The interaction between exercise induced muscle damage and fatigue on neural regulation and exercise performance during submaximal and maximal running.

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MANUSCRIPT (Journal Ready Format)

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Abstract

***Aim*:** To study the effects of muscle damage and fatigue on neuromuscular preactivation and performance during submaximal and maximal running. ***Setting*:** University of Cape Town, Sports Science Institute of South Africa. ***Methods*:** 12 male distance runners (19 - 39 years of age) with a minimum weekly training distance of 40 kilometers per week were randomly assigned to either control (n = 6) or experimental (n = 6) groups. Subjects' visited the laboratory over an 11 day period during which testing included a submaximal and maximal run (5 km time trial) on the first and last day of testing. Neuromuscular preactivation, rating of perceived exertion, heart rate and performance times were recorded during the performance trials. The intervention between performance trials included two 40 minute bouts on a treadmill at 70 % peak treadmill running speed at –10 º elevation (experimental) or horizontal (control). ***Results*:** Running performance in the 5 km time trial (5K) improved in the experimental group alone by an average of 40 seconds over 5 km ($P < 0.04$) in the presence of muscle damage and without altered neuromuscular preactivation. There was no evidence to support any interaction between altered neuromuscular activity with regard to fatigue and muscle damage during submaximal and maximal running. Evidence of muscle damage in the experimental group was supported by a significant group versus time interaction effect in subjective pain score for daily living and increased plasma creatinine kinase levels in the experimental group ($P<0.03$). A significant decrease in rating of perceived exertion (RPE) was observed in both groups during both the submaximal ($P<0.04$) and 5 km time trial ($P<0.03$) post intervention. There was an interaction effect for group versus pre-post 5K ($P<0.06$), with the post 5K RPE in the experimental group showing an average decreased RPE score of 2.6 for each kilometer and the control group an average decrease in RPE score of only 0.03. ***Conclusion*:** The research design of this study was appropriate to study the interaction between fatigue and muscle damage during submaximal and maximal running. This study suggests that there is no neuromuscular compensation after muscle damage and that EMG is regulated similarly for both fatigue and muscle damage during submaximal and maximal running. Improvement in running performance and decreased rating of perceived exertion after muscle damage is due to some unknown variable.

(Key words: neuromuscular compensation, fatigue, muscle damage, preactivation, running performance)

Introduction

Although many studies have investigated fatigue and factors limiting running performance [1-4] in humans, the understanding of fatigue remains incomplete. It was believed that running performance was limited by the capacity for aerobic muscular activity [5-8]. It was thought that limited cardiorespiratory function resulted in metabolic changes in the exercising skeletal muscle which caused fatigue [5,8]. However, this model had limitations and did not consistently explain fatigue and exercise performance. A more recent model proposed by Noakes et al [9] suggested that the central nervous system continuously modified the exercise intensity during self paced exercise by altering skeletal muscle recruitment. This model highlighted the importance of neuromuscular regulation to resist fatigue.

Changes in neuromuscular regulation have been observed following muscle damage [10-12]. The neuromuscular regulation following muscle damage and fatigue suggested that there were differences between the two conditions. For example, studies which measured muscle preactivation during short and long term exercise to fatigue, suggested a neural protective mechanism [3,13-16], whereas studies investigating exercise induced muscle damage, intimated that preactivation of the muscle did not respond to muscle damage in the same way that it does to fatigue. Several studies have suggested that a neural compensatory mechanism occurred after exercise induced muscle damage [11,12,17,18]. In this context neural compensation was defined as the increase, or the attenuated decrease, in neural activation in active skeletal muscle during a constant, absolute velocity exercise bout.

Neuromuscular models have highlighted the relationship between preactivation and fatigue, and the predominant role preactivation plays in determining efficient and superior running performance [19,20]. Paavolainen [1,21] investigated the relationship between muscle preactivation, muscle power and running performance, and showed that muscle preactivation was a sensitive and reliable marker of fatigue. Preactivation during running was calculated from electromyography (EMG) activity recorded 100 milliseconds before heel strike [21]. Muscle preactivation during running was essentially a centrally regulated, feed-forward, anticipatory mechanism [3,13,19,22-26] initiated in the brain [27]. Accordingly, preactivation prepared the lower limb muscles for landing by increasing the neural activity in the appropriate muscle, before the foot made contact with the ground [22,26,28-31]. A decrease in

preactivation lead to alterations in stiffness regulation, which played an important role in the reduction of muscle force and power [32,33] and a decrease in running efficiency [1].
Sharwood et al [3] showed that muscle recruitment, measured with surface electromyography (EMG), decreased significantly ($P < 0.0001$) towards the end of a five kilometer run, and proposed that this may be an attempt by the central nervous system to protect skeletal muscle and tendons from muscle damage, which may occur should repeated muscle contractions continue unabated [3,13-16].

The teleological explanation of these findings assumed, in accordance with the fatigue-preactivation model, that preactivation decreased significantly more after muscle damage. This change would decrease the ability of the muscle to produce force with the result of protecting already damaged muscles. This is however contrary to the findings of Byrne et al [18], who highlighted the complex role of preactivation by providing evidence to show that the stretch shortening cycle, and by implication the preactivation of the muscle, did not seem to react to muscle damage in the same way as it did to fatigue after prolonged exercise. This was supported Chambers et al [17], who showed that the decrement in muscle power was protected to some extent in movements involving preactivation, compared to movements without preactivation. These data [17,18], and others [11,12,34] suggested there was neuromuscular compensation which attenuated detrimental performance effects associated with exercise induced muscle damage, and thereby protected against a decrease in muscular performance. There was some evidence to suggest that muscle damage may cause beneficial neuromuscular compensation [28], and other studies [35] have reported an increased fatigue resistant capability in exercise involving lengthening of the muscle when compared to both concentric and isometric muscle contractions.

The interaction between these two intervening factors (fatigue vs exercise induced muscle damage), and their direct effect on muscle preactivation has not yet been studied systematically. Therefore the aim of this study was to develop a model to examine fatigue and exercise-induced muscle damage during submaximal and maximal running exercise, with particular emphasis on performance and neuromuscular regulation. This model was then used to test the theory that exercise-induced muscle damage caused neuromuscular compensation in order to attenuate a decrease in performance, and that this effect was separate from fatigue.

Methods

Subjects

Twelve healthy male distance runners (age 19 – 39 years) with a minimum training mileage of 40 kilometers per week, were recruited for this study through radio and newspaper advertising. No upper limit was placed on accumulated mileage. The exclusion criteria included no medication or analgesics within the last 12 weeks, no history of lower limb bone stress injuries, no history of muscle pathology and no viral infection within the last 12 weeks. Subjects were randomly assigned to either the experimental group (n = 6) or control group (n = 6). This study was approved by the Ethics and Research Committee of the Faculty of Health Sciences, University of Cape Town Medical School.

Study design

The testing protocol effectively took place over an eleven day period. Subjects visited the laboratory for a familiarization and pre-testing session five days prior to the start of the intervention. During this visit, the following tests were carried out for both descriptive and baseline purposes: informed consent, body composition, one repetition maximal leg press (1RM), maximal oxygen consumption test (VO_2 max), peak treadmill running speed test (PTRS) and protocol familiarization. Five days later (Day 1) subjects visited the laboratory for a subjective measurement of muscle pain, creatine kinase activity, maximal voluntary contraction (MVC), drop jumps and squat jumps (DJ/SJ), a 1.4 km constant velocity submaximal run, 40 meter sprints and a five kilometer time trial run (5K). All data recorded on day 1 represented the pre-intervention data for subsequent analysis. The following day (Day 2), was a rest day and no testing or training was carried out. Day 3 of the protocol included a pain score measurement, plasma creatine kinase analysis, and the first intervention (40 minute downhill or horizontal run). The following day (Day 4) included pain score measurements, plasma creatine kinase analysis, MVC and DJ/SJ. Day 5 of the protocol included a pain score measurement, plasma creatine kinase analysis, MVC, DJ/SJ, and second intervention (40 minute downhill or horizontal). Day 6 of the protocol was a repeat of the testing done on Day 1 and represented the post data intervention analysis. This study was conducted in conjunction with another study investigating the neuromuscular effects of the stretch shortening cycle. Both MVC and DJ/SJ were part of the other study, and all data was analyzed separately. Subjects were given 2 hours rest after the jumps before starting this study. The study design is summarized in Table 1.

DAY						
-5	1	2	3	4	5	6
Consent Info	Pain score	Rest	Pain score	Pain score	Pain score	Pain score
Anthro	CK		CK	CK	CK	CK
1RM	MVC*		40 min Downhill / Level run	MVC*	MVC*	1RM
DJ/SJ* Familiarize	DJ/SJ*			DJ/SJ*	DJ/SJ*	MVC*
	MVC				40 min Downhill / Level run	DJ/SJ*
VO_2 max	10 lap submax run					MVC*
PTRS						10 lap submax run
Track Familiarize	Sprints					Sprints
	5 Km TT					5 Km TT
	Sprints					

Table 1: *Summary of the protocol time-line. Consent information (Consent info); Anthropometry (Anthro); One repetition maximal test (1RM); Drop jumps / Squat jumps (DJ/SJ); Maximal oxygen consumption (VO_2 max); Peak treadmill running speed test (PTRS); Test familiarization (Familiarize); Creatine Kinase activity (CK); Maximal voluntary contraction (MVC); 1.4 km submaximal run (10 Lap submax run); 100m sprint (sprints); 5 kilometer time trial (TT). * Highlights the tests not part of this study.*

Experimental vs control subjects

The experimental subjects ran for 40 minutes on a motor driven treadmill (Quinton Instruments, Seattle, WA, USA) set at a -10 % elevation and a predetermined velocity of 70% of each subject's peak treadmill running speed test (PTRS). This downhill protocol induces muscle damage [36]. The control group completed the same protocol, except the treadmill was horizontal. Both subjective pain scores and plasma creatine kinase activity were used as a marker of exercise induced muscle damage.

