bone stress given. No distinction between bone stress injury and stress fracture.
Hoffman and Krishnan, 2014	retrospective, questionnaire, self-reported	1212 ultra runners (824 men, 399 women), average age 42.3 years	Injuries during last 12 months (training/competition) ULTRA study	Knee issues 24%, back injuries 12.4%, ITB 15.8, hamstring strain 11.8%, calf strain 13.1%, Achilles tendinitis or tear 10.8%, ankle sprain 10.8%, plantar fasciitis 10.6%, other foot and ankle injuries 4.5%, bunion 2.5%, stress fracture foot 3.4%, metatarsalgia 3.1% lower leg or ankle tendinitis 9.2%, pelvis or hip issues 3.7, hip flexor strain 8.7, fractures not involving the extremities 1%, upper extremity 1.4%, femur/hop stress fracture 0.5%, tibia/fibula stress fracture 1.9%, other lower leg injuries 1.5%,	
Malliaropoulos et al., 2015	retrospective, epidemiological questionnaire, convenience sample of ultra-trail runners	40 ultra runners (36 men, 4 women), average age 39.4 years	Total of 134 injuries, 90% at least one injury, mean numbers 3.38 injury/individual; 82.2% overuse; 17.7% during competition.	Diagnosed injuries (43): 22% overuse bone stress, spinal disk/low back 14%, ITB 16%, meniscal injury 14%, hamstring 12%, Achilles tendinopathy 7%, plantar fasciitis 7%, Morton's neuroma 5%, tibiofibular joint injury 2%, Adductor tendinopathy 2%; Injured areas anatomical site: 42.5% lower back, 40% knee	
Scheer et al., 2020d	retrospective survey study via questionnaire	78 runners (65 men, 13 women), average age 38.0 years	Examined adults, that participated in UER as a youth athlete (<19 years of age). Participated at first ultra at average age of 16.1 years. MSKI 23.1 %; stress fractures 6.4; 1.9 injuries/athletes	21.4 % knee pain, 17.9% ankle sprain, 14.2% ITB, 10.7% tibial stress fracture, 3.6% each: ankle tendinopathy, Achilles tendinopathy, hip flexor strain, foot stress fracture, hamstring strain, plantar fasciitis, MTSS, Morton's neuroma, bursitis.	Lifetime prevalence of stress fractures of adults that started running ultras as youth athletes: 14.1%

UER events (Krabak et al., 2011) and included a variety of pathologies, such as medial tibial stress syndrome (MTSS) and chronic exertional compartment syndrome (CECS).

Medial tibial stress syndrome [sometimes called shin soreness, shin splints, tibial stress syndrome, medial tibial periostitis and medial tibial traction periostitis (Moen et al., 2009; Reshef and Guelich, 2012)] had an incidence of between 7.8% (Fallon, 1996) and 10.1% (Hutson, 1984) in competition and a prevalence of 3.6% in youth runners (Scheer et al., 2020d).

Chronic exertional compartment syndrome affected most commonly the anterior compartment, that contain the dorsiflexor muscles of the tibialis anterior, extensor digitorum

longus, and extensor hallucis longus muscles (Fraipont and Adamson, 2003; George and Hutchinson, 2012). Diagnosis included tibialis anterior muscle pain (1.4%) (Scheer and Murray, 2011), anterior compartment pain ranging from 6.3% (Fallon, 1996) to 11% (Bishop and Fallon, 1999), and anterior compartment tendinitis (7.8%) (Fallon, 1996). Plantar flexor tendinitis/peroneal tendinitis of the lateral compartment was described in 3.1% of UER (Fallon, 1996). Gastrocnemius muscle strain occurred in 3.7% during competition (Hutson, 1984) and 13.1% (Hoffman and Krishnan, 2014) during training.

Ankle

The ankle is another frequently injured anatomical site and some investigations cite this as the most common site of injury in UER during competition, however, there was a wide range of incidences of between 16.8% (Krabak et al., 2011) to 36% (Bishop and Fallon, 1999). Tendinitis of the dorsiflexors of the foot was the most frequent diagnosis (29.6%) (Hutson, 1984) and called 'ultramarathon ankle,' a relatively specific injury to UER (Hutson, 1984; Fallon, 1996; Bishop and Fallon, 1999; Scheer and Murray, 2011). Repetitive plantar and dorsiflexion as observed during prolonged running, may cause a peritendinitis/tenosynovitis of the tendons passing under or adjacent to the extensor retinaculum of the ankle (Hutson, 1984; Fallon, 1996). Other causative factors include excessive pressure on the dorsum of the ankle due to tight fitting shoes, over-pronation, running on hard surfaces, and overstriding (Hutson, 1984; Fallon, 1996). The incidence varied and was as low as 1.4% (Scheer and Murray, 2011) and reached 29.6% (Hutson, 1984). More specifically, depending which dorsiflexor of the foot was affected the incidence varied [e.g., extensor digitorum longus 7.8% (Fallon, 1996) to 14% (Bishop and Fallon, 1999); or extensor hallucis longus 3.1% (Fallon, 1996)].

Achilles tendinopathy was another frequent MSKI with a wide range of incidences during competition, from 2.9% during a 5 day multi stage UER event (Scheer and Murray, 2011) to 7.1% in a 65 km trail race (Vernillo et al., 2016) and 7.8% in a 1005 km road UER (Fallon, 1996). The highest incidence was reported in UER events on a track with incidences of between 18.5% and 19% (Hutson, 1984; Bishop and Fallon, 1999). In training studies Achilles tendinopathy was observed in between 7% (Malliaropoulos et al., 2015) and 10.8% (Hoffman and Krishnan, 2014), and 17.9% in youth athletes (Scheer et al., 2020d). Ankle sprains were frequently observed in UER trail races (28.6%) (Vernillo et al., 2016), but were less frequent in multi-day UER events (1.4%) (Scheer and Murray, 2011). During training prevalence of ankle sprains ranged from 10.8% in adult UER (Hoffman and Krishnan, 2014) to 17.9% in youth UER (Scheer et al., 2020d). Tibio-fibular joint injuries were observed in 2% of UER (Malliaropoulos et al., 2015).

Foot

Foot injuries were also very common injuries in UER, with a wide range of incidences between 6.3% (Fallon, 1996) and 12.6% (Krabak et al., 2011). Plantar fasciitis was reported in 28.6% in trail runners during a competition (Vernillo et al., 2016), while numbers in training studies are lower, with a prevalence ranging from 7% (Malliaropoulos et al., 2015) to 10.6% (Hoffman and Krishnan, 2014) in adult UER and 3.6% in youth runners (Scheer et al., 2020d). Other pathologies included metatarsalgia (3.1%), bunion (2.5%) (Hoffman and Krishnan, 2014) and Morton's neuroma (3.6%) (Scheer et al., 2020d).

Bone Stress Injuries of the Lower Limbs

Bone stress injuries can vary in severity, with early injuries demonstrating periosteal edema and/or bone marrow edema on radiological examination, with more severe injury showing a stress fractures with radiological evidence of a fracture line (Tenforde et al., 2016).

Stress fractures are usually fatigue fractures that develop through overuse on healthy bone (Harrast and Colonno, 2010; Pegrum et al., 2012; Krabak et al., 2019; Tenforde et al., 2019). None of the studies during competition described stress fractures, however, this may be difficult to diagnose clinically. Prevalence is high in training, ranging from 6.4% in youth UER (Scheer et al., 2020d) to 22% in adults (Malliaropoulos et al., 2015). Bone stress was particularly high among female athletes (21%) (Micklesfield et al., 2007), however, it is not clear from the study if these were early bone stress injuries or actual stress fractures. Bone stress injuries in female athletes were associated with increased energy expenditure, and associated with inadequate nutrition as seen in relative energy deficiency in sport (RED-S) (Micklesfield et al., 2007; Tiller et al., 2021). Prevalence of stress fractures in adult UER were observed in different anatomical regions, such as the foot (3.4%), tibia (1.9%), and femur/hip (0.5%) (Hoffman and Krishnan, 2014) and in youth athletes tibia (10.7%), and foot (3.6%) (Scheer et al., 2020d).

Low Back

Back injuries were less frequently encountered in UER competitions (1.4%–6.3%) (Fallon, 1996; Krabak et al., 2011) and included back muscle strains (3.1% (Fallon, 1996). Back pain was more commonly encountered during training, with a prevalence of between 12–14% (Hoffman and Krishnan, 2014; Malliaropoulos et al., 2015) but not all may have been related to running, but other activities of daily life (Malliaropoulos et al., 2015).

Upper Body

Upper body injuries were also not commonly described in UER competitions with an incidence of up to 3.6% (Fallon, 1996; Krabak et al., 2011) and included the chest wall (1.5%) (Fallon, 1996) or neck sprains (7.1%) (Vernillo et al., 2016).

MSKI by Type of UER Event

MSKI During Time-Limited UER on Track

Though research is limited, two studies compared MSKI during a 6 day time-limited UER events on a track (Hutson, 1984; Bishop and Fallon, 1999). One race was held on a tartan track with a change of running direction every 12 h (Hutson, 1984), and competitors averaged 936 km over the 6 day period. The main anatomical site of injury was the ankle (48%), followed by the knee (26%), involving the extensor muscle-tendon structure of the ankle (41%) and the knee (26%), tendonitis of foot

dorsiflexors (30%) and Achilles tendonitis (19%). In comparison, the other time-limited UER event was held on a grass track, with the mean distance covered of 836 km (Bishop and Fallon, 1999) and the ankle was the anatomical site most frequently injured (36%), followed by the knee (19%). The extensor -muscle-tendon complex of the ankle (25%) and knee (22%) was less commonly affected, as well as tendonitis of the foot dorsiflexors (14%). The occurrence of Achilles tendonitis (19%) was similar (Bishop and Fallon, 1999).

MSKI During Multi-Stage, Multi-Day UER Events in Off-Road Terrain

Four studies examined MSKI during multi-day UER events on off-road terrain (Krabak et al., 2011; Scheer and Murray, 2011; Graham et al., 2012; Dawadi et al., 2020). They were all similar and comparable in length, and duration, of between 5 to 7 days, covering 219–240 km but differed in environmental conditions, with 3 of them in hot or desert environment (Krabak et al., 2011; Scheer and Murray, 2011; Graham et al., 2012) and one in cold environment, at altitude (Dawadi et al., 2020). Although two studies (Graham et al., 2012; Dawadi et al., 2020) did not provide specific diagnosis or incidences of MSKI, all injuries were minor and patterns observed were comparable to previous studies (Graham et al., 2012). The rate of MSKI were similar in three of the studies (17.0, 17.4, and 18.2%, respectively), varying between 0.7 injuries/runner and 1.8 injuries/runner (Krabak et al., 2011; Scheer and Murray, 2011; Dawadi et al., 2020). MSKI injury rates were reported in two studies but used different denominators, with a desert race reporting (minor MSKI 670.0 injuries/1000 runners; major MSKI 11.2 injuries/1000 h; overall 7.2 injuries/1000 h) (Krabak et al., 2011) and a race in the Himalaya (170 injuries/1000 runners or 1.2 cases per 1000 km) (Dawadi et al., 2020).Most MSKI were minor, occurred during the middle part of the race (around day 3 and 4) and affected the lower limbs, mainly lower leg, knee, ankle and foot (Krabak et al., 2011; Scheer and Murray, 2011). Two study showed a predominance of the lower leg/foot/ankle, whereas the other reported injuries mostly to the knee and ankle (Krabak et al., 2011; Scheer and Murray, 2011; Dawadi et al., 2020). The number of runners withdrawing from the race because of MSKI was also very similar (1.2% vs 1.4%) (Krabak et al., 2011; Scheer and Murray, 2011) with no withdrawals because of MSKI in the other two studies (Graham et al., 2012; Dawadi et al., 2020).

MSKI During Continuous UER Events

Three studies examined single, continuous UER events of different terrain and distance. While two races were held on off-road terrain, with distances of 65 km and 161 km (McGowan and Hoffman, 2015; Vernillo et al., 2016), the third was a road race over 10005 km (Fallon, 1996). The study examining the 161 km race was a retrospective analysis of medical encounters of four past edition of the same race, but did not provide specific anatomical distribution and diagnosis of MSKI, but 2.6% of all starters sought medical advice for MSKI, mostly for sprains, strains, tendinitis or muscular pain (McGowan and Hoffman, 2015). Injury rates for the 65 km UER event where high, with 614 injuries/1000 runners and 4285 injuries/1000 h (Vernillo et al., 2016). While MSKI were present in 32.8%, all were minor in nature, predominantly affecting the foot (plantar fasciitis), ankle and knee, with none of the runners having to withdrawal because of MSKI (Vernillo et al., 2016). Similarly, in the 10005 km road event, no runner had to withdrawal due to major MSKI, however, almost three quarters noticed a negative effect on performance (Fallon, 1996). Over 90% of MSKI affected the lower leg, with the main anatomical site the knee, ankle and foot (Fallon, 1996).

MSKI During Training

Four studies examined MSKI retrospectively during training, with longer observational periods via questionnaires (Micklesfield et al., 2007; Hoffman and Krishnan, 2014; Malliaropoulos et al., 2015; Scheer et al., 2020d), whereas one looked specifically at bone stress in female UER athletes (Micklesfield et al., 2007) and one at youth athletes (Scheer et al., 2020d). Bone stress in females was common and comparable to another study with mostly male athletes (21% vs 22%) (Micklesfield et al., 2007; Malliaropoulos et al., 2015), but less than in runners that started UER during their youth (14.1%) (Scheer et al., 2020d). Injury rate was between 1.9 and 3.4 injuries/athlete (Malliaropoulos et al., 2015; Scheer et al., 2020d), with the majority being overuse injuries, mainly affecting the knee, ankle and ITB (Hoffman and Krishnan, 2014; Malliaropoulos et al., 2015; Scheer et al., 2020d).

DISCUSSION

The aims of this scoping review were therefore: (1) to examine the current evidence of MSKI, providing a synthesis of the most common MSKI by anatomical region and specific diagnosis; (2) categorize MSKI by type of UER activity (competition: time-limited; multi-stage; continuous UER events and training); (3) describe knowledge gaps in the literature and provide advice on potential further research. The main findings of our review were that MSKI were mostly overuse injuries, predominantly affecting the lower limbs with different injury patterns and diagnosis between different types of UER activities.

MSKI by Anatomical Region and Diagnosis

The literature review identified 13 studies that reported on MSKI in UER. There is agreement across all studies that MSKI in UER are common, mostly overuse in nature and predominantly affecting the lower limbs (Hutson, 1984; Fallon, 1996; Bishop and Fallon, 1999; Krabak et al., 2011; Scheer and Murray, 2011; Graham et al., 2012; Hoffman and Krishnan, 2014; Malliaropoulos et al., 2015; McGowan and Hoffman, 2015; Vernillo et al., 2016; Dawadi et al., 2020; Scheer et al., 2020d). The main anatomical sites for MSKI are the lower leg, knee, ankle and foot, with the main diagnosis of MTSS, Achilles and patella tendinopathy, PFPS, ankle sprains, plantar fasciitis, ultramarathon ankle and bone stress injuries (Hutson, 1984; Fallon, 1996; Bishop and Fallon, 1999; Micklesfield et al., 2007; Krabak et al., 2011; Scheer and Murray, 2011;

Graham et al., 2012; Hoffman and Krishnan, 2014; Malliaropoulos et al., 2015; McGowan and Hoffman, 2015; Vernillo et al., 2016; Dawadi et al., 2020; Scheer et al., 2020d). Most injuries are generally minor in all types of UER events (Fallon, 1996; Bishop and Fallon, 1999; Krabak et al., 2014), but nevertheless can affect performance or lead to race withdrawal (Bishop and Fallon, 1999; Scheer and Murray, 2011; Graham et al., 2012; Krabak et al., 2014; Vernillo et al., 2016).

Definition of MSKI

One of the challenges in comparing MSKI across the different studies was, that no uniform definition of MSKI was used. Some studies provided no specific definitions of MSKI (Scheer and Murray, 2011; Graham et al., 2012; McGowan and Hoffman, 2015; Dawadi et al., 2020), whilst others defined MSKI as a disability resulting in a medical encounter (Krabak et al., 2011; Vernillo et al., 2016), affecting performance (Bishop and Fallon, 1999), based on a diagnosis of a health care provider, or via self-assessment (Micklesfield et al., 2007; Hoffman and Krishnan, 2014; Malliaropoulos et al., 2015; Scheer et al., 2020d). Classifying and using a standard reporting systems of MSKI in UER is important, as lack of a standardized definition affects the rates of injuries and hinders comparison between studies (Kluitenberg et al., 2015; Yamato et al., 2015a,b). Further, most studies include small sample sizes, and therefore possibly influencing the rate of diagnosis, as with small changes in a particular diagnosis this can lead to large percentage differences. This may also be of particular interest in diagnoses that are made clinically during competitions, such as stress fractures or CECS. CECS is an overuse injury that presents with increased pressure within one of the compartments of the lower leg, that can lead to ischemia, decreased tissue perfusion and pain (Fraipont and Adamson, 2003; George and Hutchinson, 2012). The gold standard for diagnosis CECS is with compartmental pressure testing (Fraipont and Adamson, 2003; George and Hutchinson, 2012). This is not feasible during competition and diagnosis presented in the studies are made on clinical examination and suspicion. Therefore, some MSKI may be either underrepresented (e.g., stress fractures) or overrepresented (e.g., CECS) in small samples and not reflect the true incidence. Interestingly, none of the training studies reported on CECS, but a high number of stress fractures were observed, ranging from 6–22% (Micklesfield et al., 2007; Malliaropoulos et al., 2015; Scheer et al., 2020d).

Future studies therefore should consider examining MSKI on larger populations, either in larger races, pooling data from similar events or over several editions of the same event, to provide a better understanding of the incidence of MSKI. Similarly, it is important to examine different type of events as the limited research has shown, that MSKI are distributed differently depending on type of UER.

MSKI Comparing Different UER Events

During two 6 day time-limited UER events on the track, with an average distances of 836–936 km, the main anatomical site of injury was the ankle (36–48%) and the knee (19–26%), with tendonitis of foot dorsiflexors (25–41%) and Achilles tendonitis (19%) as the most frequent diagnosis (Hutson, 1984; Bishop and Fallon, 1999). Running on a loop-course of track may put additional stress on one particular anatomical side, however, in both studies injuries were evenly distributed between the left and right limb (Hutson, 1984; Bishop and Fallon, 1999), demonstrating that a changing of the running direction while running on a track, may help in preventing lower limb injuries (Gajda et al., 2020). Although, the lower limb was also commonly injured during multi-day UER events (ranging between 219 km–240 km) the distribution of MSKI differed [lower leg (35%), ankle (16.8%), knee (13.1%), and foot (12.6%)], with 0.2–1.8 injuries/runner and overall incidence of 7.2 injuries/1000 h or 1.2 injuries/1000 km (Krabak et al., 2011; Scheer and Murray, 2011; Graham et al., 2012; Dawadi et al., 2020). However, by comparison the overall distance covered in the same time period was very different (219–240 km vs. 836–936 km), that may be result in different injury patterns.

Three studies examined continuous UER events, ranging from 65 km to 10005 km (Fallon, 1996; McGowan and Hoffman, 2015; Vernillo et al., 2016). The shortest (65 km) race reported the highest number of MSKI (32%), with injury rates of 614 injuries/1000 runners and 4285 injuries/1000 h, with the main anatomical site, the foot (28.6%), ankle (28.6%) and knee (14.3%) (Vernillo et al., 2016). This represents the highest incidence of any UER event, possibly related to the off-road (trail) environment where the race was held, with significant elevation change and prolonged uphill and downhill running sections. Ankle and knee injuries were mostly diagnosed as sprains, possibly more related to acute injuries than true overuse injuries, although no further clinical assessment or diagnostic criteria are provided (Vernillo et al., 2016). But it is also possible that due to the short nature of the race, the running speed was comparatively faster compared to the other races, making injuries more likely. During a 1005 km road race, overuse MSKI were common with over 90% affecting the lower leg (31.3% knee, 28.1% ankle) (Fallon, 1996). The 161 km does not provide specific anatomical distribution and diagnosis of MSKI, but overall 2.6% of all starters sought medical advice for MSKI, mostly for sprains, strains, tendinitis or muscular pain (McGowan and Hoffman, 2015).

MSKI During Training

Although training studies also showed a predominance for lower limb MSKI, bone stress and stress fractures were commonly reported in contrast to data from competition, ranging from 6–21% (Micklesfield et al., 2007; Malliaropoulos et al., 2015; Scheer et al., 2020d), likely due to the longer observational periods and better diagnostic possibilities. Similarly, back injuries are rarely reported during competition but during training this varies between 12.4%–14%, although it may not always be directly related to running but to other activities of daily living (Hoffman and Krishnan, 2014; Malliaropoulos et al., 2015). Injury rates during training were between 1.9 and 3.4 injuries/athlete (Malliaropoulos et al., 2015; Scheer et al., 2020d), which is higher compared to injury rates during most competitions (0.7–2.1 injuries/runner) (Bishop and Fallon, 1999; Krabak et al., 2011; Scheer and Murray, 2011), possibly demonstrating the effects of continued large training loads and/or inadequate rest days. The highest injury rates were observed in a 65 km off-road UER event

with 4.3 injuries/runner (Vernillo et al., 2016), suggesting that the demand of short and faster off-road events may have higher incidences of MSKI.

Practical Considerations

These findings may be of interest to a variety of practitioners. Firstly, medical practitioners and health care providers may be able to plan medical provisions when attending UER competitions. There is guidance on how to provide medical care at UER events (Hoffman et al., 2014), however, given the increased risk of MSKI during shorter, off-road UER events, these provisions may need to be adapted and/or increased. Similarly, medical practitioners may be able to treat training injuries more appropriately, especially considering the increased risk of bone stress injuries and back injuries during training. Secondly, coaches and athletes may benefit of the knowledge of the distribution of injuries and type of UER activity and plan their training program accordingly to try and avoid these overuse injuries.

Future Research Directions

Sports scientist and researchers may benefit from the synthesis of the available evidence and suggestions for future reference, helping them design further studies to reduce the knowledge gap. Examining different UER events, environments and surfaces may therefore also help to gain a better understanding of the underlying mechanisms of MSKI. Prior running experience and training history of the athlete as well as prior personal injury history may also be important and should be investigated. This may help to evaluate the impact of MSKI on race performance and withdrawal, as MSKI can lead to performance decrement (Fallon, 1996) and race withdrawal in 0.7%–4.8% of runners (Hutson, 1984; McGowan and Hoffman, 2015). Further, most studies include both sexes and sex difference have not been examined separately, however, it may be of interest as in non-UER females have more bone stress injuries while male runners more Achilles tendon injuries, although overall injury rates in non-UER athletes are similar (20.8 injuries/1000 females and 20.4 injuries/1000 males) (Hollander et al., 2021). Generally, there is less research in female UER, and it would be important to examine sex differences, as key aspects of female athlete physiology warrant careful consideration, e.g., RED-S that may lead to physiological impairments resulting in an increased risk of bone stress injuries (Tiller et al., 2021). Additionally, age differences in MSKI have not been examined, which is another important aspect, especially if UER could lead to negative long-term health effects on the musculoskeletal system.

Examination of risk factors leading to MSKI in UER is another important aspect as there seems to be a U-shaped pattern between the running distance and the time-loss injury (Kluitenberg et al., 2015). Especially in UER, that requires large training volumes this will be an important aspect, as well as strategies to reduce MSKI. MSKI may be reduced through online tailored advice and this has been observed in trail runners participating in distances of between 15–62 km (Hespanhol et al., 2018), however, if this is particularly applicable to UER is not known but an interesting aspect to investigate further. It may also be interesting to explore how long athletes have to refrain from UER after a MSKI or if they part take in other sports (e.g., cycling) either during rehab or stop UER altogether.

CONCLUSION

Musculoskeletal injuries in UER are common, mostly affecting the lower limb and are of overuse in nature. MSKI differ between competition and training, with multistage events, predominantly affecting the lower leg (medial tibial stress syndrome), foot, knee (patella femoral pain syndrome (PFPS), while timed events mainly affect the ankle (tendinitis of the foot dorsiflexors, Achilles tendinopathy), and knee (patellar tendinopathy). Short continuous UER events off-road have the highest incidence of MSKI, mainly affecting the foot (plantar fasciitis), ankle (ankle sprain), whereas in long continuous UER the main anatomical site of injury is the knee (PFPS), and ankle. During training the back, knee and bone stress injuries are common. Future considerations include examining MSKI in different UER events, environments and surfaces, and on larger study populations. Establishing risk factors, examining sex differences and using a standard reporting system of MSKI in UER are also important.

AUTHOR CONTRIBUTIONS

VS performed conception and design of the study, literature search and analysis, and manuscript writing and editing. BK performed literature search and analysis and manuscript writing and editing. Both authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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The Role of Environmental Conditions on Master Marathon Running Performance in 1,280,557 Finishers the 'New York City Marathon' From 1970 to 2019

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Aim: This study investigated the influence of weather conditions on running performance in female and male age group runners in the largest marathon in the world, the "New York City Marathon."

Methods: The analysis included data from 1,280,557 finishers the "New York City Marathon" from the years 1970 to 2019. Linear mixed models for men and women finishers with race time (min) as dependent variable and 5-year age groups, temperature, wind and relative humidity tertiles (low, medium, high) as independent factors and finisher as random intercept was performed. Additional models with an interaction between age groups and one weather variable each were performed.

Results: Temperature was positively associated with race time while wind speed and humidity were negatively associated ($p < 0.001$). Men were significantly greater affected wind speed and humidity than women ($p < 0.001$ for interaction) but not by temperature ($p = 0.17$ for interaction). With an average of 8 min longer race time, high temperature had the greatest effect on race time. The effect of high humidity on race time was significantly increased in 40–59 years old men and 25–65 years old women. High temperatures had an increased effect on race time in 30–64 years old men and 40–64 years old women. The inverse association between race time and high wind speed was pronounced in finishers with younger age.

Conclusion: Performance was lower on days with high temperature, low humidity and low wind speed. Men seemed to benefit more from higher humidity and wind speed than women. Aged (70 +) finishers were not greater affected by high temperatures.

Keywords: running, elderly athlete, performance, environmental conditions, weather

INTRODUCTION

Marathon running is of high popularity with increasing numbers of participants especially for master (i.e., age group) marathoners (Jokl et al., 2004) and female runners (Vitti et al., 2020). It is well known that weather conditions are likely to deteriorate and negatively impact marathon race performance (Martin, 2007). Different environmental factors such as temperature (Cheuvront and Haymes, 2001; Ely et al., 2007; Montain et al., 2007; Vihma, 2010; Knechtle et al., 2019; Nikolaidis et al., 2019b; Gasparetto and Nesseler, 2020), wind (Vihma, 2010; Knechtle et al., 2019; Nikolaidis et al., 2019b), rain (Knechtle et al., 2019; Nikolaidis et al., 2019b), and humidity (Vihma, 2010; Knechtle et al., 2019; Nikolaidis et al., 2019b) are reported to have an influence on marathon running performance.

Especially environmental temperatures seemed to have a high impact on marathon running performance (Ely et al., 2007, 2008; Vihma, 2010; El Helou et al., 2012; Knechtle et al., 2019; Gasparetto and Nesseler, 2020) where increasing air temperatures seemed to have the highest impact on marathon race times (Cheuvront and Haymes, 2001; Ely et al., 2007; Montain et al., 2007; Vihma, 2010; Gasparetto and Nesseler, 2020).

The optimal ambient temperatures for maximal running speed seemed to depend on the performance level of a marathon runner (El Helou et al., 2012). Regarding the influence of environmental temperature, differences were reported regarding the effect on performance level (Ely et al., 2007, 2008; Vihma, 2010; Gasparetto and Nesseler, 2020) where performance seems to be impaired in both faster (Ely et al., 2008; Gasparetto and Nesseler, 2020) and in slower marathon runners (Ely et al., 2007; Montain et al., 2007; Vihma, 2010). Analyses from the "Boston Marathon" showed, however, that all performance levels of marathoners were impaired with increasing temperatures (Knechtle et al., 2019). Ambient temperatures seemed also to affect the performance regarding the sex of the marathoners (Vihma, 2010). Effects of warm weather seemed to be less evident for female than male marathoners (Vihma, 2010).

Increased ambient temperatures generally reduced athletic performance (El Helou et al., 2012; Lindemann et al., 2017; Reeve et al., 2019). High ambient temperatures can lead to exertional heat illness and even to exertional heat stroke in runners (DeMartini et al., 2014). Elderly athletes (Kenny et al., 2017) and elderly active people (Kenny et al., 2015; Stapleton et al., 2015) seemed to be more affected by higher ambient temperatures. The effects of environmental conditions such as high temperatures are well-known for different groups of marathoners (i.e., female runners, male runners, elite runners, slower runners), but have not been investigated in age group (i.e., master) marathoners although their number continuously increases in large city marathons such as the "New York City Marathon" (Jokl et al., 2004; Lepers and Cattagni, 2012).

The aim of the present study was, therefore, to investigate the effect of ambient temperature on marathon running performance in master marathoners (i.e., age group runners) competing in the largest city marathon in the world, the "New York City Marathon" since its first edition in 1970. We hypothesized that performance would decrease with increasing ambient temperature, especially with increasing age of both female and male 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 participant as the study involved the analysis of publicly available data (EKSG 01-06-2010).

The Race

The "New York City Marathon" is the world's largest annual marathon with actually over 50,000 annual finishers[1]. The "New York City Marathon" first took place in 1970. Until 1975, the marathon was held in Central Park, where four laps were completed. In the first few years it was held in mid-September, from 1976 to 1985 it took place at the end of October. It has had its current date since 1986, with the exception of 1993 and 1995, when it only took place on the second Sunday in November. The race always takes place on the first Sunday in November in New York City. There was no race in 2012 due to the aftermath of Hurricane Sandy and in 2020 the race was canceled due to safety concerns resulting from the COVID-19 pandemic.

In the first marathon on September 13, 1970, 127 participants took part, of which only 55 made it to the finish. The low proportion of finishers persisted for a few years. Only since 1979 have 90% and more of the registered runners regularly crossed the finish line. The number of participants continued to increase slowly. In 1971, there were 245 runners at the start, in 1974 more than 500. This caused increasing organizational problems, as the larger the number of participants it became more difficult to count the laps for each runner. In 1976, the "New York City Marathon" was expanded to all five New York boroughs for the first time to mark the 200th anniversary of the independence of the United States.

The "New York City Marathon" is not a circuit, but goes from Fort Wadsworth on Staten Island via Brooklyn, Queens and the Bronx to Manhattan, where the finish line is in Central Park. Due to the large number of participants, the start is now in four waves (from 2008 to 2011 in three waves) with an interval of about 30 min. In each wave there are three start lanes, which are only finally united at mile 8 (12.9 kilometers). The professional runners start separately some time before the main waves, as do the participants in the wheelchair class. The professional runners start at the head of the first wave.

Data

The athlete data was downloaded from the official New York Road Runners website[2] using a web browser and a JavaScript code. Every athlete's sex, age, country of origin and final race time were thus obtained. Athletes were grouped in 5-year age groups. The weather data was obtained from

[1]https://www.nyrr.org/tcsnycmarathon

[2]https://results.nyrr.org

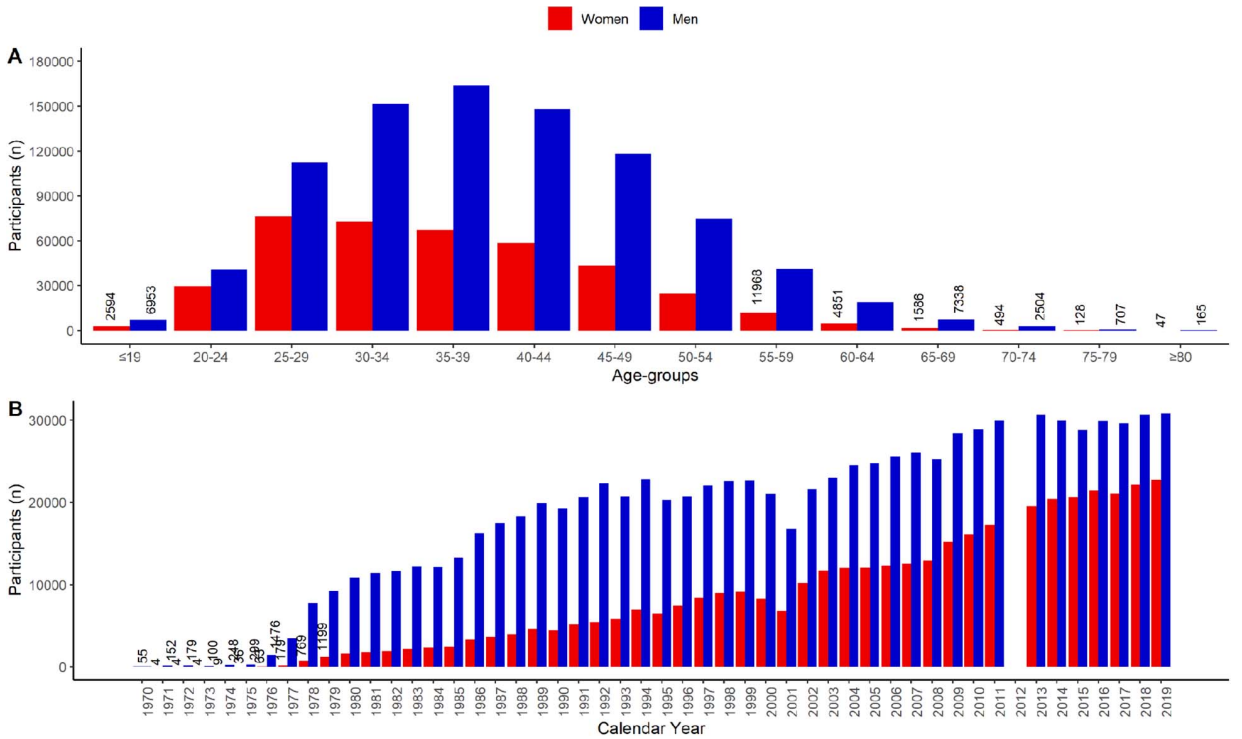

FIGURE 1 | Participants by age group **(A)** and calendar years **(B)**.

https://wunderground.com. The LaGuardia Airport Station was chosen as the most appropriate weather station because of its central location along the route of the "New York City Marathon" and its complete historic dataset. For each race day, we retrieved the on-site weather conditions at 1pm, a time at which most athletes would have been around the halfway point of their run.

Statistical Analysis

The Shapiro-Wilk and Levene's tests were applied for normality and homogeneity, respectively. The average temperature, relative humidity, and wind speed in each race day of each year were transformed into categorical variables in tertiles for low, medium, and high. Temperature: low (5.0 – 11.7°C), medium (11.8 – 17.0°C), high (17.1 – 24.5°C). Relative humidity: low (26 – 43%), medium (43.1 – 56.9%), high (57 – 100%). Wind speed: low (8.1 – 16.1 km/h), medium (16.2 – 22.5 km/h), high (22.6 – 48.3 km/h) were transformed into categorical variables in tertiles for low, medium, and high. A linear mixed model with race time (min) as dependent variable and 5-year age groups, sex, temperature, wind and relative humidity tertiles as independent factors and finisher as random intercept was performed. The mixed model was performed once including interactions between sex and weather categories and once for both sexes separately. Additionally, models for both sexes separately including an interaction between age groups and one weather variable each were performed to explore the weather effect across age groups. Further, linear mixed models were applied for a subset of top ten male and female finishers of each race. Diagnostic plots were used to assess model assumptions. The significance level was set as $p < 0.05$. All statistics were performed with R (Version 3.5.1, R Core Team, 2017).

RESULTS

This analysis included data from the "New York City Marathon" from the years 1970 to 2019, including 886,569 male participants, and 393,988 female participants. Thus, the total sample size is $n = 1{,}280{,}557$. Participants aging from 30 to 49 years old were the most prevalent for both men and women (**Figure 1**, panel A). The number of participants is increasing for both sexes across years (**Figure 1**, panel B). Although the number of women is increasing more than men throughout the years, women only represented 42% of participants in 2019. Along with the increasing number of participants, race time has increased (**Figure 2**, panel A). On the other hand, the top ten finishers improved their race time within the first decade (**Figure 2**, panel B).

Table 1 shows the weather conditions (temperature, humidity, and wind speed) in the "New York City Marathon" on race day with data from 1970 to 2019. A negative correlation was identified between average temperature and calendar year ($p = 0.001$), but not between calendar year and humidity or wind speed (**Figure 3**).

Mean (SD) race time by weather categories (temperature, humidity, and wind speed) by sex is shown for all finishers and

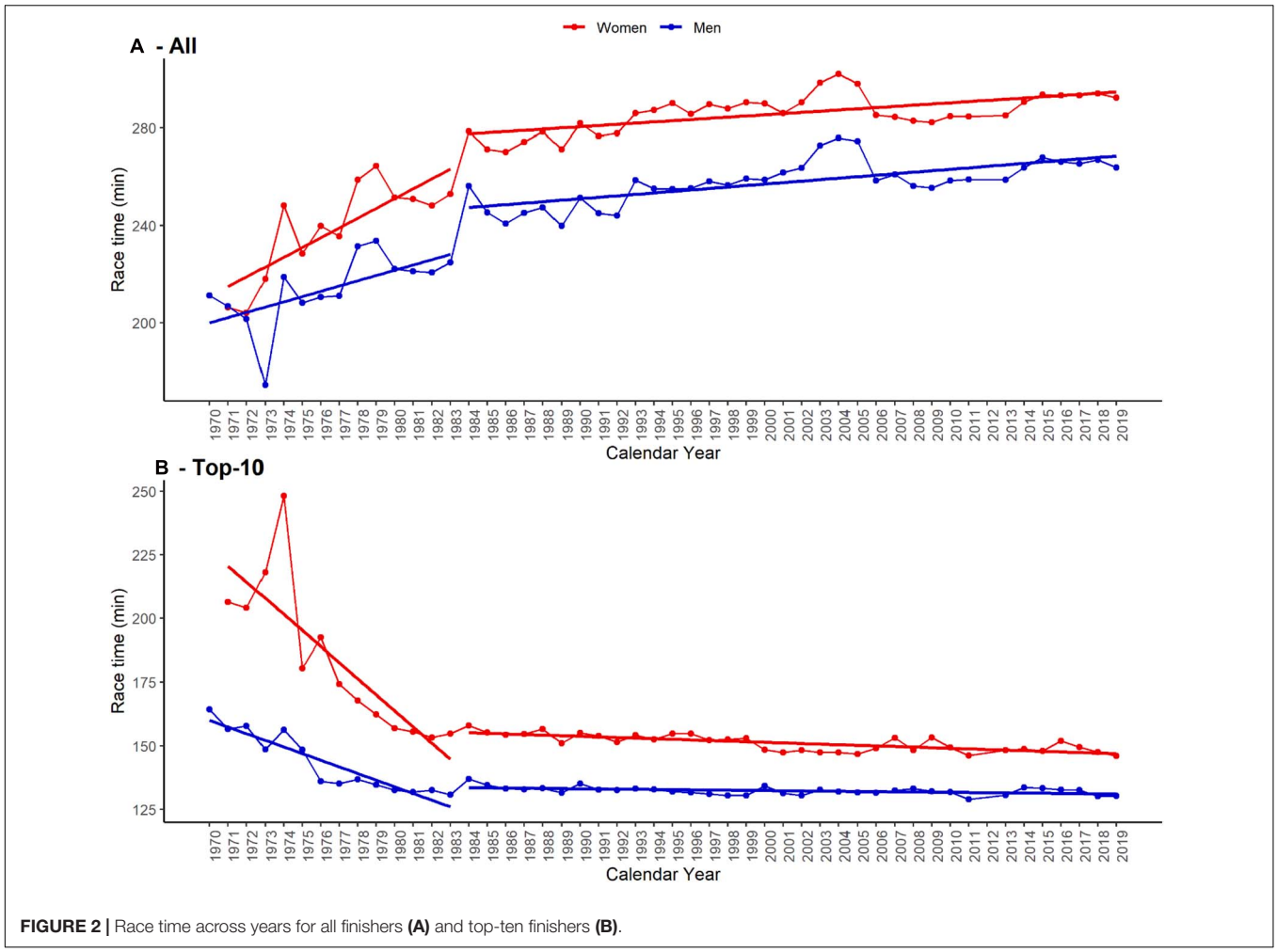

FIGURE 2 | Race time across years for all finishers **(A)** and top-ten finishers **(B)**.

top ten finishes in **Figure 4**. We found significant interactions for sex and wind speed ($p < 0.001$) as well as sex and humidity ($p < 0.001$) within the whole study cohort, but not within top ten finishers. Thereof, **Table 2** shows the linear mixed models for both sexes separately for the full cohort and the model including both, men and women, for top ten finishers. The greatest effect size was found for high temperatures in men [β 7.73 95% Confidence Interval (7.5 – 7.97)] and women [β 7.78 (7.37 – 8.29)] as well as top ten finishers [β 1.87 (0.78 – 2.97)]. For a sensitivity analyses, we included an interaction term between humidity and temperature. In men, high temperature in combination with high humidity were associated with highest race time while low temperature and high humidity were related to lowest race time. In women, highest race time was observed in high temperature and low humidity (**Figure 5**).

We observed a non-linear relation between race time and age with fastest race time in age 20–34 and increasing race time afterward. We found a significant interaction between age groups and each weather variable. The relation between age group, weather condition and race time is depicted in **Figure 6**, showing the predicted average race time and 95% confidence intervals for each age group and weather category, derived from the linear mixed models.

The effect of high humidity on race time was increased in age groups 40–59 years in men and 25–65 years in women (**Table 3**). High temperatures had an increased effect on race time in age groups 30–64 years in men and 40–64 years in women (**Table 4**). The inverse association between race time and high wind speed was pronounced in finishers with younger age and less strong in finishers in age groups 40 + (**Table 5**).

TABLE 1 | Weather conditions in the "New York City Marathon" on race day.

	Mean	SD	Min	Max
Average temperature (°C)	14.6	5.1	5.0	24.4
Relative humidity (%)	53.1	17.9	26.0	100.0
Wind speed (km/h)	21.0	8.9	8.0	48.3

Data from 1970 to 2019. SD, standard deviation.

DISCUSSION

This study investigated the effect of environmental conditions (i.e., ambient temperature, humidity, precipitation, and wind

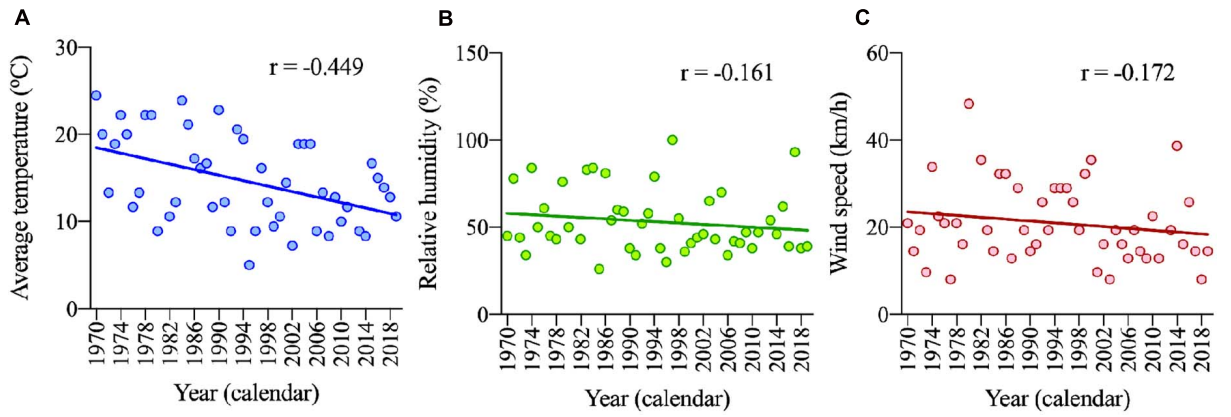

FIGURE 3 | Change in temperature **(A)**, humidity **(B)** and wind speed **(C)** across years.

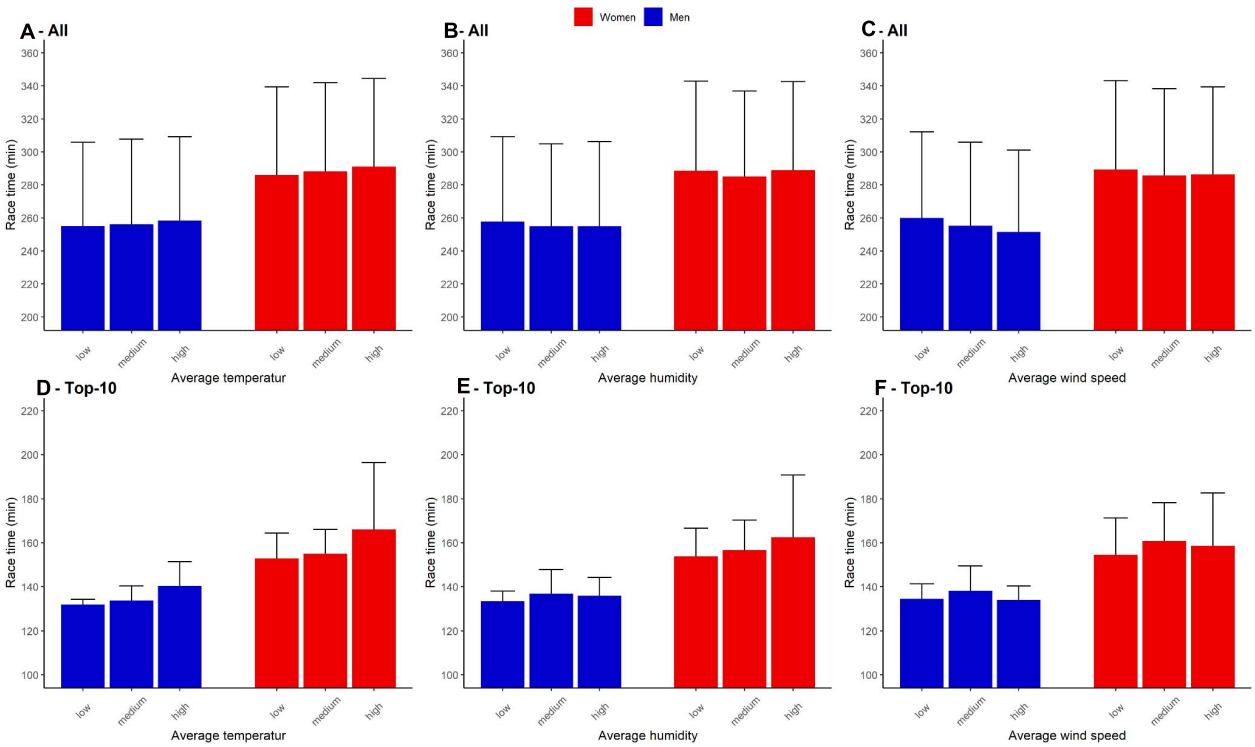

FIGURE 4 | Race time by performance level for temperature **(A)**, humidity **(B)** and wind speed **(C)** for all finishers and for top ten finishers **(D–F)**.

speed) on marathon running performance in age group marathoners competing in the largest city marathon in the world, the "New York City Marathon" with the hypothesis that performance would decrease with increasing ambient temperature, especially with increasing age of both female and male age group marathoners. The main findings were (i) temperature was positively associated with race time while wind speed and humidity were negatively associated, (ii) men were significantly greater affected by wind speed and humidity than women but not by temperature, (iii) the effects were smaller and did not differ between men and women in the top ten finishers, (iv) the effect of high humidity on race time was increased in age groups 40–59 years in men and 25–65 years in women, (v) high temperatures had an increased effect on race time in age groups 30–64 years in men and 40–64 years in women, and (vi) the inverse association between race time and high wind speed was pronounced in finishers with younger age and less strong in finishers in age groups 40 +.

We can confirm our hypothesis that increasing ambient temperatures is associated with reduced marathon race time regardless of sex. This association was found in all finishers as well as in top-10 finishers only. The effect of high temperature

TABLE 2 | Linear mixed model with random intercept for race time (min).

	Model 1 (Full cohort men)	Model 2 (Full cohort women)	Model 3 (top-10)
Medium humidity	−1.73 [−1.92; −1.54] *	−1.74 [−2.04; −1.45] *	0.08 [−0.94; 1.10]
High humidity	−2.11 [−2.31; −1.90] *	−0.90 [−1.24; −0.57] *	0.56 [−0.40; 1.52]
Medium temperature	1.19 [1.00; 1.38] *	1.38 [1.09; 1.67] *	0.04 [−0.92; 0.99]
High temperature	7.73 [7.50; 7.97] *	7.78 [7.37; 8.19] *	1.87 [0.78; 2.97] *
Medium wind speed	−3.18 [−3.39; −2.98] *	−1.90 [−2.22; −1.57] *	1.51 [0.55; 2.48] *
High wind speed	−4.92 [−5.12; −4.73] *	−0.87 [−1.18; −0.57] *	1.35 [0.35; 2.36] *
Male sex	N/A	N/A	−24.24 [−26.7; −21.8] *
Num. obs.	886471	393985	949
Num. groups: id	620308	301502	661
Var: id (Intercept)	1693.04	2044.45	236.85
Var: Residual	683.23	614.62	11.94

*Shown are β effect size with 95% confidence intervals. *$p < 0.05$, low tertile for age categories (not shown) and each weather variable. All models were adjusted for age categories (not shown).*

on race time was significantly greater in 30–65 years old men and in 40–69 years old women, but not greater in the very old (70 +) in contrast to our hypotheses. The influence of ambient temperature on marathon running performance is well-known (Ely et al., 2007, 2008; Vihma, 2010; Knechtle et al., 2019; Gasparetto and Nesseler, 2020), however, increased temperatures seemed to have different effects on different groups of runners (e.g., slower and faster runners, younger and older runners, female and male runners).

In a large city marathon such as the "New York City Marathon," the fastest race times are achieved by runners at younger ages (Nikolaidis et al., 2018b). In the present analysis, the plateau of the fastest race times ranged for both men and women from 30 to 40 years old, regardless of the decade. However, most of the runners in the "New York City Marathon" are master runners (Jokl et al., 2004) who are considerably slower than elite runners (Nikolaidis and Knechtle, 2019). Therefore, one might assume that age group marathoners would suffer more from increased ambient temperatures due to their slower race times.

Generally, slower marathoners seemed to be more affected by increased temperatures (Ely et al., 2007; Vihma, 2010). However, other studies reported that increasing temperatures slowed also faster marathoners (Ely et al., 2008). Regarding the "New York City Marathon," Gasparetto and Nesseler (2020) investigated performances of the top 1,000 runners for every year during the last twelve editions and found that the fastest runners experienced a larger decline in performance than the slower ones under identical thermal exposures. These disparate findings might be explained by the different approaches to the analyses (e.g., selection of elite and sub-elite runners, definition of high and low temperatures, sample sizes, etc.).

We also found differences regarding the sexes. A potential explanation for the disparate findings for female and male age group runners could be the lower female participation in this race (42% female finishers in 2019). Little is known in literature regarding a potential sex difference of the influence of ambient temperature in marathon running. One study found a difference in the effect of warm weather for female and male marathoners. An analysis of different weather variables on running performance in the "Stockholm Marathon" from 1980 to 2008 showed that effects of warm weather were less evident for female than for male runners (Vihma, 2010). Future studies need to investigate the sex differences regarding the influence of temperature on female and male marathon running performance.

One might also assume that a decrease in marathon running performance due to increased ambient temperatures might be due to global warming (El Helou et al., 2012). In the "New York City Marathon," however, temperatures on race day decreased across calendar years. This might be due to the fact that race date changed from September to October and later to November.

Regarding the other weather variables, we found that wind speed and humidity were negatively associated with race time (i.e., faster race times with higher wind speed and higher humidity). Men were significantly greater affected by medium and high wind speed and high humidity than women and the effects were smaller and did not differ between men and women in the top ten finishers. For the age group runners, the effect of high humidity on race time was increased in age groups 40–59 years in men and 25–65 years in women, and the inverse association between race time and high wind speed was pronounced in finishers with younger age and less strong in finishers in age groups 40 +.

The aspect of the influence of wind has been investigated in the "Boston Marathon" as a point-to-point race. In the "Boston Marathon," wind affected marathon running performance (El Helou et al., 2012; Knechtle et al., 2019). Tail wind improved performances (Knechtle et al., 2019) but increasing wind speed was also related to worsened performances in all finishers and near elite groups (Knechtle et al., 2019). Wind coming from the West, compared to wind coming from other directions, was the most favorable for performance (Knechtle et al., 2019). The difference between the "New York City Marathon" and the "Boston Marathon" regarding the influence of wind on marathon running performance are very likely explained by their setting. The "New York City Marathon" is held in a city and not a point-to-point race in contrast to the "Boston Marathon." As far as we are aware, no other study found differences regarding the influence of wind speed in marathon running between the sexes. Future studies need to investigate why men seemed to benefit more from higher humidity and wind speed than women with regard to their race time.

While we found an influence of humidity on marathon running performance, others found not. Although one might assume that humidity might affect marathon running performance, an analysis of the influence of temperature, humidity, dew point, and the atmospheric pressure at sea level in six European (Paris, London, Berlin) and American (Boston, Chicago, New York) marathon races from 2001 to 2010 through 1,791,972 participants' performances (all finishers per year and

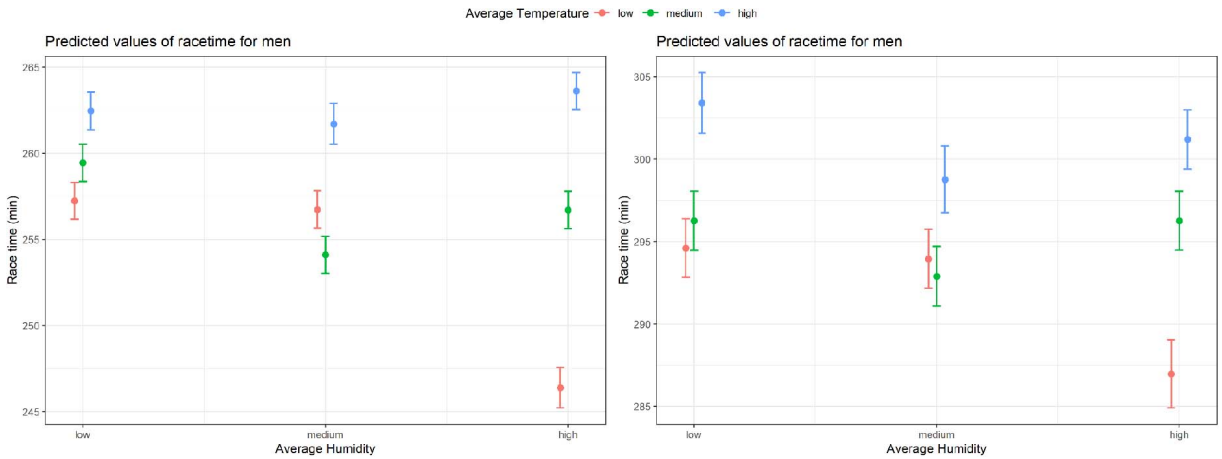

FIGURE 5 | Predicted average race time (min) with 95% confidence intervals for men and women according to temperature and humidity, derived from the linear mixed models.

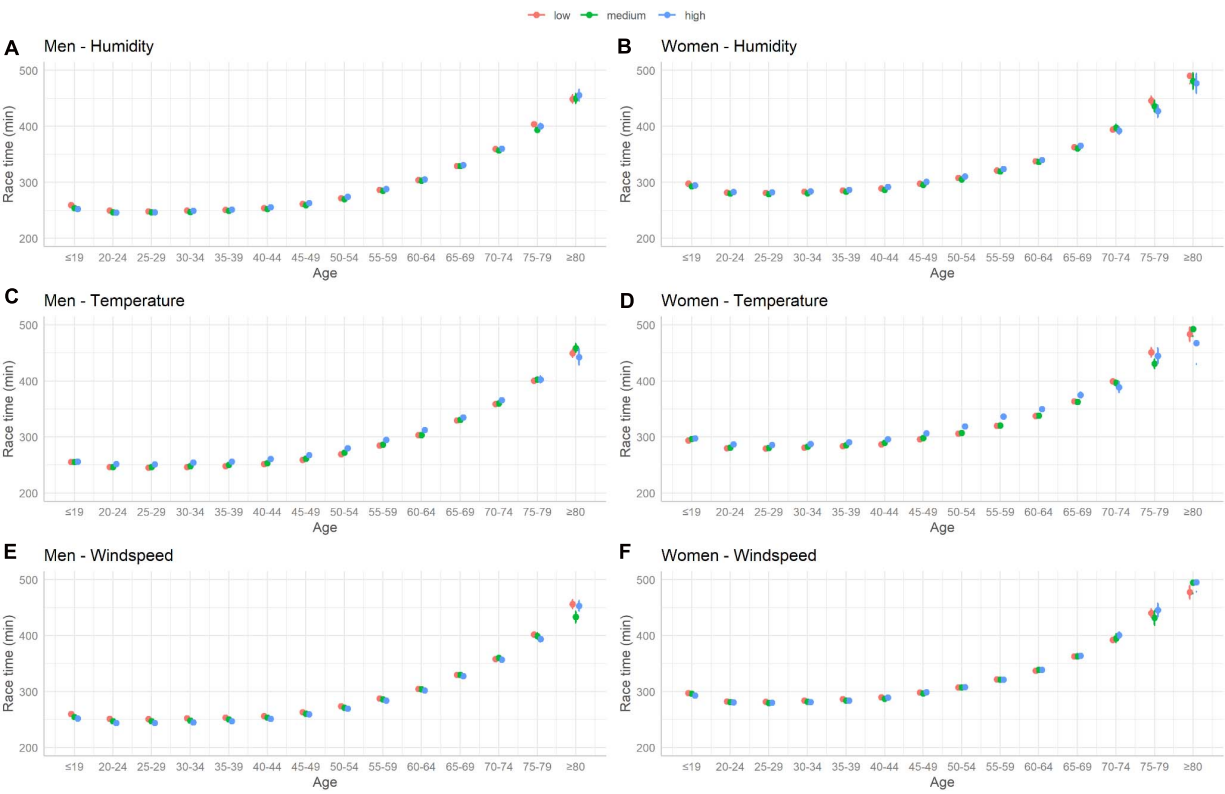

FIGURE 6 | Predicted average race time (min) with 95% confidence intervals for each age group and weather category derived from the linear mixed models. Humidity for men **(A)** and women **(B)**, temperature for men **(C)** and women **(D)**, wind speed for men **(E)** and women **(F)**.

race) showed that air temperature, but no other environmental parameters had any significant impact on marathon running performance (El Helou et al., 2012). The disparate findings might be explained by the fact that we investigated one single race held always at the same location where El Helou et al. (2012) combined data of different race locations. Future studies need to investigate the differences between female and male marathon runners regarding the effect of wind speed and humidity and their performance.

Specific physiological mechanisms might, however, also explain sex- and age-dependent influences on marathon running performance in master marathoners where age, sex, anthropometry, fitness level and training might have an influence on heat tolerance (Kazman et al., 2015; Alele et al., 2021).

TABLE 3 | Linear mixed model for race time with age group and humidity interaction.

	Men	Women
(Intercept)	298.38 [297.73; 299.02]*	334.14 [332.86; 335.42]*
Age group [S. = 19]	−39.02 [−40.64; −37.40]*	−36.38 [−39.10; −33.65]*
Age group [S.20–24]	−48.75 [−49.62; −47.87]*	−52.29 [−53.74; −50.83]*
Age group [S.25–29]	−50.09 [−50.82; −49.35]*	−53.08 [−54.42; −51.73]*
Age group [S.30–34]	−48.82 [−49.53; −48.11]*	−50.65 [−52.00; −49.30]*
Age group [S.35–39]	−47.41 [−48.11; −46.71]*	−48.65 [−50.00; −47.30]*
Age group [S.40–44]	−44.28 [−44.98; −43.58]*	−45.07 [−46.43; −43.71]*
Age group [S.45–49]	−36.96 [−37.68; −36.25]*	−36.11 [−37.49; −34.74]*
Age group [S.50–54]	−26.51 [−27.26; −25.77]*	−26.06 [−27.50; −24.62]*
Age group [S.55–59]	−12.03 [−12.83; −11.23]*	−12.63 [−14.20; −11.07]*
Age group [S.60–64]	5.96 [5.02; 6.90]*	3.64 [1.80; 5.47]*
Age group [S.65–69]	31.07 [29.84; 32.31]*	28.82 [26.22; 31.43]*
Age group [S.70–74]	61.02 [59.17; 62.87]*	60.36 [56.29; 64.42]*
Age group [S.75–79]	105.37 [102.09; 108.66]*	112.08 [105.11; 119.04]*
Medium Humidity	−2.42 [−3.31; −1.52]*	−3.02 [−4.73; −1.32]*
Age group [S. = 19]	−2.82 [−5.23; −0.40]*	−1.87 [−6.03; 2.29]
Age group [S.20–24]	−0.73 [−2.01; 0.55]	1.24 [−0.78; 3.25]
Age group [S.25–29]	0.60 [−0.44; 1.63]	0.87 [−0.95; 2.69]
Age group [S.30–34]	0.17 [−0.83; 1.16]	0.47 [−1.35; 2.29]
Age group [S.35–39]	0.68 [−0.31; 1.67]	1.13 [−0.70; 2.96]
Age group [S.40–44]	0.88 [−0.11; 1.87]	0.60 [−1.25; 2.44]
Age group [S.45–49]	0.62 [−0.40; 1.63]	0.42 [−1.47; 2.32]
Age group [S.50–54]	0.57 [−0.50; 1.64]	0.14 [−1.87; 2.15]
Age group [S.55–59]	0.84 [−0.35; 2.02]	1.06 [−1.20; 3.32]
Age group [S.60–64]	1.08 [−0.33; 2.48]	2.10 [−0.72; 4.92]
Age group [S.65–69]	2.02 [0.11; 3.94]*	1.04 [−2.99; 5.07]
Age group [S.70–74]	0.06 [−2.85; 2.97]	6.24 [−0.13;12.62]
Age group [S.75–79]	−7.45 [−12.51; −2.38]*	−7.25 [−18.69; 4.19]
High Humidity	0.09 [−0.88; 1.07]	−1.32 [−3.19; 0.55]
Age group [S. = 19]	−7.25 [−9.60; −4.91]*	−2.05 [−6.12; 2.02]
Age group [S.20–24]	−3.46 [−4.76; −2.16]*	2.09 [−0.05; 4.23]
Age group [S.25–29]	−1.57 [−2.67; −0.47]*	2.69 [0.72; 4.67]*
Age group [S.30–34]	−0.64 [−1.71; 0.42]	2.00 [0.02; 3.97]*
Age group [S.35–39]	0.19 [−0.87; 1.25]	2.66 [0.67; 4.64]*
Age group [S.40–44]	1.35 [0.29; 2.42]*	4.17 [2.16; 6.17]*
Age group [S.45–49]	1.67 [0.58; 2.76]*	4.23 [2.18; 6.28]*
Age group [S.50–54]	2.14 [1.00; 3.29]*	3.64 [1.47; 5.81]*
Age group [S.55–59]	1.88 [0.63; 3.14]*	3.99 [1.57; 6.41]*
Age group [S.60–64]	1.02 [−0.50; 2.54]	3.37 [0.41; 6.32]*
Age group [S.65–69]	1.11 [−0.93; 3.16]	3.73 [−0.45; 7.91]
Age group [S.70–74]	0.71 [−2.28; 3.70]	−0.80 [−7.56; 5.96]
Age group [S.75–79]	−3.77 [−9.10; 1.56]	−17.66 [−29.42; −5.90]*
Num. obs.	886471	393985
Num. groups: id	620308	301502
Var: id (Intercept)	1691.19	2041.32
Var: Residual	697.24	621.97

Shown are β effect size with 95% Confidence Intervals. Null hypothesis value outside the 95% confidence interval. Grand Mean as reference for age groups and Low category as reference for humidity.*

TABLE 4 | Linear mixed model for race time with age group and temperature interaction.

	Men	Women
(Intercept)	296.21 [295.56; 296.86]*	332.90 [331.64; 334.15]*
Age group [S. = 19]	−41.01 [−42.65; −39.37]*	−39.26 [−42.00; −36.53]*
Age group [S.20–24]	−50.00 [−50.89; −49.11]*	−52.97 [−54.41; −51.53]*
Age group [S.25–29]	−50.77 [−51.50; −50.03]*	−53.63 [−54.96; −52.31]*
Age group [S.30–34]	−49.96 [−50.68; −49.25]*	−51.71 [−53.03; −50.38]*
Age group [S.35–39]	−48.17 [−48.88; −47.47]*	−49.53 [−50.85; −48.20]*
Age group [S.40–44]	−44.73 [−45.43; −44.02]*	−46.20 [−47.53; −44.87]*
Age group [S.45–49]	−37.20 [−37.92; −36.49]*	−37.02 [−38.37; −35.67]*
Age group [S.50–54]	−26.74 [−27.49; −26.00]*	−26.87 [−28.28; −25.45]*
Age group [S.55–59]	−11.66 [−12.46; −10.85]*	−12.78 [−14.32; −11.24]*
Age group [S.60–64]	7.23 [6.29; 8.17]*	4.51 [2.66; 6.36]*
Age group [S.65–69]	33.15 [31.91; 34.40]*	30.50 [27.90; 33.11]*
Age group [S.70–74]	62.59 [60.72; 64.46]*	66.26 [62.17; 70.35]*
Age group [S.75–79]	104.02 [100.68; 107.37]*	118.10 [110.71; 125.49]*
Medium Temperature	1.92 [1.10; 2.75]*	−0.00 [−1.53; 1.53]
Age group [S. = 19]	−1.87 [−4.17; 0.43]	2.65 [−1.15; 6.45]
Age group [S.20–24]	−1.82 [−3.00; −0.64]*	0.82 [−0.99; 2.64]
Age group [S.25–29]	−0.87 [−1.83; 0.09]	1.03 [−0.61; 2.67]
Age group [S.30–34]	−0.41 [−1.33; 0.51]	1.44 [−0.20; 3.08]
Age group [S.35–39]	−0.03 [−0.94; 0.88]	2.01 [0.37; 3.66]*
Age group [S.40–44]	−0.04 [−0.95; 0.87]	2.64 [0.98; 4.30]*
Age group [S.45–49]	0.34 [−0.59; 1.28]	2.06 [0.36; 3.76]*
Age group [S.50–54]	0.24 [−0.75; 1.22]	1.22 [−0.58; 3.02]
Age group [S.55–59]	−0.32 [−1.41; 0.77]	0.23 [−1.78; 2.24]
Age group [S.60–64]	−1.83 [−3.14; −0.53]*	0.51 [−1.97; 2.99]
Age group [S.65–69]	−0.51 [−2.26; 1.24]	−0.94 [−4.51; 2.63]
Age group [S.70–74]	−0.85 [−3.47; 1.76]	−2.68 [−8.25; 2.88]
Age group [S.75–79]	0.60 [−4.10; 5.30]	−20.33 [−30.55; −10.11]*
High temperature	5.99 [4.76; 7.22]*	5.00 [1.94; 8.06]*
Age group [S. = 19]	−5.14 [−7.70; −2.58]*	−1.35 [−6.43; 3.72]
Age group [S.20–24]	−0.72 [−2.26; 0.82]	1.68 [−1.62; 4.97]
Age group [S.25–29]	−0.12 [−1.46; 1.23]	1.34 [−1.81; 4.50]
Age group [S.30–34]	1.82 [0.51; 3.13]*	1.34 [−1.81; 4.49]
Age group [S.35–39]	2.09 [0.79; 3.40]*	1.93 [−1.23; 5.08]
Age group [S.40–44]	3.06 [1.74; 4.37]*	3.96 [0.78; 7.14]*
Age group [S.45–49]	2.89 [1.55; 4.23]*	5.50 [2.26; 8.74]*
Age group [S.50–54]	4.58 [3.18; 5.98]*	7.74 [4.35; 11.12]*
Age group [S.55–59]	4.18 [2.65; 5.71]*	11.27 [7.61; 14.94]*
Age group [S.60–64]	3.08 [1.28; 4.89]*	7.62 [3.24; 12.00]*
Age group [S.65–69]	−0.66 [−3.12; 1.81]	6.66 [0.96; 12.36]*
Age group [S.70–74]	1.17 [−2.47; 4.81]	−15.36 [−25.00; −5.72]*
Age group [S.75–79]	−3.57 [−9.74; 2.59]	−11.48 [−26.09; 3.12]
Num. obs.	886471	393985
Num. groups: id	620308	301502
Var: id (Intercept)	1703.97	2044.41
Var: Residual	683.04	614.85

Shown are β effect size with 95% confidence intervals. Null hypothesis value outside the 95% confidence interval. Grand Mean as reference for age groups and Low category as reference for temperature.*

Heat tolerance can be influenced by heat acclimation leading to a reduction in heat stress (Garrett et al., 2014; Schleh et al., 2018). The fitness level has an influence on heat tolerance, where low cardiorespiratory fitness (Selkirk and McLellan, 2001; Lisman et al., 2014) and body fatness (Selkirk and McLellan, 2001) are associated with heat intolerance. Individuals with a

TABLE 5 | Linear mixed model for race time with age group and wind speed interaction.

	Men	Women
(Intercept)	299.88 [299.22; 300.53]*	332.77 [331.60; 333.93]*
Age group [S. = 19]	−40.16 [−41.79; −38.53]*	−35.46 [−38.12; −32.80]*
Age group [S.20–24]	−48.37 [−49.26; −47.49]*	−50.38 [−51.73; −49.03]*
Age group [S.25–29]	−49.13 [−49.88; −48.39]*	−50.98 [−52.22; −49.74]*
Age group [S.30–34]	−47.69 [−48.41; −46.97]*	−48.65 [−49.89; −47.41]*
Age group [S.35–39]	−46.35 [−47.06; −45.64]*	−46.11 [−47.34; −44.87]*
Age group [S.40–44]	−43.81 [−44.53; −43.10]*	−43.09 [−44.34; −41.85]*
Age group [S.45–49]	−36.91 [−37.63; −36.18]*	−34.70 [−35.96; −33.44]*
Age group [S.50–54]	−26.40 [−27.15; −25.65]*	−25.22 [−26.54; −23.90]*
Age group [S.55–59]	−12.29 [−13.09; −11.48]*	−11.12 [−12.56; −9.69]*
Age group [S.60–64]	4.62 [3.68; 5.55]*	4.65 [2.95; 6.36]*
Age group [S.65–69]	29.93 [28.71; 31.15]*	29.90 [27.52; 32.28]*
Age group [S.70–74]	58.37 [56.59; 60.14]*	59.36 [55.56; 63.17]*
Age group [S.75–79]	102.01 [98.96; 105.06]*	107.13 [100.74; 113.52]*
Medium Wind Speed	−3.89 [−4.90; −2.88]*	−0.03 [−2.01; 1.95]
Age group [S. = 19]	−1.21 [−3.78; 1.36]	−1.34 [−5.82; 3.14]
Age group [S.20–24]	−0.58 [−1.97; 0.82]	−1.18 [−3.47; 1.11]
Age group [S.25–29]	0.14 [−1.02; 1.29]	−1.93 [−4.02; 0.17]
Age group [S.30–34]	−0.08 [−1.19; 1.03]	−2.13 [−4.22; −0.03]*
Age group [S.35–39]	0.52 [−0.58; 1.62]	−2.52 [−4.62; −0.41]*
Age group [S.40–44]	1.38 [0.28; 2.49]*	−2.35 [−4.47; −0.24]*
Age group [S.45–49]	1.27 [0.14; 2.41]*	−1.51 [−3.68; 0.66]
Age group [S.50–54]	1.41 [0.22; 2.60]*	0.07 [−2.23; 2.36]
Age group [S.55–59]	2.39 [1.08; 3.70]*	−0.30 [−2.86; 2.26]
Age group [S.60–64]	3.60 [2.04; 5.15]*	1.18 [−1.97; 4.33]
Age group [S.65–69]	3.56 [1.46; 5.67]*	0.26 [−4.21; 4.73]
Age group [S.70–74]	5.65 [2.49; 8.80]*	2.95 [−4.83; 10.73]
Age group [S.75–79]	1.15 [−4.48; 6.79]	−8.54 [−21.25; 4.18]
High Wind Speed	−5.00 [−5.96; −4.04]*	1.45 [−0.41; 3.30]
Age group [S. = 19]	−2.84 [−5.13; −0.56]*	−6.05 [−10.04; −2.07]*
Age group [S.20–24]	−2.64 [−3.92; −1.36]*	−3.31 [−5.43; −1.19]*
Age group [S.25–29]	−1.93 [−3.01; −0.85]*	−3.34 [−5.30; −1.38]*
Age group [S.30–34]	−2.37 [−3.41; −1.32]*	−4.10 [−6.06; −2.14]*
Age group [S.35–39]	−1.62 [−2.66; −0.58]*	−4.49 [−6.46; −2.52]*
Age group [S.40–44]	0.18 [−0.86; 1.23]	−2.08 [−4.07; −0.10]*
Age group [S.45–49]	1.33 [0.26; 2.40]*	−0.73 [−2.76; 1.30]
Age group [S.50–54]	0.88 [−0.24; 2.00]	−0.92 [−3.06; 1.22]
Age group [S.55–59]	1.04 [−0.19; 2.27]	−1.66 [−4.05; 0.74]
Age group [S.60–64]	2.72 [1.25; 4.19]*	−0.07 [−3.00; 2.86]
Age group [S.65–69]	2.81 [0.84; 4.77]*	−0.36 [−4.52; 3.79]
Age group [S.70–74]	3.70 [0.79; 6.61]*	6.96 [0.63; 13.29]*
Age group [S.75–79]	−3.15 [−8.33; 2.02]	3.87 [−8.49; 16.23]
Num. obs.	886471	393985
Num. groups: id	620308	301502
Var: id (Intercept)	1683.29	2041.29
Var: Residual	695.84	623.37

Shown are β effect size with 95% confidence intervals. Null hypothesis value outside the 95% confidence interval. Grand Mean as reference for age groups and Low category as reference for wind speed.*

higher body fat have a lower heat tolerance due to a reduced capacity to store heat (Cheung et al., 2000). Training can improve heat tolerance (McLellan, 2001) where the training must be long enough to train heat tolerance (Cheung and McLellan, 1999). Women are more likely to be heat intolerant than men (Druyan et al., 2012; Kazman et al., 2015). Thermoregulation in women is affected by the menstrual cycle where body core temperature is adversely affected during the luteal phase (Pivarnik et al., 1992). Women not using oral contraceptives are at a thermoregulatory disadvantage during the luteal phase of the menstrual cycle (Cheung et al., 2000). Heat tolerance is increased during early follicular phase for non-users of oral contraceptives (Tenaglia et al., 1999).

Limitations

A limitation is the aspect that the first races were held in "Central Park" with trees and the race then was held as a city marathon with a difference course. The shadow from the trees might have had an influence on performance. Other aspects such as psychological and physiological aspects (Nikolaidis and Knechtle, 2018; Nikolaidis et al., 2018a), pre-race experience (Malchrowicz-Mośko et al., 2020), training (Piacentini et al., 2013), and nutrition (Roca et al., 2020; Knechtle et al., 2021; Methenitis et al., 2021) and pacing during the race (Cuk et al., 2019; Nikolaidis et al., 2019a) were not considered.

CONCLUSION

In 1,280,557 age group finishers the "New York City Marathon" from the years 1970 to 2019, temperature was positively associated with race time while wind speed and humidity were negatively associated. Regarding sex, men were significantly greater affected by wind speed and humidity than women but not by temperature. High temperatures had the greatest effect on race time with an average of 8 min longer race time. Regarding age, the effect of high humidity on race time was significantly increased in 40–59 years old men and 25–65 years old women. High temperatures had an increased effect on race time in 30–64 years old men and 40–64 years old women. The inverse association between race time and high wind speed was pronounced in finishers with younger age. An observational study investigating a large data set provides results from "real life" enabling athletes and coaches to better prepare for "real life conditions."

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

EV, PN, CM, OG, CS, and BK performed the material preparation and data collection. TM and CS performed the data analysis. BK, TM, and CS conducted the data interpretation. BK wrote the first draft of the manuscript. All authors contributed to the study conception and design, commented on previous versions of the manuscript, read, and approved the final manuscript.

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Death Zone Weather Extremes Mountaineers Have Experienced in Successful Ascents

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Background: Few data are available on mountaineers' survival prospects in extreme weather above 8000 m (the Death Zone). We aimed to assess Death Zone weather extremes experienced in climbing-season ascents of Everest and K2, all winter ascents of 8000 m peaks (8K) in the Himalayas and Karakoram, environmental records of human survival, and weather extremes experienced with and without oxygen support.

Materials and Methods: We analyzed 528 ascents of 8K peaks: 423 non-winter ascents without supplemental oxygen (Everest–210, K2–213), 76 ascents in winter without oxygen, and 29 in winter with oxygen. We assessed environmental conditions using the ERA5 dataset (1978–2021): barometric pressure (BP), temperature (Temp), wind speed (Wind), wind chill equivalent temperature (WCT), and facial frostbite time (FFT).

Results: The most extreme conditions that climbers have experienced with and without supplemental oxygen were: BP 320 hPa (winter Everest) vs. 329 hPa (non-winter Everest); Temp –41°C (winter Everest) vs. –45°C (winter Nanga Parbat); Wind 46 $m{\cdot}s^{-1}$ (winter Everest) vs. 48 $m{\cdot}s^{-1}$ (winter Kangchenjunga). The most extreme combined conditions of BP $\leq$ 333 hPa, Temp ≤ -30°C, Wind $\geq$ 25 $m{\cdot}s^{-1}$, WCT ≤ -54°C and FFT $\leq$ 3 min were encountered in 14 ascents of Everest, two without oxygen (late autumn and winter) and 12 oxygen-supported in winter. The average extreme conditions experienced in ascents with and without oxygen were: BP 326 $\pm$ 3 hPa (winter Everest) vs. 335 $\pm$ 2 hPa (non-winter Everest); Temp -40 ± 0°C (winter K2) vs. -38 ± 5°C (winter low Karakoram 8K peaks); Wind 36 $\pm$ 7 $m{\cdot}s^{-1}$ (winter Everest) vs. 41 $\pm$ 9 $m{\cdot}s^{-1}$ (winter high Himalayan 8K peaks).

Conclusions:

1. The most extreme combined environmental BP, Temp and Wind were experienced in winter and off-season ascents of Everest.
2. Mountaineers using supplemental oxygen endured more extreme conditions than climbers without oxygen.
3. Climbing-season weather extremes in the Death Zone were more severe on Everest than on K2.
4. Extreme wind speed characterized winter ascents of Himalayan peaks, but severely low temperatures marked winter climbs in Karakoram.

Keywords: altitude, weather, extremes, mountaineering, barometric pressure, temperature, wind

INTRODUCTION

The pioneering era to be the first climbers of 8000 meter (8K) peaks ended with the first winter ascent of K2 in January 2021; it was the last 8K peak unclimbed in winter (Benavides, 2021b). Since G. Mallory, E. Norton, and H. Somervell took their first steps above 8000 m as members of the British Everest expedition in 1922 (Somervell, 1938) it has taken climbers almost a century to reach all 14 of the world's 8K peaks without supplemental oxygen in the climbing and the winter seasons. The first 8K peak climbed without oxygen was Annapurna by the French alpinists Herzog and Lachenal, 1950 (Herzog, 1953). Mount Everest, the highest peak on Earth, was first climbed with oxygen support by Hillary and Norgay, 1953 (Hunt and Hillary, 1953) and without supplemental oxygen by Messner and Habeler, 1978 (Habeler, 1979; Messner, 1979). In 1978 the US alpinist L. Reichardt climbed K2 (8611 m), the last 8K peak unclimbed without oxygen support.

Yet more extreme winter Himalayan mountaineering was launched in February 1980 with the first winter oxygen-supported ascent of Everest by K. Wielicki and L. Cichy (Brniak and Nyka, 1981). Only one climber, Ang Rita Sherpa, has repeated their feat without supplemental oxygen, in December 1987 (West, 1993; Garrido et al., 2019; Salisbury and Hawley, 2020). Manaslu was the first 8K peak climbed in winter without oxygen, by the Polish party of Berbeka and Gajewski, 1984 (Korniszewski, 1984). K2 was the last 8K peak unclimbed in winter, but was finally climbed by a team of 10 Nepalese in January 2021 (Farooq, 2021). One climber, Nirmal Purja, reached K2's summit without supplemental oxygen (Benavides, 2021b). Between Herzog and Lachenal's success on Annapurna in 1950 and 2021 more than 6500 times 8K peaks have been climbed without supplemental oxygen: about 4500 ascents in the Himalayas (1950–2020) (Salisbury and Hawley, 2020) and about 2070 in Karakoram (1953–2009) (8000ers, 2021).

Altitude, latitude and season mainly determine climatic conditions such as barometric pressure (BP), air temperature (Temp) and wind speed (Wind) (Whiteman, 2000). The higher the altitude and the latitude, and the colder the season, the lower are BP and Temp (Brunt, 1952; West, 1996; Whiteman, 2000; West et al., 2007c). Summit bids on 8K peaks face most risk when climbers enter the Death Zone, the altitudes above 8000 m where extreme conditions threaten human survival (West et al., 2007a). BP imposes the main limit on physiological performance at high altitude because it determines the critically important partial pressure of inspired oxygen, maximum oxygen uptake and speed of vertical ascent (Pugh et al., 1964; West and Wagner, 1980; West, 1983, 2000; West et al., 1983a,b, 2007b; Sutton et al., 1988; Bailey, 2001; Matthews et al., 2020b). Low temperatures and high winds significantly compound physiological stress at extreme altitudes (Huey et al., 2001; Huey and Eguskitza, 2001; McIntosh et al., 2008; Moore and Semple, 2011, 2012; Moore et al., 2012).

A climber's maximal and sustainable power in the Death Zone drop to levels where activity becomes impossible and the body's heat generation plummets, greatly increasing the risk of hypothermia (Havenith, 2010). The combined effect of Temp and Wind on a climber can be expressed by thermal stress indices such as wind chill temperature (WCT) and facial frostbite time (FFT) (Tikuisis and Osczevski, 2003; Osczevski and Bluestein, 2005). Wind chill temperature is calculated as an air temperature that without wind would cause the same steady-state facial heat loss as occurs at a given temperature and wind speed (Osczevski and Bluestein, 2005). Facial frostbite time is defined as the time it takes facial flesh to freeze (Moore and Semple, 2011). Low BP leads to hypoxia and high-altitude illness, low temperatures with high winds determine the risk of hypothermia and frostbite: these are the main environmental factors responsible for most non-traumatic deaths of mountaineers above the base camp at Everest (Firth et al., 2008). On Everest, more than 80% of all climbers' deaths have occurred in the Death Zone in the summit bid (Firth et al., 2008).

Meteorological data for the conditions endured while climbing in the Death Zone are usually restricted to single weather factors on Everest (West et al., 1983b; West, 1999; Moore and Semple, 2004; Grocott et al., 2010; Moore et al., 2012; Moore and Semple, 2012; Matthews et al., 2020b). The estimated average climbing-season BP on Everest is 333 hPa, dropping to 323 hPa in midwinter (Matthews et al., 2020b). A BP of 329 hPa is the lowest recorded for any ascent of Everest unsupported by oxygen (West, 1993; Matthews et al., 2020b). The most extreme Temp encountered on Everest's summit was –49°C with Wind as high

as 80 m·s^{-1} (Matthews et al., 2020a). Everest's average climbing-season WCT is –45°C, FFT is 7 min; in midwinter WCT is close to –65°C and FFT is less than 1 min (Moore and Semple, 2011). In a previous article (Szymczak et al., 2021) we analyzed the weather extremes on the summits of Everest and K2 in the climbing and the winter seasons using monthly means between 1979 and 2019.

All 8K peaks have already been ascended in the climbing and the winter seasons, so here we analyze and summarize weather conditions that mountaineers have experienced in the most extreme climbs of 8K peaks to determine the environmental high-altitude extremes of human survival. We evaluate the Death Zone's weather extremes in all ascents of Everest and K2 without oxygen, as well as in all winter ascents of 8K peaks in the Himalayas and Karakoram with and without supplemental oxygen. By broadening the knowledge of the most extreme survivable environmental conditions expedition leaders, physiologists and physicians will have the facts they need to prepare climbers for extreme high-altitude weather and to maximize mountaineers' chances of reaching summits while minimizing the risks of their attempts.

MATERIALS AND METHODS

Materials

To survey all ascents in the most extreme environmental conditions, our analyses included all successful ascents without oxygen of the two highest 8K peaks: Mount Everest (8848 m) and K2 (8611 m) in the two the highest mountain ranges, the Himalayas and Karakoram. We also examined all successful winter ascents with and without supplemental oxygen of all the other 8K peaks. Our analyses covered the period from the first ascents of Everest and K2 without oxygen in 1978 to the first winter ascent of K2 in 2021.

We analyzed climatic conditions climbers have endured on the summits of 8K peaks, so we used a successful ascent as the unit of our analysis. We considered an attempt successful if a climber reached the summit, whether the climber returned to base camp or died during the descent. We excluded attempts that terminated before reaching the summit, whatever the reason. Each successful ascent was a separate data point. Winter-season ascents included the meteorological winter (December–February) and the calendar winter season from December 22 to March 20. We obtained the number, dates and times of ascents of Himalayan 8K peaks from the Himalayan Database (Salisbury and Hawley, 2020) and ascents of Karakoram's 8K peaks from mountaineering journals, books and online sources (8000ers, 2021).

We identified 528 ascents that met our criteria: 499 without oxygen (423 non-winter and 76 in winter) and 29 oxygen-supported winter ascents. Of the 423 non-winter ascents, 210 were of Everest (ME-noO_2) and 213 were of K2 (K2-noO_2). Most non-winter ascents of Everest without oxygen (93%, 196/210) and K2 (88%, 188/213) were accomplished in the climbing season, May and October for Everest (Salisbury and Hawley, 2020; Matthews et al., 2020b), and July and August for K2 (8000ers, 2021).

The altitude and the latitude of the summit determine its weather, so we divided the 76 winter ascents of 8K peaks without oxygen into five groups for our analysis: 1 of Everest (wME-noO_2), 1 of K2 (wK2-noO_2), 10 of high Himalayan 8K peaks (wH&H-noO_2), 50 of low Himalayan 8K peaks (wL&H-noO_2) and 14 of low Karakoram 8K peaks (wL&K-noO_2) (**Table 1**).

The wH&H-noO_2 ascents included winter climbs of four Himalayan 8K peaks higher than 8450 m: Kangchenjunga (8586 m), Kangchenjunga South (8494 m), Lhotse (8516 m) and Makalu (8481 m). The group of wL&H-noO_2 ascents included winter climbs of five Himalayan 8K peaks lower than 8250 m: Cho Oyu (8201 m), Dhaulagiri (8167 m), Manaslu (8156 m), Annapurna (8091 m) and Shisha Pangma (8013 m). The wL&K-noO_2 ascents included winter climbs of four Karakoram 8K peaks lower than 8250 m: Nanga Parbat (8126 m), Gasherbrum I (8068 m), Broad Peak (8051 m) and Gasherbrum II (8035 m). Nanga Parbat is a Himalayan peak but at Karakoram's latitude, so we included it in the wL&K-noO_2 group. K2 is the only high 8K peak in the Karakoram range, so we analyzed it separately as wK2-noO_2 (**Table 1**).

Of the 29 oxygen-supported ascents of 8K peaks in winter (w8000-yesO_2) 14 were of Everest (wME-yesO_2), 9 were of K2 (wK2-yesO_2), 4 were of high Himalayan peaks and 2 were of low Himalayan peaks (**Table 1**).

The 528 ascents we analyzed were accomplished over 211 days on the summits. Of these ascents 509 were by male climbers and 19 by females. Their average age was 36 $\pm$ 7 years. There were 425 ascents by lowlanders and 103 by highlanders. We included Tibetans and populations with Tibetan ancestry in the highlander group: Nepali highlanders (Sherpa, Rai, Magar, Tamang) (Cole et al., 2017) and Pakistani highlanders (Balti) (Yang et al., 2020). Forty-eight climbers died on their descents (**Table 1**).

Methods

These 8K peaks lack weather stations that can provide *in situ* data, so we used the ERA5 Reanalysis as our primary source of meteorological conditions. ERA5 is a state-of-the-art database provided by the European Centre for Medium-Range Weather Forecasts (ECMWF) (Hersbach et al., 2020). This database assimilates all available observations, including satellite data, and is provided at the high spatial resolution of 0.25 $\times$ 0.25 degrees of latitude and longitude. The calculated values of weather conditions at the summit altitude should be interpreted as averages for the roughly rectangular area defined by the reanalysis model's resolution for the location of the mountain. The rectangles have sides of about 28 km by 24 km in the Himalayas and 28 km by 22 km at the latitude of Karakoram.

We analyzed the climatic factors that most limit human performance and survival at high altitude: BP, Temp and Wind. Our research examined four meteorological variables at the 300, 350, and 400 hPa isobaric levels. We used hourly values of geopotential height, air temperature and wind vector components (u, zonal; v, meridional) at these three standard synoptic levels to calculate BP, Temp and Wind at the desired height. When the exact time of the ascent was unavailable, we estimated the environmental conditions using a Gaussian weighted average for the day of the successful climb: 11:30 Nepal

TABLE 1 | Characteristics of ascents of 8000 m peaks.

