A Decrease in Soleus Muscle Force Generation in Rats After Downhill Running

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Abstract/Résumé

The purpose of the present study was to investigate the immediate and 48-hr post-exercise effects of eccentric contraction-biased exercise on the contractile properties of the soleus muscle in situ. Adult male Wistar rats were categorised into sedentary control rats (n = 10), rats studied immediately (n = 10), and rats studied 48 hours after the exercise (n = 10). The exercise protocol consisted of a 90-min intermittent downhill running (−16°, 16 m/min) on a motor-driven treadmill. The contractile properties of the soleus muscle were recorded following i.p. chloral hydrate anaesthesia. Isometric twitch force (Pt), time-to-peak tension (TPT), half-relaxation time (1/2 RT), and tetanic force at stimulation frequencies of 40, 80, and 100 Hz were recorded. A low-frequency muscle fatigue protocol (stimulation at 4 Hz for 5 min) was applied to test for fatigability. The main findings indicated that Pt generation dropped both immediately and 48 hr after the exercise, while tetanic force was partially restored after 48 hr. Exercise-induced E-C coupling failure and contractile machinery disorganisation due to muscle injury are put forward as the main force reduction causes.

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Strenuous or unaccustomed physical activity involving eccentric contractions, such as downhill running, generates mechanical stress inducing several responses from skeletal muscle cells (Ebbeling and Clarkson, 1989). These cellular responses are considered to be indicative of muscle injury, and they include biochemical (Maughan et al., 1989; Nosaka, Clarkson, and Apple, 1992; Schwane, Johnson, Vandenakker, and Armstrong, 1983), morphological (Armstrong, Olgilvie, and Schwane, 1983; Frieden and Lieber, 1992; Ogilvie, Armstrong, Baired, and Bottoms, 1988), and functional (Davies and White, 1981; Ploutz-Snyder, Tesch, and Dudley, 1998; Warren, Jenkins, Packer, Witt, and Armstrong, 1992) alterations of the skeletal muscles involved. Morphological alterations are disorganised myofibrillar architecture including Z-line streaming and broadening, mitochondria swelling, and myofilament disruption in the sarcomeres (Frieden, Sjostrom, and Ekblom, 1983; Newham, McPhail, Mills, and Edwards, 1983). A dilation of the cisternal and longitudinal areas of the sarcoplasmic reticulum (SR) and the t-tubular system (McCutcheon, Byrd, and Hodgson, 1992) is also observed, and it has been suggested that this alteration may affect the release and reuptake process of Ca²⁺ by the SR and, consequently, the ability of a muscle to generate force.

It has been postulated that eccentric exercise causes the most severe injury at the structural level, compared to other activities (Faulkner, Brooks, and Opitceck, 1993; McCully and Faulkner, 1985). Eccentric exercise-induced muscle performance impairment has been described in terms of reduced maximal voluntary force generation (Clarkson and Ebbeling, 1988; Newham, Jones, and Clarkson, 1987; Saxton et al., 1995) or decreased stimulated tetanic force both in humans (Davies and White, 1981; Newham, Mills, Quigley, and Edwards, 1983; Saxton et al., 1995) and in animals (Brooks and Falkner, 1990; McCully and Falkner, 1985; Wood, Morgan, and Prosko, 1993). Furthermore, it has been recently proposed that measuring the electrically induced isometric force is the best way to describe muscular function deficits caused by eccentric contraction-induced muscle injury (Warren, Lowe, and Armstrong, 1999).

Downhill running is an acceptable and commonly used model for inducing skeletal muscle injury. Several studies have described the biochemical, morphological, and functional changes associated with downhill running in human and
animal models. However, only the reports by Lynch, Fary, and Williams (1997) and Warren et al. (1992) examined the contractile properties following downhill running in rodents. According to them, the tetanic force of the soleus muscle decreased after downhill running, but the force decrease time span was not the same in both cases. Therefore, there is a clear need for further research in this area.

The purpose of the present study was to look into the effects of eccentric contraction-biased exercise on the contractile properties of skeletal muscles. We studied the immediate and 48-hr post-exercise effects of downhill running on the twitch force and on the tetanic force at stimulation frequencies of 40, 80, and 100 Hz, as well as the soleus muscle fatigue rate in situ.

