Title
Sand training: Exercise-induced muscle damage and inflammatory responses to matched-intensity exercise

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Abstract

This study compared the levels of muscle damage and inflammation caused by a matched-intensity interval running session on soft sand and grass surfaces. In a counterbalanced, repeated measures and crossover design, 11 well-trained female athletes completed two interval-based running sessions one week apart on either a grass or a sand surface with exercise heart rate (HR) fixed at 83-88% of HR maximum. Venous blood samples were collected pre-, post- and 24 h post-exercise, and analysed for myoglobin (Mb) and C-reactive protein (CRP). Ratings of perceived exertion (RPE) and perceptual ratings of muscle soreness (DOMS) were recorded immediately post- and 24 h post-exercise. A significant time effect showed that Mb increased from pre- to post-exercise on grass (p=0.003) but not on sand (p=0.759). A significant interaction effect (p=0.023) showed a greater relative increase in Mb on grass compared to sand (p=0.007). No main effects (p>0.05) were seen for CRP differences between surfaces (p>0.05); however, a large effect size (d=1.35) suggested a tendency for a smaller relative change in CRP on sand compared to grass. However, RPE and DOMS scores were not significantly different between conditions. These results suggest that the level of muscle damage incurred from a matched-intensity running session can be significantly reduced by running on soft sand as compared to grass. Coaches and sport science practitioners may be able to use this information to minimize musculoskeletal stress while still incurring an equivalent cardiovascular training stimulus.

Key Words: Sand Training, Muscle Damage, Inflammation, Team Sport.
INTRODUCTION

Previous research relating to exercise on soft beach sand has focused on the decreased impact forces associated with this ground surface [1-3], and on the energetics required to move [4-6], with comparisons drawn against more traditional ground surfaces such as grass. Additionally, recent studies have used soft sand training with team sport athletes to determine the potential application for enhanced conditioning purposes [7-9].

It is well established that sand running requires ~1.2-1.6 times the energy cost (EC) of running on firm surfaces such as grass at similar speeds [5, 10]. Several factors may cause this increased EC, including reduced elastic energy recovery, decreased muscle-tendon efficiency, and increased work lost to the environment [4, 6]. Additionally, it has also been shown that there is significantly greater electromyographic activation of the lower limb muscles when running on sand compared to grass, due to increased stabilisation demands around the ankle, knee and hip joints [11]. The higher EC of sand running has also resulted in greater lactate accumulation compared to running on firm surfaces, suggesting a large anaerobic contribution to the EC of sand running, despite the relatively low velocities recorded (<13 km/h) in these studies [10].

In addition to EC differences, the low surface stiffness of sand may also reduce the ground reaction forces encountered at foot-strike [1]. With this in mind, lower levels of exercise-induced muscle damage have been recorded after sand training, as compared to firm surfaces [2], resulting in reduced next day muscle soreness ratings and performance decrements [2, 3]. However, other research found no significant differences in markers of muscle damage following interval running or sport-specific conditioning sessions conducted on sand or grass [7, 8]. Although these results suggest that the low impact forces on sand do not translate into decreased muscle damage during team sport-specific training sessions, importantly, exercise intensity was not controlled for in these investigations. In fact, these authors showed that increases in myoglobin, haptoglobin and C-reactive protein concentrations
were similar, despite a 1.1-1.6 fold increase in training intensity on sand, as compared to grass [7, 8]. Consequently, it is possible that if training intensity had been controlled for in these studies, then the resultant muscle damage markers may have also been lower, which may show sand to be an appropriate low-impact training surface for recovery-based running training or other applications where minimizing musculoskeletal stress is a primary goal. As such, the aim of this study was to assess the impact of sand as compared to grass running on markers of muscle damage, inflammation and perceptual soreness/recovery via a controlled, matched-intensity training session performed on each surface.

METHODS

Experimental approach to the problem

Participants initially completed two sessions (one per week) of sand familiarisation training. These sessions started with light jogging and walking, and increased to higher intensity activity, similar to that performed during the ensuing experimental trials. All participants therefore had equal exposure to sand training before commencing testing. Subsequently, participants completed two experimental trials, each separated by 7 days in a counterbalanced, repeated measures and crossover study design. Each trial encompassed an interval-based running session performed on either a sand or a grass ground surface (in alternate weeks), with sessions matched for time and intensity. Each session occurred at the same time of day to control for environmental conditions and circadian variation.

