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# Sex-related differences and effects of short and long trail running races on resting muscle-tendon mechanical properties

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The purpose of the study was to assess sex-related differences in resting mechanical properties and adaptations of skeletal muscles and tendons in response to trail running races of different distances using multi-site shear wave elastography assessments of the lower limb, force capacity and blood analyses. Sex differences in resting mechanical properties of knee extensor and plantar flexor muscles and tendons were characterized by shear wave velocity (SWV) measurements in healthy males ( $N = 42$ ) and females ( $N = 25$ ) trained in long-distance running. Effects of running distance on muscle and tendon properties were assessed in short ( $<60$  km,  $N = 23$ ) vs. long ( $>100$  km,  $N = 26$ ) distance races. Changes in isometric maximal voluntary contraction torque, serum C-reactive protein and creatine kinase activity were also quantified after running races. Higher SWV of relaxed *triceps surae* muscle was detected in females as compared to males before running races ( $+4.8\%$ ,  $p = 0.006$ ), but the significant increases in *triceps surae* muscle group ( $+7.0\%$ ,  $p = 0.001$ ) and patellar tendon SWV ( $+15.4\%$ ,  $p = 0.001$ ) after short-distance races were independent of sex. A significant decrease in *triceps surae* muscle SWV was found after long-distance races in the whole experimental population ( $-3.1\%$ ,  $p = 0.049$ ). Post-races increase in C-reactive protein and creatine kinase activity were significantly correlated to the relative decreases in *triceps surae* and *quadriceps femoris* skeletal muscle SWV ( $\rho = -0.56$ ,  $p = 0.001$  and  $\rho = -0.51$ ,  $p = 0.001$ , respectively). Resting mechanical properties of muscles and tendons are affected by sex, and adaptations to trail races are related to running distance. Exercise-induced changes in resting skeletal muscle mechanical properties are associated with enhanced indirect markers of inflammation and muscle damage.

## KEYWORDS

muscle damage, shear wave elastography, tendon, ultra-trail

## 1 | INTRODUCTION

The risk of skeletal muscle lesions has been reported to be higher in men as compared to women in international athletes.<sup>1</sup> A similar sex-related difference has been found for exercise-induced muscle damage (EIMD) in animals<sup>2</sup> due to a potentially strengthened microarchitecture of muscle fibers in females compared with males. However, the results obtained in human studies remain conflicting.<sup>3,4</sup> Although the growing interest in the passive/resting mechanical properties<sup>5</sup> to assess soft tissue damage,<sup>6</sup> sex-related differences have been poorly investigated and need to be further assessed in populations exposed to muscle damage such as athletes.<sup>7</sup> Sex-related differences in resting mechanical properties of tendon and skeletal muscles have been assessed in several muscle groups or tendons and results reported either no difference<sup>8</sup> or a higher shear wave modulus/velocity (i.e., index of Young's modulus assessed from shear wave elastography [SWE]) in males<sup>9,10</sup> or in females.<sup>11</sup> In addition, no subsequent EIMD was induced after the sex-related difference assessment. Yet, resting mechanical characteristics could be a potential indirect assessment of EIMD exposure considering a recent study that reported a negative correlation between a high resting shear wave modulus of the *rectus femoris* muscle before a damaging exercise and the delayed (i.e., 48 h post-exercise) relative maximal voluntary knee extension force<sup>12</sup> (i.e., maximal voluntary contraction [MVC] force loss being considered as the most reliable indirect marker of muscle damage<sup>13</sup>).

The effects of EIMD (i.e., soreness, inflammation, diminished joint range of motion and MVC force loss<sup>14</sup>) on resting skeletal muscle mechanical properties have been explored.<sup>6,15</sup> A higher increase in shear wave modulus after EIMD was found at a stretched position in comparison to a non-stretched one<sup>6</sup> suggesting heterogeneous adaptations of resting muscle mechanical properties over the joint range of motion. In addition, the transient increase in shear wave modulus 30 min after a damaging exercise was found to be correlated with the depressed MVC force assessed 2 days post-exercise.<sup>16</sup> Nevertheless, the duration and intensity of exercise led to a discrepancy in the modulation of resting muscle mechanical properties. Exercises inducing muscle fatigue were found to generate a decrease in resting muscle shear wave modulus/velocity<sup>17</sup> whereas exercises expected to induce muscle damage led to an increase in resting muscle mechanical properties.<sup>18,19</sup> However, a very long and intense exercise inducing muscle damage to generate a decrease in shear wave modulus.<sup>20</sup> The reasons for such differences remain unclear, but it has been suggested that changes in muscle shear modulus might reflect adaptative mechanisms that could prevent or limit fatigue and alterations in muscle

tissue during an ultra-marathon.<sup>21</sup> So far, sex differences in the extent of exercise-induced muscle damage and the ability to rest muscle-tendon mechanical properties to adapt after long-distance running remain unknown. Considering that the inflammatory response is attenuated in females in comparison to males following an acute eccentric exercise,<sup>3</sup> it could be expected less secondary damage exposure<sup>22</sup> for females.

The aims of the present study were to *i*) assess the initial sex-related differences in resting mechanical properties of skeletal muscles and tendons using shear wave elastography and *ii*) assess the changes in muscle and tendon resting mechanical properties and indirect markers of inflammation and muscle tissue damage after both short (i.e., <60 km) and long (i.e., >100 km) trail running races. Lower resting skeletal muscle and tendon shear wave velocity (SWV) in females compared to males leading to potential lower exposure to muscle damage was hypothesized. An increase in resting skeletal muscle and tendon shear wave velocity was also expected after shorter running races whereas a decrease was assumed after longer running races.

## 2 | MATERIALS AND METHODS

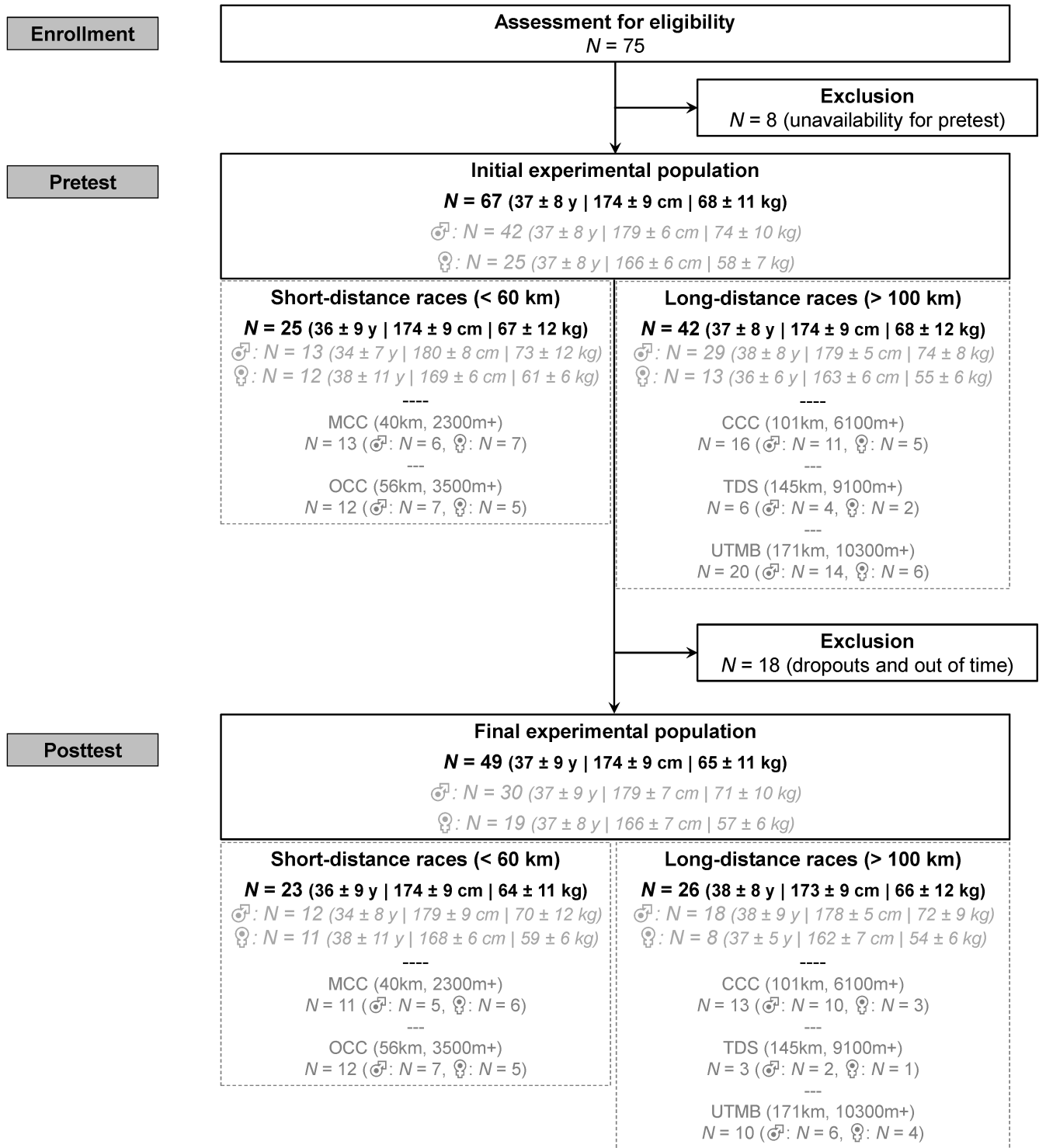
### 2.1 | Subjects

A pilot study to determine the inter-day reproducibility of the SWV measurements was performed by the same experimenter (A.F.) in 12 healthy participants (mean  $\pm$  SD; 6 males/6 females: 30  $\pm$  6 years, 171  $\pm$  9 cm, 68  $\pm$  12 kg). Two repeated testing sessions, separated by a 2-d resting period, were performed at the same time of day.