**Material and Methods**

**ANIMALS AND ANIMAL CARE**

This research project complies with the guidelines for animal use established by the American Physiological Society and was approved by the local ethical committee in accordance with EEC Council Directive 86/609. Thirty adult (8- to 10-week-old) male Wistar rats, weighing 220–270 g, were used in the study. They were housed in groups of four to five per cage in a temperature-controlled room (22–24 °C) with a 12hr:12hr light-dark cycle. Commercial rat chow and tap water were provided ad libitum. Rats were randomly divided into three groups as follows: (a) sedentary controls (Ctrl), (b) an exercised group studied immediately after the exercise (Ex0), and (c) an exercised group studied 48 hr after the exercise (Ex48).

**EXERCISE PROTOCOL**

The exercise protocol consisted of a 90-min intermittent downhill running on a motor-driven treadmill. After a few minutes, in order for the rats to become accustomed to the procedure, exercised groups performed eighteen 5-min running bouts at a speed of 16 m/min with an inclination of −16°, separated by 2-min rest periods. A similar exercise protocol has been previously shown to cause a microtrauma to the soleus muscle (Armstrong et al., 1983; Komulainen, Kytola, and Vihko, 1983; Ogilvie et al., 1988). When required, rats were encouraged to run by brushing their tails with a soft bristle brush. After the exercise, the animals were placed back in their cages with free access to food and water, until they were examined.

**SURGICAL PROCEDURE**

Animals were anaesthetised by intraperitoneal (i.p.) injections of chloral hydrate (4.5%, 450 mg/kg body weight), and the soleus muscle of the right hind limb was prepared for tension recording in situ. Skin was sectioned and a longitudinal incision was made on the lateral surface of the right hind limb over the area covered by gluteus superficialis. Adjacent muscles were opened up gently by blunt-tip forceps, and the sciatic nerve was exposed. A small incision was made on the back side of the ankle, thus uncovering the distal tendon. The gastrocnemius muscle was carefully retracted in order to avoid rupturing the blood vessels, and the intact soleus muscle was exposed. The tendon was then tied with 3/0 silk thread and distally cut.
IN-SITU ISOMETRIC TENSION RECORDING

The rat was placed prone on a stable rodent surgery table and was prepared for tension recording. Steel pins were inserted through the knee and ankle joints to stabilise the hind limb, while cloth tape was used to secure the foot perpendicularly to the lower leg. Pins were supported with magnetic stand holders throughout the experiment. The tendon of the soleus muscle was attached to a strain gauge transducer (Dynamometer UFI, Devices) by a short silk suture, and bipolar silver electrodes were placed under the sciatic nerve, which was held in a relaxed position. Isometric contractions were evoked by stimulating the sciatic nerve (Digitimer DS9A stimulator) using supramaximal (3–8 volts) square pulses of 0.5 ms duration. Tetanic twitch stimulation was set at 350 ms. The signal from the transducer was amplified by a DC transducer amplifier (Neurolog NL 107), displayed on an oscilloscope screen (Fluke PM 3380A), stored in a computer, and calculated by a data acquisition process software (FlukeView combiscope software).

The muscle was adjusted to optimal length (Lo) through a micromanipulator allowing motion on the 3 axes (Prior, England). Lo was defined as the muscle length at which maximal twitch tension was obtained. It took an average of 5–6 single twitch trials to set Lo. Once this was set, a 1-min resting period was allowed before the actual recordings. Throughout the recordings, the longitudinal axis of the muscle was in alignment with the longitudinal axis of the transducer, and both were parallel to the tibia of the examined leg. All devices during the tension-recording procedure were controlled by a pulse programmer (Digitimer D4030).

Contractile properties measured included maximal isometric single-twitch tension (Pt), time-to-peak tension (TPT), single-twitch half-relaxation time (1/2 RT), as well as tetanic tension at stimulation frequencies of 40, 80, and 100 Hz. A low-frequency muscle fatigue protocol (stimulation at 4 Hz for 5 min) was applied to test for fatigability. The decrease in tension after the 5-min stimulated contraction period was expressed as a percentage of the initial tension, denoting the fatigue index (FI). To estimate the ability for muscle recovery, an isometric single twitch was recorded 3 min after the completion of the fatigue protocol (Pt, TPT, 1/2 RT). The tension recorded after the 3-min recovery period was expressed as a percentage of the initial tension, denoting the recovery index (RI). For both FI and RI, the force at 4 Hz was considered to be the initial tension (the force of the first twitch recorded in the beginning of the 5-min fatigue protocol).