Informed consent and history information

Subjects were informed about the purpose of the study, the testing to be undertaken, the possible risks relating to the trial and their right to withdraw from the study at any stage. All subjects were required to complete an informed consent form prior to starting of the study. A full training history, which included information regarding performance and training distance over their entire running career, and current training distance was obtained from each subject.

Intervention

The experimental group completed two 40 minute sessions separated by 48 hours, running downhill (-10% elevation) on a treadmill. The control group completed the same protocol, except the treadmill run was horizontal (0% elevation).

Anthropometry

Stature, mass, and skinfold measurements were recorded before testing commenced on the first day. These measurements were taken by the same assessor using bony landmarks as reference points described by Ross et al [37]. Body fat was expressed as the sum of seven skinfolds and also as a percentage using the equation of Durnin and Womersley [38]. Lean thigh volume was calculated according to the method adapted from Katch and Katch [39], which assumes that the thigh has the shape of a truncated cone.

Active warm up

A standardized warm up which consisted of a 5 minute cycle on a stationary bike at 25 Watts was performed before testing each day. Subjects were not allowed to stretch passively.

Jump tests

Prior to the running protocol, subjects performed a MVC, five body weight-bearing squat jumps and five body-weight bearing drop jumps from a step height of 33cm. Subjects were given a two hour rest period before commencing with this study. These data are not reported in this study.

1 Repetition Maximum Test

Each subject performed a leg press one-repetition maximum test (1RM). The load assignment was standardized according to the 1RM protocol described by Kraemer et al [40]. The subject was given a two minute rest period before every attempt at a new load. The foot placement was standardized by having both feet positioned 1 ½ times their bi-acromial width apart, with the longitudinal shaft of the tibia parallel to the ground. The plate was lowered to the point where a 90° angle (measured with a goniometer and spirit level) was formed at the knee joint. The height of the foot plate along its pulley rail was measured and clearly marked from the base of the pulley rail. This measurement was recorded to ensure the subject descended to the exact knee angle in the future testing sessions, thus standardizing the test for when the subject was re-tested. Standardized encouragement was given throughout the testing. Subjects were required to wear the same shoes throughout the protocol.

Peak treadmill running speed (PTRS) and Maximal oxygen consumption (VO_2 max) test

Maximal oxygen consumption (Oxycon Alpha, Jaeger / Mijnhardt, Groningen, Netherlands) and peak treadmill running speed (PTRS) were determined using a continuous, incremental running protocol on a motor driven treadmill (Quinton Instruments, Seattle, WA, USA). Subjects began the test running at 12 km.hr $^{-1}$ on a horizontal treadmill and the speed was increased by 0.5 km.hr $^{-1}$ every thirty seconds thereafter [41]. The test continued until the subjects were unable to maintain the pace of the treadmill. The VO_2 max was defined as the highest oxygen consumption recorded during the test that was sustained over 30 seconds. PTRS was defined as the fastest running speed the subject could maintain for a total period of 30 seconds during the test. Rating of perceived exertion (RPE) was measured every 30 seconds using the Borg RPE scale [42]. Heart rate was recorded every 5 seconds using a Polar heart rate monitor (Vantage XL, Polar Electro, Kempele, Finland).

Subjective pain measurement

A subjective assessment of muscle pain was obtained by an independent assessor asking the subjects to rate the level of pain localised to their exercising skeletal muscles. Four categories were considered, and these included general pain at rest, pain during normal daily activities, pain during a passive stretch of the muscle and pain when pressure was applied to the muscle on a scale 0 –10. A score of 0 represented no pain and a score of 10

represented extreme pain. The muscle groups which were tested included: the quadriceps muscle group, the hamstring muscle group and the calf muscle group.

Plasma creatine kinase activity

At the start of day 1, 3, 5 and 6, approximately 5 milliliters of venous blood was drawn from the forearm antecubital vein and collected in an EDTA vacutainer tube. The plasma was stored at -20°C until analysis for creatine kinase. Plasma creatine kinase activity has been used as a marker of damage to muscle cell membranes and leakage of cell contents into the blood [43]. Plasma creatine kinase activity on the first day was used as the subjects' rested baseline value and all subsequent values were normalized accordingly.

Electromyographic (EMG) activity measurements

EMG preparation included the removal of hair on the subjects' right leg by shaving the site of electrode placement with a disposable razor. The outer layer of epidermal cells was then removed with industrial sand paper and finally all dirt and oil was removed from the site using an alcohol swab. The electrode positions were standardized and positioned longitudinally on the belly of the vastus medialis (VM), vastus lateralis (VL), biceps femoris (BF) and gastrocnemius (GA) muscles. Each subject was fitted with bipolar EMG electrodes (Blue sensor, Ambu skin electrodes SP-00-S, Medicotest A/S, Denmark). Electrodes were secured using 50 mm Elastoplast adhesive plaster. All EMG data were amplified and recorded telemetrically (Noraxon Telemyo, Scottsdale, Arizona, USA).

A one centimeter foot sensor (NorSwitch bilateral telemetric footswitch system, Noraxon, Arizona, USA) was placed on the posterior most surface of the heel and the anterior most part of the first toe to determine ground contact time during running. The foot sensor was secured onto the heel and toe using 12 mm Transpore dressing tape. Data from the foot switch was recorded simultaneously with the EMG signals. The foot switch was used to determine the period of preactivation during running, which encompasses the 100 milliseconds before heel strike [21].

EMG raw data activity was sampled at 2000 Hz and was amplified and recorded telemetrically (Noraxon Telemyo, Scottsdale, Arizona, USA). The raw EMG signal was filtered using a RJ-50 filter, followed by a 15 - 500 band pass filter. All data were then smoothed by taking the root mean square of the signal over 50 ms periods. The amount of

EMG activity was quantified by determining the area under the curve for EMG (μV) vs time (s), resulting in an integrated EMG (iEMG) measurement. This iEMG value during preactivation for each of the four muscle groups was normalized to both the iEMG value obtained from the MVC for each respective muscle group, and the iEMG during the 6th lap (840 m) of the constant velocity submaximal run. Subsequent analysis showed the normalization to the submaximal run to be a better representation of iEMG, and therefore all data shown was normalized to the 6th lap (840 m) of the submaximal run.

Maximum voluntary contraction (MVC)

Before the beginning of both the jump and running protocols, subjects performed a leg press maximal voluntary contraction on a leg press machine. The load for the leg press was set at 120% of each subjects' predetermined 1-repetition maximum. Foot placement on the foot plate was standardized to that of the predetermined 1RM foot placement. Subjects were required to perform a maximal voluntary contraction over a 5 second period. EMG activity was recorded throughout the 5 second period, of which the first and last second of the epoch was discarded. This was repeated twice making a total of 3 repetitions. Each subject was required to perform three attempts separated by a 2 minute rest between contractions. For analysis, the MVC with the highest EMG activity recorded was used for subsequent normalization. If the highest EMG activity recorded was greater than 10 % of the other two attempts, then the second highest EMG recorded value was used.

1.4 km submaximal run at 70% PTRS

Subjects began the running protocol by performing a 1.4 kilometer submaximal run around an indoor track at 70% of their predetermined peak treadmill running speed. The circumference of the indoor track used was 140 m and a light pacing system around the track was used to pace the athletes for 10 laps. Rate of perceived exertion, electromyographic activity, and heart rate were recorded during laps 6 (840 meters), 8 (1120 meters) and 10 (1400 meters) of the run. All data were recorded for a distance of 40 meters over the straight portion of the track. This was done in order to prevent any abnormal gait patterns that could occur from running over the corner stretch of the track. Heart rate was recorded every 5 seconds for the entire duration.

20 meter sprint test

Following the submaximal run, subjects were given a 10 minute rest period before starting the sprint test. All subjects performed three 40-meter sprints prior to the time trial on the indoor running track. Subjects were required to sprint at 130% of their PTRS. A constant velocity over the 40 m was achieved using the light pacing system. Each subject was given a 60 meter flying start in order to ensure a normal gait had been reached before the 40 meter recording period. By allowing a flying start, the exclusion of any gait changes associated with the acceleration in catching up to the light pacing system was prevented. The 40 meter sprint time was recorded using photocell gates. All data recorded on the first day were adjusted to 100 and used to represent the non-fatigued, and non-damaged pre 5 km time trial data. All subsequent data were adjusted accordingly. During the final lap of the 5 km time trial, subjects were required to sprint the last 100 meters of the time trial at 130% of their PTRS. Constant velocity during the sprint was again controlled by the light pacing system.

5 kilometer time trial run

After the submaximal sprints, subjects were given 10 minutes rest before being required to run a 5 km time trial (5K) on the 140 meter indoor track. All subjects were asked to run "as fast as they possibly could". Subjects were provided with split times and distance covered at every kilometer. All subjects were verbally encouraged using standardized verbal cues throughout the run. During the 5K, rate of perceived exertion and electromyographic activity were recorded. Data were recorded at every kilometer during the run for a distance of 40 meters, and again during the last 10 seconds of the run. All data were recorded on the straight portion of the running track. Heart rate was recorded every 5 seconds throughout the 5K. All subjective measurements were recorded by an independent assessor.

Intervention protocol

Both the experimental and control group performed a 40 minute submaximal run at 70% of their PTRS on a motor driven treadmill (Quinton Instruments, Seattle, WA, USA). Both the experimental group and control group were given a 3 minute warm up period on the treadmill at 10 $km.h^{-1}$ at a 0% decline. The treadmill was set at a -10% elevation for the experimental group, and horizontal for the control group. Both groups ran at a velocity of 70% of their PTRS. Heart rate was recorded every 5 seconds, and RPE every 5 minutes throughout the treadmill run.