The characteristics of the population and withdrawn data are displayed in [Figure 1](#). All 75 participants were recruited between July and September 2019. Participants had no known musculoskeletal, articular, or cardiovascular abnormalities. Exclusion criteria included injury in the past 3 months, the presence of chronic or central neurological diseases, use of neuroactive substances or corticosteroid medications and pregnancy.

Our prospective study was conducted in conformity with the last version of the Declaration of Helsinki.<sup>23</sup> All participants were fully informed about the nature and the aim of the study, and gave their informed written consent to participate. Approval for the project was obtained from the French Ethical Research committee (CPP Ouest VI, ethics committee agreement #19.03.14.41740). Our study was registered on [clinicaltrials.gov](https://clinicaltrials.gov) (NCT04025138).

The number of participants was chosen based on the findings of a previous study showing that a third of data was withdrawn mainly due to participant drop-out during the trail running race.<sup>21</sup> From the 49 remaining



**FIGURE 1** Inclusion table with subjects withdrawn quantification. Subjects performed either a race that was less 60 km (short: *de Marigny-Combe à Chamonix* [MCC] and *Orsières-Champex-Chamonix* [OCC]) or longer than 100 km (long: *Courmayeur-Champex-Chamonix* [CCC], *sur les Traces des Ducs de Savoie* [TDS] and the *Ultra-Trail du Mont-Blanc* [UTMB]). D+: positive altitude change

participants, variability of SWV measurements assessed from reproducibility results and a statistical power calculation ( $\alpha = 0.05$  and  $1-\beta = 0.8$ ), a change lower than 5% in the resting *triceps surae* skeletal muscle SWV after the mountain ultra-marathon can be robustly assessed.

## 2.2 | Experimental design

Each participant performed three sessions. The first session consisted of the inclusion procedures, anthropometric measurements and familiarization with MVC a few

weeks before the race. The second session was performed within 2–4 days before the mountain ultra-marathon. The third session occurred within the hour following the end of the race. During the last two sessions, blood samples were taken and muscle soreness, MVC torque and shear wave velocity of muscle and tendon tissues were assessed as described below. Runners participated either in a race that was less than 60 km (hereafter referred to as short-distance races) or a race that was longer than 100 km (hereafter referred to as long-distance races) (Figure 1).

### 2.3 | Maximal voluntary contraction torque and muscle soreness assessments

The testing procedures for MVC torque measurements and visual analog scale (VAS) score were similar to previous studies.<sup>21,24–26</sup>

Muscle soreness was assessed based on pain perception in the thigh and lower leg muscles, quantified using a VAS with a 100 mm horizontal line with “no pain” on one end (0 mm) and “extremely painful” on the other (100 mm). The question was asked at rest as the actual subjective feeling of the athlete during the interview with the physician.

For the MVC measurements, participants were seated on an isometric knee dynamometer (ARS dynamometer, S2P, Ljubljana, Slovenia) with their knees flexed at 90°. They performed a 5-min warm-up, which included a set of 10 sub-maximal knee extensions under isometric conditions (i.e., 2 s on/2 s off with increased perceived intensity from 10% to 90% of the maximum at the 10th contraction). MVC torque was quantified as the maximal value achieved across the three trials separated by a resting period of at least 30 s. A similar procedure was used to assess plantarflexion MVC torque with ankle angles fixed at 90° on a foot platform (tibia perpendicular to the foot-knee angle: 90°). Signals were analog-to-digitally converted at a sampling rate of 2 kHz by a *Powerlab* system (16/30-ML880/P, AD Instruments) and analyzed offline using *Labchart* software (v8, AD Instruments).

### 2.4 | Blood markers

An automated analyzer (Cobas C11, Roche Diagnostics) was used for the measurement of serum C-reactive protein levels (CRP, turbidimetry method) and serum creatine kinase activity (CK, photometric method). Plasma interleukin-6 level was measured by Bio-Plex Multiplex immunoassay (IL-6, Bio-rad), using the Bio-Plex Pro Human Cytokine 17-plex Assay kit and the Bio-Plex 3D platform (Bio-rad), according to the manufacturer's instructions. However, blood samples for IL-6 measurements were

only collected in 17 participants participating in the short-distance races ( $N = 9$ ) and long-distance races ( $N = 8$ ).<sup>27,28</sup>

### 2.5 | Shear wave elastography

Ultrasound explorations were performed with muscles in a relaxed state with the probe oriented parallel to the muscle fibers. An ultrasound device Aixplorer (v.2.1.0.3395, SuperSonic Imagine) coupled with a linear transducer array (5–18 MHz, SuperLinear 18–5, Vermon) was used in shear wave elastography mode. The SWE parameters were persistence medium, opacity 75%, optimization penetration and scales 7 and 16 m/s for muscle and tendon, respectively. All ultrasound acquisitions and treatments were performed by the same experimenter (A.F., with 8 years of experience in skeletal muscle and tendon ultrasound explorations). The time for SWE exploration was approximately 25 min.

Assessments of the *quadriceps femoris* (QF) muscle and the patellar tendon were conducted with participants lying supine on a customized massage table at two knee angles (180° [knee fully extended] and 90°). A horizontal plane was drawn on the thigh of the participant, corresponding to 40% of the length between the upper border of the patella and the anterosuperior iliac spine with the knee fully extended and in a relaxed state. Acquisitions were performed for two zones (dist [the most distal part of the muscle observed with B-mode ultrasonic imaging] and mid [measurement performed on the drawn horizontal plane corresponding to muscle belly]) for the lateral compartment of *rectus femoris* (RF), *vastus lateralis* (VL) and *vastus medialis* (VM). Two acquisitions were also performed for the *vastus intermedius* (VI) on the drawn horizontal plane (i.e., 40% of the length between the upper border of the patella and the anterosuperior iliac spine) below the RF and VL muscles bellies. A mid-portion patellar tendon exploration was also recorded.

Thereafter, measures of the *triceps surae* (TS) muscle and Achilles tendon were performed with participants lying in a prone position using three different ankle angles (120° [plantar flexion], 90° [tibia perpendicular to the foot] and 75° [dorsi flexion]). As for the thigh muscles, a horizontal plane was drawn on the lower leg of the participant, corresponding to 70% of the length between the calcaneus and the popliteal curve with the knee fully extended and relaxed. Acquisitions were performed for two zones (dist and mid) for the *gastrocnemius lateralis* (GL) and the *gastrocnemius medialis* (GM). Two zones were also explored for the *soleus* (SO) (dist and mid [muscle portion beneath the GM myotendinous junction]). Four zones were explored for the Achilles tendon (dist [near the insertion on the *calcaneus*], free [portion between the



*calcaneus* and the myotendinous junction with the SO], SOdist [portion above the distal part of the SO] and MTJ [at the myotendinous junction of the tendon with the GM]). Standard acoustic coupling gel was used for ultrasound explorations and the experimenter carefully exerted the less pressure as possible with the ultrasound probe on the skin. Participant positions and exploration zones were displayed in Figure 2. A single image of the SWV maps with a spatial resolution of  $1 \times 1$  mm was recorded for each exploration zones. Although the location of the probe between visits was the same for each participant, the position and the size of the SWE map were adapted for each exploration on the basis of the B-mode ultrasound image to avoid subcutaneous fat, skin, bone and fascias. Caution was taken to avoid including unfilled and saturated areas in each acquired SWE map.