To ensure that the muscle retained its Lo after repeated contractions during the 5-min fatigue protocol, an average of 2–3 single-twitch trials were applied immediately after the 3-min recovery period. Once Lo was confirmed, the actual single twitch was recorded after 1 min. Throughout tension recording, the rat was kept warm with a heating pad. The sciatic nerve and soleus muscle were kept moist during the experiment by periodically sprinkling them with Krebs solution drops at 37 °C. Immediately after tension recording, the soleus muscle of the right hind limb was excised and weighed on a 3-digit electronic balance (AND Electronic Balances, FX-300). Normalisation of the data from different-size muscles was achieved by expressing generated force in mN per gram of muscle weight.

STATISTICAL ANALYSIS

Data were analysed using the SPSS program. The Kolmogorov-Smirnov test was used to check for normal variable distribution. To evaluate group differences, the
multivariate analysis of variance (one-way MANOVA) was applied and, when the univariate F tests were significant, Tukey’s post hoc test was used to further identify differences between group pairs. The statistical significance level was set at \( P < .05 \).

**Results**

The single twitch force (Pt) of both exercised groups was significantly lower (\( p < .001 \) for Ex0 and \( p < .01 \) for Ex48, respectively), compared to the control group. However, no significant differences were found among groups for single-twitch time-to-peak tension (TPT) and half-relaxation time (1/2 RT; Figure 1).

![Graphs showing Pt, TPT, and 1/2 RT for Ctrl, Ex0, and Ex48 conditions](image)

**Figure 1.** Means ± SE values for isometric single twitch. Ctrl: control group; Ex0: immediately after exercise; Ex48: 48 hr after exercise.
Figure 2. Means ± SE values for unfused (stimulation at 40 Hz) and for fused (stimulation at 80 and 100 Hz) tetanic force. Ctrl: control group; Ex0: immediately after exercise; Ex48: 48 hr after exercise.

Both unfused (40 Hz) and fused (80 and 100 Hz) tetanic forces were (Figure 2) significantly lower only immediately after the exercise ($p < .001$ for unfused and $p < .01$ for fused tetanus, respectively). The fatigue index (FI) of the group studied immediately after the exercise was significantly lower (76% vs. 88%), compared to the controls ($p < .01$). Although non-significant, the same situation was observed in the group studied 48 hr after the exercise (80% vs. 88% of the controls; Figure 3).
Figure 3. Means ± SE values for fatigue index (FI) using a low frequency fatigue protocol (4 Hz, for 5 min). The decrease in tension after the 5-min stimulation period is expressed as a percentage of the initial tension. Ctrl: control group; Ex0: immediately after exercise; Ex48: 48 hours after exercise.

The single-twitch force (Pt) 3 min upon termination of the fatigue protocol was significantly lower than the force of the controls both immediately after (p < .001) and 48 hr after (p < .001) the exercise. No significant difference was found for time-to-peak tension (TPT) 3 min after the fatigue protocol. However, the single-twitch half-relaxation time (1/2 RT) 3 min after the fatigue protocol increased significantly only in the group studied 48 hr after the exercise (p < .01). There was no difference between the group studied immediately after the exercise and the control group (Figure 4).

There were significant differences between the exercised groups and the control group (p < .001) concerning the recovery index (RI). In both exercised groups, the single-twitch force 3 min after the fatigue protocol, expressed as a percentage of the initial tension, reached only 80% (p < .001). On the other hand, the RI of the control group increased by 20% of the initial value (Figure 5). A concise presentation of the decrease in percentage terms compared to the controls for the main contractile properties of the study is given in Table 1.

Discussion

The main findings of this study indicated that the isometric single-twitch force (Pt) decreased both immediately after and 48 hr after the exercise, while the isometric tetanic force at stimulation frequencies of 40, 80, and 100 Hz decreased immediately after, but partially recovered 48 hr after the exercise. These findings are in line with the results of previous studies reporting decrease in Po after downhill running. In the present study, isometric tetanic force decreased immediately after the exercise was significant (about 20%), compared to the controls, but did not exceed 12% 48 hr after the exercise and was not statistically significant. Warren et al. (1992), who measured contractile muscle properties in situ after downhill running in rats, found that Po decreased approximately 20% both immediately
Figure 4. Means ± SE values for isometric single twitch 3 min after the end of the fatigue protocol. Ctrl: control group; Ex0: immediately after exercise; Ex48: 48 hr after exercise.