Subjects

Eleven well-trained female team sport athletes (Age: 18 ± 2 y, Height: 172.0 ± 10.3 cm, Mass: 68.5 ± 10.4 kg, $\sum_7$ skinfolds: 90.7 ± 12.7 mm) were recruited from the Western Australian Institute of Sport Netball and Field Hockey squads to participate in this study. Before testing commenced, written consent was obtained from all participants. This study was approved by the Human Ethics Committee of the University of Western Australia (RA/4/1/6826), adhering to the declaration of Helsinki.

Procedures
The interval-based running session commenced with a 10 min warm-up, comprising a 6 min continuous jog at a self-selected low to moderate intensity, followed by 2 min of static stretching (2 x 20 s on each of the major lower limb muscle groups) and 2 min of dynamic walk-through exercises. Participants then completed the interval running session, comprising 2 x 3 min efforts with 2 min active rest, 3 x 2 min efforts with 1 min active rest, and 4 x 1 min efforts with 30 s active rest. The intensity of running bouts was matched by heart rate (HR), maintaining a running speed equivalent of 83-88% HR$_{\text{max}}$. The HR$_{\text{max}}$ was taken from the athletes’ most recent (within 4 weeks) 20 m shuttle run test, performed as part of their regular training cycle. The intensity for work intervals was determined in pilot testing through the assessment of the most practically applicable running speed on sand as the lowest speed at which participants could maintain good running form. A 6 min cool-down, comprising 3 min of walking and 3 min of static stretching concluded each trial.

Throughout each session, HR was monitored by both the athlete and the researcher via short range radio telemetry (Polar Team Sport 2 System, Polar Electro Oy, Kempele, Finland) with immediate feedback given to ensure that athletes stayed within the prescribed HR zone. Additionally, athletes wore a global position system (GPS) tracking unit (Catapult Minimaxx S4 10 Hz) to measure distance covered and movement speed. Data was extracted using Catapult Sprint software (Catapult Sports, Victoria, Australia) and incorporated data from both the interval running efforts and active rest periods. After each session, participant’s gave a rating of Perceived Exertion (RPE) using the Borg perceptual scale, encompassing the anchor points 6 (no exertion) to 20 (maximal exertion) [12].

Forearm venous blood samples were collected pre-, immediately post-, and 24 h post-training for markers of muscle damage (Myoglobin; Mb) and inflammation (C-Reactive Protein; CRP). All samples were collected after 5 min of supine rest using a 21-gauge needle into an 8.5ml SST II Gel separator tube (BD Vacutainer, Australia). Samples were allowed to clot for 60 min at room temperature, before being centrifuged at 10˚C and 3000 rpm for 10 min. The serum supernatant was divided into 1 ml aliquots and stored at -80˚C until further analysis. Serum Mb levels (pre- and post-training) were determined using an enzyme-linked immunosorbent assay (ELISA) kit for human Mb.
(Genway Biotech, Inc., San Diego, USA Lot number: 6Q1) using a BMG Labtech FLUOstar OPTIMA plate reader with a CV <10%. Serum CRP levels (pre- and 24 h post-training) were measured using an Architect analyser (c16000) and determined using a CRP Vario Reagent (Abbott Diagnostics, Abbott Laboratories, Abbott Park, IL 60064, USA). The CV for CRP determination at 5.89 and 24.76 mg/L was 2.08 and 2.03%, respectively.

At 24 h post-training, delayed onset muscular soreness (DOMS) was measured via a muscle soreness questionnaire [7], which had participants identify the degree of perceived muscle soreness of different muscle areas (quadriceps, gluteals, hamstrings, triceps surae, tibialis anterior/peroneals) on a scale from 0 (normal; without stiffness or pain) to 10 (very painful). A score was recorded for each muscle area, and then combined to give an overall muscle soreness rating for each trial.

Participants maintained normal training schedules prior to testing days, but did not engage in intensive exercise, use any recovery modalities (e.g. cold water immersion, massage, etc.) or consume alcohol or caffeine for 24 h both prior to, and after each testing session. Participants attended all sessions in a well-rested state, and recorded (and then replicated) their nutritional habits during the 24 h preceding the first experimental session, and also during the 24 h follow-up period. Water was allowed ad libitum throughout each session, up to a maximum of 600 ml. Sand sessions were completed on a level area of soft, dry beach sand away from the waters’ edge. All running sessions were conducted around a continuous circular route to control for number of decelerations/accelerations. Grass sessions were completed on a well-maintained grass sporting ground. Sand training sessions were performed barefoot, whereas running shoes were worn on the grass. Surface stiffness for grass and sand trials was determined before each experimental session using a 2.25 kg Clegg impact hammer (SD Instrumentation, Wiltshire, United Kingdom) dropped from a height of 0.457 m. Peak deceleration forces (Newtons [N]) of each training surface were calculated in accordance with methods used previously [5, 13, 14].

