

The effects of protease supplementation on skeletal muscle function and DOMS following downhill running

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Accepted 16 September 2003

Protease supplementation has been shown to attenuate soft tissue injury resulting from intense exercise. The aim of this study was to evaluate the effects of protease supplementation on muscle soreness and contractile performance after downhill running. Ten matched pairs of male participants ran at a –10% grade for 30 min at 80% of their predicted maximal heart rate. The participants consumed two protease tablets (325 mg pancreatic enzymes, 75 mg trypsin, 50 mg papain, 50 mg bromelain, 10 mg amylase, 10 mg lipase, 10 mg lysozyme, 2 mg chymotrypsin) or a placebo four times a day beginning 1 day before exercise and lasting a total of 4 days. The participants were evaluated for perceived muscle soreness of the front and back of the dominant leg, pressure pain threshold by dolorimetry of the anterior medial, anterior lateral, posterior medial and posterior lateral quadrants of the thigh, and knee extension/flexion torque and power. The experimental group demonstrated superior recovery of contractile function and diminished effects of delayed-onset muscle soreness after downhill running when compared with the placebo group. Our results indicate that protease supplementation may attenuate muscle soreness after downhill running. Protease supplementation may also facilitate muscle healing and allow for faster restoration of contractile function after intense exercise.

Keywords: contractile function, delayed-onset muscle soreness, eccentric, protease, supplementation.

Introduction

The inflammatory response is a predictable phenomenon that ensues musculoskeletal injury. While this response is a necessary component of the healing process, uncontrolled inflammation may prolong skeletal muscle recovery after intense exercise or training-induced injury. Consequently, this type of muscle injury may delay return to normal function. Previous research has suggested that oral supplementation of protease may shorten recovery time after injury. It is believed that this is accomplished through early inhibition of the arachidonic cascade resulting in accelerated healing (Donoho and Rylander, 1962; Deitrick, 1965; Woolf *et al.*, 1965; Smyth *et al.*, 1967; Seligman, 1969; Taussig, 1980; Vellini *et al.*, 1986; Taussig and Batkin, 1988; Bucci, 1995; Burke, 1997; Petry, 1997).

Protease is believed to inhibit the biosynthesis of pro-inflammatory agents while stimulating the produc-

tion of anti-inflammatory agents (Woolf *et al.*, 1965; Vellini *et al.*, 1986; Taussig and Batkin, 1988). This action is similar to that of plasmin (Cirelli, 1964; Seligman, 1969; Taussig, 1980; Taussig and Batkin, 1988); however, the site of action of protease may occur further along the cascade. The anti-inflammatory action of protease is also associated with increased tissue permeability, facilitating resorption of oedema and accelerated restructuring of the damaged tissue (Cirelli, 1964; Smyth *et al.*, 1967). Interestingly, no adverse side-effects have been noted as a result of protease utilization (Donoho and Rylander, 1962; Cirelli, 1964; Deitrick, 1965; Spaeth, 1968; Seligman, 1969), including those typically associated with use of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin (i.e. gastrointestinal disturbances) (Taussig, 1980).

Protease supplementation has been shown to elicit positive effects on a variety of clinically relevant conditions, including cataract extraction (Stauber *et al.*, 1990), thrombophlebitis (Seligman, 1969), haematoma (Cirelli, 1964; Woolf *et al.*, 1965; Petry, 1997), athletic injuries (Donoho and Rylander, 1962;

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Deitrick, 1965) and other causes of contusions, abrasions, sprains, strains and fractures (Cirelli, 1964). Protease supplementation has also been shown to be effective in the debridement of third-degree burns, enhancing the absorption of antibiotics, and interfering with the growth of malignant cells (Taussig and Batkin, 1988). It is unclear if protease supplementation would also have similar positive effects on more frequent causes of musculoskeletal pain.

Many individuals experience muscle pain after an individual exercise bout if (1) they have been previously sedentary or (2) the exercise bout was significantly greater in volume or intensity than they had previously experienced. This type of muscle pain is referred to as delayed-onset muscle soreness (DOMS). Routinely, DOMS peaks 24–72 h after an exercise bout and may last as long as 7–10 days (Nosaka et al., 2002). Delayed-onset muscle soreness tends to be more severe after exercise involving eccentric contractions (Talag, 1973; Schwart et al., 1983; Byrnes et al., 1985a,b; Jones et al., 1986, 1989; Evans, 1987; Stauber et al., 1990; Appell et al., 1992; Hasson et al., 1993; Miles and Clarkson, 1994). Interestingly, as DOMS reaches peak discomfort the muscle tissue itself is almost completely healed.