Ascents group	Number of ascents	Ascent altitude [m] ± SD	Summit days	Climbers' origin (highlander/lowlander)	Sex (female/male)	Climbers' Age ± SD (min-max)	Deaths on descent
ME-noO_2	210	8848	111	63/147	7/203	35 ± 6 (20-55)	13 (6%)
K2-noO_2	213	8611	59	17/196	10/203	38 ± 7 (22-60)	26 (12%)
wME-noO_2	1	8848	1	1/0	0/1	39	0
wK2-noO_2	1	8611	1	1/0	0/1	37	0
wH&H-noO_2	10	8504 ± 43	4	4/6	0/10	38 ± 4 (30-45)	0
wL&H-noO_2	50	8158 ± 42	19	3/47	1/49	32 ± 5 (22-47)	2 (4%)
wL&K-noO_2	14	8077 ± 38	5	1/13	1/13	39 ± 8 (28-58)	3 (21%)
w8000-yesO_2	29	8677 ± 192	11	13/16	0/29	32 ± 5 (26-46)	4 (14%)
wME-yesO_2	14	8848	7	1/14	0/14	32 ± 5 (26-45)	1 (7%)
wK2-yesO_2	9	8611	1	9/0	0/9	35 ± 6 (31-46)	0

ME-noO_2, Everest non-oxygen non-winter ascents; K2-noO_2, K2 non-oxygen non-winter ascents; wME-noO_2, Everest non-oxygen winter ascents; wK2-noO_2, K2 non-oxygen winter ascents; wH&H-noO_2, high Himalayan 8K peaks non-oxygen winter ascents; wL&H-noO_2, low Himalayan 8K peaks non-oxygen winter ascents; wL&K-noO_2, low Karakoram 8K peaks non-oxygen winter ascents; w8000-yesO_2, 8K peaks oxygen-supported winter ascents; wME-yesO_2, Everest winter oxygen-supported ascents; wK2-yesO_2, K2 winter oxygen-supported ascents.

Time (NPT) ± 3.5 h for Everest ascents, 15:30 Pakistan Time (PKT) ± 3.3 h for K2 ascents and 13:30 NPT ± 2.5 h for winter ascents of 8K peaks. The summiting hour was unavailable for 10 ascents of Everest (5%), 25 ascents of K2 (12%) and 9 winter ascents of Himalayan 8K peaks without oxygen (12%).

We had to calculate BP at the summit levels of the selected 8K peaks before interpolating Temp and Wind. We calculated BP by fitting a non-linear regression model to each instance of the air pressure-height profile. Calibrated model coefficients were then used to estimate BP at a given height. This provided the data we needed to estimate the values of other variables. Temp and Wind were derived from linear interpolation of values at predefined isobaric surfaces from ERA5. Temperature lapse rates change with season (Dillon et al., 2006), so Temp was calculated individually for each instance.

From the Temp and Wind results we estimated the WCT and FFT thermal stress indices. Our WCT and FFT calculations we based on formulas by Osczevski and Bluestein and by Tikuisis and Osczevski (Tikuisis and Osczevski, 2003; Osczevski and Bluestein, 2005; Moore and Semple, 2011):

$$\mathrm{WCT[^{\circ}C]} = 13.12 + 0.621\,\mathrm{T} - 11.37\,\mathrm{V}^{0.16} + 0.3965\,\mathrm{T}\,\mathrm{V}^{0.16}$$

$$\mathrm{FFT[min]} = [-24.5(0.667\,\mathrm{V} + 4.8) + 2111](-4.8 - \mathrm{T})^{-1.668}$$

where V is the wind speed ($km{\cdot}h^{-1}$) 10 m above the surface and T is the air temperature (°C).

Statistical Analysis

We used Statistica 13.1 (StatSoft, United States) for our calculations, testing the normality of data with the Shapiro-Wilks W-test, then the homogeneity of variance with the Brown-Forsythe test. To assess the statistical significance of differences we used analysis of variance and Tukey's *post hoc* test. Our results are expressed as means with a standard deviation (x ± SD). We measured the relative variability of all parameters using a coefficient of variation (CV) and analyzed any associations of the parameters using Pearson's correlation coefficient (r). We set statistical significance at $p < 0.05$ for all our analyses.

RESULTS

Barometric Pressure

Of all 499 ascents of 8K peaks without oxygen that we analyzed, 50 experienced BP ≤ 333 hPa (10th percentile), all on Everest (**Figure 1** and **Table 2**). The average BP of 335 ± 2 hPa in non-winter ascents of Everest without oxygen was significantly lower ($p < 0.01$) than in the other groups of oxygen-unaided ascents except for one winter ascent of Everest and one winter ascent of K2 (**Table 3**). BP of 329 hPa was the lowest endured without oxygen support and was experienced in two ascents of Everest in April (**Table 4**).

Of 29 oxygen-supported ascents of 8K peaks in winter, all 14 ascents of Everest encountered BP ≤ 333 hPa (average of 326 ± 3 hPa); 12 encountered BP < 329 hPa, the lowest recorded for ascents without oxygen support (**Figure 1** and **Tables 2**, **3**). The lowest BP recorded was 320 hPa for an ascent with supplemental oxygen (**Table 4**).

Of the 50 ascents of Everest without oxygen experiencing BP ≤ 333 hPa, 23 were by highlanders and 27 by lowlanders, 36% and 18%, respectively, of all ascents without oxygen of Everest by those groups. Highlanders endured lower BP of 334 ± 2 hPa in these climbs than lowlanders at 335 ± 2 hPa ($p < 0.01$). Ang Rita Sherpa endured BP ≤ 333 hPa in 4 of his 9 ascents of Everest without oxygen, including the one in winter.

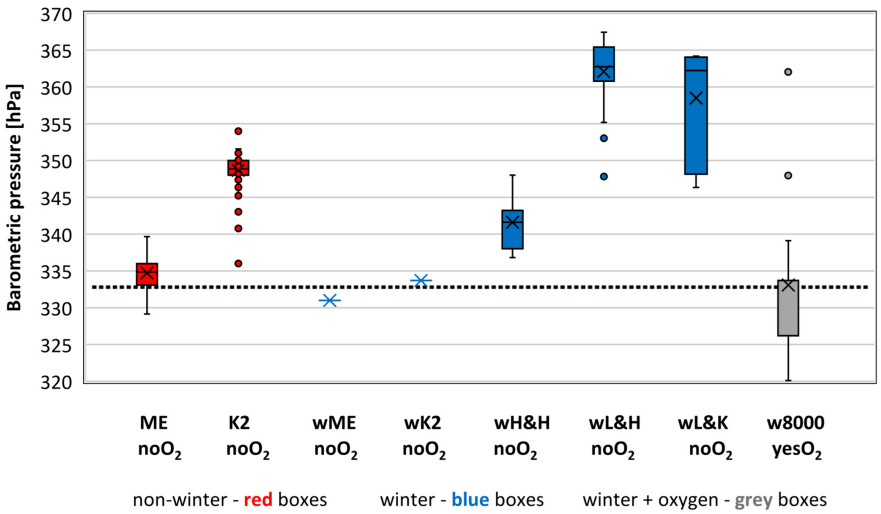

FIGURE 1 | Barometric pressure experienced on the summits we analyzed. Sample size – 499 ascents without oxygen support and 29 with oxygen support (1978–2021). Bars extend to the maximum and minimum BP, boxes span the 25th–75th percentiles, crosses inside the boxes mark mean values, horizontal lines inside the boxes represents median values, dots mark outliers, dotted line represents 10th percentile value of 333 hPa for all 499 ascents with no supplemental oxygen.

The highest average BP of 349 ± 2 hPa was encountered in non-winter ascents of K2 without oxygen, 14 hPa higher than in non-winter ascents of Everest without oxygen (**Figure 1** and **Table 3**).

Air Temperature

Air temperatures ≤−30°C (10th percentile) were experienced in 50 of the 499 ascents of 8K peaks without oxygen that we analyzed: 36 in winter. Half of the non-winter ascents with the lowest Temp were undertaken outside the typical climbing season (Everest – 4 in April, 1 in the latest-ever October ascent; K2 – 2 in October), (**Figure 2** and **Table 2**). The lowest average Temp of −38 ± 5°C was recorded in winter ascents of low Karakoram 8K peaks (wL&K-noO_2) (**Table 3**). This was significantly lower ($p < 0.01$) than the average Temp in all the other groups of ascents without oxygen except one winter ascent of K2 (**Table 3**). The lowest Temp of ≤−44°C was endured by four climbers in winter ascents of Nanga Parbat and Gasherbrum I, two low 8K Karakoram peaks (**Table 4**).

The average Temp of −36 ± 4°C in 29 oxygen-supported 8K ascents in winter did not differ statistically from the mean Temp in wL&K-noO_2 ascents (**Table 3**). Almost all oxygen-aided winter ascents (97%) were accomplished in Temp of ≤−30°C (**Figure 2** and **Table 2**). The lowest Temp of −41°C was experienced in the first oxygen-aided ascent of Everest in winter (**Table 4**).

The highest average Temp of −19 ± 3°C was recorded in non-winter ascents of K2 without oxygen. This was 6°C higher than in non-winter ascents of Everest without oxygen ($p < 0.01$) (**Figure 2** and **Table 3**).

Wind Speed

Fifty of the 499 ascents without oxygen (90th percentile) that we analyzed encountered Wind ≥ 25 m·s^{-1} (**Figure 3** and **Table 2**), 41 (82%) in winter ascents of 8K Himalayan peaks. Of the 9 non-winter ascents with Wind ≥ 25 m·s$^{-1,}$ 5 were of Everest (4 in October, 1 in May) and 4 of K2 (the earliest four summer ascents on one summit day, 13 June). The highest average Wind of 41 ± 9 m·s^{-1} was recorded in winter ascents without oxygen of high Himalayan 8K peaks (wH&H-noO_2) (**Table 3**). This Wind was significantly higher ($p < 0.01$) than all other groups of oxygen-unaided ascents that we analyzed (**Table 3**). The mean Wind value in winter ascents of low Himalayan 8K peaks (wL&H-noO_2) was twice as high as encountered in corresponding winter ascents of low Karakoram 8K peaks (wL&K-noO_2) ($p < 0.01$) (**Table 3**). Nine climbers experienced the highest Wind of ≥ 46 m·s^{-1} in winter ascents of Kangchenjunga, Kangchenjunga South and Shisha Pangma (**Table 4**). Significantly higher ($p < 0.01$) Wind of 17 ± 7 m·s^{-1} was encountered in all 13 non-winter ascents by lowlanders of Everest without oxygen that ended with deaths on their descents compared with an average Wind of 12 ± 5 m·s^{-1} in 134 ascents by lowlanders that ended safely.

Of the 29 oxygen-supported 8K ascents in winter that we analyzed, 85% in the Himalayas were climbed with Wind ≥25 m·s^{-1}. The highest Wind of 46 m·s^{-1} was experienced in a winter ascent of Everest with oxygen (**Table 4**).

The lowest Wind was recorded in non-winter ascents without oxygen of Everest (12 ± 5 m·s^{-1}) and of K2 (12 ± 5 m·s^{-1}) ($p > 0.5$) (**Figure 3** and **Table 3**).

Wind Chill Temperature

Wind chill temperature ≤−54°C (10th percentile) was experienced in 50 of the 499 ascents of 8K peaks without oxygen that we analyzed. Most (90%) of the 50 ascents with the lowest WCT were in winter (**Table 2**). All five non-winter ascents encountering a WCT of ≤−54°C were accomplished out of the typical climbing season (Everest – 2 in April, 1 – the

TABLE 2 | Number of ascents under the most severe environmental conditions of barometric pressure, ambient temperature, wind chill temperature and facial frostbite time within the 10th percentile and wind speed within 90th percentile for ascents without supplemental oxygen.

Ascents group and number	BP ≤ 333 hPa	Temp ≤ −30°C	Wind ≥ 25 m·s^{-1}	WCT ≤ −54°C	FFT ≤ 3 min
ME-noO_2 (210)	49 (23%)	12 (6%)	5 (2%)	3 (1%)	3 (1%)
K2-noO_2 (213)	0	2 (1%)	4 (2%)	2 (1%)	2 (1%)
wME-noO_2 (1)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
wK2-noO_2 (1)	0	1 (100%)	0	1 (100%)	0
wH&H-noO_2 (10)	0	6 (60%)	9 (90%)	8 (80%)	10 (100%)
wL&H-noO_2 (50)	0	14 (28%)	31 (62%)	25 (50%)	33 (66%)
wL&K-noO_2 (14)	0	14 (100%)	0	10 (71%)	1 (7%)
w8000-yesO_2 (29)	14 (48%)	28 (97%)	17 (59%)	28 (97%)	19 (66%)
wME-yesO_2 (14)	14 (100%)	14 (100%)	12 (86%)	14 (100%)	14 (100%)
wK2-yesO_2 (9)	0	9 (100%)	0	9 (100%)	0

ME-noO_2, Everest non-oxygen non-winter ascents; K2-noO_2, K2 non-oxygen non-winter ascents; wME-noO_2, Everest non-oxygen winter ascents; wK2-noO_2, K2 non-oxygen winter ascents; wH&H-noO_2, high Himalayan 8K peaks non-oxygen winter ascents; wL&H-noO_2, low Himalayan 8K peaks non-oxygen winter ascents; wL&K-noO_2, low Karakoram 8K peaks non-oxygen winter ascents; w8000-yesO_2, 8K peaks oxygen-supported winter ascents; wME-yesO_2, Everest winter oxygen-supported ascents; wK2-yesO_2, K2 winter oxygen-supported ascents.
BP, barometric pressure; Temp, temperature; Wind, wind speed; WCT, wind chill equivalent temperature; FFT, facial frostbite time.

latest October ascent; K2 – 2 in October). The lowest average WCT of −60 ± 6°C and −58 ± 6°C were recorded in winter ascents of high Himalayan 8K peaks (wH&H-noO_2) and low Karakoram 8K peaks (wL&K-noO_2) (**Table 3**). Significantly higher average WCT ($p < 0.01$) was recorded in other groups of ascents without oxygen, except one winter ascent of Everest (**Table 3**). The lowest WCT of ≤−66°C was endured by four climbers in winter ascents of Nanga Parbat and Gasherbrum I (**Table 4**).

The average WCT of −66 ± 6°C in 29 oxygen-supported winter ascents of 8K peaks did not differ statistically from the mean WCT for wH&H-noO_2 climbs (**Table 3**). All but 1 winter ascent with oxygen were accomplished in WCT ≤ −54°C (**Table 2**). The lowest WCT of −69°C was experienced in the first oxygen-aided ascent of Everest in winter (**Table 4**).

The highest average WCT of −33 ± 5°C was recorded in non-winter ascents of K2 without oxygen. This was 7°C higher than in non-winter ascents of Everest without oxygen ($p < 0.001$) (**Table 3**).

Facial Frostbite Time

We noted 50 ascents (10th percentile) with an FFT ≤ 3 min among all 499 ascents without oxygen that we analyzed (**Table 2**). Most (90%) of these 50 ascents were in winter (**Table 2**). Three of five non-winter ascents with an FFT ≤ 3 min were undertaken outside the typical climbing season: Everest – 1, the latest October ascent; K2 – 2 in October, (**Table 2**). The lowest average FFT of -2 ± 2 min was recorded in wH&H-noO_2 ascents (**Table 3**). This was significantly shorter ($p < 0.01$) than in non-winter ascents of Everest and K2, and not statistically different from other oxygen-unaided winter ascents (**Table 3**). The shortest FFT of -4 min was experienced by four climbers in winter ascents of Kangchenjunga and Shisha Pangma (**Table 4**).

Of 29 oxygen-supported ascents of 8K peaks in winter, all Himalayan climbs except 1 were accomplished in FFT ≤ 3 min. The shortest FFT of -3 min was experienced in an oxygen-supported ascent of Everest in winter (**Table 4**).

The longest average FFT of 17 ± 7 min was recorded in non-winter ascents of K2 without oxygen, 8 min longer than for non-winter ascents of Everest without oxygen ($p < 0.001$) (**Table 3**).

Combined Weather Conditions

Seventeen (3%) of the 499 ascents without oxygen that we analyzed were accomplished in combined conditions of BP ≤ 346 hPa (median) and WCT ≤ −54°C (10th percentile (**Figure 4**). Unaided assents included 4 of Everest (1 – winter, 3 – non-winter), 3 of K2 (1 – winter, 2 –non-winter), 2 of wL&K-noO_2 and 8 of wH&H-noO_2 (**Table 4**, **Figure 4**). Of the 29 oxygen-supported ascents of 8K peaks most (26, 90%) were in these extreme conditions (**Figure 4**). Only 4 ascents of the 499 we analyzed were in combined conditions of BP ≤ 333 hPa and WCT ≤ −54°C, the 10th percentile for 8K ascents without oxygen. All 4 were on Everest (1 – winter, 3 out of regular season: 1 – late October, 2 – April).

Only 2 of the 499 ascents of 8K peaks were accomplished in the combined 10th percentile for all the conditions we calculated (BP ≤ 333 hPa, Temp ≤ −30°C, Wind ≥ 25 m·s^{-1}, WCT ≤ −54°C and FFT ≤ 3 min). Both climbs were the latest in the year of all ascents of Everest without oxygen (30 October and 22 December) (**Table 4**). Of 29 oxygen-supported ascents of 8K peaks in winter, 12 of 14 ascents of Everest were in these most extreme combined conditions (**Figure 4**).

We found a strong positive correlation of BP and Temp ($r \geq 0.7$; $p < 0.05$) in most of the ascents we analyzed: non-winter ascents of Everest and K2, winter ascents of low Karakoram and Himalayan 8K peaks, and oxygen-supported ascents of Everest in winter. We observed a fairly negative correlation of BP and Wind ($r \leq$ -0.3; $p < 0.05$) and a poor negative correlation of Temp and Wind ($r \leq$ -0.1; $p < 0.05$) in non-winter ascents of Everest and K2. There was no significant correlation between BP and Wind in all winter-ascent groups, and of Temp and Wind in most of the winter groups. Temp and Wind were linked only in winter ascents of Everest with oxygen, where we noted a moderate positive correlation ($r = 0.7$; $p < 0.05$).

DISCUSSION

Low BP, low Temp and high Wind are the main environmental factors that limit human performance and survival at high

TABLE 3 | Barometric pressure, ambient temperature, wind speed and thermal stress indices: WCT and FFT for the 499 ascents without oxygen support and 29 oxygen-supported ascents of 8K peaks we analyzed: number of ascents, means ± standard deviation (SD), coefficient of variation (CV%), minimum and maximum values.

Ascent group and number	BP [hPa]	Temp [°C]	Wind [m·s^{-1}]	WCT [°C]	FFT [min]
ME-noO_2 (210)	335 ± 2 (1%) min 329, max 340	−25 ± 3 (12%) min −33, max −19	12 ± 5 (43%) min 2, max 27	−40 ± 5 (13%) min −57, max −26	9 ± 4 (39%) min 2, max 22
K2-noO_2 (213)	349 ± 2 (1%) min 336, max 354	−19 ± 3 (15%) min −36, max −14	12 ± 5 (40%) min 1, max 26	−33 ± 5(16%) min −60, max -19	17 ± 7 (39%) min 2, max 38
wME-noO_2 (1)	331	−33	26	−59	2
wK2-noO_2 (1)	334	−40	5	−55	5
wH&H-noO_2 (10)	342 ± 4 (1%) min 337, max 348	−32 ± 4 (13%) min −37, max −27	41 ± 9 (21%) min 22, max 48	−60 ± 6 (10%) min −65, max −51	−2 ± 2 (109%) min −4, max 2
wL&H-noO_2 (50)	362 ± 5 (1%) min 348, max 367	−29 ± 4 (14%) min −40, max −25	26 ± 10 (38%) min 12, max 46	−52 ± 6 (11%) min −64, max −41	2 ± 3 (141%) min −4, max 9
wL&K-noO_2 (14)	358 ± 7 (2%) min 346, max 364	−38 ± 5 (12%) min −45, max −34	12 ± 4 (35%) min 7, max 18	−58 ± 6 (11%) min −67, max -51	4 ± 1 (24%) min 3, max 5
w8000-yesO_2 (29)	333 ± 10 (3%) min 320, max 362	−36 ± 4 (11%) min −41, max −27	24 ± 14 (59%) min 5, max 46	−60 ± 6 (10%) min −69, max −47	2 ± 3 (166%) min -3, max 5
wME-yesO_2 (14)	326 ± 3 (1%) min 320, max 331	−36 ± 4 (10%) min −41, max −31	36 ± 7 (18%) min 24, max 46	−64 ± 5 (7%) min −69, max -57	−1 ± 1 (275%) min −3, max 2
wK2-yesO_2 (9)	334	−40	5	−55	5

ME-noO_2, Everest non-oxygen non-winter ascents; K2-noO_2, K2 non-oxygen non-winter ascents; wME-noO_2, Everest non-oxygen winter ascents; wK2-noO_2, K2 non-oxygen winter ascents; wH&H-noO_2, high Himalayan 8K peaks non-oxygen winter ascents; wL&H-noO_2, low Himalayan 8K peaks non-oxygen winter ascents; wL&K-noO_2, low Karakoram 8K peaks non-oxygen winter ascents; w8000-yesO_2, 8K peaks oxygen-supported winter ascents; wME-yesO_2, Everest winter oxygen-supported ascents; wK2-yesO_2, K2 winter oxygen-supported ascents.
BP, barometric pressure; Temp, temperature; Wind, wind speed; WCT, wind chill equivalent temperature; FFT, facial frostbite time.

altitude (West and Wagner, 1980; West et al., 1983a, 2007b; Huey et al., 2001; Huey and Eguskitza, 2001; Moore and Semple, 2012; Moore et al., 2012). We aimed to evaluate weather extremes in the Death Zone and to determine the most severe environmental conditions that mountaineers have experienced. No *in situ* data were available for the summits and dates we analyzed, so we derived our calculations from ERA5, a state-of-the-art meteorological database. Previously published assessments of weather on the 8K peaks were calculated from far less precise radiosonde data (West et al., 1983b) and from US National Centers for Environmental Prediction (NCEP) Reanalysis data (Moore and Semple, 2011). The NCEP Reanalysis is one of the earliest meteorological datasets with a 10-times-lower spatial resolution of 2.5 × 2.5 degrees of latitude and longitude (Kalnay et al., 1996) compared with the 0.25 × 0.25 degree resolution of the ERA5 dataset (Hersbach et al., 2020). Everest's summit BP calculated from ERA5 and corrected with *in situ* measurements from automatic weather stations installed on the mountain's South Col (7945 m) and Balcony (8430 m) as described by Matthews et al. (2020b) differed by about 1 hPa from results based on ERA5 alone as reported in our earlier article (Szymczak et al., 2021).

Barometric Pressure

Our finding that the lowest BP was experienced in winter ascents of Everest accorded with previous observations that higher altitudes and colder seasons lead to lower BP (Brunt, 1952; West et al., 1983b, 2007c; West, 1996; Whiteman, 2000). The most extreme mean BP of 326 ± 3 hPa that we recorded was experienced in oxygen-aided ascents of Everest in winter. Most (86%) of those ascents were in December, so the similarity of the calculated mean BP to Everest summit's average December values of 326 hPa (Matthews et al., 2020b) and 327 hPa (Szymczak et al., 2021) could be expected. However, the lowest BP recorded in our study of 320 hPa, experienced with oxygen support on the summit of Everest in February, was below the summit's previously reported February mean of 323 hPa (Matthews et al., 2020b) and 324 hPa (Szymczak et al., 2021). The lowest BP of 334 hPa experienced on K2's summit in January was also far from its average in January of 325 hPa (Szymczak et al., 2021). Similarly the BP of 331 hPa experienced in the only ascent of Everest without oxygen in December was higher than the peak's December's average. The difference we noted between the BP experienced by successful climbers and the monthly midwinter mean BP presented in other works (Matthews et al., 2020b; Szymczak et al., 2021) corresponded with the high daily variability recorded for BP in winter on Everest (Matthews et al., 2020b). Therefore the routine use of barometric pressure forecasts, as suggested by Moore and Semple (2011, 2012), would enable climbers to choose the most favorable summit-bid BP window and should be implemented, especially for winter expeditions.

Supplemental use of oxygen reduces the risk of high-altitude deterioration and the death of mountaineers at extreme altitudes (West, 1998; Huey and Eguskitza, 2000, 2001; Huey et al., 2001). One in 34 climbers using supplemental oxygen dies in descents from Everest compared with one in 12 without oxygen support on Everest and one in five on K2 (Huey and Eguskitza, 2000). Our result showing that BP of 320 hPa,

TABLE 4 | Mountaineers' survival records.

	Ascent group	Date	Names	BP [hPa]	Temp [°C]	Wind [ms^{-1}]	WCT [°C]	FFT [min]
1	ME-noO_2	1985.04.29	Ang Rita Sherpa	**329**	−33	7	−48	6
2	ME-noO_2	1984.04.20	Christo Ivanov Prodanov†	**329**	−31	21	−54	3
3	wL&K-noO_2 (NP)	2018.01.25	T. Mackiewicz†, E. Revol	346	**−45**	10	**-67**	3
4	wL&K-noO_2 (GI)	2012.03.09	A. Bielecki, J. Gołąb	348	**−44**	10	**−66**	3
5	wH&H-noO_2 (KG)	1986.01.11	K. Wielicki, J. Kukuczka	337	−28	**48**	−56	**−4**
6	wL&H-noO_2 (SP)	2005.01.14	P. Morawski, S. Moro	366	−28	**46**	−55	**−4**
7	wH&H-noO_2 (KS)	2012.02.15	P. Kunz, K. Chhiri Lama, L. Ongya Sherpa, N. Wangdi Sherpa, T. Dorje Sherpa	342	−35	**46**	−65	−3
8	wME-noO_2	1987.12.22	Ang Rita Sherpa	331	−33	26	−59	2
9	ME-noO_2	1985.10.30	N. Yamada	331	−32	27	−57	2
10	K2-noO_2	2021.01.16	N. Purja	334	−40	5	−55	5
11	K2-noO_2	2007.10.02	D. Urubko, S. Samoilov	336	−36	22	−60	2
12	wH&H-noO_2 (LH)	1988.12.31	K. Wielicki	338	−37	22	−63	2
13	wME-yesO_2	1980.02.17	L. Cichy, K. Wielicki	320	−41	24	−69	1
14	wME-yesO_2	1982.12.27	Y. Kato†	328	−31	46	−59	−3

Numbers in bold represent the record value.
Mountaineer survival records without supplemental oxygen:
Barometric pressure (BP) of 329 hPa: 1, 2.
Air temperature (Temp) ≤ −44°C: 3, 4.
Wind speed (Wind) ≥ 46 m·s^{-1}: 5, 6, 7.
Wind chill temperature (WCT) ≤ −66°C: 3, 4.
Facial frostbite time (FFT) of -4 min: 5, 6.
Combined conditions: BP ≤ 333 hPa, Temp ≤ –30°C, Wind ≥ 25 m·s^{-1}, WCT ≤ –54°C and FFF ≤ 3 min: 8, 9.
Combined conditions: BP ≤ 346 hPa, WCT ≤ –54°C: 1, 2, 3, 5, 7, 8, 9, 10, 11, 12.
Mountaineer survival records with supplemental oxygen: the most extreme combined conditions: 13, 14.
ME-noO_2, Everest non-oxygen non-winter ascents; K2-noO_2, K2 non-oxygen non-winter ascents; wME-noO_2, Everest non-oxygen winter ascents; wK2-noO_2, K2 non-oxygen winter ascents; wH&H-noO_2, high Himalayan 8K peaks non-oxygen winter ascents; wL&H-noO_2, low Himalayan 8K peaks non-oxygen winter ascents; wL&K-noO_2, low Karakoram 8K peaks non-oxygen winter ascents; w8000-yesO_2, 8K peaks oxygen-supported winter ascents; wME-yesO_2, Everest winter oxygen-supported ascents; NG, Nanga Parbat, GI, Gasherbrum I, KG, Kangchenjunga, SP, Shisha Pangma, KS, Kangchenjunga South, LH, Lhotse, †died during descent; BP, barometric pressure; Temp, temperature; Wind, wind speed; WCT, wind chill equivalent temperature; FFT, facial frostbite time.

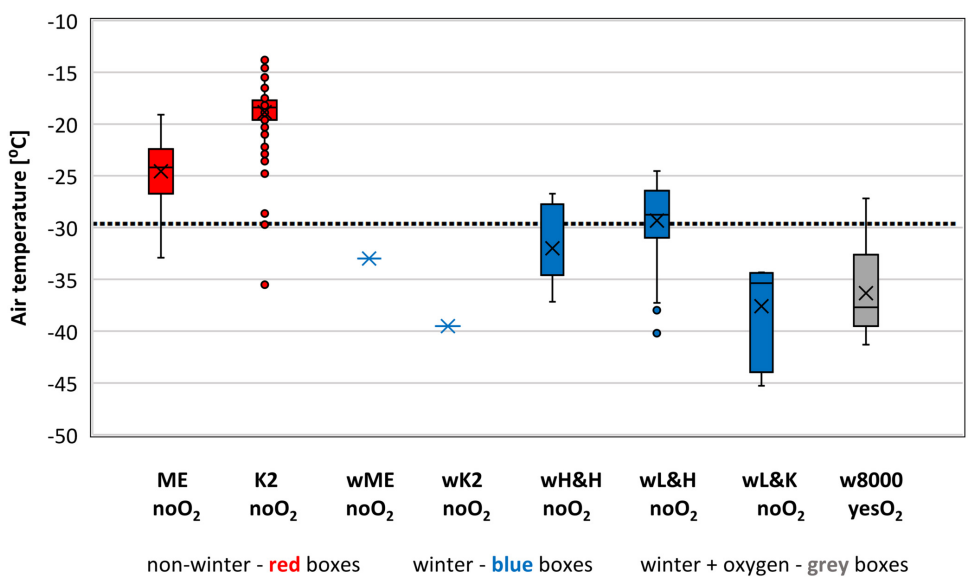

FIGURE 2 | Air temperature experienced on the summits we analyzed. Sample size – 499 ascents without supplemental oxygen and 29 with supplemental oxygen (1978–2021). Bars extend to the maximum and minimum BP, boxes span the 25th–75th percentiles, crosses inside the boxes mark mean values, horizontal lines inside the boxes represents median values, dots mark outliers, dotted line represents 10th percentile value of – 30°C for all 499 ascents without supplemental oxygen.

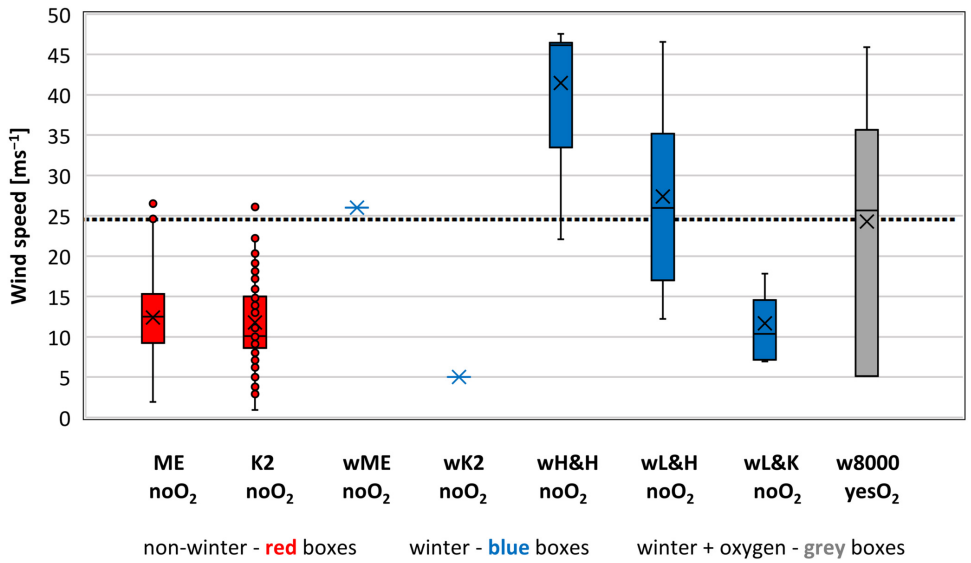

FIGURE 3 | Wind speed experienced on the summits we analyzed. Sample size – 499 ascents without supplemental oxygen and 29 with supplemental oxygen (1978–2021). Bars extend to the maximum and minimum wind speed, boxes span the 25th–75th percentiles, crosses inside the boxes mark mean values, horizontal lines inside the boxes represents median values, dots mark outliers, dotted line represents 90th percentile value of 25 $m{\cdot}s^{-1}$ for all 499 ascents without supplemental oxygen.

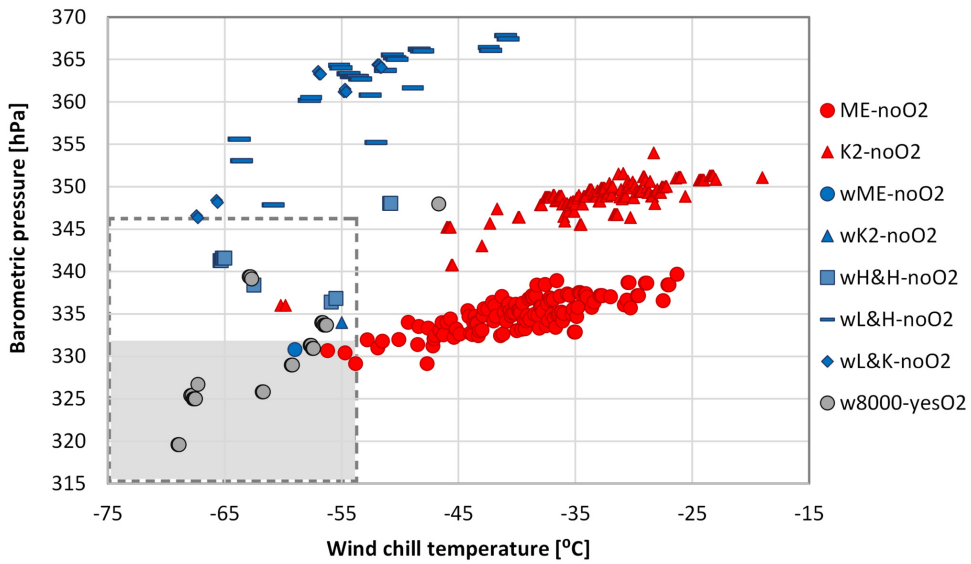

FIGURE 4 | Combined barometric pressure (BP) and wind chill temperature (WCT). Sample size – 499 ascents without supplemental oxygen and 29 with oxygen support (1978–2021). Dashed line box covers ascents in combined conditions of BP ≤ 346 hPa and WCT ≤ −54°C, gray area represents ascents in combined conditions of BP ≤ 333 hPa and WCT ≤ −54°C.

the lowest for oxygen-aided ascents, was significantly lower than the BP of 329 hPa for climbs without oxygen, strongly suggests that oxygen use enables survival of extremely low BP. A BP of 315 hPa was the lowest a human has ever survived without supplemental oxygen. It was experienced for about 20 min in a hypobaric chamber by four volunteers in the Operation Everest I study (Houston and Riley, 1947) where the BP at Everest's summit was overestimated by using a simplified formula for aviation standard atmosphere (ICAO, 1964). Considering that humans can therefore survive a BP of 315 hPa, and that Everest and K2's average midwinter BP is below 326 hPa (Matthews et al., 2020b; Szymczak et al., 2021) at the summits, with the rising interest in winter climbing at extreme altitudes (Benavides, 2021a) we expect that a new BP survival record will be established in future winter expeditions without oxygen on those peaks.

Thermal Stress

We noted that the most severe Temp in the Death Zone was experienced in winter ascents of 8K peaks in the Karakoram range. Latitude, altitude, and season mainly determine air temperature (Whiteman, 2000). Our results showed that the average Temp in winter ascents of low Karakoram 8K peaks was lower than in winter ascents of low Himalayan 8K peaks as well as high Himalayan 8K peaks that are 400 m higher. This result suggests that in winter Karakoram's latitude 8° further north has a greater effect on Temp than the 400 m altitude difference between 8K peaks. This result accords with the lower average midwinter Temp on K2's summit, the highest in the Karakoram range, than on the 237 m higher Everest further south (Szymczak et al., 2021).

The highest Wind recorded in our study occurred in winter Himalayan ascents. Wind was significantly more severe in winter ascents in the Himalayas than on Karakoram. Our calculation tallies with trends of the global jet stream analyzed by other authors (Moore and Semple, 2004; Archer and Caldeira, 2008; Pena-Ortiz et al., 2013) and the higher average midwinter Wind recorded on Everest than on K2 (Szymczak et al., 2021). The Northern Hemisphere jet stream splits over the Atlantic Ocean into the Subtropical jet stream (STJ) and the Polar Front jet stream (PFJ) (Pena-Ortiz et al., 2013). At the longitude of the Himalayas and Karakoram the STJ flows between 20° N and 35° N, above the Himalayas; the PFJ flows between 40° N and 70° N (Pena-Ortiz et al., 2013). This leaves a 5° gap between 35° N and 40° N that lies over Karakoram, which would explain the significantly lower Wind in winter ascents of Karakoram 8K peaks compared with the Himalayas.

We estimated Wind at the altitude of 8K peaks, where there is no natural wind protection by rock formations or slopes. However, wind direction and mountain topography should be considered when wind exposure is analyzed for different routes up a mountain. A route on the windward side of a mountain will expose a climber to more extreme Wind than a route on the leeward side. Jet streams are westerly winds (Pena-Ortiz et al., 2013), therefore routes on the east walls of 8K peaks are less exposed to Wind, which should be considered by winter climbers.

The combined effects of Temp and Wind can be gauged with thermal stress indices such as WCT (Osczevski and Bluestein, 2005) and FFT (Tikuisis and Osczevski, 2003). Our results show that the lowest WCT and the shortest FFT were encountered in winter ascents of 8K peaks. Our results for WCT and FFT in ascents of Everest were similar to the WCT of −45°C and the FFT of 7 min in summer and the WCT of −65°C and the FFT < 1 min in winter assessed with the NCEP Reanalysis (Moore and Semple, 2011). Wind chill temperature depends more on ambient temperature than on wind (Osczevski and Bluestein, 2005), so the extremely low temperatures of Karakoram's Death Zone in winter strongly influenced its WCT. The WCT index is limited to wind speeds below 27 $m{\cdot}s^{-1}$ (Osczevski and Bluestein, 2005), so the effect of extremely high Wind in the Himalayan winter is likely underestimated. The skin's rate of cooling is more sensitive to wind speed than to air temperature (Tikuisis and Osczevski, 2003), so higher winter Wind in the Himalayas explained the lowest average FFT that we estimated for the highest 8K peaks in this range. FFTs below 0 min signal that the extreme combined Temp and Wind in the Death Zone in winter might fall beyond the scope of the FFT index. Reduced air density at high altitude decreases convective heat loss (Huey et al., 2001). Huey et al. calculated that with the 60% decline of air density from sea level to 9000 m the convective heat loss at 9000 m decreased by about 45% compared with the loss at sea level in the same conditions at a Temp of −33.5°C and wind speeds up to 28 $m{\cdot}s^{-1}$ (Huey et al., 2001). The standard equations for WCT and FFT (Tikuisis and Osczevski, 2003; Osczevski and Bluestein, 2005) applied in our study and others (Moore and Semple, 2011) presume the sea-level densities of air and therefore likely overestimate heat loss at altitude. Considering the estimates of Huey et al. (2001), our results for WCT and FFT should be modified to show more realistic values of heat loss at high altitude. The validation of WCT and FFT for the high-altitude low-BP environment, or a more complete human heat balance index such as the Universal Thermal Climate Index (UTCI) (Blazejczyk et al., 2012), would benefit future high-altitude research and provide a more realistic assessment of thermal stress.

Combined Weather Extremes

Our results showed that the most extreme combined conditions of BP, Temp and Wind were experienced in winter and off-season ascents of Everest. This is explained by Everest's extreme altitude, which determines its lowest BP, its low winter and off-season Temp, and high Wind because of the STJ's winter activity over the Himalayas (Whiteman, 2000; Pena-Ortiz et al., 2013).

We found a strong positive correlation between BP and Temp in most of the ascents we analyzed, which agrees with similar observations by others of BP and Temp for the summits of Everest (Moore and Semple, 2011; Matthews et al., 2020b; Szymczak et al., 2021) and K2 (Szymczak et al., 2021). This also corresponds with the interrelationship of BP and Temp described by the ideal gas law (West, 1996). We noted a moderate positive correlation of Wind and Temp and no correlation of Wind and BP in winter ascents of Everest. Our results suggested that a climber in winter should expect lower Temp in a lower Wind window on Everest. Observations by Matthews et al. (2020b) that extreme low-pressure periods on Everest's summit in winter were not associated with strong winter winds would suggest that low-pressure events might occur in low Wind windows. Therefore climbers, who usually employ wind speed to identify suitable climbing weather windows (Peplow, 2004), should also focus on barometric pressure forecasts to increase their chances of success and survival, especially in the winter season.

The interrelationship of BP and Temp with Wind was more favorable in non-winter ascents of Everest and K2. We recorded a fairly negative correlation of BP and Wind in non-winter ascents of Everest and K2, which means that in a low Wind window climbers should also expect favorably higher BP. BP's positive correlation with Temp and negative correlation with Wind recorded for non-winter ascents of Everest and K2 supported the proposal by Moore and Semple (2011, 2012) for Everest climbers that summit BP can act as a simple and easily implementable predictor of the risk of hypothermia and frostbite. Huey et al. (2020) suggested that improved weather forecasting was one reason for the increased likelihood of summiting Everest and the

lower likelihood of mountaineers dying over the past 15 years compared with 1990–2005. Therefore monitoring of Temp and BP in the base camp and at higher camps, as during the first Everest expeditions almost 100 years ago (Moore et al., 2010), should become standard practice to correct more general forecasts for high-altitude expeditions.

We found that environmental conditions in the Death Zone were more severe in non-winter ascents of Everest than of K2. BP was significantly lower on Everest, which might be explained by Everest's 237 m higher altitude and the less favorable month of its climbing season. The highest BP and Temp on both peaks were recorded in July and August (Szymczak et al., 2021). Due to summer snowfalls caused by the monsoons (Moore, 2004; Moore and Semple, 2006, 2011), the main climbing season on Everest is mostly restricted to May (Matthews et al., 2020b; Salisbury and Hawley, 2020), which has lower BP and Temp than in July and August. The average BP in May on Everest's summit was 6 hPa lower than in July (Matthews et al., 2020b). K2's climbing season is in July and August (8000ers, 2021), the months with the highest BP and Temp (Szymczak et al., 2021). We found no difference in Wind for non-winter ascents of Everest and K2. Those that climb Everest and K2 in the normal season should be aware that environmental conditions in Everest's Death Zone are more severe than K2's. The higher death toll experienced on K2 (Huey and Eguskitza, 2000) is likely explained by the technically more difficult route to the summit or yet unexamined weather characteristics. K2's midwinter summit BP is similar to Everest's, but Temp and Wind are lower (Szymczak et al., 2021).

Deaths on Descent

Our analyses of 528 ascents recorded 48 (9%) that ended with the death of a climber in the descent. Most of those deaths (26) occurred on K2 in the climbing season. Three climbers reached 8K summits in the most extreme weather conditions we recorded, but died in their descent: one climber who reached Everest's summit with BP of 329 hPa, one climber who experienced Temp of −44°C and WCT of −67°C in winter on the summit of Nanga Parbat, and one mountaineer with oxygen support who reached Everest's summit in winter with Wind of 46 $m{\cdot}s^{-1}$. We did not analyze the causes of death. Only deaths on Everest have been thoroughly analyzed (Firth et al., 2008), so further research is needed for the other 8K peaks. Firth et al. (2008) observed that severe weather is the main factor responsible for about 25% of fatalities above 7000 m on Everest. Hypothermia is responsible for 16% of all deaths on Denali (McIntosh et al., 2008). Moore and Semple (2012) presented the cases of two climbers who had to bivouac above 8500 m on descent from Everest in extreme environmental conditions: the first experienced BP of 333 hPa, Temp of −31°C and Wind of 15 $m{\cdot}s^{-1}$; the second experienced BP of 338 hPa, Temp −23°C, and Wind 2 $m{\cdot}s^{-1}$. The first climber died but the second survived. Authors have attributed this death to the higher hypoxic and hypothermic stress he experienced (Moore and Semple, 2012).

The risk of hypothermia rises to critical levels when a fatigued mountaineer stops during descent or is forced to bivouac. The lower exertion a climber requires on descent reduces heat production, which falls precipitously if the climber stops (Ainslie and Reilly, 2003). Hypothermia *per se* increases fatigue because of a reduction in muscle strength and an increase in oxygen consumption for the same intensity of exercise (Hinde et al., 2017).

Tikuisis (1995) used a mathematical model to predict survival times under sedentary conditions in low Temp and high Wind. With maximum insulation of three loose layers, each 1 mm thick, the sedentary survival time is 18 h in combined Temp and Wind conditions close to the average recorded in our study for non-winter ascents of K2 (−20°C, Wind 14 $m{\cdot}s^{-1}$). The calculated survival time for typical non-winter Everest conditions (−25°C, Wind 14 $m{\cdot}s^{-1}$) is 12 h and for conditions similar to winter ascents of low Karakoram peaks (−40°C, Wind 14 $m{\cdot}s^{-1}$) survival time is only 6 h (Tikuisis, 1995). A climber forced to bivouac in winter Death Zone conditions has two to three times less survival time than in climbing season.

The level of insulation plays an important role in a climber's ability to survive thermal stress (Tikuisis, 1995). Havenith (2010) calculated that a climber without supplemental oxygen at an altitude close to that of Everest's summit and in non-winter conditions of Temp −25°C and Wind of 11 $m{\cdot}s^{-1}$, similar to those calculated in our study, needs clothing insulation of 4.5 clo to keep thermal balance. When Temp drops to −40°C, as we calculated for winter on Karakoram's 8K peaks, and Wind is 11 $m{\cdot}s^{-1}$, a climber should have insulation of 6.5 clo (Havenith, 2010). Modern high-altitude clothing provides insulation of about 5.5 clo (Havenith, 2010), which does not suffice for Death Zone conditions in winter. Given our findings of extremely low Temp and high Wind in winter ascents, those planning winter expeditions should precisely calculate the insulation properties of their climbing outfits, emergency survival bags and shelters. The choice of equipment should be based on research done for emergency medicine (Oliver et al., 2016; Haverkamp et al., 2018). Nevertheless, further research is needed for survival equipment that effectively counteracts or at least ameliorates thermal stress in the Death Zone.

Highlanders vs. Lowlanders

Our analysis of all ascents of Everest without supplemental oxygen showed that of the 64 ascents accomplished by highlanders 38% were in the 50 climbs experiencing the most severe BP. Only 18% of all 147 ascents by lowlanders were in this group. No highlanders died descending from Everest in these attempts, whereas on average every 13th successful ascent by lowlanders ended with one climber's death on the descent in these climbs. Firth et al. (2008) analyzed all ascents of Everest, with and without oxygen support, and reported that Sherpas died more than six times less frequently than other climbers on the descent from the summit. These authors concluded that the lower incidence of death among Sherpas might be due to the better acclimatization they achieve by spending more time in higher camps, by their higher chances for rescue because Sherpas work in groups, by selection to work at high altitude, and by a genetic adaptation for altitude (Firth et al., 2008).

We observed that highlanders endured worse BP than lowlanders in non-winter ascents of Everest without oxygen. Highlanders have made the only winter ascents of Everest and K2 without oxygen: Ang Rita Sherpa of Everest, and the Magar Nirmal Purja of K2. The exceptional Ang Rita Sherpa has reached the summit of Everest nine times without oxygen (Garrido et al., 2019). Three of those were in the five ascents experiencing the lowest BP ever survived by mountaineers. Nirmal Purja was the first and only person to climb all 14 of the 8K peaks within a year (Beaumont, 2021). The fact that winter ascents of Everest and K2 without oxygen have been accomplished only by highlanders and their significantly lower incidence of deaths in the Death Zone compared with lowlanders reported in our study and in others (Firth et al., 2008) suggest that highlanders possess a superior ability to survive the extreme weather at high altitude and to tolerate higher hypoxia. Tibetans have adapted to environmental hypoxia for 500 generations (Gilbert-Kawai et al., 2014). Highlanders' multitude of metabolic, physiological and biochemical adaptations to hypoxia are responsible for their performance at extreme altitude (Grocott and Levett, 2013; Gilbert-Kawai et al., 2014; Horscroft et al., 2017).

Reduced maximal oxygen uptake (VO_2max) due to extremely low BP and its detrimental effect on climbing speed are the main factors limiting ascents of the highest 8K peaks without oxygen (West, 1999; Matthews et al., 2020b). The extremely high average sea-level VO_2max of 67 $ml \cdot kg^{-1} \cdot min^{-1}$ of elite Sherpas reported by Garrido et al. (1997, 2019) compared with an average of 57 $ml \cdot kg^{-1} \cdot min^{-1}$ for lowland climbers analyzed in high-altitude scientific research (Pugh et al., 1964; West, 1983) might help explain the highlanders' higher performance and survival at extreme altitudes. Ang Rita Sherpa had VO_2max of 67 $ml \cdot kg^{-1} \cdot min^{-1}$ at the age of 53 (Garrido et al., 1997, 2019). High VO_2max at sea level should be an important factor in selecting climbers for winter expeditions to 8K peaks.

Limitations and Strengths

Our calculations of weather factors were not based on *in situ* measurements but on the ERA5 Reanalysis dataset, which has a spatial resolution of 0.25 degrees of latitude and longitude. When we calculated Wind we assumed there was no natural protection on the summits, such as rock formations or slopes. Yet mountain topography does affect climbers: a route on the windward side of a mountain would expose them to more extreme Wind than a route on the leeward side. Though we analyzed only successful ascents of 8K peaks, mountaineers might well have experienced more extreme conditions in expeditions that turned back because of weather breakdown before they reached the summit. Off-season attempts of 8K peaks lower than Everest and K2 might also subject climbers to more extreme weather conditions than those calculated in our study. The standard equations for WCT and FFT do not include the change in air density with altitude and therefore likely overestimate heat loss at altitude. Wind chill temperature and FFT should therefore be validated for the high-altitude low-BP environment, or a more complete human heat balance index needs to be devised.

This study provides the first complete assessment of the environmental factors that climbers have experienced in the most extreme successful ascents of 8K peaks. It develops the findings of our previous report (Szymczak et al., 2021), where we analyzed the weather extremes on the summits of Everest and K2 in the climbing and the winter season using monthly means between 1979 and 2019. This analysis extends the current knowledge of combined weather extremes in the Death Zone and should help future expeditions prepare for the environmental extremes they will encounter on 8K summits. Our study should also help climbers to interpret weather forecasts when planning a summit bid. This data will also help alpinist trainers, physiologists and researchers using environmental chambers. Knowledge of what to expect in the Death Zone in a particular season on a mountain range would help future mountaineers maximize their chances of reaching the summit while minimizing the risks of their attempts.

CONCLUSION AND RECOMMENDATIONS FOR MOUNTAINEERS

1. The most favorable Death Zone climatic conditions were experienced in the climbing season on the summit of K2 (July and August) and Everest (May and October), but climbing-season weather extremes in the Death Zone were more severe on Everest than on K2.
2. To find the most favorable climatic conditions, climbers planning ascents of 8K peaks without oxygen support should choose July and August for attempts of 8K peaks in Karakoram and May or October for ascents of Himalayan 8K peaks.
3. The most extreme combined environmental conditions of BP, Temp and Wind were encountered in winter and off-season ascents of Everest.
4. Climbers planning winter or off-season attempts of 8K peaks should expect worse BP, Temp and Wind in the Death Zone than in climbing season.
5. Extreme wind speed characterized winter ascents of all 8K Himalayan peaks, but severely low temperatures marked winter climbs in Karakoram.
6. Mountaineers should recognize the different characteristics of winter climatic conditions in the Death Zones of the Himalayas and Karakoram by preparing for extreme Wind in the Himalayas and severely low Temp in Karakoram.
7. Mountaineers using oxygen support survived more extreme conditions than climbers without oxygen.
8. Climbers planning winter exploration of 8K peaks should consider using oxygen support, especially when new and technically difficult routes are planned.
9. Sufficient oxygen sets should be available at the highest camps on 8K peaks for rescues during ascents and descents in the climbing and the winter seasons.
10. Expeditions to 8K peaks should regularly monitor at least BP and Temp in base and at higher camps, and also consult professional weather forecasts.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://www.himalayandatabase.com/; https://www.ecmwf.int/en/forecasts/datasets/browse-reanalysis-datasets; http://www.8000ers.com/cms/k2-general-info-192.html.

AUTHOR CONTRIBUTIONS

RS, MN, and MS: Conceptualization. RS and MM: Methodology, Validation, and Data Curation. RS, MM, and TG: Formal Analysis. RS: Investigation, Supervision, and Writing – Original Draft Preparation. RS and MS: Resources and Visualization. RS, MN, MS, MM, and TG: Writing – Review and Editing. MS: Project Administration. RS and MN: Funding Acquisition. All authors contributed to the article and approved the submitted version.

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Remote Ischemic Preconditioning Reduces Marathon-Induced Oxidative Stress and Decreases Liver and Heart Injury Markers in the Serum

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Clinical studies continue to provide evidence of organ protection by remote ischemic preconditioning (RIPC). However, there is lack of insight into impact of RIPC on exercise-induce changes in human organs' function. We here aimed to elucidate the effects of 10-day RIPC training on marathon-induced changes in the levels of serum markers of oxidative stress, and liver and heart damage. The study involved 18 male amateur runners taking part in a marathon. RIPC training was performed in the course of four cycles, by inflating and deflating a blood pressure cuff at 5-min intervals (RIPC group, $n = 10$); the control group underwent sham training ($n = 8$). The effects of RIPC on levels of oxidative stress, and liver and heart damage markers were investigated at rest after 10 consecutive days of training and after the marathon run. The 10-day RIPC training decreased the serum resting levels of C-reactive protein (CRP), alanine transaminase (ALT), γ-glutamyl transpeptidase (GGT), and malondialdehyde (MDA). After the marathon run, creatinine kinase MB (CK-MB), lactate dehydrogenase (LDH), cardiac troponin level (cTn), aspartate aminotransferase (AST), alkaline phosphatase (ALP), ALT, total bilirubin (BIL-T), and MDA levels were increased and arterial ketone body ratio (AKBR) levels were decreased in all participants. The changes were significantly diminished in the RIPC group compared with the control group. The GGT activity remained constant in the RIPC group but significantly increased in the control group after the marathon run. In conclusion, the study provides evidence for a protective effect of RIPC against liver and heart damage induced by strenuous exercise, such as the marathon.

Keywords: skeletal muscle damage, creatinine kinase MB, malondialdehyde, remote ischemic preconditioning, inflammation

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INTRODUCTION

Prolonged strenuous running, e.g., a marathon run, induces a rise in the concentrations and/or activity of biomarkers that reflect physiological stress of the skeletal muscle, liver, heart, and some other tissues (Banfi et al., 2012). Oxidative stress is one of the processes associated with tissue damage. It can be induced by exercise, especially by prolonged forms of exercise, such as the marathon and its more demanding variations (Gomez-Cabrera et al., 2006; Kawamura and Muraoka, 2018). Consequently, high-intensity or long-duration exercise can potentially lead to major changes in the markers of tissue damage that are commonly associated with pathological states (Smith et al., 2004). For instance, the concentration of serum cardiac troponin T (cTnT), one of the markers of heart muscle damage, increase after endurance events in up to 68%, and reach levels typically diagnostic of acute myocardial infarction (Fortescue et al., 2007; Eijsvogels et al., 2016). Similarly, after a long-distance run, liver injury biomarkers, such as aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), and lactate dehydrogenase (LDH) activities, and conjugated bilirubin, are elevated (Lippi et al., 2011; Shin et al., 2016). Changes in the concentration and/or activity of markers of heart and liver damage after a marathon run are transient and most return to the baseline after several days. While there is insufficient evidence to indicate any adverse effects of exercise on the heart (Kaleta-Duss et al., 2020) and liver (Lippi et al., 2011) of amateur marathon runners, further studies investigating the impact of strenuous exercise on the markers of heart and liver damage can give insight into the physiology of adaptation of these organs to a prolonged strenuous exercise.

Multiple studies aim to identify the optimal approach of reducing the impact of strenuous exercise on markers of tissue damage (for review see Kawamura and Muraoka, 2018). Currently, increasing attention is being focused on remote ischemic preconditioning (RIPC), which has been shown to be protective against ischemia-reperfusion injury (Tapuria et al., 2008) and other stressors, and can potentially increase sports performance (Caru et al., 2019). RIPC is a procedure, whereby brief cycles of limb ischemia and reperfusion are induced by inflating and deflating a blood pressure cuff. In this manner, skeletal muscle undergoes preconditioning and distal tissues are protected. RIPC reduces the increase of cardiac troponin levels (cTn) by 42% after coronary artery bypass (Venugopal et al., 2009), and by approximately 30% after valve replacement surgery (Cao et al., 2017). Similarly, it was shown that RIPC, when applied before liver resection, decreases the AST activity and bilirubin levels in comparison to control, not pre-conditioned, patients (Rakic et al., 2018; Liu et al., 2019; Wu et al., 2020). A similar trend was observed for RIPC and liver transplants (Robertson et al., 2017). Further, RIPC effectively reduces oxidative stress in some clinical scenarios, e.g., cardiopulmonary bypass and others (Chen et al., 2015; Arvola et al., 2016; Pinheiro et al., 2016) and improve peripheral endothelial function (Maxwell et al., 2019).

The reported outcomes indicate that RIPC might impact exercise-induce changes in the markers of heart and liver damage. However, to date, the data on this topic are limited and conflicting (El Messaoudi et al., 2013; Cocking et al., 2017). In the current study, we aimed to determine the effects of RIPC on the markers of heart and liver damage, and oxidative stress induced by a marathon run in amateur runners.

MATERIALS AND METHODS

Ethics Statement

The study was approved by the Bioethics Committee for Clinical Research at the Regional Medical Chamber in Gdańsk (decision number KB-24/16; Gdańsk, Poland) and was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from all participants before inclusion in the study. The participants were informed about the possibility of withdrawing their consent at any time and for any reason, and were informed about the study procedures, but not about the rationale and study aim, so as to keep them naive as to the potential effects of RIPC.

Experimental Overview

In the study, the effects of 10-day RIPC training on marathon-induced changes in the markers of oxidative stress, and liver and heart damage were evaluated. All participants were randomly assigned to two study groups undergoing either RIPC or sham-controlled intervention (RIPC vs. SHAM) for 10 consecutive days. During the first visit (pre-intervention) to laboratory, set early in the morning, basic anthropometric characteristics were measured (the subject's age, body composition, and height) and venous blood samples were drawn. On the following and subsequent days, either RIPC or SHAM procedure took place. Early in the morning 1 day after the last RIPC or SHAM training, blood samples were collected; the runners performed a marathon race on the same day. Blood samples were also collected immediately after, 24 h after, and 7 days after finishing the race.

Participants

Twenty-four male amateur runners were enrolled in the study: 12 in the RIPC group and 12 in the SHAM group. Each runner had an experience of a minimum five full marathon runs, with the completion time between 2 h 50 min and 3 h 20 min. For the study, each participant ran the 46th Dębno Marathon (Dębno, West Pomerania Province, Poland). The starting temperature on the day was 12.1°C, and the starting (and finishing) line was in the town of Dębno (40 m above sea level). The course is flat and allows the runners to achieve high running speed.

Two runners from the RIPC group and four from the SHAM group did not finish the race. Consequently, data for only 18 amateur runners were analyzed (RIPC, $n = 10$; SHAM, $n = 8$). The basic anthropometric characteristics of the groups and their performance are shown in **Table 1**. Before the experiment, a physician examined all the participants, and confirmed that they were healthy, with no history of known diseases that

TABLE 1 | Physical characteristics and the marathon run performance of the participants (mean ± SD).

Variable	Overall ($n = 19$)	RIPC ($n = 10$)	SHAM ($n = 9$)	RIPC vs. SHAM (p)
Age (year)	36.05 ± 3.25	36.70 ± 3.57	35.33 ± 2.66	0.39
Body mass (kg)	76.36 ± 7.16	72.60 ± 7.14	76.44 ± 2.66	0.16
Height (cm)	182.52 ± 3.11	182.60 ± 3.95	182.44 ± 1.77	0.91
Body mass index (kg/m^2)	22.91 ± 1.97	21.77 ± 1.60	22.96 ± 1.05	0.08
Average running speed (km/h)	11.85 ± 0.66	12.14 ± 0.57	11.57 ± 0.64	0.08
Average running time (min)	213.57 ± 12.76	208.9 ± 10.45	218 ± 13.32	0.09

RIPC, remote ischemic preconditioning training group; and SHAM, sham-controlled group.

may have affected the physical performance (examination – blood pressure, heart rate, ECG, and respiratory parameters). From the time of enrolment to the end of the study period, none of the runners reported intake of any medication or drugs, and refrained from alcohol, caffeine, guarana, theine, tea, and chocolate, as these substances may potentially influence exercise performance. Upon enrolment, the participants have adopted similar eating patterns, based on a randomized diet for their age group and physical intensity. Each participants completed survey aimed to define the training loads used during training period (divided into a periods of general preparation – 3 months and pre-start preparation – 2 months before the run; **Supplementary Material**).

RIPC Procedures

Each participant underwent 10 consecutive days (Thijssen et al., 2016) of either RIPC or SHAM conditioning before the marathon run. In both cases, the procedure was performed in the supine position, with bilateral arterial occlusion of both legs (Griffin et al., 2018; Mieszkowski et al., 2020). The occlusion cuff was positioned proximally around the thigh and inflated to 220 mmHg (to block the arterial inflow) or 20 mmHg (placebo effect) in the RIPC and SHAM groups, respectively (Paull and Van Guilder, 2019; Mieszkowski et al., 2020). Both procedures consisted of four sets of 5-min inflation, followed by 5-min deflation (Cocking et al., 2018).

The RIPC or SHAM procedure was performed at the same time (early morning) each day and under the control of color flow Doppler ultrasound (Edan DUS 60; Edan Instruments GmbH SonoTrax Basic, Langen, Germany) to ensure the full closure of the arterial inflow. All ultrasound procedures were performed according to the standards of the Polish Ultrasound Society, by a physician who had completed a training in ultrasound imaging. The participants had no knowledge of the group allocation and differences in the procedures.

Sample Collection and Inflammation Marker Determinations

The blood was collected five times: before and 24 h after the RIPC/SHAM training period (latter served also as before the marathon run measurement), immediately after, and 24 h and 7 days after the marathon race. Venous blood samples were collected into Sarstedt S-Monovette tubes (S-Monovette® Sarstedt AG&Co, Nümbrecht, Germany) containing a coagulant for blood analysis; into tubes without anticoagulant for serum separation (with coagulation accelerator); or in tubes containing EDTA for plasma isolation. Samples were centrifuged using standard laboratory methods, aliquoted, and frozen at −80°C until further analysis. The selected markers were analyzed according to the medical diagnostic procedures referenced by European Federation of Clinical Chemistry and Laboratory Medicine. EFLM by Synevo Labolatory at an accredited laboratory (Bydgoszcz, Poland; PN-EN ISO 15189) using a hematological analyzer, and immunoenzymatic and conductometric methods, as appropriate (Sysmex XS-1000i apparatus, Roche/Hitachi Cobas c. system using a Cobas c 501 analyzer, Thermo Scientific Multiscan GO Microplate Spectrophotometer produced by Fisher Scientific Finland).

The following were analyzed: (1) cardiovascular and cardiac muscle markers: creatinine kinase (CK), creatinine kinase MB (CK-MB), C-reactive protein (CRP), hemoglobin (HGB), LDH activity, myoglobin (MB), red blood cells (RBC), troponin T (TnT), and urea; (2) liver markers: albumin (ALB), alkaline phosphatase (ALP) activity, alanine transaminase (ALT) activity, AST activity, direct bilirubin (BIL-D), total bilirubin (BIL-T), GGT activity, globulin (GLB), and total protein (TP); and (3) oxidative stress markers: malondialdehyde (MDA), arterial ketone body ratio (AKBR), and conjugated dienes (CD).

Statistical Analysis

Descriptive statistics (the mean ± SD) were used for all measured variables. Two-way ANOVA with repeated measures (*group*: RIPC, SHAM × *training*: before, after) was used to investigate the difference between the effects of 10-day RIPC and SHAM training on the selected cardiac muscle, liver, and oxidative stress markers. Another two-way ANOVA with repeated measures (*group*: RIPC, SHAM; *marathon*: before, immediately after, and 24 h and 7 days after the marathon) was performed to investigate the impact of marathon running on the selected marker levels in relation to the preceding 10 days of RIPC training. In case of a significant interaction, Tukey's *post hoc* test was performed to assess differences in particular subgroups. In addition, the effect size was estimated by eta-squared statistics (η^2). Values equal to or greater than 0.01, 0.06, and 0.14 indicated a small, moderate, and large effect, respectively. All calculations and graphics were done in Statistica 12 software (StatSoft, Tulsa, OK, United States). Differences were considered statistically significant at $p \leq 0.05$. The required sample size was estimated by using GPower ver. 3.19.4 software (Faul et al., 2007).

The power analysis for interaction between analyzed factors in two-way ANOVA of repeated measures show the minimal total sample sized for the large effect size with power of 0.95 was equal to 16 subjects.

RESULTS

Effects of 10-Day RIPC Training on the Markers of Heart and Liver Damage and Oxidative Stress

The effects of 10-day RIPC training on the biomarkers of heart and liver damage, and markers of oxidative stress at rest are shown in **Table 2**.

Two-way ANOVA revealed that the levels of CK, CK-MB, LDH activity, MB, urea, ALP activity, BIL-T, BIL-D, AST activity, and CD decreased after 10 days of training irrespective of RIPC. By contrast, AKBR, ALB, and RBC levels increased after 10 days in both groups. The effect of RIPC training was only noted for CRP levels, and ALT and GGT activity, wherein the resting values of these markers decreased and those in the SHAM group remained unchanged. However, only the changes in GGT activity levels were significantly different between the groups. Further, while MDA levels were reduced in both groups, the RIPC training led to a significantly greater reduction in the levels of this marker than SHAM training. Of note, while the levels of most of the investigated markers did not differ between the groups before the intervention, the pre-training AKBR and CD levels were significantly higher and lower, respectively, in the RIPC group than those in the SHAM group. These differences were maintained after 10 days of training.

Effect of 10-Day RIPC Training on Changes in Marathon-Induced Markers of Cardiovascular System and Heart Damage

Changes in the levels of markers of heart damage following the marathon run in relation to RIPC training are presented in **Figure 1**.

The results of two-way ANOVA are shown in **Table 3**. The levels of the following markers increased in both groups immediately after the marathon run: CK, CK-MB, CRP, LDH activity, MB, TnT, and urea. The increase persisted up to 24 h after the run, except for the TnT and LDH activity levels, which returned to the approximate resting levels a day after the run. Further, the RBC and HGB levels decreased immediately after the marathon run and the reduction persisted for up to 7 days after the run. The effect of the RIPC training on TnT, CK-MB, and LDH activity levels was apparent immediately after the marathon run, in that

TABLE 2 | Changes in cardiovascular and muscle damage, hepatic and oxidative stress associated biomarkers induced by 10-day remote ischemic preconditioning training.

Biomarkers	Variable	RIPC group		SHAM group	
		Pre	Post	Pre	Post
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Cardiovascular and muscle damage	BUN (mmol/L)	5.79 ± 0.79	5.37 ± 1.04*	6.05 ± 0.69	5.63 ± 0.98*
	CK (U/L)	107.70 ± 27.68	80.30 ± 35.24*	115.42 ± 15.39	93.87 ± 17.92*
	CK-MB (ng/ml)	3.71 ± 0.54	2.95 ± 1.04*	3.77 ± 0.31	3.47 ± 0.43*
	CRP (mg/L)	2.92 ± 0.72	1.97 ± 0.92*	2.71 ± 0.65	3.03 ± 1.11
	HGB (g/dl)	15.29 ± 0.83	15.42 ± 0.80	14.74 ± 0.57	14.85 ± 0.60
	LDH (U/L)	263.08 ± 39.20	249.40 ± 43.13*	266.96 ± 29.91	237.26 ± 33.92*
	MB (ng/ml)	95.20 ± 20.32	71.80 ± 12.60*	94.65 ± 7.35	57.79 ± 16.08*
	RBC ($\times 10^6$/μl)	4.81 ± 0.28	4.95 ± 0.28*	4.53 ± 0.27	4.75 ± 0.32*
	TnT (ng/ml)	0.51 ± 0.50	0.40 ± 0.69	0.70 ± 0.45	0.49 ± 0.17
Hepatic	ALB (g/L)	41.16 ± 0.78	41.55 ± 0.88*	43.21 ± 1.17	43.30 ± 0.95*
	ALP (U/L)	132.44 ± 2.42	129.48 ± 1.96*	135.22 ± 6.67	131.96 ± 4.98*
	ALT (U/L)	29.00 ± 3.05	26.20 ± 2.89*	29.79 ± 2.70	29.03 ± 4.03
	AST (U/L)	28.61 ± 6.04	27.30 ± 6.64*	31.39 ± 2.29	29.34 ± 4.56*
	BIL-D (μmol/L)	2.67 ± 0.21	2.52 ± 0.20*	2.93 ± 0.43	2.72 ± 0.21*
	BIL-T (μmol/L)	12.43 ± 0.43	11.65 ± 0.57*	12.42 ± 0.59	11.42 ± 0.59*
	GGT (U/L)	44.00 ± 8.08	38.30 ± 7.68*#	53.12 ± 6.10	51.14 ± 6.21
	GLB (g/L)	29.73 ± 1.44	29.45 ± 1.35	28.87 ± 0.69	28.60 ± 0.86
	TP (g/L)	71.22 ± 1.02	71.59 ± 0.77	72.11 ± 0.87	71.99 ± 0.84
Oxidative stress	AKBR	0.71 ± 0.06#	0.90 ± 0.06*#	0.67 ± 0.05	0.80 ± 0.05*
	CD (abs/ml)	15.58 ± 1.88#	9.82 ± 2.36*#	17.23 ± 1.70	12.98 ± 0.88*
	MDA (μmol/L)	0.42 ± 0.18	0.07 ± 0.05*#	0.45 ± 0.10	0.25 ± 0.06*

*RIPC, 10 days of remote ischemic preconditioning; SHAM, 10 days of sham controlled intervention; BUN, blood urea nitrogen; CK, creatine kinase; CK-MB, creatinine kinase MB; CRP, c-reactive protein; HGB, hemoglobin, LDH, lactate dehydrogenase; MB, myoglobin; RBC, red blood cells; TnT, troponin T; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BIL-D, direct bilirubin; BIL-T, total bilirubin; GGT, γ-glutamyl transpeptidase; GLB, globulin; TP, total protein; MDA, malondialdehyde; AKBR, arterial ketone index; and CD, conjugated dienes.*Significant difference vs. pre at $p < 0.01$; #Significant difference vs. SHAM at $p < 0.01$.*

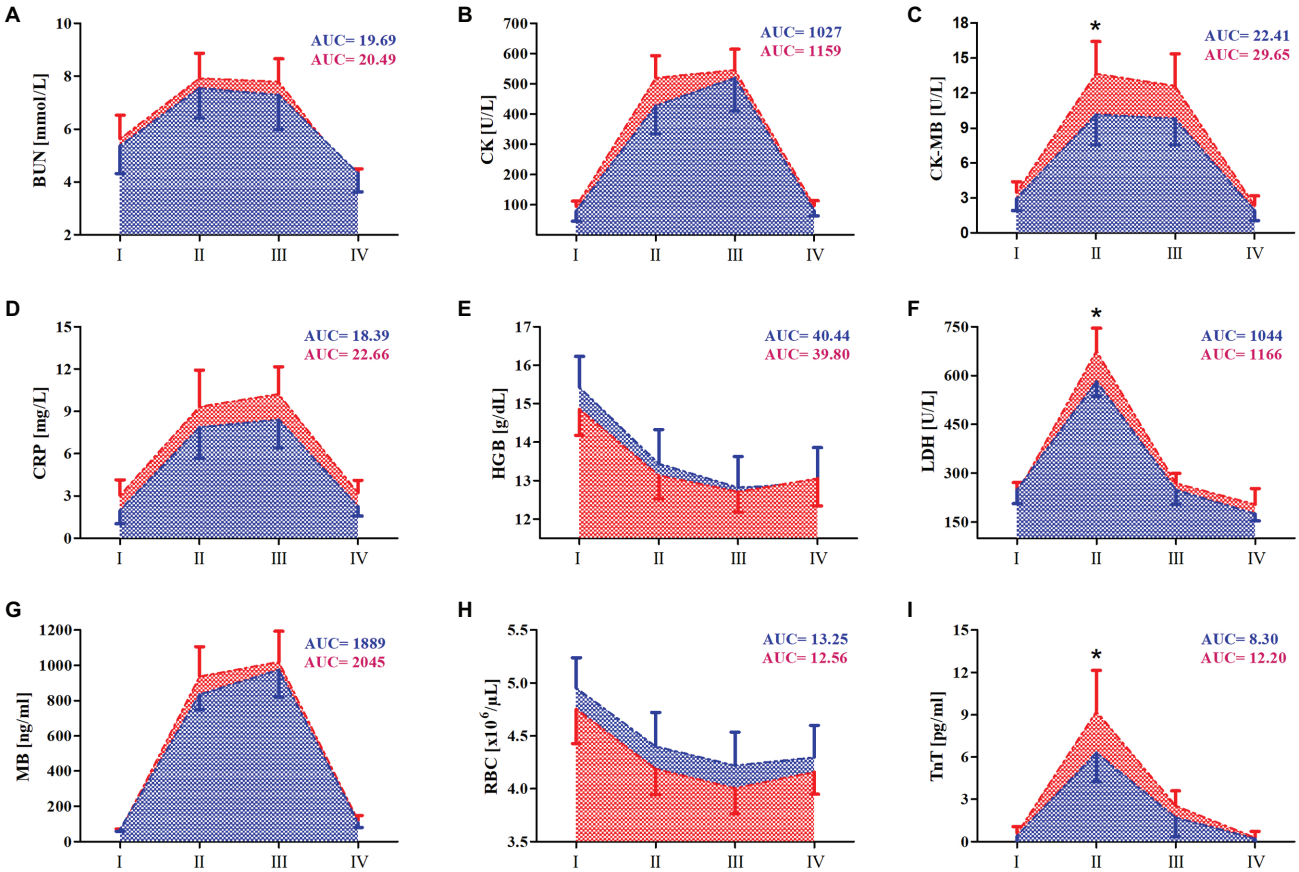

FIGURE 1 | Change in serum of selected hearth damage and cardiovascular markers after marathon run. **(A)** Blood urea nitrogen (BUN), **(B)** creatinine kinase (CK), **(C)** creatinine kinase MB (CK-MB), **(D)** C-reactive protein (CRP), **(E)** hemoglobin (HGB), **(F)** lactate dehydrogenase (LDH), **(G)** myoglobin (MB), **(H)** red blood cells (RBCs), and **(I)** troponin T (TnT). Blue color – a group after 10 days remote ischemic preconditioning training (RIPC), red color – a group after sham-controlled intervention (SHAM), AUC, area under curve, I – before the marathon run (baseline), II – immediately after marathon run, III – 24 h, and IV – 7 days after marathon. *Significant difference vs. RIPC at $p < 0.01$.

RIPC attenuated the increase of the levels of heat damage markers (**Figure 1**).

Effect of 10-Day RIPC Training on Changes in Marathon-Induced Markers of Liver Damage

The effects of RIPC training on the markers of liver damage before and after the marathon run are shown in **Figure 2**. The results of two-way ANOVA are shown in **Table 4**. ALP, BIL-D, BIL-T, and AST activity levels increased in both groups immediately after the run. In the RIPC group, the increase of ALP and AST activity, and BIL-T levels was smaller than that in the SHAM group. For ALT and GGT activity, after the marathon run, these markers only increased in the SHAM group and they remained at resting level in the RIPC group. Of note, GGT activity in the SHAM group was elevated before the marathon run and decreased to below the resting level 7 days after the run. By contrast, 24 h after the run, a decrease in ALB and TP levels was observed in both groups. The decrease was more pronounced in the RIPC group than in the SHAM group. Further, TP levels returned to resting values within next 7 days in the RIPC group but not in the SHAM group. Finally, compared with the RIPC group, GLB levels decreased immediately after and 7 days after the run in the SHAM group.

Effect of 10-Day RIPC Training on Changes in Marathon-Induced Markers of Oxidative Stress

To evaluate the effect of RIPC training on free radical generation in response to marathon run, three markers of oxidative stress were determined in the athlete serum: AKBR, MDA, and CD (**Figure 3**). The results of two-way ANOVA are shown in **Table 5**.

Immediately after and 24 h after the marathon run, the CD and MDA levels increased in both groups. The increase of MDA levels was smaller in the RIPC group than that in the SHAM group. Further, the CD levels were significantly lower in the RIPC group than in the SHAM group at all time points measured, showing no interaction with the marathon run. In turn, the AKBR levels decreased immediately after and 24 h after the marathon run in both groups. The reduction of AKBR levels was more pronounced in the SHAM group than in the RIPC group; however, the resting levels of this marker were lower in the SHAM group than those in the RIPC group.

TABLE 3 | Two-way (two groups × four repeated measurements) ANOVA tests for the cardiovascular and muscle damage markers induced by marathon run in RIPC and SHAM groups.

Variable	Effect	*F*	df	*p*	Effect size (η^2)	*Post hoc* outcome
BUN	Group	0.23	1, 16	0.63	0.01	
	Marathon	115.28	3, 48	0.01*	0.87	II, III > I, IV; IV < I, II, III
	Group × Marathon	1.73	3, 48	0.17	0.09	
CK	Group	1.53	1, 16	0.23	0.08	
	Marathon	203.05	3, 48	0.01**	0.92	III > I, II, IV; II > I, IV
	Group × Marathon	1.22	3, 48	0.31	0.07	
CK-MB	Group	6.07	1, 16	0.02*	0.27	RIPC < SHAM
	Marathon	281.30	3, 48	0.01**	0.94	II, III > I, IV
	Group × Marathon	6.56	3, 48	0.01**	0.29	II, III-IPC > I, IV-RIPC II, III-SHAM > I, IV-SHAM II-RIPC < II-SHAM
CRP	Group	4.21	1, 16	0.05*	0.21	
	Marathon	185.98	3, 48	0.01**	0.92	I < II, III; IV > II, III
	Group × Marathon	0.51	3, 48	0.67	0.03	
HGB	Group	0.48	1, 16	0.49	0.02	
	Marathon	94.53	3, 48	0.01**	0.82	I > II, III, IV; II > III
	Group × Marathon	1.68	3, 48	0.18	0.11	
LDH	Group	5.21	1, 16	0.03*	0.24	RIPC < SHAM
	Marathon	501.59	3, 48	0.01**	0.96	II > I, III, IV; IV < I. II, III
	Group × Marathon	6.20	3, 48	0.01**	0.27	II-RIPC > I, III, IV-RIPC IV-RIPC < I,III-RIPC II-SHAM > I, III, IV-SHAM II-RIPC < II-SHAM
MB	Group	1.06	1, 16	0.31	0.06	
	Marathon	576,29	3, 48	0.01**	0.97	III > I, II, IV; II > I, IV
	Group × Marathon	1.46	3, 48	0.23	0.08	
RBC	Group	1.60	1, 16	0.22	0.09	
	Marathon	60.70	3, 48	0.01**	0.79	I > II, III, IV; II > III
	Group × Marathon	0.17	3, 48	0.91	0.01	
TnT	Group	5.04	1, 16	0.04*	0.23	RIPC < SHAM
	Marathon	117.93	3, 48	0.01**	0.87	II > I, III, IV; III > I, IV
	Group × Marathon	4.20	3, 48	0.01*	0.20	II-RIPC > I, III, IV-RIPC II-SHAM > I, III, IV-SHAM II-RIPC < II-SHAM

*RIPC, group after 10 days of remote ischemic preconditioning; SHAM, group after 10 days of sham controlled intervention; BUN, blood urea nitrogen; CK, creatinine kinase; CK-MB, creatinine kinase MB; CRP, C-reactive protein; HGB, hemoglobin; LDH, lactate dehydrogenase; RBC, red blood cells; TnT, troponin T; and I, before, II, immediately after, III, 24 h after, and IV, 7 days after the marathon run. *Significant difference at $p < 0.05$; **Significant difference at $p < 0.01$.*

DISCUSSION

The main goal of the current study was to evaluate the effects of 10-day RIPC training on serum biomarkers of liver and heart damage induced by a marathon run. The observed significant increase in the levels of TnT and CK-MB, the markers of heart damage, was attenuated in runners who underwent RIPC training prior to the run. A similar effect of RIPC was observed on the levels of ALT, AST, and GGT activity, and BIL-T, the markers of liver damage.

The effects of RIPC on the induction of cTn levels by an endurance exercise have been studied before (El Messaoudi et al., 2013; Cocking et al., 2017). El Messaoudi et al. (2013) showed that RIPC before 70 min of cycling (80% maximal heart rate) and then until exhaustion (95% maximal heart rate) did not affect the cTn levels. On the other hand, Cocking et al. (2017) reported that RIPC before a 1-h time cycling trial led to an attenuation of cTn level increase after the exercise, but without any effect on the left ventricle function. In the current study, while the exercise (running vs. cycling) and load (1 vs. 3.5 h) were different from those of the study of Cocking et al. (2017), the outcomes were similar in both studies. A major factor that could explain the different outcome of the study of El Messaoudi et al. (2013) is the amount of muscle tissue involved in the RIPC procedure. In work of El Messaoudi et al. (2013), the RIPC was applied 3 × 5 min bilaterally to the upper arm, while in Cocking et al. (2017), RIPC was applied 4 × 5 min to the ipsilateral upper and lower limb, in an alternating manner. Similar to Cocking et al. (2017), in the current study, the RIPC protocol involved 4 × 5 min intervals of ischemia applied to both lower limbs, which have more muscle mass than the upper limbs. Moreover, lower limbs are mainly involved in tested activities (running, cycling), while upper arms are less involved in cycling. The observed attenuation of the increase of the exercise-induced markers of heart damage after RIPC might indicate its protective effect. However, the questions of whether an exercise-induced increase in cTn levels originates solely in the cardiac muscle and its clinical relevance remain unresolved (Stavroulakis and George, 2020).

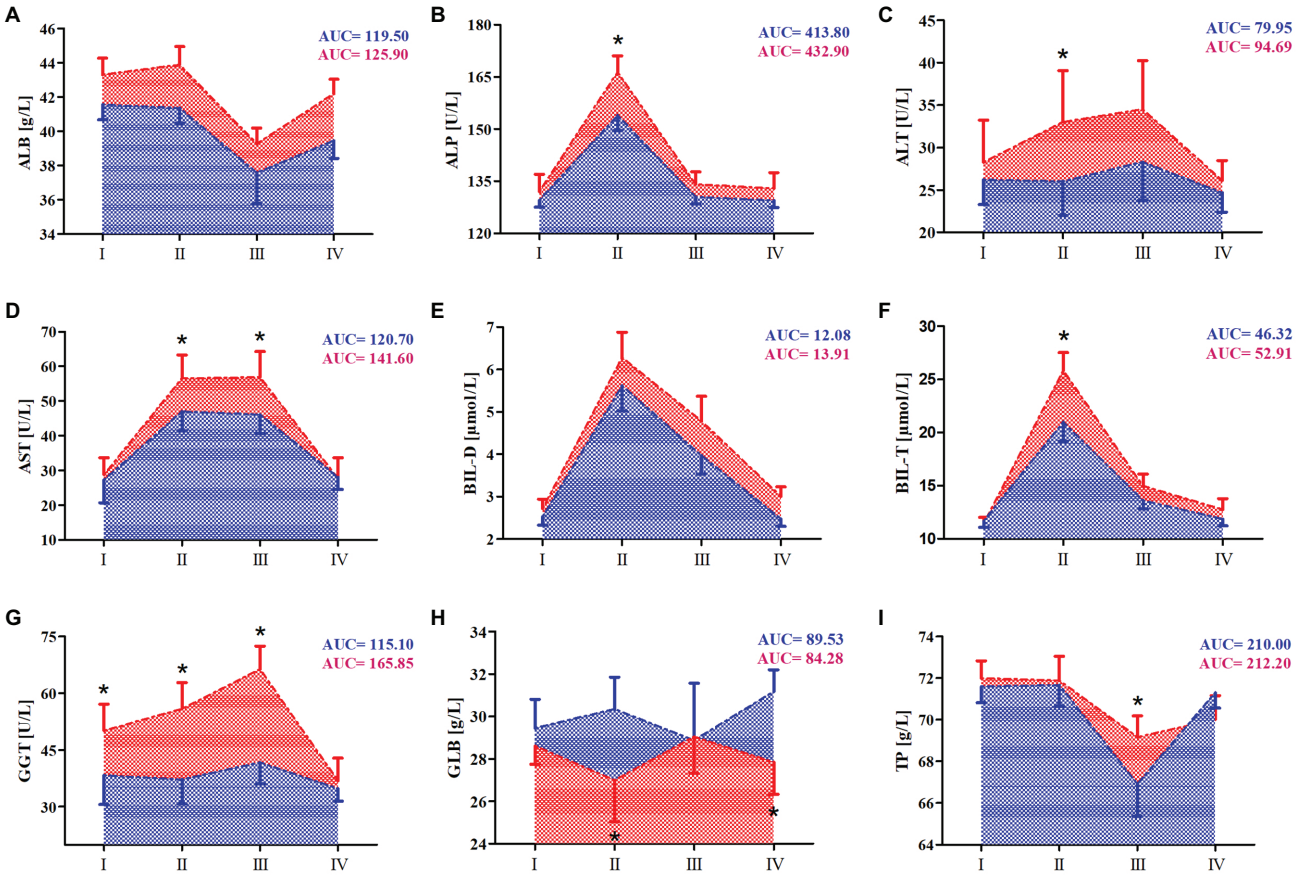

FIGURE 2 | Change in serum of selected hepatic markers after marathon run. **(A)** Albumin (ALB), **(B)** alkaline phosphatase (ALP), **(C)** alanine transaminase (ALT), **(D)** aspartate aminotransferase (AST), **(E)** direct bilirubin (BIL-D), **(F)** total bilirubin (BIL-T), **(G)** γ-glutamyl transpeptidase (GGT), **(H)** globulin (GLB), and **(I)** total protein (TP). Blue color – a group after 10 days RIPC training, Red color – a group after sham-controlled intervention (SHAM), AUC, area under curve, I – before the marathon run (baseline), II – immediately after marathon run, III – 24 h, and IV – 7 days after marathon. *Significant difference vs. RIPC at $p < 0.01$.

To the best of our knowledge, no other study investigating the effects of RIPC on exercise-induced markers of liver damage has been published. However, the data presented in the current study are in agreement with previous reports that participation in the marathon and ultra-marathon distance events results in elevated biomarkers associated with liver injury, including GGT, AST, ALT, and LDH activities (Shin et al., 2016). As most of these enzymes are also present in skeletal muscle, some authors suggest that they reflect skeletal muscle injury rather than liver injury (Lippi et al., 2011). That might be true for other enzymes but not for GGT. First of all, GGT is produced mainly in the liver, with little or no synthesis in skeletal muscle (White et al., 1985). Further, the serum GGT levels in individuals with Duchenne muscular dystrophy are in the normal range despite the substantial skeletal muscle damage indicated by high serum CK levels (Rosales et al., 2008). In the current study, we observed a significant increase in serum CK levels after the marathon run in all participants, indicating skeletal muscle damage. The RIPC training did not significantly affect the serum CK levels. On the other hand, the GGT activity was significantly increased after the run only in the SHAM group, indicating that liver activity during a prolonged strenuous exercise can be successfully impacted by RIPC.

Another marker of liver function that responds to strenuous exercise is bilirubin (De Paz et al., 1995; Shin et al., 2016). In the current study, bilirubin levels increased after the marathon run and returned to baseline over the next 7 days. This is in agreement with other studies on strenuous running exercise, including half-marathon (Lippi et al., 2011), marathon (Kratz et al., 2002), and ultramarathon (Fallon et al., 1999; Wu et al., 2004; Arakawa et al., 2016), in which an increase in bilirubin levels was also observed. The increase can be a consequence of hemolysis, which is augmented by endurance running, but also can be a result of impaired liver function in response to exercise (De Paz et al., 1995). Hence, bilirubin, as a catabolite of heme, can be a marker of hepatobiliary insufficiency. While RIPC itself did not affect bilirubin levels at rest, we observed attenuation of BIL-D increase in the RIPC group after the marathon in the current study. Hence, our assumption that RIPC would attenuate exercise-induced increase in bilirubin levels was confirmed. Importantly, low resting bilirubin levels have been associated with increased cardiovascular risk, and exercise training increases resting bilirubin levels (Swift et al., 2012; Kang et al., 2013). However, it is important to make the distinction between training and acute exercise. Collectively, the above data suggest that RIPC

TABLE 4 | Two-way (two groups × four repeated measurements) ANOVA tests for the hepatic markers induced by marathon run in RIPC and SHAM groups.