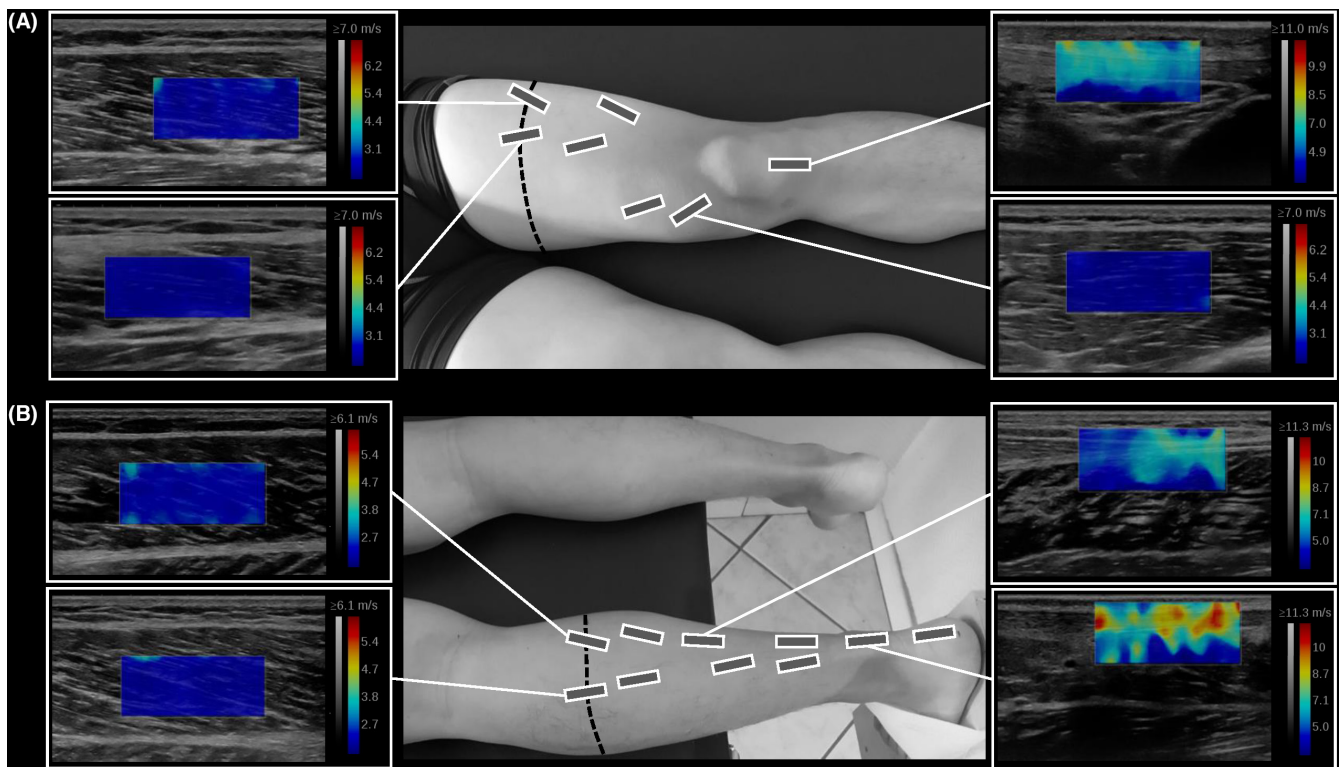
The analysis of shear wave elastography maps was performed using the Aixplorer device software (Qbox tool). Over-saturated or unfilled parts of the SWE maps were not included in the region of interest (ROI) for analysis. A single largest possible circular ROI was positioned in the center of the muscle map avoiding the borders of the SWE map and without any saturation and unfilled zones within the ROI. For tendon, a single rectangular ROI area was manually drawn from the B-mode image observation and

with the same caution about saturation and unfilled zones within the ROI. For the effects of trail running races, SWV values were averaged over the different exploration zones within each muscle or tendon.

The limb circumference was assessed using a tape measure at the levels of the drawn horizontal planes.

## 2.6 | Statistics

For the reproducibility measurements, coefficient of variation (CV) and standard error of measurement (SEM) were quantified. The normality of the data distribution was initially investigated using Shapiro–Wilk test. In the case of the non-normal distribution of the data, non-parametric tests were performed for the statistical analysis (i.e., Friedman test for the repeated measurements and Kruskal–Wallis for the main effects). Concerning SWE analyses, a three-way ANOVAs (with sex [males/females], exploration zone [dist/mid and dist/free/SOdist/MTJ for the skeletal muscles and the Achilles tendon, respectively] and joint angle [ $120^\circ/90^\circ/75^\circ$  and  $180^\circ/90^\circ$  for ankle and knee, respectively] factors) were used. Statistical analyses were performed with Statistica software (Statsoft) to assess



**FIGURE 2** Positions of the participants during the shear wave velocity assessment and related maps from elastography exploration superimposed on B-mode gray images for several exploration zones. (A) For knee extensor muscles and patellar tendon explorations. (B) For plantar flexor muscles and Achilles tendon assessments

sex differences and intramuscular variability in resting SWV across ankle and knee joint range of motion. Three-way ANOVAs with repeated measurements (race distance [short/long] factors in addition to sex and joint angle) were used to assess SWV changes in muscle and tendon tissues after trail running races (i.e., time factor [PRE/POST]). Limb circumference was assessed with a two-way ANOVA with repeated measurements (race distance and sex). Mauchly's sphericity test was performed and Greenhouse–Geisser correction was applied when necessary. A Tukey's HSD post-hoc analysis was used when appropriate. Correlations between parameters were assessed using Spearman rank correlation ( $\rho$ ) coefficients. The level of significance was set to  $p < 0.05$ . Results are reported as mean  $\pm$  SD.

### 3 | RESULTS

#### 3.1 | Reproducibility

High reproducibility of averaged SWV measurements was found (Table 1) considering the low coefficients of variation and standard errors of measurements in the SWV of

skeletal muscles (i.e., 4.5% and  $0.13 \text{ m}\cdot\text{s}^{-1}$ , respectively) and tendons (i.e., 7.1% and  $0.36 \text{ m}\cdot\text{s}^{-1}$ , respectively). A Table S1 reports the reproducibility results for each specific measurement (i.e., joint angles and exploration zones).

#### 3.2 | Sex differences and intramuscular variability

Thigh and lower leg circumferences were higher in males compared to females (thigh:  $53.6 \pm 4.3 \text{ cm}$  vs.  $51.5 \pm 2.7 \text{ cm}$ ,  $p = 0.003$ ; lower leg:  $37.6 \pm 2.6 \text{ cm}$  vs.  $35.3 \pm 1.6 \text{ cm}$ ,  $p = 0.009$ ).

A main effect for angle (i.e., increased SWV with lower values of knee flexion and dorsiflexion) was found in the SWV of each muscle and tendon ( $p = 0.001$ ). In contrast, a sex effect was only found in muscles ( $p = 0.001$ , Tables 2 and 3), except for the whole QF ( $p = 0.360$ , Table 3). A significant difference was found between males and females in muscles in the most stretched position (+10.8% for females in the GL,  $p = 0.001$ , +9.7% for females in the GL,  $p = 0.001$ , +5.4% for females in the SO,  $p = 0.001$  and +4.1% for males in the RF,  $p = 0.001$ , Tables 2 and 3, Figures S1 and S2).

Structure	Test #1 ( $\text{m}\cdot\text{s}^{-1}$ )	Test #2 ( $\text{m}\cdot\text{s}^{-1}$ )	CV (%)	SEM ( $\text{m}\cdot\text{s}^{-1}$ )
Achilles tendon	$7.33 \pm 0.40$ (7.10–7.55)	$7.29 \pm 0.51$ (7.00–7.59)	7.3	0.34
<i>Triceps surae</i>	$3.20 \pm 0.26$ (3.05–3.35)	$3.20 \pm 0.30$ (3.03–3.37)	4.5	0.14
<i>Gastrocnemius Lateralis</i>	$3.12 \pm 0.28$ (2.96–3.28)	$3.09 \pm 0.37$ (2.87–3.30)	6.5	0.20
<i>Gastrocnemius Medialis</i>	$3.63 \pm 0.38$ (3.41–3.85)	$3.64 \pm 0.36$ (3.43–3.85)	2.9	0.09
<i>Soleus</i>	$2.85 \pm 0.28$ (2.69–3.01)	$2.88 \pm 0.33$ (2.69–3.07)	6.7	0.17
Patellar tendon	$6.40 \pm 0.95$ (5.85–6.95)	$6.28 \pm 0.85$ (5.79–6.77)	6.9	0.38
<i>Quadriceps femoris</i>	$2.28 \pm 0.12$ (2.21–2.34)	$2.25 \pm 0.12$ (2.18–2.32)	2.0	0.06
<i>Rectus Femoris</i>	$2.49 \pm 0.19$ (2.38–2.61)	$2.45 \pm 0.24$ (2.31–2.59)	2.0	0.12
<i>Vastus Intermedius</i>	$2.43 \pm 0.20$ (2.31–2.54)	$2.52 \pm 0.21$ (2.39–2.64)	3.7	0.10
<i>Vastus Lateralis</i>	$2.08 \pm 0.14$ (2.00–2.16)	$2.02 \pm 0.15$ (1.93–2.10)	5.0	0.12
<i>Vastus Medialis</i>	$2.11 \pm 0.17$ (2.01–2.20)	$2.03 \pm 0.13$ (1.95–2.10)	4.4	0.08

TABLE 1 Reproducibility of shear wave velocity in Achilles tendon, *triceps surae* muscles, patellar tendon and *quadriceps femoris* muscles

Note: Data are presented as mean  $\pm$  SD (95% CI).

