

MYOCELLULAR ENZYME LEAKAGE, POLYMORPHONUCLEAR NEUTROPHIL ACTIVATION AND DELAYED ONSET MUSCLE SORENESS INDUCED BY ISOKINETIC ECCENTRIC EXERCISE

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ABSTRACT

To address the question of whether delayed onset muscular soreness (DOMS) following intense eccentric muscle contraction could be due to increased production of the arachidonic acid derived product prostaglandin E_2 (PGE_2), 10 healthy male subjects were submitted to eccentric and concentric isokinetic exercises on a Kin Trex device at 60°/s angular velocity. Exercise consisted of 8 stages of 5 maximal contractions of the knee extensor and flexor muscle groups of both legs separated by 1 min rest phases. There was an interval of at least 30 days between eccentric and concentric testing, and the order of the two exercise sessions was randomly assigned. The subjective presence and intensity of DOMS was evaluated using a visual analogue scale, immediately, following 24 h and 48 h after each test. Five blood samples were drawn from an antecubital vein: at rest before exercise, immediately after, after 30 min recovery, 24 h and 48 h after the tests. The magnitude of the acute inflammatory response to exercise was assessed by measuring plasma levels of polymorphonuclear elastase ([EL]), myeloperoxidase ([MPO]) and PGE_2 ([PGE_2]). Using two way analysis of variance, it appeared that only eccentric exercise significantly increased [EL] and DOMS, especially of the hamstring muscles. Furthermore, a significant decrease in eccentric peak torque of this muscle group only was observed on day 2 after eccentric work (- 21%; $P < 0.002$). Serum activity of creatine kinase and serum concentration of myoglobin increased significantly 24 and 48 h after both exercise tests. However, these variables reached significantly higher values following eccentric contractions 48 h after exercise. Mean [PGE_2] in the two exercise modes remained unchanged over time and were practically equal at each time point. On the basis of these findings, we conclude that the magnitude of polymorphonuclear (PMN) activation, muscle damage, and DOMS are greater after eccentric than after concentric muscle contractions. However, the hypothesized interplay between muscle damage, increased PGE_2 production, DOMS sensations, and reduced isokinetic muscle performance was not substantiated by the present results.

KEYWORDS: Isokinetic exercise, muscle damage, delayed onset muscle soreness, polymorphonuclear neutrophil, myeloperoxidase, elastase, prostaglandin.

INTRODUCTION

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The occurrence of exercise-induced muscle damage and the delayed experience of pain situated at groups of previously active muscles (delayed onset muscle

soreness, DOMS) following strenuous, unaccustomed exercise is, at present, well documented. Despite the growing body of data dealing with the damaging effects of exercise and DOMS, the underlying mechanisms of these phenomena remain poorly understood. Based on experimental studies showing that strenuous exercise triggers an inflammatory response showing some similarity with that occurring in trauma or infection, Smith (1991) put forward the hypothesis that DOMS could be a further manifestation of an exercise-induced inflammatory reaction to muscle damage. This author further suggested that muscle pain following exercise could be related to the increased production of PGE₂ by activated macrophages in injured tissue. Previous work by Salminen and Kihlström (1987) showed that indomethacin, a potent inhibitor of prostaglandin (PG) synthesis, protects mouse skeletal muscle fibers against exercise-induced injuries. More recently, Hasson *et al.* (1993) showed that another potent anti-inflammatory agent (ibuprofen), known to inhibit the production of PG, was found to significantly reduce sensations of muscle soreness after strenuous eccentric exercise. Furthermore, Hasson *et al.* (1993) found that the decline in torque production measured 24 and 48 h after eccentric testing was less marked after ibuprofen. Based on these findings, it is tempting to speculate that increased production of inflammatory mediators, derived from arachidonic acid metabolism through the cyclooxygenase pathway, could be involved in exercise-induced muscle damage and associated DOMS. It should be pointed out, however, that the effects of inhibitors of arachidonic acid metabolism on DOMS is a matter of controversy. Several authors have reported that anti-inflammatory agents failed to alleviate soreness sensation after exercise (Kuipers *et al.*, 1985; Headly *et al.*, 1986; Donnelly *et al.*, 1988; Camus *et al.*, 1993).