Variable	Effect	*F*	df	*p*	Effect size (η^2)	*Post hoc* outcome
Albumin	Group	24.71	1, 16	0.01**	0.60	RIPC < SHAM
	Marathon	135.03	3, 48	0.01**	0.89	III < I, II, IV; IV < I, II
	Group × Marathon	2.57	3, 48	0.06	0.13	
ALP	Group	30.01	1, 16	0.01**	0.65	RIPC < SHAM
	Marathon	319.38	3, 48	0.01**	0.95	II > I, III, IV
	Group × Marathon	8.40	3, 48	0.01**	0.34	II-RIPC > I, III, IV-RIPC II-SHAM > I, III, IV-SHAM II-RIPC < II-SHAM
ALT	Group	5.72	1, 16	0.03*	0.26	RIPC < SHAM
	Marathon	20.05	3, 48	0.01**	0.55	III > I, IV; II > IV
	Group × Marathon	5.90	3, 48	0.01**	0.26	III-RIPC > IV-RIPC II, III-SHAM > I, IV-SHAM II-RIPC < II-SHAM
AST	Group	6.51	1, 16	0.02*	0.29	RIPC < SHAM
	Marathon	168.68	3, 48	0.01**	0.91	II, III > I, IV
	Group × Marathon	7.18	3, 48	0.01**	0.31	II, III-RIPC > I, IV-RIPC II, III-SHAM > I, IV-SHAM II, III-RIPC < II, III-SHAM
BIL-D	Group	21.45	1, 16	0.01**	0.57	RIPC < SHAM
	Marathon	298.73	3, 48	0.01**	0.94	II > I, III, IV; III > I, IV
	Group × Marathon	2.12	3, 48	0.10	0.11	
BIL-T	Group	24.12	1, 16	0.01**	0.60	RIPC < SHAM
	Marathon	947.61	3, 48	0.01**	0.98	II > I,III, IV; I < III, VI
	Group × Marathon	38.17	3, 48	0.01**	0.70	II-RIPC > I, III, IV-RIPC I-RIPC < III-RIPC II-SHAM > I, III, IV-SHAM I-SHAM < III, IV-SHAM II-RIPC < II-SHAM
GGT	Group	30.07	1, 16	0.01**	0.65	RIPC < SHAM
	Marathon	88.02	3, 48	0.01**	0.84	III > I, II, IV; IV < I, II
	Group × Marathon	36.87	3, 48	0.01**	0.69	III-SHAM > I, II, IV SHAM IV-SHAM < I, II-SHAM I, II-RIPC < I, II-SHAM I, II, III-RIPC < I, II, III-SHAM
Globulin	Group	10.46	1, 16	0.01**	0.39	RIPC > SHAM
	Marathon	1.45	3, 48	0.23	0.08	
	Group × Marathon	6.42	3, 48	0.01**	0.28	III-RIPC > IV-RIPC II-RIPC > II-SHAM IV-RIPC > IV-SHAM
Total protein	Group	1.20	1, 16	0.29	0.07	
	Marathon	74.90	3, 48	0.01**	0.82	I, II > III, IV; III < IV
	Group × Marathon	12.50	3, 48	0.01**	0.43	I, II-RIPC > III-RIPC IV-RIPC < III-RIPC I, II-SHAM > III, IV-SHAM III-RIPC < III-SHAM

*RIPC, group after 10 days of remote ischemic preconditioning; SHAM, group after 10 days of sham controlled intervention; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BIL-T, total bilirubin; BIL-D, direct bilirubin, GGT, γ-glutamyl transpeptidase; and I, before; II, immediately after; III, 24 h after; and IV, 7 days after the marathon run. *Significant difference at $p < 0.05$; **Significant difference at $p < 0.01$.*

protects against exercise-induced liver damage, confirming clinical observations of individuals with liver disease (Rakic et al., 2018).

In eighties of twentieth century, it was demonstrated that contracting skeletal muscles generate free radicals, and that prolonged and intense exercise leads to increased free radical formation and cellular oxidative damage (Davies et al., 1982; Alessio et al., 1988; Reid et al., 1994). Prolonged exercise induces free radical damage of proteins and lipids (Turner et al., 2011; Withee et al., 2017). For example, in one study, levels of MDA, a marker of lipid peroxidation, significantly increased in men after a marathon run (Gomez-Cabrera et al., 2006). We confirmed this observation in the current study, as both CD and MDA levels significantly increased after the run. To the best of our knowledge, this is the first study demonstrating that RIPC training attenuates exercise-induced oxidative stress. The lower level of oxidative stress in RIPC trained runners than that in the SHAM control could also be associated with a lower activity of GGT. The physiological role of GGT is to counteract oxidative stress by breaking down extracellular glutathione and making cysteine available for glutathione synthesis within the cell. Conversely, cysteinylglycine, a product of a GGT-catalyzed reaction, stimulates reactive oxygen species formation and oxidative damage in cell culture in the presence of transferrin iron

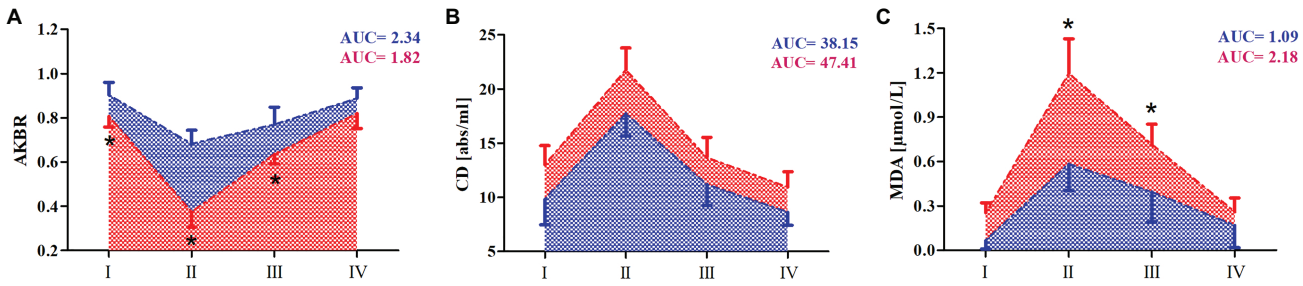

FIGURE 3 | Marathon-induced changes in markers of oxidative stress in RIPC and SHAM groups. **(A)** Arterial ketone body ratio (AKBR), **(B)** conjugated dienes (CD), and **(C)** malondialdehyde (MDA). Blue color – a group after 10 days RIPC, red color – a group after sham-controlled intervention (SHAM), AUC, area under curve, I – before, II – immediately after, III – 24 h after, and IV – 7 days after the marathon run. *Significant difference vs. RIPC at $p < 0.01$.

TABLE 5 | Two-way (two groups × four repeated measurements) ANOVA tests for the oxidative stress markers induced by marathon run in RIPC and SHAM groups.

Variable	Effect	*F*	df	*p*	Effect size (η^2)	*Post hoc* outcome
AKBR	Group	69.71	1, 16	0.01*	0.81	RIPC > SHAM
	Marathon	144.18	1, 16	0.01*	0.90	I, IV > II, III
	Group × Marathon	17.14	1, 16	0.01*	0.51	I, IV-RIPC > II, III-RIPC
						II-RIPC < III-RIPC
						II-SHAM < I, III, IV-SHAM
						III-SHAM < I, IV-SHAM
						I-RIPC > I-SHAM
						II-RIPC > II-SHAM
						III-RIPC > III-SHAM
CD	Group	23.64	1, 16	0.01*	0.59	RIPC < SHAM
	Marathon	220.03	1, 16	0.01*	0.93	II > I, III, IV; IV < I, III
	Group × Marathon	1.77	1, 16	0.16	0.09	
MDA	Group	28.86	1, 16	0.01*	0.64	RIPC < SHAM
	Marathon	168.12	1, 16	0.01*	0.91	II > I, III, IV; III > I, IV
	Group × Marathon	18.77	1, 16	0.01*	0.53	II, III-RIPC > I, IV-IPC
						II-RIPC > III-RIPC
						II, III-SHAM > I, IV-SHAM
						II-SHAM > III-SHAM
						II-RIPC < II-SHAM
						III-RIPC < III-SHAM

*RIPC, group after 10 days of remote ischemic preconditioning; SHAM, group after 10 days of sham controlled intervention; AKBR, arterial ketone body ratio; CD, conjugated dienes; MDA, malondialdehyde; and I, before, II, immediately after, III, 24 h after, and IV, 7 days after the marathon run. Significant difference at p < *0.01.*

(Drozdz et al., 1998). This may indicate some contribution of GGT to reactive oxygen species formation during a marathon.

Arterial ketone body ratio is used as an indication of the mitochondrial redox status in hepatic cells (White and Venkatesh, 2011). Decreased AKBR levels are associated with liver dysfunction and are linked to failure of other organs (Shimada et al., 1997). A marked decrease of AKBR levels often leads to a hepatic energy crisis, followed by an impairment of metabolic homeostasis. It is important to note that deleterious changes related to the low AKBR value occur several days after of heart and liver damage (Takahashi et al., 1997). In the current study, the drop in AKBR levels was temporary and the levels rapidly recovered after the marathon run, with a complete return to the baseline value after 1 week. Furthermore, the reduction in AKBR levels after the run was much smaller in the RIPC group than that in the SHAM group.

Based on the obtained results, we conclude that repeated RIPC intervention in the form of training exerts a protective effect on the heart and liver by attenuating the induction of exercise-induced markers of organ damage and reducing oxidative stress after a marathon run.

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 Bioethics Committee for Clinical Research at the Regional Medical Chamber in Gdańsk (decision number KB-24/16; Gdańsk, Poland). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JM and JA contributed to the conceptualization. JA, JM, AK, BS, BN, AB, LD-S, PB, and KS contributed to the methodology, the writing of the original draft preparation, and writing – review and editing. JM, AB, AK, BS, and BN contributed to the investigation. JM contributed to the project administration. JA contributed to the 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.2021.731889/full#supplementary-material

Supplementary Table 1 | Summary of inclusion and exclusion criteria during medical examination.

Supplementary Table 2 | Summary of training loads during normal training week in two different training periods ($n = 24$).

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REVIEW
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The Consequences of Training and Competition to the Musculoskeletal System in Ultramarathon Runners: A Narrative Review

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Ultramarathons are becoming increasingly popular every year, leading to more and more publications focusing on athletes of these endurance events. This paper summarizes the current state of knowledge on the effects of ultramarathons on the motor system. Various studies have attempted to answer questions about negative and positive effects on the musculoskeletal system, common injuries, optimal strategies, and regeneration. Considering the increasing number of ultramarathon athletes, the discoveries may have practical applications for a multitude of experts in the field of sports medicine, as well as for the athletes themselves. Acute locomotor system changes in runners as assessed by locomotor biomarkers are reversible and may be asymptomatic or painful. Injuries suffered by runners largely allow them to finish the competition and are usually overlooked. Regeneration, including regular massage and the use of supporting techniques, allows for faster convalescence. This publication is meant to be a source of knowledge for people associated with this discipline.

Keywords: ultramarathon, musculoskeletal system, endurance sport, running, injury, muscle injury

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INTRODUCTION

Ultramarathon racing has been gaining followers in recent years and its popularity has grown significantly. The sport has seen an increase in the number of competitors as well as the number of organized competitions at distances above the marathon (Kim et al., 2007; Millet et al., 2011, 2012; Krabak et al., 2013; Kupchak et al., 2014; Thompson, 2017). Ultramarathon runners are a very specific group that differs considerably from other runners (Knechtle et al., 2012b; Freund et al., 2013). These athletes are highly motivated in terms of pursuing their passion. Although, they show above-average health awareness, information about the potential detrimental effects of running on their health does not stop most of them from continuing their training (Hoffman and Krouse, 2018).

Due to the specific differences of this discipline over traditional marathon running, research that delves into the characteristics of long-term training, competitions, and effects on ultramarathoners has become important (Hoffman and Krouse, 2018). In the field of research on ultramarathons, scientific investigation is still needed, as well as counseling within the scope of training and nutrition plans (Millet et al., 2011; Krabak et al., 2013; Thompson, 2017).

The literature emphasizes that, in ultramarathon running, one should use not only the advice addressed to marathon runners (Knechtle, 2012). The characteristics of runners who run marathons and those who run longer distances vary noticeably, and most notably in terms of training sessions (Khodaee and Ansari, 2012). In addition, little research has been conducted on the long-term effects of practicing this sport (Hoffman, 2016).

Although, limited work has focused on the musculoskeletal system of ultramarathon runners, the existing reports appear relatively consistent. Current literature emphasizes that the maximal oxygen consumption, VO_2 max, the ability to generate energy, and the capacity to overcome mental resistance and fatigue are of key importance for the effective and efficient functioning of the ultramarathon runner's body (Millet, 2011; Thompson, 2017). The musculoskeletal system of ultramarathon runners is subject to enormous loads, which leads to specific adaptations for this discipline (Crenshaw et al., 1991; Harber and Trappe, 2008; Knechtle et al., 2012b). The runner's locomotor system, and its muscles in particular, is changed gradually, both at the cellular and tissue levels (Crenshaw et al., 1991; Harber and Trappe, 2008; Knechtle et al., 2012b; Ramos-Campo et al., 2016). On the other hand, training and competition strain frequently lead to injuries. Ultramarathoners deal with specific types of problems, of which those affecting the musculoskeletal system are the second most common (Khodaee and Ansari, 2012; Krabak et al., 2013, 2014; Hoffman and Krishnan, 2014; Hoffman, 2016; Vernillo et al., 2016).

In relation to the above considerations, the authors of this study attempted to characterize the impact of training and ultramarathon competitions on the movement systems of athletes. Methodical analysis of existing scientific reports will enable a more comprehensive characterization of the impact of loads on athletes and at the same time may become a guide for future scientific research by determining poorly understood areas. This work may also be a compendium of knowledge that allows athletes, coaches, doctors, and physiotherapists to increase awareness of the impact of ultramarathon training and the dangers it carries for the musculoskeletal system. For this purpose, an electronic literature search was conducted using PubMed database until October 2020. The following keywords were used: "ultra-marathon" or "ultramarathon." We identified more than 700 studies meeting our initial search criteria. Articles that described footraces performed on the ultradistance were considered eligible rather than ultra-endurance exercise in general. Similarly, studies that examined aspects of musculoskeletal system in context of ultramarathon were included in this review. The reference lists of the articles meeting our inclusion criteria were searched for additional literature.

BIOMARKERS OF THE LOCOMOTOR SYSTEM

An organism subjected to an endurance effort, such as running an ultramarathon, responds to the stress through various bodily reactions that are reflected in laboratory indicators (Khodaee et al., 2015). Most often, these changes are caused by direct organ damage (Ramos-Campo et al., 2016; Martínez-Navarro et al., 2019). However, many factors influence changes in these biomarkers, starting from individual differences to the duration and conditions in which the run is performed (Harber and Trappe, 2008; Hoffman, 2016; Hoffman et al., 2016). In addition, reference values may not provide an accurate reflection of the duress experienced under these conditions, making interpretation of certain parameters difficult. In general, changes in the level of biomarkers of muscle and cartilage damage may suggest a detrimental effect of ultramarathon distances on the musculoskeletal system, leading to degenerative changes in the future (Chilibeck et al., 1995).

Muscles

Many studies have drawn attention to the fact that completing an ultramarathon is associated with damage to muscle fibers (Kim et al., 2007, 2009; Millet et al., 2011; Krabak et al., 2014; Kupchak et al., 2014; Carmona et al., 2015; Khodaee et al., 2015; Degache et al., 2016; Hoffman et al., 2017). As with any physical activity, running also causes micro-injuries to muscle tissue, and various factors alleviate or aggravate these damages. The breakdown (catabolism) of striated muscle fibers after physical activity is a condition triggered by exercise of high total intensity (Khodaee and Ansari, 2012; Kerschan-Schindl et al., 2015; Larson et al., 2016). Eccentric contractions, which are more common in mountain ultramarathon runners, are particularly severe for myocytes. This is due to the fact that running downhill causes the muscle attachments to move apart under the influence of external force (Millet et al., 2011; Hoffman et al., 2012; Krabak et al., 2014; Carmona et al., 2015; Degache et al., 2016; Magrini et al., 2017). In the measurement of muscle mass, a noticeable loss occurs after completing the run (Knechtle et al., 2011, 2012a). The consequence of muscle microtrauma is the release of proteins such as creatine kinase (CK) into the bloodstream. CK level is widely believed to be a significant indicator of muscle injury. However, it is not fully specific for striated skeletal muscles (Fallon, 2001; Nieman et al., 2005; Kim et al., 2007; Hoffman et al., 2012; Khodaee and Ansari, 2012; Carmona et al., 2015; Magrini et al., 2017). The increase in CK depends on the degree of damage to the permeability of myocyte membranes. The level of CK increases with the extension of the ultramarathon distance (Kim et al., 2009; Carmona et al., 2015) and much more in its second half (Kim et al., 2007; Jastrzębski et al., 2015b; Son et al., 2015). Regardless, a large increase in CK due to ultramarathon running in most cases is asymptomatic and does not require hospitalization, unless there are other damaging factors (Skenderi et al., 2006; Millet et al., 2011; Kupchak et al., 2014; Magrini et al., 2017). Upon separation into its isoenzymes, the greatest increase is observed for the skeletal muscle fraction (creatine kinase isoenzyme MM, CK-MM), and a small but also noticeable increase is noted for the myocardial fraction (creatine kinase isoenzyme MB, CK-MB; Son et al., 2015). The level of the brain fraction (CK-BB) remains undetectable in these measurements. The degree of CK increase varies among athletes despite covering the same distance, an effect caused by a multitude of factors. However, training experience, years of participation

in ultramarathon distances, and the highest weekly mileage are not correlated with plasma CK levels (Hoffman et al., 2012). Only one study found a difference in CK levels between athletes of different ages. The study examined CK levels after athletes covered 25, 50, and 75 out of 100 km. Two age groups were distinguished: the older group (50.56 ± 9.7 years) showed a greater increase in CK level compared to the younger group (32 ± 5.33 years; Jastrzębski et al., 2015b). According to some authors, the correlation between finishing time and CK levels is present (161 km; Hoffman et al., 2017; 100 km; Jastrzębski et al., 2015b), and according to others, not (100 km; Knechtle et al., 2011; 160 km; Nieman et al., 2005; 161 km skyrunning; Magrini et al., 2017; 161 km; Hoffman et al., 2012; 246 km; Skenderi et al., 2006). The leading ultramarathon runners show lower initial CK values than non-leaders, and also show lower values after finishing the race (Suzuki, 2002). However, CK level is correlated with delayed-onset muscle soreness (DOMS; Nieman et al., 2005; Francisco, 2013). Runners rated pain on a 10-point Likert scale as 7.1 ± 0.3 at 1 day after the run and as 5.0 ± 0.3, 2.5 ± 0.2, and 1.6 ± 0.1 at 3, 5, and 7 days after the run, respectively (Nieman et al., 2005). The levels were the highest immediately after the run and returned to the values from before the run within 1 week (Nieman et al., 2005; Millet et al., 2011) nevertheless in 5 days after the finish they were definitely close to the initial values (Kim et al., 2009; Hoffman et al., 2017). Soreness, soreness of the lower body, and general muscle fatigue disappear at the same rate, regardless of the CK level (Hoffman et al., 2017).

Activities such as ultramarathon running can also increase levels of the heart muscle marker troponin (Carmona et al., 2015; Khodaee et al., 2015; Zebrowska et al., 2020). Unlike individuals who have suffered myocardial infarction, where cardiac troponin I (cTnI) values increase and last up to 5 days, ultramarathoners have lower cTnI values and return to normal after 24–48 h (Carmona et al., 2015).

With regard to electrolytes, which have a significant impact on muscle function, various, divergent changes in the levels of K^+ and Ca^{2+} were observed (Millet et al., 2011), as well as an inverse correlation between the concentration of Na^+ and CK measured after the run (Magrini et al., 2017). A quite common ailment such as exercise-associated hyponatremia (EAH) is also related to CK levels. During a 24-h run, the occurrence of EAH precedes an increase in CK, and possibly even increases it (Cairns and Hew, 2016).

Another marker of muscle health is myoglobin. Its level increases with the ultramarathon distance (Millet et al., 2011; Kupchak et al., 2014; Jastrzębski et al., 2015a), where in the case of running a 100-km distance, the upper norm was exceeded by a factor three-times on average. However, similar to CK, levels of this marker also show high inter-individual variability (Jastrzębski et al., 2015a). The measurements show that a 5-day recovery is sufficient to stabilize myoglobin levels within a range approaching baseline levels (Millet et al., 2011).

Myostatin is a negative regulator of muscle growth, and its concentration depends on the intensity and duration of exercise. Tested among the participants of the Spartathlon (246 km), myostatin showed a significant increase (Kerschan-Schindl et al., 2015). At the same time, the concentration of follistatin, a substance that prevents myostatin from attaching to the receptor, increased almost 4-fold immediately after completing the run, compared to the baseline values (Kerschan-Schindl et al., 2015).

The presence of fast forms of myosin (FM) in the blood serum is believed to be specific to fast skeletal muscles, whereas the slow forms of myosin (SM) may indicate damage to both skeletal muscles and, in part, cardiomyocytes. During an 85-km-long mountain run, changes were observed in the form of increased SM concentrations. Serum CK and serum SM levels are highly correlated with each other, peaking with a 1-day difference. In the case of FM, no significant changes were observed (Carmona et al., 2015).

An increase in aspartate aminotransferase (AST) is correlated with an increase in CK and myoglobin according to one study (Kupchak et al., 2014). Aminotransferases, AST, and alanine aminotransferase (ALT), do not show a uniform increase. The AST level is much more elevated than the ALT level and, combined with a lack of gamma-glutamyl transpeptidase increase, suggests that changes in the concentration of these parameters more likely result from muscle damage than liver damage caused by prolonged exercise (Skenderi et al., 2006; Kupchak et al., 2014; Jastrzębski et al., 2015b; Son et al., 2015).

Several studies have shown that the level of lactate dehydrogenase (LDH) is also associated with running ultramarathon distances and the related damage to muscle fibers. This marker rises with increasing distance traveled (Skenderi et al., 2006; Millet et al., 2011; Jastrzębski et al., 2015b) and its level returns to baseline after more than 9 days of recovery (Millet et al., 2011). After traveling 75 km and during regeneration after 100 km, an increase in the so-called liver tests and LDH it was higher for the older age group in one study (Jastrzębski et al., 2015b; **Tables 1** and **2**).

Bones and Cartilage Parts

Compared to the markers for damage to muscle fibers, fluctuations in markers for damage to cartilage tissues of the locomotor system are less noticeable in laboratory tests (Kim et al., 2009). Besides, researchers studying ultramarathoners focus their attention on cartilage less frequently than muscle tissues. The

TABLE 1 | Muscle markers after participating in ultramarathon.

CK	↑
CK-MM	↑
CK-MB	Smaller increase than CK-MM, but noticeable ↑
cTnI	Much smaller increase than in case of acute coronary syndromes, but noticeable ↑
Myoglobin	↑
Myostatin	↑
Follistatin	↑
SM	↑
FM	-
AST	↑
ALT	↑
LDH	↑

CK, creatine kinase; CK-MM, creatine kinase isoenzyme MM; CK-MB, creatine kinase isoenzyme MB; cTnI, cardiac troponin I; SM, slow myosin isoform; FM, fast myosin isoform; AST, aspartate transaminase; ALT, alanine transaminase; and LDH, lactate dehydrogenase.

TABLE 2 | Factors affecting CK increase after an ultramarathon.

Increase of the distance	↑
Second half of the distance	↑
Age	No significant impact
Sex	No significant impact
Training history	No significant impact
Experience in ultrarunning	No significant impact
Highest week running distance	No significant impact
Race finish time	Controversial factor
EAH occurrence	Possible ↑ factor

EAH, exercise-associated hyponatremia.

amounts of training of ultrarunners may have negative effects, because of altered hormone responses overriding the load-induced impact on bone formation (Chilibeck et al., 1995).

With regard to the markers of bone and cartilage components, Dickkopf 1, an antagonist of the Wnt/β-catenin pathway activating osteoblasts, decreased slightly after running. The same was true of N-terminal propeptide of type I procollagen, a marker of bone formation (Kerschan-Schindl et al., 2015). On the other hand, the level of C-terminal telopeptide of type I collagen, a bone resorption marker, increased as a result of participation in ultramarathons (Kerschan-Schindl et al., 2009, 2015). A decrease in the level of osteocalcin was also observed, the factor indicating the activity of osteoblasts, immediately after the run. These changes were no longer observed 3 days later (Mouzopoulos et al., 2007; Kerschan-Schindl et al., 2009). Receptor activator of nuclear factor kappa-β ligand (RANKL) and osteoprotegerin (OPG) proteins showed an increase in measurement after ultramarathon completion. After 3 days, the RANKL level increased further and the OPG decreased slightly, but still these markers did not reach the initial values (Kerschan-Schindl et al., 2009).

Taken together, all the markers tested indicate a change in the balance of bone metabolism. A temporary suppression of osteoblasts occurs, but this catabolic effect is short-lived and presumably related to the repair of microdamages associated with strenuous exercise, and over time bone growth resumes (Mouzopoulos et al., 2007; Kerschan-Schindl et al., 2009, 2015).

Immediately after completing a 245-km run, concentrations of bone formation markers were measured in participants. The C-terminal propeptide type I procollagen decreased and then returned to baseline levels within 3 days. Additionally, the level of the bone alkaline phosphatase (b-ALP) isoform decreased and then increased on the following day, and on the 5th day it reached pre-run levels (Mouzopoulos et al., 2007). A decrease in hydroxyproline concentration was observed after the run, which then reversed, returning to the values before the run, and then increased until the 5th day after the end of the run. However, because changes in calcium levels did not occur, the origin of these fluctuations is thought to be associated with sources other than bone, such as tendons and skin (Mouzopoulos et al., 2007).

Within the same study, the concentration of cortisol increased by up to 50% compared to the value before the run and quickly returned to normal, on the 1st day of regeneration. A negative correlation has been observed between the level of this hormone, parathyroid hormone (PTH), b-ALP levels, and osteocalcin (Mouzopoulos et al., 2007). An increase in cortisol levels is

TABLE 3 | Bone and cartilage markers after an ultramarathon.

Dkk-1	↓
P1NP	↓
CTX	↑
Osteocalcin	↓
RANKL	↑
OPG	↑
PIPC	↓
b-ALP	Firstly ↓, afterwards ↑
Hydroxyproline	Firstly ↓, afterwards ↑
Cortisol	↑
PTH	↑
COMP	↑

Dkk-1, Dickkopf-related protein 1; P1NP, N-terminal propeptide of type I procollagen; CTX, C-terminal telopeptide of type I collagen; RANKL, receptor activator for nuclear factor κB ligand; OPG, osteoprotegerin; PIPC, C-terminal propepide of type I procollagen; b-ALP, bone alkaline phosphatase; PTH, parathyroid hormone; and COMP, cartilage oligomeric matrix protein.

believed to escalate bone resorption (Mouzopoulos et al., 2007; Kupchak et al., 2014). Another important hormone in this regard is PTH, its increased levels after the run cause the suppression of osteoblasts. Normalization of PTH levels takes place until the 5th day of regeneration after the run (Mouzopoulos et al., 2007). Cartilage oligomeric matrix protein (COMP) is regarded as a sensitive and early indicator of cartilage collagen damage. An increase in this biomarker during running, especially in the second half of an ultramarathon, is now considered an indicator of damage to or hypertrophy of cartilaginous parts (Kim et al., 2007). During a 200-km run, a 1.9-fold increase in COMP in runners' serum was observed compared to pre-run concentration. This parameter returned to baseline on the 6th day of recovery after the run (Kim et al., 2009; **Table 3**).

Inflammation

In addition to the above changes, an increase in the markers of acute inflammation due to tissue damage, such as interleukin 6 (IL-6), has also been noted (Fallon, 2001; Nieman et al., 2005; Skenderi et al., 2006; Kim et al., 2007; Jastrzębski et al., 2015b; Son et al., 2015; Longman et al., 2018; Skottrup et al., 2020). Tumor necrosis factor α (TNF-α) levels remain unchanged, possibly because they are influenced by higher intensity exercise and inhibited by IL-6 (Kim et al., 2007). Many other cytokines can react to ultradistance running. During the Western States Endurance Run (WSER, 160 km), measurements of individual interleukins, chemokines, and growth factors were performed. An increase in the following parameters was demonstrated; levels of increase of a given cytokine in relation to the values obtained before the run are given in brackets: IL-6 (125x), IL-10 (24x), granulocyte colony-stimulating factor (G-CSF, 12x), IL-1RA (7x), IL-8 (5x), monocyte chemoattractant protein 1 (MCP-1, 3x), and macrophage inflammatory protein (MIP)-1β (1.2x; Nieman et al., 2005).

The situation is similar during multi-stage ultramarathons. Acute phase proteins were measured during a 6-day competition. Of the 11 subjects, a typical acute inflammatory response was observed for parameters such as iron, transferrin saturation, C-reactive protein, erythrocyte sedimentation rate (ESR), and haptoglobin. On the other hand, five subsequent

TABLE 4 | Inflammation markers after an ultramarathon.

IL-6	↑
TNF-α	-
IL-10	↑
G-CSF	↑
IL-1RA	↑
IL-8	↑
MCP-1	↑
MIP-1β	↑
hs-CRP	↑
Iron	↓
Transferrin saturation	↓
ESR	↑
Haptoglobin	↑
Transferrin	-
Albumin	-
α_1-antitrypsin	-
Complement components (C3 and C4)	-

IL-6, interleukin 6; TNF-α, tumor necrosis factor α; IL-10, interleukin 10; G-CSF, granulocyte colony-stimulating factor; IL-1RA, interleukin-1 receptor antagonist; IL-8, interleukin 8; MCP-1, monocyte chemotactic protein 1; MIP-1β, macrophage inflammatory protein 1β; hs-CRP, high-sensitivity C-reactive protein; and ESR, erythrocyte sedimentation rate.

indicators – transferrin, albumin, α1-antitrypsin, complement components (C3 and C4) – did not show such changes (Fallon, 2001). The data suggest the occurrence of an acute phase reaction in the above-mentioned cases, although, changes in specific parameters are only partially characteristic of inflammation. These discrepancies can be explained to some extent by other effort-related changes (Fallon, 2001; **Table 4**).

The observed changes in the concentration of biomarkers of the musculoskeletal system are relatively short-lived and largely dependent on the length of the distance traveled as well as many less-common factors.

MUSCLE ADAPTATION

Over time, in long-distance runners, the skeletal muscles undergo a number of adaptations. Type I and II fibers of the lower limb muscles of high-performance long-distance runners are characterized by a higher contraction rate compared to people with low activity (Crenshaw et al., 1991). Endurance running promotes the reconstruction of the mechanics of contraction of individual muscle fibers, thus optimizing their function (Harber and Trappe, 2008). The composition is dominated by type I fibers, characterized by slow-twitch but high-strength (Crenshaw et al., 1991; Harber and Trappe, 2008).

Selective hypertrophy of type I fibers can occur (63.0–78.3% of the total number of fibers; Crenshaw et al., 1991). For this reason, the SM marker seems to better reflect the degree of muscle damage during ultramarathons. To further increase specificity, cTnI was compared to SM, which revealed an increase in cTnI due to damage to cardiomyocytes. However, the increase in cTnI was so low that the SM index could be regarded as largely specific for type I myocyte damage (Carmona et al., 2015).

Noticeable changes in muscle structure also take place on a microscopic scale. In a study of the myocytes of ultramarathon runners, interesting cellular changes were found on the day following the 100-mile distance (Crenshaw et al., 1991). Centrally located muscle fibers were more densely surrounded by capillaries than peripheral fibers. Type I fibers were abundant and richly vascularized. In addition, both fiber types I and II had a 20% larger diameter compared to recreational runners (Harber and Trappe, 2008). A linear relationship was also noted between the fiber size and the associated vascular density, which actually determines the diffusion distance. The numerous mitochondria were especially densely located in the vicinity of the sarcolemma and near the vessels. On the whole, these changes are considered an adaptation to endurance effort (Crenshaw et al., 1991).

When half-marathon runners, marathon runners, and ultrarunners are compared, all runners have similar muscle mass, but the latter have a lower percentage of body fat (Knechtle et al., 2012b). Compared to marathon runners, top ultrarunners have more muscle mass in the lower limbs. This does not allow ultrarunners to develop the speed of runners in shorter distances, but greater muscle mass in the lower limbs positively influences endurance, which is important in this discipline (Millet et al., 2012). Other studies have shown that skeletal muscle mass is not correlated with the completion time of ultramarathon runners (Knechtle et al., 2012b).

The general trend is that, with age, muscle mass decreases, and so does VO_2 max. This results in poorer performance in endurance races such as ultramarathons (Trappe et al., 1996; Knechtle et al., 2012b). However, aerobic capacity does not decrease in response to exercise during ultramarathons (Efficacy et al., 2014). Loss of muscle fibers begins around the age of 50 and occurs largely in the lower extremities (Faulkner et al., 2007). This process occurs regardless of the degree of training, and while still physically active, this decline can be partially prevented (Trappe et al., 1996). Among ultramarathon runners, the type of training has not been shown to affect the percentage of muscle mass, but it has been shown to affect the percentage of fat mass (Knechtle et al., 2012b).

In studies on the influence of running a 161-km ultramarathon, a decrease in testosterone in men was shown. This change is thought to be an adaptation to regular intense exercise (Hackney et al., 2003). For ultrarunners, the reduced level of testosterone limits protein synthesis and thus the development of muscle mass, which reduces unnecessary weight and the associated energy expenditure during ultramarathons. The constantly maintained level of this hormone below the reference values may lead to a decrease in the intensity of spermatogenesis as well as a decrease in bone mineral density (Hackney, 1996; Fournier et al., 1997; Kupchak et al., 2014; Longman et al., 2018).

INJURIES AMONG ULTRAMARATHON RUNNERS

As for any physical activity, characteristic types of health problems can also be distinguished for running distances above a marathon. During the run, the following factors influence the result to a large extent: minimization of muscle damage and gastrointestinal symptoms, as well as internal motivation (Millet et al., 2012). The degree of muscle-tendon and

osteoarticular damage is very important. On the other hand, in order to avoid gastric discomfort, runners find it essential to eat foods that have already been tried by other athletes in the past while running (Costa et al., 2019). Although, ultramarathon runners can boast of above-average overall health, they are commonly affected by injuries. As many as 64% of medical visits by ultrarunners are related to injuries. Ultrarunners utilize the help of several medical care specialists mainly as: physiotherapist, 47.6%; chiropractor, 24%; podiatrist, 12.5% (Hoffman and Krishnan, 2014). The frequency of injuries is influenced by such factors as regularity of training sessions and distances covered during them, age, running experience, type of ground, intensity of and practicing activities that diversify training (Hoffman, 2016). Regardless of the source, the evidence is unambiguous – injuries are a serious problem among ultramarathoners. The majority, as many as 77% of respondents, report the occurrence of an injury in the last year, with 64.6% specifying injuries that caused a minimum 1-day break in training (Hoffman and Krishnan, 2014). In another study, this percentage is 52.2% (Hoffman and Fogard, 2011).

Many similarities exist between marathon and ultramarathon runners. However, noticeable differences occur between these two types of competitors related to the different specificity of the races themselves and common injuries and dysfunctions affecting participants of both distances (Khodaee and Ansari, 2012; Lopes et al., 2012; Millet et al., 2012; Hoffman, 2016). Hence, the results of research conducted on marathon runners cannot be fully translated to ultramarathon runners. A comparison between marathon runners and ultrarunners with regard to types of injuries shows that the former tend to undergo acute tendon pathologies more often, whereas the latter tend to suffer from chronic tendon injuries more often (Krabak et al., 2011, 2014).

The prevalence of musculoskeletal injuries during competition is unquestionable. In a study of injuries occurring during multi-day off-road ultramarathons, musculoskeletal injuries were the second most common (19%; Lopes et al., 2012; 18.2%; Schwabe et al., 2014) after skin injuries (70%; Krabak et al., 2011, 2014; Khodaee and Ansari, 2012; Vernillo et al., 2016). The studies distinguishing single-stage and multi-stage ultramarathons showed that in both cases the vast majority of injuries (locomotor system, 93%; skin, 99%) were classified as not serious enough to prevent runners from continuing (Krabak et al., 2011, 2014; Vernillo et al., 2016). The third most common was tendinitis (11.3%). In addition, sprains, tears, and bursitis were less frequent (Krabak et al., 2011). Broadly speaking, running injuries got during the competition lead to only 7.9% of dropouts, and injuries which were present before performance to 7.2% (Hoffman and Fogard, 2011).

Injuries to the motor system of ultramarathon runners are most often located in the lower limbs (92.6%; Krabak et al., 2011; Khodaee and Ansari, 2012; Hoffman and Krishnan, 2014; Vernillo et al., 2016). During the multi-stage ultramarathons, in terms of frequency of occurrence, the foot (73.7%), the shin (8.6%), the ankle (4.9%), and the knee (3.5%) show a descending pattern (Krabak et al., 2011). In contrast, one study found the knee to have the highest incidence of injuries (24%; Hoffman and Krishnan, 2014). Taking into account the different types of injuries, tendinitis is the most common, occurring as damage to the Achilles tendon (incidence 2–18.5%; Hoffman and Fogard, 2011; Lopes et al., 2012; Vernillo et al., 2016) patellofemoral pain syndrome (7.4–15.6%; Lopes et al., 2012; Hoffman and Krishnan, 2014); and extensor tendonitis (Bishop and Fallon, 1999; see **Table 5** for the full spectrum of injuries). In the case of multi-day ultramarathons, the 3rd to 4th day is observed to be a "tipping point," where injuries are most often reported by runners (Krabak et al., 2011, 2014). The time required to complete a multi-day run does not affect the frequency of injuries, generally counting and distinguishing injuries to the musculoskeletal system (Krabak et al., 2011).

Because of mountain specificity in a large number of ultramarathons, characteristic groups of muscles subject to special loads were observed (Millet et al., 2011; Hoffman et al., 2012; Degache et al., 2014). A large total number of surges, both ascents and descents, increases the involvement of the plantar flexors of the ankle joint in relation to the dorsal flexors (Degache et al., 2014).

Among the complaints affecting soft tissues within the locomotor system are exercise-associated muscle cramps (EAMCs). They are a very common affliction of ultramarathon runners and concern 19–26.2% of competitors (Schwellnus et al., 2011; Khodaee and Ansari, 2012; Schwabe et al., 2014; Vernillo et al., 2016). EAMCs account for 5% of all reasons for quitting the competition (Hoffman and Fogard, 2011; Khodaee and Ansari, 2012). Factors predisposing runners to EAMCs include: past history of EAMCs, faster covering of the first part of the distance (in the 28/56 km study), history of a fall during an ultramarathon (in this case, the probable share of accompanying neuromuscular disorders increases), prolonged duration of activity (especially during the last ¼ of the distance), and greater pain in the quadricep muscles of the thigh after running related to eccentric contractions (Schwellnus et al., 2011). In addition, putative risk factors include increased training intensity in the 3 days prior to the start and longer training units in general, more stretching exercises during training, and greater concentration of CK before the race (which may suggest greater muscle damage among the EAMC risk group; Schwellnus et al., 2011). More experienced ultramarathoners are less likely to experience problems such as muscle cramps. Male gender is a likely risk factor for EAMCs (Schwabe et al., 2014).

Muscle strains are a common problem among ultramarathoners, affecting 41% of runners (Hoffman and Stuempfle, 2016), and are also mentioned by 5% of runners as the reason for their resignation from competitions (Hoffman and Fogard, 2011). This problem most often affects the muscles most active during the run, the lower leg (13.1%), the quadriceps (11.8%), and the posterior group of the thigh (Hoffman and Krishnan, 2014; Hoffman and Stuempfle, 2016). Muscle tears are particularly common in ultramarathon runners who have suffered them in the past (Schwellnus et al., 2011; Hoffman and Stuempfle, 2016) and in runners with severe damage (elevated CK values) and with increased urea nitrogen in blood (Hoffman and Stuempfle, 2016).

Only in some studies has there been a difference between the incidence of injuries and gender. Women are injured more

TABLE 5 | Incidence of exercise-related injuries among ultrarunners.

Study	Hoffman and Krishnan, 2014	Vernillo et al., 2016	Hoffman and Fogard, 2011	Lopes et al., 2012
n	1,212	77	500	126
Injury type and/or location	**Incidence of injuries (% of affected runners)**			
Fractures not involving the extremities	1			
Upper extremity injuries (including fractures)	1.4			
Back injuries	12.4		4.2	
Iliotibial band issue	15.8		7.3	4.7
Hip flexor strain	8.7	14.3	4.2	1.0–4.7
Hamstring strain	11.8		11.1	
Stress fracture involving femur or hip	0.5		1.1	
Other leg, pelvis, or hip issues	3.7			
Knee issues	24	14.3	19.9	
Calf strain	13.1		8.8	
Achilles tendinitis or tear	9.2	7.1	11.5	2.0–18.5
Stress fracture involving tibia or fibula	1.9		3.8	
Other lower leg injuries	1.5			
Ankle sprain	10.8	28.6	9.2	5.1
Plantar fasciitis	10.6	28.6	9.6	
Stress fracture involving foot	3.4		4.6	
Morton's neuroma	3.1		1.9	
Metatarsalgia	3.1		1.5	
Great toe metatarsal phalangeal joint pain (bunion)	2.5		0.8	
Other foot and ankle injuries	4.5			
Skin wounds, blisters, and infections	1.5			
Neck/cervical spine strain		7.1		
Patellofemoral syndrome				7.4–15.6
Medial tibial stress syndrome				7.8–11.1
Patellar tendinopathy				6.3–18.5
Trochanteric bursitis				3.0–3.1
Psoas bursitis				11.1

often than men in general, but the incidence of the different groups of injuries is the same (Krabak et al., 2011; Hoffman and Krishnan, 2014; Schwabe et al., 2014). The incidence of stress fractures is a certain exception, given that women are predisposed (Hoffman and Krishnan, 2014). Anatomy as a group, ultrarunners suffer less fatigue fractures than runners of shorter distances. This difference is due in part to the fact that ultramarathon runners spend a significant fraction of their distances on hard ground, such as asphalt (Hoffman and Krishnan, 2014; Hoffman, 2016). However, the location of the injury itself differs depending on the terrain. In the case of ultramarathon runners, this type of injury affects the foot more often, which may result from the greater stress on this area when running on less level terrain and length of the distance traveled (Hoffman and Krishnan, 2014). In addition, ultramarathon runners running on soft trails more often have problems in the ankle joints, whereas ultramarathon runners running on hard ground, such as the street, are more prone to injury to the knee joints (Bishop and Fallon, 1999).

When analyzing a group of runners who suffered injuries over the last year, the individuals were statistically younger, less experienced, relatively less focused on running, spent more of their training units on high-intensity activities, and more regularly included resistance training (Krabak et al., 2011; Hoffman and Krishnan, 2014; Schwabe et al., 2014; Knechtle and Nikolaidis, 2018). Older runners have some advantage: a 10-year age difference is related to a decrease in the overall incidence of injuries by half, and of injuries within the locomotor system by 0.2 (Krabak et al., 2011). These advantages may be explained as adaptations of the ultramarathon runner's body resulting from years of practicing sports, training experience, and/or favorable genetic features that allow for many years of running (Hoffman and Krishnan, 2014).

Virtually all ultrarunners report pain after competing that is distinguished into two types, muscle soreness related to fatigue and specific injuries (Khodaee and Ansari, 2012). DOMS develops more often due to eccentric muscle contractions (Visconti et al., 2015). When DOMS affects the lower limbs, this condition is especially severe for runners, as it causes a loss of maximum contractile force and a marked discomfort. After completing the run, more than 90% of runners complain of pain in the lower limbs, with 60% indicating the calves, 23% indicating the thighs, and 8% indicating the knee area (Visconti et al., 2015). The factors correlated with the level of pain experienced include age of the competitor, experience in running ultramarathons, and CK concentration immediately after the run. Statistically, runners with CK concentrations of $\geq$28,000 UL^{-1} reported pain 1.5 times stronger than those with CK concentrations below 10,000 UL^{-1} (Hoffman et al., 2017).

One of the reasons suggested to explain how ultramarathon runners are able to cover longer distances compared to other runners is that they have a higher pain tolerance (Freund et al., 2013). The research shown that running the ultramarathon can cause partial analgetic effect induced by the effort, even

up to 30 min. Improved pain tolerance lasted the longest in the fastest runners, and was completely absent in the slower runners. Because slower runners completed the final stage at lower speeds, they may not have experienced the same effect (Hoffman et al., 2007).

Due to the prolonged effort associated with long-distance running, neuromuscular transmission is weakened. The consequence of this condition is a reduction in the maximum arbitrary muscle activation. The loss of maximal voluntary contraction (MVC, a measure of muscle strength) was determined noticeable for muscles particularly involved during a ultramarathon mountain run (Millet, 2011; Millet et al., 2011). In case of electromyography (EMG) the changes last longer (Millet et al., 2011). The decreased value of the parameters is related not only to biochemical changes occurring in the central nervous system, but also to peripheral entities involved, which induce neuromuscular weakness. This can be explained as the body's defense mechanism (Millet, 2011; Millet et al., 2011). The occurrence of the above-mentioned changes in neuromuscular transmission during single and multi-stage ultramarathons was also compared. Central nervous system changes are more noticeable in single-stage competitions, whereas peripheral nervous system changes last longer after multi-stage runs due to their greater intensity (Besson et al., 2020).

Changes in the biomechanics of running during an ultramarathon are associated with adopting a safer running technique – the movements become smoother, the energy expenditure incurred for vertical movements decreases and the frequency of steps increases (Degache et al., 2016; Thompson, 2017). Significant modifications were observed between the starting point and the middle stage of an ultramarathon. At a later stage of the course, no changes take place (Degache et al., 2016). Modifications are aimed both at reducing the sensation of pain and minimizing damage to the musculoskeletal system, especially damage caused by eccentric contractions expressed during downhills (Millet, 2011; Degache et al., 2016). The greater the variation in the length of the running stride, the greater the risk of injuries (Millet et al., 2012). Importantly, the purpose of these changes is not seen as a reduction in energy costs (Millet, 2011).

FACTORS INFLUENCING RECOVERY AND THE RISK OF INJURY

Although, a great deal of research has been conducted on how to prevent injuries to the locomotor system among runners, most studies are based on marathon runners, which cannot be clearly translated to ultramarathon runners. Thus, a clear gap exists in the research examining ultramarathons (Krabak et al., 2013).

Taking into account the experience of ultramarathon runners, attention was drawn to the fact that in order to minimize the risk of injuries, the number of starts in competitions during the season should be limited, gradually increasing the mileage during training and maintaining a balanced pace during the competition (Krabak et al., 2011). Competitors running at a statistically average pace have a smaller the risk of injuries in relation to those running faster or slower (Schwabe et al., 2014). Paying attention to the gradual increase of loads during training, and practicing activities outside of competition that are particularly demanding during competition (e.g., downhill runs), can help minimize the harmful effects of concentric and eccentric contractions on the muscles, and thus increase the resistance to this type of load (Efficacy et al., 2014). When training for a given competition, runners benefit from exercising in conditions similar to those of the target race, in terms of the type of surface, terrain, temperature, and climate (Krabak et al., 2013). A gear supply can also be a potentially important factor to help the runner finish the run successfully. Therefore, any equipment should be tested before starting a race (Krabak et al., 2013).

Although, compression socks are promoted for long-distance runners, research has shown that they provide no benefit: they do not have a positive effect on the structure and volume of the muscles of the lower limbs, nor on the results obtained. Paradoxically, runners from the research group that tested compression socks reported stronger pain after running an ultramarathon (56 km). The pain was probably due to the discomfort associated with wearing such compression elements (Geldenhuys et al., 2019).

Another factor contributing to the increase in the incidence of injuries is sleep deprivation, which is common among ultramarathon runners who cover multi-day distances (Degache et al., 2016; Larson et al., 2016; Martin et al., 2018). Research shows that sleep deprivation, in combination with other factors related to the exertion of running, impairs the control of body posture by reducing alertness, executive functions, and sensorimotor integration (Degache et al., 2014). Combined with the fact that ultramarathons are often organized in areas with difficult, uneven ground (Krabak et al., 2013, 2014; Carmona et al., 2015; Degache et al., 2016; Hoffman, 2016; Larson et al., 2016) sleep deprivation results in an increased risk of falls and injuries.

A significant difference in a short tandem-repeat polymorphism (STRP rs71746744) of the gene for the α1 chain of collagen V (COL5A1) was demonstrated among runners. Depending on the genomic variant, the competitors showed differences in flexibility and speed of finishing the run. People with the −/− genotype showed much greater flexibility, but were slower than athletes with the AGGG/AGGG and −/AGGG genotypes (Abrahams et al., 2014). Furthermore, the rs71746744 AGGG/AGGG genotype (fast, but less flexible runners) occurs much more often in people with chronic Achilles tendonitis (Abrahams et al., 2014). The above genotypic system may be one of many possible genetic adaptations to the discipline of ultramarathon running that constitute an interesting area of the future research.

Although, the use of nonsteroidal anti-inflammatory drugs (NSAIDs) before and during a run is common practice among athletes (Nieman et al., 2005; Hoffman and Fogard, 2011; Francisco, 2013; Hoffman et al., 2016; Larson et al., 2016) the drugs do not have a positive effect on the perception of muscle pain (Nieman et al., 2005; Francisco, 2013; Hoffman et al., 2016). Some researchers even indicate an increased feeling of DOMS in the first the day after the competition with the use of NSAIDs (Francisco, 2013).

Short-term amino acid supplementation, lasting between 12 and 13 h, before and during the run, did not affect the degree of muscle damage, subjective pain sensations, time to finish the run, and impaired kidney function. These results may be related to the amount of time the supplements were used. Although, the presumption is that positive effects can be expected with prolonged use, this relationship should be subjected to long-term research (Knechtle et al., 2011, 2012a; Costa et al., 2019).

Still not fully recognized, vitamin D_3 supplementation can be a significant element of the diet affecting an athlete's performance and regeneration. According to research, vitamin D_3 has a significant, beneficial effect on iron levels. In this way, it prevents the iron deficiency anemia that often affects athletes (Kasprowicz et al., 2020). In addition, a negative correlation has been noted regarding vitamin D_3 levels and markers of muscle damage. This may have a positive effect on regeneration after ultramarathon running, but no detailed recommendations are known for effective vitamin D_3 supplementation among competitors in this discipline (Zebrowska et al., 2020).

The concentration of CK after running, which is closely related to the degree of muscle damage, is considered the principal and consistent factor related to the rate of recovery (Hoffman et al., 2017). Above all, proper training has an impact on lower CK release from muscle fibers due to less damage to myocytes, and this is also related to a faster recovery rate after the run. The broadly understood adaptations resulting from the constant subjecting of the body to endurance effort are also helpful in this regard (Millet et al., 2012; Hoffman et al., 2017).

In order to accelerate regeneration, various forms of massage or pressure are used. These techniques are aimed primarily at increasing the return of lymph and blood from the lower extremities. The use of a 20-min session of specialized massage using the "effleurage" technique among ultramarathon runners was investigated, focusing on the DOMS reported after the run. Runners reported a decrease in pain sensation by 3.6 (±2.1) points on a scale of 1–10 after the procedure (Visconti et al., 2015). In another study, immediately after running an ultramarathon (161 km), a 20-min massage session and sequential intermittent pneumatic compression of the lower limbs were conducted. Subjective, immediate positive effects on muscle soreness in general and lower extremity muscles specifically were reported (Hoffman et al., 2016).

In the case of long-distance runs, an important issue is the shape and direction of the track on which the competitors move. A track length of 2,000–2,500 m is definitely preferred by runners who participate in such competitions. Moreover, the track should have a regular shape, without sharp angles, and the direction of the run should be changed at regular intervals. This is justified in practice, because overload changes of the right side, especially the knee, were observed in a 24-h race on a rectangular loop, only in the clockwise direction. Thus, failing to alter the direction of the run is a risk factor for the occurrence of injuries among runners in this type of competition (Gajda et al., 2020).

SUMMARY

Acute locomotor system changes in runners as assessed by locomotor biomarkers are reversible, can be asymptomatic or painful, and often are reversible within 1 week of the competition. Injuries suffered by runners largely allow them to finish the competition and are usually overlooked. Careful regeneration, including regular massage and the use of supporting techniques, allow for faster convalescence. Given that ultramarathon races are becoming more and more popular, this publication is mean to serve as a source of knowledge for people associated with this discipline.

LIMITATIONS OF THE STUDY

Due to the small number of studies conducted on groups of ultramarathoners, the current comparisons include ultramarathoners covering various distances, in different conditions. Each ultramarathon competition is specific, where not only distance is the factor, but also the presence of elevation gains or climate-related issues. At the current level of development of this discipline, the distances vary, resulting in few studies that compare runners only for a specific mileage.

AUTHOR CONTRIBUTIONS

AP: conceptualization and visualization. AP and ZW: writing—original draft preparation and writing—review and editing. All authors contributed to the article and approved the submitted version.

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Prolonged Sojourn at Very High Altitude Decreases Sea-Level Anaerobic Performance, Anaerobic Threshold, and Fat Mass

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Background: The influence of high altitude on an organism's physiology depends on the length and the level of hypoxic exposure it experiences. This study aimed to determine the effect of a prolonged sojourn at very high altitudes (above 3,500 m) on subsequent sea-level physical performance, body weight, body composition, and hematological parameters.

Materials and Methods: Ten alpinists, nine males and one female, with a mean age of 27 ± 4 years, participated in the study. All had been on mountaineering expeditions to 7,000 m peaks, where they spent 30 ± 1 days above 3,500 m with their average sojourn at 4,900 ± 60 m. Their aerobic and anaerobic performance, body weight, body composition, and hematological parameters were examined at an altitude of 100 m within 7 days before the expeditions and 7 days after they descended below 3,500 m.

Results: We found a significant ($p < 0.01$) decrease in maximal anaerobic power (MAP_{WAnT}) from 9.9 ± 1.3 to 9.2 ± 1.3 $W \cdot kg^{-1}$, total anaerobic work from 248.1 ± 23.8 to 228.1 ± 20.1 $J \cdot kg^{-1}$, anaerobic threshold from 39.3 ± 8.0 to 27.8 ± 5.6 $ml O_2 \cdot kg^{-1} \cdot min^{-1}$, body fat mass from 14.0 ± 3.1 to 11.5 ± 3.3%, and a significant increase ($p < 0.05$) in maximal tidal volume from 3.2 [3.0–3.2] to 3.5 [3.3–3.9] L after their sojourn at very high attitude. We found no significant changes in maximal aerobic power, maximal oxygen uptake, body weight, fat-free mass, total body water, hemoglobin, and hematocrit.

Conclusion: A month-long exposure to very high altitude led to impaired sea-level anaerobic performance and anaerobic threshold, increased maximal tidal volume, and depleted body fat mass, but had no effect on maximal aerobic power, maximal oxygen uptake, or hemoglobin and hematocrit levels.

Keywords: altitude, chronic hypoxia, physical capacity, body composition, extremes, mountaineering

INTRODUCTION

Altitude acclimatization is defined as the sum of positive changes in an organism that reduce the risk of acute altitude illness and improve performance in hypoxic conditions at altitudes above 2,500 m (West et al., 2007). The process of acclimatization focuses on increasing ventilation and raising hematocrit levels. These improvements occur within days to weeks at altitude (Lundby et al., 2004). Apart from the respiratory and hematological changes, acclimatization involves other systems that adjust to altitude over different periods, so mountaineers struggle to determine when they have been sufficiently acclimatized to high altitudes. Guidelines for mountaineers therefore include setting adequate ascent rates to higher altitudes, which are contingent on the highest altitude of sojourn being targeted (Kupper et al., 2012; Luks, 2012). Pollard and Murdoch (1997) and Broadhurst (2008) classify the range of altitudes that affect climbers as sea level (<1,500 m), moderate (1,500–2,500 m), high (2,500–3,500 m), very high (3,500–5,500 m), extreme (5,500–7,500 m), and death zone (>7,500 m). An ascent rate of 300–500 m per day increases in sleeping elevation over 2,500 m is recommended at high and very high altitudes (2,500–5,500 m; Luks, 2012). Acclimatization profiles for expeditions to extreme altitudes (>5,500 m) often involve "yo-yo tactics," where ascents to high camps are separated by rest in the base camp, usually between 3,500 and 5,500 m. Camps are usually 1,000 m in altitude apart, so overnight stays are not recommended for the first ascent to higher camps (Kupper et al., 2012).

Prolonged exposure to altitude not only involves the "positive changes" of acclimatization, but also "negative changes" of high-altitude deterioration that affect physical and mental condition (Ward, 1954; West et al., 2007). These deleterious effects include impaired physical performance (Cerretelli and di Prampero, 1985; Hoppeler et al., 1990a; Cerretelli, 1992), recovery from fatigue (Milledge et al., 1977), disturbed sleep (Weil and White, 2001), cognitive disorders (Raichle and Hornbein, 2001), and weight loss (Boyer and Blume, 1984). Deterioration becomes evident after sojourns longer than 5 weeks at extreme altitudes (>5,000–5,500 m), which are commonly endured by climbers on 8,000 m peak expeditions where the base camps are situated above 5,000 m. Sea-level maximal oxygen uptake decreases after 6 weeks above 5,200 m with some exposure to altitudes above 8,000 m (Hoppeler et al., 1990a), as does maximal anaerobic power (MAP_{WAnT}) after 5 weeks above 5,200 with ascents to higher altitudes (Cerretelli and di Prampero, 1985). Reductions in muscle mass, fiber cross-sectional area, and mitochondrial density in muscle fibers (Hoppeler et al., 2003) are the main reasons for the deterioration in physical performance after chronic hypoxia.

Typical expeditions to 6,000–7,000 m peaks usually last 3–5 weeks and site their base camps at 3,500–4,500 m (Salisbury and Hawley, 2011). Our study aimed to determine the effect of a prolonged sojourn at very high altitudes (above 3,500 m) on subsequent sea-level physical performance, body weight, body composition, and hematological parameters in alpinists who participated in expeditions to 7,000 m peaks.

MATERIALS AND METHODS

Materials

Ten Caucasian mountaineers (nine males and one female) with a mean age of 27 ± 4 years (range 20–34) participated in the study. Five mountaineers climbed Lenin Peak (7,134 m) and Chan Tengri (7,010 m) in Kyrgyzstan, and the other five climbed Korzhenevskaya Peak (7,105 m) and Somoni Peak (7,495 m) in Tajikistan. They spent 30 ± 1 days above 3,500 m at an average altitude of 4,900 ± 60 m. Both expeditions averaged 17 ± 1 climbing days and 13 rest days. We based the average altitude of their sojourn on the altitudes at which the climbers slept (**Figure 1**). Both groups were analyzed together because of the similar character of their expeditions, their plans to climb two 7,000 m peaks, the altitude of the base camps above 4,000 m, and the duration of their sojourns. Each of the climbers agreed to participate in the study, which was approved by the Regional Ethics Committee of the Medical University of Gdańsk.

Methods

We examined aerobic and anaerobic performance, body weight and composition, and hematological parameters at an altitude of 100 m within 7 days before each expedition and 7 days after the participants had descended below 3,500 m. We conducted body composition and hematological parameters measurements in the morning and on an empty stomach. On the same day, an hour after a light meal, we performed anaerobic test. The next day, in the morning, an hour after a light meal, we measured aerobic performance. All the tests were performed during the follicular phase of the female subject's menstrual cycle.

Aerobic Performance

We measured aerobic performance in an incremental exercise test to exhaustion on an ER900 cycle ergometer (Jaeger-Viasys, Germany). Aerobic performance we assessed directly with an expiratory gas analyzer (Oxycon Pro, Jaeger-Viasys, Germany) and computer software (Breath by Breath). We used a protocol of Wasserman et al. (1987) for the measurements. The main stage of the measurement was an exercise till refuse, with an incremental load of 25 W a minute, while continuously monitoring and recording oxygen uptake (VO_2) and carbon dioxide excretion (VCO_2), both in $ml \cdot kg^{-1} \cdot min^{-1}$ and $L \cdot min^{-1}$; respiratory quotient (RQ); minute ventilation (VE) as $L \cdot min^{-1}$; tidal volume (VT) in L; breath frequency (BF) as $breaths \cdot min^{-1}$; heart rate (HR) as $beats \cdot min^{-1}$; and aerobic power (MP_{VO2}) in W, $W \cdot kg^{-1}$ and $W \cdot kgFFM^{-1}$.

We measured maximal oxygen uptake (VO_2max) directly and determined anaerobic threshold (AT) using a ventilation method. The ventilation method determined anaerobic threshold when the increase in minute ventilation rose disproportionately to the increased load (Wasserman et al., 1973; Yoshida et al., 1987). This threshold has also been defined using the respiratory exchange ratio (RER; Solberg et al., 2005): RER ≥ 1 means an increase in carbon dioxide excretion, which is an indirect sign

of increased anaerobic glycolysis. We therefore expressed anaerobic threshold in $mlO_2 \cdot kg^{-1} \cdot min^{-1}$, percent of maximal oxygen uptake ($\%VO_2max$), $beats \cdot min^{-1}$, percent of maximal heart rate (%HRmax), W, and percent of maximal aerobic power ($\%MP_{VO2max}$).

Anaerobic Performance

We measured anaerobic performance during a supramaximal cycloergometric exercise for 30 s in the Wingate Anaerobic Test (WAnT; Dotan and Bar-Or, 1983; Bar-Or, 1987; Inbar et al., 1996). The test was performed on an Ergomedic E818 cycloergometer (Monark, Sweden). The load on the flywheel was set at the beginning of the exercise and was determined individually for each participant at the level of 0.075 kG per kilogram of body weight. We used MCE v 2.0 computer software to calculate the parameters we measured in the WAnT (Berry et al., 1989). The parameters we assessed were total anaerobic work (Wtot) in kJ, $J \cdot kg^{-1}$ and $J \cdot kgFFM^{-1}$; MAP_{WAnT} in W, $W \cdot kg^{-1}$, and $W \cdot kgFFM^{-1}$; time to reach maximal power (TR_{MAP}), and time of maintaining the maximal power (TM_{MAP}) in seconds (s); and the power drop index (PDI) as a percentage. The proportion between the phosphagenic ($Wana_{phosph}$) and the glycolytic ($Wana_{glycol}$) component of total anaerobic work was expressed as a percentage of total anaerobic work (%Wtot) in the WAnT (Serresse et al., 1988; Ziemann et al., 2011).

Anthropometric Measurements

Body weight (BW) and body composition were estimated using a bioelectrical impedance floor scale (TBF-300 Body Fat Monitor/Scale Analyzer, Tanita, Japan), assessing body weight (BW), fat-free mass (FFM), and total body water (TBW) in kg; fat mass (FAT) in kg and as a percentage; body mass index (BMI) in $kg \cdot m^{-2}$; body surface area (BSA) in m^2; and basic metabolic rate (BMR) in kcal.

Hematological Measurements

We express red blood cell (RBC) counts in 10^{12}/L, hematocrit (Hct) as a percentage, and blood hemoglobin concentration (Hb) as g/dl in venous blood samples collected from superficial veins of participants' upper limbs. Measurements were made with the COULTER® LH 750 Hematology Analyzer (Beckman-Coulter, United States).

Statistical Analysis

We used Statistica 13.1 (StatSoft, United States) for our statistical analyses, testing the normality of data with the Shapiro-Wilks W test, then the homogeneity of variance with the Brown-Forsythe test.

The differences between the results of physical performance, body composition, and blood parameters at sea level before and after the expeditions were analyzed using Student's *t* test (T) for parametric variables and Wilcoxon's rank test (W) for nonparametric variables.

We also calculated the effect size (partial eta^2), ranging between 0 and 1. Using Cohen's rule of thumb and the conversion table for eta^2, the interpretations of the partial eta^2 value are unequivocal. The most restricted interpretation method assigns values of partial eta^2 to the effect size as 0.1 for a small effect, 0.3 for a medium effect, and 0.5 for a large effect (Cohen, 1988; Nakagawa and Cuthill, 2007; Fritz et al., 2012).

Our results are expressed as means with a standard deviation ($M \pm SD$) for parametric variables and as medians (MD) with a lower quartile (LQ) and an upper quartile (HQ) for nonparametric variables. We set the statistical significance at $p < 0.05$ for all our analyses.

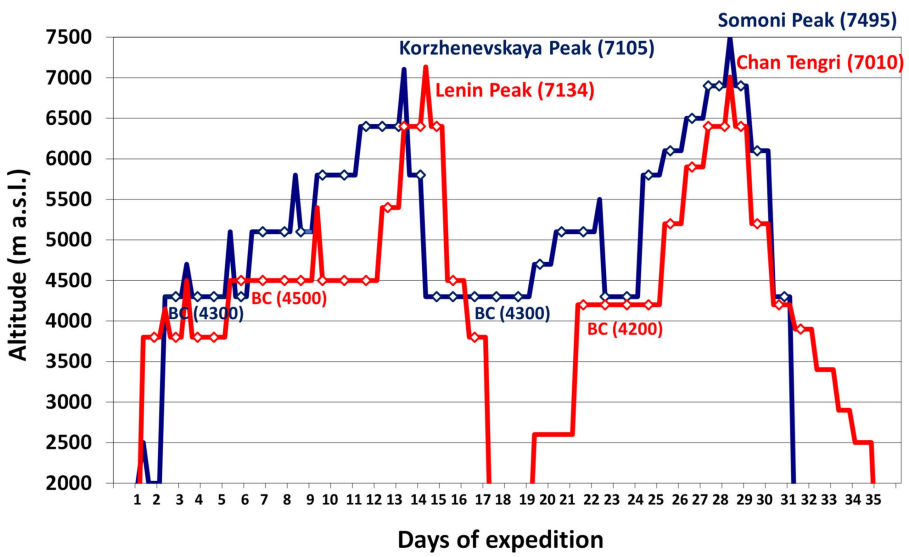

FIGURE 1 | The rate of ascent of the expeditions we analyzed. Legend: red line – Kyrgyzstan expedition to Lenin Peak (7,134 m) and Chan Tengri (7,010 m); blue line – Tajikistan expedition to Korzhenevskaya Peak (7,105 m) and Somoni Peak (7,495 m); diamonds – sleeping altitude. BC, base camp.

RESULTS

Aerobic Performance

Maximal tidal volume increased from 3.2 L before a period of chronic hypoxia to 3.5 L afterwards ($p < 0.05$, $eta^2 = 0.37$; **Table 1**). We noted an average 29% decrease in all values for anaerobic threshold after a month at very high altitude ($p < 0.01$, $eta^2 = 0.38–0.49$; **Table 1**), but we observed no significant decrease in maximal aerobic power, maximal heart rate, or maximal oxygen uptake after the period of chronic hypoxia. Maximal minute ventilation did not increase significantly and maximal breath frequency showed no changes (**Table 1**).

Anaerobic Performance

Maximal anaerobic power decreased significantly from 721.7 W before the expedition to 643.3 W after the period of hypoxia ($p < 0.001$, $eta^2 = 0.38$). Maximal anaerobic power also decreased significantly in relative values from 9.9 to 9.2 $W \cdot kg^{-1}$ ($p < 0.01$, $eta^2 = 0.33$) and from 10.9 to 9.6 $W \cdot kgFFM^{-1}$ ($p < 0.1$, $eta^2 = 0.28$; **Table 2**). Our analysis of total anaerobic work showed a significant decrease from 18.0 kJ measured before the expedition to 15.9 kJ after the sojourn at high altitude ($p < 0.001$, $eta^2 = 0.42$). Total anaerobic work decreased significantly in relative values, from 248.1 to 228.1 $J \cdot kg^{-1}$ ($p < 0.01$) and from 291.4 to 261.7 $J \cdot kgFFM^{-1}$ ($p < 0.001$, $eta^2 = 0.41$; **Table 2**).

We observed no difference before and after the period of chronic hypoxia in the time to reach maximal anaerobic power or in the time of maintaining maximal anaerobic power, in the PDI, or in the proportion of phosphagenic and glycolytic components of the total anaerobic work (**Table 2**).

Anthropometric Measurements

Chronic hypoxia induced a significant decrease in fat mass, from an average of 10.2 kg before the expedition to 8.1 kg after a month at very high altitude ($p < 0.001$, $eta^2 = 0.47$). Similarly, the percentage of fat mass decreased significantly from 14% before the expedition to 11.5% afterwards ($p < 0.001$, $eta^2 = 0.44$; **Table 3**). Fat mass in kg and % decreased in all participants. Analysis of body weight, fat-free mass, total body water, body mass index, body surface area, and basic metabolic rate did not show any significant changes after the period of chronic hypoxia.

Hematological Measurements

Our analysis of blood parameters did not show any significant changes after the period of chronic hypoxia (**Table 4**).

DISCUSSION

Dose of Hypoxia

We struggled to compare our results with those of certain authors because of the imprecisely defined methods of calculating a dose of hypoxia. The period of exposure and the level of hypoxia are different in almost all studies. The dose of hypoxia mountaineers experience on extreme-altitude expeditions to 8,000 m peaks is usually presented in days above the base camp, ignoring the altitude of the higher camps in which the alpinists spend considerable time (Boyer and Blume, 1984; Cerretelli and di Prampero, 1985; Ferretti et al., 1990b; Hoppeler et al., 1990a; Cerretelli, 1992; Reynolds et al., 1999; Mizuno et al., 2008; Doria et al., 2020). By our calculations, mountaineers on typical Mount Everest climbing itineraries spend 42 days (6 weeks) at or above the base camp (>5,300 m). Acclimatization plans usually include three rotations to higher camps before the summit bid and within the 42 days these mountaineers usually spend an equal number days of climbing and resting at an average altitude of about 6,000 m (Boukreev and DeWalt, 2002).

TABLE 1 | Parameters of aerobic performance measured before and after 1 month at very high altitude (mean altitude of 4,900 ± 60 m).

Aerobic performance parameters	Before chronic hypoxia M (±SD) or MD (LQ-HQ)		After chronic hypoxia M (±SD) or MD (LQ-HQ)		Test	*p*	Effect size
MP_{VO2max} [W]	359.0	(±57.3)	338.0	(±71.5)	T	0.12	0.09
MP_{VO2max} [$W \cdot kg^{-1}$]	4.9	(±0.6)	4.7	(±0.6)	T	0.17	0.08
MP_{VO2max} [$W \cdot kgFFM^{-1}$]	5.8	(±0.6)	5.4	(±0.7)	T	0.07	0.18
HRmax [$beats \cdot min^{-1}$]	187	(±10)	183	(±13)	T	0.08	0.19
VEmax [$L \cdot min^{-1}$]	143.5	(±39.1)	158.2	(±37.7)	T	0.18	0.06
VTmax [L]	3.2	(3.0–3.2)	3.5	(3.3–3.9)	W	0.01	0.37
BFmax [$breath \cdot min^{-1}$]	53.4	(±12.6)	53.1	(±8.1)	T	0.93	0.002
VO_2max [$L \cdot min^{-1}$]	3.7	(±0.9)	3.3	(±0.8)	T	0.24	0.06
VO_2max [$ml \cdot kg^{-1} \cdot min^{-1}$]	50.1	(±8.2)	46.8	(±7.8)	T	0.35	0.05
AT [$mlO_2\ kg^{-1} \cdot min^{1}$]	39.3	(±8.0)	27.8	(±5.6)	T	0.002	0.39
AT [$\%VO_{2max}$]	78.5	(±10.3)	59.8	(±9.2)	T	0.001	0.41
AT-HR [$beat \cdot min^{-1}$]	163	(±17)	132	(±13)	T	0.001	0.43
AT [$\%HR_{max}$]	87.2	(±7.4)	71.9	(±5.0)	T	0.001	0.38
AT [W]	275	(215–300)	150	(125–200)	W	0.005	0.49
AT [$\%MP_{VO2max}$]	73.7	(±10.7)	48.4	(±8.9)	T	0.001	0.44

Data as means (M) ± SD for parametric distribution and medians (MD) with lower quartile (LQ) and upper quartile (HQ) for nonparametric variables. Statistical tests: t test (T) for variables with parametric distribution; Wilcoxon rank test (W) for variables with nonparametric distribution. Effect size expressed as partial eta^2; maximal aerobic power (MP_{VO2max}); maximal heart rate (HRmax); maximal minute ventilation (VEmax); maximal tidal volume (VTmax); maximal breath frequency (BFmax); maximal oxygen uptake (VO_2max); and anaerobic threshold (AT).

TABLE 2 | Anaerobic performance parameters measured before and after 1 month at very high altitude (mean altitude of 4,900 ± 60 m).

Anaerobic performance parameters	Before chronic hypoxia M (±SD) or MD (LQ-HQ)		After chronic hypoxia M (±SD) or MD (LQ-HQ)		Test	*p*	Effect size
MAP_{WAnT} [W]	721.7	(±159.2)	643.3	(±162.3)	T	0.001	0.38
MAP_{WAnT} [W·kg^{-1}]	9.9	(±1.3)	9.2	(±1.3)	T	0.003	0.33
MAP_{WAnT} [W·kgFFM^{-1}]	10.9	(10.7–13.0)	9.6	(9.4–11.9)	W	0.02	0.28
TR_{MAP} [s]	4.2	(3.6–4.4)	4.4	(2.6–4.9)	W	0.7	0.12
TM_{MAP} [s]	4.4	(±1.6)	3.9	(±1.1)	T	0.3	0.14
PDI [%]	19.0	(±4.3)	18.3	(±5.5)	T	0.7	0.09
Wtot [kJ]	18.0	(±3.4)	15.9	(±3.2)	T	0.0003	0.42
Wtot [J·kg^{-1}]	248.1	(±23.8)	228.1	(±20.1)	T	0.002	0.33
Wtot [J·kgFFM^{-1}]	291.4	(±22.5)	261.7	(±20.5)	T	0.0003	0.41
$Wana_{phosph}$ [%W_{tot}]	38.6	(±10.2)	34.9	(±11.2)	T	0.6	0.11
$Wana_{glycol}$ [%W_{tot}]	83.9	(±12.1)	77.0	(±13.4)	T	0.1	0.17

Data as means (M) ± SD for parametric distribution, medians (MD) with lower quartile (LQ) and upper quartile (HQ) for nonparametric distribution of variables; Statistical tests: t test (T) for variables with parametric distribution; Wilcoxon rank test (W) for variables with nonparametric distribution. Effect size expressed as partial eta^2; Maximal anaerobic power (MAP_{WAnT}); time to reach maximal power (TR_{MAP}); time of maintaining maximal power (TM_{MAP}); power drop index (PDI); total work (Wtot); and phosphagenic ($Wana_{phosph}$) and glycolytic ($Wana_{glycol}$) component of total anaerobic work.

TABLE 3 | Anthropometric parameters measured before and after 1 month at very high altitude (mean altitude of 4,900 ± 60 m).

Anthropometric parameters	Before chronic hypoxia M (±SD) or MD (LQ-HQ)		After chronic hypoxia M (±SD) or MD (LQ-HQ)		Test	*p*	Effect size
BW [kg]	76.9	(65.5–77.3)	71.9	(67.8–78.3)	W	0.16	0.19
FAT [%]	14	(±3.1)	11.5	(±3.3)	T	0.0001	0.44
FAT [kg]	10.2	(±2.3)	8.1	(±1.8)	T	0.0001	0.47
FFM [kg]	65.8	(59.6–66.9)	63.3	(59.2–69.0)	W	0.9	0.006
TBW [kg]	48.2	(43.6–49.0)	46.3	(43.3–50.5)	W	0.9	0.007
BMI [kg·m^{-2}]	22.8	(±2.1)	22.3	(±1.8)	T	0.16	0.09
BMR [kcal]	1,825.5	(1,678–1,883)	1,758.5	(1,656–1,881)	W	0.16	0.14
BSA [m^2]	1.9	(±0.1)	1.9	(±0.1)	T	0.16	0.0001

Data are expressed as means (M) ± SD for parametric distribution, medians (MD) with lower quartile (LQ) and upper quartile (HQ) for nonparametric distribution of variables; Statistical tests: t test (T) for variables with parametric distribution; Wilcoxon rank test (W) for variables with nonparametric distribution. Effect size expressed as partial eta^2; Body weight (BW); fat mass (FAT); fat-free mass (FFM); total body water (TBW); body mass index (BMI); basic metabolic rate (BMR); and body surface area (BSA).

TABLE 4 | Blood parameters measured before and after 1 month of sojourn at a very high altitude (mean altitude of 4,900 ± 60 m).

Blood parameters	Before chronic hypoxia M (±SD) or MD (LQ-HQ)		After chronic hypoxia M (±SD) or MD (LQ-HQ)		Test	*P*	Effect size
RBC [10^{12}/L]	5.3	(4.8–5.4)	5.3	(4.9–5.4)	W	0.78	0.0003
Hct [%]	47.2	(45.0–47.8)	47.6	(43.7–49.0)	W	0.57	0.002
Hb [g/dl]	15.0	(±1.5)	15.0	(±1.1)	T	0.95	0.0001

Data are expressed as means (M) ± SD for parametric distribution, medians (MD) with lower quartile (LQ) and upper quartile (HQ) for nonparametric variables. Statistical tests: t test (T) for variables with parametric distribution, Wilcoxon rank test (W) for variables with nonparametric distribution. Effect size expressed as partial eta^2; red blood cell counts (RBC), hematocrit (Hct), and blood hemoglobin concentration (Hb).

The new metrics of hypoxic dose based on elevation and duration of exposure "kilometer hours" (Garvican-Lewis et al., 2016) or sustained duration at a given arterial saturation "saturation hours" (Millet et al., 2016) were proposed. According to a model presented by Garvican-Lewis et al. (2016), the hypoxic dose experienced by climbers in our study was ~3,500 km•h, while on typical Mount Everest expedition alpinists are exposed to the hypoxic dose of approximately 6,000 km•h. Unfortunately, the model is not widely used.

The effects of similar doses of hypoxia might differ in natural and simulated conditions. Experiments performed in simulated conditions allow researchers to control many confounding variables and enable them to focus on the effects of hypoxia on the human organism (Rose et al., 1988; Green at el., 1989; Westerterp-Plantenga et al., 1999). Mountaineering expeditions, however, experience many variables – such as low temperature, high wind, low humidity, high UV radiation, dietary restrictions, psychological stress, and great physical effort – apart from hypobaric hypoxia (Szymczak et al., 2021a,b). These confounding variables likely affect the results of these studies (Ferretti et al., 1990b; Hoppeler et al., 1990a; Reynolds et al., 1999; Mizuno et al., 2008). Authors also seldom describe the mountaineers'

climbing style and do not provide data concerning climbing intensity. The dose of hypoxia is probably different in expedition-style mountaineering and in alpine-style expeditions. In expedition-style mountaineering, the climbers leave base camp multiple times to establish higher camps and then return to base camp; alpine-style expeditions climb the mountain in a single push. Comparative studies analyzing members of mountaineering expeditions should also recognize that climbers' physical performance varies widely (Hoppeler et al., 1990a; Garrido et al., 1997). The dose of hypoxia climbers experience should be precisely defined to aid comparing different studies. Giving a dose of hypoxia in "kilometer hours" (Garvican-Lewis et al., 2016) or "saturation hours" (Millet et al., 2016) would greatly improve the comparability of the results of different studies.

Aerobic Performance

Hypoxia-inducible factor 1 (HIF-1) plays a key role in humans' adaptation to inadequate oxygen supply by stimulating erythropoiesis (Semenza, 2000), thus increasing arterial oxygen content. The HIF-1 response to hypoxia is time-dependent and down-modulated due to acclimatization (Lundby et al., 2009). Our study found no changes in the hematological parameters we measured, which might account for the unchanged aerobic performance we observed. The lung's oxygen diffusive capacity increases in hypoxic conditions, which might improve aerobic performance. This improved oxygen diffusive capacity might result from the greater number of lung capillaries that develop after prolonged exposure to hypoxic conditions (Howell et al., 2003) or because more existing capillaries are recruited during acclimatization (Capen and Wagner, 1982). The level of improved oxygen diffusive capacity seems to depend on the dose of hypoxia. Lundby et al. (2004) observed that a 2-week sojourn at 4,100 m does not increase the oxygen diffusive capacity of the lungs, whereas 8 weeks does. We did not measure the lungs' oxygen diffusive capacity, but we observed no significant changes in aerobic performance. The unchanged aerobic performance suggested that 4 weeks of hypoxia at an average altitude of 4,900 m might not have been sufficient stimulus for improving the lung's oxygen diffusive capacity, but further research is needed to confirm this speculation.

We observed no significant changes in the parameters of aerobic performance, such as VO_2max and maximal aerobic power, though a previous study described a decrease in VO_2max after 8- to 10-week sojourns at >5,200 m (Hoppeler et al., 1990a). Hypoxia has been shown to change muscle structure and so reduce sea-level aerobic performance. Hoppeler et al. (1990a) observed a 20% decrease in the cross-sectional area of muscle fiber after 8–10 weeks of exposure above 5,200 m. A similar dose of hypoxia also decreased mitochondrial volume density by 20% by deactivating mitochondrial biogenesis (Hoppeler et al., 1990b; Levett et al., 2012). HIF-1, which is induced by hypoxia and by an increase in free radicals (Askew, 2002), initiates mechanisms that decrease the quantity of free radicals in the mitochondria. The mechanisms induced by HIF-1 include more effective oxidative phosphorylation (Fukuda et al., 2007), lower pyruvate flux to mitochondria (Kim et al., 2006), the inhibition of mitochondrial biogenesis (Zhang et al., 2007), and greater mitochondrial autophagy (Zhang et al., 2008). All these mechanisms reduce oxidative stress together with the apoptosis of muscle fibers (Askew, 2002). Compensating for the negative effects of hypoxia by HIF-1, however, is limited by the dose of hypoxia. The limit seems to be crossed during a prolonged 8- to 10-week sojourn at altitudes above 5,200 m as such hypoxic dose provoked 20% decrease in the cross-sectional area of muscle fiber (Hoppeler et al., 1990a) and 20% decrease in mitochondrial volume density (Hoppeler et al., 1990b; Levett et al., 2012). The 4 weeks of hypoxia that the mountaineers in our study experienced were shorter, and the average altitude of 4,900 m was lower than the 8- to 10-week sojourn and altitude of 5,200 m that the mountaineers in a study of Hoppeler et al. (1990a) underwent. Given the insignificant changes in body weight and fat-free mass observed in our study, the dose of hypoxia that our alpinists experienced was probably too low to provoke a significant deterioration in their muscle structure, which in turn might explain the insignificant changes in their aerobic parameters. Brocherie et al. (2018) reported that repeated maximal-intensity hypoxic exercise superimposed to chronic hypoxic exposure reactivated HIF-1 and subsequent molecular downstream pathways. The hypothesis that higher climbing intensity at high altitude might improve adaptation and help to counteract muscle wasting needs further research. Unfortunately, we did not measure the level of climbing intensity in our study.

We demonstrated that 1 month at a very high altitude averaging 4,900 m significantly decreased the anaerobic threshold at sea level of the mountaineers we examined. An analysis of enzymatic activity in skeletal muscle biopsies of alpinists after an 8- to 10-week sojourn at extreme altitudes above 5,200 m revealed a 25% decrease in their aerobic potential (Hoppeler et al., 1990a). Howald et al. (1990) observed a shift from aerobic toward anaerobic metabolism after 6 weeks at extreme altitudes above 5,300 m. HIF-1 increases anaerobic metabolism to compensate for the reduction in available energy from aerobic processes by activating the genes for glycolytic enzymes, such as lactate dehydrogenase, phosphofructokinase, glucose transporters, and lactate transporters (Cerretelli et al., 2009). The significant reduction in anaerobic threshold that we observed after 1 month at the average very high altitude of 4,900 m might be explained by a shift in skeletal muscle metabolism toward anaerobic processes.

Any increased work by the respiratory muscles and the related redistribution of blood would lead to an increase in the efficiency and strength of these muscles, which might cause the greater maximum tidal volume that we observed in our study. We found no data in the literature on changes in the structure of respiratory muscles at the tissue and cellular levels after a period of chronic hypoxia, but the greater availability of oxygen, energy, and building substrates for respiratory muscles at high altitude compared with locomotory muscles might well cause different changes in their structures. This topic needs further research.

Anaerobic Performance

Our study concurred with others in finding that total anaerobic work and maximal anaerobic power decreased after 1 month at altitudes above 3,500 m (average 4,900 m). Doria et al. (2020) observed a reduction in the mechanical and the metabolic parameters of the Wingate test after a 43-day expedition in the Himalayas (23 days above 5,000 m). Cerretelli and di Prampero (1985) reported a decrease in maximal anaerobic power after 5 weeks of exposure to an altitude of 5,000 m. Their study found no changes in the parameters of anaerobic performance after 3 weeks at 5,000 m, suggesting that changes in anaerobic performance depend on the duration of hypoxia.

The reduction in the anaerobic threshold that we observed might suggest an increase in anaerobic potential, especially the glycolytic potential in the skeletal muscles, which should cause an improvement of anaerobic parameters rather than deterioration. The discrepancy we observed between decreased total anaerobic work and maximal anaerobic power and the reduction in anaerobic threshold might be explained by a hypothesis holding that changes in the level of anaerobic threshold indicate only a direction of metabolic changes in the skeletal muscle. The predominance of anaerobic processes does not necessarily indicate higher levels and greater activity of the glycolytic enzymes, but might result from less deterioration in anaerobic metabolism than in aerobic metabolism.

Hypoxia can reduce the sensitivity of skeletal muscles' cell membranes because of a reduction in the quantity and the activity of sodium-potassium adenosine triphosphatase (Na^+/K^+ ATPase), which might lead to more rapid fatigue during exercise (Aughey et al., 2005). The lower quantity and activity of Na^+/K^+ ATPase results in a deterioration of the cell membrane's sodium and potassium exchange, which would reduce impulse transmission along the muscle fibers and consequently slow the generation and transmission of impulses through the nerve fibers. Furthermore, a lower quantity and activity of Na^+/K^+ ATPase might provoke a slower impulse generation frequency in the motoneurons and reduce the fast-twitch (FT) muscle fibers' stimulation, which would likely decrease the power of the contractions they generate. This hypothesis would explain the reduced anaerobic performance observed in our study.

The average rate of ascent among climbers requires about 50% of VO_2max (West et al., 2007). Physical activity in hypoxic conditions seems to be naturally regulated and kept at the most effective level given the metabolic possibilities of the muscles. The low climbing intensity might increase the number of motoneurons of slow-twitch (ST) muscle fibers that are engaged and decrease the quantity of the engaged motoneurons of FT fibers. The reduction in the number of FT fibers recruited at altitude might result in the decrease in anaerobic performance at sea level we observed in alpinists returning from a high-altitude expedition. The lower contraction power after a high-altitude sojourn would explain the reduced maximal anaerobic power and the total anaerobic work we observed in the Wingate test of our study.

Anthropometric Data

Contrary to the results of other authors (Consolazio et al., 1968; Boyer and Blume, 1984; Macdonald et al., 2009), our study did not show a significant decrease in body weight after a sojourn at high altitude. The significant reduction in body weight that others have observed came after 2 weeks of exposure to an altitude below 5,400 m (Boyer and Blume, 1984) and to 28 days at an altitude of 4,300 m (Consolazio et al., 1968). Macdonald et al. (2009) observed a significant body weight loss of 2.4 kg after 3 weeks of trekking in the Himalayas. Our results can be explained by recovery of any lost body weight in the 7 days between the exposure to altitude and the measurements we completed at sea level. The Operation Everest III study (Westerterp-Plantenga et al., 1999) was performed in simulated extreme hypobaric conditions and reported that 63% of lost body weight was recovered within 4 days of returning to sea level, which was explained by a physiological retention of fluids. Body weight also recovered after 1 week of rest at sea level after a 1-month sojourn at 4,300 m (Consolazio et al., 1968). Less body weight will be lost with an appropriate increase in the energetic value of the food consumed at altitude to cover the increased energy demand (Butterfield et al., 1992). We did not analyze the diet followed by the alpinists in our study, but the fact that body weight did not change suggested that their energy intake was adequate to meet their energy expenditure.

Fat mass was the only component of body composition to change significantly in our study. The reduction in fat mass from 14% of body weight before the expeditions to 11.5% afterward accords with other studies that report alterations in body composition after sojourns at high altitudes below 5,400 m. Boyer and Blume (1984) reported that fat mass is the main component of body composition responsible for total body weight reduction in sojourns below 5,400 m. Macdonald et al. (2009) reported a decrease in total body weight after 21 days of trekking in the Himalayas, that loss composed 45% fat, 20% residual mass (principally protein and glycogen), and 35% of total body water.

How prolonged exposure to extreme altitudes above 5,000 m affect body composition remains unclear. Boyer and Blume (1984) found that a prolonged sojourn above 5,400 m reduced body weight: 73% to lost proteins and only 27% to a reduction in fat mass. In contrast, Reynolds et al. (1999) reported that reduced fat mass was the main change in the body composition of alpinists on an Everest expedition who spent 9 weeks above 5,300 m. Westerterp et al. (1994) observed that a 21-day sojourn at an altitude above 6,500 m reduced body weight, of which 70% was due to reduced fat mass. Reynolds et al. (1999) suggested that humans use fat reserves as an energetic substrate to cover the increased energetic demands at altitude and so spare losing muscle mass. The results reported by Boyer and Blume (1984); however, do not confirm Reynolds et al.'s suggestion.

Hematological Data

We observed no changes in the hematological parameters we examined. In contrast, Reynafarje et al. (1959) observed a 10% increase in RBC after 4 weeks at 4,500 m. Similarly, Ferretti et al. (1990a) found that a sojourn of 8–10 weeks at altitudes above 5,200 m caused a significant increase in Hb by 14.1% and Hct by 12.2%.

The 7 days between the mountaineers' descent from altitude and when we took their measurements might have affected our results, though Reynafarje et al. (1959) found that higher levels of RBC are maintained for 2–4 weeks after descent. Rice et al. (2001) observed neocytolysis-hemolysis of young circulating red blood cells (neocytes) in nine high-altitude residents with polycythemia after they descended from 4,380 m to sea level (Rice et al., 2001). The level of their RBC decreased by 7% in their first few days at lower altitude, a phenomenon that might explain the absence of changes in the hematological parameters we examined.

The unchanged hematological parameters that we observed might be partly explained by the fact that each individuals' hematopoietic system reacts differently to hypoxia: Some respond to hypoxia with an increase in their erythropoietin levels and a subsequent increase in Hb and Hct; others do not show this reaction (Chapman et al., 1998). Our alpinists might have had hematopoietic systems that did not respond to the hypoxic stimulus that was delivered.

The unchanged Hb concentration we observed might also result from an increase in red cell mass and plasma volume in a similar ratio after a month at very high altitude.

Strengths and Limitations

Our study is one of very few that analyze aerobic and anaerobic performance, body mass and composition, and hematological parameters before and after a sojourn at very high altitude. Our results expand the current knowledge of how prolonged hypoxia at very high altitude affects sea-level physical performance. Given that very high altitudes of 3,500–5,500 m include the most popular trekking and climbing areas, our work concerns a large group of people that includes mountaineers and high-altitude tourists.

Our post-expedition measurements were done within 7 days after the mountaineers descended below 3,500 m, which limits comparisons with other studies that differ in the number of days between the end of exposure to hypoxia and when sea-level measurements are taken. Mountaineers are usually examined days after they descend to sea level because they must travel from remote mountains to a suitable laboratory (Hoppeler et al., 1990a).