Statistics

Student's independent t-tests were used to compare differences in subject characteristics, training, history, plasma creatine kinase activity and body composition variables between the experimental and control groups. Friedman's two-way ANOVA was used to measure differences in subjective pain measured for each individual before and after the induction of muscle damage. The running performance, heart rate and EMG data were analyzed with a two-way analysis of variance with repeated measures. A Tukey's honest significant difference (HSD) post-hoc test was used to analyze differences when the F value of the ANOVA test was significant. Statistical significance was accepted as $P < 0.05$. All data are expressed as mean ± standard deviation.

Results

The physical characteristics and running performance data of the subjects are shown in Table 2. There were no differences between the control and experimental subjects for any of these variables. As shown in Table 2, the subjects were relatively homogenous with respect to age, sum of skinfolds, % body fat, maximal oxygen consumption, average weekly training distance and peak treadmill running speed.

The mean average weekly training distance was 60 ± 22 km and 68 ± 25 km for the control and experimental group respectively. The range of weekly training distance varied from 40 – 100 km in both groups. The range of number of years running varied from 2 – 4 and 1 – 6 for the control and experimental group respectively. Previously used predictors of performance such as peak treadmill running and maximal oxygen consumption [6,41,44-46] are shown to be relatively homogenous between the two groups. There were no statistically significant differences ($p<0.05$) between the two groups.

Variable	Control	Experimental	Control	Experimental
	Mean ± SD	Mean ± SD	Range	Range
Age (years)	23.3 ± 6.1	21.8 ± 2.1	19 - 35	20 - 25
Mass (kg)	67.2 ± 9.3	60.0 ± 14.8	59.2 – 82.4	43.3 – 87.1
Stature (cm)	175.6 ± 10.1	168.1 ± 8.1	161 – 189	156 – 179
Sum of skinfolds (mm)	58.0 ± 20.1	51.8 ± 18.9	39 – 85.6	33.2 – 87.6
% Body fat	12.9 ± 3.4	11.5 ± 3.6	9.8 – 18	8.4 – 18
Lean thigh volume (cc)	5048 ± 651	4575 ± 1388	4093 – 5740	3069 – 6956
VO_2 max ($mlO_2.kg^{-1}.min^{-1}$) #	64.6 ± 8.1	67.6 ± 5.4	54.1 – 74.8	61.4 – 73.3
PTRS ($km.h^{-1}$) *	19.3 ± 2.1	20.1 ± 0.2	16 – 22.5	20 – 20.5
Heart rate max ($b.min^{-1}$)	198 ± 7	198 ± 7	188 – 206	186 - 205
Training distance ($km.wk^{-1}$)	60.0 ± 22	68 ± 25	40 – 100	40 - 100
Training history (yrs running)	2.5 ± 0.8	3.3 ± 1.9	2 – 4	1 – 6

Table 2: *Physical characteristics and performance data of the control (n = 6) and experimental (n = 6) subjects (mean ± SD and range). There were no significant differences between the groups. * Peak treadmill running speed. # Maximal oxygen consumption.*

Intervention effects

Subjective pain

Subjective pain scores of general pain, pain during daily living, pain with pressure and pain felt during a static stretch of the relevant muscles are shown in Figure 1 and Figure 2. There was a significant increase in daily living pain (area under curve) in both the hamstrings (figure 1.e) ($P<0.05$) and gastrocnemius muscles (figure 1 f) ($P<0.04$) in the experimental group. Subjective pain tended to increase to a larger extent over time in the experimental group compared to controls for the various other subjective measures, but this was not significant.

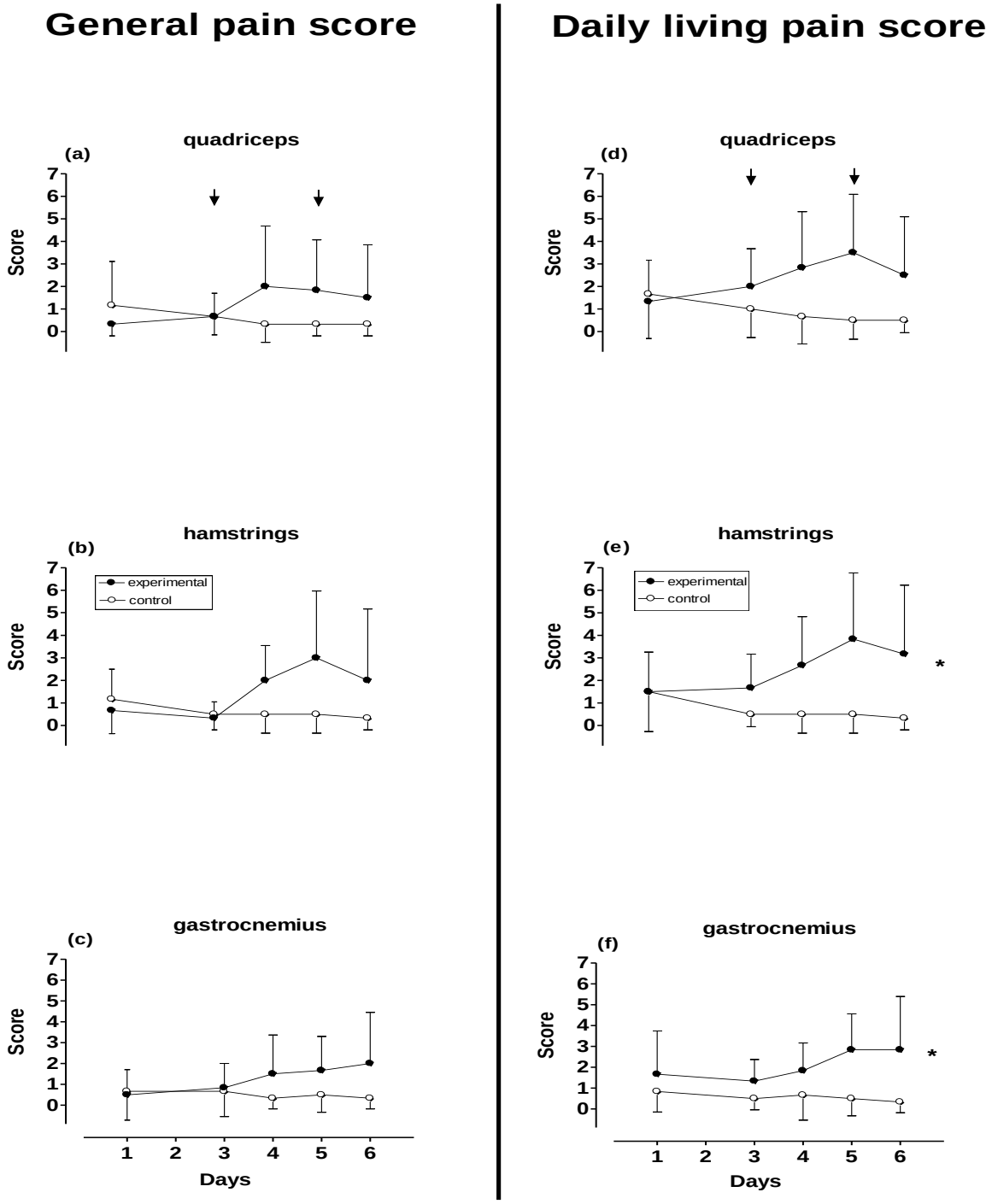

Figure 1: *Change in subjective pain scores over time in 3 muscles (a) general pain in quadriceps (b) hamstrings (c) gastrocnemius (d) pain during daily living in quadriceps (e) hamstrings and (f) gastrocnemius.* ******* *P<0.05, Significant change in area under curve for pain during daily living in hamstrings for experimental group.* ******* *P<0.04, Significant change in area under curve for pain during daily living in gastrocnemius for experimental group.* ↓ *indicates day of intervention.*