Therefore, in an attempt to assess the extent to which an exercise-induced inflammatory response and increased production of PGE₂ could be involved in DOMS and decreased muscle performance following damaging muscular work, we compared the effects of high intensity eccentric and concentric contractions on serum levels of two myocellular enzymes (taken as biochemical muscle damage indices) and blood levels of polymorphonuclear elastase ([EL]), myeloperoxidase ([MPO]) and ([PGE₂]). The rationale behind these experiments is based on the observation that eccentric contraction causes greater dam-

age to skeletal muscles and more marked DOMS than concentric contraction (Newham *et al.*, 1986; Friden *et al.*, 1986; Evans *et al.*, 1988).

MATERIAL AND METHODS

Subjects. Ten moderately active healthy male volunteers participated in this study after giving informed consent. Their mean age (\pm SEM) and body mass were 22 ± 0.4 years and 74 ± 1.5 kg, respectively. None was currently involved in lower body resistance or endurance training.

They were requested to refrain from strenuous exercise and to abstain from the consumption of any form of medication during the study period.

Exercise protocol. A standard warm-up phase consisting of 5 min cycling at 75-100 W on a bicycle ergometer (60 rpm) was followed by 5 min stretching exercises of the muscle groups subsequently used for isokinetic contractions.

All isokinetic measurements were performed on a *Kin Trex* device (*Medtronic Instruments SA*, Ecublens, Switzerland) at 60°/s angular velocity. Subjects were seated on a bench with a back rest tilted to approximately 105° of hip flexion. Their position was stabilized by means of belts placed across hips, chest and thigh.

On their first visit to the laboratory, the subjects were accustomed to the isokinetic device by performing 5 to 10 submaximal contractions of quadriceps and hamstring muscles. After a 5 min recovery phase, the eccentric and concentric maximum peak torque (PTmax) of these muscle groups were measured on both sides. During this measurement, the subjects performed 3 maximal contractions of knee flexor and extensor muscles at 60°/s in the eccentric and concentric modes. The greatest peak torque values were taken to represent PTmax. At least two weeks after this first test, the volunteers were submitted to eccentric or concentric exercise sessions carried out as follows. Each subject served as his own control, and completed two study protocols separated by at least 30 days. The order of the two exercise sessions was randomly assigned. Exercise consisted of 8 stages of 5 maximal contractions of the knee extensor and flexor muscle groups of both legs, separated by 1 min rest phases. To assess the effects of isokinetic contractions on muscle function, PT-

max was measured on day two after exercise testing, using the protocol described above.

Perception of muscle soreness and indirect evaluation of muscle damage. The subjective presence and intensity of DOMS were evaluated using a visual analogue scale graded from 0 (no pain) to 10 (very severe, intolerable pain) immediately after, 24, and 48 h after eccentric and concentric tests.

Increased serum levels of creatine kinase (CK) and myoglobin (Mb) were used as indirect indices of exercise-induced muscle damage.

Blood sampling. Before each test, an indwelling catheter was inserted into an antecubital vein and sealed. Blood samples (10 ml) were taken immediately before the isokinetic test, 10 min after catheter insertion; immediately after exercise, and after 30 min recovery. Two additional blood samples were drawn by venipuncture 24 and 48 h after exercise, respectively. The venous blood samples were divided in 2 aliquots: 5 ml whole fresh blood into a plain plastic tube, and 5 ml of whole blood into a vial containing EDTA as anticoagulant. The first portion was allowed to clot at room temperature, and the serum phase was used for the measurement of creatine kinase (CK) activity and myoglobin (Mb) concentration. The second portion was immediately centrifuged. The supernatants were kept at -70°C until analysis for plasma myeloperoxidase, elastase and PGE_2 determination.