The reduction in anaerobic threshold we observed might result from our methodology. We determined anaerobic threshold using a non-invasive method based on the ventilatory threshold, which might not correlate with the anaerobic threshold after a high-altitude sojourn in the same way as before exposure. During adaptation to high altitude, the peripheral chemoreceptors are sensitized, resulting in them responding faster to hypoxia and hypercapnia (Dempsey and Forster, 1982; Schoene et al., 1984; Sato et al., 1992). Sensitization of the peripheral chemoreceptors at altitude might provoke a quicker ventilatory response to the lower levels of arterial carbon dioxide at sea level, which might reduce the ventilatory threshold at sea level after a sojourn at high altitude. HIF-1 causes the metabolism to shift toward higher utilization of carbohydrates as energy substrates and might also provoke a higher production of carbon dioxide during exercise at the same level of VO_2max after descent to sea level. The surfeit of carbon dioxide would lead to faster ventilation and shift the ventilatory threshold to the left on the ventilation-power curve of the incremental exercise test to exhaustion.

Conclusion

The dose of hypoxia experienced in 1-month mountaineering expeditions at very high altitude averaging 4,900 m impairs anaerobic performance and the anaerobic threshold at sea level, has a neutral effect on aerobic performance and hematological parameters, and induces lower fat mass.

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 the Regional Ethics Committee of the Medical University of Gdańsk. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RS and TG: conceptualization, formal analysis, and writing – original draft preparation. RS, TG, and EZ: methodology, validation, and data Curation. RS: investigation and supervision. RS and MS: resources and visualization. RS, TG, EZ, MS, and RL: writing – review and editing. All authors contributed to the article and approved the submitted version.

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Changes in Factors Regulating Serum Sodium Homeostasis During Two Ultra-Endurance Mountain Races of Different Distances: 69 km vs. 121 km

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Overdrinking and non-osmotic arginine vasopressin release are the main risk factors for exercise-associated hyponatremia (EAH) in ultra-marathon events. However, particularly during ultra-marathon running in mountainous regions, eccentric exercise and hypoxia, which have been shown to modulate inflammation, hormones regulating fluid homeostasis (hypoxia), and oxidative stress, could contribute to serum sodium changes in a dose-dependent manner. To the best of our knowledge, the contribution of these factors, the extent of which depends on the duration and geographical location of the race, has not been well studied. Twelve male participants (11 finishers) of the short (69 km, 4,260 m elevation-gain) and 15 male participants (seven finishers) of the long (121 km, 7,554 m elevation-gain) single-stage Südtirol Ultra Sky-Race took part in this observational field study. Venous blood was drawn immediately before and after the race. Analyses included serum sodium concentration, copeptin (a stable marker for vasopressin), markers of inflammation, muscle damage and oxidative stress. Heart rate was measured during the race and race time was obtained from the race office. During the short and the long competition two and one finishers, respectively showed serum sodium concentrations >145 mmol/L. During the long competition, one athlete showed serum sodium concentrations <135 mmol/L. Only during the short competition percent changes in serum sodium concentrations of the finishers were related to percent changes in body mass ($r = -0.812$, $p = 0.002$), total time ($r = -0.608$, $p = 0.047$) and training impulse (TRIMP) ($r = -0.653$, $p = 0.030$). Data show a curvilinear (quadratic) relationship between percent changes in serum sodium concentration and body mass with race time when including all runners (short, long, finishers and non-finishers). The observed prevalence of hypo- and hypernatremia is comparable to literature reports, as is the relationship between serum sodium changes and race time, race intensity and body mass changes of the finishers of the short race. The curvilinear relationship indicates that there might be a turning point

of changes in serum sodium and body mass changes after a race time of approximately 20 h. Since the turning point is represented mainly by non-finishers, regardless of race duration slight decrease in body mass and a slight increase in serum sodium concentration should be targeted to complete the race. Drinking to the dictate of thirst seems an adequate approach to achieve this goal.

Keywords: mountain running, ultra-marathon, dysnatremia, dehydration, exercise-associated hyponatremia, hyperhydration

INTRODUCTION

Ultra-marathon running refers to any running event over marathon distances and can be performed in either a single stage or multi-stage setting (Scheer et al., 2020). In recent years, such competition have become increasingly popular in mountain areas (Stuempfle et al., 2011; Gatterer et al., 2013b, 2020; Mrakic-Sposta et al., 2015; Zanchi et al., 2016; Belinchon-deMiguel et al., 2019, 2021; Hoppel et al., 2019; Nguyen et al., 2021). The most striking features of ultra-mountain marathon running, apart from the long distance, is the difference in altitude that the participants have to overcome, as well as the possible reduced oxygen availability depending on the altitude level. The human body is challenged to the utmost during such races and health problems may occur. Common problems beside others are electrolyte disturbances, hydration imbalances, cardiocirculatory and muscular issues (Gatterer et al., 2013b; Krabak et al., 2017; Knechtle and Nikolaidis, 2018). In the literature, special attention is given to dysnatremia since both dehydration and exercise-associated hyponatremia (EAH) (serum sodium concentration < 135 mmol/L; Hew-Butler et al., 2017) can lead to serious health issues and even death (Knechtle et al., 2019). The literature on the prevalence of dysnatremia in ultra-endurance events is ample as recently reviewed (Knechtle et al., 2019). Conversely, less data is available on ultra-marathon races performed in the mountains, even though research is progressing fast. During mountain ultra-marathon races, asymptomatic hyponatremia was found in 4–8% of the ultraendurance mountain runners (Page et al., 2007; Arnaoutis et al., 2020), whereas 3% of the participants were hypernatremic (>145 mmol/L; Page et al., 2007). Page et al. (2007) furthermore showed that hypernatremia was associated with weight loss whereas hyponatremia with weight gain.

The pathogenesis and clinical representation of EAH have been recently reviewed by Knechtle et al. (2019) and Hew-Butler et al. (2017). Overdrinking and fluid retention due to non-osmotic secretion of arginine vasopressin (AVP) have been identified as the main risk factors (Speedy et al., 2000; Hew-Butler et al., 2017; Knechtle et al., 2019). Overdrinking may stem from the fact that in 1996, the American College of Sports Medicine position stand recommended to consume the maximal amount of fluids that can be tolerated (Convertino et al., 1996). Only in 2007, new recommendation emphasized the risks and consequences of both insufficient and excessive fluid consumption (Sawka et al., 2007). Since then drinking to thirst was considered best practice (Noakes, 2010; Hew-Butler et al., 2017). In addition, factors such as inflammation (linked to AVP regulation; Hew-Butler et al., 2017; Knechtle et al., 2019) and oxidative stress have been linked to the development of dysnatremia (Siegel, 2006; Hew-Butler et al., 2017). Further risk factors include exercise duration >4 h, event inexperience, pre-exercise overhydration, low body weight, weight gain during exercise, female sex, and nonsteroidal anti-inflammatory drug use (Almond et al., 2005; Knechtle et al., 2019). Moreover, intrinsic factors like physical strain, perceived exertion, sweat rate and the sensation of thirst and external factors such as race distance, temperature, altitude, humidity, availability and quality of drinks have been identified as contributing factors (O'Connor, 2006; Rosner and Kirven, 2007; Hew-Butler et al., 2008; Schenk et al., 2010; Chlibkova et al., 2018; Knechtle et al., 2019). In this regard, two special features of ultra-mountain marathon running need to be addressed. These events are held at altitude of different levels. Altitude exposure, especially when combined with exercise, induces hypoxemia with profound physiological effects. For instance hypoxia induces oxidative stress (Gatterer et al., 2013a) and inflammation (Pham et al., 2021) and affects hormones regulating fluid homeostasis (Schlittler et al., 2020; Gatterer et al., 2021). A further characteristic is the large proportion of eccentric exercise (downhill running). Similar to hypoxia eccentric exercise induces oxidative stress and inflammation (Bruunsgaard et al., 1997; Lee et al., 2002; Goldfarb et al., 2005). As outlined before inflammation, oxidative stress and dysregulation of hormones involved in fluid homeostasis have been postulated to be involved in the development of dysnatremia. Therefore, these factors must be given special attention, especially in ultra-mountain marathon competitions of varying duration held at altitude (which means different hypoxia dose and eccentric exercise load).

The present work thus aims to describe changes in hormones (e.g., copeptin as a stable marker of AVP), cytokines (e.g., IL-6), oxidative stress and metabolic byproducts (e.g., lactate, ketones) postulated to be linked to sodium regulation during a short (i.e., 69 km, 4,260 m elevation-gain) and a long (i.e., 121 km, 7,554 m elevation-gain) mountain ultra-marathon running race with different high altitude exposure levels and eccentric exercise volumes.

MATERIALS AND METHODS

Participants

Participants were recruited by public announcement and by information's provided by the race office 2 months prior to

the race. To be eligible, participants had to provide a sports medical health check (including exercise stress testing with ECG monitoring) as required by Italian regulations. Twelve healthy men participating in the short and 15 healthy males participating in the long mountain ultra-run (for details of the competition see below) agreed to take part in this observational field study and provided written informed consent. The informed consent included information's about EAH and dehydration, yet no specific recommendations were given to the participants prior to the race. Eleven participants completed the short race and seven completed the long race (runners characteristics are shown in **Table 1**). The study was approved by the Ethical Commission of the Bolzano Hospital, Italy, (No. 57-2015) and was carried out in conformity with the ethical standards of the Declaration of Helsinki.

The Race

The single-stage Südtirol Ultra Sky-Race (2015) consisted of a short (69 km, 4,260 m elevation-gain) and a long (121 km, 7,554 m elevation-gain) competition (**Figure 1**). Starting and finishing line was at Bolzano level (262 masl). The long race started in the evening (10 pm, overnight race) and the short race in the morning (7 am) the next day. On the race days, the mean temperature at Bolzano level (262 m) was 26°C (min 22°C, max 30.5°C) during day 1 and 24°C (min 19°C, max 28.5°C) during day 2 with precipitation during the last hours of the long competition.

Measurements and Instrumentation

Venous blood was collected within 2 h before the start of the race and immediately after the race from finishers and within 1 h from those who abandoned the race (research staff was present along the course mainly on huts and checkpoints). Food intake before blood collection was not prescribed, as this would have interfered with the participants' preparation for the competition.

Approximately 14 ml of blood was drawn from an antecubital vein and collected in vacutainer tubes (VACUETTE: two Serum, one EDTA). Blood was separated by centrifugation at 3500 g for 10 min at 4°C, and immediately stored in multiple aliquots at −80°C until being assayed.

Except for the reactive oxygen species (ROS) and copeptin levels, all blood parameters (i.e., white blood cell count, red blood cell count, hemoglobin concentration (Hb), hematocrit (Hct), serum sodium concentration, potassium, bicarbonate, UREA, glucose, ketones, lactate (La), creatine kinase (CK), interleukin-6, osmolality and osmole gap) were analyzed using standard laboratory procedures in the laboratory of the Bolzano hospital. ROS production rate was determined by X-band Electron Paramagnetic Resonance spectroscopy (EPR; E-Scan—Bruker BioSpin, GmbH, Germany), as described in detail elsewhere (Mrakic-Sposta et al., 2014; Vezzoli et al., 2016).

Copeptin levels were determined in duplicate using ELISA kits (MyBioSource, San Diego, United States) according to the manufacturer's instructions. Inter-assay coefficient of variation was in the range indicated by the manufacturer (CV <10%).

Serum sodium concentration of >145 mmol/L were considered to indicate hypernatremia, values between 135 and 145 mmol/l as normonatremia and concentrations <135 mmol/L as hyponatremia (Noakes et al., 2005).

Bioimpedance analyses (BIA 101 BIVA, AKERN SRL, Montacchielo, Italy) were performed before the race and after the race. All measurements have been performed according to the manufacturer's guidelines. The subjects were in a supine position with their legs and arms by their sides and values have been registered after a minimum of 5 min of rest. Subjects were required to empty their bladder immediately before assuming the supine position. Prior to the measurement, the skin was cleaned with an alcohol solution and four contact electrodes were placed on the dorsal surface of the right hand and foot. For analyses, solely raw values were used (i.e., resistance, reactance and phase angle). Resistance (R) is determined by the body's resistive (i.e., opposition to flow of current) elements and is related to water content. Reactance (Xc) is determined by the body's capacitive elements and is associated with cell size and integrity of the cell membranes (Gatterer et al., 2014; Lukaski et al., 2019; Francisco et al., 2020). The phase angle is calculated as arc tangent of Xc/R expressed in degrees and is related to the intracellular water pool and the ratio of extracellular to intracellular volumes (Gatterer et al., 2013c; Gatterer et al., 2014; Francisco et al., 2020).

Throughout the race, participants were equipped with heart rate (HR, Polar and Garmin) monitors. Data were lost for

TABLE 1 | Baseline characteristics of the participants and TRIMP during the competitions shown as mean ± SD.

	Short distance	Long distance (finishers)	Long distance (non-finishers)	value of p (ES) (short vs. long finishers)	value of p (ES) (long finishers vs. non-finishers)
Age (yr)	42.4 ± 8.8	41.0 ± 10.2	40.8 ± 5.2	0.767 (0.15)	0.952 (0.02)
Body height (m)	1.76 ± 0.64	1.82 ± 0.73	1.76 ± 0.6	0.069 (0.93)	0.118 (0.09)
Body mass (kg)	73.0 ± 7.0	79.0 ± 8.8	69.3 ± 5.4	0.126 (0.75)	0.022 (1.32)
Body mass index (kg/m^2)	23.6 ± 1.9	23.8 ± 1.3	22.3 ± 1.2	0.860 (0.12)	0.043 (1.20)
VO_{2max} (ml/min/kg)	57.0 ± 6.4	59.3 ± 4.7	59.5 ± 6.3	0.429 (0.41)	0.935 (0.04)
Finishing times (h:min:s)	11:25:53 ± 2:03:31	27:29:15 ± 3:22:41	19:34:47 ± 4:16:33	<0.001 (5.7)	0.002 (2.4)
TRIMP	1,400 ± 145	2,271 ± 321	/	<0.001 (3.50)	/

Effect size (Cohens'd), ES; maximal oxygen uptake, VO_{2max}. n = 11 and n = 7 for the short and the long distance (finishers), respectively; n = 4 for TRIMP of the long distance; n = 8 for the non-finishers of the long distance.

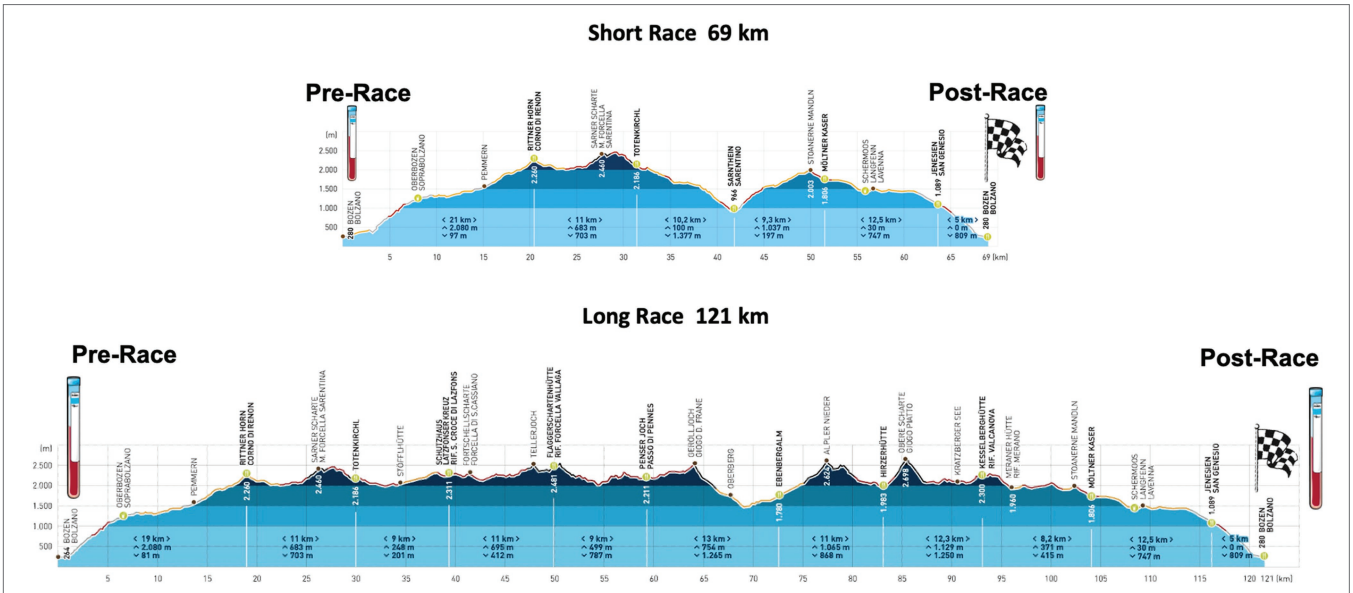

FIGURE 1 | Race profile of the Südtirol Ultra Sky-Race: in the upper part of the figure "the short race" and below "the long race" competition is shown. At pre and post, blood sampling was performed (https://www.suedtirol-ultraskyrace.it/fileadmin/user_upload/pdf/Hoeenprofil_SUSR.pdf).

three participants during the long run because of inadequate battery life. Official race times of the participants were obtained from the race office.

Pre-race Exercise Testing and Race Effort

Exercise testing was performed on a treadmill up to 2 weeks before the race as described in detail elsewhere (Gatterer et al., 2020). Briefly, the running test protocol included the following speed and incline increases: 2 min at 5 km/h walking speed with an incline of 5%, 2 min at 5 km/h walking speed with an incline of 10%, 1 min at 6 km/h walking speed with an incline of 10%, then the incline was increased by 2% per minute up to 20%, then participants started running and running speed was increased by 1 km/h per min up to exhaustion. Training impulse (TRIMP) was calculated as: LOW + MOD x 1.5 + HI x 2 + VHI x 3, where LOW corresponds to the time spent in the low intensity zone (heart rate below the ventilator threshold (VT) 1), MOD to the time spent in the moderate intensity zone (heart rate between VT1 and [(HR-VT2 − HR-VT1)/2 + HR-VT1]), HI to the time spent in the high intensity zone (heart rate values between [(HR-VT2 − HR-VT1)/2 + HR-VT1] and VT2) and VHI to the time spent in the very high intensity zone (heart rate above VT2) (Gatterer et al., 2020).

Statistical Analysis

The sample size depended on participants' willingness to respond to our call and whether participants were able to complete the race. Data were analyzed using IBM SPSS Statistics 26. Paired t-tests were used to analyze changes in the course of the short and long competition as well as of the combined race distances. For the comparison between the short and the long run, unpaired t-tests were used. Pearson correlation analyses were used to test for significant relationships between percent changes in serum sodium concentration and variables putatively related to sodium changes [correlation coefficient of $r = 0.1$ indicates a small, $r = 0.3$ a medium and $r = 0.5$ a large effect size (Cohen, 1988)]. Additionally, curve estimation was used to check for potential quadratic relationships for data of all participants (finishers, non-finishers, short and long competition). Data are presented as mean ± SD and information on effect size (ES, Cohen's d) was included, where $d = 0.2$ indicates a small, $d = 0.5$ a medium and $d = 0.8$ a large effect (Cohen, 1988). Statistical significance was set at $p < 0.05$.

RESULTS

During the short competition two out of the 11 finishers (18.2%) showed serum sodium concentrations >145 mmol/L (148 and 147 mmol/L). The single non-finisher also demonstrated a serum sodium concentration of >145 mmol/L (146 mmol/L). No cases of hyponatremia were registered. During the long competition, one athlete out of the seven finishers (14.3%) was hyponatremic (134 mmol/L) and one hypernatremic (147 mmol/L). All eight non-finishers showed serum sodium concentration in the normal range. Fluid intake per hour was 612.4 ± 187.6 ml/h during the short and 422.3 ± 197.8 ml/h during the long competition ($p = 0.057$). **Table 2** displays changes in putative factors regulating serum sodium homeostasis as well as standard clinical parameters during the short and the long competition. Whereas marked changes were registered in most of the parameters from before to after both races, different changes between the short and long competition were only found for reactance and phase angle of the bioimpedance

TABLE 2 | Changes in measured variables of the finishers during two ultra-endurance mountain races of different distances: 69 km, 4,260 m elevation-gain vs. 121 km, 7,554 m elevation-gain ($n = 11$ and $n = 7$ for the short and the long distance, respectively).

	Short distance			Long distance			*p* (ES)
	Pre	Post	%changes	Pre	Post	%changes	Differences of %changes between short and long
Body mass (kg)	73.0 ± 7.0	70.7 ± 6.7	−3.1 ± 1.3*	79.0 ± 8.8	76.9 ± 8.6	−2.7 ± 2.0*	0.553 (0.24)
Resistance (Ω)	450.9 ± 42.2	454.3 ± 41.4	0.8 ± 3.5	463.1 ± 30.4	450.2 ± 25.2	−2.5 ± 7.2	0.206 (0.58)
Reactance (Ω)	54.8 ± 6.4	60.6 ± 6.9	10.7 ± 4.3*	57.3 ± 4.0	56.0 ± 4.1	−1.7 ± 11.7	**0.005** (1.41)
Phase angle (°)	6.95 ± 0.66	7.62 ± 0.70	9.8 ± 2.7*	7.04 ± 0.26	7.20 ± 0.41	2.2 ± 4.8	**0.001** (1.95)
WBC count (10^3/μl)	6.96 ± 1.87	16.79 ± 4.04	147.5 ± 59.2*	6.38 ± 1.18	14.69 ± 2.45	131.6 ± 21.7*	0.434 (0.36)
RBC count (10^6/μl)	5.02 ± 0.33	5.23 ± 0.40	4.1 ± 4.1*	4.88 ± 0.33	4.81 ± 0.43	−1.7 ± 4.1	**0.010** (1.41)
Hb (g/dl)	14.8 ± 0.7	15.4 ± 0.9	3.6 ± 3.8*	14.5 ± 1.0	14.3 ± 1.0	−1.8 ± 3.3	**0.006** (1.52)
Hct (%)	44.7 ± 2.1	45.7 ± 2.4	2.2 ± 4.8	43.9 ± 2.2	42.7 ± 3.1	−2.7 ± 5.6	*0.067* (0.94)
Sodium (mmol/L)	142.9 ± 1.9	143.7 ± 2.1	0.6 ± 2.1	142.0 ± 1.7	142.6 ± 4.2	0.4 ± 2.7	0.870 (0.08)
Potassium (mmol/L)	3.96 ± 0.21	3.92 ± 0.42	−0.9 ± 9.1	3.97 ± 0.42	3.89 ± 0.23	−1.4 ± 9.7	0.911 (0.05)
Bicarbonate (mmol/L)	26.1 ± 2.2	23.1 ± 2.6	−10.9 ± 13.2*	25.1 ± 2.0	21.7 ± 2.8	−13.3 ± 12.4*	0.705 (0.19)
UREA (mg/dl)	30.3 ± 5.8	48.4 ± 6.0	65.2 ± 37.5*	36.7 ± 12.6	74.3 ± 31.3	106.6 ± 60.4*	*0.090* (0.82)
Glucose (mg/dl)	99.6 ± 8.6	117.4 ± 22.6	18.3 ± 23.3*	97.9 ± 5.3	109.6 ± 12.0	12.2 ± 12.8*	0.541 (0.32)
Ketones (mmol/L)	0.21 ± 0.05	0.40 ± 0.12	110.6 ± 131.3*	0.29 ± 0.11	0.57 ± 0.17	132.9 ± 122.0*	0.724 (0.18)
Lactate (mmol/L)	1.69 ± 059.	3.72 ± 1.76	142.2 ± 134.9*	1.46 ± 0.50	1.75 ± 0.76	29.3 ± 66.8	**0.032** (1.06)
Creatine kinase (U/L)	180.2 ± 102.4	1,741.7 ± 1,269.6	1,012.4 ± 827.4*	151.0 ± 57.3	5,083.6 ± 3,782.8	3,304.2 ± 2,286.4*	**0.007** (1.33)
Interleukin-6 (pg/ml)	1.92 ± 0.51	51.05 ± 19.55	2,769.1 ± 1,315.9*	2.44 ± 0.88	61.93 ± 58.12	2,208.8 ± 1,216.7*	0.378 (0.44)
Copeptin (pmol/L)	7.66 ± 2.76	27.82 ± 12.76	313.7 ± 248.6*	8.75 ± 2.13	26.28 ± 13.71	214.6 ± 155.5*	0.362 (0.48)
Reactive Oxygen Species (μmol/min)	0.172 ± 0.010	0.205 ± 0.013	19.8 ± 6.2*	0.174 ± 0.014	0.206 ± 0.008	19.2 ± 9.1*	0.870 (0.08)
Osmolality (mOsm/kg)	286.1 ± 3.0	292.2 ± 5.8	2.1 ± 2.4*	284.4 ± 3.7	293.6 ± 11.8	3.2 ± 3.7	0.465 (0.35)
Osmole gap (mOsm/kg)	9.6 ± 5.2	10.9 ± 4.1	28.4 ± 54.2	8.8 ± 1.7	9.9 ± 3.8	19.3 ± 50.1	0.724 (0.17)

*Effect size (Cohens'd), ES; hematocrit, Hct; hemoglobin concentration, Hb; red blood cell count, RBC; with blood cell count, WBC. Bold indicates a p<0.05; Italic indicates 0.05<p<0.10. *Indicates significant differences from pre to post.*

analyses, red blood cell count, Hb and La concentration and CK (**Table 2**). The four cases of hypernatremia showed a loss in body mass from 76.5 ± 3.9 to 72.4 ± 3.3 kg ($p = 0.002$) and increases in ROS (0.179 ± 0.004 to 0.200 ± 0.142 μmol/min; $p = 0.020$), IL-6 (2.25 ± 0.25 to 33.10 ± 10.56 pg./ml; $p = 0.010$), copeptin (8.4 ± 2.5 to 24.7 ± 9.3 pmol/L; $p = 0.040$) and WBC count (6.9 ± 3.0 to 15.9 ± 5.4 10^3/μl; $p = 0.005$).

During the short competition percent changes in serum sodium concentrations were negatively related to percent changes in body mass ($r = -0.812$, $p = 0.002$), total time ($r = -0.608$, $p = 0.047$) and TRIMP ($r = -0.653$, $p = 0.030$) and positively related to UREA ($r = 0.612$, $p = 0.046$). Correlations between percent changes in serum sodium concentrations and percent changes in bicarbonate ($r = -0.572$, $p = 0.066$) and osmolality ($r = 0.582$, $p = 0.060$) did not reach statistical significance even though effect size was large. During the long race, no significant correlation between percent changes in serum sodium concentration and any other measured variable was detected. When combining data of the long and the short run, significant correlations between percent changes in serum sodium concentration and changes in body mass ($r = -0.702$, $p = 0.001$) and osmolality ($r = 0.600$, $p = 0.008$) were recorded. **Table 3** displays differences in changes between finishers and non-finishers of the long competition. Data show that body mass, UREA, CK and osmolality differed between finishers and non-finishers. When including all runners (short, long, finishers and non-finishers) percent changes in serum sodium concentration and body mass showed a more curvilinear (quadratic) rather than linear relationship with race time (**Figure 2**).

DISCUSSION

The main findings of this investigation were that during the short ultra-endurance mountain race no case of hyponatremia was observed whereas during the long race one out of seven was hyponatremic, yet without showing any symptoms potentially related to serum sodium aberrations (e.g., malaise, headache; Knechtle et al., 2019). Conversely, during both races cases of hypernatremia were recorded. Serum sodium concentration changes were related to race time, race intensity (i.e., TRIMP) and body mass changes during the short race whereas none of the assessed parameters where related to the long race indicating a threshold where other factors may determine serum sodium aberrations.

It is well established that drinking habits in conjunction with hormonal dysregulation, race duration and race intensity are the main factors linked to serum sodium alterations (Rosner, 2019). Present data support these findings during the short race showing a relationship between serum sodium changes

TABLE 3 | Changes in measured variables of the finishers and non-finishers of the long mountain ultra-marathon running race ($n = 7$ and $n = 8$ for the finishers and the non-finishers, respectively).

	Finishers			Non-finishers			*p* (ES)
	Pre	post	%changes	Pre	Post	%changes	Differences of %changes between finishers and non-finishers
Body mass (kg)	79.0 ± 8.8	76.9 ± 8.6	−2.7 ± 2.0*	69.3 ± 5.4#	68.9 ± 4.8	−0.6 ± 1.5	**0.041** (1.19)
Resistance (Ω)	463.1 ± 30.4	450.2 ± 25.2	−2.5 ± 7.2	473.9 ± 23.3	458.8 ± 33.2	−3.2 ± 4.8	0.827 (0.11)
Reactance (Ω)	57.3 ± 4.0	56.0 ± 4.1	−1.7 ± 11.7	58.1 ± 5.8	58.0 ± 6.4	−0.1 ± 7.5	0.752 (0.16)
Phase angle (°)	7.04 ± 0.26	7.20 ± 0.41	2.2 ± 4.8	6.98 ± 0.52	7.16 ± 0.40	2.9 ± 5.8	0.809 (0.13)
WBC count (10^3/µl)	6.38 ± 1.18	14.69 ± 2.45	131.6 ± 21.7*	5.90 ± 1.25	11.65 ± 2.14	104.1 ± 55.1*	0.238 (0.66)
RBC count (10^6/µl)	4.88 ± 0.33	4.81 ± 0.43	−1.7 ± 4.1	5.09 ± 0.32	5.08 ± 0.34	−0.1 ± 4.9	0.513 (0.35)
Hb (g/dl)	14.5 ± 1.0	14.3 ± 1.0	−1.8 ± 3.3	14.9 ± 0.9	14.5 ± 0.7	−2.2 ± 5.9	0.881 (0.08)
Hct (%)	43.9 ± 2.2	42.7 ± 3.1	−2.7 ± 5.6	44.6 ± 2.2	44.3 ± 2.4	−0.6 ± 4.9	0.461 (0.40)
Sodium (mmol/L)	142.0 ± 1.7	142.6 ± 4.2	0.4 ± 2.7	142.5 ± 2.9	140.6 ± 2.3	−1.3 ± 1.8	0.170 (0.74)
Potassium (mmol/L)	3.97 ± 0.42	3.89 ± 0.23	−1.4 ± 9.7	3.94 ± 0.24	3.84 ± 0.38	−2.5 ± 7.9	0.817 (0.12)
Bicarbonate (mmol/L)	25.1 ± 2.0	21.7 ± 2.8	−13.3 ± 12.4*	27.4 ± 2.4	25.1 ± 2.2	−8.1 ± 5.2*	0.336 (0.55)
UREA (mg/dl)	36.7 ± 12.6	74.3 ± 31.3	106.6 ± 60.4*	37.1 ± 10.0	45.4 ± 15.9	23.8 ± 45.0	**0.010** (1.55)
Glucose (mg/dl)	97.9 ± 5.3	109.6 ± 12.0	12.2 ± 12.8*	95.8 ± 8.6	112.1 ± 23.5	16.8 ± 19.4	0.597 (0.28)
Ketones (mmol/L)	0.29 ± 0.11	0.57 ± 0.17	132.9 ± 122.0*	0.19 ± 0.04#	0.65 ± 0.70	237.5 ± 343.0	0.459 (0.41)
Lactate (mmol/L)	1.46 ± 0.50	1.75 ± 0.76	29.3 ± 66.8	1.34 ± 0.63	1.46 ± 0.64	20.8 ± 56.1	0.792 (0.14)
Creatine kinase (U/L)	151.0 ± 57.3	5,083.6 ± 3,782.8	3,304.2 ± 2,286.4*	201.3 ± 152.3	2,218.9 ± 2,045.3	1,306.8 ± 1,151.0*	**0.048** (1.10)
Interleukin-6 (pg/ml)	2.44 ± 0.88	61.93 ± 58.12	2,208.8 ± 1,216.7*	2.08 ± 0.66	24.3 ± 11.88*	1,157.3 ± 722.6*	*0.059* (1.05)
Copeptin (pmol/L)	8.75 ± 2.13	26.28 ± 13.71	214.6 ± 155.5*	4.57 ± 2.38#	25.25 ± 6.50*	599.6 ± 469.9*	*0.060* (1.10)
Reactive Oxygen Species (µmol/min)	0.174 ± 0.014	0.206 ± 0.008	19.2 ± 9.1*	0.172 ± 0.013	0.199 ± 0.018*	15.8 ± 8.5*	0.476 (0.39)
Osmolality (mOsm/kg)	284.4 ± 3.7	293.6 ± 11.8	3.2 ± 3.7	287.6 ± 5.0	285.8 ± 1.7	−0.6 ± 1.8	**0.021** (1.31)
Osmole gap (mOsm/kg)	8.8 ± 1.7	9.9 ± 3.8	19.3 ± 50.1	11.1 ± 4.3	10.4 ± 2.6	8.6 ± 50.0	0.687 (0.21)

Effect size (Cohens'd), ES; hematocrit, Hct; hemoglobin concentration, Hb; red blood cell count, RBC; with blood cell count, WBC. Bold indicates a p<0.05; Italic indicates 0.05<p<0.10.
**Indicates significant changes from pre to post.*
#Indicates significant pre-differences between groups.

and race time, race intensity and body mass changes. Interestingly no such relationship was detected for the long race. It is important to mention that hyponatremia is a condition linked to death during endurance races (Hew-Butler et al., 2017; Rosner, 2019). There was only one single case of asymptomatic hyponatremia occurring in an athlete competing in the long race. When having a closer look at this athlete, data indicate similar mechanisms as reported in the literature (Hew-Butler et al., 2017; Rosner, 2019). The athlete ran for 30 h and showed only small changes in body mass (~ −0.5 kg), whereas Hb concentration and Hct indicated hemodilution (14.9 g/dl and 46.7% before the race and 13.8 g/dl and 41.8% after the race, respectively). This athlete also showed the highest values for ROS production corrected for plasma volume changes (0.258 vs. 0.203 ± 0.017) and one of the highest post exercise copeptin values (37.2 vs. 26.7 ± 12.3). High levels of copeptin and AVP during and at the end of ultra-marathon race (160 km) despite decreases in [Na^+] (confirming non-osmotic simulation of AVP secretion) are in agreement with Hew Butler et al. (2011). Obviously, this is a single case observation prone to errors, nonetheless it supports both well-established research findings (Page et al., 2007; Rosner, 2019), as well as recent postulations stating that oxidative stress is involved in hyponatremia development (Hew-Butler et al., 2017). Changes in values of the four hypernatremia cases were similar to the ones recorded for the normonatremic competitors (finishers) of both races. This was to be expected because the serum sodium values of the hypernatremic cases were only slightly above the threshold for hypernatremia of >145 mmol/L.

Comparison of the outcomes of the short and the long run (**Table 2**) shows that race distance did not affect ROS production, interleukin-6, copeptin and serum sodium concentrations. This indicates that different hypoxic doses and eccentric exercise volumes did not have a large effect on those changes, at least in these competitions. Since muscle damaging downhill running is associated with higher CK values (Gatterer et al., 2013b), the higher values found after the long run confirm a higher proportion of eccentric exercise compared to the short race. Concerning hypoxia dose, no information is available, as oxygen saturation was not measured. However, at altitude levels of around 2,000–2,500 m (where much of the racing during the long run took place, **Figure 1**), oxygen saturation of around 90% may be expected during low intensity exercise, with individual values even around 80% (Wiseman et al., 2013). Nonetheless, it must be recognize that the physiological stress induced by this relatively low hypoxic dose may have been limited. The comparison between race distances furthermore shows that reactance and phase angle of the

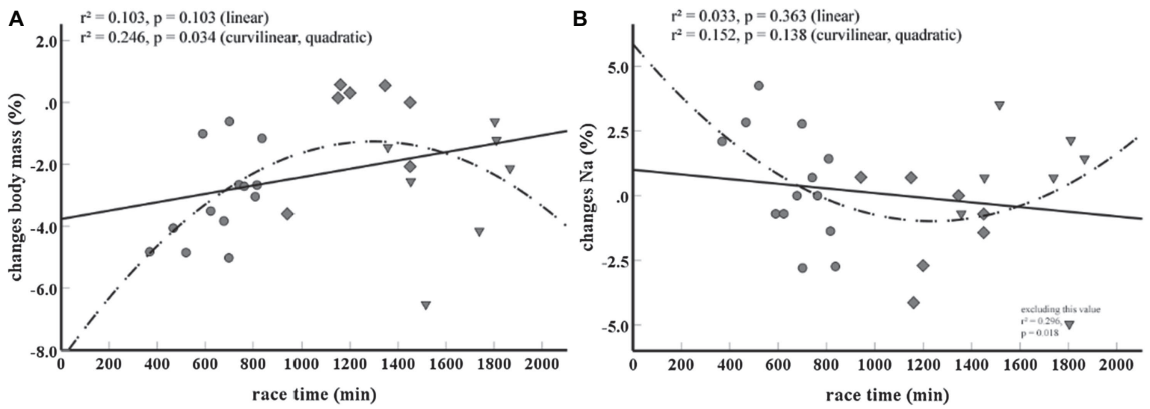

FIGURE 2 | Curvilinear (quadratic) relationship between **(A)** race time and percent changes in body mass and **(B)** race time and percent changes in serum sodium concentration. At the bottom right, **Figure 1B** shows the outcome of the analysis when excluding one outlier. Circles represent finishers of the short race, rhombus non-finishers of the long race and triangles finishers of the long race. Na, serum sodium concentration.

bioimpedance analysis are differently affected. According to recent literature, increases in phase angle indicate fluid shifts to the intracellular compartment (Francisco et al., 2020). Thus, data seem to indicate that during the short run fluid shifts to the intracellular compartment are more pronounced compared to the long run. Differences in exercise intensity between the races (Gatterer et al., 2020) may to some extent explain these findings, since race intensity was reported to be related to leg edema formation (Wilcock et al., 2006; Bracher et al., 2012). A further finding was that lactate concentration was lower after the long run. Single lactate measurements must be interpreted with caution, as they may only represent the activity prior to the measurement. However, they might also reflect a slower running speed (Gatterer et al., 2020) or muscle/liver glycogen depletion (Segal and Brooks, 1979) during the long run. No differences were observed for ketones, indicating similar free-fatty acid breakdown (Evans et al., 2017).

An interesting observation was that the percentage changes in serum sodium concentration and body mass showed a curvilinear (quadratic) rather than a linear relationship with race time when data from all athletes (short and long distance, finishers, and non-finishers) were included in the analysis (**Figure 2**). Such a curvilinear relationship was recently reported for cardiac biomarkers and race duration in marathon running (Traiperm et al., 2021). Additionally, cardiac biomarkers seemed less elevated the longer the running time in a 118 km ultra-mountain race (Martinez-Navarro et al., 2019). It was speculated that short running times may prevent excessive and prolonged volume overload and myocyte stretch (Shave et al., 2007; Serrano-Ostariz et al., 2011) whereas slow running times reflect low running pace and thus less overall strain (Traiperm et al., 2021). In the present investigation, the fastest runners of the short run showed the highest serum sodium increases and body mass reductions. With increasing race time, serum sodium concentrations seem to decrease whereas less of a body mass loss was apparent. This is in accordance to reports showing that race duration and body mass gain (or less decrease) are risk factors for hyponatremia (Rosner, 2019). Furthermore it supports the notion that the fastest runners lose the most body mass (Noakes, 2010). Interestingly, during the long run the opposite pattern was found. There the fastest runners showed the highest decreases in serum sodium concentrations and the lowest decreases in body mass. The longer the performance time the more the serum sodium increased and the more the body mass decreased. The area around the nadir (**Figure 2**) is identified at about 20 h of performance time and is mostly represented by runners that did not finish the race. In addition to showing that the shortest and the longest race times might be associated with both increases in serum sodium concentrations and losses of body mass, this graph indicates that too much of a serum sodium concentration decrease and too less of a body mass reduction are associated with a higher drop-out risk. Certainly, this observation has to be confirmed in further studies and it must be emphasized that effect sizes of the correlations were rather low, and no causal effect can be inferred. Furthermore, the reasons for a race being abandoned can be numerous (e.g., pain, fatigue, and gastrointestinal problems) as can the factors that determine race time.

Methodological Considerations

Some limitations of this observational field study have to be acknowledged. A major limitation is the limited sample size and the fact that overall changes in serum sodium concentration have been low, which limits the conclusiveness of the present data. The sample size depended on participants' willingness to respond to our call and thus was not modifiable. The low sample size, mainly of the long competition, may to some extent explain why no significant correlation during the long race was recorded between percent changes in serum sodium concentration and any other measured variable. Even though statistically not significant, large effects were found for percent changes in serum sodium concentration and body mass ($r = -0.652$, $r = 0.113$), ketones ($r = 0.706$, $p = 0.077$), osmolality ($r = 0.644$, $p = 0.118$), and copeptin ($r = -0.628$, $r = 0.131$). Since nonparametric testing (Mann–Whitney test,

Wilcoxon, and Spearman analyses) may be more robust when analyzing small sample sizes, we performed such analyses and found similar results, except for slight different p- and r-values. However, it should be mention that in the comparison between finishers and non-finishers of the long race, IL-6 and copeptin became significant ($p < 0.05$). Moreover, the correlation coefficient for changes in serum sodium concentration and total race time of the short race was slightly lower when using Spearman analysis, $r = -0.566$, $p = 0.069$. A further limitation is that data on the volume of fluid intake were obtained from personal assessments at the end of the race, which is prone to errors. In addition, diet before and during the race as well as the type of fluid intake were not assessed. However, as the participants adopted their normal race routines, this procedure should reflect real-life scenarios. It must also be considered that EAH may develop independently of body mass changes (Knechtle et al., 2019), yet body mass changes, particularly body mass gain, are still considered a surrogate for serum sodium changes (Knechtle et al., 2019). Moreover, some HR data and thus race intensity were not available because of inadequate battery life of the heart rate sensors.

In conclusion, one out of seven finishers (14%) showed asymptomatic hyponatremia after the long race whereas hypernatremia was present after the long (14%) and the short race (18%). Race duration and intensity as well as body mass changes were related to serum sodium concentrations changes during the short race. No such correlation was recorded for the long race, yet the single hyponatremic case showed hemodilution, minor body mass loss and high levels of ROS and copeptin. From a practical point of view, the observed curvilinear relationships suggest that regardless of race duration (which determines hypoxia dose and eccentric exercise load) a slight decrease in body mass and a slight increase in serum sodium concentration should be targeted to complete the race. On the other hand, to compete fast in the long run, permitting only small changes seem beneficial. Excessive increases in body mass and reductions in serum sodium concentrations should be avoided in any circumstance. According to the literature, drinking to the dictate of thirst seems an adequate approach to achieve this goal also during ultra-marathon competitions in mountain areas.

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 the Ethical Commission of the Bolzano Hospital, Italy. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KS designed, planned, and implemented the study. KS, SR, EP, KG, SM-S, and HG performed the measurements. HG and KS drafted the manuscript and analyzed the data. SR, EP, KG, and SM-S contributed in writing the manuscript. All authors contributed to the article and approved the submitted version.

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Dietary Observations of Ultra-Endurance Runners in Preparation for and During a Continuous 24-h Event

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Carbohydrate (CHO) intake recommendations for events lasting longer than 3 h indicate that athletes should ingest up to 90 g.h.$^{-1}$ of multiple transportable carbohydrates (MTC). We examined the dietary intake of amateur (males: $n=11$, females: $n=7$) ultra-endurance runners (mean age and mass 41.5 ± 5.1 years and 75.8 ± 11.7 kg) prior to, and during a 24-h ultra-endurance event. Heart rate and interstitial glucose concentration (indwelling sensor) were also tracked throughout the event. Pre-race diet (each 24 over 48 h) was recorded *via* weighed intake and included the pre-race meal (1–4 h pre-race). In-race diet (24 h event) was recorded continuously, in-field, by the research team. Analysis revealed that runners did not meet the majority of CHO intake recommendations. CHO intake over 24–48 h pre-race was lower than recommended (4.0 ± 1.4 g·kg^{-1}; 42 ± 9% of total energy), although pre-race meal CHO intake was within recommended levels (1.5 ± 0.7 g·kg^{-1}). In-race CHO intake was only in the 30–60 g·h^{-1} range (mean intake 33 ± 12 g·h^{-1}) with suboptimal amounts of multiple transportable CHO consumed. Exercise intensity was low to moderate (mean 68%HR_{max} 45%VO_{2max}) meaning that there would still be an absolute requirement for CHO to perform optimally in this ultra-event. Indeed, strong to moderate positive correlations were observed between distance covered and both CHO and energy intake in each of the three diet periods studied. Independent *t*-tests showed significantly different distances achieved by runners consuming ≥5 vs. <5 g·kg^{-1} CHO in pre-race diet [98.5 ± 18.7 miles (158.5 ± 30.1 km) vs. 78.0 ± 13.5 miles (125.5 ± 21.7 km), $p=0.04$] and ≥40 vs. <40 g·h^{-1} CHO in-race [92.2 ± 13.9 miles (148.4 ± 22.4 km) vs. 74.7 ± 13.5 miles (120.2 ± 21.7 km), $p=0.02$]. Pre-race CHO intake was positively associated with ultra-running experience, but no association was found between ultra-running experience and race distance. No association was observed between mean interstitial glucose and dietary intake, or with race distance. Further research should explore approaches to meeting pre-race dietary CHO intake as well as investigating strategies to boost in-race intake of multiple transportable CHO sources. In 24-h ultra-runners, studies examining the performance enhancing benefits of getting closer to meeting pre-race and in-race carbohydrate recommendations are required.

Keywords: multiple transportable carbohydrates, sport, continuous glucose monitors, exercise, nutrition

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INTRODUCTION

Ultra-endurance running presents the athlete with a substantial nutritional challenge. With distances up to, and in excess, of 100 miles and time limited events, such as 24-h races, it is vital that strategies are enforced to delay or minimise fatigue. Nutrition can play a key role in the preparation for, and execution of, ultra-endurance races at any level of competition. Current recommendations for ultra-endurance activities consider both pre-race diet and intake during events. Intakes of 8–12 g·kg^{-1} CHO per 24-h are recommended in the 36–48 h leading up to a prolonged endurance event to ensure well stocked muscle glycogen, with a further 1–4 g·kg^{-1} in a pre-race meal during the final 1–4 h recommended to top up liver glycogen stores (Thomas et al., 2016; Costa et al., 2019a). During prolonged exercise (>2 h), exogenous CHO ingestion can prevent hypoglycaemia, maintain high rates of CHO oxidation, and increase endurance capacity (Jeukendrup, 2014). Recommendations for endurance activities lasting >2.5–3 h, are to consume up to 90 g·h^{-1} of multiple transportable carbohydrates (MTC; Burke et al., 2011). This amount can prove challenging in ultra-endurance running events (Costa et al., 2019a,b) although higher intakes of 120 g·h^{-1} are possible and reduced exercise-induced muscle damage, in elite mountain-marathon runners (Viribay et al., 2020).

Studies investigating CHO intake of ultra-runners during competition have shown large variations in intake (25–71 g·h.$^{-1}$), at elite and non-elite levels (Glace et al., 2002; Moran et al., 2011; Stuempfle et al., 2011; Costa et al., 2014; Wardenaar et al., 2015; Stellingwerff, 2016; Martinez et al., 2018; Lavoué et al., 2020). Faster/elite runners have been shown to consume more hourly CHO than slower/amateur runners (Stellingwerff, 2016), and finishers reported to consume more than non-finishers (Stuempfle et al., 2011). From these studies, a higher CHO intake is associated with improved performance, but ultra-runners typically consume lower amounts than recommended, and less than competitors in other ultra-endurance disciplines (Pfeiffer et al., 2012). While these previous studies present evidence of actual CHO intake during ultra-endurance events, there is a lack of information on the mix of CHO sources ingested (i.e., amounts of glucose, sucrose, fructose, lactose, galactose, maltose, starch, or maltodextrin consumed), as well as frequently little indication of pre-race CHO intakes. The benefits of ingesting MTC include less gastrointestinal (GI) complaints at high CHO ingestion rates (Costa et al., 2017; Miall et al., 2018), and increased exogenous CHO oxidation rates (Wilson, 2015). The few studies examining MTC intake of runners have shown no convincing performance benefits, unless used as part of a gut-training protocol (Costa et al., 2017) although these have not specifically focused on ultra-endurance events (Pfeiffer et al., 2009; Lee et al., 2014).

Current recommendations for CHO intake rise with increasing exercise duration, but exercise intensity should also be considered. It is often reported that the rate of CHO intake should likely be reduced for those performing at lower intensities (Jeukendrup, 2014). However, for ultra-runners competing in events lasting greater than 10–12 h, it would seem that carbohydrate ingestion rates should probably match those recommended for shorter events (~3–4 h), to help meet the considerable metabolic demands of sustained activity. Although fat oxidation may provide much of the fuel utilised in events lasting up to 24 h, there will be an absolute requirement for CHO to spare muscle and liver glycogen stores, and to maintain blood glucose concentration, in order to sustain intensity of activity over that duration. Maximising CHO availability before and during such events is therefore a key to maintaining performance (Williamson, 2016). Achieving desired CHO intake during an ultra-endurance run will require intake of MTC's, and intake should be tailored to individual athletes' tolerance levels (Stellingwerff and Cox, 2014) with higher rates of MTC intake being tolerable following appropriate gut-training (Costa et al., 2017). However, no studies have closely examined both pre-race CHO intake and in-race CHO sources ingested by ultra-endurance runners over a 24-h event.

The present study therefore investigated dietary intakes of ultra-endurance runners prior to and during a competitive 24-h event. We aimed to assess pre-race CHO intake, to describe the mix of individual CHOs consumed by participants' in-race, and to evaluate the potential requirement for future MTC intervention strategies. We also aimed to assess in-race glycaemic responses in relation to feeding strategies. We hypothesised that amateur ultra-distance runners would fall short of recommended CHO intake targets before and during the event, and that, intake of MTC's could be improved during the event.

MATERIALS AND METHODS

Study Participants and Event Details

Eighteen amateur ultra-endurance runners (males: $n = 11$, females: $n = 7$) in the Glenmore-24 (G24) trail race (Aviemore, Scotland) agreed to participate in the study. G24 is a continuous undulating trail race on forest trails and tracks, over repeated laps of 4 miles (6.4 km), where the winner travels the furthest distance in 24-h. The event begins at 12 noon and ends after 24-h with runners able to change to a smaller 0.25 miles (400 m) grass field for the final hour of the event. Each large lap consists of approximately 80 m (270 feet) of ascent and descent.

Inclusion criteria for participants were: males or females; aged 18–50 years; completed at least one previous ultra-marathon event. We specifically aimed to recruit a sample that was representative of the full range of competitors at the event. Participant characteristics are shown in **Table 1**. Ethics approval was granted by University of Stirling Ethics of Research Committee. All participants gave written informed consent prior to study commencement. Of the 18 participants, 15 (11 male, four female) performed an incremental maximal treadmill test at the University laboratories to enable VO_{2max} and HR_{max} to be identified. The protocol used for the VO_{2max} test involved participants starting at 8 km/h (females) or 10 km/h (males) on a 1% gradient, increasing speed in the first few stages before increasing gradient by 2% each minute until volitional fatigue.

TABLE 1 | Participant anthropometric data, VO_{2max}, ultra-endurance running experience and nutritional composition of the pre-race diet, pre-race meal prior to the start of a 24-h ultra-endurance race, and in-race data (distance covered, pace, mass loss, heart rate, and relative $\%HR_{max}$ and $\%VO_{2max}$) by $n = 18$ participants during the Glenmore 24 trail race.

Variable	All participants ($n = 18$)	Males ($n = 11$)	Females ($n = 7$)
Age (years)	41.5 ± 5.1	39.3 ± 4.1	45.0 ± 4.7
Weight (kg)	75.8 ± 11.7	81.7 ± 6.6	66.5 ± 12.2
BMI ($kg.m^2$)	25.2 ± 2.6	25.9 ± 2.2	23.9 ± 2.8
VO_{2max} ($ml.kg.min^{-1}$)	50.7 ± 5.9 ($n = 15$)	52.0 ± 5.1 ($n = 11$)	47.1 ± 7.2 ($n = 4$)
Years of ultra running	2.7 ± 1.2	2.8 ± 1.4	2.6 ± 1.0
Ultras completed**	9 (3–27)	8 (3–15)	11 (4–27)
24 h races completed**	1 (0–3)	0 (0–3)	1 (0–1)
PRE-RACE DIET per 24 h over 2 days pre-race (n = 16)*			
Energy (Kcal)	2,730 ± 721	2,897 ± 665	2,361 ± 774
CHO (g)	296 ± 87	309 ± 86	267 ± 90
CHO ($g.kg^{-1}$)	4.0 ± 1.4	3.8 ± 1.2	4.4 ± 1.8
Total fluid [food and drinks (ml)]	2,923 ± 934	3,034 ± 954	2,923 ± 934
% Energy CHO	42 ± 9	42 ± 11	43 ± 2
% Energy PROTEIN	17 ± 4	17 ± 4	16 ± 3.0
% Energy FAT	36 ± 9	35 ± 10	39 ± 5
% Energy Alcohol	9 ± 11	11 ± 14	5 ± 5
PRE-RACE MEAL (1–4 h pre-race; n = 16)			
Energy (kcal)	878 ± 349	858 ± 317	921 ± 447
CHO (g)	110 ± 39	113 ± 25	105 ± 65
CHO ($g.kg^{-1}$)	1.5 ± 0.7	1.4 ± 0.4	1.8 ± 1.2
Protein (g)	29 ± 14	28 ± 16	33 ± 9
Fat (g)	35 ± 23	32 ± 25	41 ± 20
IN-RACE DATA			
Total race distance (miles)	80.6 ± 15.7	84.0 ± 13.5	75.3 ± 18.5
Pace (mph)	3.8 ± 0.5	3.8 ± 0.4	3.7 ± 0.6
Weight loss over race (%)	2.8 ± 2.6	3.0 ± 3.2	2.7 ± 1.7
Mean HR (bpm; $n = 7$)	124 ± 11	-	-
HRmax (%)	68 ± 5	-	-
VO_{2max} (%)	45 ± 17	-	-

**Pre-race diet data includes pre-race meal.*
*Values are mean (SD) except for **which is reported as mean (range).*

Study Design and Data Collection

The study used an observational design, examining habitual dietary intake of participants before and during a 24-h race. No dietary intervention/advice was given prior to the study. Participants were asked to follow their usual pre-race and in-race diet routines. Pre-planned in-race feeding strategy was recorded on a questionnaire sent to participants ahead of the race day. Pre-race dietary intake was recorded using a weighed food intake method. Each participant was provided with electronic scales (Salter 1036, Tonbridge, United Kingdom) to weigh and record all foods/fluids consumed and also instructed to record all timings of intake. For anything consumed away from home, participants were asked to provide a description and estimate of portion size or send photographs to the researcher. In-race dietary intake was monitored by recorders assigned to each participant. Each participant had a base in the start-finish area, and digital scales (Salter 1036) were used to weigh foods/fluids consumed, recorded to the nearest 1 g. Everything consumed at the start-finish area and food/fluid consumed during each lap was recorded along with each lap time. Water was available at the halfway point each lap; any water taken was self-reported and recorded.

Participants' interstitial glucose concentration was monitored throughout the race using indwelling continuous glucose monitors (CGM; Abbott Freestyle-Libre), inserted into the subcutaneous tissue layer of the upper arm either on the evening before, or at least 2 h before the event start on the morning of G24. CGMs automatically recorded interstitial fluid glucose concentration every 15 min throughout the event. Manual readings of interstitial glucose were also obtained from participants each lap using a hand-held scanning device linked to the CGM. This device has been validated against blood glucose readings as reported in an FDA report (FDA, 2016).

Participants body mass was obtained post-void, in minimal under clothing, 1 h prior to starting and after finishing (before any further food or fluid was consumed) to assess mass loss. A sub-set of participants ($n = 7$) wore a heart rate monitor (HRM) and/or GPS device to track intensity in relation to HRmax and VO_{2max}. All participants wore a timing chip which recorded lap times and total distance covered.

Nutritional Analysis

All dietary intakes were analysed by a Registered Dietitian (EK), using Nutritics dietary analysis software (Nutritics Limited, Dublin, Ireland). Any food/fluids not available in the database were identified from manufacturer's labels/information and added to the database. Dietary intake was analysed from 48-h pre-race weighed intake sheets to provide two sets of dietary data: (1) mean 24-h intake (pre-race diet); and (2) intake for 1–4 h prior to race start (pre-race meal). A third set of dietary intake data came from analysis of each participant's in-race diet. All macronutrients and energy intake were calculated (total, per hour, and per kg), total fluid (from foods and fluids), sodium, and caffeine. Individual intake of sugars (glucose, galactose, sucrose, fructose, maltose, and lactose) and starch or maltodextrin were also examined to investigate MTC intake. Fibre and oligosaccharide components were excluded from the individual carbohydrate analysis. Dietary intake was extrapolated from data per lap to represent nutrient intake per hour.

To investigate participants' MTC intake, individual carbohydrate components were grouped according to those absorbed *via* the Sodium-Glucose co-transporter 1 [SGLT1; glucose, galactose, 0.5 x sucrose (glucose component), maltose, lactose + starch (to include maltodextrin)], and Glucose Transporter 5 [GLUT5; fructose + 0.5 × sucrose (fructose component)] transporters.

Statistical Analysis

The main dependent variable was total distance covered, and this was regressed to independent variables including dietary intakes and other factors (VO_{2max}, mass, BMI, and gender). Pearsons correlation coefficients, linear regression analysis, and

independent *t*-tests were used to establish any associations between pre-race diet, pre-race meal, in-race diet, fitness, and ultra-running experience and distance achieved. Preliminary analyses were performed to ensure there was no violation of the assumption of normality, linearity, and multicollinearity. For independent *t*-tests, the sample was grouped according to G24 pre-race diet CHO·kg^{-1}: those who consumed ≥5 g·kg^{-1} per 24-h ($n = 3$) and those who consumed <5 g·kg^{-1} ($n = 13$). Five grams per kg was selected as the lower-level recommendation for moderate exercise (Burke et al., 2011). Another divide was made with G24 in-race CHO intake, grouping the sample into those who consumed ≥40 g·h^{-1} ($n = 6$) and <40 g·h^{-1} ($n = 12$), in line with previous hourly in-race intake of amateur ultra-runners (Stellingwerff, 2016). Statistical significance was set at $p < 0.05$, and effect sizes were measured using Hedges *g* with values of 0.2 considered a small effect, ~0.5 considered a medium effect, and > 0.8 a large effect. For correlation coefficients, >0.5 and >0.7 were used to represent moderate and strong associations, respectively. A standard multiple regression analysis was performed to assess the ability of pre-race CHO and in-race CHO intake to predict race distance. Data are reported as mean (SD). Data were analysed using SPSS (IBM SPSS Statistics for Windows, Version 23).

RESULTS

The average temperature for G24 was 16 ± 4°C (19°C maximum, 10°C minimum) with zero precipitation.

Race Distance and Intensity

The leading male and female of the G24 study group covered 110.3 (177.5 km) and 108.2 miles (174.1 km), respectively. The mean (range) distance covered by participants was 80.6 ± 15.7 (48.0–110.0) miles [129.7 ± 25.3 (77.2–177.5) km]. Ten of the participants continued moving for the full 24-h, six stopped to sleep (for between 3 and 8 h), and two were unable to continue (one female stopped after 12 h due to injury, one male after 19 h due to gastrointestinal issues). The spread of study participants ($n = 18$) within the race population was representative of participants across the field of competitors (total entrants $n = 89$; **Figure 1**).

The sub-set of participants ($n = 7$) who wore HRMs/GPS devices throughout the event exercised at a mean intensity of 68 ± 5% HR_{max}, equating to approximately 45 ± 17% VO_{2max}. (**Table 1**), thus representing low to moderate intensity exercise. Participants' ($n = 15$) VO_{2max} was positively associated with distance covered ($r = 0.58$; $p = 0.02$). To investigate VO_{2max} and distance further a linear regression was calculated, and a significant regression equation was observed [F (1,13) =6.73, $p = 0.02$] with an R^2 of 0.341.

Pre-race Diet

Sixteen of the $n = 18$ participants completed adequate pre-race dietary monitoring. Participants' mean consumption of CHO·kg^{-1} over the 2 days pre-event was 4.0 ± 1.4 g·kg^{-1} per 24-h, contributing 42 ± 9% to total energy (**Table 1**). A strong, positive association was identified between race distance and mean total CHO ingested in the pre-race diet ($r = 0.78$; $p < 0.01$) and also for CHO·kg^{-1} in the pre-race diet ($r = 0.70$; $p < 0.01$). Moderate positive associations were also observed between distance and mean energy intake ($r = 0.57$; $p < 0.05$) and energy·kg^{-1} ($r = 0.56$; $p < 0.05$). No associations were found between pre-race fluid intake and distance. An independent samples *t*-test identified a significant difference in distance covered [98.5 ± 18.7 miles (158.5 ± 30.1 km)] vs. [78.0 ± 13.5 miles (125.5 ± 21.7 km)] by participants who consumed ≥5 g·kg^{-1} CHO per 24 h in their 2-day pre-race diet vs. those who consumed <5 g·kg^{-1}; t (14) = 2.23, $p = 0.042$, Hedges' $g = 1.43$. This was also true for CHO·kg^{-1} 1-day pre-race [92.8 ± 15.9 miles (149.3 ± 25.6 km) vs. 75.2 ± 12.9 miles (121.0 ± 20.8 km)], respectively; t (14) = 2.42, $p = 0.03$, Hedges' $g = 1.32$.

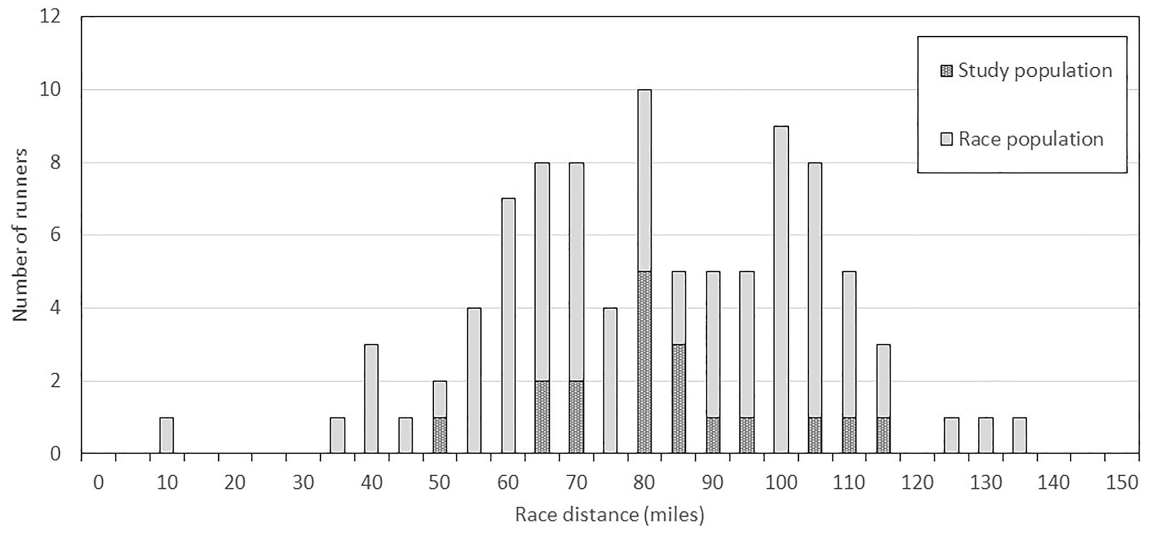

FIGURE 1 | Histogram showing the spread of study participants across the G24 race population highlighting a representative sample of participants.

Pre-race Meal

All participants consumed food and fluid in the 1–4 h pre-race with a mean energy intake of 878 ± 349 kcal and CHO intake of 1.5 ± 0.7 g.kg^{-1} contributing 49 ± 15% of total energy intake. Mean fluid intake was 940 ± 397 ml. A moderate positive association was observed between race distance and total CHO in the pre-race meal, $r = 0.68$, $p < 0.01$ and also with CHO. kg^{-1}, $r = 0.57$ $p < 0.05$.

In-Race Diet

Energy Intake

Total energy consumed, by participants was 3,907 ± 1,658 kcal with a mean of 179 ± 63 kcal·h^{-1}. Energy intake composed of 69% CHO (721 ± 326 g), 8% protein (78 ± 49 g), and 21% fat (90 ± 55 g). A wide variety of foods, fluids, and commercially available sports-nutrition products were consumed in-race (**Table 2**). Fifteen participants (83%) consumed sports-nutrition products (gels/bars/sports-drinks). In this sub-section, sports-nutrition products contributed 22 ± 14% of total energy and 28 ± 15% of total CHO intake. There were no differences in energy, macronutrients, sodium, or caffeine intake between genders. A moderate positive association was observed between distance achieved and total energy intake during the event when corrected for body mass ($r = 0.52$; $p = 0.028$).

TABLE 2 | Nutritional composition (mean ± SD) of all foods and fluids consumed by participants over the 24-h race duration (in-race diet), and actual foods, fluids, and sports-nutrition products consumed by $n = 18$ participants during the Glenmore 24 trail race.

Variable	All Participants ($n = 18$)	Males ($n = 11$)	Females ($n = 7$)
Total Energy (Kcal)	3,907 ± 1,658	4,407 ± 1,432	3,123 ± 1,788
Energy (Kcal.h^{-1})	179 ± 63	201 ± 55	144 ± 63
Energy (Kcal.kg^{-1})	52 ± 23	54 ± 17	50 ± 33
Energy-1st 12 h (Kcal.h^{-1})	207 ± 86	236 ± 73	162 ± 90
Energy-2nd 12 h (Kcal.h^{-1})	148 ± 54	156 ± 58	133 ± 49
Total CHO (g)	721 ± 326	828 ± 288	551 ± 328
CHO (g.h^{-1})	33 ± 12	38 ± 11*	26 ± 12
CHO – 1st 12 h (g.h^{-1})	39 ± 18	45 ± 16	30 ± 18
CHO – 2nd 12 h (g.h^{-1})	26 ± 9	28 ± 8	22 ± 8
CHO (g.kg^{-1})	9.6 ± 4.5	10.1 ± 3.3	8.8 ± 6.1
Total Protein (g)	78 ± 49	80 ± 46	73 ± 57
Protein (g.h^{-1})	3.6 ± 1.9	3.6 ± 1.8	3.5 ± 2.2
Total Fat (g)	90 ± 55	98 ± 55	78 ± 57
Fat (g.h^{-1})	4.1 ± 2.3	4.5 ± 2.4	3.6 ± 2.2
Total Fluid [food and drinks (ml)]	6,920 ± 2,004	8,047 ± 1,461*	5,149 ± 1,352
Fluid (ml.h^{-1})	326 ± 92	371 ± 73*	255 ± 76
Total sodium (mg)	4,217 ± 2,241	4,589 ± 1,907	3,633 ± 2,741
Sodium (mg.h^{-1})	195 ± 95	212 ± 88	169 ± 105
Sodium (mg.kg^{-1})	56.3 ± 32.7	56.3 ± 22.9	56.2 ± 46.4
Total Caffeine (mg)	247 ± 141	287 ± 133	184 ± 139
Caffeine (mg.h^{-1})	11.4 ± 6.7	13.4 ± 6.8	8.2 ± 5.7
Caffeine (mg.kg^{-1})	3.2 ± 1.6	3.5 ± 1.5	2.7 ± 1.7
FOODS, FLUIDS, and SPORTS-NUTRITION PRODUCTS CONSUMED BY G24 PARTICIPANTS IN-RACE			
Foods (savoury) consumed	Mixed nuts, bagels, quiche, corned beef hash, soup, porridge pots, pasta pots and sachets, pot noodles, Weetabix and milk, fish and chips, pork pies, ryvita, avocado, stew, cheese, ham, bread and butter, crisps, croissant, pizza, smoked sausage, fried eggs, butteries, rice cakes, and peanut butter.		
Foods (sweet) consumed	Fruit–Dried, fresh, tinned in juice, fruit and jelly pots, flapjack, rice pudding, custard pots, sweets (boiled, chewy, jelly, and fudge), mints, chocolate bars, iced buns, Eat Natural bars, iced buns, dextrose tablets, cereal bars and biscuits (chocolate and plain), cereal, yoghurt, baby food sachets, muffins, and malt loaf.		
Fluids (non-sports)	Beetroot juice, Coconut water, water, tea, coffee, cola, Irn Bru, milkshake, hot chocolate, Dioralyte, ginger ale, Sprite, Innocent smoothies, Red Bull, milkshake, Sugar free diluting juice, and homemade energy drink (13% CHO solution: maltodextrin/glucose/fructose).		
Sports nutrition products	Gels, sports beans, Shot Bloks, Tailwind, Lucozade Sport, Gatorade, SIS isotonic, Clif bars, Chia Charge bars, Power bar Energise, Powerade, Nuun electrolyte, High 5 zero, Protein shakes, Mountain Fuel Extreme, and S!Caps.		

*Data are mean ± SD. *Mean value was significantly different to female runners ($p < 0.05$).*

Total Carbohydrate Intake and Interstitial Glucose Profiles

Mean hourly intake of CHO for all participants was 33 ± 12 g·h^{-1}. During the event, CHO intake peaked in hour 5 at 49 ± 6 g with significantly lower amounts consumed in hours 1, 17, 19, 20, 22, and 24 (**Figure 2**). Individual hourly consumption varied widely with 67% of participants ($n = 12$) taking between 60 and 90 g·h^{-1} on at least one occasion, and 17% ($n = 3$) taking in excess of 100 g·h^{-1} at least once. There was a significant difference in hourly CHO intake [38 vs. 26 g; t (16) = 2.27, $p = 0.037$] between males and females, respectively, but not when corrected for body mass (0.5 ± 0.1 vs. 0.4 ± 0.2 g·kg^{-1}·h^{-1}).

Fourteen participants retained CGM sensors for the entire race duration. No association was observed between mean interstitial glucose concentration and dietary CHO intake, or with race distance. Glucose profiles were variable throughout the event. Average hourly glucose concentration for all participants ranged from 3.1 to 13.4 mmol·l^{-1}, indicating times of both hypo and hyperglycaemia during the race. Overall mean glucose for participants who retained CGM devices for the full race duration was 6.9 ± 1.2 mmol·l^{-1} (**Figure 2**). Unpublished data from our own laboratory indicate that interstitial glucose readings are elevated above blood glucose concentration during moderate intensity exercise by ~2 mmol·l^{-1} (Wilson and Galloway, unpublished observations) and readings can be influenced by exercise (FDA, 2016).

Multiple Transportable Carbohydrate Intake

Participants consumed CHO at a mean rate of 0.6 ± 0.2 g·min^{-1}, less than the 1 g·min^{-1} needed to saturate SGLT1 transporters (Jeukendrup, 2010). Estimated intake of individual CHO components was as follows: starch (12.7 ± 7.1 g·h^{-1}); sucrose (6.2 ± 4.3 g·h^{-1}); glucose (3.5 ± 1.5 g·h^{-1}); fructose (3.6 ± 2.3 g·h^{-1}); galactose (0.01 ± 0.03 g·h^{-1}); maltose (0.4 ± 0.4 g·h^{-1}); and lactose (0.9 ± 0.9 g·h^{-1}) with fibre and oligosaccharide likely making up the remaining amount. Mean intake ratio of glucose: fructose

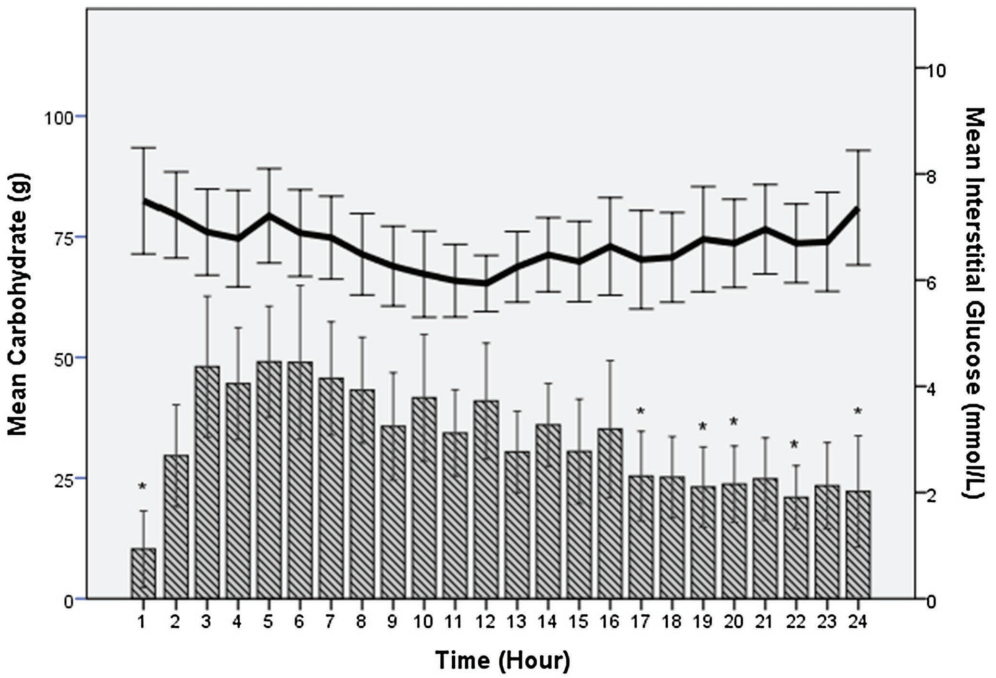

FIGURE 2 | Hourly mean interstitial glucose concentration (continuous line/right axis) and hourly mean carbohydrate (CHO) intake (bars/left axis) for participants during the G24 ultra-endurance race. Mean hourly CHO intake peaked at 49 ± 6 g in hour 5. *indicates significantly different hourly intakes to peak value. No significant differences were observed over time for mean interstitial glucose concentration.

equivalents were 3 ± 2:1, (range 2:1–8:1). Estimated mean sugars available for absorption per hour *via* SGLT1 and GLUT5 transporters were 21 ± 9 g, (range 8–39 g) and 7 ± 3 g, (range 2–15 g), respectively. Hourly transport capacity was not reached for either carbohydrate transporter with a notional remaining capacity for participants of around 39 g·h^{-1} for SGLT1 and around 22 g·h^{-1} for GLUT5.

Carbohydrate Dose and Distance

Moderate positive correlations were observed between distance and in-race total CHO, $r = 0.65$ and total CHO·kg^{-1}, $r = 0.64$ (both $p < 0.01$). A significant difference in distance was observed between those consuming ≥40 g·h^{-1} [92.2 ± 13.9 miles (148.4 ± 22.4 km)] and <40 g·h^{-1} [74.7 ± 13.5 miles (120.2 ± 21.7 km)]; t (16) = 2.56, $p = 0.021$, Hedges' $g = 1.28$. A moderate positive relationship between in-race CHO (g·h^{-1}) and distance was observed ($r = 0.57$, $p = 0.01$; **Figure 3**).

A significant regression equation was found [F (2,13) = 12.2, $p = 0.001$], with an R^2 of 0.653. In this sample of participants, both pre-race diet and in-race CHO variables were significant predictors of race distance. Participants' race distance increased by 6.6 miles (10.6 km), 95% CI, 2.16–11.1 miles (3.5–17.9 km) for each 1 g·kg^{-1} CHO in pre-race diet and 1.5 miles (2.4 km), 95% CI, 0.19–2.86 miles (0.3–4.6 km) for each 1 g·kg^{-1} CHO in-race when other variables remain constant. Although significant correlations with distance were found between $V0_2$max, pre-race energy intake, pre-race meal CHO, and in-race energy·kg^{-1}, these variables were not significant predictors of distance in the final regression model.

Ultra-Experience and Diet/Distance

For the pre-race diet, moderate positive correlations were observed between years of ultra-running and mean CHO intake, $r = 0.57$ and mean CHO.kg^{-1}, $r = 0.53$ (both $p < 0.05$). Number of ultras completed was also positively associated with pre-race diet CHO/kg, $r = 0.62$ ($p < 0.05$). For the in-race diet, no significant associations were observed between ultra-experience and CHO consumption but interestingly there was a moderate negative association between number of ultras completed and energy intake per hour $r = -0.50$ ($p < 0.05$). No association was found between ultra-running experience and distance achieved.

Fluid Intake/Dehydration:

Fluid intake (foods and fluids) per hour [371 vs. 255 ml; t(16) = 3.25, $p = 0.005$] and total fluid intake [8,047 vs. 5,149 ml; t(16) = 4.22, $p = 0.001$] were significantly different between males and females (**Table 2**), but this was not the case when corrected for body mass (4.6 ± 1.1 vs. 3.9 ± 1.2 ml·kg^{-1}·h^{-1}/100 ± 24 vs. 79 ± 26 ml·kg^{-1}). Body mass loss over the race was 2.8 ± 2.6% and no association was found between fluid intake and race distance covered.

DISCUSSION

The aims of this study were to observe the diet of ultra-endurance runners prior to and during a field-based 24-h trail race, to compare observations with current recommendations for CHO intake and to determine intake of MTC's. The main

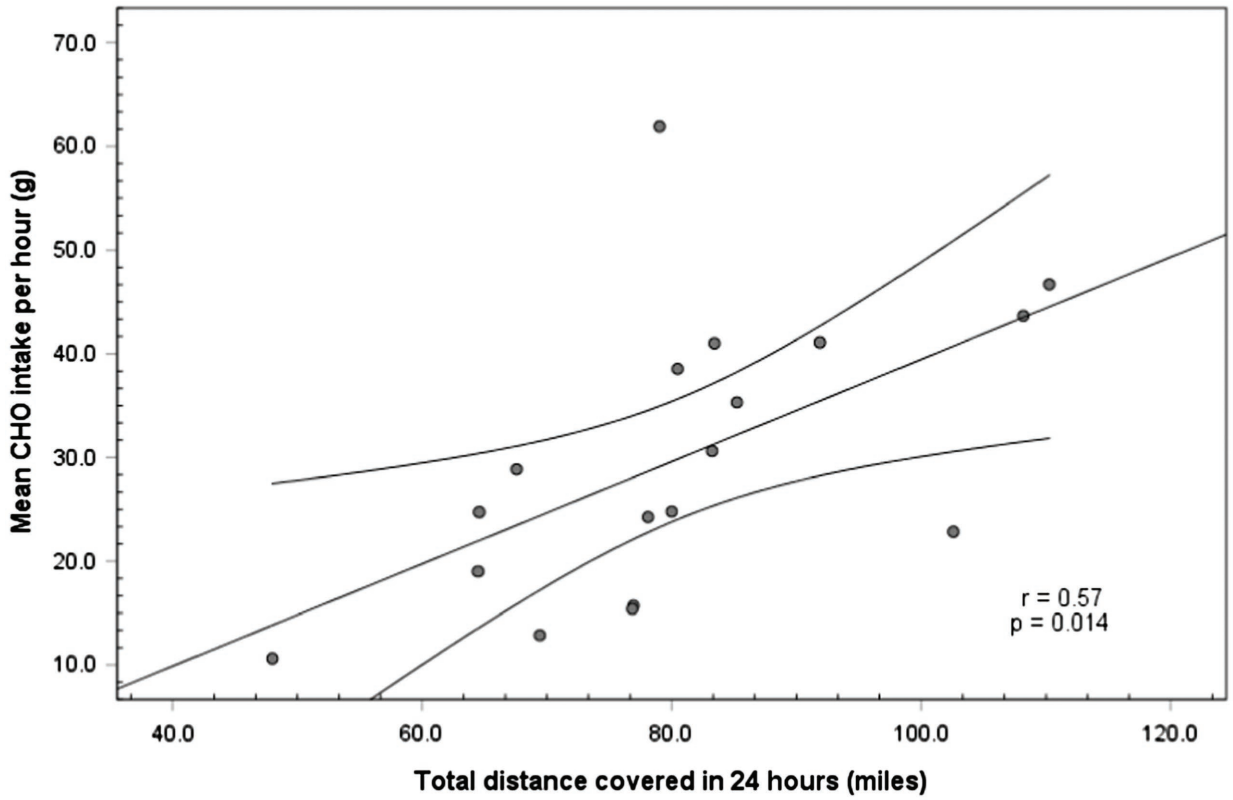

FIGURE 3 | Total race distance covered vs. CHO intake ($g \cdot h^{-1}$) during 24 h races for G24 runners showing a moderate positive association.

findings were that CHO intake was lower than current recommendations for pre-race diet and in-race intake. CHO intake was significantly related to distance achieved in the event and, based on CHO sources ingested, runners had capacity to increase their intake of MTC to help them achieve recommended CHO intakes. For both pre-race diet and in-race intake, those who consumed more CHO per kg body mass achieved greater overall race distances.

Pre-race Diet and Pre-race Meal

The pre-race diet observations demonstrate that athletes were only meeting the fuelling recommendations for short duration low intensity activities. Thus, promoting a higher CHO intake over 48-h pre-race could significantly influence race performance, although a direct cause and effect relationship cannot be confirmed from the present study due to the lack of specified intervention and control groups. Additional CHO in the region of 1–2 g/kg per 24-h in the pre-race diet represents an initial realistic and achievable adjustment to CHO intake for these ultra-runners. However, an increase of this magnitude would only take these athletes into the 5–7 g/kg per 24-h range, just more than half of the recommended CHO intake for fuelling very prolonged moderate intensity exercise events. In the present study, although ultra-running experience was associated with a higher CHO intake, it is not known if pre-race CHO intake was higher than habitual CHO intake, or if participants actively carbohydrate-loaded, but their intake was below current recommendations. These pre-race CHO intake observations are similar to those of competitors before an 85-mile mountain-marathon who consumed $4.5 \pm 1.1\ g \cdot kg^{-1} \cdot day^{-1}$ pre-event (Mahon et al., 2014), where 86% of participants planned to increase CHO over these final days. Atkinson et al. (2011) reported an intake of $5.0 \pm 1.9\ g.kg^{-1}$ CHO the day before a marathon with 68% of participants claiming to have adopted "high-carbohydrate diets." Atkinson et al. (2011) also observed that CHO content in the pre-race diet was an important predictor of marathon finishing time. Collectively, these observations could indicate that individuals do not know how to carbohydrate-load effectively, or that they have a low habitual intake of CHO. The current study supports the need for education on CHO loading strategies to help ultra-distance runners achieve more beneficial CHO intakes in their pre-race diet (Costa et al., 2014; Beck et al., 2015). For the pre-race meal, the athletes managed to meet current CHO intake guideline (Thomas et al., 2016) and intake during this 4-h pre-event period showed a significant association with race distance. However, an increased intake of CHO could still be achieved within the $1–4\ g.kg^{-1}$ recommendation. Therefore, it seems that a greater emphasis and education placed on meeting CHO intake guidelines within the pre-race diet/meal would be beneficial to their performance in ultra-running events.

In-Race Intake

In the present study, mean in-race CHO intake was in the 30–60 g·h^{-1} range for the runners, but fell short of guidance for up to 90 g·h^{-1}. Hourly in-race CHO intake was low compared to other studies on prolonged ultra-endurance running (12 h plus) including both amateur finishers (66 g·h^{-1}), non-finishers (42 g·h^{-1}; Stuempfle et al., 2011), elite-runners (71 g·h^{-1}; Stellingwerff, 2016) in 100-mile mountain races, runners in a 100-mile trail race (54 g·h^{-1}; Glace et al., 2002), and a 24-h track world championship (62 g·h^{-1}; Lavoué et al., 2020). However, Costa et al. (2014) recorded similar CHO consumption rates (37 ± 24 g·h^{-1}) to the present study for participants during the same G24 event in 2011/2012. Likewise, Martinez et al. (2018) recorded CHO intakes of 32 ± 15 g·h^{-1} in ultra-endurance mountain runners over three distances (27/41/70-miles). Lower intake (28 ± 17 g·h^{-1}) also was observed in the study by Mahon et al. (2014), which may have been due to runners carrying all food and fluids and wanting to minimise additional weight. CHO intake therefore appears to rarely reach 90 g·h^{-1} in these types of ultra-endurance running events.

Mean in-race CHO intake at 33 ± 12 g·h^{-1} in the present study would not be sufficient to saturate SGLT1 transporters and therefore intake rate would not be limiting to CHO absorption. In race CHO intake could be elevated through intake of a variety of CHO sources to push runners towards the 90 g·h^{-1} recommendation, with an increase of 10–20 g·h^{-1} from isotonic fluids, cereal bars, sports gels, incorporating maltodextrin or glucose, and fructose probably being achievable by most runners. As research knowledge builds on MTC use and more evidence emerges on ideal ratios of carbohydrate types or delivery methods for improved gut tolerance, ultra-runners would benefit from education around increasing CHO from a variety of sources into their race strategies.

Stellingwerff (2016) highlights consistent variation in CHO consumed in-race by elite (61 g·h^{-1}) and amateur ultra-runners (41 g·h^{-1}) when collectively comparing previous studies, highlighting that 20 g·h^{-1} difference can lead to substantial deficits in CHO and energy over events. Relating this to the present study, an additional 20 g·h^{-1} would amount to a difference of 480 g CHO and 1,800 kcals over 24-h. An intervention study on marathon runners demonstrated the effect of this CHO gap. Runners were grouped into those with an intervention target of 60 g·h^{-1} maltodextrin/glucose (actual intake 64.7 ± 12.3 g·h^{-1}) and those who chose CHO freely (actual intake 38.0 ± 17.5 g·h^{-1}). The intervention group demonstrated 5% faster finishing times (Hansen et al., 2014), suggesting that the extra CHO resulted in improved performance. Future intervention studies could investigate the performance effect of bridging this CHO gap in amateur ultra-runners.

Hourly energy and CHO intake fluctuated throughout the event, with lower intakes towards the end. The impact of fatigue on motivation to eat and drink was clear in the G24 runners. Experienced support crew is invaluable in helping runners to meet nutritional targets and cajole when psychologically low. This support crew can make the difference between achieving a successful outcome or not (Holt et al., 2014). Normal circadian variation also could be a factor in the decline in oral intake observed overnight (Serin and Acar Tek, 2019). Between 2 and 6 am, a circadian low is experienced, which results in a difficult time for ultra-endurance competitors. In G24, more participants (28%, $n = 5$) stopped to sleep during these hours than at other times. An interesting question would be to explore whether runners could train themselves to eat more during these hours, and whether additional food intake could influence their decisions to continue or rest, and ultimately impact upon distance achieved.

Analysis exploring the role of in-race CHO on race outcome demonstrated that those consuming ≥40 g·h^{-1} ran further than those consuming <40 g·h^{-1}. However, it is unlikely that these differences were due to CHO intake alone, as other factors such as VO_{2max} and years of ultra-marathon experience also are likely to impact on race outcome. Indeed, a moderate positive association between distance and VO_{2max} was observed in the present study suggesting that cardiovascular fitness is likely to be a confounder, with fitter runners running faster/further. Fitter/faster athletes also would have higher CHO requirements and/or be more aware of nutritional recommendations, meaning they would likely consume more CHO than slower athletes (Havemann and Goedecke, 2008).

Activity Intensity/Interstitial Glucose Concentration

A sustained intensity of 45 ± 17% VO_{2max} demonstrates that, in ultra-events, exercise intensity is low to moderate, but when sustained over 24 h this becomes a significant metabolic challenge. Other studies have reported low mean heart rates in ultra-endurance running events (Clemente-Suarez, 2015; Stellingwerff, 2016) and low pace (Glace et al., 2002; Clemente-Suarez, 2015; Ramos-Campo et al., 2016). It therefore could be suggested that in-race CHO recommendations for ultra-runners need not be high, given that endogenous fat stores will likely contribute significantly to energy requirements, and total CHO oxidation rates will be lower at lower intensities (Jeukendrup, 2014). However, an adequate amount of exogenous CHO is important to conserve muscle and liver glycogen and maintain blood glucose particularly under the challenging demands of a 24-h event. To achieve this without GI distress likely requires a good balance of MTC intake alongside other macronutrients to support total energy requirements.

Although no associations were observed between interstitial glucose levels and dietary intake, it was curious to see the variations in participants' glucose profiles. Mean glucose concentration was 7.2 mmol·l^{-1} initially, with a nadir of 5.9 mmol·l^{-1} mid-race, rising to 7.4 mmol·l^{-1} after 24 h. From **Figure 2** there appears to be an inverse relationship, with interstitial glucose concentration declining as CHO intake is higher over the first 12 h, and rising latterly as CHO intake declines. This could be a response to circadian hormonal control of glucose concentration. Ramos-Campo et al. (2016) tested runners' blood glucose pre (5.1 ± 0.5 mmol·l^{-1}) and post (5.8 ± 1.4 mmol·l^{-1}) a 54 km mountain race, showing little variation but no indication of how glucose levels responded during the race. Similar, steadier blood glucose concentrations than in the current study were

observed in runners before (5.0), during (5.4), and after (5.3 mmol·l^{-1}) a 100 mile trail race (Glace et al., 2002). To the researchers' knowledge, this is the first study to monitor interstitial glucose during a competitive ultra-endurance running event, with glucose readings reflecting the lag time between blood and interstitial glucose. Future studies using CGM devices should investigate corresponding changes in hormone concentrations such as insulin and cortisol, or monitor the effect of specific rates of CHO ingestion on glucose concentration to decipher the primary determinants of fluctuations.

Future Considerations

Whilst no firm guidance can be established from this study, the findings do support the importance of both pre-race and in-race CHO intake on performance in a 24-h race. Future research should test these experimentally under field-conditions using increased intake of MTC's, perhaps making use of newer products containing alginate hydrogel to deliver higher rates of MTC with minimal GI distress (Sutehall et al., 2018) when 'food fatigue' occurs in later stages of a 24-h race. In addition, investigating feeding strategies in ultra-endurance runners matched for VO_{2max}, would help to establish if increasing quantities of pre-race and in-race CHO result in performance improvements. It should be noted that intake of MTCs was difficult to calculate accurately in the present study due to the restricted proprietary nutritional information of specific sugar configurations in some sports-nutrition products. However, the current observations do support recommendations to increase CHO intake in preparation for, and during, ultra-endurance events, and provide insight into the range of carbohydrate sources that could be ingested to help meet target intakes of MTC's.