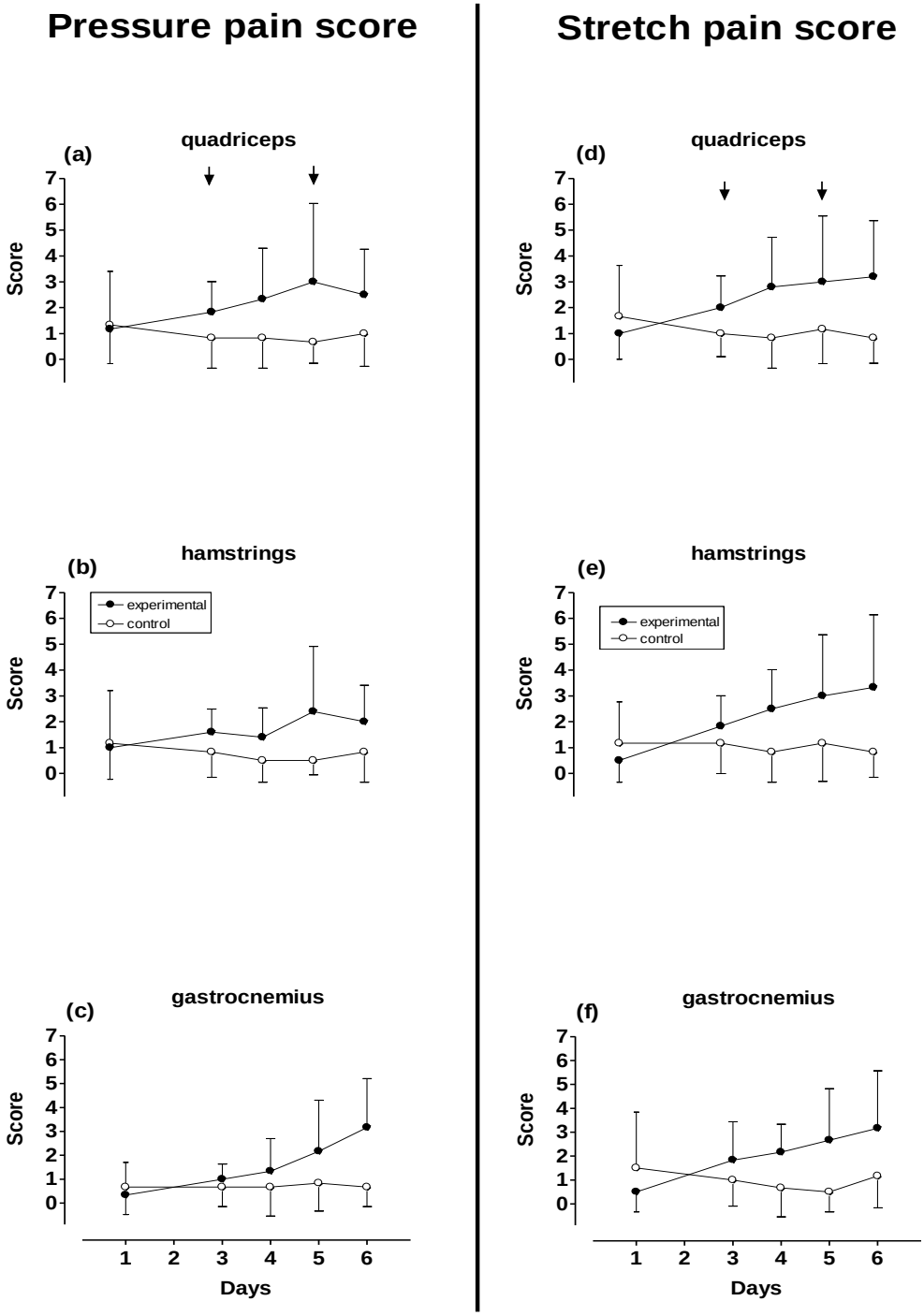

Figure 2: *Change in pain scores over time in 3 muscles (a) pressure pain in quadriceps (b) pressure pain in hamstrings (c) pressure pain in gastrocnemius (d) pain during stretch in quadriceps (e) pain during stretch in hamstrings (f) pain during stretch in gastrocnemius.*
↓ indicates day of intervention.

Creatine kinase activity

Creatine kinase activity for both experimental and control groups is illustrated in Figure 3. When normalized to percentage of the starting value of creatine kinase activity for each subject, there was a significant group vs time interaction effect (P<0.03). The creatine kinase activity of the experimental subjects increased significantly on day 4, which was approximately 24 hours after the intervention (downhill run). The two interventions are shown in Figure 3 on days 3 and 5 with an arrow (↓). Peak mean creatine kinase activity occurred on day four 294 ± 154 UI^{-1} for the experimental group and day three 145 ± 41 UI^{-1} for control subjects.

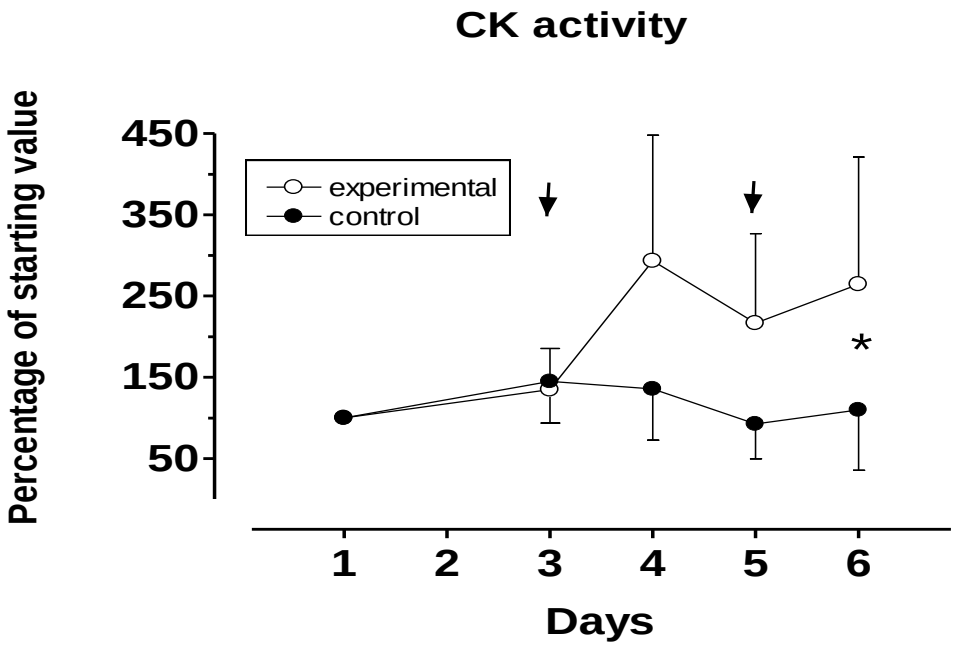

Figure 3: *Creatine kinase activity in experimental (n = 6) and control (n = 6) group over time.*

***** *P<0.03, Group vs time interaction effect.*

↓ *indicates day of intervention*

Performance

Running speed during 5K

There was a significant interaction effect between groups and condition (pre 5K – post 5K) (Figure 4) (P<0.04). Running speed after intervention did not decrease over the 5K in the control group as much as it did in the experimental group. Tukey's (HSD) post-hoc test showed that experimental post 5K (4.5 ± 0.1 $m.sec^{-1}$) was significantly different to experimental pre 5K (4.4 ± 0.2 $m.sec^{-1}$), control pre 5K (4.3 ± 1.4 $m.sec^{-1}$), control post 5K (4.3 ± 1.2 $m.sec^{-1}$). The control group showed no significant change in pre - post intervention for the 5K. There was a main effect for kilometer splits for both experimental and control groups pre 5K. In both groups pre 5K, kilometer (km) 3 and 4 were significantly different to km 1 (Figure 4) (P<0.004). Kilometer splits as main effect for km 2, 3 and 4 was also significantly different to km 1 for both groups' post 5K (P<0.002).

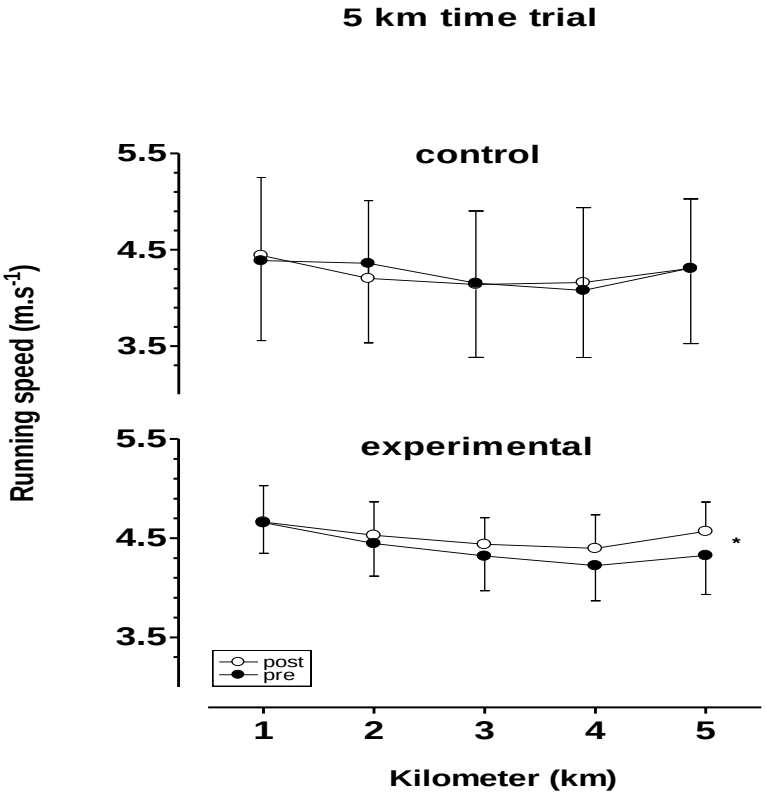

Figure 4: *Running speed ($m.sec^{-1}$) for control (n = 6) and experimental (n = 6) group during the 5 kilometer time trial (5K).*

******* *P<0.04, Significant interaction effect for group vs time (pre 5K – post 5K**).***

Mean 5K performance times of pre-intervention were 20.2 ± 3.6 minutes and 19.1 ± 1.5 minutes for control and experimental groups respectively. Post intervention times were 20.2 ± 3.5 minutes and 18.5 ± 1.2 minutes for control and experimental groups respectively. The control group pre - post intervention times were very similar, whereas the experimental group reduced their time by an average of 40 seconds over 5 kilometers, which was statsically significant (P<0.004).

Rating of perceived exertion

Submaximal run

Rating of perceived exertion (RPE) during the constant velocity submaximal run (figure 5.a and figure 5.b) showed a significant interaction effect (P<0.04) for group and main effect pre – post. A Tukey's (HSD) post-hoc analysis showed a time (meters) effect in RPE. In both groups, control (Figure 5.a) and experimental (Figure 5.b), RPE after 840 meters (m) was significantly different to RPE measured after 1120 m and 1400 m (P<0.005). 1400 m was also significantly higher than 1120 m (P<0.03) for both groups.

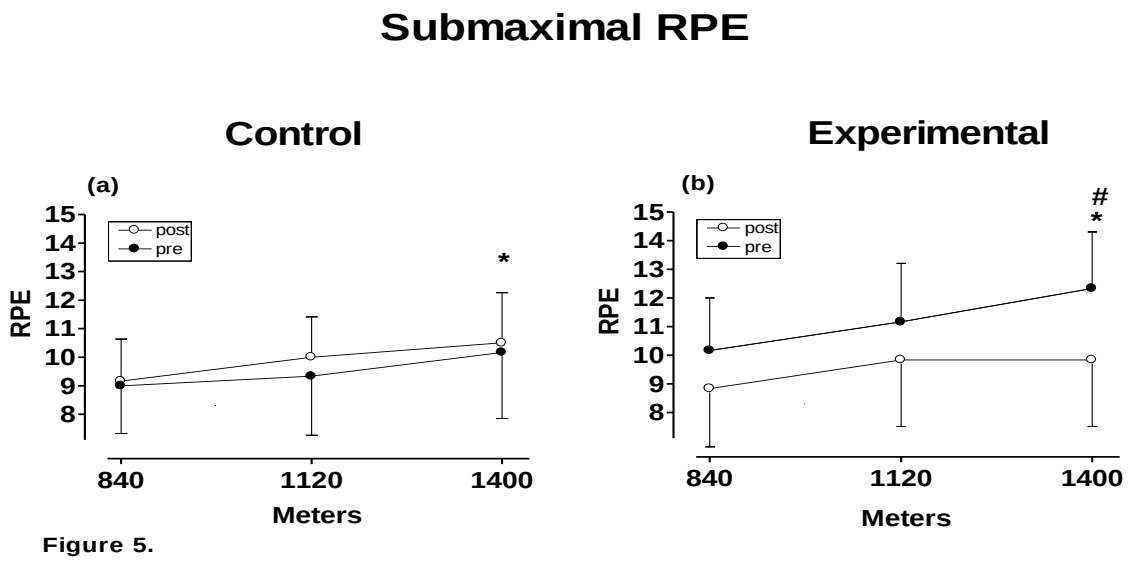

Figure 5.