Data are averaged over the different measurements (i.e., exploration zones within muscle/tendon and joint angles). For detailed data, see Table S1.

Abbreviations: CV, coefficient of variation; SEM, standard error of measurement.

A significant interaction effect for the exploration zone related to joint angle was found in the SWV of the Achilles tendon ( $p = 0.001$ , Table 1, Figure S1), the VL ( $p = 0.001$ ) and the VM ( $p = 0.001$ ) (Table 3, Figure S2). In addition, a sex difference was observed for the VL with a higher SWV in the belly of females compared with males (+20.3%,  $p = 0.001$ , Table 3).

A significant interaction effect between sex, angle and exploration zone was observed in the RF ( $p = 0.023$ ) with an SWV difference between the distal part and muscle belly for males in the knee flexed position (+9.5% in muscle belly,  $p = 0.033$ , Figure 2, Table 3) and between the muscle belly in the knee flexed position across sex (+1.4% for males,  $p = 0.033$ , Figure S2).

### 3.3 | Effects of mountain ultra-marathon

Maximal voluntary contraction torque decrease was independent of sex and race distance (Table 4). The decrease in knee extension MVC torque was observed for both short (−24%,  $p = 0.001$ ) and long (−36%,  $p = 0.001$ ) distance races. Plantar flexion MVC torque similarly decreased in both males (−30%,  $p = 0.001$ ) and females (−27%,  $p = 0.001$ ).

A significant time effect was found for soreness (i.e., VAS score) in the thigh and lower leg muscles ( $p = 0.001$ ) that was unrelated to sex and race distance (Table 4).

A significant time effect was observed for thigh (−0.8 ± 1.9%,  $p = 0.001$ ) and lower leg (−1.2 ± 2.5%,  $p = 0.001$ ) circumference independent of sex and race distance (Table 4).

Both short and long-distance races led to increased blood markers of inflammation and damage (Table 4). However, the long-distance races caused higher inflammation and muscle damage than the short-distance races. CRP increased significantly in both males and females after the longer races ( $p = 0.001$ ), but the increase was higher in females ( $p = 0.001$ , Table 2). The longer races resulted in significant increases in IL-6 concentrations ( $p = 0.002$ ) and CK activity ( $p = 0.001$ ), independently of sex (Table 4).

A significant increase in the SWV of Achilles tendon ( $p = 0.035$ ), patellar tendon ( $p = 0.008$ ) and VM muscle ( $p = 0.022$ ) was found after trail running races independently of distance, sex and joint angle. SWV increased in the whole TS muscle after the shorter races independently of sex and joint angle (Table 4 and Figure 3). A significant increase in muscle SWV after short-distance races was noted for the GL (+5.1%,  $p = 0.018$ ), the GM (+5.2%,  $p = 0.050$ ), the SO (+11.6%,  $p = 0.005$ ), the TS (+7.0%,  $p = 0.001$ , Table 4) and the patellar tendon (+15.4%,  $p = 0.001$ , Figure S3), independently of sex and

joint angle. A specific decrease in dorsiflexed GL SWV was noted for females involved in the long-distance races (−12.4%,  $p = 0.036$ , Figure 3).

### 3.4 | Correlations

The relative decrease in skeletal muscle SWV (i.e., TS and QF muscles independently of sex and joint angle) was correlated with the relative increases in CRP ( $\rho = -0.56$ ,  $p = 0.001$ , Figure 4A), IL-6 ( $\rho = -0.54$ ,  $p = 0.025$ ,  $N = 17$ , Figure 4B) and CK activity ( $\rho = -0.51$ ,  $p = 0.001$ , Figure 4C).

No correlation was found between the relative decrease in MVC torque and SWV of the related muscles, independently of sex and joint angle ( $p = 0.208$  for plantar flexors and  $p = 0.588$  for knee extensors, Figure S4A,B). Additional significant correlations were found between the relative increases in SWV of the *gastrocnemii* muscles and the patellar tendon, independently of sex and joint angle, as well as between the relative decrease in patellar tendon SWV (i.e., independently of sex and joint angle) and the increased CK activity (Figure S4C,D).

## 4 | DISCUSSION

The present study aimed to assess sex-related differences in resting mechanical properties and adaptations of skeletal muscles and tendons in response to trail running races of different distances. In contrast to the initial hypothesis, the resting plantar flexor muscles SWV was higher in female than in male trail runners. However, sex did not appear to considerably influence post-race resting muscle and tendon SWV. The shorter trail races (i.e., races <60km) lead to an increased SWV of the resting *triceps surae* muscle and patellar tendon, whereas resting *triceps surae* muscle SWV was decreased after the longer races (i.e., races >100km). The relative decreases in the resting muscles and patellar tendon SWV were negatively correlated with the relative increases in indirect markers of inflammation and muscle damage.

### 4.1 | Sex differences and intramuscular variability

The SWV values reported in the present study were in accordance with values reported in previous studies for thigh and calf skeletal muscles<sup>9,19,20</sup> as well as for patellar and Achilles tendons.<sup>19,29</sup> We also observed high reproducibility in our study, with CVs lower than 7.5%, as previously found for resting muscle and tendon SWV assessments.<sup>30,31</sup>



**TABLE 2** Shear wave velocity in *triceps surae* muscles and Achilles tendon with ankle plantarflexed (120°), at the neutral angle (90°), dorsiflexed (75°) and the average value across the joint positions and exploration zones for males and females

	Shear wave velocity (m.s <sup>-1</sup> )							
	120°		90°		75°		Males	Females
	Males	Females	Males	Females	Males	Females		
Achilles tendon	7.2 ± 2.1 <sup>a,b</sup>	7.4 ± 2.1 <sup>a,b</sup>	11.6 ± 2.4	11.2 ± 2.6	11.1 ± 1.7	10.7 ± 1.7	9.9 ± 2.9	9.8 ± 2.7
Dist	7.1 ± 1.9	7.0 ± 1.7	12.2 ± 2.4	12.2 ± 1.9	11.2 ± 2.0	11.0 ± 1.8	10.1 ± 3.1	10.1 ± 2.9
Free	7.2 ± 1.6	8.3 ± 1.8	12.4 ± 1.5	12.4 ± 1.7	11.8 ± 1.3	11.6 ± 1.4	10.4 ± 2.8	10.8 ± 2.4
SOdist	9.1 ± 1.6	9.2 ± 1.6	12.8 ± 1.5	12.6 ± 1.6	11.4 ± 1.6	10.6 ± 1.0	11.1 ± 2.2 <sup>d</sup>	10.8 ± 2.0
MTJ	5.4 ± 1.4	5.3 ± 0.9	8.9 ± 1.6	7.6 ± 1.5	10.0 ± 1.6	9.3 ± 1.6	8.1 ± 2.5 <sup>d,e,f</sup>	7.4 ± 2.2 <sup>d,e,f</sup>
<i>Triceps surae</i>	2.5 ± 0.4 <sup>a,b</sup>	2.5 ± 0.2 <sup>a,b</sup>	3.0 ± 0.3 <sup>a</sup>	3.1 ± 0.4 <sup>a</sup>	4.3 ± 0.5	4.7 ± 0.5 <sup>c</sup>	3.3 ± 0.8	3.4 ± 1.0 <sup>c</sup>
<i>Gastrocnemius Lateralis</i>	2.3 ± 0.3 <sup>a,b</sup>	2.2 ± 0.3 <sup>a,b</sup>	3.0 ± 0.3 <sup>a</sup>	3.1 ± 0.4 <sup>a</sup>	4.0 ± 0.6	4.4 ± 0.7 <sup>c</sup>	3.1 ± 0.8	3.2 ± 1.0 <sup>c</sup>
Dist	2.3 ± 0.3	2.2 ± 0.3	3.0 ± 0.4	3.1 ± 0.4	3.9 ± 0.6	4.4 ± 0.7	3.1 ± 0.8	3.2 ± 1.0
Mid	2.2 ± 0.3	2.2 ± 0.2	3.0 ± 0.3	3.1 ± 0.4	4.0 ± 0.6	4.4 ± 0.7	3.1 ± 0.9	3.2 ± 1.0
<i>Gastrocnemius Medialis</i>	2.4 ± 0.4 <sup>a,b</sup>	2.3 ± 0.4 <sup>a,b</sup>	3.3 ± 0.3 <sup>a</sup>	3.5 ± 0.5 <sup>a</sup>	5.3 ± 0.9	5.8 ± 1.0 <sup>c</sup>	3.7 ± 1.4	3.9 ± 1.6 <sup>c</sup>
Dist	2.4 ± 0.3	2.4 ± 0.4	3.4 ± 0.3	3.5 ± 0.6	5.3 ± 0.9	5.6 ± 0.9	3.7 ± 1.4	3.8 ± 1.5
Mid	2.3 ± 0.4	2.2 ± 0.3	3.3 ± 0.3	3.6 ± 0.4	5.3 ± 0.8	6.0 ± 1.0	3.6 ± 1.4	3.9 ± 1.7
<i>Soleus</i>	2.9 ± 1.1 <sup>a,b</sup>	3.1 ± 0.8 <sup>a,b</sup>	2.8 ± 0.7 <sup>a</sup>	2.8 ± 0.5 <sup>a</sup>	3.6 ± 0.7	3.8 ± 0.6 <sup>c</sup>	3.1 ± 0.9	3.2 ± 0.8 <sup>c</sup>
Dist	2.9 ± 0.5	2.8 ± 0.5	2.7 ± 0.4	2.7 ± 0.3	3.7 ± 0.7	4.1 ± 0.6	3.1 ± 0.7	3.2 ± 0.8
Mid	3.0 ± 1.5	3.4 ± 1.5	2.9 ± 0.9	2.9 ± 0.7	3.6 ± 0.7	3.6 ± 0.5	3.2 ± 1.1	3.3 ± 0.8