CONCLUSION

In this study, the amount of ingested CHO both during the pre-race diet and in-race was lower than current recommendations. Given the duration of the event, despite a low to moderate intensity of exercise, total energy requirements are very high. Therefore, ultra-endurance athletes need to consider ways to increase energy and CHO intake prior to and during these types of events. Our analysis suggests that this can most easily be achieved through increasing pre-race diet carbohydrate intake, and working on strategies to enhance intake of MTC's up to 90 g/h in-race. Strategies could include improved education on carbohydrate loading in the days prior to an ultra-endurance event and/or the incorporation of additional sports nutrition products composed of maltodextrin/fructose in-race. Making use of novel products containing alginate hydrogels, especially in the later stages of a 24-h event when dietary intake is most difficult could prove beneficial.

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 Stirling ethics committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EK and SG conceived the study, undertook data collection and analysis, and contributed to writing the manuscript. All authors contributed to the article and approved the submitted version.

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Heat Balance When Climbing Mount Everest

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Background: Mountaineers must control and regulate their thermal comfort and heat balance to survive the rigors of high altitude environment. High altitudes feature low air pressure and temperatures, strong winds and intense solar radiation, key factors affecting an expedition's success. All these climatic elements stress human heat balance and survival. We assess components of human heat balance while climbing Mt. Everest.

Materials and Methods: We calculated climbers' heat balance using the Man-ENvironment heat EXchange model (MENEX-2005) and derived meteorological data from the National Geographic Expedition's *in situ* dataset. Three weather stations sited between 3810 and 7945 m a.s.l. provided data with hourly resolution. We used data for summer (1 May–15 August 2019) and winter (16 October 2019–6 January 2020) seasons to analyze heat balance elements of convection, evaporation, respiration and radiation (solar and thermal).

Results: Meteorological and other factors affecting physiology—such as clothing insulation of 3.5–5.5 clo and activity levels of 3–5 MET—regulate human heat balance. Elevation above sea level is the main element affecting heat balance. In summer two to three times more solar radiation can be absorbed at the summit of the mountain than at the foot. Low air pressure reduces air density, which reduces convective heat loss at high altitude by up to half of the loss at lower locations with the same wind speed and air temperature.

Conclusion: 1. Alpinists face little risk of overheating or overcooling while actively climbing Mt. Everest, despite the potential risk of overcooling at extreme altitudes on Mt. Everest in winter. 2. Convection and evaporation are responsible for most of the heat lost at altitude. 3. Levels of physical activity and clothing insulation play the greatest role in counteracting heat loss at high altitude.

Keywords: heat balance, thermal stress, mountain bioclimate, altitude, extremes, mountaineering, Everest

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INTRODUCTION

High-altitude tourism and mountaineering is becoming ever more popular, especially on the world's highest mountains in the Himalayas. More than 47,000 climbers have participated in expeditions to 8,000 m peaks in the Himalayas since the first ascents in 1950 until 2021, and 19,000 of them have reached 8,000 m summits (Salisbury and Hawley, 2020). Rarely did more than 100 climbers a year attempt Himalayan peaks above 6,000 m between 1950 and 1969. Thereafter the numbers increased gradually and more than 1500 climbers a year have been attempting these peaks in the twenty-first

century (Salisbury and Hawley, 2007). Commercial climbing gained popularity in the early 1990s, when commercial expeditions contributed about 20% of all climbers attempting peaks over 6,000 meters; by 2006 climbers in commercial expeditions constituted almost 75% of attempts (Salisbury and Hawley, 2007). Two of the four most popular commercial climbing routes are on Mt. Everest and the number of climbers attempting this peak has risen 60% over the past 15 climbing seasons (Huey et al., 2020). Winter mountaineering is also gaining popularity (Benavides, 2021).

Knowledge of the Himalayan climate remains incomplete. Long-term weather stations do not exist and planners therefore lack extended observational series. Temporal meteorological observations are still made mostly during short climbing expeditions. The most important research on how Himalayan climate affects human physiology involves barometric pressure, air temperature and wind speed, the meteorological parameters that most limit human performance and survival at high altitudes. These parameters are derived from direct assessments at high altitude (West et al., 1983b; West, 1999), radiosonde data (West, 1996) and lately reanalysis data such as those from the US National Centers for Environmental Prediction (NCEP) (Kalnay et al., 1996; Moore and Semple, 2011) and the ERA5 from the European Centre for Medium-Range Weather Forecasts (Hersbach et al., 2020; Matthews et al., 2020a; Szymczak et al., 2021a,b). The state-of-the-art ERA5 data have a spatial resolution of 0.25° (about 28 km at the equator) at hourly intervals. Meteorological variables are interpolated from nearby stations and provide only an approximated picture of conditions on the mountain. A recent breakthrough project has provided detailed *in situ* values of many meteorological parameters with the installation of five automatic weather stations on the slopes of Everest by the National Geographic expedition in 2019 (Matthews et al., 2020a,b; National Geographic, 2021).

Barometric pressure determines the partial pressure of inspired oxygen (PiO_2) that is critical for physiological performance, maximum oxygen uptake (VO_2max), and the speed of vertical ascent at extreme altitude (West and Wagner, 1980; West et al., 1983a, 2007b; Bailey, 2001; Matthews et al., 2020a). Low air temperature and high wind speed mainly determine the risk of hypothermia and frostbite (Huey and Eguskitza, 2001). Firth et al. (2008) observed that severe weather is responsible for about 25% of fatalities above 7,000 m during ascents of Everest (Firth et al., 2008). Hypothermia is responsible for 16% of all deaths on Denali (McIntosh et al., 2008).

The levels and changes in barometric pressure and PiO_2 at different high-altitude locations has been explored extensively (West et al., 1983b; West, 1996; Matthews et al., 2020a,b; Szymczak et al., 2021a,b). Precise calculations of barometric pressure and PiO_2 at different altitudes have enabled analyses of how levels of hypobaric hypoxia affect humans in high-altitude expeditions (Grocott et al., 2010; Milledge, 2010; West, 2010) and in simulated conditions (Houston et al., 1987; Richalet et al., 1999).

Knowledge of the influence of hypobaric hypoxia on humans can be considered satisfactory, but hypothermic stress at high altitude has been studied only with simple parameters such as temperature, wind speed, wind chill temperature (WCT), and facial frostbite time (FFT) (Moore and Semple, 2011; Szymczak et al., 2021a,b). The standard equations for WCT (Osczevski and Bluestein, 2005) and FFT (Tikuisis and Osczevski, 2003) do not include variables that significantly determine high-altitude heat balance, such as solar radiation (Pugh, 1962) and air density (Huey et al., 2001). As air temperature, wind speed, WCT and FFT provide only general estimates of the hypothermic stress at high altitude, climbers need a more complete human heat balance analysis index, which would foster research on high-altitude thermal stress and enable more precise assessments of hypothermic stress (Szymczak et al., 2021a,b).

High-altitude climatic and physiological data that has recently become available enables more precise assessments of hypothermic stress by using models of human heat balance that include variables such as metabolic heat production, radiation balance, heat exchange by convection and conduction, and heat lost by evaporation and respiration. Our study thus aimed to analyze human heat balance at high altitudes during the active phase of climbing Mt. Everest in different weather conditions and seasons.

MATERIALS AND METHODS

Materials

We calculated heat balance characteristics of climbing Mt. Everest in different seasons using meteorological data from May 2019 to December 2020 collected by five automatic weather stations installed on the mountain between 3810 m (Phortse) and 8430 m (Balcony) (**Figure 1**) during a National Geographic expedition (Matthews et al., 2020a,b; National Geographic, 2021). The data included air temperature (Ta,°C), air vapor pressure (vp, hPa), relative air humidity (RH,%), mean wind speed (v, $m{\cdot}s^{-1}$), maximum wind speed (vmax, $m{\cdot}s^{-1}$), air pressure (ap, hPa), global solar radiation (Kglob, $W{\cdot}m^{-2}$), back (sky) longwave radiation (La, $W{\cdot}m^{-2}$), and outgoing ground longwave radiation (Lg, $W{\cdot}m^{-2}$) (**Tables 1**, **2**). The data represent the hourly average values of the measured meteorological parameters and the maximum wind speed.

The stations started and finished their measurements on different dates and recorded different variables (**Table 2**). The longest and most complete series of data were provided by stations in Phortse, Camp 2 and South Col. Fewer parameters were measured at Base Camp and at the highest station of Balcony, where the measurements ended on 20 January 2020. Balcony's wind speed measurements became unreliable after 25 October 2019 because of the extreme conditions and the anemometer barely indicated air movement from mid-December 2019. The station's air humidity sensor malfunctioned on 20 December 2019. Wind speeds recorded at the South Col station after 6 January 2020 are also doubted (Matthews et al., 2020b; **Table 2**).

Meteorological conditions clearly differed at the stations between May 2019 and May 2020 based on their altitudes. Mean air pressure ranged from about 355 hPa at the highest station (Balcony) to 646 hPa at the lowest (Phortse). Mean

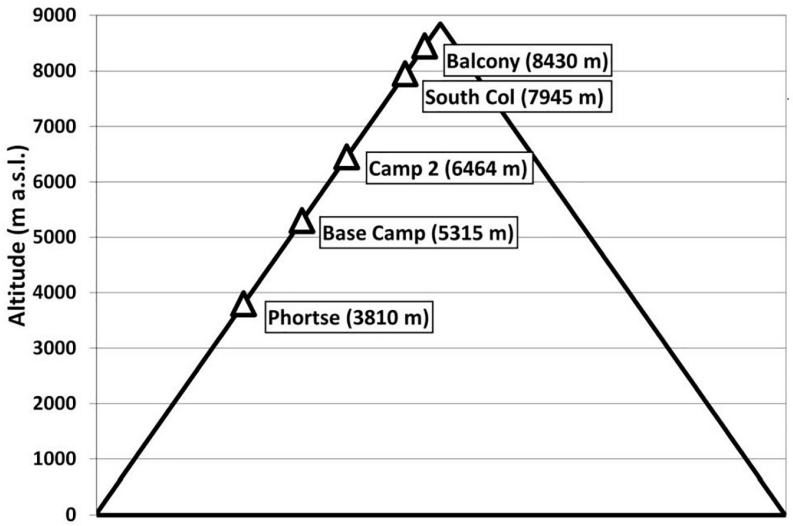

FIGURE 1 | Altitude and location of meteorological stations on Mt. Everest's Southeast Ridge Route installed during the National Geographic expedition.

air temperature ranged from –23°C at Balcony to 4°C at Phortse. Temperature extremes also ranged widely. The highest temperature (17°C) was registered at Phortse and the lowest (–45°C) at Balcony. Global solar radiation increased with altitude, with the highest momentary values ranging from 1,306 $W \cdot m^{-2}$ at Phortse to 1,692 $W \cdot m^{-2}$ at South Col. Wind speed was highest at the highest stations (South Col and Balcony). The highest mean hourly values of wind speed reached 26 $m \cdot s^{-1}$ at the exposed South Col site, which also recorded the most extreme wind gusts of about 44 $m \cdot s^{-1}$.

Large seasonal variations were recorded for air temperature, solar radiation and wind speed. The air temperature ranged over 15°C at Phortse from the warmest in August 2019 to the coldest in January 2020. The annual range at the South Col station was 19°C. Clear seasonal differences were observed in the total daily sum of global solar radiation, with the widest range of about 30 MJ at South Col between May and December 2019. Seasonal differences were smaller at other stations, ranging over about 20 MJ at Camp 2 and over 10 MJ at Phortse. Large seasonal changes in wind speed were recorded at the elevated stations: South Col showed the greatest variability in wind speed (SD = 6 $m \cdot s^{-1}$) and maximum wind speed (SD = 10 $m \cdot s^{-1}$) (**Table 3**).

Given the doubts about the accuracy of some observational data, mainly wind speed, and the large seasonal differences measured in most of the weather parameters, we selected data from three stations (Phortse, Camp 2, South Col) over periods representing summer and winter for our detailed analysis of heat balance and bio-meteorological indicators. We selected 1 May to 15 August 2019 to represent the summer season (Camp 2 from 8 May, South Col from 21 May) and 16 November 2019 to 6 January 2020 for the winter season. These periods correspond with the climbing and winter seasons in the Himalayas and Karakoram, so the dates have practical significance for climbers.

Significant intraseasonal variation of radiation and wind led to complex permutations of meteorological data and human heat balance, so we divided summer and winter season data into four broad weather ranges to improve the utility of our analysis: (1) cloudy and weak wind, (2) cloudy and strong wind, (3) sunny and weak wind, (4) sunny and strong wind.

Hourly average values of wind speed and solar radiation from the National Geographic weather stations (National Geographic, 2021) were averaged for each day and then season (summer, winter) for each station. Mean values of wind speed and global solar radiation calculated individually for each season and each station were adopted as thresholds separating the categories of wind speed (weak or strong) and solar radiation (cloudy or sunny). Daily values below the thresholds were categorized as weak (for wind speed) and cloudy (for solar radiation); those above the thresholds were categorized as strong and sunny. Each day at each station was then assigned into one of the four weather groups.

We averaged hourly values of the meteorological variables (v, Kglob, ap, Ta, vp, and RH) for the day and then for four weather categories for each station in each season (**Table 4**), then used these values in our calculations of the components of heat balance. In most cases these values differed significantly at p = 95%. At Phortse and Camp 2, weak and strong wind speed did not differ between cloudy and sunny days, in summer and in winter.

Methods

We used the Man-Environment Heat Exchange Model (MENEX-2005) (Błażejczyk, 1994, 2005a,b; Błażejczyk et al., 2012) to calculate the components of human heat balance. The model is sensitive to changes in basic meteorological elements, but unlike models such as the Universal Thermal Climate Index (UTCI) Fiala model (Fiala et al., 2012), the Munich Energy-balance

Model for Individuals (MEMI) (Hoppe, 1993) and the Klima-Michel-Model (KMM) (Jendritzky et al., 1990), MENEX-2005 accounts for the physical parameters of air that characterize the high-altitude environment: air density and air oxygen content at different altitudes above sea level (Błażejczyk et al., 2012). The model can also be used in different bioclimatic applications such as recreation, tourism, climatotherapy, health prophylaxis, and urban climatology, or in thermophysiological applications such as working conditions and sports physiology.

The MENEX_2005 model calculates the basic components of heat balance under given environmental conditions in non-stationary conditions. The method of calculating individual components of human heat balance are described by Błażejczyk and Matzarakis (2007) and Błażejczyk and Kunert (2011) and in our **Annexure**.

The general equation of heat transfer between humans and the environment used in the MENEX_2005 model is:

$$M + Q + C + E + Res = S \quad (1)$$

where: M is metabolic heat production, including basic metabolic rate (BMR) and metabolism related to physical activity; Q is radiation balance in humans; C is convective heat exchange; E is evaporative heat loss; Res is respiration heat loss; S is heat transfer balance or changes in the body's heat content. Radiation balance in humans is the sum of absorbed solar radiation (R) and net long-wave radiation (L) (**Figure 2**). All heat fluxes are expressed in $W \cdot m^{-2}$. The model will not account for heat losses to conduction because of the low values of this heat flux in a moving, upright human.

The model's inputs include meteorological and physiological variables. The meteorological information it requires include air temperature (Ta,°C), wind speed (v, $m \cdot s^{-1}$), water vapor pressure (vp, hPa), relative air humidity (RH,%), atmospheric pressure (ap, hPa) and total solar radiation (Kglob, $W \cdot m^{-2}$). Meteorological parameters are estimated at the height of a standing person's torso, about 1.2 m above ground level (Jendritzky et al., 2012). The physiological data the model requires include metabolic heat production (M, $W \cdot m^{-2}$), thermal insulation of clothing (Icl, clo), clothing albedo (ac,%), speed of movement (v', $m \cdot s^{-1}$), average skin temperature (Tsk,°C) and skin moisture content (w, without dimension). Physiological parameters such as average skin temperature and skin moisture content were calculated using empirical formulas (**Annexure**). We used values for these variables that were appropriate to the conditions we analyzed: metabolic heat production 190 and 290 $W \cdot m^{-2}$; clothing insulation of 3.5, 4.5, and 5.5 clo; albedo equal to 30; and an average speed of movement while climbing of 0.05 $m \cdot s^{-1}$.

The daily energy expenditure of climbers on high-altitude expeditions depends on altitude. Energy expenditure averages about 14.7 MJ in climbs between 2,500 and 4,800 m (Westerterp et al., 1992), and ranges from 13.6 to 20.6 MJ on Himalayan 8,000 m peak expeditions while climbing above 5,000 m (Pulfrey and Jones, 1985; Westerterp et al., 1992; Reynolds et al., 1999; West et al., 2007c). The steeper slopes that climbers encounter at higher elevations, which require more effort, might partially explain the higher energy expenditures at these altitudes (Watts et al., 1999; Bertuzzi et al., 2007; Ainsworth et al., 2011; Sas-Nowosielski and Wycislik, 2019). BMR is the largest component of daily energy expenditure at high altitude because periods of intense activity seldom last long (West et al., 2007c). Based on the experience of climbers in the Himalayas, we assumed that in a typical day climbers spend 8 h climbing and 16 h resting and sleeping. BMR of acclimatized alpinists at 5,800 m is 10% higher than at sea level (Gill and Pugh, 1964). We estimated climbers' daily energy expenditure at 15 MJ for locations at or below the Everest base camp (Phortse—3810 m, Base Camp—5,315 m) and 20 MJ for higher locations (Camp 2—6,464 m

TABLE 1 | List of abbreviations in alphabetical order.

ac, clothing albedo (%)
ap, air pressure (hPa)
BMR, basic metabolic rate ($W \cdot m^{-2}$)
C, convective heat exchange ($W \cdot m^{-2}$)
E, evaporative heat loss ($W \cdot m^{-2}$)
Epot, potential values of evaporative heat loss ($W \cdot m^{-2}$)
FFT, facial frostbite time (min)
h, height of the Sun (°)
hc, coefficient of heat transfer by convection ($K \cdot W^{-1} \cdot m^{-2}$)
hc', coefficient of heat transfer by conduction within clothing ($K \cdot W^{-1} \cdot m^{-2}$)
he, coefficient of heat transfer by evaporation ($hPa \cdot W^{-1} \cdot m^{-2}$)
Icl, thermal insulation of clothing (clo)
Ire, coefficient reducing convective and radiative heat transfer due to clothing (dimensionless)
Ie, coefficient reducing evaporative heat transfer due to clothing (dimensionless)
Kglob, total global solar radiation ($W \cdot m^{-2}$)
Kt, global solar radiation of the cloudless sky ($W \cdot m^{-2}$)
L, net long-wave radiation ($W \cdot m^{-2}$)
La, reverse radiation of the atmosphere, back (sky) longwave radiation ($W \cdot m^{-2}$)
Lg, thermal radiation emitted by the surface, outgoing ground longwave radiation ($W \cdot m^{-2}$)
Ls, radiation emitted by the surface of the body/clothing ($W \cdot m^{-2}$)
M, metabolic heat production ($W \cdot m^{-2}$)
Mrt, mean radiant temperature (°C)
Ov, oxygen volume ($g \cdot m^{-3}$)
PiO_2, partial pressure of inspired oxygen (hPa)
Q, radiation balance ($W \cdot m^{-2}$)
R, absorbed solar radiation ($W \cdot m^{-2}$)
Res, respiration heat loss ($W \cdot m^{-2}$)
RH, relative humidity of air (%)
S, heat transfer balance or changes in the body's heat content ($W \cdot m^{-2}$)
SW, water loss due to sweating ($g \cdot h^{-1}$)
Ta, air temperature (°C)
Tsk, average skin temperature (°C)
w, degree of skin moisture (dimensionless)
WCT, wind chill temperature (°C)
v, mean wind speed ($m \cdot s^{-1}$)
v', speed of movement ($m \cdot s^{-1}$)
vmax, maximum wind speed ($m \cdot s^{-1}$)
VO_2max, maximum oxygen uptake ($mlO_2 \cdot kg^{-1} \cdot min^{-1}$)
vp, water vapor pressure in the ambient air (hPa)
vp', vapor pressure equal to 5% of RH (hPa)
vps, water vapor pressure on the surface of the skin (hPa)

and South Col—7,945 m). Given these assumed daily energy expenditures, the characteristics of a typical climbing day, and a 10% increase of BMR at altitude, we calculated the values of metabolic heat production during active climbing to be 3 METs (≈190 $W \cdot m^{-2}$) for lower altitudes and 5 METs (≈290 $W \cdot m^{-2}$) for higher altitudes.

The thermal insulation of clothing used when climbing in the Himalayas was adopted on the basis of ISO 9920 (2007) and ISO 11079 (2007), Havenith's research (2010) and research conducted during the creation of the UTCI index (Havenith et al., 2012). The clothing insulation values we adopted were verified by IREQ model (Holmér, 1998) and information from participants in Himalayan expeditions organized by Polish Mountaineering Association. Clothing with 3.5 clo insulation is suitable mainly for the summer season in areas with relatively high air temperature, so we assumed it was enough to sustain thermal balance while climbing lower areas of Everest: Phortse (summer and winter) and Camp 2 (summer). Thick clothing with 4.5 clo insulation is mainly used in winter and when air temperatures drop below –15°C, as at Camp 2 in winter. Clothing with a thermal insulation of 5.5 clo is used in the winter season while climbing in the sub-peak zone of Mt. Everest, such as South Col, because of the extremely low temperatures and high wind speeds.

We determined human heat balance at different altitudes by using the values of heat transfer fluxes (convective, evaporative and respiration heat losses, absorbed solar radiation, and net long-wave radiation) and the water lost to sweating. We based our calculations of these variables on mean daily values of meteorological parameters that we calculated for each station, season, and weather category.

The characteristics of the human heat balance were complemented by oxygen volume (Ov, $g \cdot m^{-3}$). Oxygen volume determines the weight of oxygen in the air, which depends on air temperature, water vapor pressure, and atmospheric pressure, which changes with altitude above sea level. Oxygen volume is a bio-meteorological indicator used to assess the load on the respiratory system (Wojtach, 2003; Lecha Estela, 2018). Oxygen volume was calculated using the Błażejczyk and Kunert's (2011) equation.

$$Ov = [80.51 \cdot ap/(Ta + 273)] \cdot (1 - vp/ap) \quad (2)$$

Statistical Analysis

The statistical significance of the different heat balance characteristics calculated for each station, season and type of weather was verified with Stragraphics Centurion XVI, version 16.2.04, at the 95% confidence level.

RESULTS

General Characteristics of Human Heat Balance Components

Given the average values of the characteristic human heat balance for the observation period (1 May 2019 to 6 January 2020) that we calculated for various combinations of metabolic heat production and clothing insulation, the largest statistically significant differences between the stations involved convective heat loss, which increased with altitude. At the highest station of South Col, convective heat loss was about 4–5 times larger than at Phortse. Net long-wave radiation and respiration heat loss also increased with altitude. The flux of absorbed solar radiation increased with altitude and had a positive value, causing heat gain. Little heat was absorbed in this way, however.

Altitude had no significant effect on the intensity of evaporative heat loss and the amount of water lost to sweating: these variables depended on the level of physical activity. With a metabolic heat production of 290 $W \cdot m^{-2}$ they were significantly 1.5–2 times greater than at 190 $W \cdot m^{-2}$. Increased physical activity also resulted in more respiration heat loss. Physical activity had little effect on the amount of heat lost to convection, net long-wave radiation or absorbed solar radiation: these variables depended on the degree of clothing insulation. Clothing with an insulation of 4.5 clo was significantly more effective in protecting the body against losses to convection and long-wave radiation, but also significantly reduced the amount of absorbed solar radiation (**Table 5**).

The structure of heat loss fluxes changed with increased altitude. Heat lost to evaporation clearly dominated at the altitude of Phortse and Camp 2, where they reached 40–60%, depending on clothing insulation and metabolic heat production. At the highest station of South Col the convection flux contributed significantly more. Convection accounted for 41% of heat loss at a clothing insulation of 3.5 clo with metabolic heat production of 190 $W \cdot m^{-2}$. With increased effort (M = 290 $W \cdot m^{-2}$) and with thicker insulation (4.5 clo), convective heat loss at South Col (31%) was similar to evaporative heat loss (38%) (**Table 5**).

Components of Heat Balance

The components of heat balance with the clothing insulation and physical activity we assumed were clearly differentiated by the altitude above sea level, the season and the type of weather. When considering days with different weather types in summer (1 May-15 August 2019) and winter (16 October 2019 to 6 January 2020) the greatest variation occurs in convection. Convection ranged from –16 $W \cdot m^{-2}$ at Phortse in summer with cloudy and weak wind to –182 $W \cdot m^{-2}$ at South Col in winter with cloudy and strong wind. Net long-wave radiation in humans ranged from about –15 $W \cdot m^{-2}$ at Phortse in both seasons and in all-weather categories to –38 $W \cdot m^{-2}$ at South Col in winter with cloudy and strong wind. Absorbed solar radiation values ranged from 2.8 $W \cdot m^{-2}$ at Phortse in summer with cloudy and weak wind to 11.1 $W \cdot m^2$ at South Col in summer with sunny and strong wind. The evaporative heat loss differed clearly between Phortse at about –53 $W \cdot m^{-2}$ and Camp 2 and South Col at about –93 $W \cdot m^{-2}$. These large differences resulted from the metabolism values we adopted of 190 $W \cdot m^{-2}$ for Phortse and 290 $W \cdot m^{-2}$ for Camp 2 and South Col. The amount of water lost by sweating, which ranged from 135 to 144 $g \cdot h^{-1}$ at Phortse to 240–243 $g \cdot h^{-1}$ at Camp 2 and South Col resulted from evaporative heat loss and metabolic heat production (**Table 5**).

The contrasts within components of heat flux in the weather categories we compared increased with higher altitude. For example, convection was higher during strong wind days at

TABLE 2 | Characteristics of the meteorological stations installed on Everest by the National Geographic expedition.

Station	Latitude (°N)	Longitude (°E)	Elevation (m a.s.l.)	Period of observations start → end	Measured variables
Phortse	27.8456	86.7472	3810	25 Apr 2019→ 31 Dec 2020	Ta, vp, RH, v, vmax, ap, Kglob, La, Lg
Base camp	27.9952	86.8406	5315	10 Oct 2019→ 31 Dec 2020	Ta, vp, RH, ap
Camp 2	27.9810	86.9023	6464	8 May 2019→ 31 Dec 2020	Ta, vp, RH, v, vmax, ap, Kglob, La, Lg
South col	27.9719	86.9295	7945	21 May 2019→ 31 Dec 2020	Ta, vp, RH, v*, vmax*, ap, Kglob, La, Lg
Balcony	27.9826	86.9292	8430	22 May 2019→ 20 Jan 2020	Ta, vp, RH$^{\#}$, v$^{\&}$, vmax$^{\&}$, ap

Ta, air temperature; vp, air vapor pressure; RH, relative air humidity; v, mean wind speed; vmax, maximum wind speed; ap, air pressure; Kglob, global solar radiation; La, back (sky) longwave radiation; Lg, outgoing ground longwave radiation.
**Data questionable after 6 Jan 2020; $^{\#}$data available till 20 Dec 2019; $^{\&}$data available till 24 Oct 2019.*

TABLE 3 | Meteorological parameters at Mt. Everest stations between 1 May 2019 and 31 May 2020: means ± standard deviation (SD), (minimum; maximum values).

Station	Air pressure (hPa)	Air temperature (°C)	Air vapor pressure (hPa)	Relative humidity (%)	Global solar radiation (W·m^{-2})	Wind speed (hourly mean, m·s^{-1})	Wind gust (m·s^{-1})
Phortse	646 ± 3 (637; 654)	4 ± 5 (–12; 17)	7.0 ± 3 (1; 13)	78 ± 17 (7; 78)	216 ± 68 (0; 1306)	1 ± 0 (0; 6)	3 ± 1 (–; 13)
Base Camp*	531 ± 3 (520; 540)	–7 ± 4 (–21; 6)	1.8 ± 1 (0.1; 6)	45 ± 27 (3; 45)	–	–	–
Camp II	460 ± 4 (446; 468)	–11 ± 6 (–31; 4)	1.7 ± 1 (0.1; 5)	48 ± 27 (4; 48)	249 ± 92 (0; 1527)	3 ± 3 (0; 23)	7 ± 5 (–; 35)
South Col	377 ± 6 (358; 387)	–22 ± 8 (–40; –1)	0.8 ± 1 (0.1; –3)	52 ± 24 (3; 52)	334 ± 139 (0; 1692)	9$^{\&}$ ± 6 (0; 26)	16$^{\&}$ ± 10 (–; 44)
Balcony**	355 ± 6 (334, 362)	–23 ± 9 (–45; –1)	1.0 ± 1 (0.1; 2)	71 ± 13 (5; 70)	–	7$^{\#}$ ± 2 (0; 19)	8$^{\#}$ ± 4 (–; 34)

**10th Oct 2019–31st May 2020; ** 22nd May 2019–17th Jan 2020; $^{\#}$22nd May–24th Oct 2019; $^{\&}$22nd May 2019–6th Jan 2020.*

TABLE 4 | Mean values of meteorological variables in particular weather categories at different stations and seasons during summer (1 May–15 August) and winter (16 October–6 January) seasons.

			Wind speed (m·s^{-1})		Global solar radiation (MJ·day^{-1})		Air pressure (hPa)		Air temperature (°C)		Relative humidity (%)		Air vapor pressure (hPa)	
Station	Season	Weather type	Weak wind	Strong wind	Weak wind	Strong wind	Weak wind	Strong wind	Weak wind	Strong wind	Weak wind	Strong wind	Weak wind	Strong wind
Phortse	Summer	Cloudy	1.1	1.5	15.1	16.6	645.8	645.3	9.5	6.8	94.7	89.8	11.2	9.9
		Sunny	1.2	1.7	23.1	25.8	646.7	646.5	10.7	8.3	93.0	80.6	11.9	8.9
	Winter	Cloudy	0.9	1.5	9.6	12.5	646.3	644.4	–3.6	–3.2	76.9	58.4	4.8	3.1
		Sunny	1.0	1.4	17.3	17.3	646.0	647.8	–2.2	–1.8	64.2	48.5	4.1	3.6
Camp 2	Summer	Cloudy	0.9	2.1	23.8	19.9	463.3	462.6	–3.8	–8.6	85.6	59.5	4.1	2.0
		Sunny	1.0	1.9	30.9	32.3	464.0	462.6	–3.5	–6.4	77.7	45.2	3.8	1.8
	Winter	Cloudy	3.5	8.5	11.6	9.1	458.3	455.4	–17.6	–19.6	30.3	32.0	0.6	0.5
		Sunny	3.5	8.8	16.9	14.6	461.1	458.3	–16.6	–17.6	21.3	15.0	0.5	0. 3
South Col	Summer	Cloudy	2.3	8.5	29.4	40.2	383.8	382.2	–10.9	–14.3	88.1	57.1	2.4	1.2
		Sunny	2.9	7.8	44.4	46.5	383.6	381.6	–11.5	–15.4	72.6	52.2	1.9	1.0
	Winter	Cloudy	3.9	15.6	9.2	10.4	370.5	374.7	–33.5	–30.2	60.4	65.6	0.3	0.5
		Sunny	3.2	14.9	17.3	18.3	370.9	375.9	–34.0	–28.4	27.7	32.2	0.2	0.3

Phortse in winter by about 11% on sunny days to 16% on cloudy days than on winter days with weak wind. Convection on windy days at South Col in winter was as much as 4.6 times greater on cloudy days to 4.8 times greater on sunny days than on weak wind days. Contrasts in absorbed solar radiation increased with altitude on sunny and cloudy days. At Phortse the seasonal difference in absorbed solar radiation in summer was 1.5 (weak wind) to 1.6 (strong wind) times greater on sunny days than on cloudy days, and in winter 1.5 (strong wind) to 1.95 (weak wind) times greater on sunny days. At South Col the relationship of absorbed solar radiation on sunny and cloudy days ranged from 1.2 (strong wind) to 1.7 (weak wind) times greater on sunny summer days and 2.2 (strong wind) to 2.6 (weak wind) times greater on sunny winter days than on cloudy days.

Total Values and Structure of Heat Loss Fluxes

The level of heat loss with the clothing insulation values we selected and physical activity clearly differed with season and altitude. Wind and radiation conditions played an important role with greater heat loss in winter than in summer. At the highest station of South Col heat loss reached 400 W·m^{-2} in winter in cloudy and strong wind days; in summer heat loss ranged from 180 to 215 W·m^{-2}. At the lowest point, Phortse, total heat loss

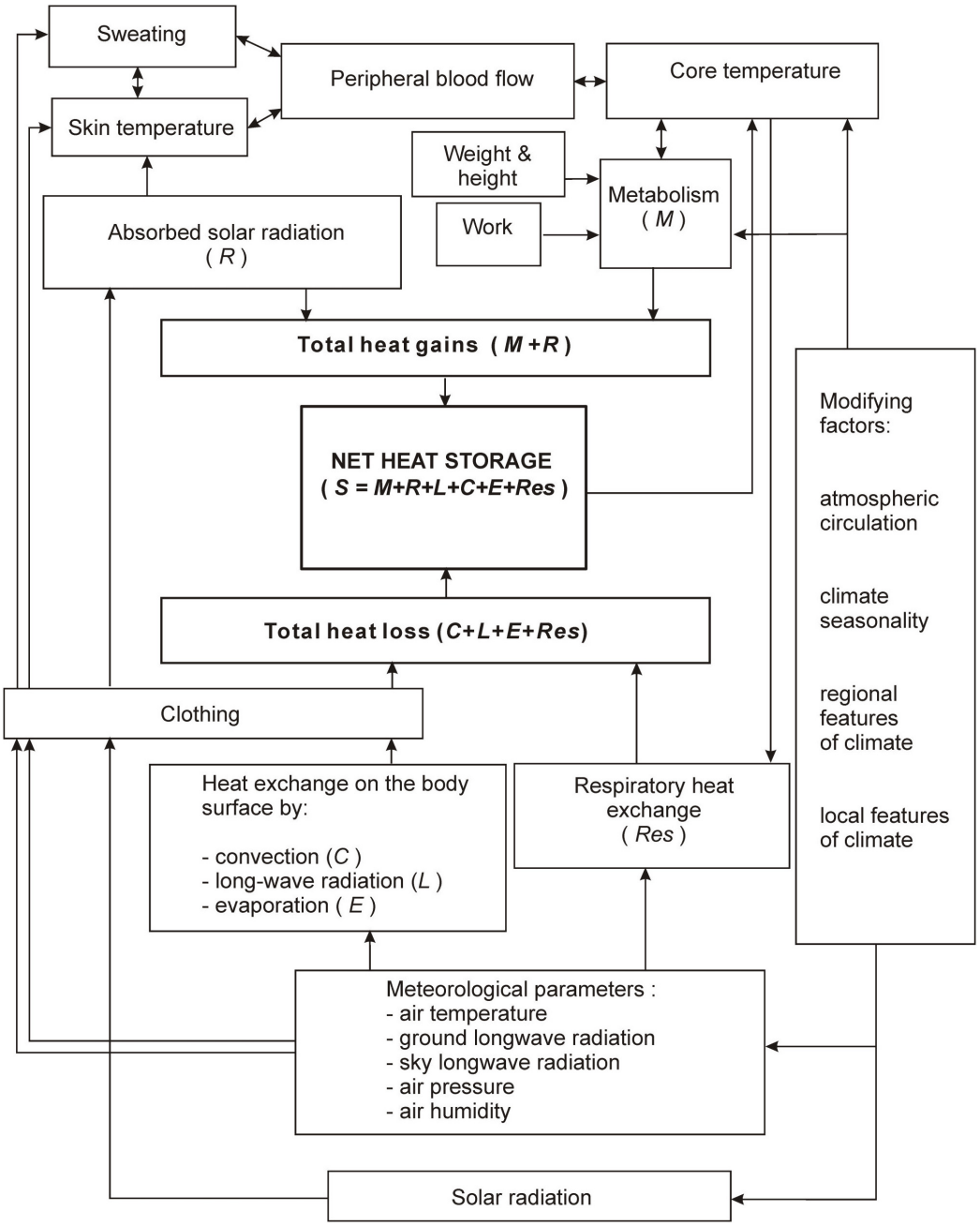

FIGURE 2 | Relationships between meteorological and physiological factors of Man-Environment Heat Exchange Model (MENEX-2005). Adapted from Błażejczyk (1994).

varied from about 110 W·m^{-2} in summer to about 130 W·m^{-2} in winter, depending on wind and radiation (**Figure 3**).

Net Heat Storage

The effect of heat exchange between the human body and the environment was a heat storage value that indicated whether heat was accumulated in the body or was eliminated, leading to the body cooling. In general, climbing caused as much as 124 W·m^{-2} of heat to accumulate in the human body, which might led to overheating as an effect of intensive activity with much internal heat production linked to the high thermo-insulating properties of mountaineering clothing. Only at the highest parts of Mt. Everest in winter were alpinists risk of overcooling, even while climbing intensively in heavy clothing (**Table 6**). Our calculations showed that alpinists faced little risk of overheating or overcooling while actively climbing. Our calculations consider only alpinists as they undergo physical exertion; the heat balance of climbers who rest in a tent or

TABLE 5 | Mean values of human heat balance variables at different stations 1 May 2019 to 6 January 2020 for different combinations of metabolic heat production and clothing insulation.

Metabolic heat production	Heat balance variables	Phortse	Camp 2	South Col	Phortse	Camp 2	South Col
			Icl = 3.5 clo			Icl = 4.5 clo	
M = 190 ($W \cdot m^{-2}$)	C ($W \cdot m^{-2}$)	–20	–32	–92	–16	–26	–77
	E ($W \cdot m^{-2}$)	–53	–51	–51	–53	–51	–51
	L ($W \cdot m^{-2}$)	–13	–18	–29	–11	–14	–24
	Res ($W \cdot m^{-2}$)	–24	–29	–33	–24	–29	–33
	R ($W \cdot m^{-2}$)	4	5	10	4	4	8
	SW (g/hour)	141	134	133	141	134	133
M = 290 ($W \cdot m^{-2}$)	C ($W \cdot m^{-2}$)	–20	–32	–92	–16	–26	–77
	E ($W \cdot m^{-2}$)	–96	–94	–93	–96	–94	–93
	L ($W \cdot m^{-2}$)	–13	–18	–30	–11	–14	–25
	Res ($W \cdot m^{-2}$)	–37	–45	–50	–37	–45	–50
	R ($W \cdot m^{-2}$)	4	5	11	4	4	9
	SW (g/hour)	251	243	242	251	243	242

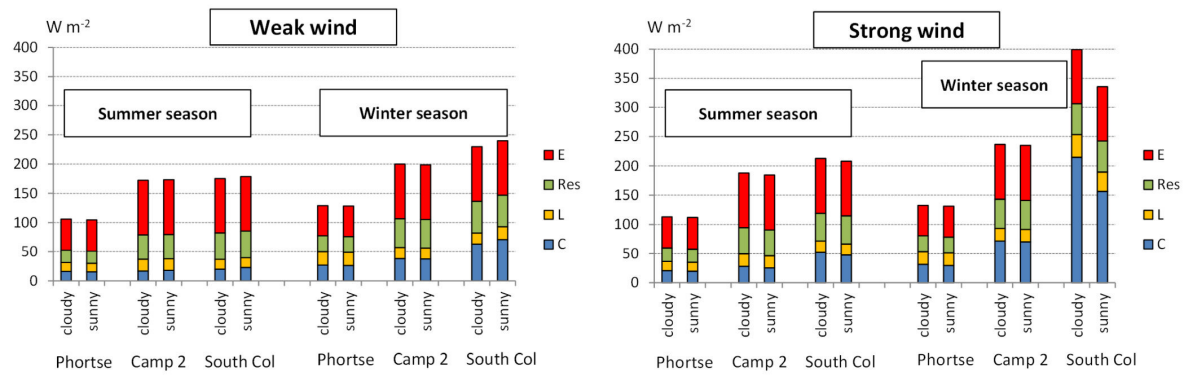

FIGURE 3 | Totals and structure of heat loss fluxes in different seasons and weather categories at particular stations. C, convective heat loss; L, net long-wave radiation in humans; Res, respiration heat loss; E, evaporative heat loss.

are forced to bivouac—unplanned camping, usually without a tent, because of bad weather or ill health—needs further scientific exploration.

Effects of Air Pressure and Solar Radiation on Elements of Heat Flux

The differences we observed in the characteristics of the heat balance at each station resulted from the complex interaction of meteorological elements. The altitude-related decrease in atmospheric pressure and air density, along with the increased solar radiation at height, significantly affected the values that we calculated for convective heat loss and absorbed solar radiation. For example, with Icl = 5.5 clo and M = 290 $W \cdot m^{-2}$, the average value of convective heat loss at South Col in the period between 1 May 2019 and 6 January 2020 (ap = 380 hPa, Ta= –18.9°C and v = 8.8 $m \cdot s^{-1)}$ was about 54 $W \cdot m^{-2}$ (**Table 5**). At Phortse (ap = 650 hPa), the convection heat loss would be 1.8 times greater with the same temperature and wind speed. The values of convective heat loss observed at higher stations (Camp 2, South Col) were smaller than if the atmospheric pressure were the same as at Phortse had the air temperature and wind speed been unchanged. At altitudes between 6,500 and 8,000 m convective heat losses are relatively lower than at an altitude of 3,800 m (**Figure 4**).

As with convective heat loss, absorbed solar radiation also increased with height: at the highest part of Mt. Everest it was 1.7 times the comparable value at Phortse.

Oxygen Volume

An important problem of a sojourn at high altitude is the amount of oxygen in the air, which decreases with altitude. Oxygen volume is 277 $g \cdot m^{-3}$ under the reference conditions of 1,000 hPa, a temperature of 15°C and water vapor pressure of 8 hPa. At the stations we analyzed on the route up Mt. Everest mean oxygen volume values decreased from 186 $g \cdot m^{-3}$ at Phortse to 141 $g \cdot m^{-3}$ at Camp 2 and 121 $g \cdot m^{-3}$ at South Col. These values represented 67, 51, and 44% of the values determined under the reference conditions. Climbers compensate for the reduced amount of oxygen in the air by breathing more intensely, which

TABLE 6 | Average values of heat balance components and net heat storage of climbers in different weather scenarios at particular stations during summer (1 May–15 August) and winter (16 October–6 January) seasons.

			Convection ($W \cdot m^{-2}$)		Long-wave radiation ($W \cdot m^{-2}$)		Absorbed solar radiation ($W \cdot m^{-2}$)		Respiration ($W \cdot m^{-2}$)		Evaporation ($W \cdot m^{-2}$)		Sweating ($g \cdot h^{-1}$)		S – net heat storage ($W \cdot m^{-2}$)	
Station (M) ($W \cdot m^{-2}$)	**Season (Icl) (clo)**	**Weather category**	**Weak wind**	**Strong wind**	**Weak wind**	**Strong wind**	**Weak wind**	**Strong wind**	**Weak wind**	**Strong wind**	**Weak wind**	**Strong wind**	**Weak wind**	**Strong wind**	**Weak wind**	**Strong wind**
Phortse	Summer	Cloudy	–16	–21	–15	–16	3	3	–22	–23	–53	–53	142	141	**87**	**80**
M = 190	Icl = 3.5	Sunny	–15	–20	–15	–16	4	5	–21	–23	–54	–54	144	144	**90**	**83**
	Winter	Cloudy	–27	–31	–23	–22	3	4	–28	–28	–52	–52	135	137	**64**	**61**
	Icl = 3.5	Sunny	–26	–29	–23	–22	6	7	–27	–27	–52	–53	137	138	**68**	**65**
Camp 2	Summer	Cloudy	–17	–28	–20	–22	5	4	–42	–45	–93	–93	243	243	**122**	**106**
M = 290	Icl = 3.5	Sunny	–18	–26	–20	–21	7	7	–42	–44	–94	–94	244	244	**124**	**113**
	Winter	Cloudy	–38	–72	–19	–22	3	3	–49	–50	–93	–93	242	242	**93**	**56**
	Icl = 4.5	Sunny	–37	–70	–19	–21	5	5	–49	–50	–93	–94	242	243	**96**	**60**
South Col	Summer	Cloudy	–20	–52	–17	–20	6	10	–46	–48	–93	–94	242	243	**120**	**87**
M = 290	Icl = 4.5	Sunny	–23	–48	–17	–19	9	11	–46	–48	–93	–93	242	243	**120**	**93**
	Winter	Cloudy	–40	–182	–25	–38	3	4	–56	–55	–93	–93	240	241	**63**	**–105**
	Icl = 5.5	Sunny	–32	–152	–27	–34	7	9	–56	–54	–92	–93	240	241	**56**	**–35**

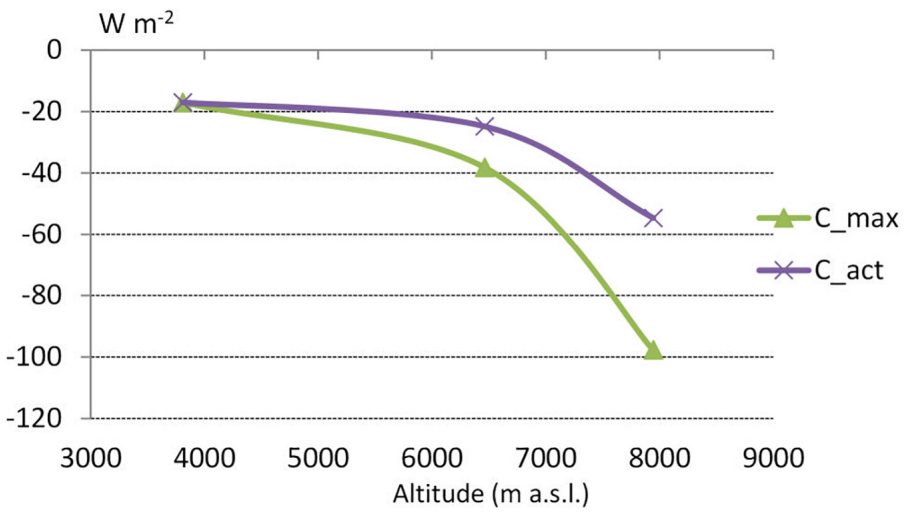

FIGURE 4 | The dependence of the convective heat loss (C) value on the level of atmospheric pressure at different stations 1 May 2019 to 6 January 2020 for metabolic heat production of 290 $W \cdot m^{-2}$ and clothing insulation 5.5 clo. C_act—actual C values observed at particular stations, C_max—potential C values from air pressure recorded at Phortse station.

became evident when we compared the amount of heat lost to respiration under different metabolic conditions. At a metabolic rate of 290 $W \cdot m^{-2}$ we found that 1.6 times more heat was lost to respiration under the same thermal and humidity conditions than at M = 190 $W \cdot m^{-2}$ (**Table 6**). This marked increase in respiration heat loss resulted from greater exertion and therefore a higher breathing rate (**Annexure**, formula 23).

Oxygen volumes varied considerably between seasons and in different weather. In summer they were slightly higher than in winter, on cloudy days they were higher than on sunny days, and in strong winds they were higher than in light winds (**Table 7**). These differences were mainly caused by differences in air temperature (**Table 4**).

DISCUSSION

Meteorological Conditions

Throughout the troposphere the higher the altitude and the colder the season, the lower are barometric pressure and air temperature at a given latitude (Brunt, 1952; West, 1996; Whiteman, 2000). Global solar radiation increases as elevation increases because of the reduced optical atmospheric mass at altitude, where diffused solar radiation therefore increases. Solar radiation is also reflected from the ice and snow covering the higher south slopes around the measuring stations as reported for Everest at South Col (Matthews et al., 2020b) and for Tatry Mountains (Błażejczyk et al., 2013). The large seasonal differences that we found in

TABLE 7 | Average values of oxygen volume in different weather scenarios at particular stations during summer (1 May–15 August) and winter (16 October–6 January) seasons.

			Oxygen volume ($g \cdot m^{-3}$)	
	Season	**Weather Category**	**Weak wind**	**Strong wind**
Phortse	Winter	Cloudy	181	183
		sunny	180	183
	Summer	Cloudy	191	191
		Sunny	190	191
Camp 2	Winter	Cloudy	137	140
		Sunny	138	139
	Summer	Cloudy	144	144
		Sunny	143	143
South Col	Winter	Cloudy	117	119
		Sunny	118	119
	Summer	Cloudy	124	123
		Sunny	122	123

wind speed at higher stations, with much higher winds in winter, accords with the seasonal trend in the global jet streams. The Subtropical component of the Northern Hemisphere jet stream flows between 20°N and 35°N, above the Himalayas in winter; this jet stream weakens in summer and shifts northwards (Archer and Caldeira, 2008; Pena-Ortiz et al., 2013).

We observed the most severe weather conditions that adversely affect human heat balance—low air temperature, high wind speed, and low solar radiation— at the highest station in winter. Alpinists should expect the lowest air temperatures, the lowest daily global solar radiation, and the strongest wind at the highest altitudes in winter. Those parameters of weather not only fall with altitude but their seasonal amplitudes also increase with altitude (National Geographic, 2021).

Convective Heat Loss

Alpinists must contend with a large increase in convective heat loss with rising altitude as they climb Mt. Everest. Convective loss is 4–5 times higher at 8,000 m than at 4,000 m, which can be explained by the significant drop in air temperature and the higher wind speeds at higher altitudes. Convective heat loss is the parameter closely linked to altitude, season, and weather. Our results accord with the findings of other authors, who have calculated the lowest values of WCT and FFT on Everest's summit in winter (Moore and Semple, 2011; Szymczak et al., 2021b). Climbers should also be aware that convective heat loss changes with the different weather categories they experience, especially in winter, along with altitude. At South Col in winter convective heat loss is almost 5 times higher in strong wind than in weak wind. This shows how abrupt changes in the weather can significantly affect human survival, as other authors have noted. Moore and Semple (2012) suggested that higher hypoxic and hypothermic stress due to weather changes is often responsible for climbers' deaths (Moore and Semple, 2012). They presented the cases of two climbers who had to bivouac above 8,500 m on descent from Everest in extreme conditions: the first experienced air pressure of 333 hPa, air temperature of –31°C and wind speed of 15 $m \cdot s^{-1}$; the second 338 hPa, –23°C, and 2 $m \cdot s^{-1}$. The first climber died but the second survived. Moore and Semple (2012) attributed his death to the higher hypoxic and hypothermic stress he underwent.

Convective Heat Loss and Air Pressure

Air density decreases at high altitudes because of the lower air pressure. Lower density provides better insulation in the near-body air layer, which reduces convective heat loss (Kandjov, 1997), as we observed in our results. With lower air pressure at higher altitudes convective heat loss will be lower than at lower altitudes with the same air temperature and wind speed. As presented in our results, at 8,000 m the lower air pressure reduced convective heat loss by almost 50% compared with convective loss at 4,000 m in the same air pressure and temperature. Convective heat loss that increases with altitude because of decreasing air temperature and increasing wind speed is partly ameliorated by the lower barometric pressure encountered at height. Our results concur with Huey et al. (2001) who calculated that with the 60% decline of air density from sea level to 9,000 m the convective heat loss at 9,000 m decreases by about 45% compared with the loss at sea level in the same conditions at a temperature of –33.5°C and wind speeds up to 28 $m \cdot s^{-1}$. The standard equations for WCT and FFT (Tikuisis and Osczevski, 2003; Osczevski and Bluestein, 2005) applied in other studies that analyzed hypothermic stress at high altitude (Moore and Semple, 2011; Szymczak et al., 2021a,b) presume sea-level densities of air and therefore significantly overestimate heat loss at altitude. Climbers usually use wind speed to identify suitable climbing weather windows (Peplow, 2004), but should therefore also consider the relationship between convective heat loss and air pressure to interpret weather forecasts at high altitude. The same wind speed causes less convective heat loss at 8,000 m than at 4,000 m.

Clothing Insulation

Clothing insulation is one of the few easily controllable parameters that enable alpinists to limit convective heat loss and to maintain thermal equilibrium (Tikuisis, 1995; Havenith et al., 2012). Proper clothing insulation determines survival time in low air temperatures and high winds. Tikuisis (1995) used a mathematical model to predict survival times under sedentary conditions. These calculations determined that in environmental conditions similar to those at the South Col on an average winter day (air temperature –20°C, wind speed 14 $m \cdot s^{-1}$) a climber wearing one loose layer 1 mm thick would survive 3 h and a climber wearing three loose 1 mm layers of clothing would survive 12 h (Tikuisis, 1995).

Our calculations determined that clothing insulation of 4.5 clo would enable a climber to counteract the potential heat loss due to environmental conditions at all the stations, in

all seasons, and in all-weather apart from the strong wind on South Col in winter (air temperature ∼ –30°C and wind speed of ∼ 15 $m \cdot s^{-1}$). In cloudy, windy, winter days at South Col we derived a net heat balance value of ∼ –70 $W \cdot m^{-2}$. Our results are similar to those published by Havenith (2010). He calculated the clothing insulation that a climber required in different air temperature and wind conditions for thermal equilibrium in a summit bid on Everest. The energy production during climbing the summit of Everest was assumed as 5 METs (Havenith, 2010). He calculated that clothing insulation of 3.5 clo was enough to maintain a climber's thermal equilibrium in conditions from an air temperature of –30°C at a low wind speed of 2 $m \cdot s^{-1}$ to an air temperature of –10°C with high wind speed of 11 $m \cdot s^{-1}$. The environmental conditions that could be balanced with clothing insulation of 4.5 clo lay between Ta –40°C with v 2 $m \cdot s^{-1}$ and Ta –20°C with v 11 $m \cdot s^{-1}$, and for 5.5 clo a range between Ta < –45°C with v 2 $m \cdot s^{-1}$ and Ta –30°C with v 11 $m \cdot s^{-1}$ (Havenith, 2010). Considering these calculations, clothing insulation of 4.5 clo should maintain a climber's thermal balance throughout ascents of Everest in the normal season (May, October), but 5.5 clo would be required for winter ascents (Szymczak et al., 2021a). Modern mountaineering clothing for extreme altitudes can provide insulation of about 5.6 clo (Havenith, 2010). Given the importance of clothing insulation to maintain thermal equilibrium and thus ensure survival, climbers should precisely calculate the insulation properties not only of their climbing outfits but also of their emergency survival bags and the shelters they need for bivouacs. Emergency insulation and protection from wind with a survival bag or shelter might help alpinists survive unforeseen situations. It should be taken into account that the very high insulation properties are needed to conserve thermal equilibrium at rest. Cena and Tapsell (2000) and Cena et al. (2003) observed that the insulation of clothes together with a sleeping bag needed to keep a climber's thermal sensation in tent between neutral and slightly warm at 5,000 m might be as high as 7 clo. Emergency equipment should be chosen based on research for emergency medicine (Oliver et al., 2016; Haverkamp et al., 2018), nevertheless, this field needs further development, which would likely include the use of external chemical or electrical heaters.

Proper clothing insulation not only significantly decreases convective heat loss but also the heat loss to long-wave radiation. The reduction of heat lost to long-wave radiation is minor when compared to convection.

Physical Activity Level

Levels of physical activity play an important role in human heat balance (Bligh and Johnson, 1973). Greater physical activity generates more metabolic heat, but also provokes heat loss to evaporation and respiration. We observed that when metabolic heat rises so does the net heat value despite the simultaneous increase in evaporation and respiration heat losses. When the metabolic heat production was increased by 100 $W \cdot m^{-2}$ from 190 to 290 $W \cdot m^{-2}$ at altitudes above 6,000 m while heat lost to evaporation and respiration increased by about 60 $W \cdot m^{-2}$ from 80 to 140 $W \cdot m^{-2}$, the net heat gain was about 40 $W \cdot m^{-2}$. Climbers need to understand the relationship between physical activity and heat balance. Situations that force a climber to stop or bivouac—such as fatigue, weather deterioration, sunset, or trauma—provoke a large decrease in metabolic heat production that is difficult to counteract in severe high-altitude conditions. Ainslie and Reilly (2003) suggested that the risk of hypothermia rises to critical levels when a fatigued mountaineer stops during descent or is forced to bivouac. The lower exertion that a climber requires on descent also reduces heat production, which falls precipitously if the climber stops (Ainslie and Reilly, 2003). Hypothermia also increases fatigue by reducing muscle strength and increasing oxygen consumption for the same intensity of exercise (Hinde et al., 2017). The message for a climber is: "If you stop, the hypothermia starts."

Respiration Heat Loss

The increase in respiration heat loss with altitude that we observed was less significant than the heat lost to convection or long-wave radiation. Respiration heat loss depends on the temperature of inhaled air, so any way of raising the temperature of inhaled atmospheric air or its mixture with oxygen from a container would decrease heat loss. Nevertheless, less advantage is gained from ameliorating respiration heat loss than from decreasing convective heat loss with proper clothing. Determining the ventilation level at any altitude is difficult, especially above 8,000 m where respiratory acidosis due to extreme hyperventilation determines the arterial partial pressure of oxygen necessary for survival (West et al., 2007a). Respiration level depends not only on activity level but also acclimatization, supplemental oxygen use and individual reaction to hypoxia. MENEX-2005 calculates respiration heat loss mainly on physical activity, so our results might underestimate the level of respiration heat loss, especially at extreme altitudes.

Evaporative Heat Loss and Water Loss

The significant part of the total heat loss is due to evaporation, which increases with higher levels of physical activity. At an activity level of 5 MET with metabolic heat production of 290 $W \cdot m^{-2}$ evaporation was responsible for about 50% of the total heat lost at the altitudes of Phortse and Camp 2. By comparison, convection was responsible for less than 20% of the heat lost at those altitudes. Only at the altitude of South Col in winter in weak wind were evaporation and convective heat loss comparable, with each being responsible for 30–40% of total heat loss. Convective heat loss dominated at the altitude of South Col in windy winter conditions and was responsible for 50% of the total heat loss; evaporative heat loss accounted for 25%. Evaporation from the human body is generally influenced by metabolic heat production, air and skin temperature (which activate sweat glands), wind speed (which accelerates evaporation of sweat), and the clothing barrier

(Błażejczyk and Kunert, 2011). Our research confirmed that high metabolic values dominated evaporation.

The amount of water a climber loses mostly depends on evaporation, which is mainly influenced by level of physical activity. Our calculations showed that alpinists lost about 2 liters of water to evaporation in 8 h of climbing at altitudes above 6,000 m. Substantial water was lost when considering the additional water lost through increased respiration. Climbers should drink 4–5 liters of fluids daily at altitude to maintain water equilibrium (Pollard and Murdoch, 1997).

Solar Radiation Heat Gain

Despite the significant increase of global solar radiation and absorbed solar radiation with altitude, we found they played a negligible role in heat balance. The insulation of clothing used at high altitude decreased the role of absorbed solar radiation. The intense solar radiation we observed at high altitude warrants further research on developing clothing materials that would be able to store solar energy and return it as heat when needed.

Limitations and Strengths

The MENEX-2005 model calculates respiration heat loss without considering the increase of ventilation due to hypoxia, which might therefore underestimate respiration heat loss, especially at extreme altitudes. We calculated metabolic heat production and levels of physical activity at different altitudes based on the daily energy expenditure of climbers observed by other authors. We understand that the values we assumed might be too general and over-simplified. The level of a climber's physical activity depends on many variables, including the difficulty of the route, whether the climber is belaying or climbing, and the number of climbers on the route especially now that queues form on Everest. Yet the most important factor limiting climbers remains the level of oxygen, especially at extreme altitudes. Climbing speed and metabolic heat production change considerably while climbing. Our calculations did not range above 8,000 m, which is below the highest altitudes that climbers experience in the Himalayas.

This study provides the first complete assessment of human heat balance at altitudes between 4,000 and 8,000 m in active climbing. We based our calculations on *in situ* measurements rather than reanalysis, which provides only approximate values of weather factors. Our results should provide important practical benefits for climbers by helping them to better interpret weather forecasts and correctly choose the insulation properties of their clothing and emergency equipment.

Human heat balance at altitude needs further research. We concentrated on the active phase of climbing, but the cessation of movement puts climber at great risk of hypothermia. Further research is needed on the precise assessment of heat balance in climbers at rest during the static phase of climbing. The future task is also better representation of respiratory heat loss due to forced ventilation and reduced oxygen volume in the air.

CONCLUSION

1. Alpinists face little risk of overheating or overcooling while actively climbing Mt. Everest, despite the potential risk of overcooling at high altitude on Mt. Everest in winter.
2. Convection and evaporation are responsible for most of the heat lost at altitude.
3. Levels of physical activity and clothing insulation play the greatest role in counteracting heat loss at high altitude.
4. Air pressure greatly influences convective heat loss, reducing its effect at higher altitudes.
5. The significant increase in solar radiation with altitude has little effect on the heat balance of climbers.
6. Respiration heat loss and heat balance in the static phase of climbing needs further research.

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 author/s.

AUTHOR CONTRIBUTIONS

RS and KB contributed to conceptualization, methodology, validation, data curation, formal analysis, investigation, supervision, writing—original draft preparation, resources, visualization, writing—review, and editing. Both authors contributed to the article and approved the submitted version.

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ANNEXURE

Radiation balance (Q)

Radiation balance (Q, $W \cdot m^{-2}$) is the sum of absorbed solar radiation (R) and net long-wave radiation (L):

$$Q = R + L \tag{1}$$

The SolGlob model developed by Błażejczyk (Błażejczyk, 1994, 2005a,b; Błażejczyk et al., 2012) based on empirical research was used to calculate the absorbed solar radiation. Given the information on the intensity of total solar radiation (Kglob), the formulas for calculating the value of R have various forms, depending on the height of the Sun (h) and the theoretically possible value of total radiation in a cloudless sky (Kt), which is calculated as follows:

$$Kt = -0.0015 \cdot h^3 + 0.1796 \cdot h^2 + 9.6375 \cdot h - 11.9 \tag{2}$$

The formulas for calculating the absorbed solar radiation have the following form:
- for $h \leq 12°$.

$$R = (0.0014 \cdot Kglob^2 + 0.476 \cdot Kglob - 3.8) \cdot (1 - 0.01 \cdot ac) \cdot Irc \tag{3}$$

- for $h > 12°$ and $Kglob/Kt \leq 0.8$

$$R = 0.247 \cdot Kglob^{0.9763} \cdot (1 - 0.01 \cdot ac) \cdot Irc \tag{4}$$

- for $h > 12°$ and Kglob/Kt from 0.81 to 1.05

$$R = 3.692 \cdot Kglob^{0.5842} \cdot (1 - 0.01 \cdot ac) \cdot Irc \tag{5}$$

- for $h > 12°$ and Kglob/Kt from 1.06 to 1.2

$$R = 43.426 \cdot Kglob^{0.2326} \cdot (1 - 0.01 \cdot ac) \cdot Irc \tag{6}$$

- for $h > 12°$ and $Kglob/Kt > 1.2$

$$R = 8.928 \cdot Kglob^{0.4861} \cdot (1 - 0.01 \cdot ac) \cdot Irc. \tag{7}$$

In the equations above, Irc is the dimensionless coefficient of the attenuation of heat flow through the clothing (for absorbed solar radiation, long-wave radiation and convection), which depends on coefficient of heat transfer by convection (hc) and coefficient of heat transfer by conduction within clothing (hc'). Irc is calculated as follows:

$$Irc = hc'/[hc' + hc + 21.55 \cdot 10^{-8} \cdot (Ta + 273)^3] \tag{8}$$

$$hc = (0.013 \cdot ap - 0.04 \cdot Ta - 0.503) \cdot (v + v')^{0.4} \tag{9}$$

$$hc' = (0.013 \cdot ap - 0.04 \cdot Ta - 0.503) \cdot 0.53/\{Icl \cdot [1 - 0.27 \cdot (v + v')^{0.4}]\} \tag{10}$$

The long-wave (L) radiation balance consists of radiation emitted by the surface of the body/clothing (Ls) and thermal radiation emitted by the surface (Lg) and the reverse radiation of the atmosphere (La):

$$L = (0.5 \cdot Lg + 0.5 \cdot La - Ls) \cdot Irc \tag{11}$$

$$\text{where : } Ls = 5.38 \cdot 10^{-8} \cdot (273 + Tsk)^4. \tag{12}$$

The skin temperature (Tsk) is calculated from the empirical formula below:

$$Tsk = (26.4 + 0.0214 \cdot Mrt + 0.2095 \cdot Ta - 0.018 \cdot RH - 0.01 \cdot v) + 0.6 \cdot (Icl - 1) + 0.00128 \cdot M \tag{13}$$

where: mean radiant temperature (Mrt) is calculated as follows:

$$Mrt = [(R/Irc + Lg + La)/(5.38 \cdot 10^{-8})]^{0.25} - 273 \tag{14}$$

Evaporative Heat Loss (E)

Evaporative heat loss (E, $W \cdot m^{-2}$) depends on the difference in water vapor pressure on the surface of the skin (vps) and in the ambient air (vp) and coefficient of heat transfer by evaporation (he). The degree of skin moisture (w), metabolic heat production (M) and dimensionless coefficient of the attenuation of heat flow through the clothing by evaporation (Ie) are also taken into account:

$$E = he \cdot (vp - vps) \cdot w \cdot Ie - [0.42 \cdot (M - 58) - 5.04] \quad (15)$$

$$\text{where : } vps = e^{(0.058 \cdot Tsk + 2.003)} \quad (16)$$

$$w = 1.031/(37.5 - Tsk) - 0.065 \quad (17)$$

$$he = [Ta \cdot (0.00006 \cdot Ta - 0.00002 \cdot ap + 0.011) + 0.02 \cdot ap - 0.773)] \cdot 0.53/\{Icl \cdot [1 - 0.27 \cdot (v + v')^{0.4}]\} \quad (18)$$

$$Ie = hc'/(hc' + hc) \quad (19)$$

The information on evaporative heat loss is supplemented by the determination of water loss due to sweating (SW, in $g \cdot h^{-1}$). Its calculation is based on the potential values of evaporative heat loss (Epot). Epot is derived from the MENEX_2005 model taking into account 5% level of relative humidity of air (RH):

$$SW = -2.6 \cdot Epot \quad (20)$$

where Epot is calculated the same way as E (see equation 15) but with vp replaced by vp'. vp' is vapor pressure equal to 5% RH as follows:

$$vp' = 6.112 \cdot 10^{[7.5 \cdot Ta/(237.7 + Ta)]} \cdot 0.05 \quad (21)$$

Convective Heat Exchange (C)

Convective heat exchange (C, $W \cdot m^{-2}$) depends on the difference between the average skin temperature (Tsk) and the air temperature (Ta), on the speed of air movement, and on its density and heat capacity (these variables are included in the coefficient hc):

$$C = hc \cdot (Ta - Tsk) \cdot Irc \quad (22)$$

Respiration Heat Loss (Res)

Respiration heat loss (Res, $W \cdot m^{-2}$) depends on the difference between the air temperature (Ta) and the exhaled air temperature (it is assumed to be 35.0°C) and on the difference between the water vapor pressure in the atmosphere (vp) and the water vapor pressure in the exhaled air (equal to 56.24 hPa):

$$Res = 0.0014 \cdot M \cdot (Ta - 35) + 0.0173 \cdot M \cdot [0.1 \cdot (vp - 56.24)] . \quad (23)$$

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Thermal Strain During Open-Water Swimming Competition in Warm Water Environments

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Open-water swim racing in warm water is associated with significant physiological strain. However, existing international policy that governs safe participation during competition relies only on a fixed water temperature threshold for event cancellation and has an unclear biophysical rationale. The current policy does not factor other environmental factors or race distance, nor provide a stratification of risk (low, moderate, high, or extreme) prior to the threshold for cancellation. Therefore, the primary aim of this *Perspectives* article is to highlight considerations for the development of modernized warm-water competition policies. We highlight current accounts (or lack thereof) of thermal strain, cooling interventions, and performance in warm-water swimming and opportunities for advancement of knowledge. Further work is needed that systematically evaluate real-world thermal strain and performance during warm water competition (alongside reports of environmental conditions), novel preparatory strategies, and in-race cooling strategies. This could ultimately form a basis for future development of modernized policies for athlete cohorts that stratifies risk and mitigation strategies according to important environmental factors and race-specific factors (distance).

Keywords: swimming, water temperature, heat, thermal strain, policy

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INTRODUCTION

Open-water swimming was first introduced as a medal event (25 km) at the Fédération Internationale de Natation (FINA) World Championships in 1991 (Shaw et al., 2014). In 2008, the marathon event (10 km) was introduced at the Olympic Games. Since its first appearance in 1991, open-water events have become a major discipline in FINA aquatic sports, including a separate 7-day world championships competition schedule including 5 km, 10 km, 25 km, and 5 km mixed relay events (FINA, 2019). Additionally, yearly marathon and ultramarathon race series are held at numerous locations around the world over a 10-month period. This has led to open-water racing competition becoming extremely diverse from a geographical and environmental perspective.

Currently, FINA has a defined scope of environmental conditions within which an open-water event can be undertaken. Regulations are associated with minimal and maximal water

temperatures (T_w) and quality and include recommendations of swimwear use for T_w below 20°C (Fédération Internationale de Natation (FINA), 2020). However, little coordinated attention has been given to interventions and policies in warm T_w (≥29°C; Bradford et al., 2013) to underpin the advised maximum temperature threshold. Prior to 2011, there was not a well-enforced maximal T_w that defined extreme risk of exertional heat illness. However, a cut-off was developed as a consequence of the tragic heat-related death of open-water swimmer Fran Crippen in 2010, which was attributed to competing in T_w above 31°C (Macaluso et al., 2013). A suggested T_w threshold for cancellation of 31°C was proposed in early 2011, but even after this recommendation, FINA completed events in environments with documented T_w around or above 31°C (Munatones, 2021). Finally, FINA updated T_w limits in 2013 to mandate the cancellation threshold of 31°C. However, the biophysical rationale justifying this threshold remains unclear.

Concerningly, numerous open-water races have been reported to be completed in T_w above 31°C as non-sanctioned races, or races where the rules have been ignored. For example, the World University Games in Taiwan (Keith, 2017) and the World Beach Games in Doha (Lord, 2019) completed open-water races in T_w that was reported to be above the 31°C threshold. Recently, during the Tokyo 2020 men and women's Olympics open-water marathon swimming competition, the start time was rescheduled for 2 h earlier to ensure T_w was below 31°C. The discussion around T_w and swimmer health is of significant interest with the upcoming 2023 FINA world open-water championships to be held in Doha (Qatar), where documented T_w are in excess of 31°C.

Although there has been significant scientific research into the consequences of hot environmental conditions on athletes' health and performance during terrestrial sporting activities and the development of subsequent large-scale protective guidelines (Armstrong et al., 2007), comparatively little attention has been paid to the safety and performance implications of competing in hot aquatic environments (Macaluso et al., 2011; Hue et al., 2013, 2015). Similar to cases in terrestrial sports during hot and humid conditions (Gamage et al., 2020), aquatic competition in high-risk conditions can have tragic consequences. This perspective article discusses current research and challenges in this context to stimulate the development of further research and policies to support athlete health during open-water swimming competition.

PHYSIOLOGICAL AND PERFORMANCE CONSEQUENCES

In comparison to terrestrial exercise, the key change in heat loss dynamics during swimming is the blunting of evaporative heat loss. In the midst of only a small contribution of evaporative heat loss (McMurray and Horvath, 1979) and the greater heat capacity of water compared to air, convection becomes a primary heat loss avenue during swimming in warm water (Nadel et al., 1974). Thus, the skin-to-water temperature gradient is a critical factor that determines the rate of body heat loss, with the gradient becoming narrower (i.e., less conducive for heat loss) at warmer T_w (McMurray and Horvath, 1979). A high metabolic rate (oxygen consumption [$\dot{V}O_2$] often exceeding 3.2 l/min at typical competition pace [Holmér, 1972; Toussaint et al., 1990; Zacca et al., 2020]) is also evident in elite open-water swimmers for long durations (≥60 min). Moreover, prolonged swimming in warm water is associated with elevated sweat rates (often exceeding 1 l/h; Macaluso et al., 2011; Hue et al., 2013). Elevated skin temperature during warm water swimming (Costill et al., 1967; McMurray and Horvath, 1979) is accompanied by a peripheral vasodilatory response, creating further competition for blood volume with the contracting muscles (Sawka et al., 2011). This competition for blood supply and concurrent loss of bodily fluid volume induces meaningful cardiovascular strain during exercise (Sawka et al., 2011).

More coordinated research has focused on low core temperature responses during cold water swimming exposure (T_w of ~10–16°C; Saycell et al., 2018). Conversely, a high core temperature response to exercise increases the risk of exertional heat illness (Armstrong et al., 2007). Some published reports suggest that rectal (T_{re}) and gastrointestinal (T_{gi}) temperature may not be consistently dangerous during simulated 5 km performance in T_w of 32.0°C (end-event T_{re} of 38.0°C; Macaluso et al., 2011) and competitive 10 km performance in T_w of 28.1°C (end-event T_{gi} 38.3°C; Hue et al., 2015), with either none or very little in-race cold fluid consumption. Bradford and colleagues (Bradford et al., 2013) reported that the end-event T_{re} during a simulated 120 min (similar duration to a 10 km event) swim in 32°C water was on average, 38.4°C for 22 competitive swimmers. The variation in the dataset (38.4 ± 0.8°C) highlighted that some athletes well exceeded a peak T_{re} of 39.0°C. While it is acknowledged that these core temperatures may not be problematic for some elite athletes (Racinais et al., 2019), it is still generally considered to be high risk for exertional heat illness (Roberts et al., 2021). Additionally, there are other published case reports of elite swimmers exceeding a T_{gi} of 39.0°C, and even recording close to 40.0°C during competitive swims ranging from 5 to 10 km in T_w up to 32°C (Bradford et al., 2019). However, methodological issues relating to appropriate depth of T_{re} sensors (Macaluso et al., 2011) as opposed to common recommendations (Lee et al., 2010), inconsistent lead-in time for the consumption of T_{gi} sensors (Hue et al., 2015) against common guidelines (Wilkinson et al., 2008), or the reporting of minimal study details (conference proceeding; Bradford et al., 2013) make it challenging to draw consistent conclusions regarding the core temperature response to warm-water competitive swimming. Future research that reports the thermal strain of athletes during competition in warm water will help move the field forward by informing and calibrating ongoing heat policy work.

While the understanding of hot and humid conditions effect upon terrestrial endurance performance is well-established (Guy et al., 2015), the effect of warm-water upon open-water swimming performance in the scientific literature is not well reported. There is some evidence that swim performance in competitive male athletes is degraded in 32°C T_w compared to 27°C T_w (~4%; Macaluso et al., 2011). Baldassarre et al. (2019) recently

reported the pacing profiles of the most successful open-water swimmers in major international 10 km races showing a negative pacing profile over the course of a race, with a significant increase in speed over the last quarter of races. Mean speed over a 10 km race was 1.48 m/s and 1.38 m/s for men and women, respectively (Baldassarre et al., 2019). However, this data set did not examine the effect of T_w. Of interest is the official results from the 2020 Olympic Games in Tokyo where official T_w 2 h prior to race start were 29.3°C and 29.5°C for women and men races, respectively. These data do not tend to demonstrate the same extent of negative pacing as observed in Baldassarre et al. (2019), although the prevailing T_w did not appear to confer substantial decrements in pace across the race. However, a much larger dataset would be required to empirically confirm the effect of T_w upon prolonged open-water swimming performance.

MEASURES TO IMPROVE SWIMMER HEALTH DURING COMPETITION IN WARM WATER

Pre-race

Preparatory Swimming in Warm Water

Repeated exposure to environmental heat during exercise training is considered the best preparation for terrestrial competition in hot conditions (Armstrong et al., 2007; Périard et al., 2015). Extensive research is available about the beneficial effects on thermoregulatory, cardiovascular, metabolic, perceptual, and performance outcomes following running and cycling training in the heat (Périard et al., 2015). However, very little is known about the adaptations associated with repeated active swim training in warm water. Hue et al. (2007) reported that 8 days of swim training in warm water (30°C) did not significantly change 400-m time-trial performance 10 days after the final training session, but there was a significant improvement (10%) 30 days after the final session. Bradford et al. (2015) failed to observe clear physiological adaptations or performance changes in competitive swimmers following seven swim training sessions in warm water (33°C). The authors suggested that a lack of cardiovascular adaptations could relate to the lower orthostatic stress in comparison to upright exercise (running and cycling) during the short-term program (Bradford et al., 2015). Future studies likely need to manipulate training parameters to facilitate potential positive adaptations, since the adaptation process to repeated heat training is sensitive to the duration and intensity of both environmental exposure and exercise protocol (Chalmers et al., 2014). Although untested, swimmers may obtain greater heat-related adaptations from a cross-training approach (i.e., cycling or running; Bradford et al., 2015), but the benefit of this approach for swimming-specific performance is uncertain. While an expansion in plasma volume (hallmark acclimation adaptation) is likely to be beneficial for cardiovascular stability during warm-water swimming, the increase in sweat rate that typically accompanies heat acclimation protocols (Périard et al., 2015) is unlikely to be meaningful for swimming in warm water since evaporative heat loss only plays a lesser role in comparison to terrestrial sports. Therefore, the cost–benefit tradeoff of cross-training must be carefully considered. Post-exercise hot water immersion/sauna may be an attractive means for swimmers to induce some heat acclimation protective adaptations while having less disruption to regular training (Casadio et al., 2017). This suggestion, however, has yet to be empirically tested in a swim training context.

Pre-race Risk Stratification

Current practice for determining the risk of heat illness during competitive open-water swimming is based only on event cancellation once a maximum allowable T_w is recorded (Fédération Internationale de Natation (FINA), 2020). An overarching international policy is set by FINA, but local governing organizations can choose to be guided by their own policy when hosting events or when their representative athletes are competing internationally. The level of protection associated with race cancellation that current FINA international (T_w of 31°C) or local governing body [e.g. a T_w of 29.45°C (Bradford et al., 2019)] is either unclear or not reported. While T_w is the predominant environmental factor that determines thermal stress during swimming, it neglects other environmental factors (air temperature, humidity, wind speed, and solar radiation) that might contribute to thermal stress to some extent. Moreover, in response to observed T_w, current policy does not implement graded risk stratification categories (i.e., low, moderate, high, or extreme) and risk-mitigation strategies prior to the race cancellation threshold, as is commonly done in many other sport and exercise settings (Armstrong et al., 2007; Sports Medicine Australia (SMA), 2021). Quantifying the level of risk would facilitate decisions by organizations and athletes to take necessary precautions. These could consist of adding cooling interventions, changing the start time to effect T_w, or shortening the course lap length to provide more opportunity for visually checking athletes and provide greater access to cold fluids at feeding stations. It is also reasonable to expect that the level of risk associated with different T_w may be different during 5, 10, and 25 km events due to differences in exposure duration and metabolic heat production, but this is not currently considered by the current international policy (Fédération Internationale de Natation (FINA), 2020).

Stratified risk for competition cohorts can be determined through biophysical modeling that estimates human heat transfer in response to both environmental and typical individual (i.e., exercise intensity) factors based upon readily available environmental and race-specific details. These biophysical modeling tools require foundational data relating to heat transfer avenues (convection, evaporation, radiation, conduction) and typical individual factors (metabolic rate, clothing, body size). In some instances, these factors are less well-established or understood in comparison to common terrestrial activities. Briefly, a primary challenge during biophysical modeling for open-water swimming is determining the most appropriate convective heat transfer coefficient (Nadel et al., 1974; Boutelier et al., 1977; Yermakova and Montgomery, 2018). The comparative effect of different T_w upon self-paced swimming speed by trained athletes during

competition (simulated or actual) over distances relevant for international competition (≥5 km) also remains underreported (Macaluso et al., 2011). The absence of these data influences the modeling of metabolic rate (i.e., heat production) during biophysical modeling, alongside the known relationships between speed, VO_2, and efficiency (Holmér, 1972; Toussaint et al., 1990; Zacca et al., 2020). Conceivably, T_w could be publicly reported by FINA alongside official race results. Moreover, the effect of environmental solar radiation on the prevailing heat load of an athlete during open-water swimming remains uncertain, influencing the biophysical modeling of radiative heat transfer. A pilot laboratory study reported that an additional radiant heat load (~400–800 W/m^2) did not have a meaningful impact upon T_{re} in comparison to the absence of additional radiant load, although this was based on between-study comparisons (Bradford et al., 2013). Between-study comparisons are limited by confounding variables related to, but not limited to, differences in body size, metabolic heat production during self-paced performance, and participant sex. Future research and reporting in these areas will help advance the field of biophysical modeling in open-water swimming.

The effective implementation of any policy that is determining risk before or during an event is constrained by the reliability and validity of measured parameters. Current international practice is to standardize the measurement of T_w at a depth of 40 cm (Fédération Internationale de Natation (FINA), 2020), but there is no standardization of measurement instrument, which can have important implications for the determination and categorization of risk in a sport and exercise setting (Cooper et al., 2017). This is important since the management of heat illness in open-water swimming competition occurs arguably between only a ~4°C zone of T_w (29°C–33°C), indicating that T_w changes of even 0.1°C may conceivably have meaningful implications.

In-race Cooling

In-race hydration is a common and feasible intervention during open-water swimming. Course feeding stations offer key strategic rehydration opportunities but require swimmers to make decisions between the necessary time required to consume cold beverages and the additional performance improvement or cooling effect that it may confer. While not largely reported, there appears to be a reluctance by some elite open-water swimmers to undertake substantial fluid replacement during competition (T_w of ~28.1°C; Hue et al., 2015). This is possibly due to athletes not valuing the additional time required to consume cool fluids (Hue et al., 2015) or gastrointestinal comfort concerns, but little attention has been paid to this question.

A review by Jay and Morris (2018) highlighted that the independent effect of cold water or ice slurry ingestion upon core temperature during terrestrial exercise is dependent on the prevailing environmental conditions. Cold fluid stimulates thermoreceptors in the stomach that may ultimately reduce the sweat rate response to exercise (Jay and Morris, 2018). Therefore, cold beverages may not always result in a net cooling benefit during exercise due to the potential for a concurrent reduction in sweat rate that reduces evaporative heat loss to at least the equivalent amount of the additional internal heat loss with the ingested cold fluid (Jay and Morris, 2018). However, in the context of swimming, evaporative heat loss plays a lesser role in comparison to terrestrial sporting events (McMurray and Horvath, 1979), and therefore, it is likely that the internal cooling benefit of drinking cold water during a swim race will outweigh the potential for any reductions in evaporative potential. Moreover, since much of the skin is continuously wet during swimming, any reductions in physiologically driven skin wettedness (*via* a reduction in sweating) that might occur with cold fluid ingestion may not have any meaningful effect upon evaporative potential during a race. An important consideration for warm water races could be to manipulate the length of each course lap, which will ultimately provide more regular access to feeding pontoons. This might encourage swimmers to consume small regular boluses of cold fluid, reducing the risk of gastric discomfort associated with potential reductions in gastric emptying rates (Anvari et al., 1995).

Like all cooling strategies, scientific evidence in the swimming literature is sparse. Hue et al. (2013) observed nine internationally-ranked swimmers during four 5 km training swims at a fixed pace while consuming either 190 ml of neutral (27°C) or cold fluid (1°C) each 1 km. The cold-fluid intervention mitigated the rise in T_{gi} (≥ ~0.4°C) in comparison to the neutral-water trial during evening training, but not morning training. The authors theorized that this could be due to the athletes experiencing slightly greater thermal strain (starting T_{gi}) and thermal stress (higher T_w of 29.1°C vs. 29.9°C) during the evening (Hue et al., 2013). Importantly, the athletes were only provided the ingestible temperature sensor 3 h prior to the training session (Hue et al., 2013), which is much shorter than commonly recommended [~8 h; (Wilkinson et al., 2008)] to avoid measurement issues with cold fluid consumption.

Hue et al. (2015) also examined the effect of fluid consumption in eight internationally-ranked open-water swimmers during two 5 km trials at a fixed race pace in T_w of ~28.6°C. The trials involved the swimmers ingesting either 190 ml of neutral (28°C) or cold (1°C) fluid at each 1 km interval (5 drinks in total). The rate of T_{gi} change was higher during the 5 km trial in the neutral-fluid consumption group in comparison to the cold-fluid consumption group (difference of ~1°C/h). The reason for such a large between-group discrepancy is unclear. Moreover, the same swimmers were observed during a sanctioned 10 km event in warm-water (T_w of 28.1°C) where they could consume neutral temperature (28°C) water *ad-libitum* at every 2 km interval. Notably, during the sanctioned competition event, athletes freely consumed only ~10% of the fluid that was consumed during the intervention trials (113 ml vs. 950 ml). This is an important consideration for the implementation of heat-related risk mitigation strategies, since when given a choice, open-water swimmers may not choose or be well-practiced at drinking larger volumes of cold fluid during a race that can result in meaningful changes to core temperature. This might relate to race tactics and the potential time-loss associated with the rehydration process.

Using known partitional calorimetry principles for determining change in body heat storage (Cramer and Jay, 2019), we predict

that a heat-stressed (core temperature of 39.0°C) elite swimmer during a 10 km event will need to consume ~400 *g* of 5°C fluid to elicit a meaningful reduction in core temperature (≥0.2°C) in comparison to not drinking. This assumes that the cold fluid consumption will have a negligible impact upon evaporative heat loss potential. This volume of fluid seems very achievable when consumed in small boluses, especially if a course is manipulated to allow more regular access to feeding pontoons. Further systematic research is needed that marries an ecologically valid volume of fluid consumption during simulated controlled conditions. Due to the relative contextual difficulty and consequences of rehydrating during competitive swimming in comparison to running and cycling, athletes may disregard in-race hydration cooling recommendations that are not mandatory if individual performance is perceived to be substantially impacted. In this instance, a compulsory hydration stop could address this issue.

Post-race Cooling

While the literature is sparse in a swimming-specific context for post-race cooling, insights can be gleaned from comprehensive reviews (McDermott et al., 2009) and position statements (Armstrong et al., 2007; Casa et al., 2015) from other sports. The post-race context is generally more generic in comparison to the in-event context across many sports. Cold-water immersion is considered the best strategy for quickly cooling a heat-stressed athlete (Casa et al., 2015).

CONCLUSION

This perspective article highlights the challenges and opportunities to develop evidence-based policies that mitigate risk during warm-water swimming competition. Future work is needed to advance knowledge on thermal strain and performance during warm-water competition, as well as evaluation of preparatory and in-race cooling strategies. Open-water swimming warm-water race policies would benefit from risk stratification (low, moderate, high, extreme) for a given race distance according to a holistic representation of prevailing environmental conditions, rather than just a single T_w threshold for the cancellation of all events.

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 author.

AUTHOR CONTRIBUTIONS

SC, GS, IM, and OJ contributed to the conception and writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Feeding Tolerance, Glucose Availability, and Whole-Body Total Carbohydrate and Fat Oxidation in Male Endurance and Ultra-Endurance Runners in Response to Prolonged Exercise, Consuming a Habitual Mixed Macronutrient Diet and Carbohydrate Feeding During Exercise

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Using metadata from previously published research, this investigation sought to explore: (1) whole-body total carbohydrate and fat oxidation rates of endurance (e.g., half and full marathon) and ultra-endurance runners during an incremental exercise test to volitional exhaustion and steady-state exercise while consuming a mixed macronutrient diet and consuming carbohydrate during steady-state running and (2) feeding tolerance and glucose availability while consuming different carbohydrate regimes during steady-state running. Competitively trained male endurance and ultra-endurance runners ($n = 28$) consuming a balanced macronutrient diet (57 ± 6% carbohydrate, 21 ± 16% protein, and 22 ± 9% fat) performed an incremental exercise test to exhaustion and one of three 3 h steady-state running protocols involving a carbohydrate feeding regime (76–90 g/h). Indirect calorimetry was used to determine maximum fat oxidation (MFO) in the incremental exercise and carbohydrate and fat oxidation rates during steady-state running. Gastrointestinal symptoms (GIS), breath hydrogen (H_2), and blood glucose responses were measured throughout the steady-state running protocols. Despite high variability between participants, high rates of MFO [mean (range): 0.66 (0.22–1.89) g/min], Fat_{max} [63 (40–94) % $\dot{V}O_{2max}$], and Fat_{min} [94 (77–100) % $\dot{V}O_{2max}$] were observed in the majority of participants in response to the incremental exercise test to volitional exhaustion. Whole-body total fat oxidation rate was 0.8 ± 0.3 g/min at the end of steady-state exercise, with 43% of participants presenting rates of ≥1.0 g/min, despite the state of hyperglycemia above resting homeostatic range [mean (95%CI): 6.9 (6.7–7.2) mmol/L]. In response to the carbohydrate feeding interventions of 90 g/h 2:1 glucose–fructose formulation, 38%

of participants showed breath H_2 responses indicative of carbohydrate malabsorption. Greater gastrointestinal symptom severity and feeding intolerance was observed with higher carbohydrate intakes (90 vs. 76 g/h) during steady-state exercise and was greatest when high exercise intensity was performed (i.e., performance test). Endurance and ultra-endurance runners can attain relatively high rates of whole-body fat oxidation during exercise in a post-prandial state and with carbohydrate provisions during exercise, despite consuming a mixed macronutrient diet. Higher carbohydrate intake during exercise may lead to greater gastrointestinal symptom severity and feeding intolerance.