Although the exact process that precipitates DOMS is not clearly understood, the most widely accepted model is based on the idea that extreme tension generated by skeletal muscle leads to damage of the structural proteins within the muscle fibres and connective tissue resulting in the perception of pain (Appell et al., 1992; Hasson et al., 1993). The inflammatory process follows this disruption. Due to structural disruption of the sarcolemma, there is an influx of calcium into the cells. These calcium ions accumulate in the mitochondria and inhibit cellular respiration. Concurrently, proteolytic enzymes that were released after the injury clear the injured cell of damaged or dysfunctional internal structures. This breakdown is accompanied by diffusion of intracellular components into the interstitium and plasma where they attract monocytes that convert to macrophages that phagocytize the area. In addition to the tissue injury, oedema, caused by the influx of fluid, increases the intramuscular pressure. This oedema is thought to activate type IV sensory neurons resulting in the sensation of dull, diffuse pain commonly associated with DOMS (Appell et al., 1992; Hasson et al., 1993). The tissue damage and subsequent debridement of the affected cellular constituents may result in the loss of organization within the muscle cell resulting in diminished function.

The aim of this study was to examine the effects of protease supplementation on DOMS after downhill running. We hypothesized that protease supplementa-

tion would reduce the intensity and duration of muscle soreness experienced by individuals after eccentric exercise. We also hypothesized that individuals receiving protease supplementation would recover contractile capabilities sooner than those receiving placebo.

Methods

Participants

Twenty healthy males (10 pairs) aged 18–29 years participated in the study. Each pair consisted of individuals matched for age, height and weight. One member of each pair was randomly assigned to a treatment group receiving protease supplementation ($n=10$), while their matched counterpart received a placebo ($n=10$). Before taking part in the study, all participants read and signed an informed-consent form approved by Elon University's Institutional Review Board for Human Subjects. The participants also completed a health history questionnaire designed to identify the degree of risk for cardiovascular or orthopaedic complications during exercise. All procedures used in this study were in accordance with guidelines established by the American College of Sports Medicine.

Protease supplementation

All participants in the experimental group received protease supplementation over a 4-day period. During this period, they consumed two protease capsules four times a day. These participants were instructed to consume the supplement capsules on an empty stomach, 30 min before meals, with 8 oz of water. Each capsule contained 325 mg pancreatic enzymes, 75 mg trypsin, 50 mg papain, 50 mg bromelain, 10 mg amylase, 10 mg lipase, 10 mg lysozyme and 2 mg chymotrypsin. The participants in the placebo group received capsules of similar size and colour containing 3500 mg sucrose. They were given identical instructions for consumption. All capsules were prepared and provided by Enzymatic Therapy Corporation (Green Bay, WI). The administration of treatment and placebo was blinded to both the participants and the investigators. At the end of the study, a representative of Enzymatic Therapy Corporation revealed the supplement code to the researchers. There were no side-effects reported by the participants as a result of the supplementation.

Data collection protocol

Twenty-four hours after the ingestion of the initial capsule, the participants reported to the laboratory for

baseline testing. They fasted for 10 h before the exercise session with the exception of their morning supplement. During this session, heart rate and ratings of perceived exertion were monitored at rest, every 5 min during exercise and 10 min into recovery. The participants mounted a level motorized treadmill and warmed up for 5 min at a self-selected pace. After the 5-min warm-up, treadmill speed was increased until a heart rate of 80% of predicted maximal heart rate was achieved. The participants maintained this pace for 5 min. At this time, the treadmill grade was adjusted to -10%. The participants maintained this workload for 30 min, after which they completed a 5-min active cool-down at a self-selected pace and a 5-min seated passive recovery period. During the 24 h preceding the exercise session and the 72 h after the exercise session, the participants were asked not to participate in any other physical activity. This protocol has been shown to be effective in precipitating muscle soreness (Byrnes *et al.*, 1985a,b).

The effect of the protease supplementation was assessed by evaluating muscle soreness, muscle strength and functional performance. Each procedure was implemented immediately before and 24, 48 and 72 h after the exercise session.