Abbreviations: Dist, distal zone; Free, free tendon zone; Mid, muscle belly zone; MTJ, myotendinous junction zone; SOdist: soleus distal zone.

<sup>a</sup>Significantly different from 75°.

<sup>b</sup>Significantly different from 90°.

<sup>c</sup>Significantly different from males.

<sup>d</sup>Significantly different from Dist.

<sup>e</sup>Significantly different from Free.

<sup>f</sup>Significantly different from SOdist.

In the present study, thigh and lower leg circumferences were greater in males than in females. This can indirectly reflect a sex difference in muscle volume.<sup>32,33</sup> It is reinforced by a larger thickness of subcutaneous adipose tissue in females in comparison to males.<sup>34</sup> The relative intramuscular adipose tissue has been reported to be similar in young healthy males and females<sup>35</sup> whereas a slightly higher muscle fat fraction was shown in middle-aged females in comparison to males.<sup>34</sup> It could be considered that even if a sex-related difference exists in intramuscular fat, it should be minimal in well-trained athletes.<sup>36</sup> However, no information is available concerning a potential sex-related difference in intramuscular connective tissue quantity and quality, that could influence the resting skeletal muscle SWV measurements.

Indeed, our findings showed that the SWV of resting *triceps surae* muscle was higher in females than in males. These results contrast with the findings of Miyamoto et al. reporting a higher resting muscle shear modulus in males compared with females.<sup>9</sup> Although the sex-related

difference was only reported for a fixed dorsiflexed angle in both studies, the discrepancy could be related to the characteristics of the experimental population given that little information was reported about the training status of the participants. It should be noteworthy that the fixed ankle joint in the study of Miyamoto et al. represented approximately 50% of the maximal range of motion in females and 60% in males. Therefore, it constitutes a potential effect of overstretching in males considering the lack of sex-related difference at the maximal range of motion in the same study.<sup>9</sup> As the chronic repetition of stretch-shortening cycles can increase ankle range of motion,<sup>37</sup> it would have been of high interest to determine the maximal range of motion in our experimental population, but this parameter was not assessed and led to a limited comparison of our results with the previous studies.

As reported in previous studies and independently of sex, our results showed that both muscle and tendon SWV increased with joint flexion (i.e., stretched position).<sup>9,30</sup> Furthermore, one spatial difference was reported inside

**TABLE 3** Shear wave velocity in *quadriceps femoris* muscles and patellar tendon with knee fully extended (180°), flexed (90°) and the average value across the joint positions and exploration zones for males and females

Muscle	Shear wave velocity (m.s <sup>-1</sup> )					
	180°		90°		Males	Females
	Males	Females	Males	Females		
Patellar tendon	4.2 ± 1.1	4.0 ± 0.6	9.4 ± 2.1	9.2 ± 2.1	6.8 ± 3.1	6.6 ± 3.0
<i>Quadriceps Femoris</i>	2.3 ± 0.2	2.3 ± 0.2	2.8 ± 0.2	2.8 ± 0.2	2.5 ± 0.3	2.6 ± 0.3
<i>Rectus Femoris</i>	2.2 ± 0.2 <sup>a</sup>	2.3 ± 0.4 <sup>a</sup>	3.2 ± 0.5	3.1 ± 0.5 <sup>b</sup>	2.7 ± 0.6	2.7 ± 0.6 <sup>b</sup>
<i>Dist</i>	2.2 ± 0.3 <sup>a</sup>	2.2 ± 0.3 <sup>a</sup>	3.0 ± 0.5	2.8 ± 0.5	2.6 ± 0.6	2.5 ± 0.5
<i>Mid</i>	2.1 ± 0.2 <sup>a</sup>	2.3 ± 0.5 <sup>a</sup>	3.3 ± 0.5 <sup>c</sup>	3.3 ± 0.5 <sup>b</sup>	2.7 ± 0.7	2.8 ± 0.7
<i>Vastus Intermedius</i>	2.8 ± 0.5	2.6 ± 0.5	3.1 ± 0.5	2.9 ± 0.4	2.9 ± 0.5	2.8 ± 0.5 <sup>b</sup>
<i>Lat</i>	2.8 ± 0.4	2.6 ± 0.5	3.2 ± 0.5	2.9 ± 0.4	3.0 ± 0.5	2.8 ± 0.5
<i>Med</i>	2.8 ± 0.5	2.7 ± 0.4	3.0 ± 0.4	2.9 ± 0.5	2.9 ± 0.5	2.8 ± 0.5
<i>Vastus Lateralis</i>	2.1 ± 0.4	2.4 ± 0.6	2.5 ± 0.4	2.8 ± 0.5	2.3 ± 0.4	2.6 ± 0.6 <sup>b</sup>
<i>Dist</i>	2.2 ± 0.3	2.2 ± 0.4	2.7 ± 0.4	2.8 ± 0.5	2.4 ± 0.4	2.5 ± 0.5
<i>Mid</i>	2.1 ± 0.5	2.5 ± 0.7	2.3 ± 0.3	2.8 ± 0.6	2.2 ± 0.4 <sup>c</sup>	2.7 ± 0.7 <sup>b</sup>
<i>Vastus Medialis</i>	2.0 ± 0.2	2.1 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	2.2 ± 0.3	2.2 ± 0.3 <sup>b</sup>
<i>Dist</i>	2.0 ± 0.2	2.1 ± 0.3	2.3 ± 0.2	2.3 ± 0.1	2.1 ± 0.3	2.2 ± 0.3
<i>Mid</i>	1.9 ± 0.2	2.0 ± 0.2	2.5 ± 0.3	2.6 ± 0.3	2.2 ± 0.3	2.3 ± 0.4

Abbreviations: Dist, distal measure; Lat, lateral zone (under the vastus lateralis); Med, medial zone (under the rectus femoris); Mid, muscle belly measure.

<sup>a</sup>Significantly different from 90°.

<sup>b</sup>Significantly different from males.

<sup>c</sup>Significantly different from Dist.

the RF, as reported previously.<sup>38</sup> This intramuscular inhomogeneity was clearly related to the stretch effect of the hip joint fully extended in the present study, leading to increased stress in the mid part as compared to the distal one within this bi-articular muscle. Overall, SWV measurements within skeletal muscles remained relatively homogeneous. Nevertheless, high variability was observed among the exploration zones along the Achilles tendon, with a lower SWV at the myotendinous junction with the GM in comparison to the more distal parts, especially in the plantarflexed position. As previously reported,<sup>39</sup> the whole Achilles tendon SWV was unchanged at ankle angles from 90° to 75°, whereas *triceps surae* muscle SWV increased. This illustrates the primary contribution of the Achilles tendon to the passive tension generated by the plantar flexor muscle-tendon unit with passive dorsiflexion.<sup>40</sup>

## 4.2 | Effects of mountain ultra-marathon

Different changes in resting SWV of muscles and tendons were found after short-distance races compared to the long-distance races, despite similar decreases in MVC torque in both races. In the present study, no correlation between relative changes in resting muscle SWV and MVC

torque was found, but a significant negative correlation was observed between the relative change in skeletal muscle SWV and blood markers of inflammation and muscle damage. Therefore, a change in resting muscle SWV could provide a more specific marker of exercise-induced muscle damage than a change in MVC.