Keywords: feeding intolerance, malabsorption, gastrointestinal symptoms, blood glucose, skeletal muscle, running

INTRODUCTION

Prolonged endurance and ultra-endurance activities (e.g., ~3 h sustained workload) place unique energy demands on individuals, considering that endogenous and exogenous energy substrate is required to maintain work rate over multiple hours of continuous exercise (Costa et al., 2018; Burke et al., 2019; Scheer et al., 2020). Limited availability of endogenous carbohydrate (CHO) stores prompts athletes to attempt dietary interventions aimed at maximizing oxidation of endogenous fat substrate, at exercise intensities relevant to prolonged endurance and ultra-endurance competition (Phinney et al., 1983; Volek et al., 2016; Burke et al., 2020). It has been shown that acute or chronic low-carbohydrate high-fat (LCHF) dietary interventions [e.g., ≤1 gCHO/kg body mass (BM)/day] increase whole-body fat oxidation during prolonged aerobic exercise in both highly trained and recreationally competitive athletes compared to typical carbohydrate dietary provisions (e.g., ~6 gCHO/kgBM/day; Burke et al., 2018; Russo et al., 2021b). Maximum fat oxidation (MFO) rate of 1.54 vs. 0.67 g/min during an incremental graded exercise test, and MFO occurring at a greater percentage of $\dot{V}O_{2max}$ (Fat_{max}; 70.3 vs. 54.9%), has been reported when comparing long-term low- vs. high-carbohydrate diets, respectively, albeit through self-reported dietary log methods (Volek et al., 2016). Additionally, during a 180 min submaximal run of a similar intensity to ultra-marathon competition (i.e., 64% $\dot{V}O_{2max}$), total fat oxidation was significantly greater in a LCHF (1.21 g/min) compared with a high-carbohydrate (0.76 g/min) dietary group, when only water was consumed during exercise (Volek et al., 2016). However, such metabolic adaptations may be at the expense of altering gastrointestinal functional responses through downregulating intestinal carbohydrate transporters (Jeukendrup, 2017; Costa et al., 2017a), and/or suppressing carbohydrate aerobic oxidative pathways through glycolytic enzyme downregulation (Stellingwerff et al., 2006), irrespective of dietary carbohydrate provision upon reintroduction and/or increased carbohydrate provisions during exercise. Both of these carbohydrate tolerance outcomes may have implications in impairing exercise performance, from the perspective of gastrointestinal symptom induction (Costa et al., 2017a; Miall et al., 2018), and skeletal muscle metabolism fuel kinetics (Burke et al., 2017, 2020, 2021).

It is well established that dietary modification can alter fuel kinetics during prolonged endurance exercise. For example, adaptations to high whole-body fat oxidation during endurance exercise have been repeatedly observed in elite race walkers (i.e., ≥1.0 g/min) after a period of LCHF dietary intervention, but not observed with sustained high carbohydrate dietary intake (e.g., 8.6 gCHO/kgBM/day; Burke et al., 2017, 2021), noting the high-intensity and short-endurance duration of the exercise model (e.g., 10 and 25 km race walk competition simulation). Regardless of dietary choice, endurance and ultra-endurance athletes regularly train and compete beyond the point of metabolically stressed endogenous carbohydrate stores (i.e., ≥3 h; Alcock et al., 2018), with carbohydrate intake during exercise rarely meeting the energy expenditure of the respective exercise bout, despite the ability to maintain a sustained exercise workload (Costa et al., 2018). It is therefore plausible that such athletes would develop adaptations to optimize fat oxidation, even when consuming a mixed macronutrient diet, due to the frequent low-carbohydrate availability state encountered toward the end of prolonged exercise training sessions undertaken on consecutive days. In accordance with this plausibility, it has recently been suggested that adjusting carbohydrate availability to match the training demands will allow for optimal training completion in adjunct with desired training adaptations (Impey et al., 2018). A recent systematic literature review, however, suggested that such dietary carbohydrate adjustment, "*carbohydrate periodization*," does not translate into improved performance outcomes (Gejl and Nybo, 2021). It is, however, important to highlight that the review's focus was on the restriction of dietary carbohydrate intake in conjunction with relatively short bouts of steady-state and/or intense (i.e., performance test) endurance exercise (<3 h), and not on increasing exercise load while consuming habitual dietary carbohydrate, as per typical ultra-endurance training practices (i.e., consecutive days of ≥3 h per session). Nevertheless, despite the large variation in carbohydrate restriction and exercise protocols between studies, the dietary interventions employing greater carbohydrate intake variation, and longer duration experimental exercise models, appeared to result in the largest performance difference favoring carbohydrate periodization (Marquet et al., 2016a,b). Taken together, these theoretical conceptualizations suggest LCHF dietary interventions and/or carbohydrate periodization may not necessarily be required to

obtain a high rate of whole-body total fat oxidation during sustained aerobic exercise to support optimal endurance exercise outcomes, if endurance and ultra-endurance athletes are undertaking training sessions "*over-and-above*" the duration considered to stress muscle glycogen stores, irrespective of glucose availability. With the broader research focusing on LCHF diets and carbohydrate periodization protocols, it is important to highlight that other strategies that may enhance fat oxidation efficiency during steady-state exercise (e.g., dietary choice and/or training load) require further exploration and substantiation in the recreational endurance and ultra-endurance population commonly encountering exercise bouts ≥3 h duration at lower intensities.

From a carbohydrate intake and oxidation during prolonged endurance exercise perspective, it is clear from the available research that tolerance to high intake rates of carbohydrate during prolonged endurance and ultra-endurance exercise enhances performance in a dose-dependent manner (e.g., 9–78 g/h), with optimal intake range reported at 68–88 g/h, and diminishing performance enhancing effects with intake rates of >78 g/h (Stellingwerff and Cox, 2014). Although broad-spectrum guidelines and recommendations are advised targeted at high carbohydrate intake rates during exercise ≥3 h (e.g., up to 90 g/h multiple-transportable carbohydrates), and taking into consideration the exercise scenario (e.g., duration and ambient conditions) and individualism (e.g., fitness status and tolerance; Thomas et al., 2016); concerns have been raised in professional practice, especially within ultra-endurance running event nutritional support, regarding feeding tolerance issues arising in practice. Sports Dietetic and/or Nutrition Practitioners, supporting both recreational and elite-level endurance and ultra-endurance athletes, consistently report intolerance to high carbohydrate intake rates during exercise among the majority of the competitive endurance and ultra-endurance runners and may contribute to the performance debilitating gastrointestinal symptoms (GIS) consistently reported, especially in ultra-marathon events (Costa et al., 2017b). Such anecdotal observations from practitioners have been supported by previous field (e.g., multi-stage and single-stage ultra-marathon competition), laboratory-controlled research (e.g., gut challenge protocol), and clinical cases (Costa et al., 2016, 2017a; Alcock et al., 2018; Gaskell et al., 2021c). It is important to also note that the broad-spectrum recommendations for high intake rates of carbohydrate, through multiple-transportable carbohydrate forms, during exercise stem from the potential saturation of intestinal epithelial carbohydrate transporters (i.e., SGLT-1 and GLUT5) and maximal transport activity, speculating that intestinal epithelial carbohydrate transporters are the prime rate limiting factor for circulatory glucose availability, for subsequent muscle glucose uptake and oxidation during high-intensity prolonged endurance exercise (e.g., >70% $\dot{V}O_{2max}$), generally investigated in homogenous populations (e.g., highly trained and elite male cyclists; Jeukendrup, 2014, 2017). These broad-spectrum intake values appear to exceed whole-body total carbohydrate oxidation rates of many endurance and ultra-endurance populations at their respective competition exercise workload (Costa et al., 2017a). Personalized carbohydrate intake rates during exercise according to needs (e.g., relative to athlete's body mass, ~1.0 g/kgBM/h) may provide sufficient carbohydrate fuel to support whole body carbohydrate oxidation while mitigating GIS incidence during moderate intensity prolonged endurance and ultra-endurance exercise (e.g., ~60% $\dot{V}O_{2max}$). As such, updated carbohydrate intake guidelines and recommendations for endurance running have been proposed in accordance with the World Athletics (formally the IAAF) consensus statement (Burke et al., 2019; Costa et al., 2019b).

With this in mind, the current study aimed to utilize metadata from previously published research (Costa et al., 2017a; McCubbin et al., 2020; Gaskell et al., 2021a) to: (1) explore fuel kinetics of endurance and ultra-endurance runners in response to an incremental exercise test to volitional exhaustion and (2) explore feeding tolerance and GIS (i.e., incidence and severity), glucose availability, and whole-body total carbohydrate and fat oxidation rates, in response to differing carbohydrate intake protocols during prolonged strenuous exercise protocols in competitively trained male endurance (e.g., half and full marathon) and ultra-endurance (>full marathon) runners consuming a habitual mixed macronutrient diet. Based on the current literature, it was hypothesized that: (1) endurance and ultra-endurance runners would exhibit high MFO in response to the incremental exercise test to volitional exhaustion and (2) proportionally higher carbohydrate feeding rates during prolonged steady-state exercise would result in greater feeding intolerance and GIS (i.e., incidence and severity), but greater blood glucose availability and maintenance of whole-body total carbohydrate oxidation. In addition, it was also hypothesized that participants would present high fat oxidation rates (i.e., ≥1 g/min), typically assumed only possible following LCHF ketogenic diets, during prolonged steady-state exercise despite habitually consuming a mixed macronutrient diet.

TABLE 1 | Participant characteristics.

n **= 28**	**P1 (*n* = 13)**	**P2 (*n* = 7)**	**P3 (*n* = 8)**	***P***
Age (y)	36 (32–43)	46 (36–55)	35 (30–38)	0.017
Height (m)	1.80 (1.77–1.83)	1.76 (1.72–1.79)	1.80 (1.76–1.83)	0.334
Body mass (kg)	76.3 (72.2–80.4)	75.9 (70.2–81.6)	75.3 (72.3–78.4)	0.953
Body fat mass (%)	11.7 (9.8–13.7)	16.7 (14.0–19.4)	14.7 (10.6–18.7)	0.059
$\dot{V}O_{2max}$	58.9 (55.2–62.7)	54.9 (55.2–62.7)	59.2 (49.3–60.5)	0.444
Steady-state running speed*	10.6 (10.1–11.2)	9.4 (9.1–9.7)	9.9 (9.0–10.9)	0.011
Three hour protocol distance covered (km)**	34.4 (32.7–38.1)	28.1 (27.0–29.3)	29.8 (26.2–34.3)	<0.001
Training volume (min/week)	460 (377–543)	501 (400–603)	498 (408–590)	0.764

*Mean (95% CI). *Running speed at 60% $\dot{V}O_{2max}$.*
***Distance covered across the three distinct 3 h running exercise protocols.*

MATERIALS AND METHODS

Participants

Twenty-eight competitively trained male runners volunteered to participate in the study (**Table 1**). Participants identified as either recreationally trained endurance (e.g., half and/or full marathon) and/or ultra-endurance (i.e., >marathon) runners based on competition or event participation. All participants gave written informed consent, which received local ethics (Monash University Human Research Ethics Committee) approval (ethics approval numbers: CF13/3645-2013001874, 15012, and 18587) and conformed to the Helsinki Declaration for Human Research Ethics. Standard exclusion criteria have previously been defined in Costa et al. (2017a). In addition, participants were also excluded if reporting adhering to macronutrient modification dietary practices (e.g., LCHF, ketogenic, and/or glycogen manipulation diets) within 1 month before the experimental protocol. All participants reported consuming a standard varied macronutrient diet on training and non-training days [mean ± SD (% energy contribution): 11.8 ± 2.8 MJ/day (149 ± 44 g/day protein (21 ± 6%), 403 ± 115 g/day carbohydrate (57 ± 16%), 68 ± 29 g/day fat (22 ± 9%), and 3.1 ± 0.9 L/day water)], which was confirmed by dietary assessment and analysis similar to previously reported procedures (Costa et al., 2013, 2014). All participants reported having some exposure in consuming carbohydrate (i.e., solid, semi-solid, and/or fluid) during training and/or competition, but no participant reported being accustomed to consuming ≥90 g/h. In addition, all participants reported having experienced a mild to severe GIS episode during training and/or competition. As part of standard experimental procedures, participants refrained from strenuous exercise in the days leading up to (i.e., 24–48 h) any exercise testing session. To minimize participant artifact errors potentially associated with low level of fitness status and training load, despite participants identifying themselves as endurance or ultra-endurance runners, participants with measured $\dot{V}O_{2max}$ < 45.0 ml/kgBM/min were excluded from the study.

Experimental Procedures

Incremental Exercise Test to Volitional Exhaustion

The incremental test was deliberately undertaken 2 h post-prandial (e.g., 2.9 ± 1.0 MJ, 29 ± 12 g protein, 97 ± 37 g carbohydrates, 18 ± 6 g fat, and 414 ± 235 ml water; Russo et al., 2021a) to reflect endurance athlete behavior before longer training sessions or competition (Costa et al., 2013, 2014). Baseline stature and BM were measured, and body fat mass determined using multi-frequency bioelectrical impedance analysis (mBCA 515, Seca, Ecomed, Hamburg, Germany). An incremental running test was performed to volitional exhaustion on a motorized treadmill to determine $\dot{V}O_{2max}$, MFO, Fat_{max}, and minimal fat oxidation (Fat_{min}) through breath-by-breath indirect calorimetry (Vmax Encore Metabolic Cart, Carefusion, San Diego, California, United States), in 20–22°C ambient temperature (T_{amb}) and 45–55% relative humidity (RH). The exercise test began with a treadmill speed of 6 km/h; then, running initiated at 8 km/h at 1% inclination. Speed was increased by 2 km/h every 3 min until reaching 16 km/h, at which point inclination was increased by 2.5% every 3 min until the participant reached volitional exhaustion (Costa et al., 2009). Criteria for attaining $\dot{V}O_{2max}$ included the participants reaching volitional exhaustion (e.g., rating of perceived exertion 19–20), a heart rate within 10 beats/min of predicted maximal heart rate, respiratory exchange ratio (RER) of ≥1.10, and/or no further increases in $\dot{V}O_{2max}$ observed with increasing workload. During the incremental exercise test, two fans were placed one meter from the treadmill at a dual fan speed of 10.6 km/h. Whole-body total carbohydrate and fat oxidation rates were calculated from the last min of each increment, using non-protein respiratory quotient values as published by Péronnet and Massicotte (1991):

$$\text{Total CHO oxidation} : \left(4.585 \times \dot{V}CO_2\right) - \left(3.226 \times \dot{V}O_2\right)$$

$$\text{Total Fat oxidation} : \left(1.695 \times \dot{V}O_2\right) - \left(1.701 \times \dot{V}CO_2\right)$$

MFO was calculated as the highest rate of fat oxidation achieved at any interval, Fat_{max} the % $\dot{V}O_{2max}$ attained at MFO, and minimum fat oxidation (Fat_{min}) the % $\dot{V}O_{2max}$ when fat oxidation ceased [i.e., respiratory exchange ratio (RER) = 1.000]. From the $\dot{V}O_2$ – work rate relationship, the treadmill speed at 60% $\dot{V}O_{2max}$ and 1% gradient was extrapolated and verified and used to determine the running speed for the steady-state exercise (10.1 ± 1.2 km/h).

Endurance Exercise Test

On a separate occasion, ≥1 week after the incremental exercise test, participants reported to the laboratory (0800 h), having consumed a standardized breakfast (2.3 ± 0.2 MJ, 17 ± 2 g protein, 94 ± 8 g carbohydrate, and 13 ± 3 g fat, 499 ± 207 ml water) 2 h prior to exercise initiation (0700 h), in a euhydrated state [plasma osmolality (P_{Osmol}) ≤300 mOsmol/kg and/or total body water ≥55%]. Within 30 min of beginning the test, participants voided before pre-exercise body mass was measured. As part of exercise gastroenterology intervention studies reported elsewhere (Costa et al., 2017a; McCubbin et al., 2020; Gaskell et al., 2021a), participants undertook one of three endurance exercise protocols:

Protocol 1 (P1; Costa et al., 2017a): after consuming a habitual varied macronutrient diet (*ad libitum* with recorded intake) in the lead-up days before the main experimental trial, participants then undertook the experimental endurance exercise test. This consisted of 2 h steady-state treadmill running (n = 13: T_{amb} 23 ± 1°C, 54 ± 7% RH, dual-fan wind speed 10.6 km/h, heart rate 139 ± 6 bpm, and RPE 12 ± 1) at the previously determined treadmill speed corresponding to 60% $\dot{V}O_{2max}$, while consuming a formulated gel-disc containing 30 g carbohydrate with 300 ml water (10% *w/v*, 90 g/h, 2:1 glucose–fructose, 316 mOsmol/kg), at 0 min and every 20 min thereafter; followed by 1 h distance test (heart rate 164 ± 6 and RPE 15 ± 2) with water provisions *ad libitum* (270 ± 215 ml). Total distance over the 3 h protocol was 34.4 ± 3.0 km. Exercise-associated BM loss and post-exercise P_{Osmol} were 2.3 ± 0.9% and 300 ± 7 mOsmol/kg, respectively.

Protocol 2 (P2; Gaskell et al., 2021a): after consuming a habitual varied macronutrient diet (*ad libitum* with recorded

intake) in the lead-up days before the main experimental trial and a provided standardized 24-h low FODMAP diet (i.e., total FODMAP 2 ± 0 g/day; Gaskell et al., 2020), participants then undertook the experimental endurance exercise test. This consisted of 3 h steady-state treadmill running ($n = 7$: T_{amb} 23 ± 1°C, 44 ± 6% RH, dual-fan wind speed 10.6 km/h, heart rate 134 ± 9, and RPE 13 ± 1), at the previously determined treadmill speed corresponding to 60% $\dot{V}O_{2max}$, while consuming a beverage containing 25 ± 3 g carbohydrate (10% *w/v*, 76 ± 8 g/h, 509 mOsmol/kg; equivalent to 1.0 g/kgBM/h; Costa et al., 2017a; Miall et al., 2018) at 0 min and every 20 min thereafter for the first 2 h, then water provisions *ad libitum* for the 3rd h (170 ± 122 ml), apart from a 150 ml solution containing 20 g of lactulose (Actilax, alphapharm, QLD, Australia) at 150 min as part of the OCTT procedure. Total distance over the 3 h protocol was 28.1 ± 1.2 km. Exercise-associated BM loss and post-exercise P_{Osmol} were 1.1 ± 0.4% and 294 ± 5 mOsmol/kg, respectively. To avoid participant duplication bias, one participant was fully removed from the data set due to participation in P1.

Protocol 3 (P3; McCubbin et al., 2020): after consuming a habitual varied macronutrient diet (*ad libitum* with recorded intake) in the lead-up days before the main experimental trial, participants then undertook the experimental endurance exercise test. This consisted of 3 h steady-state treadmill running ($n = 8$: T_{amb} 24 ± 1°C, 39 ± 9% RH, dual-fan wind speed 10.6 km/h, heart rate 138 ± 5, and RPE 11 ± 2), at the previously determined treadmill speed corresponding to 60% $\dot{V}O_{2max}$, while consuming a beverage containing 45 g carbohydrate (16% *w/v*, 90 g/h, 2:1 glucose–fructose, 460 mOsmol/kg), at 0 min and every 30 min thereafter. Additional water was provisioned *ad libitum*, but not consumed by participants (0 ± 0 ml). Total distance over the 3 h protocol was 29.8 ± 4.2 km. Exercise-associated BM loss was 2.2 ± 0.7%, while P_{Osmol} was not determined on this occasion. To avoid participant duplication bias, one participant was fully removed from the data set due to participation in P1.

Capillary blood glucose was measured every 30 min with a handheld glucometer (Accuchek, Roche, Basel, Switzerland). $\dot{V}O_2$, $\dot{V}CO_2$, and RER were measured every 20 min in P1, and every 30 min in P2 and P3, using breath-by-breath indirect calorimetry, as previously described. Whole-body total carbohydrate and fat oxidation were calculated from the last 5 min of each collection point, as previously described. Heart rate (Polar Electro, Kempele, Finland) and rating of perceived exertion (RPE; 6–20 scale; Borg, 1982) were recorded every 10 min on P1, and every 15 min on P2 and P3, during the endurance exercise test. Due to the nature of the experimental design, GIS and feeding tolerance were recorded every 10 min for the first 120 min and again at 180 min (post distance test) on P1, and every 15 min continuously for 180 min on P2 and P3, during the endurance exercise test. A validated and reliability checked, exercise specific, modified visual analogue (mVAS) scale was used to assess gut discomfort, total-GIS, upper-GIS (i.e., gastro-esophageal symptoms: belching, heartburn, stomach bloating, upper abdominal pain, urge to regurgitate, and/or actual regurgitation), lower-GIS [intestinal symptoms: flatulence, lower abdominal bloating, lower abdominal pain, urge to defecate, and abnormal defecation (e.g., loose watery stools, diarrhea, and/or fecal blood loss)], and other related symptoms that include transient abdominal pain (stitch) and nausea, during exercise (10-point rating scale, each point indicative of 10 mm; Gaskell et al., 2019). Participants were educated and advised to complete the GIS rating scale as follows: 1–4 indicative of mild GIS (i.e., sensation of GIS, but not substantial enough to interfere with exercise workload) and increasing in magnitude, 5–9 indicative of severe GIS (i.e., GIS substantial enough to interfere with exercise workload), and 10 indicative of extremely severe GIS warranting exercise reduction or cessation. If no GIS were reported by participants, this was recorded as a 0, and subsequently no GIS severity rating was assessed. Considering GIS, such as regurgitation and defecation, results in complete or temporary reduction or cessation of exercise, these GIS are presented as 0 and 10 rating only. Additionally, a 10-point Likert-type rating scale was used to quantify self-reported perceptive feeding tolerance, with 0 indicating no tolerance to 10 indicating extremely high tolerance (five indicative of moderate tolerance; Miall et al., 2018). For consistency, all measurements and samples were recorded and collected before carbohydrate feeding at each respective time point. In addition, breath samples were obtained pre-exercise, during (every 1 h and 30 min, respectively) and throughout recovery (every 30 min until 4 h post-exercise and every 15 min until 2 h post-exercise, respectively) and analyzed in duplicate for breath H_2 determination (BreathTracker Digital MicroLyzer; Quintron, Milwaukee, WI, United States) on P1 and P3. Breath samples were collected in accordance with clinical gastroenterology guidelines (Bate et al., 2010), whereby participants were instructed to expire normally twice into a 250 ml breath collection bag that included a mouthpiece and residue bag (Wagner Analysen Technick, Bremen, Germany). The breath sample was collected on the 2nd expiration. Breath H_2 determination on P2 was used to detect gastrointestinal transit in accordance with orocecal transit time procedures (Gaskell et al., 2021a) and, therefore, is not possible to be used for detecting any malabsorption of the carbohydrate feeding regime during exercise.

Statistical Analysis

Confirmation of adequate statistical power *a priori* for the primary research is previously described (Costa et al., 2017a; McCubbin et al., 2020; Gaskell et al., 2021a). Participants and researchers at the time of data collection were unaware that the combined metadata would be used for analysis of feeding tolerance, GIS, blood glucose availability, and whole-body total carbohydrate and fat oxidation rates in response to various exertional stress protocols with differing carbohydrate feeding regimes during exercise. Based on the statistical test, mean, SD, and effect size, and applying a standard alpha (0.05) and beta value (0.80), the current participant sample size is estimated to provide adequate statistical power (power* 0.80–0.99) for detecting significant exercise and feeding associated differences within protocols, but not between protocols (G*Power 3.1, Kiel, Germany). Descriptive data in text are presented as mean ± SD. Primary and secondary variable data in text and tables are presented as mean and 95% CI, unless otherwise indicated.

For clarity, data in figures are presented as mean ± SEM. All data were checked for normal distribution (Shapiro–Wilk test of normality) by calculating skewness and kurtosis coefficients. General linear mixed model with *post hoc* was used to determine differences in oxidation rates during the incremental exercise test. Variables with singular data points were examined using independent sample *t* tests or nonparametric Mann–Whitney U test, when appropriate. Variables with multiple data points were examined using a one-way ANOVA or nonparametric Kruskal–Wallis test, when appropriate, with Tukey's *post hoc* HSD. Pearson's or Spearman's rank correlation coefficient was used to assess associations between variables. Statistics were analyzed using SPSS statistical software (V.26.0, IBM Corp, Armonk, NY, United States) with significance accepted at $p < 0.05$.

RESULTS

Participants

Participant characteristics between exercise protocols are depicted in **Table 1**. Age ($p = 0.017$), steady-state running speed ($p = 0.011$), and distance covered in the 3 h exercise test ($p < 0.001$) were significantly different, whereby an older participant cohort was recruited in P2 [46 (36–55) y] compared with P1 [36 (32–43) y] and P3 [35 (30–38) y]. In addition, steady-state running speed in P1, and distance covered in P1 due to the inclusion of a 1 h distance test in the 3rd h of exercise, was higher (10.6 km/h and 34.4 km, respectively) compared with P2 (9.4 km/h and 28.1 km, respectively) and P3 (9.9 km/h and 29.8 km, respectively).

Incremental Exercise Test

As exercise intensity increased, whole-body total carbohydrate oxidation increased proportionally ($p < 0.001$), and whole-body total fat oxidation decreased until reaching Fat_{min} ($p < 0.001$). MFO observed in the incremental exercise test was 0.66 g/min (range: 0.22–1.89 g/min) with Fat_{max} occurring at 63% of $\dot{V}O_{2max}$ (range: 40–94%) and the cessation of fat oxidation occurring at 94% of $\dot{V}O_{2max}$ (range: 77–100%). No significant differences were observed between exercise protocol groups.

Substrate Oxidation Rates During Steady-State Exercise

Whole-body energy expenditure and whole-body total carbohydrate and fat oxidation rates during steady-state exercise are presented in **Figure 1**. No change in whole-body energy expenditure was observed on P1 ($p = 0.853$), P2 ($p = 0.966$), and P3 ($p = 0.996$). Whole-body total carbohydrate oxidation during steady-state exercise did not significantly change along the exercise protocol on P2 ($p = 0.571$) and P3 ($p = 0.082$); however, a significant reduction at 3 h in P1 ($p = 0.001$) was observed following the cessation of carbohydrate feeding. Whole-body total fat oxidation during steady-state exercise did not significantly change along the exercise protocol on P2 ($p = 0.064$) and P3 ($p = 0.329$); however, a significant increase at 3 h in P1 ($p < 0.001$) was observed following the cessation of carbohydrate feeding. Across the three exercise protocols, 46% of runners presented whole-body total fat oxidation rates during steady-state exercise ≥1.0 g/min ($n = 13/28$). No significant correlations were observed between whole-body total carbohydrate and fat oxidation rates during steady-state and end of exercise with BM, training volume, and fitness status ($\dot{V}O_{2max}$). No significant correlations were observed between whole-body total carbohydrate oxidation during steady-state or end of exercise with absolute dietary energy, protein, carbohydrate, and fat intake. Analysis conducted on relative (corrected for BM) dietary intake revealed a significant positive association between carbohydrate intake and whole-body total carbohydrate oxidation during steady-state exercise ($r = 0.415$, $p = 0.049$). A significant positive association between absolute dietary fat intake with whole-body total fat oxidation during steady-state exercise [fat ($r_s = 0.465$, $p = 0.025$)] was observed. In addition, significant positive associations between absolute dietary energy ($r_s = 0.445$, $p = 0.033$), protein ($r_s = 0.480$, $p = 0.020$), and fat ($r_s = 0.594$, $p = 0.003$) intakes with whole-body total fat oxidation at the end of exercise were observed, but not for dietary carbohydrate intake ($r = 0.029$, $p = 0.894$). Analysis conducted on relative (corrected for BM) dietary intake revealed significant positive association between protein ($r = 0.459$, $p = 0.028$) and fat ($r_s = 0.508$, $p = 0.013$) intake and whole-body total fat oxidation at the end of exercise.

Blood Glucose During Steady-State Exercise

Blood glucose responses are presented in **Figure 2**. From baseline, blood glucose concentration increased in response to exercise and the respective carbohydrate feeding procedures in P1 ($p < 0.001$), P2 ($p < 0.001$), and P3 ($p = 0.015$).

Breath H_2

No significant changes were observed for breath H_2 responses to the carbohydrate feeding intervention during exercise. A significant increase in breath H_2 ($p < 0.001$) was observed during the recovery period, whereby values increased from 2 (1–3) ppm pre-exercise baseline to 8 (3–12) ppm between 30 and 120 min post-exercise ($p < 0.01$). In response to the carbohydrate feeding intervention on P1 and P3 (90 g/h 2:1 glucose–fructose formulation), 38% of participants showed breath H_2 responses indicative of carbohydrate malabsorption (≥10 ppm; Bate et al., 2010).

Gastrointestinal Symptoms

Gastrointestinal symptoms are presented in **Figure 3**. In response to the carbohydrate feeding intervention during exercise, incidence of minor and severe GIS was reported on P1 (100 and 46%, respectively), P2 (100 and 57%, respectively), and P3 (89 and 22%, respectively). Increases in gut discomfort were observed on P1 ($p < 0.001$), and P3 ($p = 0.005$), but not P2 ($p = 0.155$), as the exercise progressed (**Figure 3A**). Increases in total-GIS and upper-GIS were observed on P1 ($p = 0.003$

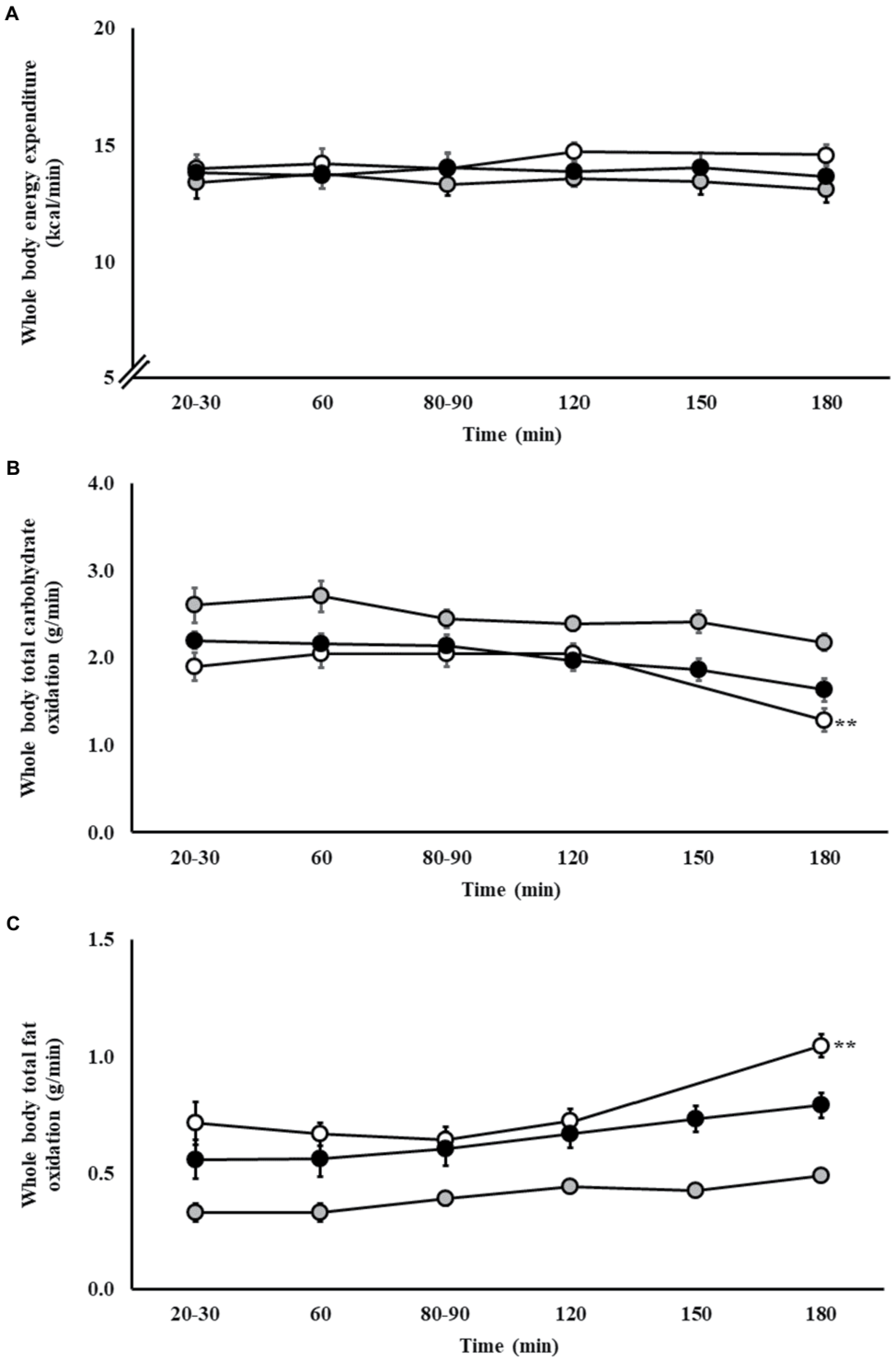

FIGURE 1 | Whole body energy expenditure **(A)**, total carbohydrate **(B)** and fat **(C)** oxidation rates during steady-state running with carbohydrate (CHO) provision on P1 (○), P2 (◍), and P3 (●). Mean ± SEM ($n = 28$): $^{**}p < 0.01$ vs. 20 min. P1: formulated gel-disc containing 30 g CHO with 300 ml water (10% *w/v*, 90 g/h, 2:1 glucose–fructose, 316 mOsmol/kg), at 0 min and every* 20 min thereafter for 120 min; followed by water provisions *ad libitum* for the 3rd h (270 ± 215 ml). Oxidation rates measured every 20 min for 120 min, then at 180 min. P2: CHO beverage containing 25 ± 3 g CHO (10% *w/v*, 76 ± 8 g/h, 509 mOsmol/kg; equivalent to 1.0 g/kgBM/h) at 0 min and every 20 min thereafter for 120 min, then water provisions *ad libitum* for the 3rd h (170 ± 122 ml). Oxidation rates measured every 30 min for 180 min. P3: CHO beverage containing 45 g CHO (16% *w/v*, 90 g/h, 2:1 glucose–fructose, 460 mOsmol/kg), at 0 min and every 30 min thereafter for 180 min. Water provisioned *ad libitum*, but not consumed by participants (0 ± 0 ml). Oxidation rates measured every 30 min for 180 min.

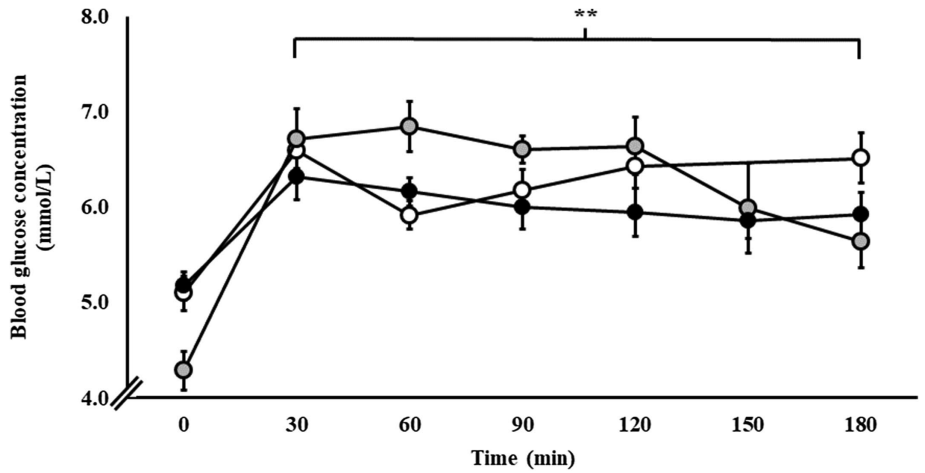

FIGURE 2 | Blood glucose responses during steady-state running with CHO provision on P1 (○), P2 (◍), and P3 (●). Mean ± SEM ($n = 28$): $^{**}p < 0.01$ vs. 0 min. P1: formulated gel-disc containing 30 g CHO with 300 ml water (10% *w/v*, 90 g/h, 2:1 glucose–fructose, 316 mOsmol/kg), at 0 min and every 20 min thereafter for 120 min; followed by water provisions *ad libitum* for the 3rd h (270 ± 215 ml). P2: CHO beverage containing 25 ± 3 g CHO (10% *w/v*, 76 ± 8 g/h, 509 mOsmol/kg; equivalent to 1.0 g/kgBM/h) at 0 min and every 20 min thereafter for 120 min, then water provisions *ad libitum* for the 3rd h (170 ± 122 ml). P3: CHO beverage containing 45 g CHO (16% *w/v*, 90 g/h, 2:1 glucose–fructose, 460 mOsmol/kg), at 0 min and every 30 min thereafter for 180 min. Water provisioned *ad libitum*, but not consumed by participants (0 ± 0 ml).

and $p = 0.014$, respectively) and P3 ($p = 0.008$ and $p = 0.036$, respectively), but not P2 ($p = 0.120$ and $p = 0.148$, respectively; **Figures 3B,C**). There were no significant changes in lower-GIS (**Figure 3D**) and nausea on P1 ($p = 0.254$ and $p = 0.091$, respectively), P2 ($p = 0.381$ and $p = 1.000$, respectively), and P3 ($p = 0.079$ and $p = 1.000$, respectively).

Feeding Tolerance

Feeding tolerance markers are presented in **Figure 4**. Perception of appetite and thirst was reported as low (≤5.0) throughout all exercise protocols, and no significant difference was observed throughout exercise on P1 ($p = 0.250$ and $p = 0.929$, respectively), P2 ($p = 0.440$ and $p = 0.729$, respectively), and P3 ($p = 0.250$ and $p = 0.696$, respectively). Interest in food and drink ("I want to eat and drink") was also low (<5.0) throughout all exercise protocols, and no significant difference was observed throughout exercise on P1 ($p = 0.739$ and $p = 0.762$, respectively), P2 ($p = 0.151$ and $p = 0.953$, respectively), and P3 ($p = 0.684$ and $p = 0.626$, respectively). However, tolerance to food and drink ("I could eat and drink") significantly decreased as exercise progressed on P1 ($p = 0.013$ and $p = 0.046$, respectively), but not P2 ($p = 0.894$ and $p = 0.986$, respectively) and P3 ($p = 0.292$ and $p = 0.118$, respectively).

DISCUSSION

The current study aimed to utilize metadata from previously published research to explore: (1) fuel kinetics of endurance and ultra-endurance runners in response to an incremental exercise test to volitional exhaustion and (2) gastrointestinal feeding tolerance and GIS, glucose availability, and whole-body total carbohydrate and fat oxidation rates, in response to differing carbohydrate intake protocols during prolonged strenuous exercise protocols in competitively trained male endurance and ultra-endurance runners consuming a habitual mixed macronutrient diet. Firstly, the data show a vast range of MFO rates during both the incremental exercise test (i.e., 0.22–1.89 g/min) and steady-state exercise (0.35–1.29 g/min at 180 min), with an average MFO of 0.8 and ≥1 g/min observed in $n = 13/28$ participants at the end (i.e., 180 min) of the experimental endurance exercise test, despite consuming carbohydrate during exercise and presenting elevated blood glucose concentrations. These data suggest that a high whole-body total fat oxidation rate can be attained without dietary carbohydrate abstinence or restriction, and even when carbohydrate is provided during prolonged endurance exercise (i.e., up to 3 h). Secondly, the data suggest that gastrointestinal feeding tolerance to ingestion of 90 g/h carbohydrate during prolonged steady-state running was poorer compared with relative carbohydrate provisions (1.0 g/kgBM/h, 76 g/h), with greater feeding intolerance observed when exercise intensity is increased (i.e., performance distance test), as evidenced by lower feeding tolerance markers, high incidence and severity of GIS, and carbohydrate malabsorption.

Previous research has explored whole-body total carbohydrate and fat oxidation rates in the post-prandial period. Following a meal containing ~141 g of carbohydrate in a low and high glycemic index from 3 h prior to exercise, average whole-body total carbohydrate oxidation rates of 2.70–3.16 g/min and fat oxidation rates of 0.14–0.33 g/min in response to a running time to exhaustion performance test at 70% $\dot{V}O_{2max}$, in recreational runners, have been reported (Wu and Williams, 2006). While previous research investigated whole-body total carbohydrate and fat oxidation rates with carbohydrate feeding during 150 min

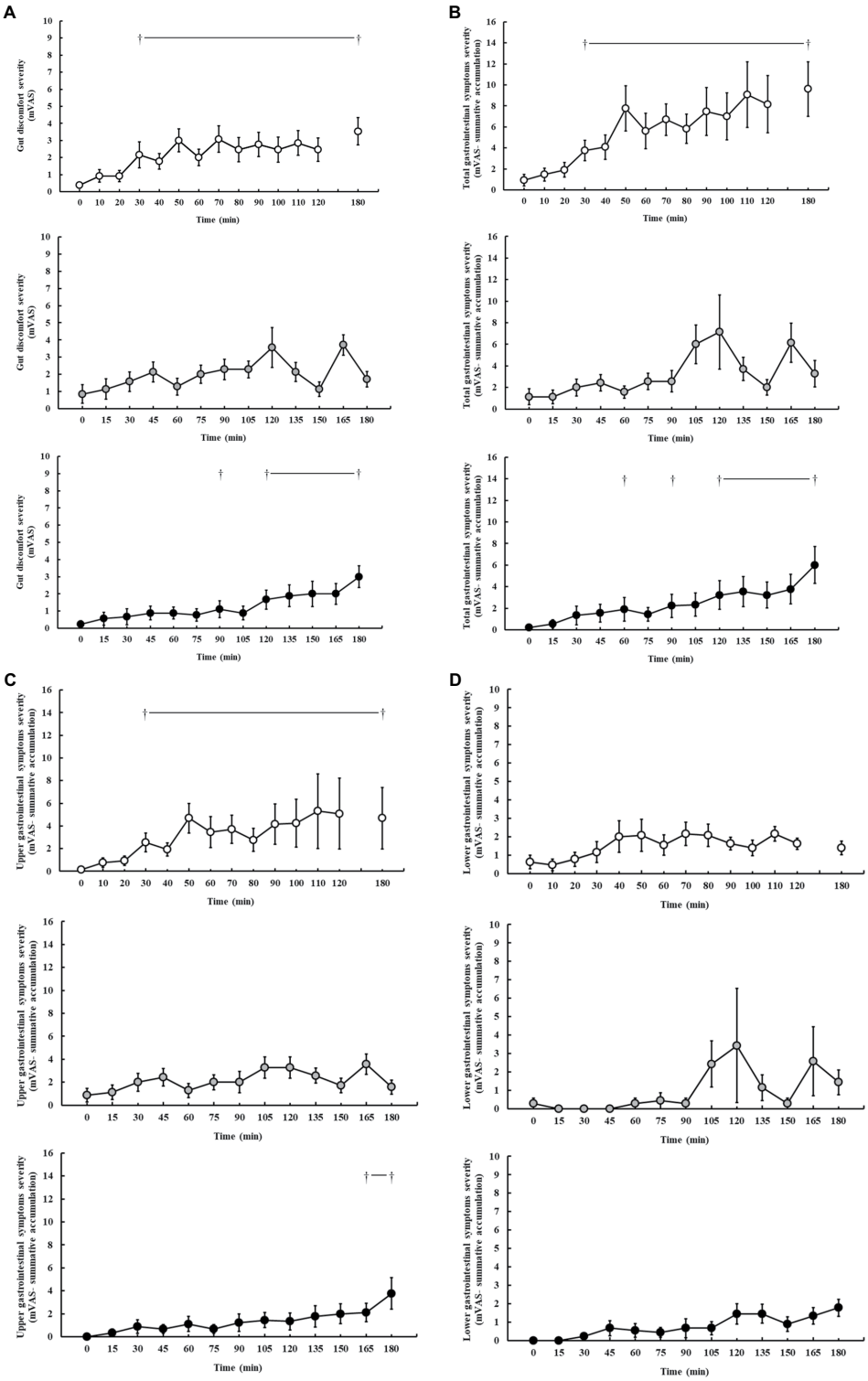

FIGURE 3 | Gut discomfort **(A)**, total **(B)**, upper **(C)**, and lower **(D)** gastrointestinal symptom severity rated on a exercise specific mVAS for gastrointestinal symptoms (Gaskell et al., 2019), during steady-state running with CHO provision on P1 (○), P2 (◍), and P3 (●). Mean ± SEM ($n = 28$): $^{\dagger}p < 0.05$ vs. pre-exercise (0 min) resting GIS. P1: formulated gel-disc containing 30 g CHO with 300 ml water (10% *w/v*, 90 g/h, 2:1 glucose–fructose, 316 mOsmol/kg), at 0 min and every 20 min thereafter for 120 min; followed by water provisions *ad libitum* for the 3rd h (270 ± 215 ml). P2: CHO beverage containing 25 ± 3 g CHO (10% *w/v*, 76 ± 8 g/h, 509 mOsmol/kg; equivalent to 1.0 g/kgBM/h) at 0 min and every 20 min thereafter for 120 min, then water provisions *ad libitum* for the 3rd h (170 ± 122 ml). P3: CHO beverage containing 45 g CHO (16% *w/v*, 90 g/h, 2:1 glucose–fructose, 460 mOsmol/kg), at 0 min and every 30 min thereafter for 180 min. Water provisioned *ad libitum,* but not consumed by participants (0 ± 0 ml).

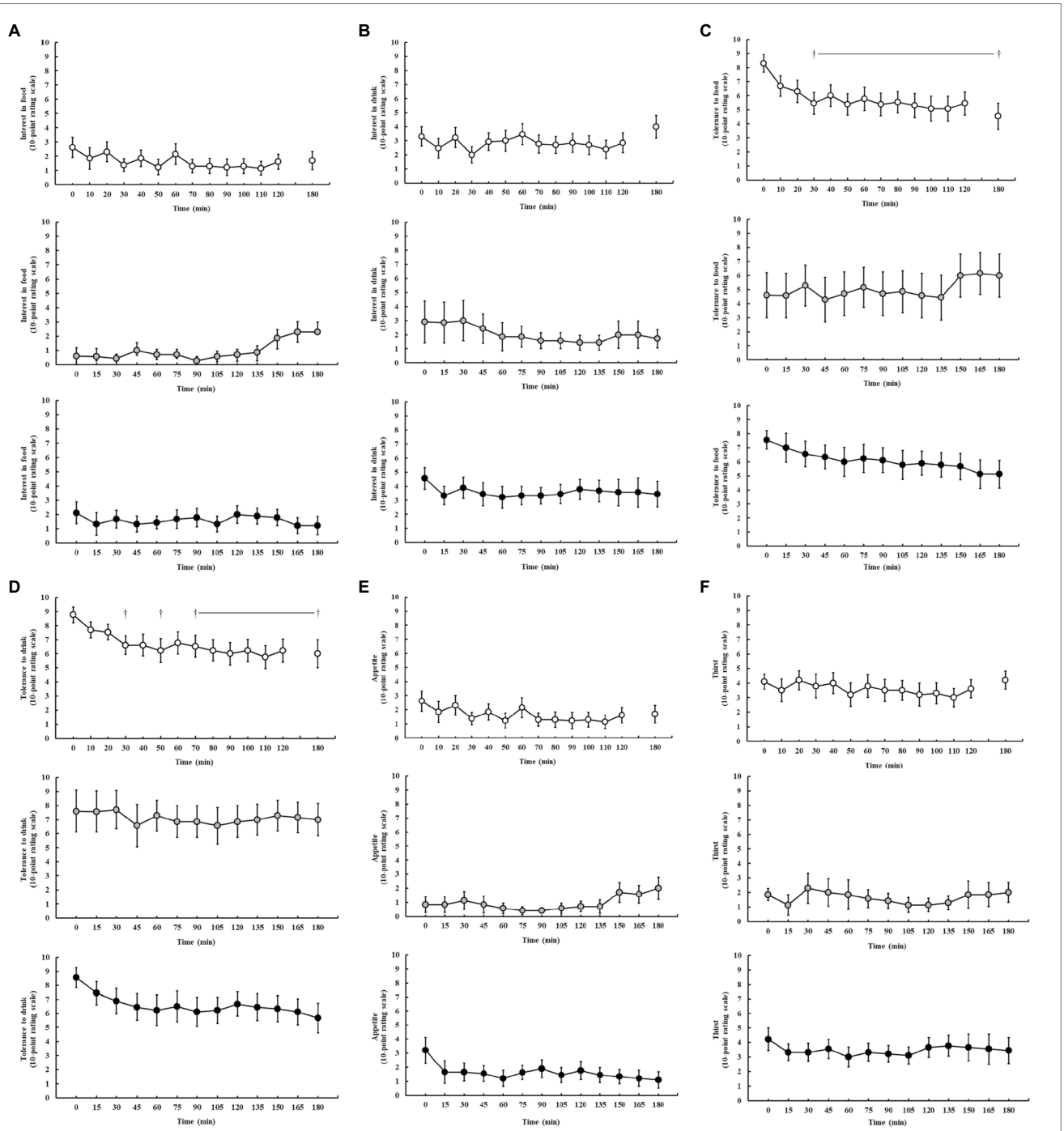

FIGURE 4 | Feeding tolerance markers, including interest in food **(A)**, interest in drink **(B)**, tolerance to food **(C)**, tolerance to drink **(D)**, appetite **(E)**, and thirst **(F)**, rated on a 10-point Likert-type rating scale (Miall et al., 2018), during steady-state running with CHO provision on P1 (○), P2 (◍), and P3 (●). Mean ± SEM ($n = 28$): †$p < 0.05$ vs. pre-exercise (0 min) resting feeding tolerance. P1: formulated gel-disc containing 30 g CHO with 300 ml water (10% *w/v*, 90 g/h, 2:1 glucose–fructose, 316 mOsmol/kg), at 0 min and every 20 min thereafter for 120 min; followed by water provisions *ad libitum* for the 3rd h (270 ± 215 ml). P2: CHO beverage containing 25 ± 3 g CHO (10% *w/v*, 76 ± 8 g/h, 509 mOsmol/kg; equivalent to 1.0 g/kgBM/h) at 0 min and every 20 min thereafter for 120 min, then water provisions *ad libitum* for the 3rd h (170 ± 122 ml). P3: CHO beverage containing 45 g CHO (16% *w/v*, 90 g/h, 2:1 glucose–fructose, 460 mOsmol/kg), at 0 min and every 30 min thereafter for 180 min. Water provisioned *ad libitum*, but not consumed by participants (0 ± 0 ml).

cycling exercise at 50% W_{max}, in highly trained cyclists ($\dot{V}O_{2max}$: 68.1 ml/kgBM/min), with provision of 1.2 g/min glucose and 2.4 g/min glucose/fructose 1:1 ratio resulting in average whole-body total carbohydrate oxidation rates of 2.23–2.64 g/min and fat oxidation of 0.50–0.70 g/min (Jentjens and Jeukendrup, 2005), the current study using a more modest

pre-exercise meal (94 g, 2 h prior to exercise) and carbohydrate provisions during exercise (1.3–1.5 g/min) showed lower whole-body total carbohydrate oxidation rates [1st 2 h steady state: 2.16 g/min, and end of exercise (3 h): 1.61 g/min], but moderately higher whole-body total fat oxidation rates [1st 2 h steady state: 0.58 g/min, and end of exercise (3 h): 0.83 g/min]. These results, however, are in accordance with an ultra-endurance exercise cycling protocol (i.e., 5 h) while consuming carbohydrate during exercise (i.e., 1.5 g glucose or glucose/fructose solutions) reporting whole-body total carbohydrate and fat oxidation rates of ~2.0 and ~0.5 g/min, respectively. The whole-body total fat oxidation rates observed in the current study are surprising considering that the consumption of carbohydrate in the hours prior to strenuous endurance exercise (e.g., <1 h) is frequently reported to reduce fat oxidation of the subsequent exercise bout (Montain et al., 1991; Coyle et al., 1997; Achten and Jeukendrup, 2003). Moreover, unlike the majority of previous investigations, a unique aspect to the current experimental procedures (i.e., incremental exercise test to volitional exhaustion and steady-state exercise with carbohydrate feeding up to 3 h of exercise) and assessment of a cluster of up- and downstream exercise specific energy metabolism primary outcomes (i.e., feeding tolerance, GIS, glucose availability, and total whole body carbohydrate and fat oxidation rates) were the undertaking of running exercise in the post-prandial condition and with carbohydrate provisions during exercise. This dual feeding scenario mirrors real-life practices of athletes, which is generally an uncommon application in experimental designs exploring fuel kinetics in athlete populations, but of high translational research relevance. The authors acknowledge that the current data set uses metadata extrapolated from previously published research that focused on markers of exercise-induced gastrointestinal syndrome (EIGS; Costa et al., 2017b, 2020b), including GIS and feeding tolerance (Costa et al., 2017a; McCubbin et al., 2020; Gaskell et al., 2021a). Therefore, a limitation of the current study was the inability to statistically compare data between exercise test protocols (i.e., P1–P3), considering the paralleled experimental procedure, and limited participant numbers in each exercise protocol required to reach statistical power. Nevertheless, sufficient statistical power (e.g., G*Power: 0.89–0.99 for primary variables) was established for within-exercise test protocol comparisons, thus providing a unique opportunity to understand how the participant groups responded to differing feeding regimes during endurance running. Moreover, the authors acknowledge that the current study did not assess exogenous carbohydrate oxidation using the ^{13}C stable isotope method, aligned with whole-body total carbohydrate oxidation. Although this information may have provided an insight into the magnitude to which consumed carbohydrates during exercise contributed to fuel provisions, such analysis was outside the scope of the primary research outcomes and raises two key discussion points for not warranting such analysis: (1) methodological limitation of applying the ^{13}C stable isotope method for detecting exogenous carbohydrate oxidation that includes experimental preparation – glycogen depletion exercise protocol (e.g., depletion of ^{13}C glycogen stores prior to application of stable isotope) and rigorously controlled dietary provision (e.g., to eliminate and avoid consumption of ^{13}C-rich foods/fluids) in the days leading into the exercise trial (Jeukendrup and Jentjens, 2000; Jentjens et al., 2004a,b, 2006); and (2) irrespective of exogenous carbohydrate oxidation, measurement of whole-body total carbohydrate oxidation during prolonged endurance exercise (≥3 h) to the point of stressed muscle glycogen stores provides an estimated upper-limit for carbohydrate intake tolerance (Costa et al., 2017a; Alcock et al., 2018), to which there is no practical application of over-riding the upper limit. In the current study, the observations provide evidence of high mean MFO (0.66 g/min), Fat_{max} (64% $\dot{V}O_{2max}$), and Fat_{min} (94% $\dot{V}O_{2max}$) during the incremental exercise test to volitional exhaustion in trained endurance and ultra-endurance runners, especially considering the non-fasted testing protocol. Previous studies of MFO and Fat_{max}, in participants consuming a regular mixed diet and tested 2–4 h in the post-prandial period, have observed MFO of 0.45 and 0.55 g/min at 52 and 64% $\dot{V}O_{2max}$, respectively, in highly trained male cyclists (González-Haro, 2011; Schwindling et al., 2014), 0.39 g/min at 52% $\dot{V}O_{2max}$ in male short-course triathletes (González-Haro, 2011), and 0.40 g/min at 56% $\dot{V}O_{2max}$ in a group of endurance-trained female athletes in comparison with 0.32 g/min at 53% $\dot{V}O_{2max}$ in an untrained healthy female control group (Stisen et al., 2006). Interestingly, the mean value obtained for MFO in the current cohort (0.66 g/min) is similar to that observed by Volek et al. (2016) in male ultra-marathon runners consuming a habitually high-carbohydrate diet, who completed the incremental test in the afternoon, following a 4 h fast (0.67 g/min). In contrast, Fat_{max} (mean 64 ± 5% $\dot{V}O_{2max}$) was substantially higher than that observed in the high-carbohydrate group of Volek et al. (2016) (55 ± 8% $\dot{V}O2_{max}$), and more closely resembled that of the LCHF ketogenic group (70 ± 6% $\dot{V}O_{2max}$). It is important to note, that previous research has reported lower MFO and Fat_{max} when an incremental exercise test is performed in the post-prandial state compared to fasted (Bergman and Brooks, 1999; Achten et al., 2002a,b; Achten and Jeukendrup, 2003). For example, 75 g glucose given 45 min before the incremental cycling exercise bout resulted in an MFO of 0.33 g/min at Fat_{max} 52% $\dot{V}O_{2max}$, compared with 0.46 g/min at Fat_{max} 60% $\dot{V}O_{2max}$ on placebo (Achten et al., 2002b). Taken together, the results from the current study suggest large athlete group variation in MFO and Fat_{max} in response to an incremental exercise test and also suggest the current group of endurance and ultra-endurance runners present high MFO and Fat_{max} compared with previous groups, despite consuming a habitual mixed macronutrient diet and performing the incremental exercise test in the post-prandial state.

During steady-state exercise, whole-body total fat oxidation rates of ≥1.0 g/min are generally not consistently reported other than in athletes following a LCHF ketogenic diet (Volek et al., 2016; Burke et al., 2017, 2020), and it has been assumed that this type of dietary pattern is required to upregulate fat oxidation to this extent. In the current data set, mean whole-body total fat oxidation rates of 0.6 ± 0.2 g/min were observed throughout the 180 min exercise protocol, and a final whole-body total fat oxidation rate of 0.8 ± 0.3 g/min at 180 min. Irrespective of the large individual variation in oxidation rates among the study

participants, of interest, almost half ($n = 13/28$) of participants were observed to have a fat oxidation rate at 180 min of ≥1.0 g/min. These fat oxidation rates are despite a habitual mixed macronutrient diet, having consumed carbohydrate in the 2–3 h of running (i.e., P1, P2, and P3) and presenting a mean blood glucose concentration of 6.3 ± 0.5 mmol/L throughout all three protocols. Although the average whole-body fat oxidation rates at the end of the 180 min exercise protocols were lower than previous reported oxidation rates after following a LCHF diet, it was surprising and unexpected that values would approach those reported for athletes following such diets (Volek et al., 2016; Webster et al., 2016; Burke et al., 2017, 2020), with a reasonable number of individual study participants showing similar oxidation rates (e.g., 1.0–1.3 g/min). Moreover, it was interesting to observe the positive correlations between dietary intake and oxidation rates, whereby relative (/kgBM) dietary carbohydrate intake correlated with steady-state whole-body total carbohydrate oxidation, and dietary fat intake (absolute and relative) correlated with steady state and end of exercise whole-body total fat oxidation. The observed correlation between energy and protein intake is likely to be reflective of the dietary fat contribution to total energy intake, and the selection and consumption of fat containing animal protein foods and/or fluids, respectively. Although the correlations observed are small in nature, these findings highlight the importance of pre-exercise dietary choices on during-exercise fuel kinetics, more so than other sub-group correlations analyzed (e.g., BM, training volume, and fitness status). In addition, the current results may likely reflect pre-exercise starting muscle glycogen status resulting from dietary intake (Costill et al., 1971; Sherman et al., 1993), which poses a limitation in the current study, whereby muscle glycogen levels were not assessed to ascertain the habitual dietary intake of participants on pre-exercise muscle glycogen stores and subsequent fuel kinetics during exercise in the post-prandial period and with carbohydrate provision throughout exercise.

A recent meta-analysis suggests that the point of stressed muscle glycogen stores, in athletes of similar fitness to the current study, exercising at an intensity of 60–70% $\dot{V}O_{2max}$ and with a habitual dietary carbohydrate intake as assessed from participant food diaries, is around 2–2.5 h, and that the effect of carbohydrate intake during exercise on muscle glycogen depletion is minimal (Areta and Hopkins, 2018). In this scenario, frequent long-duration training sessions (e.g., ≥3 h) or twice-a-day training is common in endurance and ultra-endurance athletes and is likely to result in frequently depleted muscle glycogen despite consuming a mixed macronutrient diet. It is therefore perhaps unsurprising that these athletes have an ability to sustain high fat oxidation rates without the need for specific low-carbohydrate dietary interventions (e.g., long term LCHF diets, or acute carbohydrate restriction within carbohydrate periodization models), given their regular exposure to low carbohydrate availability during the end stages of prolonged endurance and ultra-endurance training sessions. These observations have substantial practical relevance considering the consistent negative performance outcomes observed with LCHF dietary approaches within controlled experimental procedures (Burke et al., 2017, 2020), in which suppressed pyruvate dehydrogenase enzyme activity at the terminal section of glycolysis appears to be a key mechanistic culprit (Stellingwerff et al., 2006); albeit in response to high-intensity endurance exercise (e.g., ~80% $\dot{V}O_{2peak}$) within a competitive and/or simulated competitive setting (i.e., race walking). Performance implications of more prolonged exercise bouts using time to exhaustion, time trial, and/or ultra-endurance experimental models have reported equivocal outcomes (Phinney et al., 1983; Carey et al., 2001; Lambert et al., 2001; Havemann et al., 2006). Therefore, additional research is warranted to comprehensively assess the interaction between other factors that may impact performance in response to LCHF dietary adherence, aside from the consistently proposed implications from enhancing fat fuel utilization. For example, the implications of such dietary fat intake behavior and associated luminal originated pathogenic translocation reported during dietary lipid digestion and absorption activity (Bowser et al., 2020; Mohammad and Thiemermann, 2021), and effects on EIGS and GIS that have been linked to performance decrements (Costa et al., 2017a; Miall et al., 2018).

In the current study we did not observe correlations between either whole-body total carbohydrate and fat oxidation with BM at the conclusion of the 3 h run, which is dissimilar to previous reports comparing male and female recreational endurance athletes ($r = 0.510$ and $r = 0.594$, respectively; Costa et al., 2017a). This is largely to be expected, considering the relatively homogeneous BM of the study cohort and subsequent energy cost and specific fuel kinetics of exercise in proportion with BM. Specific assessments using isotope tracers suggest that while whole-body total carbohydrate oxidation may correlate with BM, exogenous carbohydrate oxidation does not (Jeukendrup, 2010). It is noteworthy that 39% of participants had an average whole-body total carbohydrate oxidation rate of less than their exogenous carbohydrate intake rate during the 2 h steady-state run. In this scenario, which represents a typical exercise intensity encountered in many recreational endurance and ultra-endurance training and competitive activities, broad-spectrum guidelines and recommendations, anecdotally employed by many sport and exercise nutrition and dietetic support practitioners, of up to 1.5 g/min multi-transportable carbohydrate intake for exercise ≥3 h (Thomas et al., 2016), may be unnecessary for some athletes. Consumption of carbohydrate at rates greater than total carbohydrate oxidation may serve little purpose and may increase the logistical and gastrointestinal burden on the athlete (Costa et al., 2018, 2019b), as was the case in the current study. For example, carbohydrate consumption at 90 g/h in a 2:1 glucose–fructose ratio over a 3 h running exercise protocol resulted in greater incidence and severity of GIS and greater feeding intolerance, compared with carbohydrate feeding at rates of 76 g/h (i.e., 1 g/kgBM/h). Increasing the exercise intensity in the 3rd h of exercise (i.e., greater exercise stress load 1 h performance distance test) further increased GIS and feeding intolerance burden, despite withdrawal of carbohydrate feeding regime (P1), whereas withdrawal of carbohydrate feeding regime in the 3rd h, while maintaining steady-state exercise reduced the GIS burden (P2). It is noteworthy to report that reduced GIS in the 3rd h of exercise on P2 may likely be due to individual and/or combined factors including: the lower carbohydrate intake rate in the first 2 h, the pre-exercise

24-h FODMAP controlled diet, withdrawal from carbohydrate intake in the 3rd h of running, and/or the more modest exertional stress (e.g., lower running speed and less distance covered over the 3 h), compared with P1 and P3. In addition, the rapid rise in GIS seen at 165 min on P2 likely reflects the 150 ml lactulose solution given at 150 min as part of the OCTT assessment procedure.

From a translational research and professional practice perspective, athletes who regularly train and compete at submaximal intensities (e.g., endurance and ultra-endurance sports), individual assessment of carbohydrate oxidation rates while challenged with 1.5 g/min multi-transportable carbohydrate intake would allow for individualization of intake targets, which are likely to be scaled at least partially by body mass (e.g., carbohydrate feeding rates at 1 g/kgBM as a starting point; Costa et al., 2017a; Gaskell et al., 2021c). Another supporting factor is the evidence of increased blood glucose concentration without increased whole-body total carbohydrate oxidation. In fact, at the point of reduced total carbohydrate oxidation (i.e., 2.4 g/min at initiation of steady state to 1.6 g/min at 3 h) and increased total fat oxidation (i.e., 0.5 g/min at initiation of steady state to 0.8 g/min at 3 h), blood glucose remained stable and consistent with the initial peak value that occurred within 30 min of exercise commencement (i.e., pre-exercise 4.9–6.1 mmol/L). Previous reports suggested that intestinal absorption, and subsequent increases in blood glucose availability (e.g., increased blood glucose concentration), appears as a rate limiting factor for increased exogenous carbohydrate utilization during exercise (Jeukendrup et al., 2006; Cox et al., 2010; Jeukendrup, 2010). With the focus on whole-body fuel kinetics in the current study, the increased blood glucose in response to the feeding regimen did not result in increased whole-body total carbohydrate oxidation, which appears predominantly dependent on the skeletal muscle uptake of circulatory glucose. This observation raises an important question about the barriers or limiters of carbohydrate oxidation in skeletal muscle during endurance exercise.

The frequent citation that intestinal absorption is the primary limiting factor to exogenous carbohydrate oxidation in skeletal muscle during exercise has led to recommendations for the ingestion of multiple transportable carbohydrate sources up to 1.5 g/min during endurance exercise ≥3 h, in order to take advantage of both SGLT-1 and GLUT-5 transporters in the intestinal epithelium (Jeukendrup, 2010), increasing total carbohydrate uptake into the blood and reducing carbohydrate malabsorption. Purposeful "*gut training*" to presumably increase transporter abundance and function (Costa et al., 2017a) can have a similar effect. It is proposed that the upregulation of intestinal carbohydrate absorption occurs through stimulation of intestinal nutrient sensing molecules (e.g., T1R3 and α-gustducin) that are expressed in enteroendocrine cells along the intestinal epithelium and prompt the mRNA expression and protein synthesis of SGLT-1 through gut hormones regulating pathways (e.g., GIP and GLP-1; Margolskee et al., 2007; Shirazi-Beechey et al., 2011). However, such strategies to increase carbohydrate intestinal absorption and blood glucose availability have not universally resulted in increased whole-body total carbohydrate oxidation rates (Costa et al., 2017a). These findings suggest that at least in some athletes (possibly recreational vs. elite), the glucose uptake into skeletal muscle and/or oxidation of carbohydrate within the mitochondria may represent a limiting factor for total carbohydrate oxidation and possibly limit total energy production and exercise performance. Thus, based on the current presented data, we describe a principal four-layered compartment of rate limiting factors of carbohydrate to skeletal muscle glucose availability (**Figure 5**): (1) intake behavior – determined by real-time tolerance and GIS, (2) gastrointestinal – determined by gastrointestinal transit and regulation of glucose absorption, (3) circulatory – determined by skeletal muscle uptake and metabolic gradient of blood glucose bioavailability, and (4) skeletal muscle metabolism – conversion of glucose to acetyl CoA through glycolysis, the action of pyruvate dehydrogenase enzyme, and/or the skeletal muscle production and intramuscular cytosol concentration of lactate, with impacts on mitochondrial function (Stellingwerff et al., 2006; Costa et al., 2016, 2017a; San-Millan and Brooks, 2018; Hargreaves and Spriet, 2020). The theoretical concept of such a model has recently been thoroughly and elegantly reviewed by Malone et al. (2021).

Withholding carbohydrate intake in the final hour of running on P1 and P2 did not result in a substantial reduction in blood glucose concentration. This could theoretically be due to: (1) continued absorption of carbohydrate as a result of luminal trafficking and continued oversaturation of SGLT-1 transporter induced by the carbohydrate intake of the first 2 h; (2) saturation of the GLUT-4 transporter at the skeletal muscle plasma membrane resulting in a rate liming uptake of circuiting glucose into skeletal muscle; and/or (3) hepatic glucose release as a result of gluconeogenesis, potentially the predominate cause in P1 (i.e., in response to the 1 h performance test in the 3rd h). Interestingly, the lower carbohydrate intake in P2, compared with P1 and P3, did start to show reductions in total carbohydrate oxidation as exercise progressed from the 2nd to 3rd h, suggesting that 1.5 gCHO/min better supports carbohydrate availability, but potentially at the expense of greater GIS. Nevertheless, the lower carbohydrate intake in P2 did not result in an absent GIS incidence, as 100% of participants still reported at least one minor GIS incidence during the steady-state exercise protocol, highlighting the potency of exercise stress *per se* in inducing GIS incidence (Snipe et al., 2017, 2018a,b). A heterogeneous participant response was observed for GIS type and severity across all three included studies, confirming the large individual variation in exercise-associated GIS previously reported (Costa et al., 2020b). These types of responses have also been observed in field research with substantial heterogeneity between exercise modes, durations, intensities, and carbohydrate intakes during exercise (Pfeiffer et al., 2012). However, a common theme is that the higher performers consumed more carbohydrates during exercise, but also reported greater GIS. Conversely, a recent field study reported no GIS in elite ultra-endurance runners that underwent "*gut-training*" beforehand, and consuming either 120, 90, or 60 g/h of a 2:1 glucose–fructose gel formulation during a mountain marathon with ~4,000 m cumulative slope in 10°C and 60% relative humidity ambient conditions (Viribay et al., 2020). Reported within, three participants withdrew from the event due to gastrointestinal issues, but the participant group/s of these withdrawals were not reported and no formal

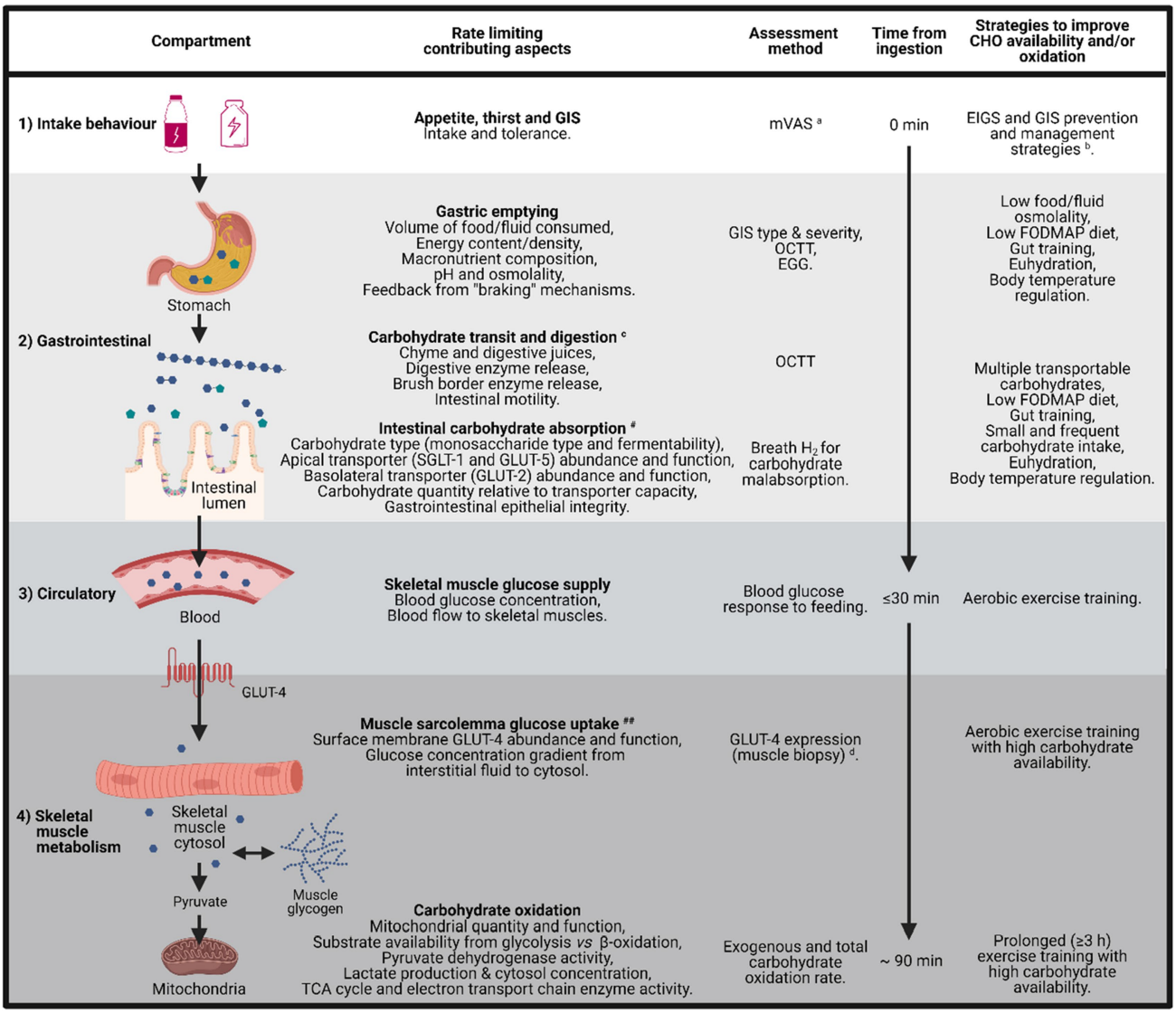

FIGURE 5 | Schematic illustration of the barriers and limitations to total carbohydrate availability and oxidation, from mouth to mitochondria, including the time course for carbohydrate uptake and oxidation, factors that limit each step, assessment method for quantification, and potential strategies to reduce these limitations. [a]Modified visual analogue scale (mVAS; Gaskell et al., 2019). [b]EIGS and GIS prevention and management strategies (Costa et al., 2017b, 2020b). [c]Gastrointestinal brake mechanisms: nutritive and non-nutritive residue along the small intestine, and inclusive of terminal ileum, results in neural and enteroendocrine negative feedback to gastric activity (Layer et al., 1990; Van Citters and Lin, 2006; Shin et al., 2013; van Avesaat et al., 2015; Miall et al., 2018). [d]GLUT-4 detection methods (McGee and Hargreaves, 2006; Flores-Opazo et al., 2020). [#]dependent on taste receptors (e.g., T1R3 and α-gustducin expressed in epithelial enteroendocrine cells) and gut hormones (e.g., GIP and GLP-1 that are activated by taste receptor stimulation – nutrient presence along the intestinal lumen) that regulate the SGLT-1 protein synthesis and translocation to the apical border of enterocytes (Rozengurt, 2006; Margolskee et al., 2007; Shirazi-Beechey et al., 2011). [##]Dependent on magnitude of skeletal muscle blood perfusion, sarcolemma GLUT-4 concentration, GLUT-4 saturation, cytosol Ca^{2+} flux, glycolytic enzyme concentration and activity (i.e., intramuscular glucose metabolism gradient; Hargreaves and Spriet, 2020). EGG, electrogastrography and OCTT, orocecal transit time.

measure of GIS and/or feeding tolerance assessment in real-time or retrospectively was reported. It is important to note that the primary outcomes were not gastrointestinal related, but rather exercise-induced muscle damage (i.e., exercise recovery); and therefore, no valid assessment of GIS (e.g., validated assessment tool, real-time verification, and GIS vs. performance outcomes analysis) and feeding tolerance markers were adequately and robustly undertaken, so caution is warranted in using such an experimental design to interpret the impact of 60–120 g/h carbohydrate intake during running on GIS and feeding tolerance. Nevertheless, such broadly stated outcomes highlight and provide some discussion around either, (1) the ability of elite athletes to cope (i.e., gastrointestinal tract, circulatory glucose availability, glucose uptake by skeletal muscle uptake, and carbohydrate oxidation) with high rates of carbohydrate intake, (2) the efficacy of gut-training, and/or (3) the importance of using valid and reliable GIS and feeding tolerance assessment tools. In controlled laboratory settings with the ability to reduce confounding factors

that may impact gastrointestinal integrity and/or function, and correctly applying a validated and reliable GIS assessment tool in real-time, it is clear to suggest that exercise stress, heat stress, and intake volume all contribute to increase the risk for GIS incidence and severity (Snipe et al., 2017, 2018a,b; Snipe and Costa, 2018; Costa et al., 2019a; Gaskell et al., 2019, 2020, 2021c; Russo et al., 2021a). Despite it not being possible to determine carbohydrate malabsorption on P2 due to including an OCTT assessment procedures (Gaskell et al., 2021a), 38% of participants in P1 and P3 receiving 1.5 g/min multi-transportable carbohydrate presented with breath H_2 values indicative of carbohydrate malabsorption of clinical significance in the recovery period (Bate et al., 2010). An important observation to note was that the incidence and magnitude of carbohydrate malabsorption did not translate to GIS type, incidence, or severity. This observation is consistent with a previous study comparing a 24-h low (<5 g) and high (42 g) fermentable oligo-di-mono-saccharides and polyols (FODMAP) dietary intervention, which despite substantially reduced breath H_2 before exercise following the low FODMAP diet, exercise-associated GIS incidence was similar to the high FODMAP trial, and severity was only modestly lower and not abolished on the low FODMAP trial. Together these study outcomes support the multifactorial and inter-dynamic causal pathways and exacerbation factors of EIGS and associated GIS that are not necessarily limited to carbohydrate intake type, concentration, and volume (Gaskell et al., 2021b,c).

CONCLUSION

The presented metadata from an incremental exercise test to exhaustion and three 3-h running exercise protocols with differing carbohydrate feeding regimes during exercise suggests the following: (1) A large proportion of endurance and ultra-endurance runners can attain relatively high rates of whole-body fat oxidation during exercise in a post-prandial state (particularly after 2 h of exercise), despite consuming a mixed macronutrient diet, and consuming carbohydrate during steady state exercise. (2) Carbohydrate feeding tolerance and GIS appear to be dependent on total load of carbohydrate consumed during exercise and the exercise intensity [e.g., steady-state vs. race pace (performance test)]. Taken together, the outcomes of the metadata analysis suggest future research is warranted in assessing the practical feasibility of using whole-body substrate oxidation data to tailor during exercise carbohydrate intake quantity and quality, with the aim of reducing the risk of unnecessary intake that may overburden the gastrointestinal tract leading to performance decremental GIS.

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 Monash University Human Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RC was the chief investigator of this research and responsible for the original research idea. RC, SG, and AM contributed to the development of the various experimental designs (i.e., P1, P2, and P3). All authors contributed to the various aspects of data and sample collection and analysis. CR contributed to the initial manuscript draft. All authors contributed to the various aspects of the manuscript preparation and review. All authors read and approved the final manuscript.

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Post-marathon Decline in Right Ventricular Radial Motion Component Among Amateur Sportsmen

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Moderate physical activity has a positive impact on health, although extreme forms of sport such as marathon running may trigger exercise-induced cardiac fatigue. The explicit distinction between the right ventricular (RV) physiological response to training and maladaptive remodeling has not yet been determined. In this study, we aimed to analyze the impact of running a marathon on RV mechanics in amateur athletes using three-dimensional (3D) echocardiography (ECHO) and the ReVISION method (RV separate wall motion quantification). A group of 34 men with a mean age of 40 ± 8 years who successfully finished a marathon underwent ECHO three times, i.e., 2 weeks before the marathon (stage I), at the marathon finish line (stage II), and 2 weeks after the marathon (stage III). The ECHO findings were then correlated with the concentrations of biomarkers related to myocardial injury and overload and also obtained at the three stages. On finishing the marathon, the amateur athletes were found to have a significant ($p < 0.05$) increase in end-diastolic (with a median of 51.4 vs. 57.0 ml/m^2) and end-systolic (with a median of 24.9 vs. 31.5 ml/m^2) RV volumes indexed to body surface area, reduced RV ejection fraction (RVEF) (with a median of 51.0% vs. 46.0%), and a decrease in RV radial shortening [i.e., radial EF (REF)] (with a mean of 23.0 ± 4.5% vs. 19.3 ± 4.2%), with other RV motion components remaining unchanged. The post-competition decrease in REF was more evident in runners with larger total volume of trainings ($R^2 = 0.4776$, $p = 0.0002$) and higher concentrations of high-sensitivity cardiac troponin I ($r = 0.43$, $p < 0.05$) during the preparation period. The decrease in REF was more prominent in the training of marathoners more than 47 km/week. At stage II, marathoners with a more marked decrease in RVEF and REF had higher galectin-3 (Gal-3) levels ($r = -0.48$ and $r = -0.39$, respectively; $p < 0.05$). Running a marathon significantly altered the RV performance of amateur athletes. Transient impairment in RV systolic function resulted from decreased radial shortening, which appeared in those who trained more extensively. Observed ECHO changes correlated with the concentrations of the profibrotic marker Gal-3.

Keywords: marathon run, amateur runners, revision method, right ventricular motion components, right ventricular dysfunction (RV dysfunction), 3D echocardiography, galectine-3, overtraining

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INTRODUCTION

Regular physical activity has many well-documented health benefits (Nystoriak and Bhatnagar, 2018), particularly a reduction in all-cause mortality (Pelliccia et al., 2021). In recent years, the growing popularity of long-distance running competitions has been observed (Nikolaidis et al., 2018). Although the minimum weekly amount of moderate- and vigorous-intensity exercise for healthy adults is known (Pelliccia et al., 2021), the beneficial upper level of sports activity is not defined. Undoubtedly, preparing to run a marathon requires many hours of intense training. Not only a challenging training regimen but also participating in the competition itself is exhausting and can result in cardiac fatigue (Oxborough et al., 2010).

During extreme training, due to the different compensatory capacities of the pulmonary vascular bed and systemic circuit, the increase in pulmonary artery pressure is disproportionately greater, and exercise-induced overload predominantly affects the right ventricle (RV) (La Gerche et al., 2014). Among acute manifestations, impaired relaxation and decreased contractility of the RV have been observed in marathon runners after competing (Elliott and La Gerche, 2015; Lewicka-Potocka et al., 2020). Some researchers even suggested that there is a risk of developing "exercise-induced arrhythmogenic RV cardiomyopathy," among possible long-term consequences of repetitive bouts of intensive training (Heidbuchel et al., 2012). Nevertheless, there is still ongoing discussion about the boundary between physiological adaptation to exercise and pathological RV remodeling (Leischik et al., 2020).

Due to the complex anatomy and mechanics of contraction, the RV remains a diagnostic challenge. Following the anatomical axes, overall RV systolic movement can be divided into three components, namely, longitudinal (displacement of the tricuspid annulus toward the apex), anteroposterior (stretching the RV wall by the contraction of the septum), and radial (internal relocation of the RV free wall) (Lakatos et al., 2017). Each of these components and their contraction can be precisely assessed with the advanced ECHO technique, i.e., the ReVISION method (RV separate wall motion quantification) (Lakatos et al., 2017). This approach overcomes the limitations of conventional parameters and allows precise estimation of all RV motion components and their contribution to global RV function.

Normally, the relative participation of these motion components in global RV function is rather equal, although it may vary depending on age and underlying diseases (Lakatos et al., 2017, 2020). Although current literature on this topic is scarce, it seems that changes in RV motion contribution or reduction in even single motion displacement can be an early marker of myocardial damage.

On the biochemical level, the increased concentrations of biomarkers reflecting myocardial overload and injury can be found after a marathon run (La Gerche et al., 2012). We observed a post-run increase in the plasma levels of high-sensitivity cardiac troponin I (hs-cTnI), heart-type fatty acid binding protein (H-FABP), N-terminal proatrial natriuretic peptide (NT-proANP), B-type natriuretic peptide (BNP), growth differentiation factor 15 (GDF-15), and galectin-3 (Gal-3) (Kaleta-Duss et al., 2020).

In this study, we aimed to analyze the impact of running a marathon on the mechanics of RV contraction in amateur athletes using three-dimensional (3D) echocardiography (ECHO) and the ReVISION method. We hypothesized that intense endurance exercise results in cardiac dysfunction that predominantly affects the RV and may be affected by the prerace training intensity and reflected by the increment of cardiac biomarkers of myocardial injury and overload.

MATERIALS AND METHODS

Participants

Runners, who planned to attend the 2nd PZU Marathon in Gdańsk, Poland, were recruited to the study *via* invitations sent to local sports clubs. Due to the fact that there are known significant gender-linked differences in the structure of the heart of both male and female athletes (Di Paolo and Pelliccia, 2007) to avoid eventual non-homogeneity of the studied group, we decided to include only men. In the pre-participation screening, training habits and medical history were collected from every volunteer. Eligible candidates had to be aged between 20 and 55 years with no concomitant chronic diseases. We included runners who trained $\leq$11 h/week.

Study Design

The study was conducted in three stages. Physical, ECG, and echocardiographic examinations were performed at each stage, and venous blood samples were collected to test the concentration of the selected laboratory parameters and cardiovascular biomarkers. The analysis took place 2 weeks before the marathon (stage I), at the marathon finish line (stage II), and 2 weeks after the marathon (stage III). Additionally, at stage I, a cardiopulmonary exercise test (CPET) was performed, and the methodology has been presented in detail previously (Lewicka-Potocka et al., 2021). The study protocol was approved by the Independent Bioethics Commission for Research of the Medical University of Gdansk (NKBBN/104/2016), and all participants gave written informed consent.

Measurements

The ECHO was performed with a Vivid E9 apparatus (GE Healthcare, Horten, Norway) equipped with a 4VD transducer. In line with the current recommendations (Lang et al., 2015), standard 2D views and measurements were obtained and analyzed off-line with echocardiographic quantification software (EchoPac 201, GE Healthcare). The methodology for the echocardiographic measurements, such as 2D parameters reflecting the RV systolic function, has been described in detail previously (Lewicka-Potocka et al., 2020, 2021). In those manuscripts, we analyzed the marathon impact on conventional RV measures such as tricuspid annular plane systolic excursion (TAPSE), fractional area change (FAC), tricuspid lateral annular systolic velocity (S' wave), four-chamber longitudinal strain, and diastolic RV function including the RV isovolumetric relaxation time.

All participants underwent 3D ECHO in accordance with the guidelines (Lang et al., 2012), and ECG-triggered multiple-beat full-volume 3D data sets for the left ventricle (LV) and RV were acquired from the apical view while participants held their breath for 5 s. Further analysis was performed off-line using dedicated commercially available systems on an EchoPac workstation with integrated software for LV and RV analysis (4D RV-Function 2, TomTec, Imaging GmbH, Unterschleissheim, Germany). The end-diastolic and end-systolic volumes (EDV and ESV) of the LV and RV indexed to body surface area were calculated together with the stroke volume (SV) and ejection fraction (EF). Additionally, a detailed analysis of RV mechanics was performed using the ReVISION method (Lakatos et al., 2017). Along known RV motion directions, three volumetric components were specified in the semiautomatically exported 3D RV beutel, and their volume changes over time were calculated following orthogonal anatomical axes. The volumes contributing to only one motion direction were measured at each time, while the others were blocked. Thus, the global RV function was decomposed and separate EFs such as longitudinal (LEF), radial (REF), and anteroposterior (APEF) were obtained. The relative contributions of the motion components to global RVEF were also determined and presented as indexes such as LEFi (LEF/RVEF), REFi (REF/RVEF), and APEFi (APEF/RVEF).

Biochemical Analysis

The concentrations of selected cardiovascular biomarkers, which were proved to be elevated after marathon run (Kaleta-Duss et al., 2020), such as hs-cTnI, H-FABP, NT-proANP, BNP, GDF-15, and Gal-3 were used to perform correlations with echocardiographic parameters. The methodology description of obtaining these biomarkers has been presented in detail previously (Kaleta-Duss et al., 2020).

Statistical Analysis

All statistical analyses were performed using Statistica 13.3 software (StatSoft Inc., Tulsa, OK, USA) and R version 4.0.4 (https://cran.r-project.org/). The normal distribution of data was checked using the Shapiro–Wilk test. The normally distributed data are presented as mean ± SD, while those diverging from normal distribution by means of the median with respective interquartile range (IQR).

A comparison between the three stages of the study, such as LV and RV volumes and EFs, was performed using ANOVA and Tukey's *post hoc* test for normally distributed data or a Friedman test and *post hoc* test for non-normally distributed variables.

A comparison of RV motion components and their ratios among the study stages was performed using repeated-measures ANOVA with subsequent Tukey's *post hoc* test for multiple comparisons. The assumptions of the analysis were checked using Mauchly's test of sphericity. The linear regression analysis was performed to determine the relationship of ECHO findings with the cardiovascular biomarkers obtained at each stage of the study (I–III), such as hs-cTnI, H-FABP, NT-proANP, BNP, GDF-15, and Gal-3. The relationships between the difference of REF at stages I and II and the training volume (expressed as either the number of kilometers or hours per week) were modeled using the linear regression or non-linear second-order function estimation, and the goodness of fit was inferred based on the respective coefficient of determination (R^2). To provide results reflecting a larger population, the relationship analysis of the ECHO findings with the training habits was performed excluding the definitely outstanding values. A p-value < 0.05 was considered significant.

RESULTS

In total, we enrolled 34 eligible amateur runners who successfully finished the marathon competition. The participants were Caucasian men with a mean age of 40 ± 8 years. The characteristics of the study group have been previously presented thoroughly, such as their training habits and results of CPET (Lewicka-Potocka et al., 2021). The mean achieved marathon finishing time was 3.7 ± 0.4 h. When preparing for a marathon, the mean reported training distance was 56.5 ± 19.7 km/week, and the mean total training time was 6.48 ± 2.3 h/week, whereas the peak oxygen uptake (VO_2peak) was 53.7 ± 6.9 ml/kg/min (Lewicka-Potocka et al., 2021). The results on the biochemical analysis between study stages and changes in the concentrations of cardiovascular biomarkers (hs-cTnI, Gal-3, H-FABP, NT-proANP, BNP, and GDF-15) expressed as mean ± SD have been described in detail recently (Kaleta-Duss et al., 2020). Concerning correlations between biomarkers and ECHO parameters presented in this study, the mean concentration of Gal-3 at stage I was 8.53 ± 3.04 ng/ml vs. 10.65 ± 2.33 ng/ml at stage II. The mean level of H-FABP obtained after the marathon was 13.57 ± 9.63 ng/ml. At stage I, the mean concentration of hs-cTnI was 0.01 ± 0.01 ng/ml with 0.06 ± 0.09 ng/ml at the marathon finish line (Kaleta-Duss et al., 2020).

Table 1 presents 3D ECHO RV and LV parameters obtained in the three stages of the study, such as volumes and EFs. A detailed analysis of the RV motion components is shown in **Table 2**. The comparison of results from stages I and III revealed no differences (**Tables 1**, **2**). After the run, a significant increase in the RV volumes, such as RVEDV and RVESV, was observed with a reduction in LVEDV (**Table 1**). The marathon competition resulted in significant deterioration of RV systolic function, with no influence on LVEF (**Table 1**). The post-run decrease in RV radial contraction, expressed by REF, was the main contributor to the exercise-induced drop in global RVEF (**Table 2**; **Figure 1**). No significant post-run changes in longitudinal or anteroposterior EFs were noticed (**Figure 1**). The reduction in radial shortening was transient and normalized within a 2-week interval. The contraction pattern and relative contribution of all RV motion components to global RV systolic function, quantified as LEFi, REFi, and APEFi, did not differ between study stages (**Table 2**).