**Statistical Analyses**
Statistical Analyses

Determination of the sample size was attained via a power analysis using customized computer software (GPOWER Version 3.1, Department of Psychology, Bonn University, Bonn Germany). The effect sizes used to generate the sample size estimation were attained from the serum myoglobin responses to an interval-based running session on sand and grass surfaces (Binnie, Dawson, et al., 2013a, 2013b). Based on such data, the power analysis for a-priori: two-way ANOVA for a within and between group analysis suggested a sample size of 8 (critical F-value: 3.15; expected power: 0.96; alpha-level: p<0.05). Results are expressed as mean and standard deviation (±SD). Data normality was assessed via a Shapiro-Wilk test. The CRP data was found to be non-normally distributed and was analyzed using non-parametric statistics. Differences in HR, RPE, DOMS, GPS variables and surface stiffness were determined via a one-way ANOVA. The effect of the two training surfaces (sand and grass) on the subsequent pre- and post-exercise Mb responses was quantified by a two-way repeated measures ANOVA. Post-hoc (Fisher's LSD) paired samples t-tests were subsequently applied where appropriate to determine specific differences between the surfaces. Differences in the relative change of Mb was compared between conditions using a one-way ANOVA. Finally, the effect of the two training surfaces (sand and grass) on the subsequent pre- and post-exercise CRP responses (and the relative change) was quantified via a Wilcoxon signed-rank test. Significance was set at p<0.05.

RESULTS

Surface stiffness measures showed peak impact deceleration forces on sand (286.1 ± 137.1 N) were significantly lower (p=0.001) than for grass (1090.0 ± 94.2 N).

The distance covered in running intervals was significantly lower (p=0.001) on sand (1963 ± 288 m) compared to grass (2881 ± 487 m). Also, mean running speed was significantly lower (p=0.001) on sand (5.31 ± 0.78 km/h) compared to grass (7.78 ± 1.32 km/h).
Training responses for HR, RPE and DOMS were 163 ± 8 bpm, 11 ± 1 and 10 ± 5 on sand, and 164 ± 7 bpm, 11 ± 1 and 12 ± 6 on grass, respectively. No significant differences were found for HR (p=0.896), RPE (p=0.695) or DOMS (p=0.237) between the sand and grass trials.

A significant time effect (p=0.003) showed that Mb changed from pre- to post-exercise. Post-hoc analysis showed this increase occurred after training on grass only (p=0.003), and not on sand (p=0.759) (Figure 1). A significant interaction (p=0.023) was also evident, with the relative change in Mb levels showing a significantly greater (p=0.007) increase after grass training, as compared to sand.

Figure 1 here

No significant time (p=0.887) or interaction (p=0.433) effect was evident between or within each training surface for CRP responses (Figure 2). Additionally, there were no differences in the relative change of CRP from pre- to post-exercise (p=0.444). However, a large effect size (d=1.35) suggested a tendency for the relative change in CRP to be greater on grass as compared to sand.

Figure 2 here

DISCUSSION

The results of this investigation showed that running on soft sand has the potential to decrease exercise-induced muscle damage and inflammation. This may be achieved while attaining an equivalent aerobic training stimulus compared to a similar session performed on a more traditional training surface such as grass.

During this study, exercise intensity was closely matched between the sand and grass training surfaces, with no differences in HR or RPE evident between trials. This experimental design is unique when compared to previous sand running investigations that have matched running speed [4, 6, 11], and/or prescribed work intervals [7, 8], seeking to demonstrate that significantly higher training
intensities are attainable on sand versus grass surfaces for an equivalent absolute exercise output. When relative intensity is considered however, the rise in myoglobin from pre- to post-training was only significant following the grass session. Such findings may suggest that a greater degree of muscle damage occurred during exercise on grass as compared to sand. Since the training surface was the only variable manipulated in this study, the different surface conditions encountered may explain the difference in these post-exercise blood markers. Such an outcome may be attributed to the shock absorbing qualities of soft sand, which could potentially reduce the amount of stress placed on the muscle-tendon complex during impact with the ground surface [2]. In support of this, the surface stiffness measures for the sand and grass venues used here showed that peak impact deceleration forces were approximately 3.8 times greater on the grass. These findings are similar to previous investigations, which have also reported decreased deceleration forces associated with sand training, thus having the potential to reduce muscle damage [5, 7, 11].