Assessing muscle soreness

Perceived muscle soreness was evaluated for each participant daily upon their arrival at the laboratory. These data were gathered via a Muscle Soreness Questionnaire (MSQ) and a pressure threshold meter (dolorimeter). The MSQ asks participants to rate their general soreness on a scale of 1 (normal) to 10 (very, very sore) for the right front thigh and right back thigh.

A dolorimeter was used to assess deep muscle pain and trigger point tenderness of the thigh resulting from inflammation. The reliability and validity of the dolorimeter has been established previously (Keele, 1954; Merskey and Spear, 1964; Fischer, 1986, 1987; Miles and Clarkson, 1994). The dolorimeter was calibrated in $\text{kg} \cdot \text{cm}^{-2}$ with a range of 11 kg with 100-g divisions. Pressure threshold measurements were made at the approximate mid-point of four muscles: the vastus lateralis, vastus medialis, medial head of the biceps femoris, and lateral head of the biceps femoris. These points were marked and remarked with an indelible marker after each measurement to determine tenderness over the same point each time. Measurements were taken with the participant lying supine (vastus lateralis and vastus medialis) and prone (medial head of the biceps femoris and lateral head of the biceps femoris) on a matted treatment table. All measurements were taken from the right thigh.

The pressure threshold measurements consisted of the following steps. First, the following explanation was given to each participant: 'I am going to measure pressure threshold; that is, how much pressure will induce discomfort. I am going to increase pressure slowly with this device. Say "Yes" when you start to feel pain or discomfort. I will stop the pressure as soon as you say "Yes", so it will not hurt you. It is important that you understand that this is a test of sensitivity, not a test of endurance. Do you understand or have any questions?' After the explanation was given, the dolorimeter was placed exactly over the indelible mark and perpendicular to the muscle under observation. Pressure was increased continuously 1 kg per second until the participant said 'Yes'. At this time, the pressure was stopped, the meter was removed from the skin, and the measurement was recorded.

Muscular strength and power

Muscular strength and power were assessed for each participant using a Biodex System 2 isokinetic dynamometer. Each participant was asked to perform five repetitions of maximal knee extension and flexion contractions at three different speeds (1.6, 3.1 and 5.2 $\text{rad} \cdot \text{s}^{-1}$). The test speeds were administered in random order for each participant to eliminate any potential ordering effects of the testing. The peak strength and power measurements were recorded for each speed and direction.

Agility

The participants were asked to perform shuttle runs to assess any detriment to sport performance caused by muscle soreness. The shuttle run test involved a series of two sprints between two markers approximately 9.12 m (30 feet) apart. Each participant began at the first marker and sprinted to the second marker. Upon arrival at the second marker, he bent down and picked up a wooden block. He then sprinted back to the first marker, placed the block on the ground, turned around and sprinted back to the second marker, retrieved a second block and returned to the first marker. This was a timed test.

Statistical analysis

Comparisons between groups and submaximal exercise treatments for all variables were made using a multivariate analysis of variance (MANOVA) with a repeated measures procedure. Significance was set at $P < 0.05$. Standard contrast procedures were performed when main effects were found.

Results

The data reported here were serial in nature. Initial torque and power measurements were somewhat variable (Table 1). Therefore, all data were converted to and reported as gain scores. This was done in an attempt to demonstrate any differences in recovery time from acute muscle damage between the experimental and placebo groups.

Physical characteristics

There were no significant differences in height, weight or age between the groups (Table 2).

Contractile performance

The experimental group demonstrated lower reductions in torque production than the placebo group for knee flexion (Fig. 1) measured at $1.6 \text{ rad} \cdot \text{s}^{-1}$ 2 days after exercise and for knee extension (Fig. 2) measured at $3.1 \text{ rad} \cdot \text{s}^{-1}$ both on the day immediately after exercise and 2 days after exercise. The experimental group also demonstrated superior power production for knee flexion (Fig. 3) measured at $1.6 \text{ rad} \cdot \text{s}^{-1}$ on both the day after and 2 days after exercise. There were no differences in knee

extension power measurements (Fig. 4) between the groups.