Based on the outcomes of regression modeling, participants reporting higher training distances (expressed as the number of running kilometers per week) showed a greater decline in REF (higher values of the REF change between stages I and II); nevertheless, the relationship was clearly non-linear, following a distinct U-shaped curve with the minimum at around 47 km/week (predicted change of REF between stages I and II with

TABLE 1 | Three-dimensional (3D) echocardiographic parameters of right and left ventricle obtained in amateur marathon runners.

Parameter	Stage I	Stage II	Stage III	ANOVA	*Post-hoc P*-value	
	Mean ± SD[a] or Median (1st; 3rd quartile)[b]			*P*-value	Stage I vs. II	Stage I vs. III
RVEDV [ml/m^2]	51.4 (44.9;58.5)	57.0 (52.0;61.6)	52.6 (47.5;58.4)	∧ **<0.05**	**<0.05**	>0.05
RVESV [ml/m^2]	24.9 (21.3;29.1)	31.5 (27.5;34.4)	26.8 (23.0;29.2)	∧ **<0.05**	**<0.05**	>0.05
RVSV [ml]	53.0 (46.0;59.0)	53.0 (48.8;57.0)	53.0 (46.0;59.0)	∧ >0.05	–	–
RVEF [%]	51.0 (50.0;53.0)	46.0 (43.0;48.3)	51.0 (48.0;53.0)	∧ **<0.05**	**<0.05**	>0.05
LVEDV [ml/m^2]	57.2 ± 10.4	52.0 ± 8.7	57.2 ± 11.0	* **<0.05**	**<0.05**	>0.05
LVESV [ml/m^2]	25.1 (20.6;28.0)	22.9 (21.2;26.5)	24.4 (20.9;27.8)	∧ >0.05	–	–
LVSV [ml]	63.7 ± 11.7	55.0 ± 7.8	63.8 ± 13.4	* **<0.05**	**<0.05**	>0.05
LVEF [%]	56.4 ± 3.6	54.9 ± 4.6	56.4 ± 3.4	* >0.05	–	–

Stage I, 2 weeks before the marathon run; Stage II, at the finish line; Stage III, 2 weeks after the competition; SD, standard deviation; RVEDV, right ventricular end-diastolic volume indexed to body surface area; RVESV, right ventricular end-systolic volume indexed to body surface area; RVSV, right ventricular stroke volume; RVEF, right ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume indexed to body surface area; LVESV, left ventricular end-systolic volume indexed to body surface area; LVSV, left ventricular stroke volume; LVEF, left ventricular ejection fraction. Statistically significant values are marked with bold.

**ANOVA with post hoc Tukey's test if applicable; ^Friedman-ANOVA with post hoc average rank test if applicable; [a]when normally distributed; [b]when non-normally distributed.*

TABLE 2 | Right ventricular mechanics and 3D motion components in amateur marathon runners.

Parameter	Stage I	Stage II	Stage III	ANOVA#	*Post-hoc P*-value	
	Mean ± SD			*P*-value	Stage I vs. II	Stage I vs. III
LEF [%]	22.9 ± 3.2	20.4 ± 3.8	22.2 ± 3.7	>0.05	–	–
REF [%]	23.0 ± 4.5	19.3 ± 4.2	21.2 ± 4.5	**<0.05**	**<0.05**	>0.05
APEF [%]	17.3 ± 3.5	16.4 ± 4.2	17.3 ± 3.5	>0.05	–	–
LEFi	0.46 ± 0.05	0.45 ± 0.08	0.46 ± 0.07	>0.05	–	–
REFi	0.46 ± 0.06	0.42 ± 0.08	0.44 ± 0.07	>0.05	–	–
APEFi	0.35 ± 0.06	0.36 ± 0.07	0.36 ± 0.06	>0.05	–	–

#Repeated-measures ANOVA with subsequent Tukey's post hoc test for multiple comparisons; Stage I, 2 weeks before the marathon run; Stage II, at the finish line; Stage III, 2 weeks after the competition; LEF, longitudinal ejection fraction; REF, radial ejection fraction; APEF, anteroposterior ejection fraction; LEFi, longitudinal ejection fraction index; REFi, radial ejection fraction index; APEFi, anteroposterior ejection fraction index. Statistically significant values are marked with bold.

a value of 1.04 ± 5.26). The calculated second-order model explained around 48% of observed REF change and was highly statistically significant ($R^2 = 0.4776$, $p = 0.0002$). Compared to kilometers/week, the hours/week variable was a worse predictor of REF decline ($R^2 = 0.1934$, $p = 0.0290$) since, for this parameter, it was not possible to find a model that explained more than 20% of the variation in the change of REF between stages I and II. A greater decline in REF after the competition was also observed in subjects with higher levels of hs-cTnI at stage I ($r = 0.43$, $p < 0.05$).

Participants who reported a higher training distance (expressed as running hours per week or as a number of running kilometers during a week) showed higher LEF values ($r = 0.39$ and $r = 0.38$ respectively, $p < 0.05$) at the marathon finishing line. Opposite tendencies were found for the RV radial and longitudinal motion components after the run (**Figure 2**). There was a negative correlation between REFi and LEFi values ($r = -0.68$, $p < 0.05$) at stage II. Runners with a greater drop in REF at stage II had higher LEFi and LEF after the run as well ($r = 0.57$ and $r = 0.43$, respectively, $p < 0.05$).

These observations were mirrored by changes in Gal-3 concentrations. After the marathon, higher levels of Gal-3 were found not only in runners with lower RVEF ($r = -0.48$, $p < 0.05$) (**Figure 3**) but also in those with lower REFi or REF ($r = -0.44$ and $r = -0.39$, respectively, $p < 0.05$) (**Figure 4**) and higher LEFi at stage II ($r = 0.43$, $p < 0.05$). Greater increases in post-run Gal-3 concentrations were found in less-fit amateurs with lower VO_2peak values ($r = -0.47$, $p < 0.05$) (**Figure 5**) and in those who needed more time to finish the competition ($r = 0.47$, $p < 0.05$). After the marathon, positive correlations between LEF or LEFi and hs-cTnI obtained at stage II ($r = 0.45$ and $r = 0.43$, respectively, $p < 0.05$) and between LEFI and H-FABP obtained at stage II ($r = 0.38$, $p < 0.05$) were found. Also, runners with more marked change in LEF had higher raise in hs-cTnI ($r = 0.62$, $p < 0.05$) and H-FABP concentration ($r = 0.86$, $p < 0.05$). The contraction pattern and relative contribution of all RV motion components to global RV systolic function, quantified as LEFi, REFi, and APEFi, did not differ between study stages (**Table 2**). No significant and strong correlations were found between the other biomarkers collected at stage II and major echocardiographic marathon-induced alterations (the decrease in REF and RVEF).

DISCUSSION

In this study, we performed a detailed analysis of the impact of running a marathon on RV mechanics in amateur athletes. To the best of our knowledge, this is the first study comparing RV

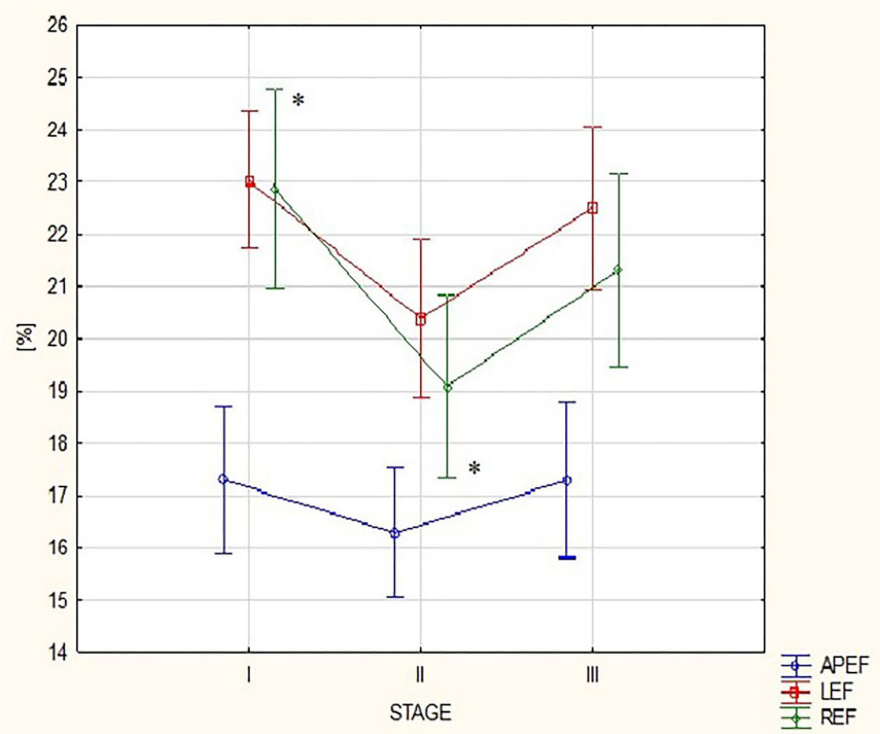

FIGURE 1 | Changes in right ventricular (RV) motion components: anteroposterior ejection fraction (EF), longitudinal EF, and radial EF between the three study stages. APEF, anteroposterior ejection fraction; LEF, longitudinal ejection fraction; REF, radial ejection fraction; Stage I-2 weeks before marathon; Stage II-at marathon finish line; Stage III-2 weeks after marathon. *For REF: ANOVA p-value < 0.05, *post hoc* Tuckey test p-value < 0.05.

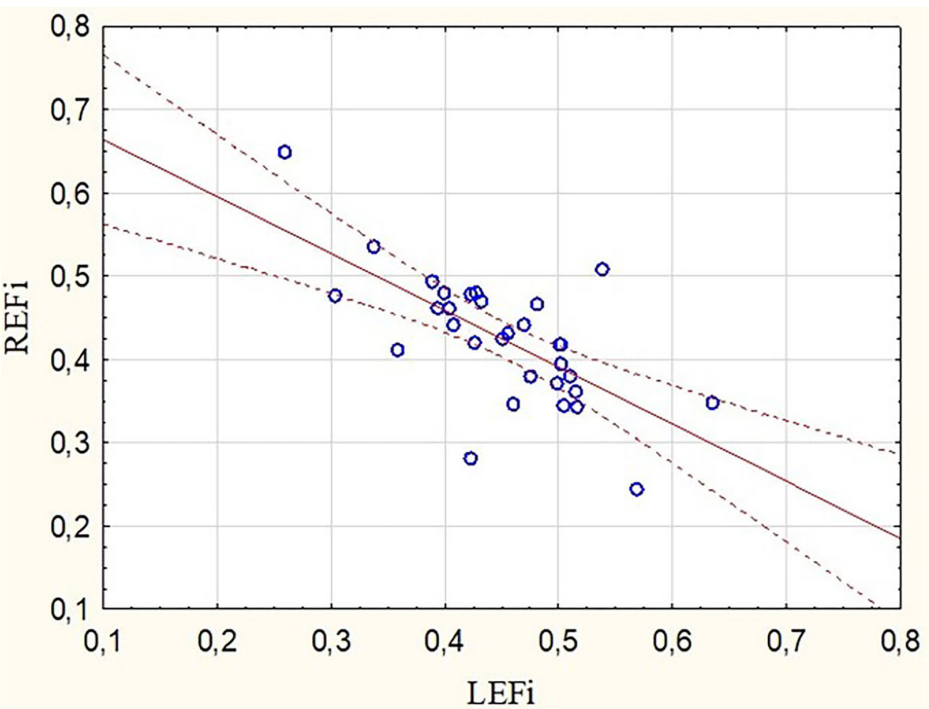

FIGURE 2 | Correlation between radial and longitudinal RVEF indexes obtained after the marathon run ($r = -0.68$, $p < 0.05$). REFi, radial ejection fraction index; LEFi, longitudinal ejection fraction index.

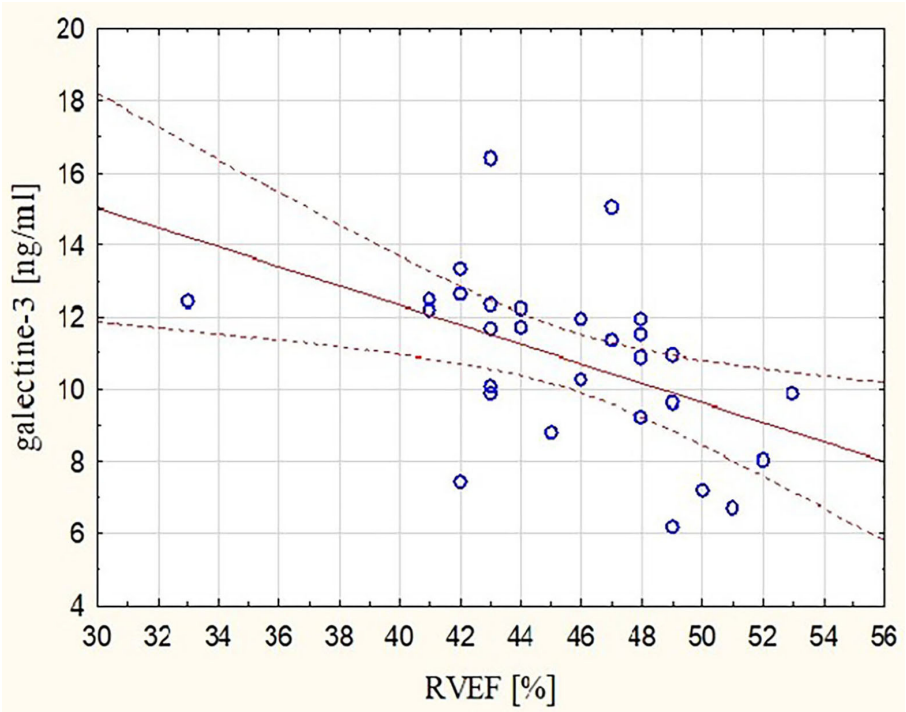

FIGURE 3 | Correlation between post-run RVEF and concentration of galectin-3 (Gal-3) obtained at marathon finishing line ($r = -0.48$, $p < 0.05$). RVEF, right ventricular ejection fraction.

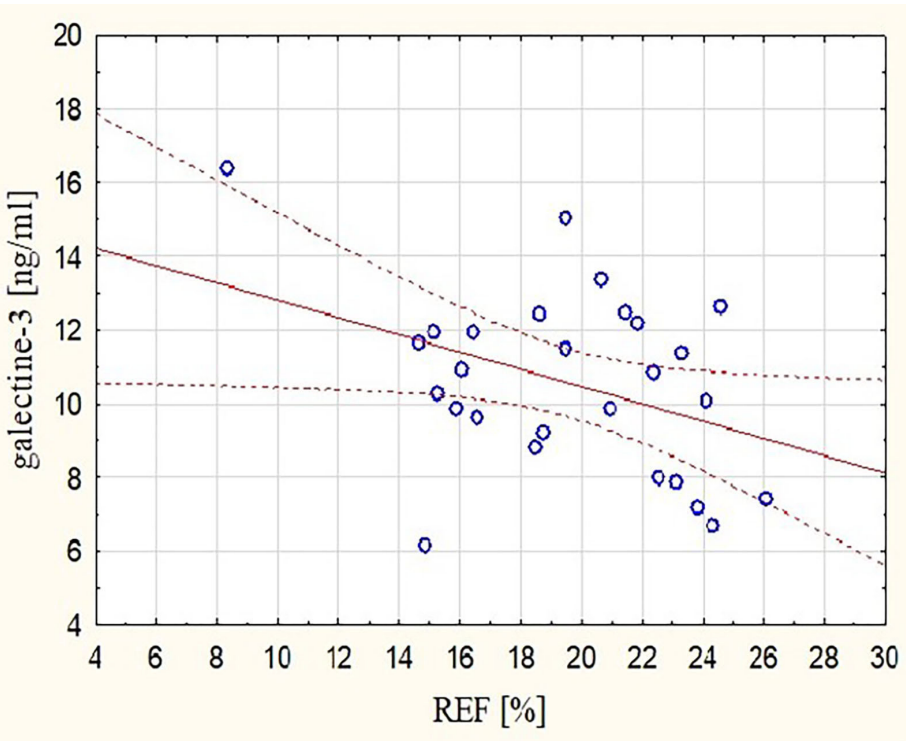

FIGURE 4 | Correlation between post-run RV radial EF and concentration of Gal-3 obtained at marathon finishing line ($r = -0.39$, $p < 0.05$). REF, radial ejection fraction.

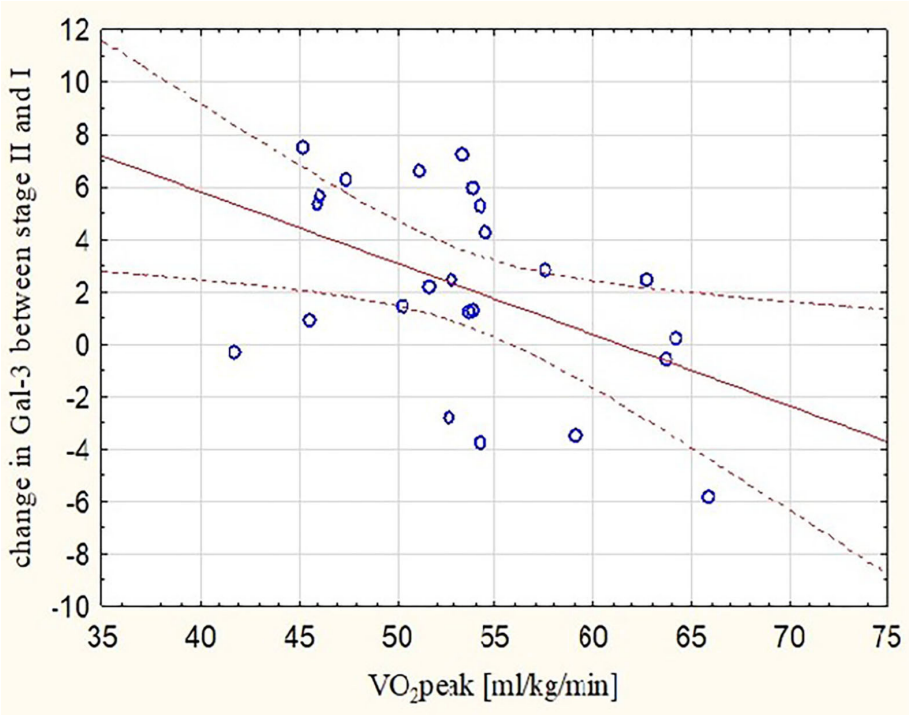

FIGURE 5 | Correlation between peak oxygen uptake and increases in post-marathon Gal-3 concentrations ($r = -0.47$, $p < 0.05$). Gal-3, galectine-3; Stage I-2 weeks before marathon; Stage II-at marathon finish line; VO_2peak, peak oxygen uptake.

function and its relative motion components before and after running a marathon using the echocardiographic ReVISION method. After the competition, the study participants were found to have a transiently enlarged RV with significantly reduced systolic function. Separate quantification of the RV motion components revealed decreased radial shortening as the main contributor to reduced RV global function with preserved longitudinal and anteroposterior movements. We were able to determine the precise amount of training above which the decrease in radial contraction appeared. The echocardiographic findings of the modified RV mechanics were also reflected on the biochemical level, as significant correlations were found with the profibrotic marker of cardiac remodeling, Gal-3. The post-run deterioration of the radial motion contributor may serve as a novel marker of changed RV systolic function among amateur marathon runners.

While the effects of exercise on the LV have been extensively investigated, the RV has long been called "the dark side of the moon" and has remained unexplored. The variety of 2D ECHO parameters used for RV evaluation in everyday practice refers mainly to longitudinal motion (Lakatos et al., 2017), and measuring RV contractility with these conventional measures seems unrepresentative for the entire RV function. Due to technological advances in recent years, more diagnostic options have become available to evaluate RV functioning; nevertheless, the pathophysiology of the competing RV is still not well understood.

It has previously been shown that various conditions that overburden the RV can induce significant changes in its contractile pattern and that one motion contributor may become more important to the EF than others. Similarly, in patients with tricuspid or pulmonary valve regurgitation, an increased longitudinal RV motion following volume overload was reported (Kovács et al., 2019). Therefore, there may be a greater decrease in one motion component over the others in some situations, reflecting a marker of RV dysfunction, as in pulmonary hypertension or pulmonary embolism where a pressure overload causes a greater reduction in radial vs. longitudinal shortening, which becomes supernormal (Lakatos et al., 2017, 2020).

There are some similarities between patients with elevated pulmonary arterial systolic pressure (PASP) and subsequent RV overload and marathon runners in terms of the reduced radial shortening observed in both groups.

While running a marathon, the cardiac output (CO) increases even up to 40 L/min, which is seven times higher than in normal resting conditions (La Gerche et al., 2014). Bearing in mind the simplified Poiseuille's equation that illuminates the functioning of the circulatory system, such an increase in CO must result in increased vascular pressure unless it is lowered by a drop in resistance (La Gerche et al., 2014). The RV normally pumps blood against a low-resistance and low-pressure pulmonary circuit;

however, its load changes dramatically during intense exercise, as the drop in pulmonary vascular resistance seems to be relatively smaller than in the systemic circulation (La Gerche et al., 2014). Consequently, as PASP increases significantly, pressure overload is added, proportionally with augmented CO, and a subsequent increase in RV wall stress is observed (La Gerche et al., 2011, 2014). Although the raise in PASP is transient and normalizes briefly after the exercise (Wierzbowska-Drabik et al., 2019), it causes increased RV work, and as shown in this study, the acute effects following a marathon run manifest as an increase in RV volumes, a decrease in radial contraction, and a deterioration in global RV systolic function. Overall, the impact of running a marathon is mainly on the RV, whereas the LV seems to cope well.

Among the amateur athletes, the post-run impairment of RV function was transient and completely normalized in 2 weeks of observation. Nevertheless, in cases of sustained, repetitive exercising, functional RV changes can consolidate into RV remodeling that is not entirely physiological. In a rat model, after 4 months of forced training, the exercise-induced changes in ventricular function resulted in increased fibrosis in the RV and both atria followed by increased susceptibility to ventricular arrhythmias (Benito et al., 2011).

The marathon effect was also manifested by noticeably elevated concentrations of hs-cTnI, BNP, and stress-related biomarkers, such as GDF-15 and Gal-3 (Kaleta-Duss et al., 2020). Often considered a determinant of myocardial injury, such a "biochemical and biomarker storm" may prompt the creation of fibrotic deposits, mainly in the most strained cardiac chambers. Although the direct proarrhythmic significance in humans has not been proved yet, the incidence of fibrotic changes was higher in marathon runners than in age-matched controls (12 vs. 4%) (Breuckmann et al., 2009). It is noted that the prevalence of myocardial fibrosis, which was found in 50% of veteran endurance runners, was related to the years of training and the number of completed endurance competitions (Wilson et al., 2011). Athletes with delayed gadolinium enhancement in the interventricular septum near the RV attachment had greater RV volume, lower RVEF, and a longer history of endurance sports participation (La Gerche et al., 2012). Interestingly, ventricular arrhythmias recorded in athletes usually originated from the RV (Heidbüchel et al., 2003). Although the data on the eventual proarrhythmic effect of endurance exercise are limited, the high prevalence of atrial fibrillation is a well-known phenomenon among athletes in whom the incidence of arrhythmia is associated with left atrial remodeling and, typically, increased atrial volume (Elliott et al., 2018). Unquestionably, sports activity evokes arrhythmias in individuals with preexisting cardiac disease, such as RV arrhythmogenic cardiomyopathy or hypertrophic cardiomyopathy (Pelliccia et al., 2021).

Nevertheless, the highest risk of acute events, such as cardiac arrest, exercise-related collapse, and chest pain, is linked with middle-aged male athletes who finish marathon runs within 3–4 h (Sharma, 2017). Although frequently exhibiting a cardiovascular risk typical of their peers, the middle-aged group of amateur athletes typically aims to achieve their best results during sports competitions. The question is whether they are adequately prepared to run a marathon in terms of cumulative RV fatigue and the risk of being overtrained.

In this study, higher baseline hs-cTnI levels were associated with a greater decline in post-marathon RV radial shortening. Moreover, we proved that the amount of training in the preparation period is important in terms of observed postrace REF changes. Exercising of more than 47 km/week was connected with a prominent postrace reduction in RV radial contraction. However, not only too much but also very minimal training is meaningful and best described by the "U-shaped curve" (**Figure 6**). Marathon runners who train less are at high risk of marathon-induced myocardial injury because they develop higher pulmonary pressure, greater RV dysfunction, and elevated cardiac troponin T and N-terminal pro-brain natriuretic peptide (Neilan et al., 2006). In our study, those who were less fit (who had a lower VO_2peak in CPET) had higher post-competition plasma concentrations of Gal-3.

An increased concentration of Gal-3 involved in the processes of fibrosis and inflammation is found in acute and chronic heart failure (Coburn and Frishman, 2014). In patients with reduced LVEF, it correlates with the geometry and function of RV and PASP (Zaborska et al., 2020). Gal-3 is considered to be an important factor contributing to cardiopulmonary remodeling in pulmonary hypertension (Barman et al., 2019). In the general population, Gal-3 concentrations predict all-cause mortality (de Boer et al., 2012). In the studied amateur runners, the post-marathon Gal-3 measurements accurately reflected the RV performance, and higher Gal-3 concentrations were found in marathoners with lower global RV function and decreased radial shortening at stage II. These results may suggest a possible link between exercise-induced RV fatigue and myocardial fibrosis, although more research is needed to prove such a relationship.

As many studies have reported preserved RV function in athletes (Leischik et al., 2020), it is likely that a sufficiently long low-intensity preparation period determines the RV response to endurance exercise. The time required for the RV to adapt to the increased overload is not known and possibly depends on the individual. The increased RV dimensions with preserved RVEF are known cardiac adaptive changes called the "athlete's heart" features (Sanz-de la Garza et al., 2020).

In this study, we have shown that among amateur athletes, exhausting exercise acutely but reversibly alters RV radial shortening. It is possible that the long-term RV adaptation also involves persistent changes in the RV contractile pattern and the constant redistribution of motion components. Undoubtedly, the decrease in one systolic contributor can be compensated for by the increased shortening of others, and the global RV function may be preserved. Similarly, heart-transplant recipients, in whom radial shortening is usually superior to longitudinal and reduced 2D parameters, do not indicate RV failure (Lakatos et al., 2018b). Interestingly, after the marathon, we observed different trends in radial and longitudinal motion and negative correlations between them, which was

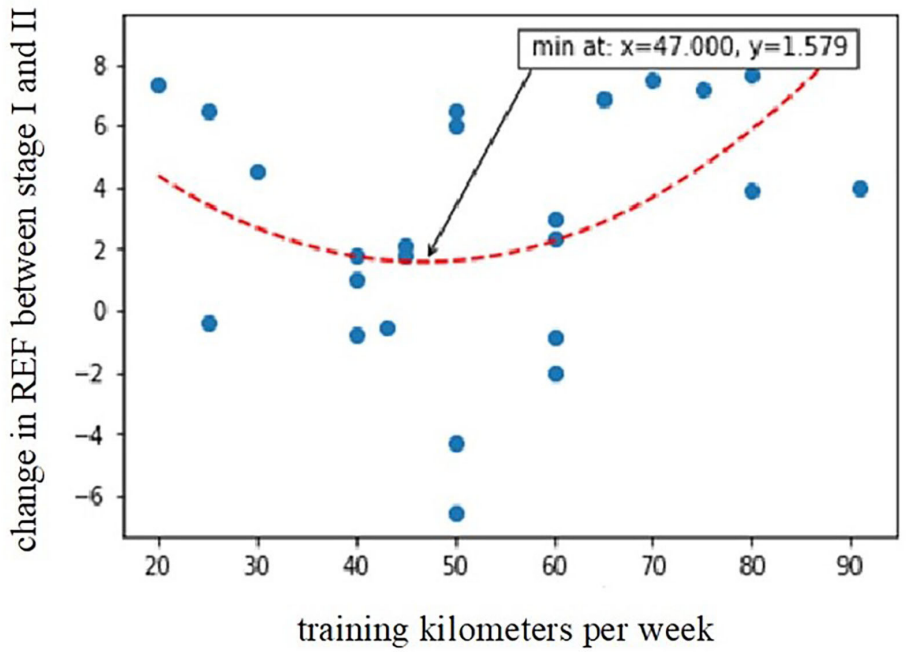

FIGURE 6 | Relationship between training kilometers per week during the preparation period and post-marathon decline in radial EF ($R^2 = 0.4776$, $p = 0.0002$). REF, radial ejection fraction; Stage I-2 weeks before marathon; Stage II-at marthon finish line.

also reflected in Gal-3 concentrations. In another study performed only in resting conditions, top-level water polo players were compared with sedentary controls, and a significant shift in RV contraction pattern was reported with increased longitudinal vs. radial motion (Lakatos et al., 2018a). The more trained the players, the more evident the RV motion changes they presented (Lakatos et al., 2018a). Therefore, the question arises whether such a permanent change in the mechanics of RV contraction may also develop in the group of recreational runners; however, this requires follow-up observation. Further studies are also necessary to determine whether the reduction in the radial motion contributor is a new feature of the "athlete's heart" or a marker of the onset of RV dysfunction.

CONCLUSION

Completing marathon results in significant RV changes in amateur athletes, with decreased RV contractility and increased RV volumes, and less if any effect on the LV function. The drop in global RV function occurs due to a transient decline in its radial shortening, with preserved longitudinal and anteroposterior motion components. The ReVISION method enables a comprehensive analysis of the mechanics of RV contraction and allows insight into the physiology of the competing heart. The observed post-competition macroscopic remodeling of the RV is also reflected at the biochemical level, as the echocardiographic findings correlate with the changes in concentrations of a so-called marker of fibrosis, Gal-3, and a more profound RV dysfunction appears in runners with higher Gal-3 levels. The importance of adequate training preparation for marathon participation seems unquestionable. The thesis of cumulative RV damage is supported here, as a decrease in radial contraction occurred in those who have been probably under- and overtrained, and higher Gal-3 levels after the run were found in those who were less fit.

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 Independent Bioethics Commission for Research of the Medical University of Gdansk. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ZL-P, AK-D, EL, and AD-K: contributed to the conceptualization and design of the study. ZL-P, AK-D, EL, MK, AF, PS, RG, GR, and AD-K: managed investigation, analysis,

and methodology. ZL-P, AK-D, and AD-K: organized the database. ZL-P and AD-K: performed the statistical analysis. ZL-P: wrote the first draft of the manuscript. EL and AD-K: supervised writing the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Sympathovagal Balance Is a Strong Predictor of Post High-Volume Endurance Exercise Cardiac Arrhythmia

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Regular physical activity is important for cardiovascular health. However, high-volume endurance exercise has been associated with increased number of electrocardiogram (ECG) abnormalities, including disturbances in cardiac rhythm (arrhythmias) and abnormalities in ECG pattern. The aim of this study was to assess if heart rate variability (HRV) is associated with ECG abnormalities. Fifteen participants with previous cycling experience completed a 21-day high-volume endurance exercise cycle over 3,515 km. Participants wore a 5-lead Holter monitor for 24 h pre- and post-exercise, which was used to quantify ECG abnormalities and export sinus R-to-R intervals (NN) used to calculate HRV characteristics. As noise is prevalent in 24-h HRV recordings, both 24-h and heart rate collected during stable periods of time (i.e., deep sleep) were examined. Participants experienced significantly more arrhythmias post high-volume endurance exercise (median = 35) compared to pre (median = 12; $p = 0.041$). All 24-h and deep sleep HRV outcomes were not different pre-to-post high-volume endurance exercise ($p > 0.05$). Strong and significant associations with arrhythmia number post-exercise were found for total arrhythmia (total arrhythmia number pre-exercise, $\rho = 0.79$; age, $\rho = 0.73$), supraventricular arrhythmia (supraventricular arrhythmia number pre-exercise: $\rho = 0.74$; age: $\rho = 0.66$), and ventricular arrhythmia (age: $\rho = 0.54$). As a result, age and arrhythmia number pre-exercise were controlled for in hierarchical regression, which revealed that only deep sleep derived low frequency to high frequency (LF/HF) ratio post high-volume endurance exercise predicted post total arrhythmia number ($B = 0.63$, $R^2\Delta = 34\%$, $p = 0.013$) and supraventricular arrhythmia number ($B = 0.77$, $R^2\Delta = 69\%$, $p < 0.001$). In this study of recreationally active people, only deep sleep derived LF/HF ratio was associated with more total and supraventricular arrhythmias after high-volume endurance exercise. This finding suggests that measurement of sympathovagal balance during deep sleep might be useful to monitor arrhythmia risk after prolonged high-volume endurance exercise performance.

Keywords: sympathovagal balance, autonomic imbalance, cardiac, arrhythmia, endurance

INTRODUCTION

Regular aerobic exercise is important for cardiovascular (CV) health (Hower et al., 2018) and decreases the risk of CVdiseases (CVD), such as myocardial infarction and heart failure (Channon, 2020). It also leads to positive chronic structural and functional adaptations to the heart (Sharma, 2003) which are more apparent if the recommended volume of exercise per week is exceeded (George et al., 2012). This has led to an increasing proportion of the general population performing aerobic exercise on a weekly basis for periods of time that greatly exceed exercise recommendations for health (Piercy et al., 2018) (termed high-volume endurance exercise). However, links between high-volume endurance exercise and adverse CV changes are increasingly being reported (Williams and Thompson, 2014; Elliott and La Gerche, 2015), such as sudden cardiac death (Tsuji et al., 1996) and electrocardiogram (ECG) abnormalities (Ector et al., 2007; Abdulla and Nielsen, 2009; Wundersitz et al., 2019). ECG abnormalities include disturbances in the cardiac rhythm (arrhythmias) and abnormalities in the ECG pattern. Arrhythmias can be further classified as those originating in the ventricles (ventricular arrhythmias) or in the atria and other regions of the heart above the ventricles (supraventricular arrhythmias). For example, a 10-fold increase in cardiac arrhythmias was found in adults pre-to-post high-volume endurance exercise performance previously. High-volume endurance exercise is also being performed by people with varied training histories and across the lifespan, which may also be of concern for older adults performing this form of exercise as age is related to ECG abnormality (Zorzi et al., 2018b; Mannina et al., 2021). An ability to understand when the heart is acutely under excess stress from the chronic performance of high-volume endurance exercise would be beneficial for safe high-volume endurance exercise performance for those in the general population, as well as reducing heart injury risks that can be severe and potentially life threatening.

Imbalances in autonomic control are increasingly being linked to chronic ECG abnormality in the literature (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Sareban et al., 2020), especially in clinical populations (e.g., Smith, 1982; Binkley et al., 1991; Bigger et al., 1992; Algra et al., 1993; Kocovic et al., 1993; Hartikainen et al., 1996; de Oliveira et al., 2014). A relatively simple and non-invasive method that has shown promise for monitoring autonomic control is through heart rate variability (HRV) assessment, which is used to measure changes in intervals between successive heartbeats and is a non-invasive reflection of the health of the cardiac automatic nervous system (ANS) (Rajendra Acharya et al., 2006). A healthy ANS normally displays variability in heart rate and a stressed system is usually less variable (Kleiger et al., 1987; Nolan et al., 1998; La Rovere et al., 2003). Although, elevations in atrial fibrillation (AF) is strongly associated with increased risk of mortality (Bigger et al., 1992) and arrhythmias like AF can lead to elevations in HRV (e.g., low frequency to high frequency [LF/HF] ratio Lombardi et al., 2001).

It has been previously demonstrated that high-volume endurance exercise changes HRV in athletes (Zehender et al., 1990; Carter et al., 2003; Hynynen et al., 2006; Swanson, 2006; Seiler et al., 2007; Seidl and Asplund, 2014). For example, Seiler et al., 2007 found that highly trained athletes displayed changes in HRV, lasting 90 min after performing 60 to 120 min of exercise, and Hynynen et al. (2006) found chronic overtraining in athletes lead to significantly decreased HRV characteristics. Although, HRV analysis appears to provide a useful evaluation of autonomic control in athletes, reflecting the hearts ability to recover and respond to changing exercise-related stimuli (Acharya et al., 2007) a potential limitation of this method is that any activity during waking hours, such as physical movement (Moody, 1992; Saponznikov et al., 1992; Tobaldini et al., 2013) can introduce errors or noise within the signal. Hence, potentially important changes in ANS control might be missed. A more reliable method of assessing HRV, is to section and analyze data for periods where participants heart rate is stable, such as during deep sleep (Clifford et al., 2005; Herzig et al., 2017a,b; Kim and Shin, 2017). Deep sleep refers to sleep periods where there is regular breathing, high delta wave activity and low variation in HRV characteristics (Herzig et al., 2017b). Previous authors have reported that deep sleep HRV is more time accurate and reliable when compared to waking hours and other restful periods (Catcheside et al., 2001).

Deep sleep HRV has been assessed in several studies of athletes, but only shortly after an acute bout of exercise (Al Haddad et al., 2009; Myllymaki et al., 2012; Herzig et al., 2018). For example, Al Haddad et al., 2009 assessed the effect of supramaximal intermittent exercise to exhaustion on deep sleep HRV and found post-exercise vagal-related HRV characteristics significantly changed immediately and 12 h after exercise (i.e., LF/HF ratio expressed as a percentage significantly higher, mean RR significantly lower), but had returned to normal after 36 h. To date, however, no study has assessed the effect of high-volume endurance exercise on deep sleep HRV or investigated if changes in HRV are related to ECG abnormalities in response to high-volume endurance exercise. Therefore, the aim of this study was to assess the effect of high-volume endurance exercise in recreationally active people on HRV characteristics and if changes in HRV characteristics are related to ECG abnormalities in response to high-volume endurance exercise.

MATERIALS AND METHODS

Participants and Protocol

This study performed a secondary analysis on previously published data (Wundersitz et al., 2019). The study was approved by the Bendigo Health Care Group Human Research Ethics Committee and the La Trobe University Human Ethics Committee (HREC/17/BHCG/9). Participants or legal guardians of participants aged under 18 years provided written informed consent prior to participating. Fifteen (14 male) recreationally active people (mean ± SD: age, 34.7 ± 19.3 years; height, 1.79 ± 0.08 m; body mass, 78.3 ± 17.6 kg) with 8.4 ± 12.8 years of recreational cycling experience took part in the 'Make a difference, change our world' charity bicycle ride (MADRIDE)[1]. The ride covered 3,515 km from Bunbury in Western Australia to

[1]https://madcow.org.au/event/mad-ride/

Bendigo in Victoria (similar in distance to the Tour De France) (Marijon et al., 2013). Prior to completing the ride, participants completed screening (Balady et al., 1998) and training history questionnaires. Participants reported training for 8.7 (95%CI 4.9 – 12.5) months and covering 3.6 (3.1 – 4.2) sessions per week. On average during each session they trained for 2.9 (2.1 – 3.6) hours and covered 78 (63.5 – 92.5) km. Participants cycled for 21 consecutive days, rode for 6.4 (5.8 – 6.9) hours a day and on average covered 167 (150.9 – 183.8) km/day at 26.3 (25.2 – 27.4) km/h.

To determine HRV characteristics and ECG abnormality number, a 5-lead Holter monitor (SEER Light; GE Healthcare, Horten, Norway) was placed on each participants chest by a cardiac technician and worn for 24 h (9.2 ± 3.2 days pre-exercise). Whilst wearing the Holter monitor, participants were instructed to avoid exercising and to limit intense activities of daily living during this time. Participants again wore the same Holter monitor after completing the 21-days of high-volume endurance exercise (3.0 ± 1.8 days post-exercise). The availability of Holter monitors for research in a public hospital setting limited the ability to test all participants immediately post multi-day high-volume endurance exercise and was added as a covariate to analyses. Holter monitoring was chosen as it is the gold standard method of HRV measurement (Akintola et al., 2016; Georgiou et al., 2018).

Data Analysis

Manufacturer software (version 8, MARS Holter Analysis System; GE Healthcare, Horten, Norway) was used to process raw Holter monitor data throughout both 24-h wear periods. Holter data were screened to identify ECG abnormalities by an experienced cardiac technician and verified by a cardiologist who were both blinded to participant identity and measurement time point. R-to-R intervals between QRS complexes (RR intervals) were then exported using QRSDK software (MARS Holter Analysis System version 8; GE Healthcare, Horten, Norway) to allow further analyses. Abnormal RR intervals (non-sinus rhythm) were excluded from the analysis and those normal intervals between QRS complexes not excluded (NN intervals) were saved for further analyses in LabView software (version 2016; National Instruments, United Kingdom). Specifically, the NN signal was visually displayed in LabView and then deep sleep periods were identified and exported. To be classified as a deep sleep period, a 10-min duration where NN intervals were stationary was required and this inactive period was considered to be when the NN interval had less than a 1% change in slope (Brandenberger et al., 2005). The middle 5-min within this 10-min window was then used to calculate time and frequency domain HRV outcomes. Specifically, time domain variables included: heart rate (HR) mean, NN mean, standard deviation of the NN intervals (NNSD), proportion derived by dividing NN50 by the total number of NN intervals (PNN50) and square root of the mean squared difference of successive NN intervals (RMMSD). Frequency domain variables included: Total power (the sum of the four spectral densities; low-frequency component [LF], high-frequency component [HF], ultra-low frequency [ULF] and very-low frequency component [VLF]), normalized power in the low frequency (LF norm), normalized power in the high frequency (HF norm) and LF/HF ratio. Time and frequency domain variables were analyzed according to recommendations by Malik et al. (1996) In addition, Poincaré SD1 (length of the transverse line of the Poincaré plot area) was calculated to quantify changes in parasympathetic nervous system activity and Poincaré SD2 (length of the longitudinal line of the Poincaré plot area) was calculated to quantify changes in sympathetic nervous system activity (Brennan et al., 2001). Then the ratio of the two (SD1/SD2) was calculated to represent the balance between parasympathetic and sympathetic nervous system activity. Poincaré analyses can be used to detect irregularities that may otherwise be difficult to determine with conventional time and frequency domain variables (Laitio et al., 2000).

Statistical Analyses

SPSS for Windows (version 25; IBM Corporation, Armonk, NY, United States) was used to perform all statistical analyses. Shapiro-Wilks tests were performed to assess normality of data. If data were not-normally distributed, the data were log transformed. To assess the effect of 21-days of high-volume endurance exercise on HRV, one-way analysis of variance (ANOVAs - within factor: measurement time) were performed. Mauchly's test was consulted for all ANOVA's and Greenhouse Geisser corrected p-values are presented when the assumption of sphericity was violated. If significant main effects were found, pairwise comparisons with Bonferroni correction were performed. To explore the effect 'participant age' and the 'measurement time point post cycling' that data were captured, both were added as a covariates and additional ANCOVAs performed if significant Pearson's correlation coefficients were identified between the covariate and dependent variable (Kim, 2018). Data were conveyed as the mean and 95% confidence interval unless data were not normally distributed (then the data were presented as median and interquartile range). Effect sizes (partial eta-squared statistic; η^2_p) are presented and statistical significance was set as $p < 0.05$.

To identify if there were factors that might predict post high-volume endurance exercise arrhythmia response, associations between participant characteristics (i.e., age, pre-exercise arrhythmia, training history, weekly training distance, measurement time point, gender) and all 24 h and deep sleep HRV pre/post characteristics were assessed with Spearman Rho correlations (ρ). Next, a two-step hierarchical regression was performed to identify which HRV characteristics were most associated with arrhythmia response (total arrhythmia, as well as supraventricular and ventricular arrhythmia responses) post multi-day endurance exercise. For the arrhythmia data, three (1 pre, 2 post) scores were 1.96 standard deviations above the mean and positively skewed the variables. Given that outlier adjustment is preferable to whole variable transformation in samples with 1 or 2 outliers, we winsorized (Rivest, 1994) the outliers whereby scores were adjusted down, while maintaining their rank. The pre and post multi-day endurance exercise arrhythmia data were normally distributed after winsorizing.

RESULTS

ECG abnormalities that were not classified as arrhythmias were noted in seven participants, three pre (2nd degree AV block [two] and ST wave elevation) and four post (1st degree AV block, 2nd degree AV block, and ST-T wave changes [two]). In addition, single beat abnormalities were noted in three participants (Pre: bundle branch block, non-conducted atrial ectopic and ventricular escape beat; Post: junctional escape beat). Participants experienced significantly less total arrhythmias pre high-volume endurance exercise (median = 12, interquartile range = 4 – 62) compared to post (median = 35, interquartile range = 4 – 231; $F_{1, 14} = 5.038$, p = 0.041, $\eta^2_p = 0.265$). However, the differences pre to post high-volume endurance exercise in supraventricular (pre: median = 37, interquartile range = 3 – 40; post: median = 53, interquartile range = 4 – 57; $F_{1, 14} = 0.903$, p = 0.358, $\eta^2_p = 0.061$) and ventricular (pre: median = 6, interquartile range = 0 – 6; Post: median = 20, interquartile range = 0 – 20; $F_{1, 14} = 1.060$, p = 0.321, $\eta^2_p = 0.07$) arrhythmias did not reach significance.

24 h and Deep Sleep HRV Pre and Post High-Volume Endurance Exercise

There was an average of 5.8 (95%CI: 4.7 to 6.9) deep sleep periods per person identified at pre and 5.9 (95%CI: 4.8 to 6.9) identified post high-volume endurance exercise ($F_{1, 14} = 0.006$, p = 0.938, $\eta^2_p < 0.001$). 24-h and deep sleep HRV characteristics pre and post high-volume exercise are shown in **Tables 1**, **2**. There was no statistically significant effect of measurement time on 24-h ($p > 0.05$, $\eta^2_p \leq 0.139$) or deep sleep ($p > 0.05$, $\eta^2_p \leq 0.110$) HRV characteristics measured.

Factors Associated With Arrhythmia Response Post High-Volume Endurance Exercise

Age was the only factor to strongly correlate with total, supraventricular and ventricular arrhythmia post high-volume endurance exercise ($\rho \geq 0.54$, $p < 0.05$). Arrhythmia pre strongly correlated with total and supraventricular arrhythmia post high-volume endurance exercise ($\rho \geq 0.66$, $p < 0.01$), but not ventricular arrhythmia ($\rho = 0.27$, $p < 0.05$). All other factors were not significant ($p > 0.05$) and displayed moderate or lower correlations ($\rho \leq 0.43$) with arrhythmia response post-exercise (**Table 3**). Deep sleep and 24-h HRV correlations are provided in **Supplementary Data**.

Does HRV Predict Post High-Volume Endurance Exercise ECG Predict Total Arrhythmia, Supraventricular and Ventricular Number

In the final hierarchal regression model that used the stepwise entry method at step 2, only deep sleep derived LF/HF ratio post high-volume endurance exercise was included in the final model for total arrhythmia and supraventricular arrhythmia analyses after controlling for age and pre-exercise arrhythmia number (**Table 4**). Specifically, higher LF/HF ratio post high-volume endurance exercise was strongly associated with higher counts of arrhythmia (total arrhythmia: B = 0.80, $p = 0.012$). The full model with all variables included was also a significant predictor of post-exercise total arrhythmia, $R^2 = 0.64$, $F(3, 10) = 6.01$, $p = 0.013$ and supraventricular arrhythmia $R^2 = 0.69$, $F(3, 10) = 22.13$, $p < 0.001$. Post exercise ventricular arrhythmia during deep sleep and the 24-h data (total arrhythmia, supraventricular and ventricular arrhythmia post exercise), were not associated with participant age or arrhythmia pre-exercise and no additional variables were entered at step 2.

Additional Findings

We removed the one female participant from the regression analyses as controlling for gender with such an imbalance is not appropriate. However, to confirm if the trends observed would remain with the additional female participant, we re-ran the analysis and controlled for gender. In this model, the findings mirrored those from total arrhythmia, with higher LF/HF post-exercise only associated with all post-exercise ECG abnormality ($B = 0.80$, $p = 0.012$) and supraventricular arrhythmia ($B = 1.07$, $p < 0.001$) during deep sleep.

DISCUSSION

This was the first study to assess the relationship between deep sleep HRV characteristics, using a controlled measurement technique, and number of ECG abnormality in a recreational population of cyclists after performing multi-day high-volume endurance exercise. The main finding was that during deep sleep LF/HF ratio was a strong predictor of post-exercise total and supraventricular arrhythmia after controlling for age and arrhythmia number pre-exercise. We also confirmed that baseline arrhythmia number, in particular total and supraventricular arrhythmia number, and age were strongly correlated with post-exercise arrhythmia response, and that HRV characteristics were not significantly changed after performing multi-day high-volume endurance exercise.

Results from the current study suggest that post multi-day high-volume endurance exercise total arrhythmia and supraventricular number were related to baseline arrhythmia number and participant age. This result supports the widespread understanding that age is related to ECG abnormality, both in athletes (Andersen et al., 2013; Zorzi et al., 2018a,b) and the general population (Furberg et al., 1994; Lok and Lau, 1996; Mannina et al., 2021). This result is likely influenced by cardiovascular system deterioration with increasing age (Hatch et al., 2011) and accumulated demand on the heart from cumulative years of exercise training (Myrstad et al., 2014). The association between baseline total arrhythmia number with post-exercise total arrhythmia response suggests that multi-day high-volume endurance exercise is not likely to influence ECG abnormality in people with baseline arrhythmias.

Supraventricular arrhythmia number post high-volume endurance exercise was also related to baseline supraventricular number. This result is not unexpected, considering that high

TABLE 1 | Time domain HRV characteristics calculated pre and post high-volume endurance exercise (n = 15).

HRV variable	Pre (95%CI)	Post (95%CI)	Mean difference (95%CI)	Effect size (η^2)	*P* value
24-h HRV					
pNN50 (%)	28.0 (20.1 – 36.0)	27.7 (19.5 – 36.0)	−0.30 (−3.83 – 3.23)	0.002	0.857
RMSSD (ms) #	63.9 (48.9 – 79.0)	64.0 (49.9 – 78.0)	0.06 (−5.08 – 5.20)	0.001	0.927
HR mean (bpm)	70.6 (66.5 – 74.7)	68.3 (63.3 – 73.3)	−2.31 (−5.26 – 0.63)	0.168	0.114
NNSD (ms)	73.6 (62.3 – 84.9)	74.3 (63.3 – 85.3)	0.69 (−3.49 – 4.87)	0.009	0.937
NN mean (ms)	906 (853 – 958)	932 (868 – 997)	26.9 (−11.5 – 65.3)	0.139	0.156
Deep Sleep HRV					
pNN50 (%)	45.0 (33.3 – 56.7)	40.5 (29.9 – 51.1)	−4.5 (−12.4 – 3.4)	0.097	0.240
RMSSD (ms)	79.8 (61.3 – 98.3)	73.1 (57.8 – 88.4)	−6.7 (−18.8 – 5.4)	0.092	0.254
HR mean (bpm)	55.2 (51.6 – 58.9)	56.0 (52.6 – 59.4)	0.8 (−2.2 – 3.8)	0.021	0.589
NNSD (ms)	79.5 (64.1 – 95.0)	78.2 (64.9 – 91.4)	−1.4 (−11.6 – 8.9)	0.006	0.781
NN mean (ms)	1116 (1038 – 1195)	1099 (1037 – 1161)	−17.3 (−80.9 – 46.3)	0.024	0.569

Data are presented as mean (95%CI). Note: #, data were not normally distributed; η^2_p, partial eta-squared statistic; bpm, beats per minute; HR, heart rate; ms, millisecond; NN mean, mean of normal-to-normal intervals between QRS complexes; NNSD, standard deviation of the NN intervals; pNN50, proportion derived by dividing NN50 by the total number of NN intervals; RMSSD, the square root of the mean of the sum of the squares of differences between adjacent normal R-R intervals SD, standard deviation; SDNN, SD of all NN intervals.

TABLE 2 | Frequency domain HRV characteristics calculated pre and post high-volume endurance exercise (n = 15).

HRV variable	Pre (95%CI)	Post (95%CI)	Mean difference (95%CI)	Effect size (η^2)	*P* value
24-hour HRV					
Total power (ms^2) #	5516 (3896 – 7136)	5540 (4049 – 7030)	23.5 (−590 – 638)	0.011	0.697
LF norm	67.9 (62.1 – 73.6)	67.6 (62.1 – 73.1)	−0.24 (−3.25 – 2.76)	0.002	0.865
HF norm	28.7 (23.2 – 34.1)	29.1 (23.9 – 34.3)	0.45 (−2.17 –3.08)	0.010	0.717
LF/HF ratio	2.77 (2.03 – 3.50)	2.68 (1.99 – 3.37)	−0.09 (−0.41 – 0.24)	0.015	0.646
Poincaré SD1 (ms) #	45.3 (34.6 – 55.9)	45.3 (35.4 – 55.2)	0.04 (−3.59 – 3.67)	0.001	0.926
Poincaré SD2 (ms)	272 (237 – 307)	254 (225 – 282)	−18.4 (−47.6 – 10.9)	0.115	0.199
SD1/SD2 ratio	0.16 (0.14 – 0.19)	0.17 (0.15 – 0.20)	0.01 (−0.01 – 0.03)	0.102	0.228
Deep sleep HRV					
Total power (ms^2)	6145 (4040 - 8250)	6590 (4243 - 8936)	444 (−991 – 1880)	0.031	0.518
LF norm	55.2 (43.6 - 66.7)	58.8 (49.2 - 68.5)	3.67 (−5.9 – 13.2)	0.045	0.423
HF norm	41.3 (30.3 - 52.2)	37.9 (28.7 - 47.1)	−3.4 (−11.8 – 5.1)	0.050	0.406
LF/HF#	1.40 (0.71 – 1.65)	1.36 (1.16 – 1.66)	0.10 (−0.20 – 0.40)	0.062	0.352
Poincaré SD1 (ms)	56.6 (43.5 - 69.7)	51.9 (41.1 - 62.7)	−4.7 (−13.3 – 3.8)	0.091	0.256
Poincaré SD2 (ms)	112.7 (93.8 - 131.6)	120.2 (100.1 - 140.4)	7.5 (−12.0 – 27.0)	0.047	0.422
SD1/SD2 ratio #	0.72 (0.65 – 0.77)	0.68 (0.61 – 0.77)	−0.04 (−0.11 – 0.03)	0.110	0.210

Data are presented as mean (95%CI). #, data were not normally distributed; η^2_p, partial eta-squared statistic; HF norm, normalized power in the high frequency; LF norm, normalized power in the low frequency; LF/HF, ratio of LF to HF power; Poincaré SD1, length of the transverse line of the Poincaré plot area; Poincaré SD2, length of the longitudinal line of the Poincaré plot area; SD1/SD2 ratio, Poincaré SD1 to Poincaré SD2 ratio.

volume endurance exercise is accepted as a factor contributing to the development of supraventricular arrhythmias (D'Souza et al., 2019), including bradyarrhythmia, atrioventricular block (Baldesberger et al., 2008) and atrial fibrillation (Andersen et al., 2013; Myrstad et al., 2014). Further, supraventricular arrhythmias can have serious quality of life and sport participation consequences in athletes (D'Souza et al., 2019), although in the general population it has been suggested that they are common and rarely life threatening (Blomstrom-Lundqvist et al., 2003). The current findings highlight the importance of screening programs being performed prior to high-volume endurance exercise to identify unknown and potentially serious cardiac issues (Corrado et al., 2006; Mont et al., 2020).

Controlling for age and baseline arrhythmia number, the current results showed that higher LF/HF ratio post-exercise, when recorded during deep sleep, was a strong predictor of post-exercise total arrhythmia and supraventricular arrhythmia number. This finding was not seen when LF/HF ratio data were analyzed over a 24-h period or when ventricular arrhythmias were assessed. To our knowledge, researchers have not previously assessed the effect of endurance exercise in humans on HRV measures (during deep sleep or 24 h) and used these to predict ECG abnormality response. There has been HRV and ECG abnormality research in one study of horses performing endurance exercise (Flethøj et al., 2016), as well as animal and clinical populations performing single (e.g., Guiraud et al., 2013; Broux et al., 2017; Frick et al., 2019) or multiple (training studies; e.g., Deligiannis et al., 1999; Andrade et al., 2017) sessions of short duration exercise. In these studies, mixed findings were found between LF/HF ratio and ECG abnormality. For example,

TABLE 3 | Correlations comparing arrhythmia, age, days post-ride measurement performed, riding years and weekly training distance.

	Arrhythmia	1	2	3	4	5	6	7
(1). Arrhythmia Post^{WIN}	Total	1						
	Supraventricular	1						
	Ventricular	1						
(2). Arrhythmia Pre^{WIN}	Total	0.79**	1					
	Supraventricular	0.66**	1					
	Ventricular	0.27	1					
(3). Age	Total	0.73**	0.67**	1				
	Supraventricular	0.74**	0.69**	1				
	Ventricular	0.54*	0.18	1				
(4). Measurement time	Total	−0.19	−0.13	−0.39	1			
	Supraventricular	−0.14	−0.09	−0.39	1			
	Ventricular	−0.23	−0.01	−0.39	1			
(5). Riding years	Total	−0.26	−0.11	0.01	−0.21	1		
	Supraventricular	−0.33	−0.09	0.01	−0.21	1		
	Ventricular	0.00	−0.27	0.01	−0.21	1		
(6). Distance per week	Total	−0.19	−0.06	−0.37	−0.15	0.15	1	
	Supraventricular	−0.15	−0.06	−0.37	−0.15	0.15	1	
	Ventricular	−0.05	0.07	−0.37	−0.15	0.15	1	
(7). Gender	Total	0.43	0.37	0.31	−0.27	0.03	−0.09	1
	Supraventricular	−0.06	0.06	0.31	−0.27	0.03	−0.09	1
	Ventricular	0.44	0.49	0.31	−0.27	0.03	−0.09	1

** $p < 0.05$; ** $p < 0.01$; ^{WIN}, outliers were winsorized whereby scores were adjusted down, while maintaining their rank.*

TABLE 4 | Hierarchical regression with a Stepwise solution after step 1 assessing if measures of 24-h and deep sleep HRV are associated with post exercise ECG abnormality and arrhythmia after controlling for age and pre-exercise abnormality (n = 14).

		Step summary					
		DS HRV			24-H HRV		
	Predictor	*Beta*	$R^2\Delta$	*p*	*Beta*	$R^2\Delta$	*p*
Total arrhythmia number	Step 1		0.30	0.136		0.18	0.334
	Age	−0.25			0.36		
	Arrhythmia pre-exercise	0.23			0.30		
	Step 2		0.34	0.013		–	–
	LF/HF post exercise	0.80*			–		
Supraventricular arrhythmia	Step 1		0.18	0.334		0.18	0.344
	Age	−0.33					
	Arrhythmia pre-exercise	0.25			0.35 0.31		
	Step 2		0.69	< 0.001		–	–
	LF/HF post exercise	1.07**			–		
Ventricular arrhythmia	Step 1		0.02	0.887		0.02	0.887
	Age	0.12			0.12		
	Arrhythmia pre-exercise	−0.10			−0.10		

** $p < 0.05$; ** $p < 0.01$; $R^2\Delta = R^2$ change; DS HRV, heart rate variability derived during deep sleep; 24-H HRV, heart rate variability derived during 24-h recording.*

similar to the current study Guiraud et al. (2013), found an association between ECG abnormality and LF/HF ratio, with a decrease in ECG abnormality strongly related to a decrease in LF/HF ratio in people with chronic heart failure performing high intensity exercise (r = 0.66; $p < 0.01$). However Broux et al. (2017), found that LF/HF ratio was significantly lower at rest and during exercise (lunging test) in horses with atrial fibrillation ($p < 0.001$). Although few exercise studies are available, there is an abundance of research in clinical populations demonstrating that either a higher (Lombardi et al., 2001; Rizzo et al., 2015) or lower (Nortamo et al., 2018; Sagnard et al., 2020) LF/HF ratio is associated with ECG abnormality. There are a number of potential factors alone or in combination that might contribute to inconsistent findings in the literature, such as the duration

over which HRV was assessed (Furlan et al., 1990), as well as the population (i.e., animal or healthy/clinical human) and type of ECG abnormality (Lombardi et al., 2001) under investigation. For example, mechanisms responsible for heart rate control are not likely to, and cannot, be considered stable with longer term recordings, such as over 24-h (Furlan et al., 1990). Performing moderate physical activity (Moody, 1992; Saponznikov et al., 1992; Tobaldini et al., 2013), emotional circumstances/mental stress (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), and changing respiration (Malliani et al., 1991) or blood pressure (i.e., hypotension) can all induce changes in LF or HF power, hence LF/HF ratio, across longer term recordings. These should be controlled for and the results of the current study support this assumption, suggesting that measurement of HRV during deep sleep are more sensitive to detect ECG abnormalities than those calculated from 24-h recordings.

Regardless of the inconsistencies in the literature, the results of the current study suggest that there is potential to use HRV measures, such as LF/HF ratio, to acutely monitor responses to high-volume endurance exercise. If LF/HF ratio increases post high-volume endurance exercise, this could be used to indicate liability to increased ECG arrhythmia number. Further wearable devices (i.e., phones, fitness tracking watches, etc.) offer a cost-effective way to monitor exercise within 24-h of performance (i.e., whenever they go to sleep) and non-invasively as long as they produce the HRV measure LF/HF ratio during sleep. Future research should continue to assess the relationship between LF/HF ratio and ECG abnormality number after performing high-volume endurance exercise in a large cohort of people, especially those who perform exercise for health benefit. Future studies should endeavor to identify a potential threshold value where any increase in LF/HF ratio above this is of concern.

HRV measures assessed during deep sleep were unchanged as a result of high-volume endurance exercise. Recovery from acute, shorter duration exercise is normally rapid (e.g., Kaikkonen et al., 2007; Michael et al., 2016) and complete recovery might take at least 48 h after high-intensity exercise (Stanley et al., 2013). Our results extend this work and confirm that after 21-days of high-volume endurance exercise participants displayed no differences when measured 72 h after.

Strengths and Limitations

A strength of this study was that a controlled measurement technique (i.e., 5-lead Holter monitor) was used to quantify outcomes in the study and HRV measures were measured during deep sleep (and over 24-h) rather than averaged over the 24-h monitoring period. There are, however, a number of limitations that should be acknowledged. First, as data were only captured pre- and post-high-volume endurance exercise, application of HRV measures during exercise as a potential predictor of endurance exercise produced ECG abnormality are not known. What is known is that HRV characteristics are substantially reduced during exercise (Michael et al., 2017), so any change in LF/HF ratio might or might not be related to ECG abnormality in this situation and more research is needed. Second, LF/HF ratio is a non-invasive measure that is typically used as an indirect measure of sympathovagal balance (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), however, this is controversial in the literature (Billman, 2013; Michael et al., 2017). Higher LF/HF ratio post-exercise being a strong predictor of post-exercise ECG arrhythmia number suggest that high-volume endurance exercise was associated with low vagal activation and sympathetic predominance (Eckberg, 1997) leading to increased ECG arrhythmia number. Sympathetic predominance is typically seen in people under chronic life stress (e.g., Lucini et al., 2005) suggesting that high-volume endurance exercise can lead to a similar situation. However, measures of stress were not captured in this study, so it was not possible to assess the influence of this potential confounding variable.

CONCLUSION

High-volume endurance exercise is growing in popularity across the lifespan due to perceived health benefits. However, there is a body of evidence in professional athletes suggesting that exercise might have negative CV consequences when the amount of exercise performed greatly exceeds recommendations. Results from the current study in recreationally active people found that baseline ECG arrhythmia number, in particular supraventricular arrhythmia number, was associated with post high-volume endurance exercise ECG arrhythmia number. After controlling for baseline ECG arrhythmia number, the HRV characteristic LF/HF ratio measured during deep sleep was shown to be strongly associated with ECG arrhythmia number after performing high-volume endurance exercise for 21 consecutive days. LF/HF ratio offers promise as a non-invasive measure associated with high-volume endurance exercise-induced ECG abnormality.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are available online (doi: 10.26181/1914974) included in **Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Bendigo Health Care Group Human Research Ethics Committee and La Trobe University Human Ethics Committee (HREC/17/BHCG/9). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MK, BG, and CL conceptualized the project. DW and KN performed data collection. DW, KN, and SP analyzed the data.

DW, MK, and BW statistically analyzed the data. SP, DW, and BG drafted the first draft. DW, MK, CL, and BG adapted the draft to the final 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.2022.848174/full#supplementary-material

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Road to Tokyo 2020 Olympic Games: Training Characteristics of a World Class Male Triathlete

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There is a growing interest in the scientific literature for reporting top-class endurance athletes training programs. This case study reports on the training program of a world-class male triathlete preparing to compete in the Tokyo 2020 Olympic Games. A macrocycle of 43 weeks is presented. The triathlete performed 14.74 ± 3.01 h of weekly endurance training volume. Training intensity distribution (TID) was 81.93% ± 6.74%/7.16% ± 2.03%/10.91% ± 6.90% for zones 1 (low intensity, <VT1), 2 (moderate intensity, VT1-VT2) and 3 (high intensity, >VT2) respectively. Pyramidal TID model is observed during the initial stages of the periodization and Polarized TID model is observed at the end of the macrocycle. The triathlete's peak $\dot{V}O_2$ was increased by 20% on cycling and by 14% on running. Peak power was increased by 3.13% on cycling test and peak speed by 9.71% on running test. Finally, the triathlete placed 12th in Olympic distance and 10th in Mixed Relay in Tokyo 2020 Olympic games.

Keywords: triathlon, training load, ECOS, training periodization, endurance training

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INTRODUCTION

The summer Olympic Games are organized every 4 years with a great socioeconomic impact, since they have an audience of more than three billion viewers around the world (Yan, 2020). Triathlon is an endurance sport that combines swimming, road cycling and distance running performed in that order (ITU, 2021). This sport has been part of the Olympic program since Sydney 2000 with the Olympic distance discipline (1.5 km swimming, 40 km cycling and 10 km running). Tokyo 2020 has been the first edition to include the mixed relay format, where the four members of each participating team (i.e, two women and two men) each complete a full super sprint distance triathlon (0.3 km swimming, 8 km cycling and 2 km running). 55 triathletes participated in the Olympic distance of the Tokyo 2020 Olympic Games, previously qualified according to different criteria 2 years before the event. A maximum of three triathletes from the same country can take part in the individual event in both male and female categories. Regarding to the mixed relay race, a total of 18 teams took part in the premiere of this modality in the Olympic Games (ITU, 2021). Besides, Tokyo 2020 Olympic Games were considered particularly hard due to the extreme weather conditions. Meteorological data indicated that weather condition were both hot (26–28°C) and humid (87–89%) during the triathlon races. Such conditions affect the endurance performance and increase the risk of exertional heat illness (Racinais et al., 2015a).

There is a growing interest in the scientific literature for reporting top-class endurance athletes' successful programs (Ingham et al., 2012; Tnønessen et al., 2014; Rønnestad and Hansen, 2018; Tjelta, 2019; Haugen et al., 2021; Kenneally et al., 2021). Variables such as a total training volume, exercise intensity and training intensity distribution (TID) have been commonly analyzed in this kind of researches. TID is defined as the time of the exercise that an athlete spends at the three different zones of

training intensity (Stöggl and Sperlich, 2014): zone 1, at or below the first ventilatory threshold (<VT1); zone 2, between first and second ventilatory threshold (VT1-VT2); zone 3, at or beyond the second ventilatory threshold (>VT2) (Skinner and McLellan, 1980). Polarized model, which is based on a high percentage of time at zone 1 and greater percentages at zone 3 than at zone 2, has been presented as the optimal model of TID to enhance the performance of endurance athletes (Seiler and Kjerland, 2006; Neal et al., 2013; Stöggl and Sperlich, 2014). However, not all findings on polarized TID point to its superiority (Treff et al., 2017). Besides, other TID models as threshold or pyramidal, which accumulate a greater percentage of time at zone 2 than polarized model, has also been presented as effective (Plews and Laursen, 2017; Selles-Perez et al., 2019; González-Ravé et al., 2021). On the other hand, fewer studies analyze training load using specific training load quantification methods for endurance sports (Esteve-Lanao et al., 2017; Selles-Perez et al., 2019).

Physiological profiles of elite endurance athletes have been also reported in several research (Ingham et al., 2012; Bell et al., 2017). VO_{2max}, ventilatory thresholds, as well as power or speed at which these variables occur, these have been common parameters used by coaches to assess fitness levels and prescribe training plans. Even more, it is particularly interesting to describe the dynamics of these variables along the macrocycle (Legaz Arrese et al., 2005; Legaz and Eston, 2005; Sellés-Pérez et al., 2019).

There are limited reports about training characteristics and physiological performance of top-level triathletes (Millet and Vleck, 2000; Millet et al., 2003). Mujika (2014) described training characteristics of a female triathlete in her preparation to the London 2012 Olympic Games. Later, this author presented the physiological profile and the training plan for a world-champion male paratriathlete (Mujika et al., 2015). However, to the best of our knowledge, no case report has been published of world class male triathlete preparing for the Olympic Games. Thus, the aim of this case study is to describe the training characteristics and the physiological profile of a world-class male triathlete who participated in both the Olympic distance event and the mixed relay event at the Tokyo 2020 Olympic Games.

MATERIALS AND METHODS

Characteristics of the Triathlete

This case study received the authorization from the Alicante University Ethics Committee (UA-2017-04-11 expedient). Written consent was obtained from a 29 years-old male triathlete who has competed in world triathlon series since 2012 obtaining 20 podiums and four victories in International Triathlon Union (ITU) races. He finished in fourth position of the World Triathlon Series (WTS) ranking in 2019, obtaining the qualification for the Tokyo 2020 Olympic Games. He also competed in Rio 2016, where he placed 18th.

Physiological Testing, Anthropometric Measures and Training Zone Settings

Swimming test were performed on a 25 m homologated swimming pool through an incremental multistage test consisting of seven repetitions of 200 m every 5 min (Sweetenham and Atkinson, 2003; Muñoz et al., 2014). The first repetition of the protocol was 20 s slower than his personal best in 200 m registered the previous week in a training session and then every repetition was 4 s faster until the sixth, and for the seventh repetition the triathlete was told to perform to swim as fast as possible. Blood lactate (bLA; mmol/l) samples from the ear lobe were analyzed with a portable lactate analyzer (Lactate Scout® 4, EKF Diagnostics, Germany). The criteria to determine training zones were follows in swimming: a blood lactate 0.5 mMol/L increase for first lactate threshold (LT_1), >1 mmol/l increase for second lactate threshold (LT_2) and 8–9 mmol/l for maximal aerobic speed (MAS) (Beneke, 2003; Jamnick et al., 2020).

Incremental tests of volitional exhaustion were used to determine training zones in cycling and running. A ramp protocol was used for cycling on a roller (Cycleops® The Hammer, United States) starting at 150 W and increasing 5 W each 12 s (Muñoz et al., 2014). The triathlete used his own bike (BH®, Aerolight, Spain) and his power meter (ROTOR®, 2inpower Road, Spain) to perform the test. The cycling tests were performed in the same room with the same temperature (20°C). The running test was performed on a 400 m homologated track (University of Alicante facilities). The triathlete started at 13.9 km/h and increased 0.3 km/h every 200 m (Brue, 1985). The triathlete uses the same running shoes in both test (ASICS®, Metaspeed Edge, Japan). Weather conditions were a temperature of 19°C and a wind of 7.5 km/h for the first running test, while they were a temperature of 15°C and a wind of 5.4 km/h for the second test.

Running and cycling tests were conducted using a portable gas-exchange analyzer (Cosmed® K4b 2, Italy). The following variables were measured during the test: oxygen uptake ($\dot{V}O_2$); pulmonary ventilation ($\dot{V}E$); ventilatory equivalent for oxygen/ $\dot{V}E/\dot{V}O2$); ventilatory equivalent for carbon dioxide ($\dot{V}E/\dot{V}CO_2$); and end-tidal partial pressure of oxygen ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$). Maximal oxygen uptake ($\dot{V}O_{2max}$) was recorded as the highest $\dot{V}O_2$ value obtained for any continuous 1 min period. The first ventilatory threshold (VT1) was determined using the criteria of an increase in both $\dot{V}E/\dot{V}O_2$ and $P_{ET}O_2$ with no increase in $\dot{V}E/\dot{V}CO_2$, whereas the second ventilatory threshold (VT2) was determined using the criteria of an increase in both $\dot{V}E/VO_2$ and $\dot{V}E/\dot{V}CO_2$ and a decrease in $P_{ET}CO_2$. Two independent observers identified VT1 and VT2. Heart rate (HR) was continuously monitored during the test using a heart rate meter (SUUNTO®, Spartan, Finland). Later, a range of HR and power or velocity for each training zone was established. Eight training zones were calculated to be more precise prescribing the intensity of the training sessions. These training zones reported both internal load (HR) and external load (speed or power) data (Cejuela and Esteve-Lanao, 2011, 2020). Besides, a RPE scale (1–10) was related to these training zones. Additionally, the Skinner and McLellan triphasic model with three training zones was followed to present TID and training load distribution (TLD) data.

Anthropometric measurements were performed following standard protocols adopted by the international society for the

Advancement on Kinanthropometry (ISAK) (Ross and Marfell-Jones, 1991) by the same researcher with ISAK certification level 3. All measurements were taken on the day of the cycling test under basal conditions, in the same room with the same temperature (22°C). Height and body mass were measured on portable set scales (models 213 and 707, Seca®, Deutschland) to the nearest 0.1 cm and 0.01 kg, respectively. The thickness of six skinfolds (subscapular, triceps, supraspinale, abdominal, front thigh and medial calf) were measured using a caliper calibrated to the nearest 0.2 mm (Holtain®, United Kingdom). Four girths (relaxed arm, flexed arm, thigh and calf) were performed using flexible anthropometric steel tape (Holtain®, United Kingdom). The sum of skinfolds was calculated, as well as muscular mass was determined using the method of Lee et al. (2000).

Control of the Training Load

Cycling power data were measured using a power meter located on the crank (ROTOR®, 2inpower Road, Spain). The triathlete recorded all cycling and running training sessions with his HR monitor (SUUNTO®, Spartan, Finland) and after the data were uploaded to a specific data analysis software (TrainingPeaks®,WKO5, United States). HR and RPE were used mainly for low intensity workouts in running and cycling. Speed and power were also used to control moderate and high intensity workouts in running and cycling. RPE and the medium pace for 100 m were used to control swimming workouts, considering the different training zones obtained in the swimming lactate test. Besides, the triathlete was filling personal training logs with the information recorded regarding the amount of time spent in each training zone and with other subjective information such as hours of sleep and with the subjective load scale (ECSs in Spanish) from 0 to 5 (Cejuela and Esteve-Lanao, 2011; Cejuela and Esteve-Lanao, 2020). Execution speed was controlled in strength training using a linear encoder (VITRUVE®, Spain). Most of the training workouts was supervised by the coach of the triathlete (RC) or an assistant coach (SS or AT).

The objective load scale (ECOs in Spanish) training load quantification method was applied (Cejuela and Esteve-Lanao, 2011; Cejuela and Esteve-Lanao, 2020). Briefly, the ECOs were calculated by multiplying the time (minutes) that the triathlete spends in every training zone (1–8) during the workout by a scoring value between 1 and 50 (depending on the training zone) and by a specific factor of 1.0, 0.75 or 0.5 for running, swimming or cycling respectively. This methodology seems the most appropriate for triathlon because it compares different endurance activities, attending the different degrees of muscle damage, energy cost, effort densities, and differences at the ability of maintaining technique in the three segments (Cejuela and Esteve-Lanao, 2011; Cejuela and Esteve-Lanao, 2020). A specific software (All in your mind Training system®, Mexico) was used to calculate the ECOs throughout the training plan.

Main Characteristics of the Training Period

The triathlete trained with the same methodology in the 2019 and 2020 seasons. The goal was competing in the WTS in 2019, where he ranked fourth. Due to COVID-19 there were no WTS in 2020. This season he was Spanish championship.

The training period for the Tokyo Olympic Games consisted out of 43 weeks, which were grouped in a total of 14 mesocycles. The main part of the training was developed in Alicante (Spain) or in Talavera de la Reina (Spain). Besides, two training camps of 4 weeks were performed in the high-altitude performance center of Sierra Nevada (2,340 m altitude, Spain). Simulated altitude sessions (iAltitude®, Spain) were performed at three times of the season (daily from week 8–10, from week 14–16 and from week 36–40). The duration of the sessions was from 45 to 90 min and the range of altitude exposures was from 3,500 to 6,000 m. The first physiological tests took place at week 6 (swimming) and 7 (cycling and running test). The second physiological tests were performed at week 24. A traditional periodization was used in the first part of the season. The general preparatory period was performed from week 1–14. These weeks were characterized by an increasing of training volume progressively, being the most part of the training sessions around VT1. The specific preparatory period was performed from week 15–30, including the two training camps in high-altitude performance center. The first one was from week 18–21 and the second one was from week 27–30. The main goal of training period consisted in develop higher training zones, nearer to race intensity (VO_{2max} and VT2). Usually, transitions trainings were performed twice weekly with intensities nearer to the competition (around VT2). The triathlete also took part in two competitions during this period in order to training, one sprint duathlon at week 24 (1st) and one Olympic triathlon at week 28 (1st). The first competitive period lasted 5 weeks (from week 31 to week 35) and included two Olympic-distance triathlon world series (WTS). The first one in Yokohama (week 32) where the triathlete ranked at 14th position and the second one in Leeds (week 35) where the triathlete ranked at 4th position. A Mixed relay Olympic qualification event were also performed at week 33 at Lisbon, where the team of Spain ranked at 6th. A tapering period took place during this competitive period. The aim goal of these weeks was reduced training load to promote the supercompensation of the triathlete and improve the recovery. This was mainly done through the training volume reduction. High intensity training sessions were maintained during this period. A 5 weeks training block (from week 36 to week 40) of specific preparation to Tokyo Olympic Games was conducted after these three competitions. Training load was increased again during this period. Tapering period to the Olympic games was performed during weeks 41 and 42, through the decrease of the training load in an exponential manner, in order to promote supercompensation. Besides, these weeks included the adaptation period to the Tokyo jet lag. Finally, the triathlete competed in both the Olympic distance triathlon event and the mixed relay triathlon event at week 43.

The triathlete also performed strength training throughout his preparation to the Olympic games. As a general rule, two weekly strength training sessions were performed during the most part of the season. Multi-joint exercises, both upper and lower body, were performed by the triathlete. Training loads progressed from 55% to 75% of 1RM, performing from two to four sets per exercise. The rep range was from four to eight reps per set, always working away from muscle failure and with a loss of speed not greater than 15% within the set. Furthermore,

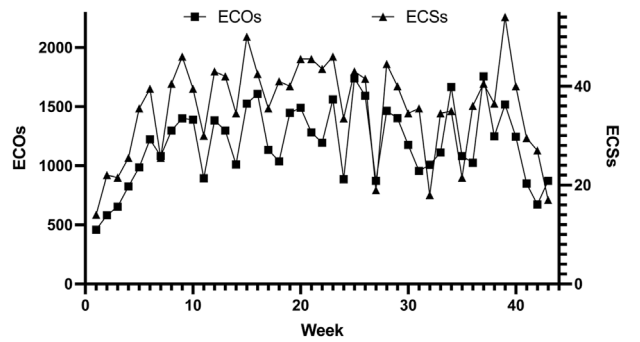

FIGURE 1 | Weekly subjective training load (ECSs) related with the objective training load (ECOs).

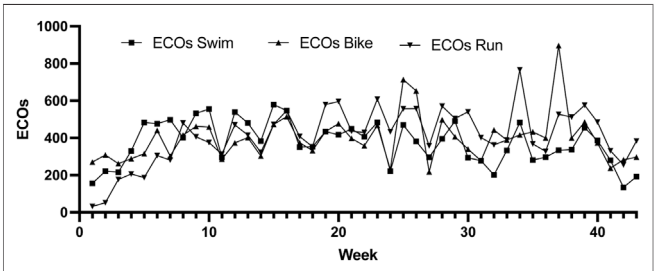

FIGURE 2 | Weekly training load per discipline.

complementary exercises were carried out three or 4 days a week, after the swimming training sessions. These exercises included hip, ankle and thoracic mobility, core training, Achilles tendon prevention exercises or exercises with elastic bands for the shoulder stability.

Polarization index was calculated to quantify the level of polarization every week (Treff et al., 2019). TLD, defined as the percentage of objective training load (ECOs) that the triathlete performs in zones 1, 2 and 3, was also calculated every week.

Heat Adaptation Protocol

A heat adaptation protocol was carried out in order to minimize the impact on performance of the adversely expected weather conditions in Tokyo (high humidity and temperature). On the one hand, the triathlete performed a heat acclimation using the passive method of sauna from week 18 to the begin of the first competitive period in alternate weeks. The sauna session was performed at the end of the training day. The triathlete was exposed for 20–30 min to a sauna bath with a temperature of 70–80°C (Racinais et al., 2019). On the other hand, the weather of Alicante was used to do the acclimatization from week 34 to the Olympic Games. Thus, the triathlete was gradually exposed to training at intensities close to the competition in the middle hours of the day. These training sessions were performed with a temperature from 25 to 32°C and with a humidity from 70% to 80%. A specific non-invasive sensor to assess core body temperature (CORE®, green TEG, Switzerland) was used in this training sessions (Verdel et al., 2021). A special focus on hydration was done during this period. Thus, recovery drinks with sodium were used to compensate for the sweat losses, while maintaining the optimal requirements of carbohydrates and protein to optimize recovery (Racinais et al., 2015a). Finally, the triathlete spent 10 days in Tokyo before the first competition performing several training sessions to maintain the heat adaptation.

RESULTS

Figure 1 shows the weekly subjective training load (ECS) reported by the triathlete and its relationship with the objective training load (ECOs). Peak of objective training load was performed at week 37 (1757 ECOs). Training load of the competitions was included in the summary. Peak of subjective training load was reported at week 39 (ECS), 1 month before to Tokyo Olympic Games. This subjective training load is related to one of the weeks with more objective training load (week 39, 1517 ECOs).

Figure 2 shows the weekly training load (ECOs) for swim, bike and run respectively. It is observed the peak training load for swimming at week 15 (579 ECOs), for cycling at week 37 (896 ECOs) and for running at week 34 (766 ECOs).

Training intensity distribution, training volume, and polarization index of the weeks are presented in **Table 1**. After 4 weeks of low intensity training (>90% of training volume in zone 1) a pyramidal model is shown from week five to week 18, with the exception of week 12 (polarization index = 2.2). From 19 until the end of the training period a polarized training intensity distribution were carried out by the triathlete the majority of the weeks. **Figure 3** shows the training load distribution. It is observed that 50.6% ± 15.5% of the training load was performed at zone 1 and 40.7% ± 16.8% was performed at zone 3.

Anthropometric measurements are presented at **Table 2**. A decrease in total body mass and sum of skinfolds are shown. However, few losses in muscular mass are reported. The change in physiological measures and performance during the season is observed at **Table 3**. The performance of the athlete was increased considerably in the three segments.

Lactate blood concentration during two running training sessions in heat condition is presented in **Figure 4**. Training session 1 (week 36, 31°C and 68% of humidity) consisted in twenty repetitions of 400 m in a track (20 × 400). Training session 2 (week 40, 27°C and 74% of humidity) consisted in twenty-four repetitions of 400 m in the same track (24 × 400). The rest between repetitions was 20 seconds. The lactate was measured every four repetitions. Despite the fact that in the second training session the triathlete performed four repetitions more, a lower lactate concentration is observed. In addition, body core temperature reached was lower in the second training session (from 37.4 to 38.3°C) compared to the first (from 37.5 to 38.9°C).

The performance in the races of the 2021 season is shown in **Table 4**. **Table 5** shows the power data of cycling segment during

TABLE 1 | Weekly training volume and training intensity distribution (triphasic model).

Week	Volume (hours)	% Z1	% Z2	% Z3	P.I.	Week	Volume (hours)	% Z1	% Z2	% Z3	P.I.
1	10.4	95	5	0	0.0	23	18.3	83	4	13	2.4
2	11.6	95	5	0	0.0	24	13.6	78	6	16	2.3
3	12.3	93	7	0	0.0	25	20.1	71	3	26	2.8
4	12.1	92	7	1	1.1	26	18.7	79	7	14	2.2
5	13.3	86	9	5	1.7	27	13.4	82	8	10	2.0
6	17	84	12	4	1.4	28	17.9	78	6	16	2.3
7	12.9	89	6	5	1.9	29	14.1	80	6	14	2.3
8	15.9	83	11	6	1.7	30	12.5	78	7	15	2.2
9	17.9	85	9	6	1.8	31	11.6	82	7	11	2.1
10	16.2	85	7	8	2.0	32	10	75	5	20	2.5
11	13.7	89	9	2	1.3	33	15.4	77	7	16	2.2
12	16.4	86	5	9	2.2	34	17.3	71	11	18	2.1
13	16.1	84	7	9	2.0	35	9.6	66	7	27	2.4
14	13.9	88	6	6	1.9	36	16.6	82	10	8	1.8
15	17.9	83	9	8	1.9	37	17.1	77	7	16	2.2
16	18.5	84	8	8	1.9	38	15	75	2	22	2.7
17	13	84	8	8	1.9	39	17.6	77	7	16	2.2
18	15.2	86	9	5	1.7	40	15.3	85	5	10	2.2
19	17.5	84	6	10	2.1	41	10	75	8	17	2.2
20	18	84	5	11	2.3	42	10.1	82	9	9	1.9
21	16.6	82	8	10	2.0	43	7.3	64	10	26	2.2
22	16	85	7	8	2.0	Average	14.7 ± 3.0	81.9 ± 6.7	7.2 ± 2.0	10.9 ± 6.9	1.9 ± 0.6

Z, zone; P.I., polarization index.

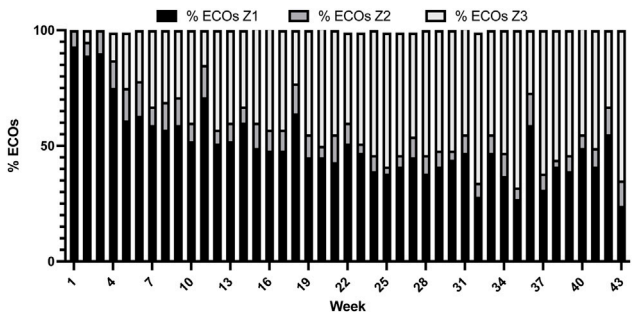

FIGURE 3 | Weekly training load distribution (ECOs distributed at triphasic model).

the Olympic distance races. It is shown the average power, the average normalized power and the number of high intensity power peaks (from 550 to 1,050 W). The average time of these power peaks was 4.2 s.

DISCUSSION

The main goal of this research was to describe the training process of a world-class triathlete to prepare the Tokyo Olympic Games. The average of training volume carried out by the triathlete was less than 15 h, and the peak of training volume was almost 19 h. This training volume is less than the training volume reported by a female elite triathlete (more than 20 weekly average training hours) in her Olympic preparation for London 2012 (Mujika, 2014), but higher than the training volume reported by amateur long-distance triathletes (Neal et al., 2011; Muñoz et al., 2014; Esteve-Lanao et al., 2017; Selles-Perez et al., 2019). It is important to note that the training volume referred in our study is the active time and it does not include pauses and the time of the strength training. Compared with other endurance sports, Rønnestad and Hansen (2018) reported around 12 h of average weekly training for an elite cyclist. Kenneally et al. (2021) reported around 9 h (140 km weekly) of weekly training volume for a case of study of a world-class 5000 m athlete. As a consequence, the triathlete reported more total training volume (weekly hours) than in other cases of study about endurance athletes of other sport disciplines. Despite the fact that the volume per discipline is less than the specialists in each sport, triathletes must develop the performance of three sport modalities. Therefore, a greater number of weekly training hours would be necessary.

TABLE 2 | Anthropometric measurements.

Variable	Anthropometry 1	Anthropometry 2
Body height (cm)	179.0	179.0
Body mass (kg)	68.8	66.3
∑ 6 Skinfolds	34.0	26.5
Muscular Mass (kg)	31.1	30.9

∑ *6 Skinfolds, Sum of six skinfolds; Muscular Mass (kg) (Lee et al., 2000).*

There are not too many studies which have analyzed the training load developed by high performance endurance athletes. TRIMPS (training impulse) have been a method to report training load in different endurance sports such as running (Esteve-Lanao et al., 2007; Stellingwerff, 2012; Muñoz et al., 2013) or cycling (Earnest et al., 2004; Rodríguez-Marroyo

TABLE 3 | Change in physiological measures and performance during the season.

		LT1			LT2			MAS/Peak HR		
		Test 1	Test 2	% Change	Test 1	Test 2	% Change	Test 1	Test 2	% Change
Swimming	**Speed (m/s)**	1.40	1.42	+1.4%	1.49	1.51	+1.3%	1.56	1.61	+3.2%
	Lac	3.5	2.8	–20%	6.5	4.1	–36.9%	10.5	10	–4.8%
	HR	155	145	–6.5%	170	160	–5.9%	181	178	–1.7%
		VT1			**VT2**			**VO_{2Max}/Peak power/Peak Speed/ Peak HR**		
		TEST 1	**TEST 2**	**% Change**	**TEST 1**	**TEST 2**	**% Change**	**TEST 1**	**TEST 2**	**% Change**
Cycling	**P**	240	280	+16.7%	365	405	+11.0%	480	495	+3.1%
	P/BM	3.5	4.2	+22.0%	5.3	6.1	+16.0%	7.0	7.50	+7.8%
	VO_2	44.2	57.1	+7.8%	61.3	72.2	+17.8%	70.5	84.0	+19.2%
	HR	140	140	0%	170	168	–1.2%	186	185	–0.5%
Running	**Speed (km/h)**	15.8	16.2	+2.5%	19.4	20.2	+4.1%	20.6	22.6	+9.7%
	VO_2	43.2	54.8	+26.9%	57.6	69.5	+20.7%	72.0	81.8	+13.6%
	HR	152	153	+0.7%	172	173	+0.6%	190	191	+0.5%

LT, lactate threshold; P, Power (watts); P/BM, Power/Body Mass (w/kg); VO_2, Oxygen uptake (ml/kg/min); Lac, Blood lactate (mmol/L); HR, Heart Rate (bpm); % Change, Percentage of change between test one and test two.