Figure 5: *Rating of perceived exertion of submaximal run for (a) control (n = 6) and (b) experimental (n = 6) groups. # P<0.04, Significant interaction for group x pre-post.*

** P<0.005, Significant time effect 840 m vs 1120 m vs 1400 m.*

RPE during 5K

There was a significant main effect of pre vs post (Figure 6.b) (P<0.03). There was an interaction effect for group vs pre-post (P<0.06), with the post 5K RPE in the experimental group showing an average decreased RPE score of 2.6 for each kilometer when compared to experimental pre 5K. The control group showed an average decrease in RPE score of 0.03. This was calculated from the sum of RPE score over the 5K for each subject. The groups total was then calculated by adding the total individual scores. This was done for both pre 5K RPE and post 5K RPE. The RPE difference was then calculated by subtracting the total RPE of the groups pre 5K from the post 5K value. The total groups' difference was then divided by the group number to attain a group mean value. This group mean value was then divided by 5 to attain a mean difference in RPE per kilometer. In both groups, there was a significant time (km) effect (Figure 6.a and Figure 6.b) (P<0.04) for km 1 vs km 2 vs km 3 vs km 4 vs km 5.

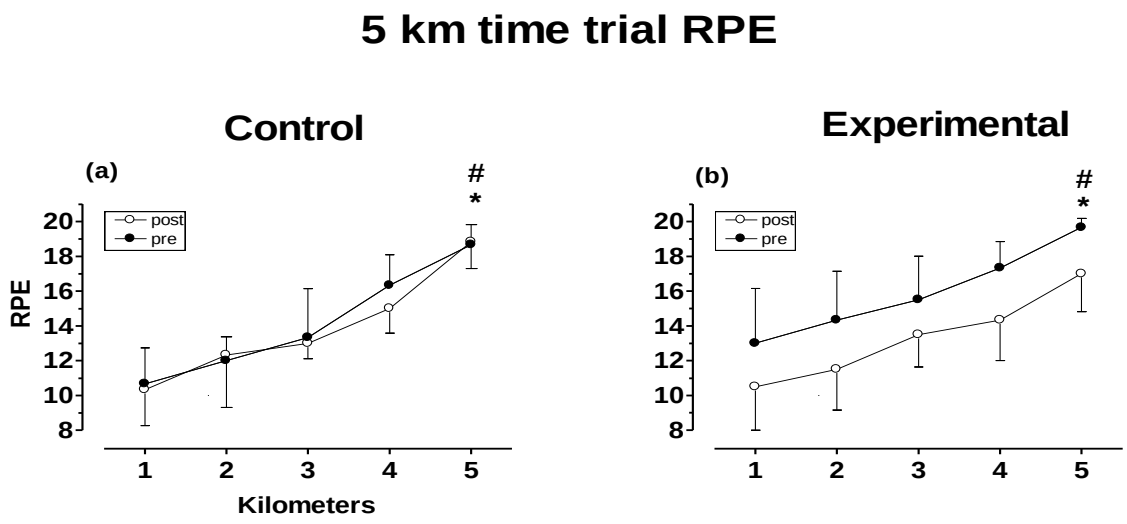

Figure 6.

Figure 6: *Rating of perceived exertion of 5 kilometer time trial (5K) for (a) control (n = 6) and (b) experimental (n = 6) groups.* **#** *P<0.03, Significant main effect pre vs post.* ***** *P<0.04, Significant time effect km 1 vs km 2 vs km 3 vs km 4 vs km 5.*

Heart rate

Submaximal run

During the constant velocity submaximal (1.4 km) run, heart rate increased significantly over time (Figure 7.a and 7.b) (P<0.007) for both groups. A Tukey's (HSD) post-hoc analysis showed a significant difference in heart rate for 840 m vs 1120 m and 1400 m. There was a significant pre versus post effect (Figure 7.a and 7.b) (P<0.03) for both groups, with post submaximal heart rates being significantly lower. Although not significant, the experimental group's mean post submaximal heart rate tended to be lower than both pre-experimental, and pre-post submaximal control group's mean heart rate (Figure 7.b).

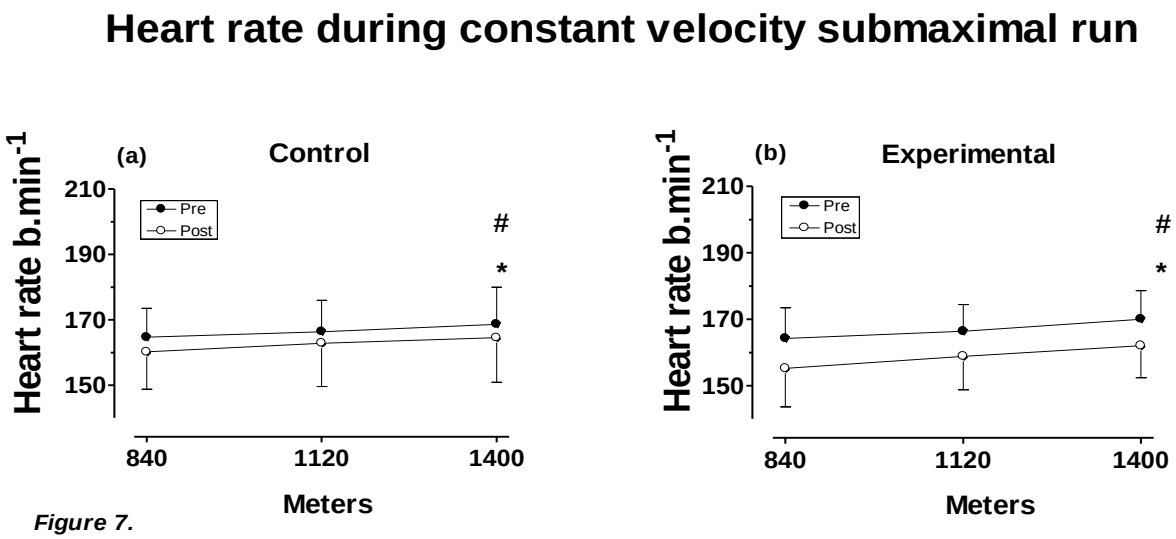

Figure 7.

Figure 7: *Heart rate during the constant velocity submaximal run (a) control (n =6) and (b) experimental (n = 6) group. # P<0.03, Main effect pre vs post for both groups.* ******* *P<0.007, 840 m vs 1120 m vs 1400 m for both groups.*

Heart rate during 5K

Heart rate during the 5K, was analyzed as heart rate during km 1 to 4 (Figure 8.a and Figure 8.b), and heart rate during km 5 (data not shown). There was a significant increase in heart rate in both control and experimental groups from km 1 to 4 (Figure 8.a and Figure 8.b) (P<0.005). A Tukey's (HSD) showed a significant difference in heart rate for km 1 vs km 2 vs km 3 vs km 4. Mean heart rate during km 5 of the pre 5K were 194 ± 6 b. min $^{-1}$ and 193 ± 10 b. min $^{-1}$ for the control and experimental group respectively. The mean post 5K heart rates were 194 ± 7 b.min^{-1} for the control group and 197 ± 6 b.min^{-1} for the experimental

group. There were no significant differences in heart rate during the last km between groups, or pre versus post 5K.

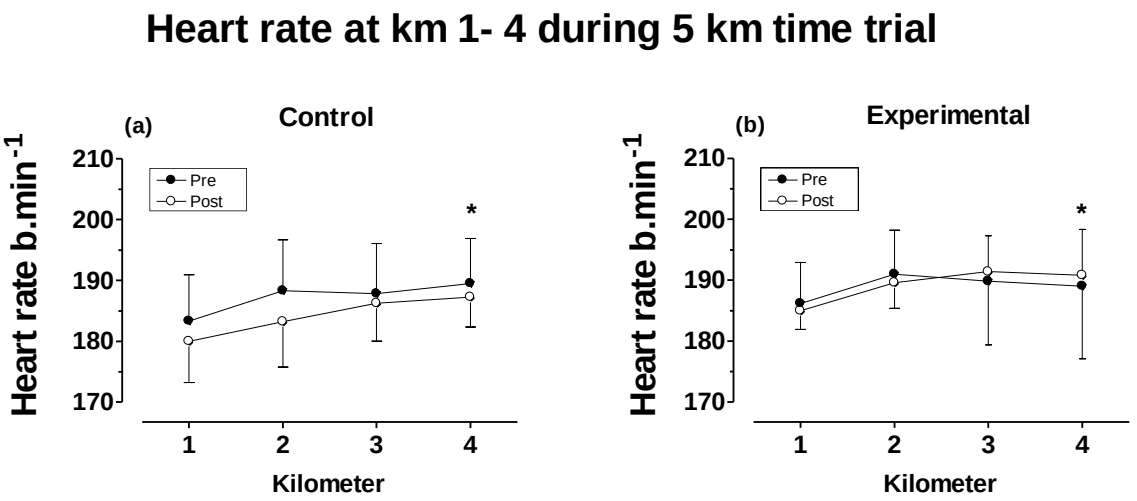

Figure 8.