Pressure pain threshold

The experimental group perceived that they were less sore after the exercise intervention according to the results of the Muscle Soreness Questionnaire. This was especially evident on days 1 and 2 post-exercise for the anterior aspect of the thigh and on all days post-exercise for the posterior aspect of the thigh (Fig. 5). These results were consistent with the quantification of pressure pain threshold measured by dolorimetry. This effect was most profound for the anterior lateral quadrant of the thigh for the experimental group. The experimental group was able to tolerate more pressure on the anterior lateral quadrant than the placebo group on all days following the exercise intervention (Fig. 6). This effect was also seen 3 days after exercise for the posterior medial quadrant of the thigh (Fig. 7). The remaining differences in pressure pain threshold were not significant (Figs. 6 and 7).

Functional performance

There were no differences in agility times between the experimental and placebo groups (Fig. 8).

Table 1. Initial torque and power values

| | Speed | Protease | Placebo |
|-----------------------------|---------------------------------------|------------------|------------------|
| Knee extension torque (N·m) | $1.6 \text{ rad} \cdot \text{s}^{-1}$ | 248.8 ± 37.0 | 219.0 ± 57.4 |
| | $3.1 \text{ rad} \cdot \text{s}^{-1}$ | 182.7 ± 21.0 | 182.9 ± 24.5 |
| | $5.2 \text{ rad} \cdot \text{s}^{-1}$ | 144.7 ± 17.5 | 136.4 ± 16.8 |
| Knee flexion torque (N·m) | $1.6 \text{ rad} \cdot \text{s}^{-1}$ | 126.9 ± 20.3 | 127.3 ± 25.3 |
| | $3.1 \text{ rad} \cdot \text{s}^{-1}$ | 103.9 ± 17.9 | 101.6 ± 17.7 |
| | $5.2 \text{ rad} \cdot \text{s}^{-1}$ | 83.1 ± 21.6 | 75.3 ± 15.1 |
| Knee extension power (W) | $1.6 \text{ rad} \cdot \text{s}^{-1}$ | 234.9 ± 39.3 | 232.8 ± 37.0 |
| | $3.1 \text{ rad} \cdot \text{s}^{-1}$ | 325.7 ± 38.7 | 320.1 ± 62.9 |
| | $5.2 \text{ rad} \cdot \text{s}^{-1}$ | 334.7 ± 71.1 | 303.6 ± 79.2 |
| Knee flexion power (W) | $1.6 \text{ rad} \cdot \text{s}^{-1}$ | 132.1 ± 26.7 | 135.5 ± 32.8 |
| | $3.1 \text{ rad} \cdot \text{s}^{-1}$ | 204.5 ± 69.5 | 185.0 ± 53.0 |
| | $5.2 \text{ rad} \cdot \text{s}^{-1}$ | 183.8 ± 61.8 | 160.2 ± 64.2 |

Table 2. Physical characteristics of the participants (mean \pm s; $n = 20$)

| Group | | Age (years) | Height (m) | Body mass (kg) |
|----------|--------------|----------------|-----------------|----------------|
| Protease | ($n = 10$) | 22.1 ± 1.0 | 1.83 ± 0.02 | 83.7 ± 3.0 |
| Placebo | ($n = 10$) | 22.0 ± 0.9 | 1.82 ± 0.02 | 84.5 ± 3.9 |

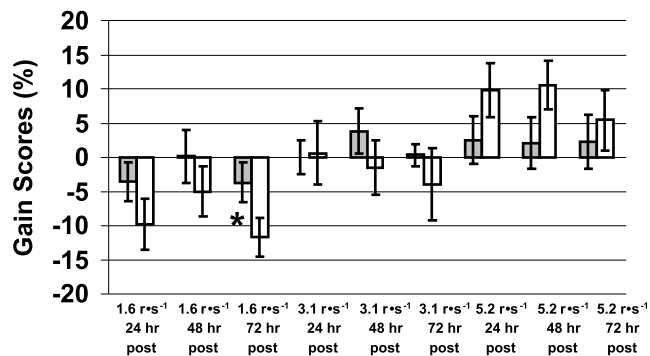


Fig. 1. Changes in knee flexion torque after downhill running. Grey bars = protease-supplemented group, white bars = placebo group. * $P < 0.05$.