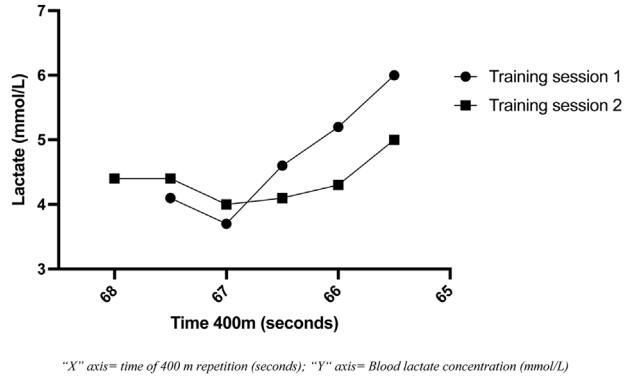

FIGURE 4 | Blood lactate concentration of two running training sessions in heat condition.

TABLE 4 | The performance in the races during the season 2021.

Event	Time (min)				
	Swimming	**T1**	**Cycling**	**T2**	**Running**
Tokyo OG	18.33	0.63	56.15	0.55	30.7
Leeds WTS	17.9	1.16	54.36	0.31	30.46
Yokohama WTS	18.6	0.93	53.71	0.4	30.43
Tokyo OG Mixed Relay	3.83	0.78	9.78	0.35	4.78
Lisbon Mixed Relay WTS	4.08	0.65	9.85	0.43	5.53
Event	**Speed**				
	Swimming	**T1**	**Cycling**	**T2**	**Running**
Tokyo OG	1.36		42.74		19.54
Leeds WTS	1.4		44.15		19.7
Yokohama WTS	1.34		44.68		19.72
Tokyo OG Mixed Relay	1.3		42.94		21.34
Lisbon Mixed Relay WTS	1.22		41.42		21.7

Time, minutes; Speed in swimming, meter per second; Speed in cycling and running, kilometer per hour. OG, olympic games; WTS, world triathlon series.

et al., 2011). It has also been used occasionally in triathlon (Neal et al., 2011). However, this method does not differentiate between sports disciplines with its consequent energy cost or muscle damage (Cejuela and Esteve-Lanao, 2011). It is also based on the triphasic model of training zones (Skinner and McLellan, 1980), not considering that the time to maintain the intensity of the exercise decrease exponentially from the second ventilatory threshold (Billat et al., 2012). Thus, the ECO-method seems to be more specific to quantify the training load in triathlon (Cejuela and Esteve-Lanao, 2011). Weekly average ECOs completed by the triathlete was 1,186.26 ± 320.86 ECOs. These data were in line with the ECOs reported by elite international triathletes (Olaya-Cuartero and Cejuela, 2021), but higher than those reported by elite national triathletes (Saugy et al., 2016), amateur long-distance triathletes (Esteve-Lanao et al., 2017; Selles-Perez et al., 2019) or amateur marathon runners (Esteve-Lanao et al., 2017).

TID followed by this triathlete was mainly polarized. Several previous studies have shown the effectiveness of polarized TID in elite endurance athletes (Billat et al., 2001; Fiskerstrand and Seiler, 2004; Stöggl and Sperlich, 2014, 2015). It is also observed a pyramidal TID in the first part of the season. Some other cases of study have found this TID organization over the season, involving an evolution from a more pyramidal TID during the preparatory period to a more polarized TID during the competitive period (Tjelta, 2019; Kenneally et al., 2021). Both TID models (polarized and pyramidal), present the emphasis in zone 1 (≈80%), being the remaining ≈20% of training volume distributed mainly in zone 3 in polarized TID or mainly in zone 2 in pyramidal TID (Stöggl and Sperlich, 2015). There is no clear consensus on which of these models has greater effects on performance, since few experimental studies have been carried out in this regard. Treff et al. (2017) did not report significantly

TABLE 5 | The power profile in the cycling segment of the races during the season 2021.

Event	Avg P	Avg NP	Rep 550–1050 W	Segment orography
Tokyo OG	338	362	125	Flat and technical
Leeds WTS	307	368	87	Hills and technical
Yokohama WTS	319	347	175	Flat and technical

Avg P, watts average; Avg NP, normalized watts average; Rep, Number of repetitions between 580 and 1050 W.

differences between polarized and pyramidal training groups in elite rowers. Furthermore, Selles-Perez et al. (2019) observed how both TID were effective to improve the performance in amateur long-distance triathletes, but no clear differences between groups were found.

TLD has not been analyzed in previous studies. In this sense, even though most of the training was performed in zone 1, the triathlete completed several training weeks with more than 50% of training load in zone 3. Therefore, the analysis of training load distribution could be a new variable to be incorporated in future research, since it can provide more information about the intensity performed by endurance athletes. A ratio of 50% of training load in Zone 1 and 50% in zones two and three could represent a general guideline. Besides, the triathlete was exposed to stressful training situations such as hypoxia and heat (Racinais et al., 2015a; Flaherty et al., 2016). Thus, the weeks of training camps in altitude and the training sessions to acclimation to the Tokyo's weather represented an added stress that must be considered.

The current consensus recommendations on training and competing in the heat (Racinais et al., 2015a) was followed to design the heat adaptation protocol. Heat acclimatization improves thermal comfort and submaximal as well as maximal exercise performance in warm-hot conditions (Racinais et al., 2015b). The first part of the acclimatization consisted on sauna baths post-exercise. Previous studies have shown the effectiveness of sauna bathing on heat acclimation (Kissling et al., 2020; Kirby et al., 2021). On the other hand, 8 weeks of progressive heat acclimatization in natural environment was performed by the triathlete previous the week of competition in Olympic Games. Training sessions with heat progressed in intensity (from low intensity training sessions to simulated competition training sessions) during these weeks, as well as the weather conditions (temperature and humidity) were harder progressively. As it is recommended in previous research, the main acclimatization block was performed the 2 weeks prior to travel, with 10 days of re-acclimation after arrival to Tokyo (Racinais et al., 2019).

Training camps at high performance center in altitude had a duration of 4 weeks. This period of time seems to be optimal to inducing accelerated erythropoiesis whereas 18 days are long enough for beneficial changes in economy or muscle buffering capacity (Millet et al., 2010). Besides, the altitude training camps were developed at an altitude of 2340 m which is defined as the optimal altitude to living high and training high (2,200–2,500) (Millet et al., 2010).

According to physiological measurements, an improvement in the triathlete's performance is observed in the three disciplines. Relative peak $\dot{V}O_2$ value reported by the triathlete in the second test was higher than the data reported about other elite endurance athletes such as rowers (Treff et al., 2017), swimmers (Fernandes et al., 2008), cyclists (Rønnestad and Hansen, 2018) or runners (Balsalobre-Fernández et al., 2018), and similar to other elite triathletes (Millet et al., 2003). Only some studies with top-class endurance athletes have shown similar values of VO_{2max} (Rønnestad and Hansen, 2018; Jones et al., 2021). The improvement observed in peak $\dot{V}O_2$ was in line with the case study reported by Rønnestad and Hansen (2018), where a world-class elite cyclist had an improvement of 17% after 58 training weeks using a block periodization. However, peak $\dot{V}O_2$ improvements in both cycling and running are much higher than other changes in $\dot{V}O_{2max}$ reported in elite and well-trained athletes after a training period (García-Pallarés et al., 2010; Stoøren et al., 2012; Rønnestad et al., 2014). The triathlete performed the first test after transition period and after the first weeks of the season, where the training sessions were mainly at low intensity. So, the detraining of high intensity training zones should be considered to interpret these improvements. After the first tests, a greater amount of high intensity training sessions were prescribed in the three disciplines, which had a greater impact on the triathlete's peak $\dot{V}O_2$. Additionally, the decrease in total body mass (kg) and fat mass must be considered to read into the large change observed in peak $\dot{V}O_2$ (Bassett and Howley, 2000; Mooses and Hackney, 2017) Both absolute and relative mechanical peak power output, as well as mechanical power output at ventilatory thresholds during cycling test were similar than the values reported for a world-class cyclist (Rønnestad and Hansen, 2018). It is important to know that in the cycling segment of the WTS and OG, many high power peaks are demanded, conditioned by the orography and the tactics of the race. Finally, the peak speed reported and ventilatory threshold during running test were also in line with the data reported by other elite running athletes (Ingham et al., 2012; Kenneally et al., 2021) and with the performance obtained in the races.

Finally, some limitations should be considered. Tests were only performed at the beginning and in the middle of the season. Therefore, the physiological changes produced from the middle to the end of the season are not shown. The competitions, as well as the dynamics of specific training sessions for Tokyo Olympic Games, did not allow a new test week to be scheduled at the end of the macrocycle. In addition, the manuscript shows a case study on a training macrocycle of a world-class triathlete, so the training program and the results obtained cannot be extrapolated to another type of population.

Despite these possible limitations, this research may be interesting for coaches and researches because helps to know

the training characteristics of a world-class triathlete in his preparation for one of the main world sporting events. Future research with endurance athletes of this level of performance is needed.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion 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 The UA-2017-04-11 expedient. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

RC is the athlete's coach. Therefore, he designed the training plan and he was present in most of the training sessions. SS-P is the assistant coach and he advised on the design of strength training. He was also present at numerous training sessions. The manuscript was written by both authors simultaneously.

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Effect of an Ultra-Endurance Event on Cardiovascular Function and Cognitive Performance in Marathon Runners

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Background: Ultra-marathon running participation has become increasingly more popular in recent years; however, there is inconclusive evidence concerning the effects of participation on cognition and cardiovascular function. The purpose of this study was to examine alterations in cardiovascular function and cognitive performance and their association in ultra-marathon runners prior to and following an ultra-endurance event.

Methods: In total, 24 runners (19 males and 5 females) participated in an ultra-marathon race (FatDog120) held in British Columbia, Canada. Participants competed in varying races distances [48 km (n = 2), 80 km (n = 7), 113 km (n = 3), and 193 km (n = 12)]. Cognition was assessed prior to and upon race completion using simple reaction time, choice reaction time, discrimination reaction time, and recognition memory (% correct). Cardiovascular function was assessed prior to and upon race completion using radial applanation tonometry for diastolic pulse contour examination.

Results: Cognitive performance displayed significantly ($p < 0.001$) slower reaction times post-race for simple (30.2%), discrimination (22.7%), and choice reaction time (30.5%), as well as a significant ($p < 0.05$) reduction in memory test performance (−8.2%). A significant association between systemic vascular resistance and choice reaction time was observed post-race ($r = 0.41$, $p < 0.05$). Significant changes in post-race cardiovascular function were observed in resting heart rate (31.5%), cardiac output (27.5%), mean arterial blood pressure (−5.6%), total systemic resistance (−17.6%), systolic blood pressure (−7.0%), pulse pressure (−11.2%), and rate pressure product (22.4%). There was evidence of enhanced cardiovascular function being associated with improved cognitive performance before and after the ultra-endurance event.

Conclusion: Ultra endurance running is associated with marked impairments in cognitive performance that are associated (at least in part) with changes in cardiovascular function in healthy adults.

Keywords: cardiovascular function, cognition, endurance exercise, ultra-marathon, race performance

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INTRODUCTION

Ultra-marathons are running events that extend beyond the traditional marathon length of 42.2 km. Depending on the event, this style of marathon may be limited to a fixed race time completion or by completing the required distance (irrespective of time). These events often involve changes in elevation, where participants are required to make multiple ascents and descents greater than 2,000 m while running over challenging terrain (Bonsignore et al., 2017).

Current systematic reviews examining the physiological and psychological demands of ultra-marathons have demonstrated the importance of elevated and sustained cardiovascular function and cognitive performance (Garbisu-Hualde and Santos-Concejero, 2020; Roebuck et al., 2018). This is important because ultra-marathon runners have indicated that both race strategy and race management are critical components for running a successful race (Simpson et al., 2014). Moreover, ultra-endurance athletes are often required to make critical decisions while navigating potentially life-threatening terrain and environmental conditions. As such, the necessity for sustained cognitive performance throughout an ultra-endurance event is required to support athletes in critical decision making at any period of the race. Limited research has been conducted in this field. However, Hurdiel et al. (2015) demonstrated a significant impairment in post-race cognitive function when compared to pre-race in ultra-marathon runners that occurred irrespective of race length. Ultra-marathons continue to attract experienced marathon runners of all ages seeking new experiences in testing their physical and mental capacity. Recent evidence has shown that speed of processing and decision making, measured by simple and choice reaction times, becomes more variable through adulthood and decreases linearly at the age of 50 (Der and Deary, 2006). This is particularly salient given the average ages of participants engaging in ultra-endurance events and the large percentage of runners who are over the age of 50 (Nikolaidis and Knechtle, 2018).

It is well known that regular exercise is an effective primary and secondary preventative strategy for at least 25 chronic medical conditions (Warburton et al., 2006). Moreover, physical activity and/or exercise have been shown to be protective against cognitive decline in aging populations (Barnes et al., 2003). However, continued research has demonstrated the potential adverse effects of extreme exercise, such as the effect of ultra-endurance events on cardiovascular function (Burr et al., 2012; Bonsignore et al., 2017) and acute measures of cognition (Hurdiel et al., 2015). For example, there is evidence that participation in ultra-marathon events may lead to an acute and transient increase in resting arterial stiffness (Burr et al., 2014), impaired left ventricular systolic and diastolic function (Burr et al., 2012), a reduction in large artery compliance (Bonsignore et al., 2017), and increased reaction time with the greatest decrements occurring when performing the most complex cognitive task (Burrows et al., 2014). Given the important health- and performance-related consequences of cardiovascular and cognitive function, this research has important implications for individuals that engage routinely in this form of extreme exercise (ultra-endurance events). The potential for cognitive and cardiovascular impairment during and following ultra-endurance events, and a paucity of studies understanding cardiovascular and cognitive function need to be reconciled to understand the overall health implications of ultra-endurance events.

Accordingly, the purposes of this investigation were to determine: (1) if cognitive and cardiovascular function are reduced following an ultra-endurance event, and (2) the association between cardiovascular and cognitive function following an ultra-endurance event. We hypothesized that there would be a significant association between cognitive performance and cardiovascular function following an ultra-endurance event and cognitive performance (as measured primarily by various reaction time tasks) would be slower post-race compared to pre-race.

MATERIALS AND METHODS

Participants

A total of 24 experienced ultra-marathon runners, who were registered in an ultra-endurance trail race, were recruited and provided written informed consent to participate in the research. Participant characteristics are provided in **Table 1**. Each participant was recruited 1 day preceding the start of the event. Participants completed the Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) to ensure they were ready to participate in physical activity (Warburton et al., 2011). Each participant self-reported they had not smoked nor participated in strenuous exercise 24-h prior to baseline testing. In addition, each participant confirmed through self-report that they had refrained from any form of caffeine from 2 h prior, and had not consumed alcohol from 12-h prior to baseline testing. This investigation was approved by and executed in exact accordance with the Clinical Research Ethics Board at the University of British Columbia in accordance with the ethical standards established by Declaration of Helsinki and its later amendments.

Study Design

This was an observational study that examined cardiovascular function and cognitive performance prior to and upon completion of an ultra-marathon and their associations in marathon runners. Race distances were: 30 miles (48.3 km), 50 miles (80.5 km), 70 miles (112.7 km), and 120 miles (193.1 km). The ultra-endurance (FatDog120) event was held in the Cascade Mountains in southwestern British Columbia in mid-August with an average temperature of 17.5°C (range 8.7–26.3°C). The race course included a total elevation gain of 3,276 m in the 80.5-km event and 8,673 m in the 193.1-km event. The highest ascent on the 195-km course was 2,300 m and the lowest ascent was 600 m. The highest ascent on the 48.3- and 80.5-km course was 2,200 m and the lowest ascent was 500 m (Bonsignore et al., 2017). The challenging terrain for each event included technical climbs and non-technical flat trails. Nutrition and hydration throughout the study period was at *ad libitum* at the discretion of each runner. Baseline measures

TABLE 1 | Participant characteristics.

Participants			Age	Weight	Height	BMI	Waist circumference	Body fat	Ultra-marathon experience	Mean weekly training distance
Male	**Female**	***(n)***	**(years)**	**(kg)**	**(cm)**	**(kg·m^{-2})**	**(cm)**	**(%)**	**(years)**	**(km)**
19	5	24	38.6 ± 9.8	70.8 ± 9.8	174.2 ± 9.0	23.2 ± 1.9	81.6 ± 7.2	17.5 ± 5.7	4.0 ± 1.0	87.0 ± 1.0

Values are displayed as a mean (±SD).

for participants were assessed 24-h before the start of the race and within 45-min of completing the ultra-marathon. Both pre- and post-race assessments were conducted in a controlled thermoneutral environment (located immediately adjacent to the race start/finish line).

Anthropometric Measures

Anthropometric assessments were recorded without shoes. Body weight and body composition was examined via bioelectrical impedance (Tanita Model TBF 300A, Arlington Heights, IL, United States) utilizing a single frequency analysis for estimating body fat percentage. Height (cm) and weight (kg) were used to calculate the body mass index of each participant (kg·m^{-2}).

Cognitive Measures

Cognition, more specifically, speed of processing, decision making, and recognition memory, was assessed using four tasks: simple reaction time (ms), choice reaction time (CRT; ms), discrimination reaction time (ms), and a recognition memory test (% correct). Tasks were administered using E-prime software Version 3.0 (Psychology Software Tools, Sharpsburg, PA, United States) on an ASUS tablet (AsusTek, Taipei, Taiwan). All tasks were supervised by researchers and participants received both an instruction screen and had three practice trials for each task prior to testing. Each reaction time task consisted of 20 stimuli that appeared at random inter-stimulus intervals between 500 and 2,500 ms. For simple reaction time, participants were shown a stimulus (blue dot) on a screen and instructed to respond as fast as possible by pressing the "v" key (which also had a blue dot placed on the key for reference). For discrimination reaction time (also referred to in the literature as recognition reaction time or go/no-go reaction time), participants were randomly shown a blue, green, or red stimulus (dot on the screen) and instructed to respond only to the green dot by pressing the "n" key as fast as possible. In choice reaction time, participants were randomly shown a blue or green stimulus and instructed to respond as fast as possible by pressing the "v" key when the blue stimulus appeared on the screen or the "n" key when the green stimulus appeared on the screen. During the recognition memory task, participants observed a group of playing cards that were shown on the screen for a total of 4 s. Following this period, a single card appeared on the screen in which participants were instructed to respond as quickly as possible if the single card was previously shown in the group of playing cards. Participants responded by pressing the "v" key if the card was previously shown in the group of playing cards or the "n" key if the card was not previously shown. Data were time and date stamped and manually extracted from E-prime for analysis.

Cardiovascular Measures

After a 10-min stabilization period, cardiovascular assessments were conducted laying in a supine position while blindfolded and wearing noise canceling ear protection in a thermoneutral environment. All cardiovascular measures were conducted three times with the mean value used for analysis. Heart rate (bpm) and blood pressure (mmHg) were recorded using an automated blood pressure cuff monitor (BPM-100, BpTru®, VSM MedTech Ltd, BC, Canada) positioned over the brachial artery of the left arm. Pulse pressure (mmHg), rate pressure product (mmHg.bpm), and mean arterial pressure (mmHg) were calculated. Applanation tonometry (HDI/Pulsewave CR-2000) (Hypertension Diagnostics Inc., Eagan, MN, United States) was used to non-invasively measure small (C_2), large (C_1) arterial compliance, and total systemic vascular resistance (Saugel et al., 2015). Participants were instructed to lie quietly with the right wrist constrained with a wrist stabilizer in the effort to enhance the signal quality of radial artery pulse-waves. After palpation, the sensor was positioned over the area of maximal radial artery pulsation. Blood pressure indices were recorded using an automated blood pressure cuff positioned on the upper extremity of the arm. Change in right arterial waveforms were examined over a 30-s period. Indices of cardiac function such as, cardiac output (L·min^{-1}), stroke volume (mL·beat^{-1}) and cardiac index (L·min^{-1}·m^{-2}) were concurrently estimated. The standardization from utilizing automated measurement techniques required minimal skill from the technician, thereby enhancing the reliability in measures in healthy participants while yielding a high accuracy (Zimlichman et al., 2005).

Data Analysis

Data was analyzed using Microsoft® EXLSTAT 5.1. All data in text, tables and figures are displayed as means (±SD). Normality of data sets were examined using a Shapiro-Wilk's test for normality ($p > 0.05$). A two-tailed paired sample t-test was utilized to examine variation in pre-and post-race cardiovascular and cognitive measures. Significance was declared using a probability of $p < 0.05$. A Pearson Product correlation coefficient (r) was employed to examine the strength of association between each cardiovascular and cognitive performance measure during the pre-race and post-race time period. The following principles were applied for identifying the strength of correlation; 0.10–0.29 small, 0.30–0.49 moderate, and >0.50 large (Cohen, 1988).

RESULTS

Cardiac output (and index) was significantly increased post-race as a result of significant increases in resting heart rate (31.5%) with a small non-significant decrease in stroke volume post-race (−4.8%, $p = 0.28$; **Table 2**). There were significant reductions in systolic blood pressure (−7.0%), diastolic pressure (−4.4%), mean arterial blood pressure (−5.6%), pulse pressure (−11.2%), and systemic vascular resistance (−17.6%; **Table 2**). Rate pressure product was significantly increased (22.4%) following the race. Large arterial compliance (8.0%, $p = 0.25$) and small arterial compliance (−6.8%, $p = 0.69$) were not statistically changed following the ultra-endurance event (**Table 3**).

Pre- and post-race cognitive performance measures are illustrated in **Figure 1**. Post-race simple reaction time was slower by 30.2% (258.3 ± 37.5 vs. 336.3 ± 54.8 ms, $p < 0.001$). Post-race discrimination reaction time was slower by 22.7% (326.6 ± 51.5 vs. 400.8 ± 61.1 ms, $p < 0.001$) and choice reaction time was slower by 30.5% (406.0 ± 90.1 vs 530.0 ± 146.6 ms, $p < 0.001$). Recognition memory performance (% correct), deteriorated by 8.2% post-race (88.9 ± 8.7 vs. 81.6 ± 13.5%; $p < 0.05$). The association between indices of cognitive performance and cardiovascular function are displayed as a correlation matrix with heat map for both the pre-race period (**Table 4**) and post-race period (**Table 5**). Pre-race, the strongest relationships were seen between cognitive reaction time measures (i.e., simple, discrimination reaction time, and choice reaction time) and total systemic resistance and heart rate (**Table 4**). Post-race, the strongest relationship was observed between choice reaction time and systemic vascular resistance ($r = 0.41$).

TABLE 2 | Pre- and post-race resting cardiac function.

Variable	Pre-race	Post-race	% Change	*p*
Resting heart rate (bpm)	61.4 ± 8.6	80.7 ± 13.3	31.5%	0.00
193 km (*n* = 12)	60.4 ± 10.6	77.1 ± 13.8	27.6%	
113 km (*n* = 3)	67.3 ± 2.3	82.7 ± 3.2	22.8%	
80 km (*n* = 7)	62.5 ± 5.1	87.6 ± 15.1	40.1%	
48 km (*n* = 2)	53.8 ± 5.9	74.7 ± 5.7	38.7%	
Cardiac output ($L \cdot min^{-1}$)	4.1 ± 0.82	5.3 ± 1.3	27.5%	0.00
193 km (*n* = 12)	4.4 ± 0.57	5.2 ± 1.4	17.2%	
113 km (*n* = 3)	3.4 ± 1.3	4.8 ± 0.19	42.2%	
80 km (*n* = 7)	4.2 ± 0.45	5.7 ± 1.5	34.8%	
48 km (*n* = 2)	3.0 ± 1.4	4.7 ± 0.24	58.1%	
Stroke volume ($mL \cdot beat^{-1}$)	68.1 ± 15.3	64.8 ± 7.5	−4.8%	0.28
193 km (*n* = 12)	74.6 ± 12.1	66.9 ± 8.3	−10.3%	
113 km (*n* = 3)	50.2 ± 19.6	57.9 ± 1.5	15.4%	
80 km (*n* = 7)	67.7 ± 5.5	64.5 ± 6.6	−4.7%	
48 km (*n* = 2)	57.1 ± 33.1	63.3 ± 8.1	10.9%	
Cardiac index ($L \cdot min^{-1} \cdot m^{-2}$)	2.3 ± 0.52	2.8 ± 0.64	22.1%	0.00
193 km (*n* = 12)	2.4 ± 0.34	2.6 ± 0.46	7.2%	
113 km (*n* = 3)	1.87 ± 0.79	2.64 ± 0.25	41.3%	
80 km (*n* = 7)	2.5 ± 0.35	3.3 ± 0.84	34.1%	
48 km (*n* = 2)	1.5 ± 0.62	2.34 ± 0.03	60.8%	

Values displayed as means (±SD).

DISCUSSION

The purpose of this investigation was to examine cognitive performance and cardiovascular function prior to and upon completion of an ultra-endurance event (i.e., the Fatdog 120). The main findings of this study were as follows: (1) cognitive performance was significantly diminished post-race, and (2) cardiovascular function displayed significant associations with indices of cognitive performance during both the pre-race and post-race period, with total systemic resistance and heart rate exhibiting the strongest relationships with measures of reaction time at baseline (i.e., simple reaction time, discrimination reaction time, and choice reaction time), and total systemic vascular resistance displaying the strongest association with cognitive performance (i.e., choice reaction time) during the post-race period. Our findings support and extend the work of Hurdiel et al. (2015) demonstrating significantly slower reaction times for simple reaction time, discrimination reaction time, and choice reaction time along with a significant reduction in memory test performance after an ultra-endurance event.

Our current study revealed important insight into the effects of prolonged strenuous exercise on arterial compliance. There is inconclusive evidence concerning the effects of participation in ultra-endurance events. Some studies have revealed reduced measures of arterial compliance (or increased arterial stiffness) after prolonged strenuous exercise (Burr et al., 2012, 2014; Bonsignore et al., 2017), while others have revealed no significant changes (Vlachopoulos et al., 2010). Our current findings revealed no significant change in arterial compliance as assessed by applanation tonometry. This is contrary to previous findings wherein increased arterial stiffness (Burr et al., 2014) and reduced arterial compliance (Burr et al., 2012; Bonsignore et al., 2017) were observed after an ultra-endurance event. However, our findings are consistent with those of others (Vlachopoulos et al., 2010).

Importantly, in the current investigation markers of systemic vascular resistance were also decreased alongside other markers supporting post-exercise hypotension (i.e., reduced systolic and diastolic blood pressure). It is plausible that the unchanged arterial compliance was the result of the post-exercise hypotension observed in our trial (Vlachopoulos et al., 2010; Tomoto et al., 2015) or the effect of exercise-related systemic inflammation (Tomoto et al., 2015).

The majority of literature in the area has focused on shorter duration aerobic exercise in well controlled laboratory conditions. Cardiac parasympathetic reactivation following short-term aerobic exercise is well-established (Stanley et al., 2013). Stanley et al. (2013) have argued that metaboreflex stimulation (e.g., muscle and blood acidosis) is a key determinant of parasympathetic reactivation seen shortly post-exercise (e.g., 0–90 min). Moreover, they argue that time for complete cardiac autonomic recovery following high-intensity exercise is at least 48 h. However, there is comparatively limited evidence related to sympathetic and parasympathetic control/balance following prolonged strenuous exercise (in particular ultra-endurance events) (Hautala et al., 2001; Scott et al., 2009; Foulds et al., 2014; Martínez-Navarro et al., 2019). Although

TABLE 3 | Pre- and post-race vascular function.

Variable	Pre-race	Post-race	% Change	*p*
Systolic blood pressure (mmHg)	120.2 ± 16.6	111.7 ± 9.6	−7.0%	0.02
193 km (*n* = 12)	121.3 ± 1 2.0	112.6 ± 10.8	−7.2%	
113 km (*n* = 3)	105.3 ± 1 9.9	105.1 ± 8.6	−0.2%	
80 km (*n* = 7)	125.9 ± 17.6	112.2 ± 9.4	−10.9%	
48 km (*n* = 2)	115.5 ± 34.2	114.5 ± 1.2	−0.9%	
Diastolic blood pressure (mmHg)	74.2 ± 9.0	70.9 ± 6.2	−4.4%	0.06
193 km (*n* = 12)	71.8 ± 8.6	70.5 ± 7.6	−1.8%	
113 km (*n* = 3)	73.4 ± 6.0	70.1 ± 5.8	−4.5%	
80 km (*n* = 7)	78.3 ± 11.4	71.3 ± 5.3	−8.9%	
48 km (*n* = 2)	75.7 ± 4.7	73.5 ± 3.5	−2.9%	
Mean arterial pressure (mmHg)	89.5 ± 10.7	84.5 ± 7.0	−5.6%	0.02
193 km (*n* = 12)	88.3 ± 9.0	84.5 ± 8.4	−4.3%	
113 km (*n* = 3)	84.1 ± 10.1	81.8 ± 6.7	−2.7%	
80 km (*n* = 7)	94.2 ± 13.4	84.9 ± 6.3	−9.8%	
48 km (*n* = 2)	88.9 ± 14.5	87.2 ± 2.0	−2.0%	
Pulse pressure (mmHg)	46.0 ± 11.9	40.8 ± 5.6	−11.2%	0.04
193 km (*n* = 12)	49.5 ± 8.3	42.1 ± 5.7	−14.9%	
113 km (*n* = 3)	31.9 ± 15.5	35.0 ± 2.9	9.8%	
80 km (*n* = 7)	47.6 ± 7.3	40.9 ± 5.9	−14.1%	
48 km (*n* = 2)	39.8 ± 29.5	41.0 ± 4.7	2.9%	
Total systemic resistance (dyne.s.cm^{-5})	1,365.5 ± 180.4	1,124.8 ± 239.3	−17.6%	0.00
193 km (*n* = 12)	1,379.0 ± 199.0	1,177.3 ± 261.6	−14.6%	
113 km (*n* = 3)	1,259.0 ± 244.3	913.7 ± 140.3	−27.4%	
80 km (*n* = 7)	1,387.3 ± 126.2	1,150.6 ± 229.9	−17.1%	
48 km (*n* = 2)	1,368.5 ± 241.1	1,037.0 ± 144.2	−24.2%	
Rate pressure product (mmHg.bpm)	7,357.9 ± 1392.4	9,006.7 ± 1720.4	22.4%	0.00
193 km (*n* = 12)	7,326.5 ± 1444.4	8,618.6 ± 1356.4	17.6%	
113 km (*n* = 3)	7,067.4 ± 1130.7	8,706.9 ± 1040.2	23.2%	
80 km (*n* = 7)	7,890.7 ± 1438.1	9,932.1 ± 2478.8	25.9%	
48 km (*n* = 2)	6,117.1 ± 1159.3	8,546.0 ± 559.7	39.7%	
Large arterial compliance (mL.mmHg^{-1} × 10)	17.6 ± 2.5	19.1 ± 6.3	8.0%	0.25
193 km (*n* = 12)	17.9 ± 4.4	19.4 ± 5.5	8.2%	
113 km (*n* = 3)	18.3 ± 0.6	24.9 ± 9.2	36.5%	
80 km (*n* = 7)	16.9 ± 3.9	15.2 ± 4.6	−10.3%	
48 km (*n* = 2)	17.5 ± 1.2	21.7 ± 7.2	24.4%	
Small arterial compliance (mL.mmHg^{-1} × 100)	9.0 ± 2.4	8.4 ± 5.6	−6.8%	0.69
193 km (*n* = 12)	9.0 ± 2.6	9.1 ± 5.7	1.8%	
113 km (*n* = 3)	9.6 ± 1.1	12.9 ± 9.9	34.3%	
80 km (*n* = 7)	8.6 ± 2.5	5.5 ± 2.7	−35.9%	
48 km (*n* = 2)	10.0 ± 4.0	8.0 ± 1.8	−20.5%	

Values displayed as means (±SD).

limited in number, there is compelling evidence of blunted parasympathetic activity following prolonged ultra-endurance events requiring considerable time for full cardiac autonomic recovery. For instance, Hautala et al. (2001) revealed that cardiac vagal outflow (as assessed by heart rate variability) was blunted for several hours following prolonged (75 km) vigorous cross-country skiing race (Hautala et al., 2001). Previously, we (Foulds et al., 2014) revealed that recreational ultra-endurance athletes demonstrated significantly greater sympathetic modulation and lower parasympathetic modulation (reflecting increased sympathovagal balance) following an ultra-endurance mountain event (involving 120 and 195 km race lengths). Martínez-Navarro et al. (2019) revealed that vagally-mediated heart rate variability was decreased and cardiac autonomic modulation was less complex following a 118 km mountain ultra-marathon. Similar to our current findings, a significant increase in heart rate (58.8 ± 7.9 vs 76.3 ± 9.6 bpm, respectively) was also observed following the ultra-endurance event (Martínez-Navarro et al., 2019). These authors also revealed that the athletes with the higher baseline overall and vagally-mediated heart rate variability achieved faster race finishing times. A recent study by Swart and Constantinou (2020) revealed that ultra-endurance

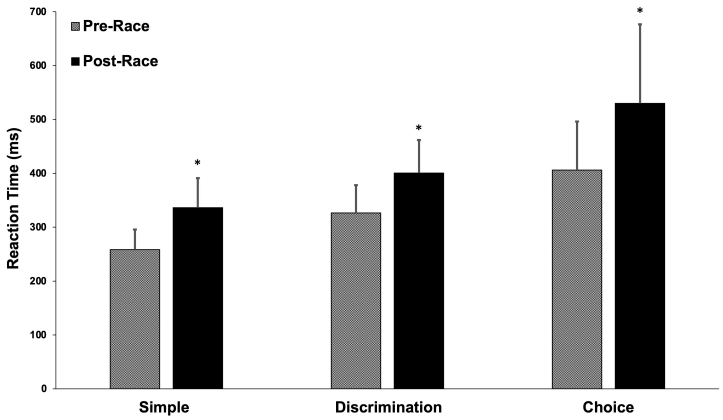


FIGURE 1 | Pre- and post-race reaction time. Values are displayed as a mean (±SD). *$p < 0.0001$.

TABLE 4 | Correlation matrix: pre-race associations between indices of cardiovascular function and cognitive performance and displayed as Pearson correlation coefficient.

Variable	Cardiovascular measure												Cognitive measure			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Systolic Blood Pressure (mmHg)	1															
2. Diastolic Blood Pressure (mmHg)	0.74*	1														
3. Mean Arterial Pressure (mmHg)	0.92*	0.94*	1													
4. Pulse Pressure (mmHg)	0.83*	0.24	0.55*	1												
5. Total Systemic Resistance (dyne·sec·cm^{-5})	0.34	0.1	0.23	0.40*	1											
6. Rate Pressure Product (mmHg·bpm)	0.61*	0.78*	0.75*	0.24	−0.09	1										
7. Large Arterial Compliance (mL·mmHg^{-1} × 10)	0.11	0	0.06	0.16	0.28	−0.05	1									
8. Small Arterial Compliance (mL·mmHg^{-1} × 100)	0.27	0.13	0.21	0.29	0	0.15	0.35	1								
9. Heart Rate (bpm)	−0.19	0.27	0.06	−0.50*	−0.44*	0.66*	−0.17	−0.07	1							
10. Stroke Volume (mL·beat^{1})	0.43*	0.01	0.22	0.61*	0.04	0.52*	0.05	0.24	0.22	1						
11. Cardiac Output (L·min^{-1})	0.44*	−0.27	0.07	0.87*	0.35	−0.14	0.15	0.22	−0.62*	0.62*	1					
12. Cardiac Index (L·min^{-1}·m^{-2})	0.18	−0.12	0.02	0.36	0.13	0.39	−0.01	0.19	0.3	0.85*	0.44*	1				
13. Simple Reaction (ms)	0.06	−0.13	−0.05	0.2	0.39	−0.23	−0.1	0.1	−0.35	0.03	0.26	0.12	1			
14. Discrimination Reaction Time (ms)	0.16	−0.14	0	0.35	0.50*	−0.19	0.24	0.09	−0.41*	0.13	0.41*	0.18	0.70*	1		
15. Choice Reaction Time (ms)	0.08	−0.08	0	0.18	0.56*	−0.23	0.39	0.13	−0.38	−0.04	0.23	0	0.67*	0.69*	1	
16. Memory (% Correct)	−0.37	−0.05	−0.21	−0.50*	−0.19	−0.19	−0.17	−0.3	0.13	−0.44*	−0.47*	−0.24	−0.32	−0.47*	−0.36	1

*$p < 0.05$.

Strength of correlation: -1.0 -0.40 0 0.40 1.0

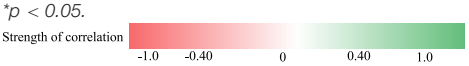
mountain bike participation led to diminished vagal activity (evaluated by heart rate variability) and a shift to sympathetic dominance (Swart and Constantinou, 2020). Collectively, our current findings and that of several other studies support the hypothesis that ultra-endurance events can lead to a transient shift to sympathetic dominance. However, this finding may not be uniform as Scott et al. (2009) revealed no significant changes in autonomic function (as evaluated by heart rate variability) following a 160 km ultra-endurance trail run (Scott et al., 2009).

The increase in heart rate in the face of a reduction in systemic vascular resistance and relative modest post-exercise hypotension reflects the complex adjustments in cardiovascular control and the potential interplay of several factors (MacDonald, 2002; Halliwill et al., 2014; Romero et al., 2017). As reviewed by Romero et al. (2017), arterial blood pressure is the product of cardiac output (arterial inflow) divided by systemic vascular conductance [inverse of systematic vascular resistance (often referred to as total peripheral resistance)] (Romero et al., 2017). In our current study, there was a significant increase in cardiac output (mediated by an increase in heart rate). Systematic vascular resistance is determined by the level of vasoconstriction or vasodilation of vascular beds (Romero et al., 2017). Key potential mechanisms underlying the modest post-exercise hypotension

TABLE 5 | Correlation matrix: post-race associations between indices of cardiovascular function and cognitive performance and displayed as Pearson correlation coefficient.

Variable	Cardiovascular measure												Cognitive measure			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Systolic Blood Pressure (mmHg)	1															
2. Diastolic Blood Pressure (mmHg)	0.82*	1														
3. Mean Arterial Pressure (mmHg)	0.94*	0.97*	1													
4. Pulse Pressure (mmHg)	0.78*	0.28	0.52*	1												
5. Total Systemic Resistance (dyne·sec·cm^{-5})	0.24	0.29	0.28	0.08	1											
6. Rate Pressure Product (mmHg·bpm)	0.39	0.2	0.3	0.44*	−0.2	1										
7. Large Arterial Compliance (mL·mmHg^{-1} × 10)	−0.04	−0.14	−0.1	0.08	0.05	0.34	1									
8. Small Arterial Compliance (mL·mmHg^{-1} × 100)	−0.35	−0.3	−0.34	−0.25	0.19	−0.44*	0.02	1								
9. Heart Rate (bpm)	−0.03	−0.16	−0.11	0.12	−0.36	0.90*	0.36	−0.35	1							
10. Stroke Volume (mL·beat^{1})	0.08	−0.33	−0.16	0.50*	−0.33	0.81*	0.38	−0.28	0.85*	1						
11. Cardiac Output (L·min^{-1})	0.23	−0.36	−0.11	0.79*	−0.13	0.29	0.16	−0.06	0.23	0.70*	1					
12. Cardiac Index (L·min^{-1}ŒEm^{-2})	−0.02	−0.34	−0.21	0.33	−0.13	0.77*	0.34	−0.25	0.86*	0.93*	0.54*	1				
13. Simple Reaction (ms)	−0.28	−0.24	−0.27	−0.22	0.13	−0.18	0.14	−0.14	−0.07	−0.1	−0.08	−0.11	1			
14. Discrimination Reaction Time (ms)	−0.1	−0.01	−0.05	−0.15	0.36	−0.35	−0.25	−0.05	−0.33	−0.34	−0.15	−0.28	0.72*	1		
15. Choice Reaction Time (ms)	−0.29	−0.09	−0.19	−0.37	0.41*	−0.35	−0.06	−0.12	−0.24	−0.34	−0.31	−0.2	0.77*	0.78*	1	
16. Memory (% Correct)	−0.03	0.03	0.01	−0.09	−0.19	0.08	0.08	−0.08	0.1	0.05	−0.09	0.14	−0.42*	−0.44*	−0.18	1

*$p < 0.05$.



observed in our study include baroreflex and thermoreflex resetting, vasodilatation (dependent upon the activation of histamine H_1- and H_2-receptors), and pre-synaptic inhibition of sympathetic vasoconstrictor nerves (Halliwill et al., 2014; Romero et al., 2017). Other factors that likely affect post-exercise hypotension include fluid/blood volume status, ambient heat, and orthostatic stress (Halliwill et al., 2014). However, the small non-significant decrease in both stroke volume and body weight suggests that marked hypovolemia and a reduction in venous return were likely absent following the event.

Physiological measures such as maximal aerobic power and its determinants (in particular cardiac function) have shown to be important factors in race completion time (Davies and Thompson, 1979; Gledhill et al., 1999). High cardiorespiratory fitness has also been associated with improved cognitive performance measures (such as attention and memory) (Hillman et al., 2008). This association in ultra-marathon runners has most recently been demonstrated by Cona et al. (2015) where runners with faster race completion times displayed better inhibitory control than slower runners supporting the role of fitness level on cognitive performance. The contextual relationship between cerebral blood flow and cognitive function has been well reviewed (Ogoh and Tarumi, 2019). Cerebral blood flow has shown to be well maintained with minimal changes in perfusion irrespective of significant alterations in mean arterial blood pressure (Lassen, 1959). This phenomenon has been characterized as cerebral autoregulation, a homeostatic mechanism that regulates cerebral blood flow to maintain cerebral perfusion, using alterations in vasomotor response to control cerebral vascular resistance (Aaslid et al., 1989). Current reviews around the relationship between arterial blood pressure and cerebral blood flow have emphasized the complicated nature between each regulatory mechanism; however, there remains compelling evidence for both direct and indirect effects of arterial blood pressure on cerebral blood flow regulation (Ogoh and Tarumi, 2019). Specifically, cardiac output is thought to directly influence cerebral blood flow in healthy individuals during exercise and rest, with the latter demonstrating a greater association (Ogoh et al., 2005), suggesting enhanced cardiac function would potentially improve cognitive performance. Furthermore, cerebral vascular perfusion may be independent of cardiac output as long as blood pressure remains stable (Van Lieshout et al., 2003).

In our current study, we examined resting measures of cardiovascular function and the association with markers of cognitive performance. There was evidence of enhanced cardiovascular function being associated with improved cognitive performance at rest and after an ultra-endurance event. We hypothesize that the redistribution of cardiac output to skeletal muscle as a response to the metaboreflex for meeting metabolic demands (Laughlin et al., 2011), in addition to various physiological responses causing post-exercise hypotension (MacDonald, 2002), may have sufficiently altered cerebral vascular blood flow thereby influencing and decreasing cognitive performance (Van Lieshout et al., 2003). This, however, was likely tempered by the global increase in cardiac function (particularly heart rate) potentially attenuating the decline observed in cognitive performance. Further research in this area is warranted to fully elucidate the mechanisms responsible for our findings.

The immediate response after completing an ultra-marathon involves alterations in energy balance, increased hormone

secretion, musculoskeletal damage, and/or immunosuppression (Knechtle and Nikolaidis, 2018). It is not fully clear how this multifaceted response to exercise affects cognitive function. However, the lack of association between several of the indices of cognition and cardiovascular function in the current study may have been the result of complex physiological mechanisms occurring post-race. It is also plausible that the age of our participants (37.0 ± 9.7 y) may have influenced our cognitive findings. Cognitive measures (such as choice and simple reaction time) have been shown to decrease significantly after the age of 50 (Der and Deary, 2006). It is plausible that our findings may have been accentuated in older participants. Further research is clearly warranted owing to the increasing participation rates in older adults within ultra-endurance events (Scheer, 2019). Age and sex are becoming increasingly known factors influencing ultra-marathon race performance. For instance, a recent study showed that sex differences were attenuated in ultra-endurance performance with increasing distance and age (Waldvogel et al., 2019). The participants in our study were around 20% female. Females have been shown to have slower and more accurate reaction time (Der and Deary, 2006; Dykiert et al., 2012). Further research should examine the potential influences of sex (Scheer, 2019).

Additionally, we suggest that the reduced cognitive performance seen in our study may have been from a combination of physical and mental fatigue, as well as extended wakefulness. Many participants in our study had been awake for >24 h and completed the race, and subsequent cognitive testing, in the early morning hours (between 12 and 6 a.m.). This is a well-established time when cognitive performance reaches a nadir (Durmer and Dinges, 2005; Van Dongen and Dinges, 2005). Performance decrements on cognitive testing have shown to be greatest in the early morning hours following an ultra-endurance event (Doppelmayr et al., 2005; Hurdiel et al., 2018). Owing to the influence of extreme physical and psychological demands of participating in an ultra-marathon event, we expected cognitive performance would decrease post-race. The current study contradicts previous work displaying prolonged physical fatigue may in fact result in a reduction of psychomotor and neuromuscular function; however, it does not necessarily have to be associated with mental fatigue and a reduction in cognitive functioning (Wollseiffen et al., 2016; Krokosz et al., 2020). These studies showed no significant change in cognitive performance following prolonged exercise suggesting that enhanced physical conditioning may have an impact on the cognitive performance post-race; however, further empirical evidence is required.

LIMITATIONS

A potential limitation in the current study was the absence of measures of blood volume that may have accounted for the reduction in stroke volume and blood pressure. However, the non-significant reduction in post-race body weight (kg) (−0.8%, $p = 0.10$) is suggestive that hypovolemia was likely absent, as ultra-marathon runners are habitually trained to consume water *ad libitum* in an effort to maintain total body water, hematocrit, and hemoglobin (Robach et al., 2014; Ramos-Campo et al., 2016; Belinchon-deMiguel and Clemente-Suarez, 2018).

We recognize that the small sample size in each race length limited our ability to examine more closely the temporal changes that occur with both cardiovascular function and cognitive performance across different race lengths. Furthermore, participant recruitment was controlled by the availability and desire of registered runners. This may have resulted in a selection bias, affecting the findings of this study. Further research with larger sample sizes is warranted in this area to fully elucidate the effects of race distance on cardiovascular function and cognitive performance.

We acknowledge that cerebral vascular blood flow was not examined in the current study, and as such, we encourage further inquiry into the association between cerebral blood flow and cognitive performance after completing an ultra-endurance event to expand on our current findings. We also encourage future studies to include ultra-endurance athletes > 50 years of age, as they represent an increasing demographic in the sport and may provide further insight into age, cognition, and prolonged exercise.

CONCLUSION

This study revealed significant reductions in cardiovascular function and cognitive performance in healthy runners after completing an ultra-marathon event. There were additional associations between indices of cognitive performance and cardiovascular function during the pre-race period when compared to the post-race period. This study revealed emerging evidence that systemic vascular resistance is inversely associated with cognitive performance prior to and upon completing an ultra-endurance event. It remains unclear the exact mechanisms responsible for these findings. Future research is recommended to examine the association between cerebral blood flow and cognitive performance after performing an ultra-endurance event.

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 Clinical Research Ethics Board at the University

of British Columbia. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SB and DW contributed to conception, design, implementation of the study and secured funding for the study. KK, SB, and DW organized the race event and related research study. AP performed the statistical analyses. AJ, AP, and SB wrote the first draft of the manuscript. SB, AP, AJ, ES, and DW wrote sections of the manuscript. RM served as the first nations advisor for this research. All authors contributed to the revision of the manuscript and approved the final submitted version.

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Exercise-Induced Atrial Remodeling in Female Amateur Marathon Runners Assessed by Three-Dimensional and Speckle Tracking Echocardiography

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Endurance athletes have an increased risk of atrial remodeling and atrial arrhythmias. However, data regarding atrial adaptation to physical exercise in non-elite athletes are limited. Even less is known about atrial performance in women. We aimed to elucidate exercise-induced changes in atrial morphology and function in female amateur marathon runners using three-dimensional (3D) echocardiography and two-dimensional (2D) speckle tracking echocardiography (STE). The study group consisted of 27 female (40 ± 7 years) amateur athletes. Right (RA) and left atrial (LA) measures were assessed three times: 2–3 weeks before the marathon (stage 1), immediately after the run (stage 2), and 2 weeks after the competition (stage 3). Directly after the marathon, a remarkable RA dilatation, as assessed by RA maximal volume (RAVmax, 31.3 ± 6.8 vs. 35.0 ± 7.0 ml/m^2; $p = 0.008$), with concomitant increase in RA contractile function [RA active emptying fraction (RA active EF), 27.7 ± 8.6 vs. 35.0 ± 12.1%; $p = 0.014$; RA peak atrial contraction strain (RA PACS) 13.8 ± 1.8 vs. 15.6 ± 2.5%; $p = 0.016$] was noticed. There were no significant changes in LA volumes between stages, while LA active EF (34.3 ± 6.4 vs. 39.4 ± 8.6%; $p = 0.020$), along with LA PACS (12.8 ± 2.1 vs. 14.9 ± 2.7%; $p = 0.002$), increased post race. After the race, an increase in right ventricular (RV) dimensions (RV end-diastolic volume index, 48.8 ± 11.0 vs. 60.0 ± 11.1 ml/m^2; $p = 0.001$) and a decrease in RV function (RV ejection fraction, 54.9 ± 6.3 vs. 49.1 ± 6.3%; $p = 0.006$) were observed. The magnitude of post-race RV dilatation was correlated with peak RA longitudinal strain deterioration ($r = -0.56$, $p = 0.032$). The measured parameters did not differ between stages 1 and 3. In female amateur athletes, apart from RV enlargement and dysfunction, marathon running promotes transient biatrial remodeling, with more pronounced changes in the RA. Post-race RA dilatation and increment of the active contraction force of both atria are observed. However, RA reservoir function diminishes in those with post-race RV dilation.

Keywords: marathon running, atrial remodeling, 3D echocardiography, 2D speckle-tracking echocardiography, female amateur athletes, endurance training

INTRODUCTION

Physical activity is gaining popularity in the general population, with a growing number of both male and female amateur athletes. Endurance training promotes structural, electrical, and functional changes in the heart, with significant gender discrepancies (Di Paolo and Pelliccia, 2007; Pelliccia and Adami, 2017). Until the first half of the 20th century, women were considered physiologically unable to perform sports at a competitive level, and most publications on exercise-induced cardiac remodeling pertain to male athletes. Recently, however, the participation of women in marathons increased from 10% in 1980 to 43% in 2013 (Roberts, et al., 2013). Despite the growing number of female athletes, little is known about the long-term cardiac effects of prolonged exercise in this population.

Traditionally, the ventricles are the main focus of clinical research, but the importance of atrial morphology and function should not be underestimated. Exercise-induced atrial dilatation, as a physiologic adaptation to exercise conditioning, has been previously described (Pelliccia et al., 2005; Brugger et al., 2014). According to Gabrielli et al. (2014), the atria of athletes function at lower strain, larger volumes, and higher atrial wall stress. Indeed, the evaluation of atrial performance plays a fundamental role in the assessment of an athlete's heart, differentiating physiologic adaptation to exercise from pathologic changes (D'Ascenzi et al., 2018). Recent reports have shown that exercise-induced atrial remodeling may contribute to development and perpetuation of atrial fibrillation (AF) (Abdulla and Nielsen, 2009; Aizer et al., 2009; Mont et al., 2009). However, the aforementioned outcomes regard mainly male athletes, with scarce data on atrial adaptation to endurance training in women (Pelliccia et al., 2005; D'Ascenzi et al., 2014).

The majority of studies describing atrial volumes and function in athletes are based on two-dimensional (2D) echocardiographic assessment, but 2D methods rely strongly on correct positioning and angulation of imaging planes, complicated in the case of atria. Real-time, three-dimensional (3D) echocardiography may overcome all limitations of conventional echocardiography, without any angulation issues or geometric assumptions about atrial shape (Artang et al., 2009; Caselli et al., 2010).

In addition, speckle tracking echocardiography (STE) allows objective and quantitative evaluation of global and regional atrial deformation. The atrial longitudinal 2D strain is considered the most useful parameter for functional analysis of both atria due to its significant feasibility and reproducibility (Padeletti et al., 2012). An accurate assessment of atria in endurance athletes is crucial to provide a comprehensive evaluation of biatrial remodeling and its relation to exercise capacity (Nielsen et al., 2021).

Therefore, the aim of our study was to assess echocardiographic parameters of biatrial performance at rest and directly after the marathon run in female amateur athletes and investigate whether this prolonged training correlates with improvement or worsening of atrial function. We hypothesized that endurance of physical activities, such as marathon run, may lead to corresponding cardiovascular changes in amateurs, as in elite athletes.

MATERIALS AND METHODS

Study Design and Participants

We enrolled 27 female Caucasian marathon runners aged between 28 and 57 years, who participated in the XXIV Orlen Solidarity and 5th Gdansk Marathon. The participants were healthy, in sinus rhythm, without cardiovascular comorbidities or other chronic diseases, and had a negative family history of cardiac disease or sudden cardiac death.

The study protocol consisted of three stages: 2–3 weeks before the start of the race (stage 1), immediately after the marathon run, on the finish line (stage 2), and 2 weeks after the competition (stage 3). At each stage, physical examination with anthropometric data (height, weight, body mass index, and blood pressure), electrocardiography (ECG), and transthoracic echocardiography was performed. Furthermore, baseline assessment included the intensity of training, defined in hours and distance per week; number of completed marathons; cardiopulmonary exercise test on a treadmill; and 24-h Holter ECG monitoring. The index race performance time was also noted. During the competition, participants were allowed to rehydrate *ad libitum*, and no food intake restrictions were advised. Detailed study information was provided to all volunteers, and written consent was obtained from all participants prior to the study. The study protocol was approved by the Bioethics Committee of the Medical University of Gdansk, Poland (No. NKBBN 104/2016).

3D Echocardiographic Assessment

Standard and 3D echocardiographic examination was performed using a commercially available system (Vivid E9 and E95, GE Healthcare, Horten, Norway), following the current recommendations of the European Association of Preventive Cardiology (EAPC) and European Association of Cardiovascular Imaging (EACVI) (Kou et al., 2014; Pelliccia et al., 2018). The subjects were studied in the steep left-lateral position. Data sets of both ventricles and atria were obtained from the apex from six cardiac cycles, by means of electrocardiographically gated full-volume 3D echocardiography. Off-line data analysis was performed by two researchers, using echocardiographic quantification software (EchoPac 201, GE Healthcare, Norway). The blood–tissue interface was automatically initialized by the software and afterward manually corrected frame-by-frame by tracing the endocardium from the 2-, 3-, and 4- chamber and short-axis view. The atrial data set alignment, identifying atrial

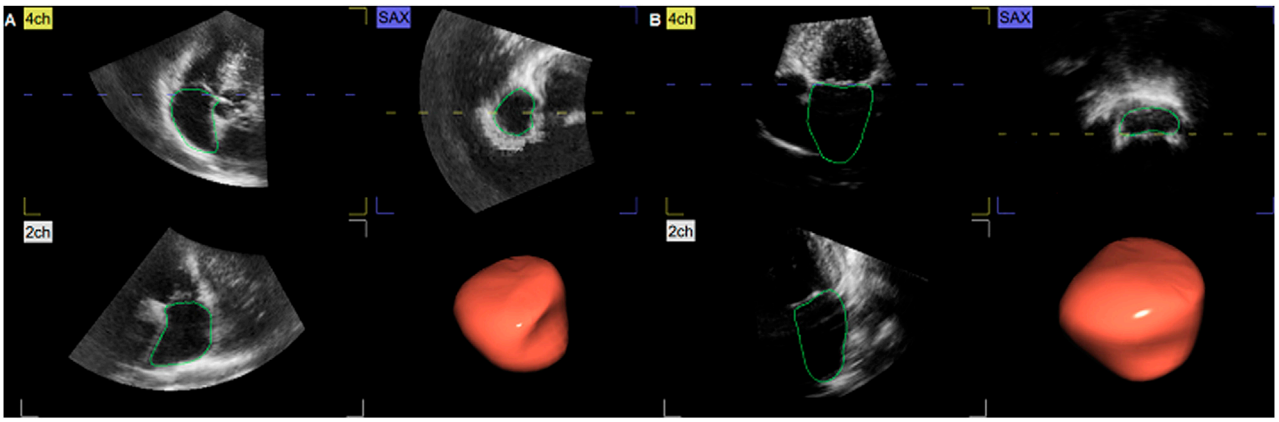

FIGURE 1 | Three-dimensional (3D) echocardiographic assessment of the right atrium (RA) **(A)** and left atrium (LA) **(B)**. From the apical view, both transversal and longitudinal visualization of atrial volume changes during the cardiac cycle was obtained, with endocardial contouring and cast reconstruction. 2ch, two-chamber view; 4ch, four-chamber view; SAX, short-axis view.

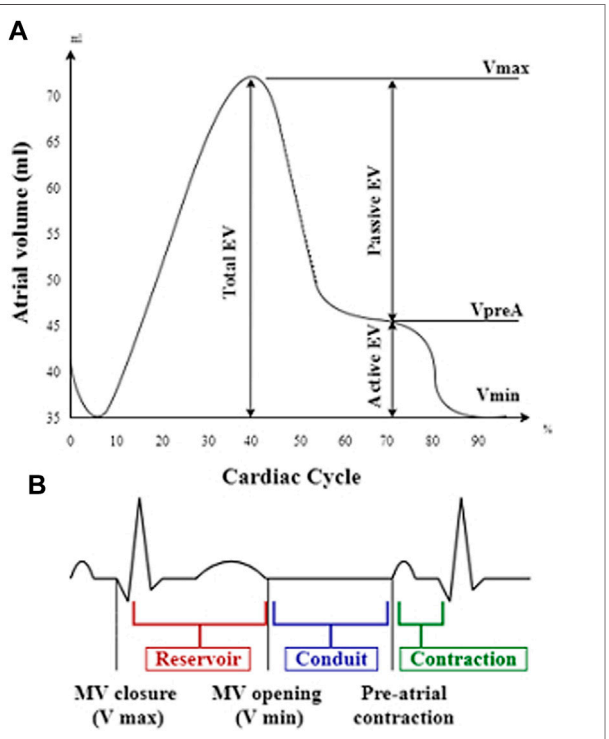

FIGURE 2 | Atrial volume–time curve presenting atrial volume changes during the cardiac cycle **(A)** in relation to the reservoir, conduit, and contraction phases on electrocardiogram **(B)**. EV, emptying volume; Vmax, maximal volume; VpreA, pre-A-wave volume; Vmin, minimal volume; MV, mitral valve.

volumes throughout the cardiac cycle, is demonstrated in **Figure 1**.

To quantify ventricular 3D morphology, we determined ventricular end-diastolic volume (EDV) and ventricular end-systolic volume (ESV), indexed to body surface area (BSA). In the case of left ventricular (LV) function, indexed LV stroke volume (LVSV) and LV ejection fraction (LVEF) were measured. While considering right ventricular (RV) contractility, RV ejection fraction (RVEF) and RV fractional area change (RVFAC) were calculated.

Volumetric changes of both atria were measured at three stages: maximal volume (Vmax, at the end of ventricular systole just before mitral valve opening), minimal volume (Vmin, at the end of ventricular diastole just before mitral valve closure), and atrial volume before contraction (VpreA). The atrial volumes were indexed to BSA. Total emptying volume (EV) was defined as Vmax-Vmin. Using these volumetric data, we computed the total emptying fraction (EF) as ([Vmax-Vmin]/Vmax) x 100, the passive EF as ([Vmax-VpreA]/Vmax) x 100, and the active EF as ([VpreA-Vmin]/VpreA) x 100, as parameters of the atrial reservoir, conduit, and contraction function, respectively (**Figure 2**). According to the current EAPC/EACVI guidelines (Pelliccia et al., 2018), the normal ranges for left atrial (LA) volume are 36 ml/m^2 in men and 33 ml/m^2 in women, while the upper limits for right atrial (RA) volume are 33.8 ml/m^2 in men and 29.3 ml/m^2 in women.

2D Speckle Tracking Echocardiography

Atrial myocardial deformation was measured with the use of 2D STE during breath-holding with a stable ECG tracing. Three consecutive heart cycles were recorded and averaged. The frame rate was set between 60 and 80 frames per second. LA strain parameters were obtained from the apical 4-chamber and 2-chamber views, whereas RA strain was calculated from the apical 4-chamber view. The endocardial surface of both atria was manually traced by a point-and-click approach. Then, the epicardial surface tracing was automatically generated by the system, creating a region of interest (ROI). After manual adjustment of ROI, the software automatically divided the atrial wall into six equidistant segments and analyzed it.

Finally, the longitudinal strain curves for each segment and a mean curve of all segments were generated (**Figure 3**). Before the

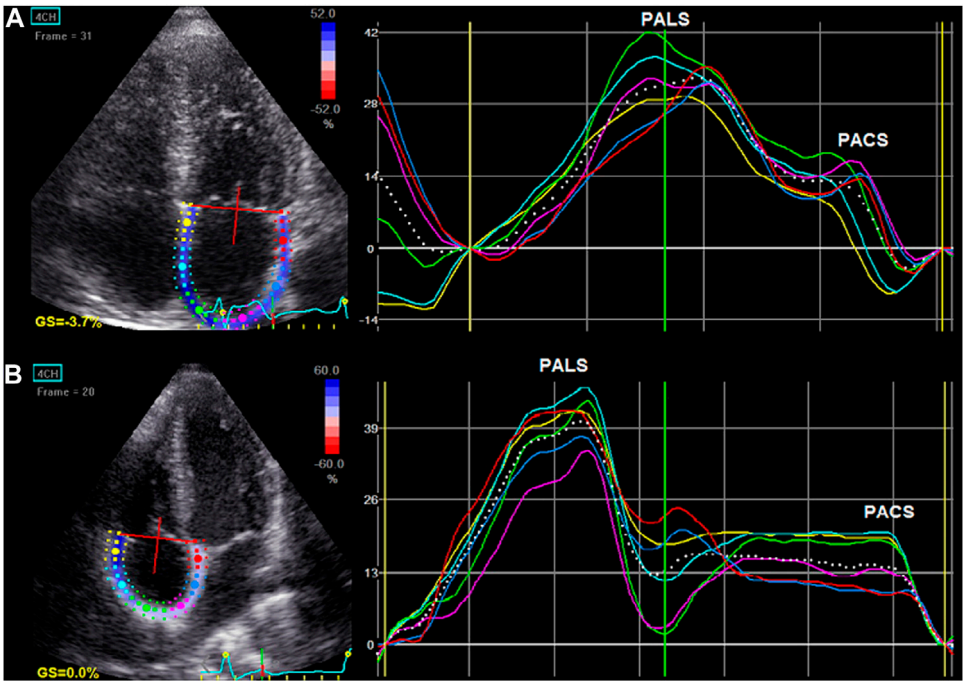

FIGURE 3 | Left atrial **(A)** and right atrial **(B)** longitudinal myocardial deformation by two-dimensional speckle tracking echocardiography (2D STE). The dashed curve depicts the average strain. The first positive peak of the curve is the peak atrial longitudinal strain (PALS), measured at the end of the reservoir phase. The second peak, just before the active atrial contraction, represents peak atrial contraction strain (PACS).

opening of the mitral valve, when the atrium fills and stretches until its peak in systole, positive atrial reservoir strain (peak atrial longitudinal strain, PALS) was measured. After the opening of the mitral valve, the atrium empties quickly and shortens, and the strain decreases up to a plateau, followed by a second positive peak, which corresponds to the atrial contractile phase (peak atrial contraction strain, PACS). Reference values for LA and RA strain parameters were used according to current recommendations (Padeletti et al., 2012; Nielsen et al., 2021).

Statistical Analysis

Data analysis was performed using Statistica 13.3 software (Statsoft Inc., Tulsa, Oklahoma, United States). The normal distribution of all continuous variables was examined using the Shapiro–Wilk test, and data were presented as mean ± standard deviation (SD). The comparison between the three stages was performed with repeated measures ANOVA analysis and the post-hoc Tukey test for normally distributed data. Non-normally distributed measurements were compared with Friedman ANOVA and post-hoc for Friedman ANOVA. Post-hoc analysis was performed for stages 1 and 2 and 1 and 3. In addition, the generalized eta-squared was provided as the recommended parameter when reporting effect sizes for ANOVA and Kendall's W for the parameters where Friedman's ANOVA test was applied (**Supplementary Material**). Correlations were measured using the Spearman and Pearson method, as appropriate for data distribution. A p-value < 0.05 was considered statistically significant.

TABLE 1 | Physical characteristics and intensity of training of the studied group.

Variable	Women (N = 27)
Age, years	40 ± 7
Weight, kg	59 ± 8
Height, cm	166 ± 5
BMI, kg/m^2	22 ± 3
BSA, m^2	1.65 ± 0.11
VO_2max, ml/kg/min	42.8 ± 5.1
Training intensity	
Hours of running/week	8.1 ± 3.5
Distance running/week, km	60.7 ± 27.8
Number of completed marathons	5.3 ± 4.0
Marathon performance time, min	253.0 ± 32.5

BMI, body mass index; BSA, body surface area; VO2max, maximal oxygen uptake.

RESULTS

Baseline Characteristics

All participants completed the marathon; however, due to poor imaging, both pre- and post-race RA measures were obtainable only in 16 runners. LA parameters were successfully assessed in the whole study group. Basic demographics and morphometric data of the marathoners are listed in **Table 1**. The mean age of 27 female amateur marathon runners was 40 ± 7 years. As a parameter of aerobic capacity, maximal oxygen uptake (VO_2max) was measured at stage 1, and it reached 42.8 ± 5.1 ml/kg/min. Systolic and diastolic blood pressure values were within the normal ranges, both at rest and after the run.

TABLE 2 | Biventricular measures assessed by three-dimensional echocardiography in female amateur athletes.

Parameter	Female (N = 27)			ANOVA *p*-Value	Post-Hoc *p*-Value	
	Stage 1	Stage 2	Stage 3		S1 vs. S2	S1 vs. S3
LVEDV index, mL/m^2	66.2 ± 7.9	60.1 ± 9.5	65.3 ± 7.5	<0.001	<0.001	0.278
LVESV index, mL/m^2	25.4 ± 4.3	22.4 ± 4.2	24.8 ± 3.6	<0.001	<0.001	0.480
LVSV index, mL/m^2	40.8 ± 5.8	36.9 ± 7.5	40.4 ± 5.7	<0.001	<0.001	0.396
LVEF, %	61.6 ± 4.5	62.8 ± 3.7	62.1 ± 3.7	0.059	-	-
RVEDV index, mL/m^2	48.8 ± 11.0	60.0 ± 11.1	51.5 ± 8.3	0.002	0.001	0.402
RVESV index, mL/m^2	22.2 ± 6.5	30.7 ± 7.0	23.8 ± 5.0	<0.001	<0.001	0.310
RVSV index, mL/m^2	26.6 ± 6.4	29.3 ± 6.1	27.8 ± 6.0	0.468	-	-
RVEF, %	54.9 ± 6.3	49.1 ± 6.3	54.3 ± 5.9	0.004	0.006	0.869
RVFAC, %	47.2 ± 5.9	42.6 ± 5.3	48.2 ± 2.9	0.009	0.037	0.897

LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVSV, left ventricular stroke volume; LVEF, left ventricular ejection fraction; RVEDV, right ventricular end-diastolic volume; RVESV, right ventricular end-systolic volume; RVSV, right ventricular stroke volume; RVEF, right ventricular ejection fraction; RVFAC, right ventricular fractional area change. Data are presented as mean ± standard deviation.

TABLE 3 | Left atrial parameters assessed by three-dimensional and two-dimensional speckle tracking echocardiography in female amateur athletes.

Parameter	Female (N = 27)			ANOVA *p*-Value	Post-Hoc *p*-Value	
	Stage 1	Stage 2	Stage 3		S1 vs. S2	S1 vs. S3
LAVmax index, mL/m^2	32.7 ± 6.8	31.2 ± 8.6	32.7 ± 7.0	0.314	-	-
LAVmin index, mL/m^2	14.0 ± 3.8	12.7 ± 4.0	13.7 ± 3.9	0.056	-	-
LAVpreA index, mL/m^2	21.4 ± 5.8	21.1 ± 6.5	20.9 ± 5.6	0.820	-	-
LA total EV index, mL/m^2	18.9 ± 4.2	18.8 ± 5.6	19.1 ± 3.8	0.893	-	-
LA passive EV index, mL/m^2	11.4 ± 3.0	10.4 ± 3.9	12.0 ± 3.0	0.086	-	-
LA active EV index, mL/m^2	7.5 ± 2.5	8.4 ± 3.4	7.0 ± 2.5	0.087	-	-
LA total EF, %	57.8 ± 5.5	59.8 ± 5.7	58.4 ± 5.2	0.116	-	-
LA passive EF, %	35.4 ± 8.1	33.2 ± 8.2	36.0 ± 8.7	0.158	-	-
LA active EF, %	34.3 ± 6.4	39.4 ± 8.6	34.0 ± 7.5	0.010	0.020	0.995
LA PALS, %	37.4 ± 3.2	38.2 ± 3.2	37.1 ± 3.2	0.150	-	-
LA PACS, %	12.8 ± 2.1	14.9 ± 2.7	12.6 ± 2.2	<0.001	0.002	0.935

LAVmax, left atrial maximal volume; LAVmin, left atrial minimal volume; LAVpreA, left atrial pre-A-wave volume; LAEV, left atrial emptying volume; LAEF, left atrial emptying fraction; LA PALS, peak left atrial longitudinal strain; LA PACS, peak left atrial contraction strain. Data are presented as mean ± standard deviation.

The female athletes completed 5.3 ± 4.0 marathons, with a current average training duration of 8.1 ± 3.5 h per week and mean running distance of 60.7 ± 27.8 km per week. The marathon performance time reached 253.0 ± 32.5 min.

In 24-h Holter monitoring, the underlying baseline rhythm was sinus with a minimal heart rate (HR) of 40.3 ± 4.4 bpm, maximal HR of 136.8 ± 18.6 bpm, and average HR of 64.0 ± 6.3 bpm. Both premature supraventricular complexes (SVPCs) and premature ventricular complexes (PVCs) were rarely observed within the recording time.

Echocardiographic Measurements

Table 2 summarizes exercise-induced 3D echocardiographic changes of RV and LV. After the marathon, there was a significant increase in the RV, with a decrease in LV dimensions. RV systolic contractility, assessed by RVEF and RVFAC, significantly decreased at stage 2, while LVEF did not differ remarkably post race. We did not notice any remarkable differences in the RV or LV parameters between stage 1 and stage 3.

A comparison of echocardiographic parameters of LA between stages is shown in **Table 3**. When analyzing the atrial size, there were no significant changes in 3D LA volumes between the stages, which, however, have tendency to decrease after the marathon run. Taking present EAPC/EACVI recommendations (Pelliccia et al., 2018) into consideration, LA enlargement was observed at baseline in 48% of female athletes, in 41% after the training, and in 44% of study participants at stage 3 (no differences between the stages). Functional measures of LA, such as LA total EF and LA passive EF, remained within the same range, while LA active EF increased post race (34.3 ± 6.4 vs. 39.4 ± 8.6%; $p = 0.020$). The outcomes were consistent with 2D STE values. Regarding LA reservoir function, there were no significant differences in LA PALS between stages, while the active phase of atrial contraction increased immediately after the marathon (LA PACS, 12.8 ± 2.1 vs. 14.9 ± 2.7%; $p = 0.002$) and returned to baseline during the recovery period.

Table 4 summarizes exercise-induced changes in RA echocardiographic parameters. There were no significant differences in any RA measures between stage 1 and stage 3. In contrast to LA morphological remodeling, female athletes showed a significantly higher RAVmax index (31.3 ± 6.8 vs. 35.0 ± 7.0 ml/m^2; $p = 0.008$), along with the RAVpreA index

TABLE 4 | Right atrial parameters assessed by three-dimensional and two-dimensional speckle tracking echocardiography in female amateur athletes.

Parameter	Female (N = 16)			ANOVA *p*-Value	Post-Hoc *p*-Value	
	Stage 1	Stage 2	Stage 3		S1 vs. S2	S1 vs. S3
RAVmax index, mL/m^2	31.3 ± 6.8	35.0 ± 7.0	31.1 ± 5.9	0.002	0.008	0.986
RAVmin index, mL/m^2	14.4 ± 4.0	16.4 ± 3.8	14.3 ± 3.4	0.039	0.081	0.983
RAVpreA index, mL/m^2	20.5 ± 5.7	25.3 ± 6.1	20.5 ± 4.8	<0.001	<0.001	0.999
RA total EV index, mL/m^2	16.9 ± 4.7	18.7 ± 4.9	16.8 ± 4.7	0.015	0.030	1.000
RA passive EV index, mL/m^2	10.5 ± 3.2	9.9 ± 2.8	10.5 ± 3.0	0.795	-	-
RA active EV index, mL/m^2	5.9 ± 2.8	9.0 ± 4.4	6.0 ± 2.5	<0.001	<0.001	0.996
RA total EF, %	53.9 ± 8.3	53.7 ± 7.7	53.5 ± 8.4	0.984	-	-
RA passive EF, %	33.7 ± 9.1	29.5 ± 7.0	33.0 ± 6.7	0.066	-	-
RA active EF, %	27.7 ± 8.6	35.0 ± 12.1	27.9 ± 7.8	0.007	0.014	0.994
RA PALS, %	37.6 ± 3.6	36.4 ± 3.6	37.6 ± 3.2	0.224	-	-
RA PACS, %	13.8 ± 1.8	15.6 ± 2.5	13.9 ± 1.6	0.010	0.016	0.972

RAVmax, right atrial maximal volume; RAVmin, right atrial minimal volume; RAVpreA, right atrial pre-A-wave volume; RAEV, right atrial emptying volume; RAEF, right atrial emptying fraction; RA PALS, peak right atrial longitudinal strain; RA PACS, peak right atrial contraction strain. Data are presented as mean ± standard deviation.

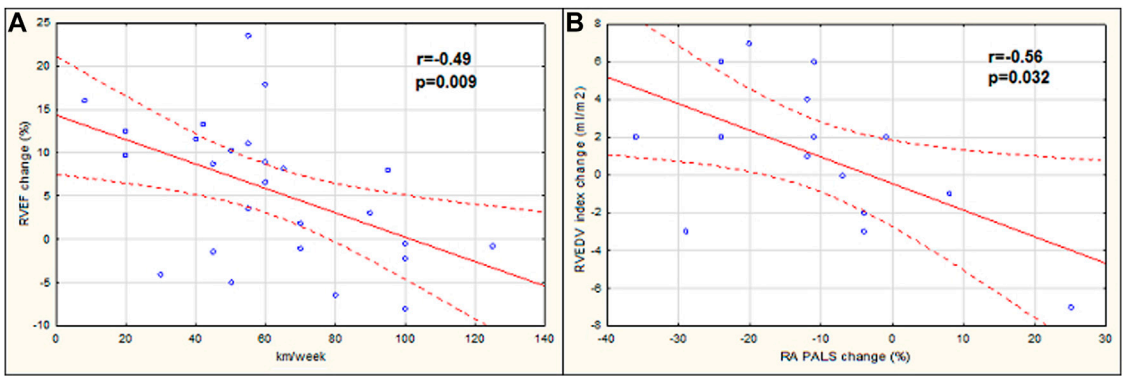

FIGURE 4 | Correlation between the distance of running per week with the right ventricular ejection fraction (RVEF) change **(A)**, the peak right atrial longitudinal strain (RA PALS) change with the right ventricular end-diastolic volume (RVEDV) index change **(B)**. RVEF change, the difference in RVEF stage 1 vs. stage 2; RA PALS change, the difference in RA PALS stage 1 vs. stage 2; RVEDV index change, the difference in RVEDV index stage 1 vs. stage 2.

(20.5 ± 5.7 vs. 25.3 ± 6.1 ml/m^2; $p < 0.001$), RA total EV index (16.9 ± 4.7 vs. 18.7 ± 4.9 ml/m^2; $p = 0.030$), and RA active EV index (5.9 ± 2.8 vs. 9.0 ± 4.4 ml/m^2; $p < 0.001$) after the marathon run. Sixty-nine percent of our athletes exceeded the upper limit of 3D RAVmax at rest, whereas the number of women with RA enlargement after the race equaled 94% ($p = 0.070$, stage 1 vs. stage 2). At stage 3, RA dimensions exceeded the upper limit in 75% of runners ($p = 0.694$, stage 1 vs. stage 3). RA contractile function, namely, RA active EF (27.7 ± 8.6 vs. 35.0 ± 12.1%; $p = 0.014$) increased post race, while RA reservoir and passive EF did not differ remarkably between stages. The aforementioned 3D RA differences were accompanied by a significant increment in RA PACS (13.8 ± 1.8 vs. 15.6 ± 2.5%; $p = 0.016$), as assessed by 2D STE.

Taking RA size into consideration, we divided the study participants into two groups according to the presence of RA dilatation at baseline. In athletes with RA volumes above upper limits at rest (N = 11), a significant post-exercise RV dilatation (RVEDV; 51.9 ± 9.5 vs. 63.2 ± 8.2 ml/m^2; $p = 0.012$) and reduction in function (RVEF; 59.3 ± 7.4 vs. 54.8 ± 5.7%; $p = 0.027$), with concomitant decrease in the LV dimensions (LVEDV; 69.9 ± 6.5 vs. 59.1 ± 9.2 ml/m^2; $p = 0.025$), was observed. Both RA and LA volumes did not differ remarkably between stages, while RA active EF increased after the marathon (38.7 ± 8.9 vs. 46.6 ± 10.4%; $p = 0.007$). In the group without atrial enlargement before the race, there were no significant post-exercise changes in any of the studied parameters.

When analyzing relations between the studied parameters, hours of training per week were positively related to the age of marathoners (r = 0.52, $p = 0.006$), while the distance of running per week inversely correlated with the exercise-induced change of RVEF (r = −0.49, $p = 0.009$) (**Figure 4A**). The post-race increase in RVEDV was negatively associated with RA PALS change (r = −0.56, $p = 0.032$) (**Figure 4B**).

DISCUSSION

The present study provides a comprehensive evaluation of exercise-induced biatrial remodeling in female amateur marathon runners, assessed by 3D echocardiography and 2D STE. We proved that endurance exercise alters echocardiographic

parameters of both atrial morphology and function in amateurs, similar to previously described alternations in elite athletes. Although numerous studies have already investigated the effect of marathon running on cardiac structure and function (Kaleta et al., 2017), most are restricted to male athletes or concern resting conditions, with limited data on acute remodeling in female counterparts (Pelliccia et al., 1996; D'Ascenzi et al., 2014).

Women, however, exhibit different cardiac adaptations to exercise than men (Di Paolo and Pelliccia, 2007). There are several biochemical, physiological, and psychological factors that determine sex-dependent cardiac response (Colombo and Finocchiaro, 2018). Women, on average smaller, have lower mean body mass, different autonomic tone, and hormonal profile than men. While taking the hormonal system into consideration, testosterone in men directly stimulates myocardial hypertrophy, while estrogens in women inhibit this process with an opposed effect on cardiac remodeling. According to recent reports, female endurance athletes adapt primarily by increasing ventricular dimensions rather than wall thickness (Pelliccia et al., 1996). It has been proved that the right chambers of the heart are particularly altered. Indeed, female athletes show increased RV cavity size, along with RA dimensions, in comparison to non-athlete participants in baseline conditions (Pelliccia and Adami, 2017). In individuals without cardiovascular disease, RVEF is higher in women than men, which may allow sportswomen to better tolerate exercise-induced RV dilatation. Finally, female athletes are less prone to sudden cardiac death (SCD) mainly because of lower sympathetic activation during endurance training and hormonal protection (Rajan et al., 2021).

To the best of our knowledge, our study is the first one among Polish female amateur athletes that evaluates the acute effect of marathon running on biatrial performance by using 3D echocardiography and 2D STE. In our relatively small study group, we noticed a significant increase in RA size, with no relevant decrease in LA dimensions after the race. Indeed, acute dilatation of RA and RV, but not of LA and LV, has been demonstrated in amateur runners immediately after the marathon race (Trivax et al., 2010; Lewicka-Potocka et al., 2022). In our recent research, which included female marathon runners (Lasocka et al., 2021), we observed a significantly greater number of female athletes meeting the ECG criteria for RA enlargement after the run compared to baseline, with no difference in the incidence of LA enlargement between the stages. Wilhelm et al. (2012a, 2012b) examined the change in proatrial natriuretic peptides before and after the marathon. A remarkable increase of the cardiac biomarker postrace was observed, both in professional and amateur runners, which might reflect higher propensity of exercise-induced atrial dilatation in the study groups.

Along with morphological changes, the atrial functional parameters also evolve with increasing effort in athletes. The application of atrial phasic volumes and strain analysis enables better characterization of myocardial deformation of the atria, distinguishing pathological dysfunction from physiological atrial adaptation to exercise. Most of the studies compare atrial performance and sedentary controls in athletes under resting conditions. According to Lakatos et al. (2020), elite sportsmen had lower reservoir and contractile function, as assessed by 3D LA total EF and LA active EF, while LA passive EF did not differ between the groups. Similar outcomes were described in another study on 3D volumetric changes (Nemes et al., 2017). When taking 2D STE into consideration, a recent analysis revealed that reservoir and contraction strains of either RA or LA were decreased in athletes at rest than in non-sportive subjects (Gabrielli et al., 2014). These findings are consistent with those of Sanchis et al. (2017), who found lower biatrial deformation values in athletes, with significantly higher RA and LA contractile function in women than men at rest.

However, data are scarce regarding atrial functional measures during or directly after endurance training. In our study, female amateur runners showed a remarkable increase in LA active EF and PACS post-race, while LA reservoir and conduit function remained unchanged. In RA, prolonged exercise induced a significant increment in atrial contractile function, as assessed by 3D and 2D STE parameters. When it comes to volumetric data, Wright et al. (2015) showed that both LA passive and reservoir EV did not change during moderate exercise, while LA pump function significantly increased. Similar outcomes were obtained by Cavigli et al. (2022), who described increased LA PACS directly after an ultra-marathon run, without changes in reservoir or conduit function using strain analysis. These observations, based also on female athletes, agree with our findings and could be explained by LA contribution to maintain LV filling according to the Frank–Starling mechanism. Although other authors confirmed the post-exercise increment in LA PACS (Oxborough et al., 2010; Gabrielli et al., 2014), all of these studies are based on the male population. The extent of atrial adaptation to exercise changes during the training period Sanz-de la Garza et al. (2016) showed an increase in atrial reservoir and contractile function in distances up to 35 km, with a further decrease post 56 km. Only a small group of post 56 km runners proved to increase atrial contractile function. That demonstrates different atrial responses to the training stimulus among athletes.

Atrial remodeling might be a physiologic adaptation to volume overload, permitting a greater volume delivery and increased cardiac output. The rise of preload and afterload during exercise seems to particularly involve the RV in terms of chamber dilatation and lower deformation, without impairment in LV function (La Gerche et al., 2012), which can be a result of increased pulmonary pressure during the race. Indeed, we noticed a significant increase in RV dimensions and concomitant reduction in RV function among study participants after the marathon. In the recent analysis of male amateur athletes, Lewicka-Potocka et al. (2022) also observed a transiently enlarged RV with reduced contractility, as assessed by a decrease in RV radial shortening. The reduction in RV systolic function correlated with the intensity of the preparation period, in the form of a "U-shaped curve." Both athletes with very minimal training and those with more than 47 km running per week presented a greater decrease in RV contractility post race. On the contrary,

in our study, the greater training volume per week was associated with lower RV dysfunction, especially among participants with more than 60 km running per week. These findings are supported by Neilan et al. (2006). In the research on 60 non-elite marathon runners, both men and women, the training mileage of the study participants was inversely related to RV contractility, pulmonary pressures, and level of cardiac biomarkers. Compared with athletes training above 45 miles per week, those who run less than 35 miles per week demonstrated increased pulmonary pressures, greater RV dysfunction, and increased cardiac troponin T level and N-terminal pro-brain natriuretic peptide. As reported, the extent of the preparation period determines cardiac response to endurance training, such as a marathon run, and should be individually counted.

Secondary to RV changes, the impairment of RA may also be observed. When ventricular afterload increases, atrial reservoir function is primarily affected. In contrast, atrial contractile function is maintained or even increased. Although in our research, RA reservoir function did not differ significantly between stages, in athletes with a greater post-race increase in RVEDV, a lower increment in RA PALS was observed. As all of the abnormalities were transient and not reported in the control examination, they should be classified as physiological aspects of the "athlete's heart" rather than pathological alternations.

However, repetitive stretching of the atria may predispose to chronic structural changes in response to the recurrent volume overload and excessive cardiac strain (Pluim et al., 2000; Maron and Pelliccia, 2006). Król et al. (2016) examined 114 international-level rowers and described LA enlargement in nearly half of the enrolled athletes (43%), with higher frequency in men than in women (52.5% vs. 32.1%; $p < 0.05$), while Wilhelm et al. (2012) demonstrated an independent effect of repeated marathon running on biatrial size, with larger RA and LA volumes in endurance athletes compared to non-marathon runners. In subjects who participated in six or more marathons, right and left atrial enlargements were present in 60% and 74% of athletes, respectively. In our study, at baseline, 48% of the study participants exceeded the upper limit of LA volume, while RA enlargement was present in 69% of women. Moreover, after dividing the study participants according to the initial presence of RA dilatation, we noticed a significant post-exercise RV dilatation and reduction in systolic function, along with decrease in the LV dimensions in those with RA size above the upper limits at rest. Therefore, the baseline increase in atrial volumes may indicate a higher prevalence of exercise-induced changes, which, over a long time period, may result in persistent cardiac remodeling.

Atria working at higher wall stress are more susceptible to the development of atrial fibrosis, along with incidental atrial arrhythmia (Gabrielli et al., 2014). Recent reports have shown a relationship between endurance training and AF (Abdulla and Nielsen, 2009; Aizer et al., 2009; Mont et al., 2009). According to the latest analysis, reduced strain values of LA, especially reservoir strain, are strong identifiers of AF (Sørensen et al., 2021). It has been proven that regardless of athletic status, both reservoir and contractile strains are lower in those with AF. The possible mechanisms explaining the association remain speculative. Atrial remodeling, vagal tone, and atrial ectopic triggers are found to contribute to increased incidence of AF in non-elite endurance athletes (Wilhelm et al., 2011). Although our post-race cardiac monitoring did not reveal any increase in any form of supraventricular arrhythmia, this observation cannot correlate to possible arrhythmias that occurred during the race itself. RA dilatation, which was high both at rest and after the run in our study group, could be the substrate for arrhythmias (Mont et al., 2009), especially in the long-term follow-up. Further research is needed to fully understand the mechanism underlying AF development in sportsmen.

This study is the first one that assessed and compared atrial remodeling at rest and after a marathon run in female amateur athletes by using 3D echocardiography. Compared with 2D assessment, 3D echocardiography provides more accurate measurements of atria and has superior prognostic ability (Artang et al., 2009; Caselli et al., 2010). Moreover, 3D echocardiography offers an additional capability to predict cardiovascular events (Badano et al., 2016). The availability of 3D reference values will help clinicians identify exercise-induced atrial remodeling and differentiate it from potential atrial dysfunction.

Several limitations of our study should be noticed. First, the study was carried out in a relatively small sample and involved only white female amateur athletes living in the Pomeranian Voivodeship, Poland, limiting its statistical power and generalizability. Moreover, the number of female participants with adequate RA images both at rest and after the run was low because during the marathon in 2018, major attention was given to ventricular function. Second, it lacks a control group of non-amateur marathon runners matched by sex, age, height, and weight, or male amateur runners to detect gender differences in atrial remodeling in resting conditions. In addition, the study focuses on the comparison of cardiac changes before and directly after endurance training, without any data obtained during the exercise itself. Therefore, the observed atrial remodeling may not fully extrapolate to the acute exertion phase. Finally, there is a lack of long-time follow-up, which makes it impossible to confirm whether the observed atrial performance predisposes to a higher prevalence of AF among study participants in the future.

CONCLUSION

Long-term physical training promotes biatrial remodeling in female non-elite athletes, with more pronounced changes in the RA. After the marathon, a significant dilatation of both RV and RA and a concomitant decrease in RV contractility were observed. These changes were especially present in women with RA enlargement in baseline conditions. When analyzing left heart chambers, LV dimensions decreased post race, and no relevant reduction in LA size was observed, without any functional impairment. Post race, the active contraction phase

of both atria increased. However, considering atrial reservoir function, greater RV dilatation was observed post race and lower increase in RA PALS was detected. Although the observed changes were proved reversible, the long-term consequences of repeated strenuous training remain to be clarified. A better understanding of atrial adaptation to a marathon run among amateurs is clinically relevant for clarifying the risk of exercise-induced arrhythmias. Moreover, our study suggests that appropriate preparation before a marathon is important to protect against greater RV dysfunction.

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 author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Bioethics Committee of the Medical University of Gdansk, Poland. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization, ZL and AD-K; formal analysis, ZL and AD-K; methodology, ZL, AD-K, and EL; validation, ZL, AD-K, AK-D, ZL-P, AF, LD-S, RN, DK, LK, and EL; investigation, ZL, AD-K, ZL-P, AF, LD-S, RN, DK, LK, EL, and GR; data curation, ZL, AD-K, ZL-P, AF, LD-S, RN, DK, and EL; statistical analysis, ZL and AD-K; writing—original draft preparation, ZL; writing—review and editing, AD-K and EL; visualization, ZL; supervision, AD-K, EL, and GR; project administration, AD-K and GR; funding acquisition, AD-K, EL, and GR. All authors have read and agreed to the published version of the manuscript.

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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.2022.863217/full#supplementary-material

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