Post-activation potentiation increases recruitment of fast twitch fibers: a potential practical application in runners

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Abstract
To determine the relationship between fatigue and post-activation potentiation, we examined the effects of sub-maximal continuous running on neuromuscular function tests, as well as on the squat jump and counter movement jump in endurance athletes. The height of the squat jump and counter movement jump and the estimate of the fast twitch fiber recruiting capabilities were assessed in seven male middle distance runners before and after 40 min of continuous running at an intensity corresponding to the individual lactate threshold. The same test was then repeated after three weeks of specific aerobic training. Since the three variables were strongly correlated, only the estimate of the fast twitch fiber was considered for the results. The subjects showed a significant improvement in the fast twitch fiber recruitment percentage after the 40 min run. Our data show that submaximal physical exercise determined a change in fast twitch muscle fiber recruitment patterns observed when subjects performed vertical jumps; however, this recruitment capacity was proportional to the subjects’ individual fast twitch muscle fiber profiles measured before the 40 min run. The results of the jump tests did not change significantly after the three-week training period. These results suggest that pre-fatigue methods, through sub-maximal exercises, could be used to take advantage of explosive capacity in middle-distance runners.

Key words: squat jump, middle distance running, lactate threshold, fibers, recruitment

Introduction
Neuromuscular function tests, such as a squat, counter movement and drop jumps, maximal isometric voluntary contraction (MVIC) and isokinetic maximal concentric contractions (Abernethy et al., 1995; Cometti et al., 2011; Wilson and Murphy, 1996), are important tools for monitoring fatigue in athletes (Raeder et al., 2016; Sams et al., 2017); however, there is no standardized test to measure muscle fatigue in different sports (Baar and Mcgee, 2008). Several studies have shown a decrease in maximal voluntary contraction (MVC) after long duration physical activities such as marathons or ultra-marathons (Giovanelli et al., 2016; Millet et al., 2002; Millet and Lepers, 2004). Moreover, it is known that maximal strength is produced by the neuromuscular recruitment of both fast and slow twitch fibers (Bosco and Komi, 1979); therefore, measuring the MVC peak does not allow to discriminate among the relative contributions of different types of fibers. Bosco and Komi demonstrated that subjects with a high percentage of fast twitch fibers (FTF) in their lower limbs muscles achieved better results in vertical jump tests: the squat jump (SJ) and counter movement jump (CMJ) (Bosco and Komi, 1979; Bosco et al., 1983). Hence, the intervention of phasic units in the maximal execution of this ballistic exercise is dominant over tonic units. Moreover,
according to Bosco (1999), the SJ and CMJ, performed at the maximum power output, allow to obtain an indirect estimate of the percentage of FTF recruited, with an error of less than 5%.

The effect of fatigue induced by running exercises on jumping ability is not well understood. While some authors have reported an acute enhancement of jumping ability (Boullosa et al., 2011; García-Pinillos et al., 2015; Latorre-Román et al., 2014; Vuorimaa et al., 2006) after running protocols in runners, others (Boullosa and Tuimil, 2009) have not found post-activation potentiation (PAP) effects in non-runners after a similar stimulus.

The term of PAP refers to the significant enhancement of muscular twitch force after voluntary contractile activity (Mettler and Griffin, 2012), but the mechanism responsible for this muscle potentiation has not been fully elucidated (Wilson et al., 2013; Golaś et al., 2016). There are several suggested mechanisms behind PAP, including the recruitment of higher order motor units, an increase in the pennation angle, and the phosphorylation of myosin regulatory light chains (Healy and Comyns, 2017; Tillin and Bishop, 2009). PAP also appears to occur at the spinal level, through increased synaptic efficacy between Ia afferent terminals and α-motoneurons of the homonymous muscle (Hodgson et al., 2005). Regardless of the mechanism involved, the aim of incorporating PAP protocols into an athlete’s training program is to elicit an acute enhancement in performance.