To determine the possible influence of warming-up and stretching exercise on the baseline variables under investigation, blood samples were drawn in four subjects at rest before warm-up. Blood analyses revealed no effects of warm-up on baseline values. Hematocrit and hemoglobin measurements showed that the exercise protocol used in the present study did not induce any significant change in plasma volume (less than 3%).

Biochemical Assays. Plasma elastase was measured using an immunoactivation method (Merck, Kit 11332). Plasma myeloperoxidase and PGE_2 were assessed using the radioimmunological method described by Pincemail *et al.* (1991) and an enzyme immunoassay (Cayman Chemical, Kit 514016), respectively. Spectrophotometric techniques were used to measure the serum of creatine kinase activity [CK] and myoglobin concentration [Mb].

Statistical analysis. Values are expressed throughout this study as mean \pm SEM. For each variable, differences between mean values obtained at each time point in the two experiments were evaluated using a paired Student's *t* test. A two-way analysis of variance for repeated measures design (Two-way *Anova* test) was used to assess changes over time. Where appropriate, comparisons with baseline values were performed using paired *t*-tests with Bonferroni's adjustment. A *P* value < 0.05 was considered to represent statistical significance.

RESULTS

Because there was no significant difference between the peak torque of left and right limbs, mean PTmax values attained by knee flexor and extensor muscles during eccentric and concentric exercise at $60^{\circ}/\text{s}$ were calculated by pooling the values measured on both sides. As expected, the mean peak torque of extensor muscles (concentric mode: 225 ± 11.8 Nm; eccentric mode: 328 ± 18.8 Nm) was significantly greater than that of flexor muscles (concentric mode: 130 ± 8.8 Nm; eccentric mode: 161 ± 10 Nm; $P < 0.001$). It also appeared that the highest mean peak torque values were obtained in the eccentric mode ($P < 0.001$).

The effects of isokinetic exercise sessions on the rating of muscle soreness are shown in Figure 1. While concentric exercise did not induce muscle soreness, eccentric muscular work was followed by severe muscle pain in the knee extensor and flexor muscle groups 24 h after exercise. Maximal soreness sensations was noted 48 h postexercise, especially at the hamstring muscles.

Changes in serum CK and Mb values following both exercise conditions are illustrated in Figures 2 and 3, respectively. These variables increased progressively as a function of time in both experimental conditions. Following concentric exercise, [CK] reached a peak value of 1150 ± 364 IU/l after 24 h recovery. Eccentric exercise was followed by a more marked increase of [CK]. Maximal [CK] values of 8485 ± 2683 IU/l were measured on day 2 after eccentric isokinetic trials, and were significantly greater than those reached following concentric contractions. A progressive increase of [Mb] over time was also observed. After concentric exercise, [Mb] peaked after 30 min recovery (102 ± 32 ng/ml) and then decreased towards values slightly higher (45 ± 14

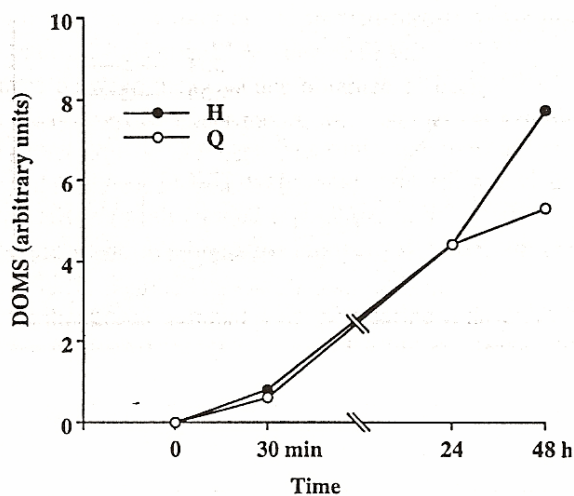


Fig. 1. MEAN SORENESS RATING SCORE (ARBITRARY UNITS) OF THE QUADRICEPS (Q) AND HAMSTRING (H) MUSCLE GROUPS BEFORE AND AFTER CONCENTRIC AND ECCENTRIC EXERCISE BOUTS. 0 : Pre-exercise; 30 min: immediately after exercise; 24 h, 48 h: 24 h and 48 h after exercise.