after as well as 48 hr after the exercise, during the phagocytic phase. The less pronounced Po decrease found in our study 48 hr after the exercise may be attributed to the different volume of the training protocol. The exercise protocol we used, although similar in slope (−16° vs. −17°) to that of Warren et al. (1992), was of lower intensity (16 m/min vs. 25 m/min) and shorter duration (90 min vs. 150 min). The 48-hr post-exercise downhill running-induced Po decrease has been also confirmed by Lynch et al. (1997), who reported a significant decrease in soleus muscle Po but no difference 24 hr after the exercise.
Figure 5. Means ± SE values for recovery index (RI) using a low frequency fatigue protocol (4 Hz, for 5 min). The tension change after the 3-min recovery period is expressed as a percentage of the initial tension. Ctrl: control group; Ex0: immediately after exercise; Ex48: 48 hr after exercise.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pt</th>
<th>P40 Hz</th>
<th>P80 Hz</th>
<th>P100 Hz</th>
<th>Pt1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex0</td>
<td>20.9***</td>
<td>19.2***</td>
<td>19.6**</td>
<td>17.7**</td>
<td>39.8***</td>
</tr>
<tr>
<td>Ex48</td>
<td>10.3*</td>
<td>9.7</td>
<td>11.4</td>
<td>7.2</td>
<td>31.5***</td>
</tr>
</tbody>
</table>

*p < .05, **p < .01, ***p < .001 compared to the control group; Pt: isometric twitch force; P40, 80, 100 Hz: isometric tetanic force; Pt1: isometric twitch force 3 min after fatigue; Ex0: immediately after exercise; Ex48: 48 hr after exercise.

A decrease in force generation may be attributed to various mechanisms. It has been suggested that the main cause for this decrease after eccentric contraction is an excitation-contraction (E-C) coupling failure. Ingalls, Warren, Williams, Ward, and Armstrong (1998) found an approximately 50% decrease in Po immediately after a muscle injury induced by 150 eccentric contractions, which remained unchanged for 5 days. They considered that the E-C coupling failure could explain at least 75% of Po decrease immediately after eccentric contraction. Although the contribution of E-C coupling failure to the decreased Po gradually diminished over time, it was still responsible for at least 57% of force reduction in 5 days; even after 14 days the recovery of Po was still incomplete. They suggested that the E-C coupling impairment probably occurred at or below the level of the voltage sensor in the t-tubule and that it might be located on the t-tubule SR Ca++ release channel interface. Balnave and Allen (1995) also attributed Po deficit after stretch-induced muscle injury to the reduction in free cytosolic Ca++ concentration due to the E-C coupling failure. They reported a 58% decrease in Po, which coincided with a 60% reduction in tetanic-free cytosolic Ca++ concentration, after the
application of 28 tetani. Additionally, several other researchers have also attributed reduced muscle force generation to the same mechanism (Vergara, Rapoprot, and Nassar-Gentina, 1977; Warren et al., 1993; Westerblad and Allen, 1991).

A possible explanation of the findings in the present study is that of the E-C coupling failure theory. The decline in Pt and tetanic force immediately after the exercise might be mainly due to the impaired E-C coupling process, which is likely to reduce the amount of Ca++ release from the SR, thus reducing muscle force generation. It has been demonstrated that, in a single twitch, the SR Ca++ channels open with depolarisation and close rapidly once the membrane is repolarised, allowing a very short time for Ca++ release (Eusebi, Miledi, and Takahashi, 1980). The consequence of this is a reduced Ca++ availability in Pt compared to the tetanic force characterized by a train of stimuli. In this regard, Pt seems to be a more sensitive indicator of a normal Ca++ transient process than the tetanic force, since even a minor muscle fibre injury could impair the Ca++ transient process and consequently, decrease Pt more easily than the tetanic force. Our findings demonstrate that 48 hr after the exercise, Pt was still depressed, while the tetanic force partially recovered. We propose that, although the injury was sustained and probably even exacerbated 48 hr after the exercise, the high-frequency stimulus applied in order to generate tetanic force may compensate for the possible E-C coupling process failure, which was not the case with the single Pt stimulus.