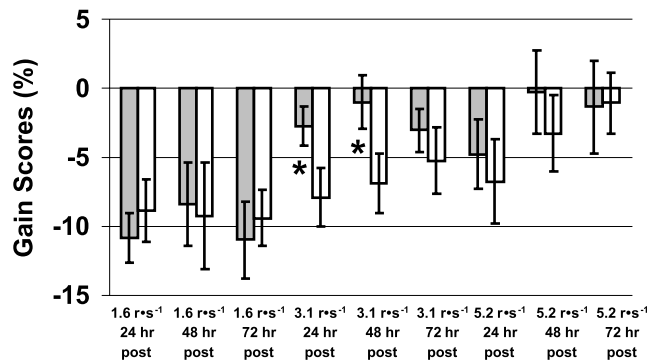


Fig. 2. Changes in knee extension torque after downhill running. Grey bars = protease-supplemented group, white bars = placebo group. * $P < 0.05$.

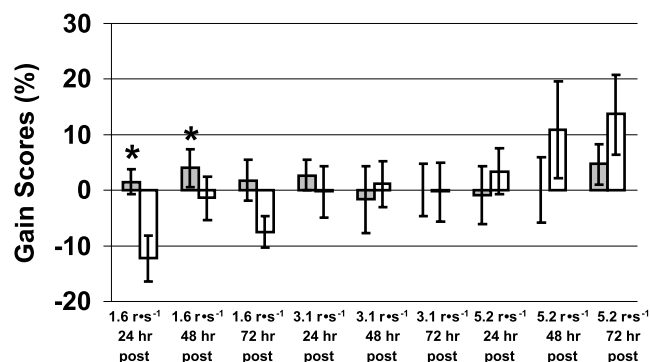


Fig. 3. Changes in knee flexion power after downhill running. Grey bars = protease-supplemented group, white bars = placebo group. * $P < 0.05$.

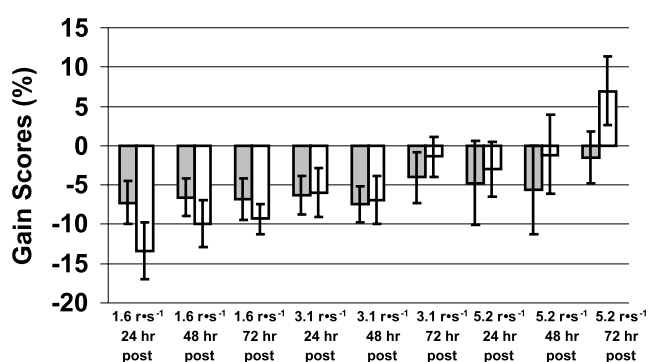


Fig. 4. Changes in knee extension power after downhill running. Grey bars = protease-supplemented group, white bars = placebo group. * $P < 0.05$.

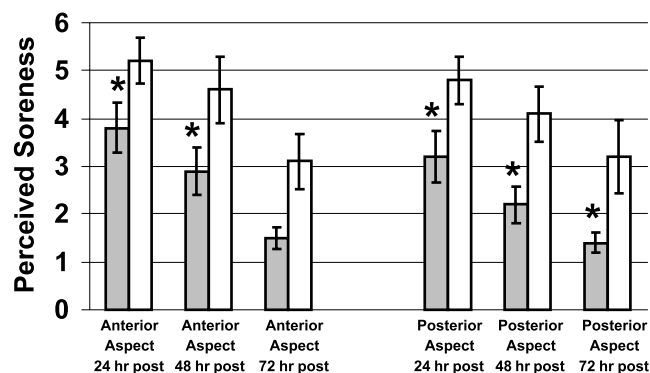


Fig. 5. Perceptions of soreness after downhill running. Grey bars = protease-supplemented group, white bars = placebo group. * $P < 0.05$.

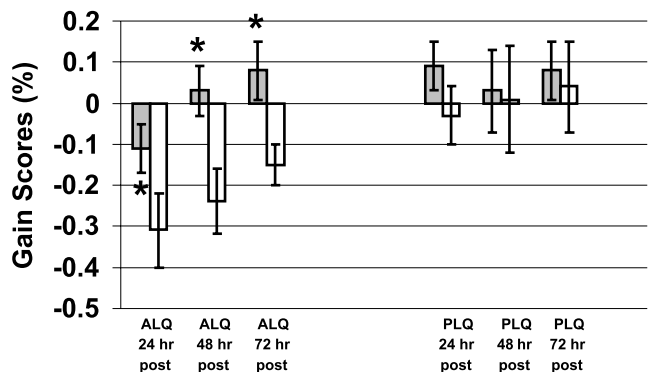


Fig. 6. Changes in dolorimetry in the lateral quadrant of the thigh after downhill running. ALQ = anterior lateral quadrant, PLQ = posterior lateral quadrant, grey bars = protease-supplemented group, white bars = placebo group. * $P < 0.05$.