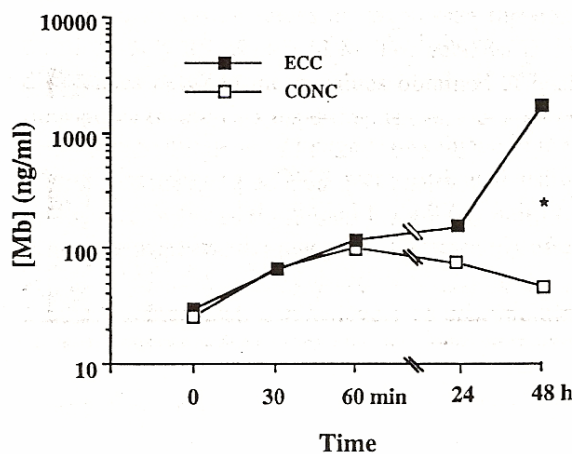


Fig. 3. MEAN SERUM CONCENTRATION OF MYOGLOBIN (LOGARITHMIC SCALE) AS A FUNCTION OF TIME IN CONCENTRIC AND ECCENTRIC EXERCISE TRIALS. (See Fig. 2). * Significant difference between eccentric and concentric isokinetic tests; $P < 0.05$

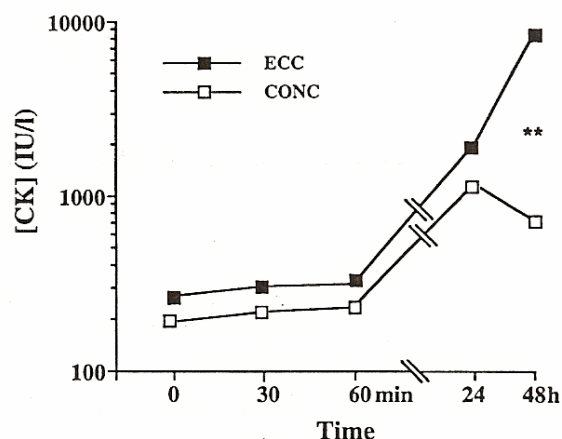


Fig. 2. MEAN SERUM ACTIVITY OF CREATINE KINASE (LOGARITHMIC SCALE) AS A FUNCTION OF TIME IN CONCENTRIC (CONC) AND ECCENTRIC (ECC) EXERCISE TRIALS. 0 : Pre-exercise; 30 min: immediately after exercise; 60 min: after 30 min recovery; 24 h, 48 h: 24 h and 48 h after exercise. ** Significant difference between eccentric and concentric isokinetic tests; $P < 0.001$.

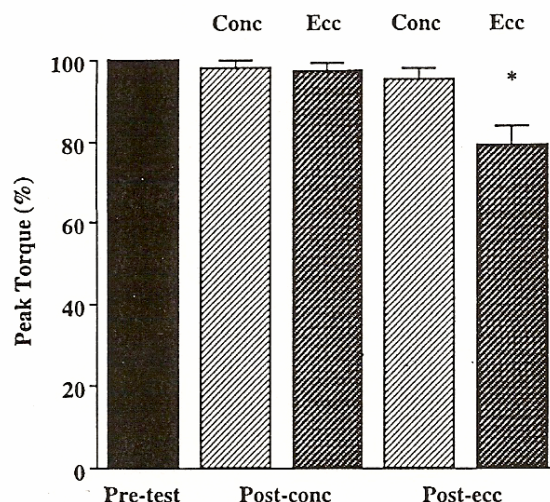


Fig. 4. PEAK TORQUE (IN % OF PRE-TEST VALUE) PRODUCED BY HAMSTRING MUSCLE GROUP IN ECCENTRIC AND CONCENTRIC MODES 2 DAYS AFTER EITHER MAXIMAL CONCENTRIC OR ECCENTRIC TESTS. * Significant difference between pre- and post-test values, $P < 0.002$.

ng/ml) than baseline on day 2 postexercise. While the changes of [Mb] induced by eccentric exercise were similar to those observed after concentric contractions up to 30 min post-test, this variable continued to increase, reaching top levels of 1634 ± 517 ng/ml after 48 h recovery. At this time point, the difference between the [Mb] values measured in the

two experimental conditions was significant ($P < 0.05$).