Figure 8: *Heart rate for kilometers 1 – 4 of 5 kilometer time trial (a) control and (b) experimental group.* ******* *P<0.005, km 1 vs km 2 vs km 3 vs km 4 for control and experimental groups.*

Neuromuscular EMG

Submaximal run

Neuromuscular preactivation during constant velocity submaximal run for both control and experimental groups is illustrated in Figure 9.a and Figure 9.b respectively. EMG preactivation from four muscles (vastus lateralis obliques (VMO), vastus medialis obliques (VMO), biceps femoris (BF) and medial gastrocnemius (MG)) was recorded. There was a significant pre versus post effect (Figure 9.a and Figure 9.b) (P<0.005) for both groups recorded in BF, with a mean increase in percentage preactivation from pre to post of 9 % (88 ± 12 % – 97 ± 14 %) in the control group and 19 % (90 ± 12 % – 109 ± 10 %) in the experimental group. There was a significant (P<0.04) pre versus post effect for MG, with a mean increase in post submaximal percent preactivation of 7 % (88 ± 11 % – 95 ± 10 %) for the control group and 11 % (94 ± 11 % – 106 ± 15 %) for the experimental group. Vastus lateralis showed a pre versus post difference (Figure 9.a and Figure 9.b) (P<0.0509) for both groups.

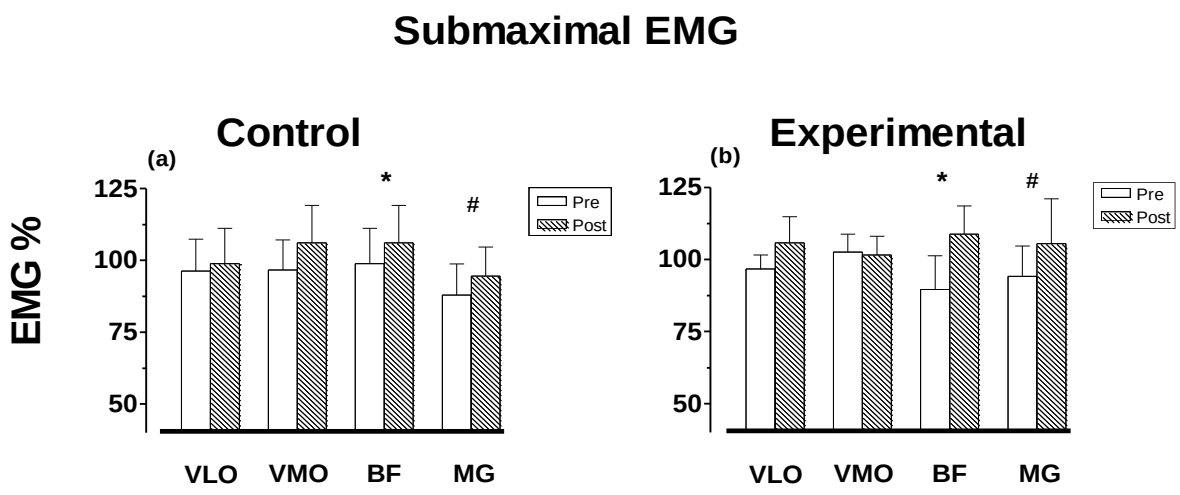

Figure 9.

Figure 9: *Pre and post intervention preactivation recorded in four muscles [vastus lateralis obliques (VLO), vastus medialis obliques (VMO), biceps femoris (BF), medial gastrocnemius (MG)] during constant velocity submaximal run for control (figure 9.a) and experimental (figure 9.b).* ******* *P < 0.05, Main effect pre vs post in both groups for muscle (BF). # P < 0.04, Main effect pre vs post in both groups for muscle (MG).*

Preactivation during 5K

Preactivation was analyzed separating km 1 to 4 (Figure 10.a to h) and constant velocity sprint (Figure 11.a to f) during km 5 for the four muscles (VLO, VMO, BF and MG). There was a significant variance (Figure 10.c and Figure 10.d) (P<0.009) in the experimental group's pre versus post VMO. There were no differences between groups or condition for all three other muscles (VLO, BF and MG). There was a significant time difference effect per km (Figure 10.g and Figure 10.h) (P<0.03) for both groups, with km 3 and 4 being significantly different to km 1 in the MG muscle.

Analysis of the constant velocity sprint during the last 40 meters of the 5K (Figure 11.a to f) showed that there were no significant interactions for groups and conditions in muscle VLO, BF and MG. There was a significant difference in preactivation in pre versus post (Figure 11a to d) (P<0.0003) for VMO, however both groups responded similarly with a decrease in preactivation post 5K. There were no differences in recorded preactivation between groups during pre intervention - after sprint, and post intervention - after sprint (Figure 11.e and figure 11.f).

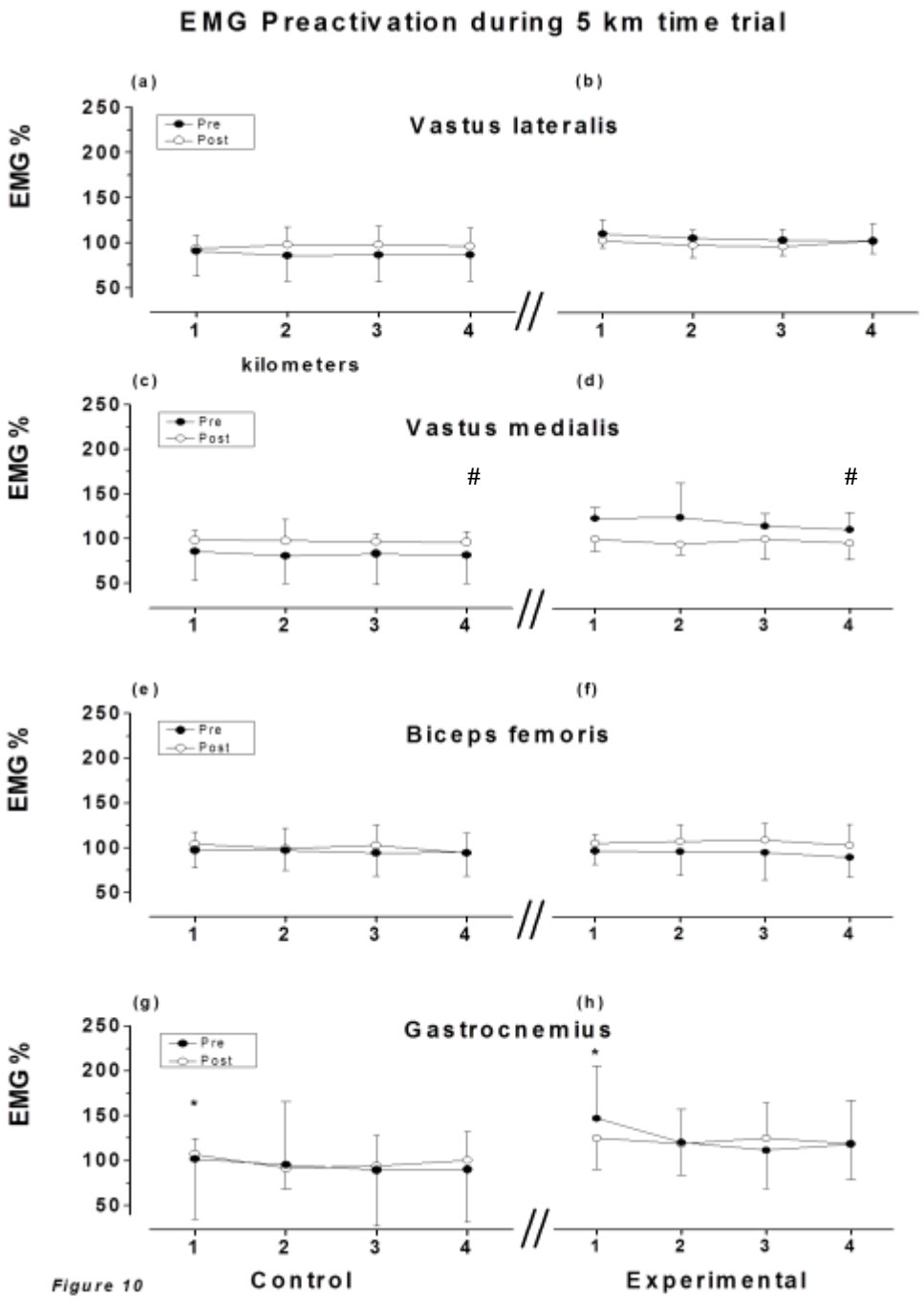

Figure 10

Figure 10: *Preactivation of four muscles during km 1 to 4 of 5 kilometer time trial (5K), (a) control group vastus lateralis obliques (VLO), (b) experimental group VLO, (c) control group vastus medialis obliques (VMO), (d) experimental group VMO, (e) control group biceps femoris (BF), (f) experimental BF, (g) control group medial gastrocnemius (MG) and (h) experimental group MG. # P<0.009, Interaction of experimental group pre-post (10.c and 10.d).* ******* *P<0.03, Time effect for km 1 vs km 3 and km 4 (10.g and 10.h).*