There is also a lack of information on the mechanism responsible for the PAP phenomenon induced by running exercises in endurance runners. Among the studies that found PAP after submaximal exercise, Vuorimaa et al. (2006) showed that 40 min of running at a speed corresponding to 80% VO₂max produced significant improvement in power performance of elite long-distance runners. However, the authors gauged intensity by maximum oxygen uptake, without taking into account the individual lactate threshold of the subjects. In this regard, a study performed by Meyer et al. (1999) has shown that, due to the large variability of individual values of VO₂max and HR at the lactate threshold, for the control of elite athletes or for research purposes, it is important to calculate intensity using a percentage of the lactate threshold and not VO₂max or HRmax.

The first aim of our study was to evaluate vertical jumping ability and to estimate the percentage of FTF involved in the jump using the Bosco’s test (Bosco, 1999), before and after 40 min of running on a treadmill at an intensity equal to the speed of the individual lactate threshold, namely LT2 (4.0 mmol/l). The second aim was to test whether this effect changed after 3 weeks of specific training.

Methods

Experimental Approach to the Problem

This study tested the hypothesis that a standardized submaximal run at an intensity equal to the speed of LT2, able to affect a selective glycogen depletion in the STF (Seiler and Kjerland, 2006), could improve the FTF recruitment ability under pre-fatigued muscle conditions.

Subjects

Seven male athletes were enrolled, age 26.6 ± 6 years; body mass 66.7 ± 7 kg; body height 177.8 ± 8 cm, with training experience in the extended middle-distance events of 4 ± 1 years. The subjects provided written informed consent in accordance with the ethics committee of the University of Urbino. The study was approved by the Department of Biomolecular Science of the Urbino University and was conducted in agreement with the Declaration of Helsinki (1975).

Procedures

Each athlete was tested to determine his LT2 and individual training zones. Training intensity zones for each athlete were based on the following lactate concentration values: zone 1 (< LT1), < 2.0 mmol/L, zone 2 (> LT1 < LT2), > 2.0 and < 4.0 mmol/l, zone 3 (> LT2), > 4.0 mmol/l, as proposed by Seiler and Kjerland (2006).

The protocol to determine the LT2 consisted of a minimum of 5 and a maximum of 9 incremental 4 min stages. The intensity of the first step was calculated according to the individual athletic level, and the speed of the treadmill was increased by 1 km/h every 4 min as indicated in the guidelines (Winter, 2006). Lactate concentration was measured by micro-withdrawals from the fingertip of the forefinger with a Lactate Pro lactate meter (ARKRAY, Kyoto, Japan) (Baldari et al., 2009; Tanner et al., 2010) before the test and within the 30 s of the end of each stage. The running speed at LT2 (4.0 mmol/l) was determined using a validated algorithm by Bentley et al. (2007) and implemented using specifically designed software by Newell et al. (2007). Approximately 20 min after the lactic threshold tests, the athletes were shown the proper execution of
the SJ and CMJ. The athletes returned to the lab for the experimental phase of the study 48 hours after the initial meeting.

The subjects performed five SJs and CMJs, before ($t_0$) and 3 min after ($t_1$) the 40 min run on the treadmill at LT2 speed. Performance was measured using an Ergojump contact platform (Ergojump, Psion XP, MA.GI.CA., Rome, Italy) as described by Bosco et al. (1983). This Jump-mat system provides trustworthy measurements for monitoring changes in jump height (Pueo et al., 2016). Before performing the 40 min run at LT2, the athletes completed a 10 min warm up at a speed of 10 km/h. After 3 weeks of specific training, the SJ and CMJ tests were repeated before ($t_2$) and after ($t_3$) a 40 min run at LT2, newly defined using the same protocol as in the first test. Differences in speed at LT2 before and after the training period are shown in Figure 1.

**Statistical Analyses**

The measured variables, tested at the beginning of the experiment and after the three-week training period, were: height of the SJ and CMJ, estimate of FTF percentage recruitment (%FTF) (Bosco, 1992) using the height of the jump tests, running speed at LT2.