Taken overall, statistical analysis of these data indicates that while there was no significant difference for either [CK] or [Mb] at rest, immediately after, 30 min, and 24 h postexercise between the two experimental conditions, elevations of these biochem-

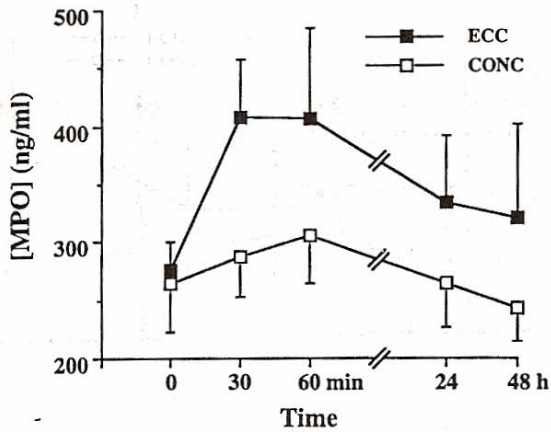


Fig. 5. CHANGES IN MEAN PLASMA CONCENTRATION OF MYELOPEROXIDASE INDUCED BY MAXIMAL CONCENTRIC AND ECCENTRIC CONTRACTIONS (see Fig. 2). Vertical bars are SEM.

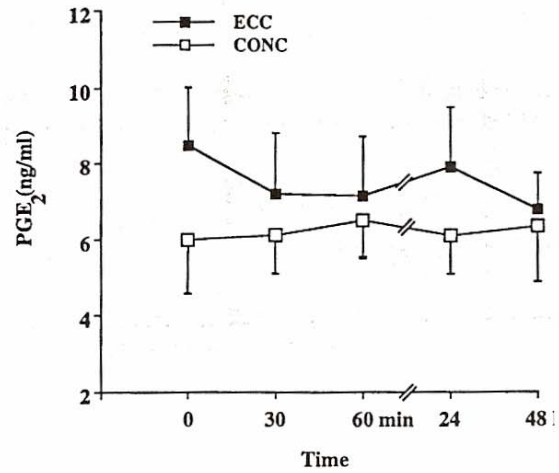


Fig. 7. PLASMA CONCENTRATION OF PROSTAGLANDIN E₂ ([PGE₂]) AS A FUNCTION OF TIME IN THE TWO EXPERIMENTS (see Fig. 2). Vertical bars are SEM.

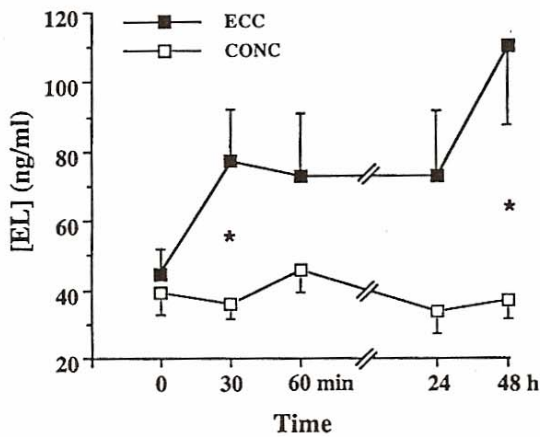


Fig. 6. CHANGES IN MEAN PLASMA CONCENTRATION OF ELASTASE INDUCED BY MAXIMAL CONCENTRIC AND ECCENTRIC CONTRACTIONS (see Fig. 2). Vertical bars are SEM.