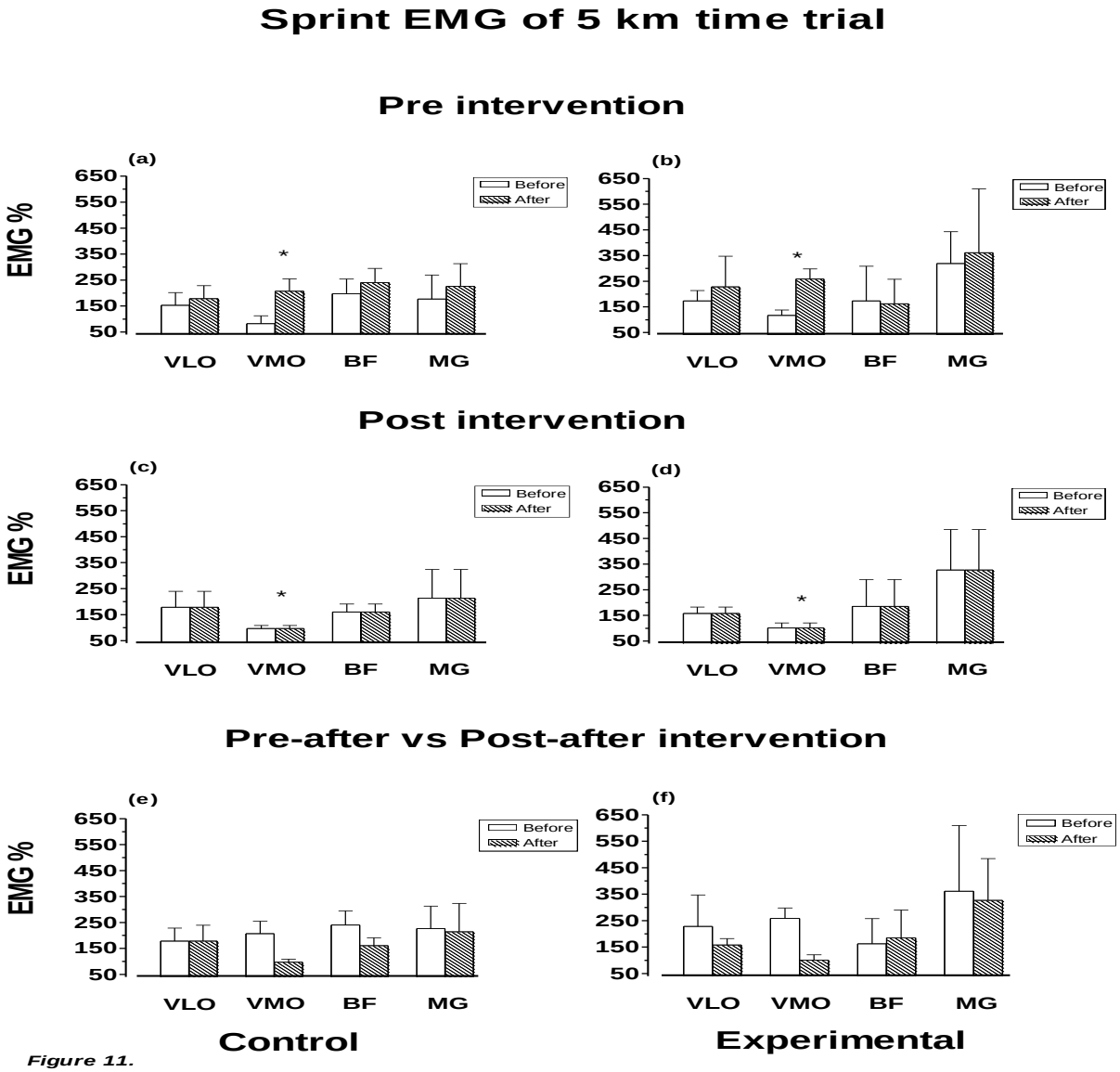

Figure 11.

Figure 11: *Preactivation measured in four muscles [vastus lateralis obliques (VLO), vastus medialis obliques (VMO), biceps femoris (BF), medial gastrocnemius (MG)], during pre-sprint and post-sprint of 5 km time trial, pre intervention (Figure 11.a and Figure 11.b), post intervention (Figure 11.c and Figure 11.d) and pre-after sprint (before intervention) vs post-after sprint (after intervention) (Figure 11.e and Figure 11.f).*

** P < 0.003, Significant difference in VMO main effect pre vs post intervention.*

Discussion

The first important finding was that the research design of this study was appropriate to study the interaction between fatigue and muscle damage during submaximal and maximal running. In this study, there was evidence to suggest that muscle damage occurred in the experimental group as a result of the downhill intervention. This was supported by both subjective pain scores for daily living (Figure 3.e and Figure 3.f) and increased plasma creatine kinase activity (Figure 3) in the experimental group. This suggests that both groups reacted differently to the intervention, with the experimental group incurring muscle damage as a result of the downhill intervention.

The data also suggested that fatigue had taken place in both groups. This was supported by a significant main effect of time for RPE (Figure 5 and Figure 6) increasing in both groups during submaximal and maximal running. A possible explanation for the observed increase in EMG in both BF and MG during the post intervention submaximal run (Figure 9.a and Figure 9.b) for both groups, was that there may have been altered recruitment as a result of fatigue [47-49]. This interpretation was based on the assumption that if there was an increased EMG activity and by implication muscle recruitment for the same power output during a constant velocity submaximal run, then there must have been a degree of fatigue occurring.

There was also evidence to suggest that fatigue had also taken place during the 5K run. This was supported by a significant increase in RPE (Figure 6.a and Figure 6.b) in both groups after each kilometer throughout the 5K. Previous studies [1,3] investigating fatigue over a 5K illustrated a decreased preactivation in VLO, VMO and MG. This study supported this idea through a significant decrease in preactivation in the MG during the 5K run. However, both control and experimental groups reacted the same, which suggested that there were no differences in neural regulation during the preactivation phase as a result of fatigue and muscle damage.

In the next phase of this study, the theory that exercise-induced muscle damage causes neuromuscular compensation to attenuate a decrease in performance was tested, and this was separate to the effects of fatigue.

The main finding of this study was that there was no evidence to support any interaction between altered neuromuscular activity with regard to fatigue and muscle damage during

submaximal (Figure 9) and maximal (Figure 10) running. Studies investigating exercise induced muscle damage have intimated that preactivation of the muscle does not respond to muscle damage the same way as it does to fatigue [17,18]. Neuromuscular compensation following exercise induced muscle damage has previously been proposed as a protective mechanism against a decrement in performance [11,12,28,34]. In this study, contrary to initial expectations, performance over a 5K was improved by an average reduction in time of 40 seconds in the experimental group. This effect was statistically significant ($P<0.04$) and practically relevant as all the subjects were experienced runners. This study therefore supported the findings of an attenuated decrease in performance after exercise predominated by muscle lengthening actions; however, it suggested that this mechanism may have its origin apart from neuromuscular regulation.

This was supported through the similar change in preactivation for both the control group and experimental group during the 5 kilometer time trial. Should neuromuscular compensation have occurred to prevent a decrease in performance after muscle damage, one would have expected a significant difference in preactivation between the 'fatigued' control group and the 'muscle damaged' experimental group. This was however contrary to the findings of this study, which showed a significant interaction effect in 5K performance between group and condition (pre 5K - post 5K), with no evidence of altered neuromuscular activity. The only significant interaction in neuromuscular preactivation during maximal exercise was seen in the vastus medialis obliques, which showed a decrease in preactivation during the 5K in the experimental group after intervention, and therefore could not have accounted for the improvement in performance.

The precise mechanism therefore for the attenuated decrease in performance following muscle lengthening exercise remains unclear. This study suggested that there was some other mechanism beyond neuromuscular regulation that was responsible for the differences seen in performance. In the understanding of the mechanistic regulation of muscle functioning, and that the change in muscle function is occurring distal to the action potential, this would suggest that the change in muscle function may be related to changes in calcium dynamics, and would therefore not alter EMG activity. An increase in either calcium release, or calcium uptake by the sarcoplasmic reticulum, would lead to an improved muscle function [50]. A limitation to this study is that no metabolic or hormonal changes were measured, and thus such a mechanism is speculation.

Other possible factors that may contribute to altered performance following muscle lengthening activities may include altered muscle-tendon properties or running biomechanics [51]. Alterations in muscle-tendon stiffness regulation play an important role in the reduction of muscle force and power [32,33] and a decrease in running efficiency [1]. One could speculate as to the possibility of muscle lengthening exercise causing altered muscle-tendon stiffness which distributes an increased transfer of energy to the tendon unit, thus sparing already damaged muscle fibers. An increase in tendon elastic recoil during running, could explain an improvement in performance without alterations to EMG. Stride parameters such as a change in stride length and ground contact time have been shown to change over a 5 kilometer time trial [3], and therefore may contribute to a change in running efficiency. Future studies should investigate the interaction effect of these variables and muscle damage and fatigue.

The third major finding of this study was the significant decreased RPE (Figure 6b) observed in both groups during both the submaximal and 5K post intervention. This effect occurs in the experimental group despite the presence of muscle damage and a significant improvement in running performance over a 5 kilometer time trial, and therefore suggests that there may be some variable that is both altered through and unique to muscle lengthening exercise. Further research into the possible mechanisms that may result in a decrease in RPE after muscle lengthening exercise which causes muscle damage should be investigated.

Based on the assumption that heart rate during a submaximal constant velocity bout of exercise decreases as aerobic fitness improves [52], the fourth finding of this study was that there was a tendency for physiological adaptions to occur within 5 days of applied stressor, as shown by the decrease in heart rate (Figure 7) and RPE (Figure 5 and Figure 6) in both groups after intervention.

Conclusion

In conclusion, this study showed that this research design was effective in studying the interaction between fatigue and muscle damage during submaximal and maximal running. Using this study design, there was an improvement in performance over a 5K in the presence of muscle damage. This improvement in performance occurred with no consistent neuromuscular changes, suggesting that muscle recruitment was not altered.