From a functional point of view, the decrease in MVC is not specific to the severity of muscle tissue damage and can be influenced by impairments of the nervous system.<sup>41</sup> In contrast, the relative decrease in resting muscle SWV was correlated with the increases in blood indirect markers of muscle damage (i.e., CK activity, CRP). However, it remains to further assess the muscle passive elastic properties change across the different physiological steps related to exercise-induced muscle damage.<sup>22,42</sup> Indeed, EIMD is related to the mechanical constraints imposed by exercise on muscle tissue but also to the physiological events following the initial damage leading to additional structural alterations.<sup>22,42</sup> Therefore, the changes in the skeletal muscle biochemical environment<sup>43</sup> and microstructural organization<sup>7</sup> could contribute to the decrease in resting skeletal muscles' mechanical properties. The duration of the exercise appears to be a key factor to explain the contrasting results assessed in the present study after short and long races. It has mainly been observed that after fatiguing exercises, muscles tend to be more compliant<sup>17</sup>

**TABLE 4** Inflammation and muscle damage blood markers, thigh and lower leg circumference, maximal isometric torque, soreness of knee extensors and plantar flexors and shear wave velocity assessed, independently of joint angle, in Achilles tendon, ankle plantar flexor muscles, patellar tendon and knee extensor muscles in males and females before (PRE) and after (POST) short (<60 km) and long (>100 km) distance races

Length	Parameter	Males		Females		All participants		N ( $\delta/\bar{q}$ )
		PRE	POST	PRE	POST	PRE	POST	
		Short						
	Knee extension torque (N.m)	289 ± 71	208 ± 65 <sup>c</sup>	190 ± 23	152 ± 19 <sup>c</sup>	244 ± 74	183 ± 56 <sup>c</sup>	22 (12/10)
	Plantar flexion torque (N.m)	160 ± 34	116 ± 37 <sup>c</sup>	117 ± 26	92 ± 21 <sup>c</sup>	140 ± 37	105 ± 33 <sup>c</sup>	22 (12/10)
	Knee extensors soreness (mm)	8 ± 7	56 ± 22	3 ± 5	27 ± 27	5 ± 7	42 ± 28	23 (12/11)
	Plantar flexors soreness (mm)	11 ± 10	53 ± 20 <sup>b</sup>	2 ± 5	29 ± 32 <sup>a</sup>	7 ± 9	42 ± 28	23 (12/11)
	Thigh circumference (cm)	52.5 ± 4.5	52.0 ± 4.2	52.1 ± 2.5	52.0 ± 3.1	52.3 ± 3.6	52.0 ± 3.7 <sup>c</sup>	23 (12/11)
	Lower leg circumference (cm)	36.6 ± 2.8	35.6 ± 2.8	35.6 ± 1.6	35.2 ± 1.6	36.1 ± 2.3	35.4 ± 2.3 <sup>c</sup>	23 (12/11)
	CRP (mg.l <sup>-1</sup> )	1.2 ± 0.3	2.0 ± 1.4 <sup>b</sup>	1.1 ± 0.1	2.3 ± 1.3 <sup>b</sup>	1.1 ± 0.3	2.1 ± 1.4 <sup>b</sup>	22 (12/10)
	CK activity (IU.l <sup>-1</sup> )	142 ± 69	724 ± 412	107 ± 70	789 ± 624	126 ± 70	754 ± 507 <sup>b</sup>	22 (12/10)
	IL-6 (pg.ml <sup>-1</sup> )	3.4 ± 1.9	23.3 ± 9.2	4.8 ± 1.8	17.8 ± 3.1	4.1 ± 1.9	20.8 ± 7.3 <sup>b</sup>	9 (5/4)
	Achilles tendon SWV (m.s <sup>-1</sup> )	9.8 ± 2.2	10.0 ± 1.8	9.8 ± 1.8	10.1 ± 1.8	9.8 ± 2.0	10.0 ± 1.8	23 (12/11)
	<i>Triceps surae</i> SWV (m.s <sup>-1</sup> )	3.4 ± 0.9	3.7 ± 0.8	3.4 ± 1.0	3.5 ± 0.9	3.4 ± 0.9	3.6 ± 0.9 <sup>b,c</sup>	23 (12/11)
	<i>Gastrocnemius Lateralis</i>	3.2 ± 0.9	3.4 ± 0.9	3.1 ± 1.0	3.2 ± 1.1	3.2 ± 0.9	3.3 ± 1.0 <sup>b,c</sup>	23 (12/11)
	<i>Gastrocnemius Medialis</i>	3.7 ± 1.5	4.0 ± 1.4	3.9 ± 1.6	4.0 ± 1.6	3.8 ± 1.6	4.0 ± 1.5 <sup>b,c</sup>	23 (12/11)
	<i>Soleus</i>	3.2 ± 0.6	3.6 ± 0.7	3.1 ± 0.6	3.3 ± 0.5	3.1 ± 0.6	3.5 ± 0.6 <sup>b,c</sup>	23 (12/11)
	Patellar tendon SWV (m.s <sup>-1</sup> )	6.4 ± 2.9	7.3 ± 3.5	6.1 ± 2.6	7.0 ± 3.2	6.3 ± 2.8	7.2 ± 3.3 <sup>c</sup>	23 (12/11)
	<i>Quadriceps femoris</i> SWV (m.s <sup>-1</sup> )	2.6 ± 0.3	2.7 ± 0.4	2.6 ± 0.3	2.6 ± 0.4	2.6 ± 0.3	2.6 ± 0.4	23 (12/11)
	<i>Rectus Femoris</i>	2.7 ± 0.6	2.7 ± 0.6	2.8 ± 0.6	2.8 ± 0.7	2.7 ± 0.6	2.7 ± 0.6	23 (12/11)
	<i>Vastus Intermedius</i>	3.0 ± 0.5	3.0 ± 0.4	2.7 ± 0.4	2.8 ± 0.5	2.9 ± 0.5	2.9 ± 0.5	23 (12/11)
	<i>Vastus Lateralis</i>	2.4 ± 0.4	2.7 ± 0.6 <sup>b</sup>	2.7 ± 0.6	2.6 ± 0.6	2.6 ± 0.5	2.6 ± 0.6	23 (12/11)
	<i>Vastus Medialis</i>	2.2 ± 0.3	2.4 ± 0.4 <sup>b</sup>	2.3 ± 0.3	2.3 ± 0.3	2.3 ± 0.3	2.3 ± 0.4	23 (12/11)

TABLE 4 (Continued)

Length	Parameter	Males		Females		All participants		N ( $\delta/\bar{q}$ )
		PRE	POST	PRE	POST	PRE	POST	
		Long	Knee extension torque (N.m)	284 ± 47	177 ± 51 <sup>c</sup>	170 ± 35	117 ± 36 <sup>c</sup>	
	Plantar flexion torque (N.m)	167 ± 25	114 ± 30 <sup>c</sup>	113 ± 28	73 ± 21 <sup>c</sup>	149 ± 36	100 ± 33 <sup>c</sup>	24 (16/8)
	Knee extensors soreness (mm)	5 ± 9	44 ± 23	8 ± 13	48 ± 28	6 ± 10	46 ± 24	26 (18/8)
	Plantar flexors soreness (mm)	6 ± 10	29 ± 21	2 ± 3	23 ± 21	5 ± 9	27 ± 21	26 (18/8)
	Thigh circumference (cm)	53.9 ± 3.9	53.4 ± 4.1	51.3 ± 3.6 <sup>a</sup>	50.8 ± 3.4 <sup>a</sup>	53.1 ± 3.9	52.6 ± 4.1 <sup>c</sup>	26 (18/8)
	Lower leg circumference (cm)	37.9 ± 2.4	37.7 ± 2.3	35.7 ± 1.5 <sup>a</sup>	35.4 ± 1.6 <sup>a</sup>	37.2 ± 2.4	37.0 ± 2.3 <sup>c</sup>	26 (18/8)
	CRP (mg.l <sup>-1</sup> )	1.1 ± 0.3	20.0 ± 16.0 <sup>c</sup>	1.9 ± 2.3	38.0 ± 24.4 <sup>a,c</sup>	1.4 ± 1.3	25.6 ± 20.3 <sup>c</sup>	26 (18/8)
	CK activity (IU.l <sup>-1</sup> )	155 ± 117	9768 ± 8313	114 ± 60	4850 ± 3236	142 ± 103	8255 ± 7435 <sup>c</sup>	26 (18/8)
	IL-6 (pg.ml <sup>-1</sup> )	3.4 ± 1.5	59.9 ± 45.9	1.7 ± 1.2	39.9 ± 35.8	2.5 ± 1.5	49.9 ± 39.6 <sup>c</sup>	8 (4/4)
	Achilles tendon SWV (m.s <sup>-1</sup> )	10.0 ± 2.3	10.1 ± 2.4	9.6 ± 2.1	10.2 ± 2.1	9.9 ± 2.2	10.1 ± 2.3	26 (18/8)
	<i>Triceps surae</i> SWV (m.s <sup>-1</sup> )	3.2 ± 0.8	3.1 ± 0.7	3.4 ± 1.0	3.1 ± 0.8	3.2 ± 0.9	3.1 ± 0.7 <sup>c</sup>	26 (18/8)
	<i>Gastrocnemius Lateralis</i>	3.0 ± 0.8	3.0 ± 0.8	3.2 ± 1.0	2.9 ± 0.8	3.1 ± 0.8	3.0 ± 0.8	26 (18/8)
	<i>Gastrocnemius Medialis</i>	3.5 ± 1.2	3.5 ± 1.2	3.7 ± 1.5	3.5 ± 1.3	3.6 ± 1.3	3.5 ± 1.2	26 (18/8)
	<i>Soleus</i>	2.9 ± 0.6	2.9 ± 0.5	3.2 ± 0.7	2.9 ± 0.5	3.0 ± 0.6	2.9 ± 0.5	26 (18/8)
	Patellar tendon SWV (m.s <sup>-1</sup> )	7.0 ± 3.1	6.9 ± 3.1	7.2 ± 3.4	7.4 ± 3.4	7.2 ± 3.1	7.4 ± 3.2	26 (18/8)
	<i>Quadriceps femoris</i> SWV (m.s <sup>-1</sup> )	2.4 ± 0.3	2.5 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	26 (18/8)
	<i>Rectus Femoris</i>	2.6 ± 0.6	2.6 ± 0.6	2.5 ± 0.6	2.5 ± 0.6	2.5 ± 0.6	2.5 ± 0.6	26 (18/8)
	<i>Vastus Intermedius</i>	2.8 ± 0.4	2.8 ± 0.3	2.7 ± 0.3	2.6 ± 0.3	2.7 ± 0.4	2.6 ± 0.3	26 (18/8)
	<i>Vastus Lateralis</i>	2.2 ± 0.3	2.3 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	2.4 ± 0.4	2.4 ± 0.3	26 (18/8)
	<i>Vastus Medialis</i>	2.1 ± 0.3	2.1 ± 0.4	2.2 ± 0.4	2.3 ± 0.4	2.2 ± 0.3	2.3 ± 0.4	26 (18/8)