In order to evaluate the strength of the correlation among the SJ, CMJ and %FTF, regression analysis was performed. Since the variables SJ, CMJ and %FTF were strongly correlated (Figure 2), only the %FTF variable was considered.

The %FTF data are provided in a scatter plot: we calculated the linear regression from the values at $t_0$ and $t_1$, and the same was done for the values at $t_2$ and $t_3$. In order to highlight the significance of the differences between $t_0 - t_1$, and between $t_2 - t_3$, the statistic of the difference between the slopes and the intercepts of the regression lines was used. The slope of the regression line between $t_0$ and $t_1$ was then compared to the null hypothesis ($b = 1$) to determine if there was a significant change in the percentage of fast fibers recruited; the same procedure was performed for $t_2$ and $t_3$. Moreover, the two intercepts and the two slopes between the straight lines of linear regression $t_0 - t_1$ and $t_2 - t_3$ were compared (the first error type was set at 0.05). Finally, 2-tailed paired sample t-tests were used to determine differences in running speed at LT2 before and after the three-week training period.

**Results**

During the training period (3 weeks), each athlete had a total of 21 training sessions. Based on the total time-in-zone method, 73.7% (566 min) of all training time was spent below the LT1 intensity (zone 1), 9.8% (75 min) of endurance training time was between LT1 and LT2 (zone 2), and 16.5% (127 min) was over LT2 (zone 3). The training program is shown in Table 1, and training intensity distribution is shown in Figure 3.

After 3 weeks of training, the running speed at LT2 varied significantly: 14.1 ± 0.7 pre-training vs 14.6 ± 0.6 post-training ($p < 0.001$), which accounted for an increase of 3.9% (Figure 1). We did not find significant differences ($p > 0.05$) for the intercepts and the slopes or when comparing the two lines of linear regression $t_0 - t_1$ and $t_2 - t_3$. This means that after 3 weeks of training pre-post %FTF recruited did not change (Figure 4). With regard to the intercepts (expected value $a = 0$) (indicator of systematic increase), there were no significant differences between before and after 40 min of running, regardless of the training period ($t_0-t_1$ versus $t_2-t_3$). In contrast, the slopes are significantly different from $b = 1$ (in dashed line), between $t_0$ and $t_1$ there is a proportional increase rate of 29% ($p = 0.043$) and an increase rate of 14% between $t_2$ and $t_3$ ($p = 0.056$). In fact, in the latter case we are at the limit of significance, and this is probably due to the low sample size. It should be emphasized that the bivariate distributions considering $t_0$ and $t_1$ and $t_2$ and $t_3$ are strictly linear ($r = 0.97, r = 0.99$, respectively) (Figure 4).

**Discussion**

PAP has not been explored thoroughly enough in scientific literature, and the causes of this phenomenon have not yet been clarified. García-Pinillos et al. (2016) investigated whether kinematic data during the CMJ might explain the PAP phenomenon after an exhausting running test; they also hypothesized that improvements observed in CMJ performance despite high fatigue levels, might be related to the manner in which the athletes jumped (jumping kinematic). However, they concluded that the CMJ kinematics did not explain the PAP phenomenon after an exhausting running test in endurance runners and
rejected the hypothesis that kinematic variables were determinant of the PAP phenomenon in those subjects. Therefore, the causes of PAP must be further investigated under different experimental conditions.

The causes of loss of strength can be divided into central and peripheral causes. With regard to the peripheral causes, the depletion of muscle glycogen is certainly among the most important. It is known that prolonged exercise performed at submaximal intensity causes a selective depletion of muscle glycogen in proportion to the intensity of the exercise (Gollnick et al., 1974). In particular, Gollnick et al. (1974) clearly showed that differential rates of glycogen depletion occurred in human skeletal muscle fibers during exercise with workloads requiring less than 100% of VO2max: slow twitch fibers (STF) were the first to lose glycogen, whilst with supramaximal loads (over 100% VO2max), the depletion of glycogen occurred first in the FTF. In a more recent study, Krustup et al. (2004) demonstrated that previous exercise that induced STF glycogen depletion could be used as a model to enhance FTF recruitment during subsequent moderate-intensity exercise. Furthermore, current literature states that there is an increase in the activity of the central nervous system (CNS) proportional to the duration and intensity of the endurance exercise (Baar and Mcgee, 2008).