References of Manucript

1. L. M. Paavolainen, A. T. Nummela, and H. K. Rusko, Neuromuscular characteristics and muscle power as determinants of 5-km running performance. Med. Sci. Sports Exerc. 31, 124-130 (1999).
2. L. Paavolainen. Neuromuscular characteristics and muscle power as determinants of running performance in endurance athletes. 1999. University of JYVÄSKYLÄ. Thesis/Dissertation
3. K. Sharwood. The effects of endurance training on neuromuscular characteristics in masters runners. 2003. Thesis/Dissertation
4. J. Brewer, C. Williams, and A. Patton, The influence of high carbohydrate diets on endurance running performance. Eur. J. Appl. Physiol Occup. Physiol 57, 698-706 (1988).
5. M. J. Joyner, Modeling: optimal marathon performance on the basis of physiological factors. J. Appl. Physiol 70, 683-687 (1991).
6. D. R. Bassett, Jr. and E. T. Howley, Maximal oxygen uptake: "classical" versus "contemporary" viewpoints. Med. Sci. Sports Exerc. 29, 591-603 (1997).
7. A. V. Hill and H. Lupton, Muscular exercise, lactic acid, and the supply and utilization of oxygen. Quarterly Medical Journal 16, 135-171 (1923).
8. D. R. Bassett, Jr. and E. T. Howley, Limiting factors for maximum oxygen uptake and determinants of endurance performance. Med. Sci. Sports Exerc. 32, 70-84 (2000).
9. T. D. Noakes, J. E. Peltonen, and H. K. Rusko, Evidence that a central governor regulates exercise performance during acute hypoxia and hyperoxia. J Exp. Biol. 204, 3225-3234 (2001).
10. R. G. Eston, S. Finney, S. Baker, and V. Baltzopoulos, Muscle tenderness and peak torque changes after downhill running following a prior bout of isokinetic eccentric exercise. J. Sports Sci. 14, 291-299 (1996).
11. T. Hortobagyi, J. Barrier, D. Beard, J. Braspennincx, P. Koens, P. DeVita, L. Dempsey, and J. Lambert, Greater initial adaptations to submaximal muscle lengthening than maximal shortening. J. Appl. Physiol 81, 1677-1682 (1996).
12. T. Hortobagyi, J. P. Hill, J. A. Houmard, D. D. Fraser, N. J. Lambert, and R. G. Israel, Adaptive responses to muscle lengthening and shortening in humans. J. Appl. Physiol 80, 765-772 (1996).

13. A. Gollhofer, P. V. Komi, N. Fujitsuka, and M. Miyashita, Fatigue during stretch-shortening cycle exercises. II. Changes in neuromuscular activation patterns of human skeletal muscle. Int. J. Sports Med. 8 Suppl 1, 38-47 (1987).
14. R. M. Enoka and D. G. Stuart, Neurobiology of muscle fatigue. J. Appl. Physiol 72, 1631-1648 (1992).
15. T. C. Chen, Effects of a second bout of maximal eccentric exercise on muscle damage and electromyographic activity. Eur. J. Appl. Physiol 89, 115-121 (2003).
16. A. J. Fuglevand and D. A. Keen, Re-evaluation of muscle wisdom in the human adductor pollicis using physiological rates of stimulation. J. Physiol 549, 865-875 (2003).
17. C. Chambers, T. D. Noakes, E. V. Lambert, and M. I. Lambert. Time course of recovery of vertical jump height and heart rate versus running speed after a 90-km foot race. Journal of Sports Sciences 16[7], 645-651. 1998. E & FN Spon Ltd.
18. C. Byrne and R. Eston, The effect of exercise-induced muscle damage on isometric and dynamic knee extensor strength and vertical jump performance. J. Sports Sci. 20, 417-425 (2002).
19. A. Gollhofer, D. Schmidtbleicher, and V. Dietz, Regulation of muscle stiffness in human locomotion. Int. J. Sports Med. 5, 19-22 (1984).
20. A. Mero, P. V. Komi, and R. J. Gregor, Biomechanics of sprint running. A review. Sports Med. 13, 376-392 (1992).
21. L. Paavolainen, A. Nummela, H. Rusko, and K. Hakkinen, Neuromuscular characteristics and fatigue during 10 km running. Int. J. Sports Med. 20, 516-521 (1999).
22. V. Dietz, D. Schmidtbleicher, and J. Noth, Neuronal mechanisms of human locomotion. J. Neurophysiol. 42, 1212-1222 (1979).
23. A. Gollhofer, P. V. Komi, M. Miyashita, and O. Aura, Fatigue during stretch-shortening cycle exercises: changes in mechanical performance of human skeletal muscle. Int. J. Sports Med. 8, 71-78 (1987).
24. T. Hortobagyi and P. DeVita, Muscle pre- and coactivity during downward stepping are associated with leg stiffness in aging. J. Electromyogr. Kinesiol. 10, 117-126 (2000).
25. T. Moritani, L. Oddson, and A. Thorstensson, Electromyographic evidence of selective fatigue during the eccentric phase of stretch/shortening cycles in man. Eur. J. Appl. Physiol Occup. Physiol 60, 425-429 (1990).

26. O. Aura and P. V. Komi, Effects of prestretch intensity on mechanical efficiency of positive work and on elastic behavior of skeletal muscle in stretch-shortening cycle exercise. Int. J. Sports Med. 7, 137-143 (1986).
27. B. Libet, C. A. Gleason, E. W. Wright, and D. K. Pearl, Time of conscious intention to act in relation to onset of cerebral activity (readiness-potential). The unconscious initiation of a freely voluntary act. Brain 106 (Pt 3), 623-642 (1983).
28. R. M. Enoka, Eccentric contractions require unique activation strategies by the nervous system. J. Appl. Physiol 81, 2339-2346 (1996).
29. D. G. Allen, Eccentric muscle damage: mechanisms of early reduction of force. Acta Physiol Scand. 171, 311-319 (2001).
30. J. Avela, P. M. Santos, and P. V. Komi, Effects of differently induced stretch loads on neuromuscular control in drop jump exercise. Eur. J. Appl. Physiol Occup. Physiol 72, 553-562 (1996).
31. A. Mero and P. V. Komi, Electromyographic activity in sprinting at speeds ranging from sub-maximal to supra-maximal. Med. Sci. Sports Exerc. 19, 266-274 (1987).
32. J. Avela, H. Kyrolainen, P. V. Komi, and D. Rama, Reduced reflex sensitivity persists several days after long-lasting stretch-shortening cycle exercise. J. Appl. Physiol 86, 1292-1300 (1999).
33. T. Horita, P. V. Komi, C. Nicol, and H. Kyrolainen, Interaction between pre-landing activities and stiffness regulation of the knee joint musculoskeletal system in the drop jump: implications to performance. Eur. J. Appl. Physiol 88, 76-84 (2002).
34. P. A. Tesch, G. A. Dudley, M. R. Duvoisin, B. M. Hather, and R. T. Harris, Force and EMG signal patterns during repeated bouts of concentric or eccentric muscle actions. Acta Physiol Scand. 138, 263-271 (1990).
35. D. Kay, G. A. St Clair, M. J. Mitchell, M. I. Lambert, and T. D. Noakes, Different neuromuscular recruitment patterns during eccentric, concentric and isometric contractions. J. Electromyogr. Kinesiol. 10, 425-431 (2000).
36. L. Schutte and M. I. Lambert, Delayed-onset Muscle Soreness: Proposed Mechanisms, Prevention, and Treatment. Int. J. Sports Med. 2, 1-4 (2001).
37. W. D. Ross and M. J. Marfell-Jones, Kinanthropometry. In Physiological testing of the high-performance athlete ed. by J. D. MacDougall, H. A. Wenger, and H. S. Green, pp 223-308. Human Kinetics Books, Champaign, (1991).

38. J. V. Durnin and J. Womersley, Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br. J. Nutr. 32, 77-97 (1974).
39. V. L. Katch and F. I. Katch, A simple anthropometric method for calculating segmental leg limb volume. Research Quarterly American Association for Health and Physical Education 45, 211-214 (1974).
40. W. J. Kraemer and A. C. Fry, Strength Testing: Development and evaluation of Methodology. In Physiological Assessment of Human Fitness ed. by P. J. Maud and C. Foster, Champaign, Illinois (1995).
41. T. D. Noakes, K. H. Myburgh, and R. Schall, Peak treadmill running velocity during the VO2 max test predicts running performance. J. Sports Sci. 8, 35-45 (1990).
42. G. Borg, Borg's perceived exertion and pain scales. In Human Kinetics Champaign (IL), (1998).
43. S. Sorichter, A. Koller, C. Haid, K. Wicke, W. Judmaier, P. Werner, and E. Raas, Light concentric exercise and heavy eccentric muscle loading: effects on CK, MRI and markers of inflammation. Int. J. Sports Med. 16, 288-292 (1995).
44. L. V. Billat and J. P. Koralsztein, Significance of the velocity at VO2max and time to exhaustion at this velocity. Sports Med. 22, 90-108 (1996).
45. D. L. Costill, H. Thomason, and E. Roberts, Fractional utilization of the aerobic capacity during distance running. Med. Sci. Sports 5, 248-252 (1973).
46. C. Foster, D. L. Costill, J. T. Daniels, and W. J. Fink, Skeletal muscle enzyme activity, fiber composition and VO2 max in relation to distance running performance. Eur. J Appl. Physiol Occup. Physiol 39, 73-80 (1978).
47. G. Hellsing and L. Lindstrom, Rotation of synergistic activity during isometric jaw closing muscle contraction in man. Acta Physiol Scand. 118, 203-207 (1983).
48. S. C. Gandevia, Spinal and Supraspinal Factors in Human Muscle Fatigue. Physiol. Rev. 81, 1725-1789 (2001).
49. I. Zijdewind, M. J. Zwarts, and D. Kernell, Fatigue-associated changes in the electromyogram of the human first dorsal interosseous muscle. Muscle Nerve 22, 1432-1436 (1999).
50. D. A. Jones and J. M. Round, Skeletal muscle in health and disease: A textbook of muscle physiology, Manchester University Press, Manchester, UK (1990).

51. C. L. Brockett, D. L. Morgan, and U. Proske, Human hamstring muscles adapt to eccentric exercise by changing optimum length. Med. Sci. Sports Exerc. 33, 783-790 (2001).
52. J. H. Wilmore, P. R. Stanforth, J. Gagnon, A. S. Laon, D. C. Rao, J. S. Skinner, and C. Bouchard, Endurance exercise training has a minimal effect on resting heart rate: The HERITAGE Study. Med. Sci. Sports Exerc. 28, 829-835 (1996).