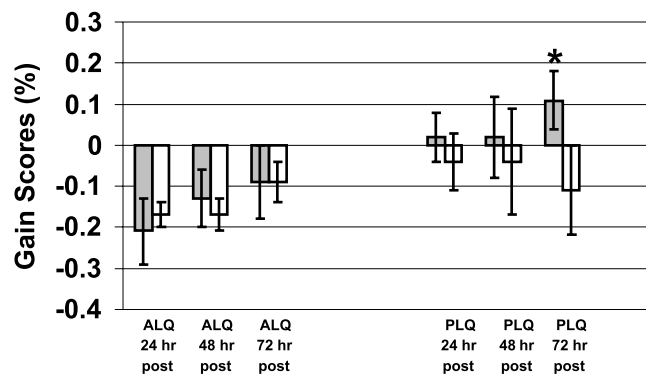


Fig. 7. Changes in dolorimetry in the medial quadrant of the thigh after downhill running. ALQ = anterior lateral quadrant, PLQ = posterior lateral quadrant, grey bars = protease-supplemented group, white bars = placebo group. * $P < 0.05$.

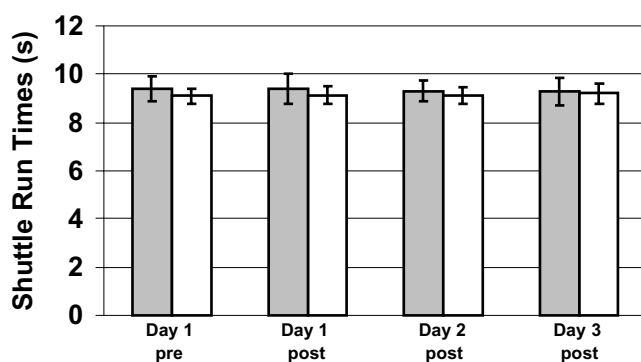


Fig. 8. Shuttle run times before (pre) and after (post) the downhill run. Grey bars = protease-supplemented group, white bars = placebo group.

Discussion

The results of this study suggest that protease supplementation hastens recovery of contractile capabilities and attenuates increases in perception of pain after downhill running.

Downhill running has consistently been found to result in increased muscular tenderness (Byrnes *et al.*, 1985b; Eston *et al.*, 1996). In fact, previous research has shown a potentiation of muscle soreness beginning immediately after downhill running and steadily increasing for the next 48 h. In the present study, the increases in pain perception after downhill running were less pronounced in the experimental group than in the control group. The Muscle Soreness Questionnaire revealed that the anterior and posterior thigh of the experimental group felt more normal than that of the placebo group subsequent to the downhill running treatment. This finding may be explained by the potential attenuation of inflammation believed to result

from protease supplementation. A reduction of the inflammatory process would lead to less oedema and a diminished recruitment of pain receptors in those individuals in the experimental group (Cirelli, 1964; Smyth *et al.*, 1967).

Dolorimetric analysis of the quadriceps and hamstrings were consistent with the results of the MSQ. This was most pronounced in the anterior lateral quadrant on all 3 days after downhill running. The comparison of the dolorimetric measures for the remaining quadrants appeared to be superior for the experimental group; however, these data were not statistically significant. The differences seen in the anterior lateral quadrant may be related to muscle activation patterns utilized during downhill running, as the anterior aspect of the thigh may accept a greater portion of the individual's body weight as their weight is shifted onto the swing leg at the end of the gait cycle during downhill running. Such a muscle utilization pattern may result in greater eccentric forces and corresponding muscle damage. The attenuation of pain experienced by the experimental group may be an indication of reduced inflammation inside the muscle as well as a sign of facilitated healing. Previous studies have demonstrated an increasing pattern of pain perception for 48 h after downhill running (Byrnes *et al.*, 1985b; Evans, 1987). In fact, peak pain seemed to be reached 48 h after exercise (Byrnes *et al.*, 1985b; Evans, 1987). By contrast, those receiving protease supplements in this study began to experience a reduction of pain between 24 and 48 h after downhill running.