Another purpose of the present study was to look into the effects of downhill running on the fatigue rate of the soleus muscle. Based on the low-frequency stimulation protocol used in this study, the fatigue index (FI) dropped both immediately after and 48 hr after the eccentric exercise, thus substantiating a possible reduction in the SR Ca++ release rate. This result is in agreement with earlier studies reporting a more pronounced force decrease at low stimulation frequencies after eccentric contractions, compared to isometric (Balnave and Allen, 1995) and concentric contractions (Davies and White, 1981; Edwards, Mills, and Newham, 1981) because of a low SR Ca++ release rate (Jones, 1981; Jones, Newham, and Torgan, 1989). In the present study the decreased FI probably cannot be attributed only to the fatigue per se that is induced by the low-frequency protocol but also to the muscle injury that is caused by downhill running, since the decreased FI of the controls was only 12% compared to the exercised groups studied immediately after (24%) and 48 hr after the exercise (20%). Pt, which was recorded 3 min after the fatigue protocol (Pt) in our study, dropped significantly both immediately after (40%) and 48 hr after the exercise (31%), compared to the controls. Those findings may further add to the SR Ca++ reduced release rate hypothesis.

Another finding of the present study was the 48-hr post-exercise increase in the 1/2 RT recorded 3 min after the completion of the fatigue protocol (83 vs. 71 ms for controls). Given the prolonged—albeit in a non-significant way (77 vs. 70 ms for controls)—pre-fatigue 1/2 RT, it is proposed that post-fatigue 1/2 RT prolongation is due both to the fatigue of the low-frequency protocol plus the injury of downhill running, possibly through the limited SR Ca++ reuptake process. A depression in the rate of Ca++ uptake by the SR has been attributed to either a reduced Ca++-stimulated ATPase activity observed after exhaustive exercise (Luckin, Favero, and Klug, 1991; Yasuda et al., 1999) or to mechanical damage of the SR induced by contractions of stretched muscle fibres (Balnave and Allen, 1995). Other studies report higher resting Ca++ concentrations for an extended period of time after
repeated contractions, with (Balnave and Allen, 1995) or without muscle stretch (Westerblad and Allen, 1991), proposing a reduced rate of Ca\(^{++}\) reuptake capacity for the SR (Balnave and Allen, 1995; Byrd, Bode, and Klug, 1989; Fitts, Courtright, Kim, and Witzmann, 1982). Furthermore, a decreased SR Ca\(^{++}\) uptake rate over time was also reported by Ingalls et al. (1998), who found modest reductions in the rates of SR Ca\(^{++}\) release and uptake immediately after eccentric contractions and even higher reductions 2 days after the contractions, which reached their peak from the third up to the fifth day. SR function resumed normality 14 days after the injury.

The ability for recovery, as expressed by the recovery index (RI), was limited both immediately after and 48 hr after the exercise due to sustained muscle injury inhibiting recovery. On the other hand, the RI of the control group not only reached its initial value but exceeded it by 20%, thus demonstrating the viability of the preparation. It seems that, although appropriate for detecting exercise-induced muscle dysfunction, the low-frequency fatigue protocol (stimulation at 4 Hz, for 5 min) used in the present study cannot cause any considerable amount of fatigue to non-exercised muscles (control group); rather, it facilitates muscle contraction. The tension recordings of the control group muscles showed a staircase effect (trappe phenomenon) that may reflect the increased availability of Ca\(^{++}\), which was not the case with exercised muscles.

Another possible mechanism contributing to decreased force generation could be the structural damage of the myofibrils. It has been postulated that disorganisation of the contractile machinery due to mechanical disruptions in force-generating and force-transmitting elements could possibly explain the reduced muscle force (Balnave & Allen, 1995; Ingalls et al., 1998; Morgan and Allen, 1999). Another such factor relating to muscle force deficits immediately after the exercise could be lower myofibrillar Ca\(^{++}\) sensitivity levels due to increased myoplasmic levels of certain metabolites, such as hydrogen ions and inorganic phosphate (Allen, Lee, and Westerblad, 1989; Lee, Westerblad, and Allen, 1991; Metzger and Moss, 1990). However, the relative role of each of the above muscle injury mechanisms is not known yet; further research will, undoubtedly, provide the answer.

**Conclusions**

In conclusion, the main findings of this study indicate that Pt is reduced both immediately after and 48 hr after the exercise, while isometric tetanic force is partially restored 48 hr after downhill running. Partial tetanic force recovery 48 hr after the exercise may be attributed to the high-frequency stimulus applied, which may compensate for the possible SR operational dysfunction masking the injury imposed by downhill running. It is proposed that the E-C coupling failure and the disorganisation of the contractile machinery are the main causes for the force deficit. However, further research is needed to determine the precise decreased force generation mechanisms associated with eccentric contractions.

**References**