In light of these findings, we hypothesized that a pre-fatigue state obtained by a sub-maximal exercise might yield a selective glycogen depletion of STF, while maintaining high levels of glycogen in FTF. In our study, the tested athletes showed a significant improvement in the estimated recruitment percentage of FTF (Bosco, 1992) during the jump tests following 40 min of running at LT2 speed. After a 3-week training period, despite an increase in running speed at LT2, the results of the jump tests did not change significantly. Hence, we can speculate that the significant increase in the recruitment percentage of FTF observed in our subjects may have been triggered firstly, by an unfavorable energetic condition of STF caused by a selective glycogen depletion following the 40 min of running at LT2 speed and, secondly, by an increased activity of the CNS resulting in better recruitment of FTF at a high activation threshold. Finally, it should be pointed out that the recruitment of FTF occurs in proportion to the percentage of each subject’s FTF. Therefore, subjects with more FTF experience better recruitment of those fibers immediately after the 40-min run, as is apparent from the values reported in the graph (Figure 4). Moreover, our results are confirmed by some studies showing that the most important muscle characteristic affecting PAP is the fiber type; muscles with the shortest twitch contraction times and highest proportion of fast twitch (Type II) fibers show the greatest PAP. The greater PAP in Type II fibers is likely related to their greater capacity for MLC phosphorylation in response to high frequency activation (Hamada et al., 2000).

Despite the limitations of this investigation stemming from the small sample size and the lack of analysis of muscle glycogen measurement, our findings may lay the groundwork for further studies designed to explore and test our hypotheses more thoroughly.

**Practical Applications**

Often, during prolonged long-distance races (5000 m, 10000 m), the last lap is run at speeds that require greater recruitment of FTF, and there are many examples of top level athletes who manage to run that final lap in a time that approaches their personal best in the 400 meters. Our study shows that “pre-fatigue” can be used before workouts that require use of FTF (such as explosive force, or even sprinting), in order to improve the final part of a competition or a change of pace. It might therefore be useful, during fractionated training, to include the fastest fractions in the final part of the session (eg. 10000 m. fractionated: 3 x 2000 m + 3 x 1000 m + 5 x 200 m).
References
Bosco C. *Strength assessment with the Bosco's test*. Rome: Italian society of sport science; 1999


Figure 1. Effect of three weeks of training on running speed at LT2; bars error represent standard deviation.

Figure 2. Correlation between SJ, CMJ and % of FT fibers recruited.
Figure 3. Training intensity distribution in the three zone model during the three-week training period.

Figure 4. Percentage of FT fibers pre- and post- 40 min run, before and after three weeks of training.

Table 1. Training program

<table>
<thead>
<tr>
<th>Week</th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
</table>

% FT fibers at t0-t1

% FT fibers at t2-t3

y = 1.2998x + 7.2168
R² = 0.95501

y = 1.4789x + 1.5529
R² = 0.98218
<table>
<thead>
<tr>
<th></th>
<th>First Training Week</th>
<th>Second Training Week</th>
<th>Third Training Week</th>
<th>Assessment week</th>
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<tbody>
<tr>
<td>Assessment week</td>
<td>40 min Zone 1</td>
<td>30 min Zone 1</td>
<td>30 min Zone 1</td>
<td>50 min Zone 1</td>
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<tr>
<td>Lactate Threshold</td>
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<td>30 min Zone 1</td>
<td>30 min Zone 1</td>
<td>30 min Zone 1</td>
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<tr>
<td>Assessment</td>
<td>30 min Zone 1</td>
<td>30 min Zone 1</td>
<td>30 min Zone 1</td>
<td>30 min Zone 1</td>
</tr>
<tr>
<td>Experimental Protocol</td>
<td>40 min Zone 1</td>
<td>40 min Zone 1</td>
<td>40 min Zone 1</td>
<td>40 min Zone 1</td>
</tr>
</tbody>
</table>