After eccentric exercise, damage to the contractile element of the muscle leads to the occurrence of DOMS. This is seen in the ultrastructure of the muscle as streaming of the Z-lines (Evans, 1987). This modification to the contractile mechanism is often associated with a reduction in the tension-generating abilities of the muscle (Evans, 1987; Appell *et al.*, 1992; Hasson *et al.*, 1993). These changes in the muscle structure have been reported to last for several weeks after eccentric exercise (Byrnes *et al.*, 1985b; Evans, 1987; Appell *et al.*, 1992; Hasson *et al.*, 1993). Together with the streaming of the Z-lines, the muscle also experiences oedema, which can both hinder function and potentiate pain (Appell *et al.*, 1992; Hasson *et al.*, 1993). Protease supplementation has been shown to stop the inflammatory process and facilitate the debridement of damaged tissue; therefore, it is reasonable to hypothesize that the differences in pain reported by the experimental and placebo groups were due to the supplementation.

Downhill running has been shown to elicit pronounced muscle damage and DOMS. Associated with muscle damage and pain is a reduction in the tension-

generating capability of skeletal muscle (Byrnes *et al.*, 1985b; Evans, 1987). Similar results were noted in the present study. The recovery of contractile capabilities after training-induced muscle damage may fall within two areas that are intrinsically linked as they pertain to performance – one being the return of a feeling of well-being (i.e. a reduction in pain) and the other being the restoration of the muscle structure itself. This result may be at least partially attributable to the attenuation of muscular pain resulting from ingestion of oral protease. But, a further physiological explanation for the recovery of contractile performance may lie with accelerated restructuring of the muscle cell leading to a faster recovery of functional abilities.

The rate of recovery for training-damaged skeletal muscle appears to range from several days to several weeks (Evans, 1987). However, after a moderate bout of exercise, one may assume that the rate of recovery would be closer to the lower end of that range, probably near the 48-h mark (Evans, 1987). In the present study, the loss of contractile function after downhill running was less for the experimental group and the subsequent recovery was faster. This finding was seen during low-speed ($1.6 \text{ rad} \cdot \text{s}^{-1}$) to moderate-speed contractions ($3.1 \text{ rad} \cdot \text{s}^{-1}$). The recovery of contractile capabilities occurring at low to moderate speeds rather than at higher speeds ($5.2 \text{ rad} \cdot \text{s}^{-1}$) may best be explained by recognizing that the forces generated inside skeletal muscle will be higher during contractions performed at slower isokinetic speeds. This is consistent with the force–velocity relationship of skeletal muscle. When muscle contraction speeds are limited to low or moderate speeds, typically higher tensions are generated. A muscle experiencing higher amounts of damage would not be capable of generating as much tension as when it is in a less damaged state. Consequently, it is a fair assertion that a muscle experiencing accelerated healing and reduced inflammation would recover functional capabilities sooner than a muscle recovering even at normal rates.

As a result, one would not expect to see as pronounced a recovery during high-speed contractions as during low- and moderate-speed contractions. This finding was consistent for the results of the agility test as well. The lack of significant differences for the agility times may be best explained by examining the type of contractions seen during sprinting. Muscle contractions utilized during sprinting rely heavily on fast contractile velocities. As a result, the act of sprinting would involve very rapid, relatively low-force contractions. Consequently, if one were to accept the notion that the force–velocity relationship of skeletal muscle underlies the differences seen in the isokinetic measures, then the results of the agility test would be in line with the isokinetic trials, as the contractile velocities seen during

sprinting are similar to those seen during high-speed ($5.2 \text{ rad} \cdot \text{s}^{-1}$) isokinetics.

These findings have several potential clinical applications. Individuals beginning an exercise programme or beginning rehabilitation from surgery or an illness often withdraw from their exercise programme due to discomfort stemming from DOMS. The supplementation of protease may provide a reasonable treatment for these individuals. It could be argued that individuals taking protease supplements may actually show better training-related gains than those not receiving protease. This increased training effect could be attributable to the potential attenuation of the pain response and the accelerated rate of healing seen with protease supplementation. By coupling the effects of protease supplementation, a more rapid recovery of the contractile mechanism and decreased discomfort with a challenging training programme, an individual may be able to achieve higher training volumes than could be performed without supplementation. This may result in a faster recovery from illness or injury and a more rapid return to normal function.

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