Abbreviations: CK, creatine kinase; CRP, C-reactive protein; IL-6, interleukin-6; SWV, shear wave velocity.

<sup>a</sup>Significantly different from males.

<sup>b</sup>Significantly different from long.

<sup>c</sup>Significantly different from PRE.

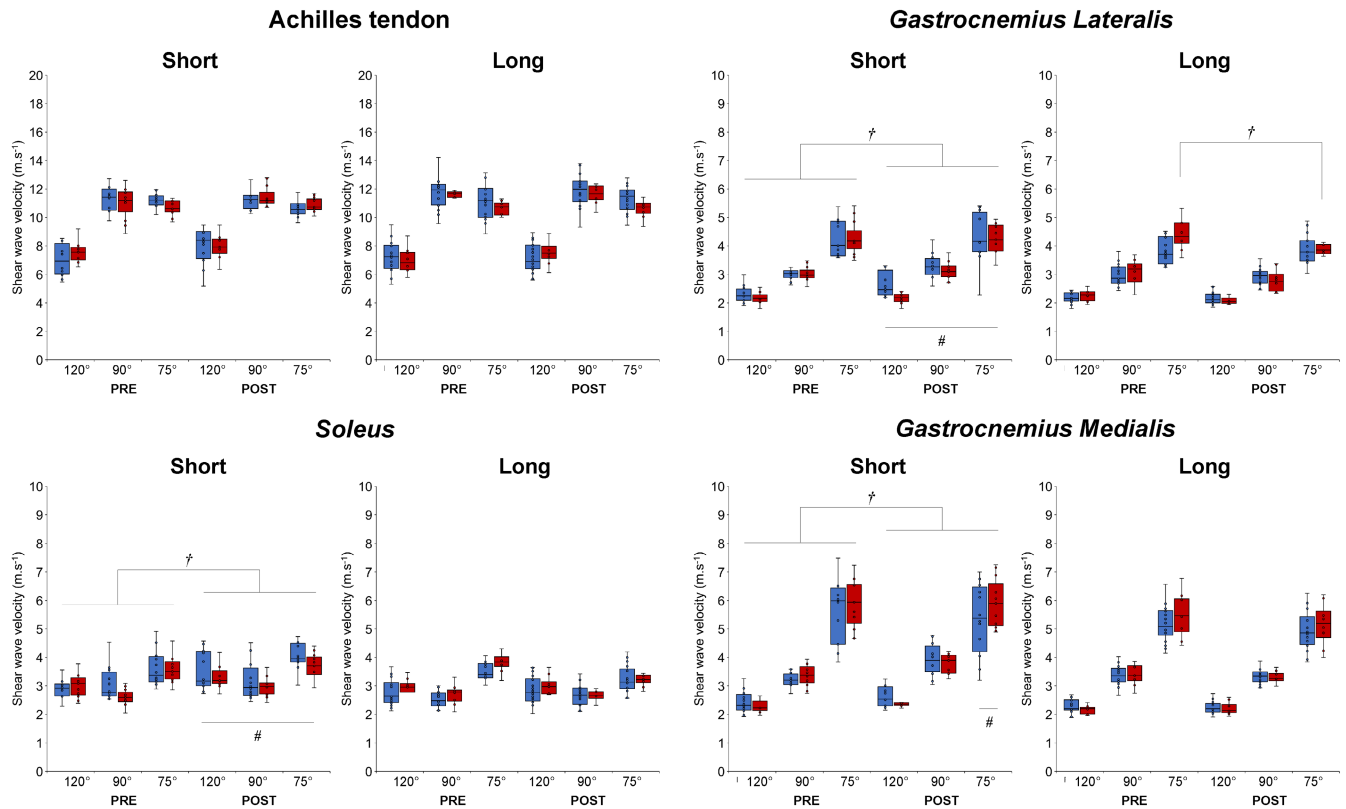


FIGURE 3 Shear wave velocity of Achilles tendon and *triceps surae* muscles (*gastrocnemius lateralis* [GL], *gastrocnemius medialis* [GM] and *soleus* [SO]) for different ankle angles (120°/90°/75°) in males (blue) and females (red) before (PRE) and after (POST) long (>100 km) and short (<60 km) running races. †: significant difference between PRE and POST, #: significant difference between short and long

whereas muscles become stiffer after more damaging exercises.<sup>44</sup> However, very intense and long exercise led to more compliant muscle.<sup>20</sup>

Several physiological hypotheses have been reported in previous works studying skeletal muscle fiber structure alterations<sup>6,20</sup> to explain the increase in passive/resting skeletal muscle stiffness, shear modulus or velocity after short races. For instance, it has been suggested a titin-actin binding<sup>45</sup> in the presence of high intracellular calcium concentration, as reported after lengthening contractions exercise and stretch-induced injury.<sup>46,47</sup> Moreover, the water diffusion in active muscle areas during and after the exercise<sup>48</sup> could lead to increased stiffness of collagen fibrils<sup>49</sup> within the muscle extracellular matrix (ECM). The composition of the skeletal muscle ECM can have a major influence on skeletal muscle mechanical characteristics.<sup>50–52</sup> Although no increase in muscle circumference was found in our study after short races, suggesting a lack of change in intramuscular water content, it has been reported a spatial variability of muscle cross-sectional area change along with the muscle after a resistance exercise<sup>53</sup> that could have been missed by a single measurement of limb circumference performed in the present study.