An alternative explanation for our results might be that there was a lower absolute running speed and distance covered in the sand trial of the current investigation (sand: 1962 m vs. grass: 2881 m), and that the higher velocity in the grass session may have resulted in a greater number of ground contacts that could have increased the degree of muscle damage incurred. Although possible, this investigation was primarily focused on matching exercise intensity, and therefore, even if a greater degree of muscle damage was the result of altered running kinematics, an attenuated blood marker response for an equivalent training intensity stimulus was still the primary outcome on the sand.

In contrast to the post-exercise muscle damage response, there was no significant change in CRP from pre- to 24 h post-training on either surface, suggesting no substantial increases in inflammation. This result differs from previous team sport investigations, where significant increases in CRP are seen from pre- to 24 h post-training on both sand and grass surfaces [7, 8]. Our findings may be attributed to the lower relative intensity and shorter duration of the training session examined here, which may not have provided sufficient stimulus to produce a significant inflammatory response. Despite this, a large effect (d=1.35) suggested a tendency for a greater relative increase in CRP levels following
grass training compared to sand. Therefore, similar to the post-exercise muscle damage response, it is possible that the low impact nature of the sand surface acted to negate the rise in inflammation incurred from the training session. With this in mind, the blood markers measured here may suggest that a sub-maximal aerobic training session performed on sand (as opposed to grass) may minimise both the degree of muscle damage experienced and the subsequent inflammatory response; however, it remains to be seen whether these results would still be present at higher running speeds, training intensities, and/or session durations.

In addition to these blood markers, the athletes’ perceptual ratings of lower limb muscle soreness (DOMS) were also measured at 24 h post-training, with no differences between the two trials recorded. The results from the aforementioned team sport studies [7, 8] showed similar post-exercise DOMS ratings between surfaces despite significantly higher training intensities on sand, suggesting a lesser degree of perceived muscle soreness on the sand surface. Furthermore, results from a recent longitudinal training study showed moderate effect sizes to suggest lower ratings of muscular soreness resulting from team sport training on sand versus grass, despite relatively more total work in the sand training group [9]. However, in comparison to these studies, the DOMS measures recorded here were substantially lower on both surfaces, most likely due to the decreased duration and/or training intensity of the exercise bout. Given these lower relative DOMS scores, it is possible that the measure was not sensitive enough to detect any differences between surfaces. Alternatively, it has also been shown that running on sand versus grass at similar speeds requires a significantly greater activation of muscles that control hip and knee motion during the stance and swing phase of running gait, with the greatest differences observed at slower speeds [i.e. 8 km/h [11]]. This is due to the increased need for stability on sand, and also to account for the energy lost to the compliant training surface. As such, it is possible that a substantially higher degree of muscular engagement and activation on sand may have increased the athletes’ lower-limb muscle awareness and perception of fatigue, and in turn masked the aforementioned benefits indicated by the lower levels of muscle damage. As such, it is possible that this conflicting relationship of muscle damage and perceptual muscle soreness on sand
should be examined further, particularly at lower running speeds, where the significant increases in muscle activation on sand may somewhat limit the perceptual benefits of the soft training surface.

PRACTICAL APPLICATIONS

The results of this investigation suggest that using soft sand as a training surface has the potential to reduce the muscle damage and inflammation incurred from a training session compared to grass, when sessions are matched for intensity and duration. Practically, these findings could apply to an athlete returning from injury (or reduced training load) seeking to undertake a conditioning session, while minimising the degree of muscle damage experienced. As such, soft sand training may be a useful/alternative tool for sub-maximal aerobic conditioning. However, further research is needed to determine whether this effect between surfaces is still present at higher training intensities and/or durations.
REFERENCES


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Figure 1. Mean (±SEM) pre- and post- exercise (and relative change) serum myoglobin (Mb) concentrations for sand and grass trials.

* Significantly different to grass (p<0.05)

‡ Significantly different to pre-exercise (p<0.05)
Figure 2. Mean (±SEM) pre- and 24 h post- exercise (and relative change) serum C-reactive protein concentrations for sand and grass trials.