| First Training Week      | 50 min Zone 1       | 60 min Zone 1        | 60 min Zone 1       | 60 min Zone 1   |
| 20 min Zone 1            | 20 min Zone 1       | 20 min Zone 1        | 20 min Zone 1       | 20 min Zone 1   |
| 8 x 400                  | 10 min Zone 1       | 10 min Zone 1        | 10 min Zone 1       | 10 min Zone 1   |
| 10'40"                   | 60 min Zone 1       | 20 min Zone 2        | 60 min Zone 1       | 60 min Zone 1   |
| Zone 3                   | Zone 2              | Zone 1               | Zone 1              | Zone 1          |
| Rest 1 min               | 5 x 4min Zone 3     | 10 min Zone 2        | 5 x 4min Zone 3     | 5 x 4min Zone 3 |
| Recovery                 | Rest 3 min Zone 1   | 10 min Zone 1        | Rest 3 min Zone 1   | Rest 3 min Zone 1 |
| 10' Zone 1               |                     |                      |                     |                 |

| Second Training Week     | 60 min Zone 1       | 60 min Zone 1        | 60 min Zone 1       | 60 min Zone 1   |
| 20 min Zone 1            | 20 min Zone 1       | 20 min Zone 1        | 20 min Zone 1       | 20 min Zone 1   |
| 10 x 400                 | 13'20" Zone 3       | 25 min Zone 2        | 60 min Zone 1       | 60 min Zone 1   |
| 13'20"                   | 60 min Zone 1       | 10 min Zone 2        | 60 min Zone 1       | 60 min Zone 1   |
| Zone 3                   | Zone 1              | Zone 1               | Zone 1              | Zone 1          |
| Rest 1 min               | 5 x 4min Zone 3     | 10 min Zone 2        | 5 x 4min Zone 3     | 5 x 4min Zone 3 |
| Recovery                 | Rest 3 min Zone 1   | 10 min Zone 1        | Rest 3 min Zone 1   | Rest 3 min Zone 1 |
| 10' Zone 1               |                     |                      |                     |                 |

| Third Training Week      | 60 min Zone 1       | 60 min Zone 1        | 60 min Zone 1       | 60 min Zone 1   |
| 20 min Zone 1            | 20 min Zone 1       | 20 min Zone 1        | 20 min Zone 1       | 20 min Zone 1   |
| 12 x 400                 | 16' Zone 3          | 30 min Zone 2        | 60 min Zone 1       | 60 min Zone 1   |
| 16' Zone 3               | 60 min Zone 1       | 10 min Zone 2        | 60 min Zone 1       | 60 min Zone 1   |
| Rest 1 min               | 6 x 4min Zone 3     | 10 min Zone 2        | 6 x 4min Zone 3     | 6 x 4min Zone 3 |
| Recovery                 | Rest 3 min Zone 1   | 10 min Zone 1        | Rest 3 min Zone 1   | Rest 3 min Zone 1 |
| 10' Zone 1               |                     |                      |                     |                 |

| Assessment week          | 50 min Zone 1       | 40 min Zone 1        | 30 min Zone 1       | 30 min Zone 1   |
| Lactate Threshold        | 30 min Zone 1       | 30 min Zone 1        | 30 min Zone 1       | 30 min Zone 1   |
| Assessment               | 30 min Zone 1       | 30 min Zone 1        | 30 min Zone 1       | 30 min Zone 1   |
| Experimental Protocol    | 40 min Zone 1       | 40 min Zone 1        | 40 min Zone 1       | 40 min Zone 1   |