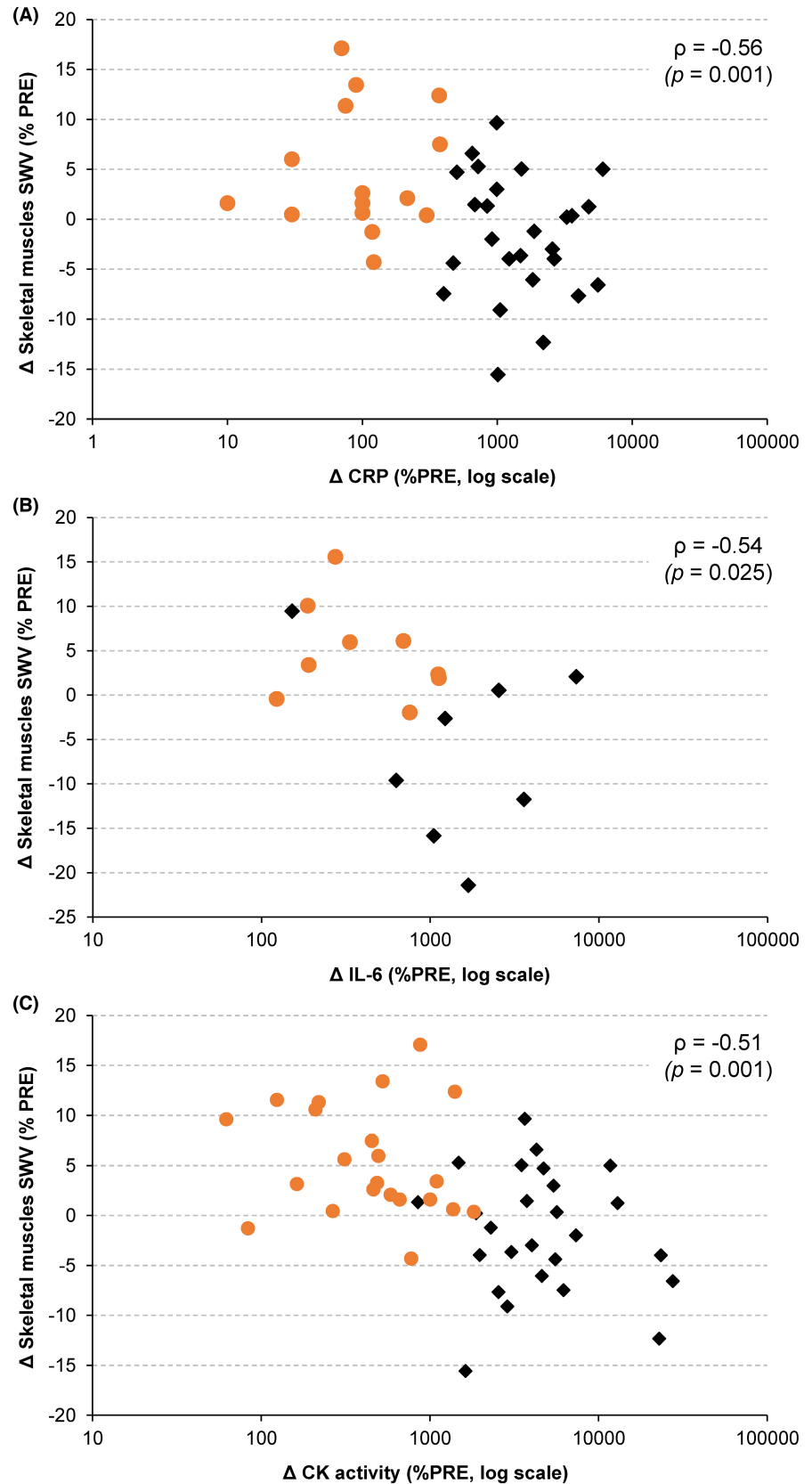
In contrast to the increase in resting muscle SWV after the short-distance races, the long-distance races resulted

in decreased *triceps surae* SWV. From a physiological point of view, the decrease in resting skeletal muscle SWV after long-distance races could be related to the large alteration of the structural organization. The increase in IL-6 found in our study was related to the increase in exercise duration and a concomitant matrix metalloproteinase (MMP) release<sup>54</sup> contributing to the collagen breakdown. These markers indicate the initiation/occurrence of connective tissue remodeling in response to mechanical loading.<sup>50</sup> Muscle edema generated by alterations of muscle and connective tissues could have been limited by large dehydration suggested by a large decrease in blood hematocrit previously reported.<sup>27,28</sup> In addition, the delayed effect between the onset of MMP release and post-race measurements could have led to a difference in the structural status of skeletal muscle connective tissues after short races (from 4.5 to 12.5 h after the beginning of the races) and long ones (from 15.5 to 44.5 h after the beginning of the races). These physiological phenomena could partly explain the discrepancy in resting muscle SWV measurements changes after short and long races.

The initial hypothesis of a sex-related difference in the adaptation of resting skeletal muscle elastic properties has not been verified except for the specific decrease in the GL SWV in dorsiflexed position after the long-distance races



**FIGURE 4** (A) Correlation between relative changes in C-reactive protein concentration ( $\Delta$ CRP) and shear wave velocity ( $\Delta$ SWV) of resting skeletal muscles (i.e., including *triceps surae* and *quadriceps femoris* muscles independently of sex and joint angle) after short (<60 km—light circles) and long (>100 km—dark diamonds) distance races (changes less than 1% were not reported on the graph due to the log scale but were included in Spearman rho coefficient calculation). (B) Correlation between relative changes in interleukin-6 concentration ( $\Delta$ IL-6) and  $\Delta$ SWV of resting skeletal muscles after short (MCC race: 40 km—light circles) and long (UTMB race: 171 km—dark diamonds) distance races ( $N = 17$ ). (C) Correlation between relative changes in creatine kinase activity ( $\Delta$ CK) and  $\Delta$ SWV of resting skeletal muscles after short (<60 km—light circles) and long (>100 km—dark diamonds) distance races



only observed in female runners. It was assumed that females could be less exposed to muscle damage than males. In the present study, CRPs increase after long races to a lower extent in females in comparison to males, similar to

what has been reported after an acute eccentric exercise.<sup>3</sup> It has been reported that estrogen and relaxin regulate the inflammation processes related to exercise-induced muscle damage.<sup>55,56</sup> In animals, estrogen attenuates leucocyte

infiltration and modulates satellite cells activation and proliferation.<sup>57-59</sup> In addition, relaxin can reduce the recruitment of neutrophils and enhance the migration of mononuclear leucocytes to the inflammation site.<sup>56</sup> As a consequence, the release of MMP could have been lower in females in comparison to males as observed in peritendinous tissues after an acute resistance exercise<sup>60</sup> leading to potentially less secondary damage related to inflammation<sup>22,42</sup> and then a less alteration of the structural arrangement of ECM. However, the potential sex-related differences in muscle tissue alteration after long-duration races was not detected by the SWV measurements in the present study.

Overall, the change in resting SWV assessed after both short and long races were mainly observed in plantar flexor muscles and patellar tendon. These specific localizations can be partly related to the specific muscle-tendon interaction in plantar flexors and knee extensors and the experimental conditions specific to the present study. Indeed, previous studies reported that adaptations of passive/resting mechanical properties were higher in stretched positions.<sup>6</sup> The patellar tendon and plantar flexor muscles described the highest SWV increases with joint flexion (see Figures S1 and S2) partly related to the relatively short length of patellar tendon and *gastrocnemii* muscles within the whole muscle-tendon unit length leading to a rapid strain with joint flexion.

### 4.3 | Methodological considerations and limitations

Despite the very good reproducibility of the SWV measures, our study does not come without limitations. The lack of passive torque assessment during SWV measurements constitutes the main limitation, due to the high dependence of SWV measurements on tension modulation within the muscle and tendon tissues. In addition, the characterization of the maximal joint range of motion would have been of great interest to further discuss our results. However, joint angles were controlled during the assessments of the mechanical properties of resting muscle and tendon tissues, and covered the main joint range of motion used in trail running. Furthermore, no preconditioning was performed before the SWE explorations that can generate potential thixotropy phenomena.<sup>61</sup> However, the same conditions were observed for all the participants before and after the running races which can limit the inter-individual variability already taken into account in the reproducibility assessment. Considering the sex-related differences, it would have been of interest to control the menstrual cycle of females to discriminate the potential influence of hormonal fluctuation even if no consequence on tissue mechanical properties have been previously reported.<sup>9</sup>

## 5 | PERSPECTIVES

Shear wave elastography revealed sex differences in resting skeletal muscle mechanical properties measured in a population of experienced trail runners. SWV measurements within the skeletal muscle are overall homogeneous even if a potential spatial difference can be observed in the stretched position of bi-articular muscles. However, the relative homogeneity of intramuscular SWV measurements remains to be assessed during muscle contraction considering the influence of muscle activation on SWV measurements,<sup>62</sup> the potential inhomogeneity of activation in bi-articular muscles<sup>63</sup> and in the context of muscle damage.<sup>64</sup> In experienced trail runners, races generate changes in resting mechanical properties mainly localized in the patellar tendon and *triceps surae* muscle that differ between short and long-distance, while the effects of sex are moderate. The correlations between the relative changes in skeletal muscle shear wave velocity, inflammation and damage markers confirm that shear wave elastography might be a useful tool for specific diagnosis of muscle tissue alterations.

### AUTHOR CONTRIBUTION

A.F., T.B., L.F. and G.Y.M. conceived and designed the research, A.F., T.B., E.S., S.C.S., J.B., L.F. and P.C. performed experiments, analyzed data and interpreted results of experiments. A.F. prepared figures and drafted the manuscript. A.F., T.B., E.S., S.C.S., J.B., P.C., C.A.H. and G.Y.M. edited and revised the manuscript and approved the final version of it.

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### CONFLICT OF INTEREST


The authors declare no conflict of interest, financial or otherwise.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.




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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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