* Significant difference between eccentric and concentric contractions, $P < 0.05$.

ical indices of muscle damage were significantly greater after eccentric than after concentric work 48 h post-test.

Concentric exercise did not cause any change in isokinetic muscle performance. Despite the soreness and marked increases of serum CK and Mb levels following eccentric contractions, there was no significant change in PTmax of the knee extensor muscles. However, eccentric contractions of the flexor muscles induced a significant decrease (-21%; $P <$

0.002) of the performance of the hamstrings in the eccentric mode only (Fig. 4).

The effects of maximal isokinetic contractions in the two exercise modes on [MPO] and [EL] are shown in Figures 5 and 6, respectively. There was no significant change in these variables over time. Plasma concentrations of MPO were maximal at the end of exercise and remained practically unchanged 30 min later. A slight decrease of mean [MPO] was observed subsequently. Two-way analysis of variance with repeated measurements showed that there was no significant change of [MPO] over time.

By paired t -test, it appeared that mean [MPO] values did not differ significantly between the two groups at each time interval.

Eccentric contractions also resulted in an increase of mean [EL] values. When compared to baseline, mean [EL] reached significantly higher values immediately after exercise and 48 h later ($P < 0.05$). On the other hand, there was a significant difference between mean [EL] measured under the two experimental conditions at these two sampling times.

Mean [PGE₂] measured at rest was 5.8 ± 1.4 and 8.5 ± 1.5 ng/ml before concentric and eccentric tests, respectively. As shown in Figure 7, the two exercise bouts did not elicit any significant change in [PGE₂]. Mean [PGE₂] values were not significantly different, at each time point, during the two experiments.

DISCUSSION

The concept of DOMS refers to the sensation of pain and discomfort accompanied by stiffness and weakness occurring in skeletal muscle after strenuous exercise, especially when high peak forces or eccentric contractions are involved (Friden *et al.*, 1986; Donnelly *et al.*, 1988; Smith, 1991; Hasson *et al.*, 1993).

Despite the considerable amount of data that has accumulated on DOMS following muscular exercise, the mechanisms responsible for this syndrome are poorly understood. It has been hypothesized that muscle damage triggers an inflammatory response (Smith, 1991; Camus *et al.*, 1993, 1994c; Cannon *et al.*, 1994) which is ultimately responsible for the delayed sensation of pain at the level of previously active muscle (Smith, 1991; Camus *et al.*, 1994c).

The present study was designed to verify the hypothesis that DOMS following intense eccentric contractions could be related to increased PGE₂ production in response to exercise-induced muscle damage. For that purpose, we compared the time course of serum levels of CK and Mb in subjects submitted to intensive concentric and eccentric isokinetic exercise. In addition, we studied the changes of plasma levels of PGE₂, polymorphonuclear-derived EL and MPO over time.

The rationale behind these experiments was based on the results of previous work showing that unaccustomed intense eccentric contractions cause greater muscle damage (Newham *et al.*, 1986), and are followed by more severe DOMS symptoms than concentric exercise (Friden *et al.*, 1986). Furthermore, it has been shown that muscle function was impaired to a greater extent after eccentrically rather than concentrically biased exercise (Newham *et al.*, 1986; Friden *et al.*, 1986).

In agreement with these data, we found that while concentric exercise did not induce muscle pain or loss of force, eccentric contractions were followed by marked DOMS in the quadriceps and hamstring muscle groups. Within the study period, these painful sensations reached a maximal intensity 48 h post-exercise. Consistent with the hypothesis of exercise-induced muscle damage as an etiologic factor of DOMS is the finding that peak levels of [CK] and [Mb] were also reached on day two after exercise.

As pointed out by Smith (1991), several algogenic substances such as potassium, calcium, kinins, and prostaglandins could be involved in the genera-

tion of DOMS. Among these, PGE₂ could play an important role in sensitizing the afferent nerve endings responsible for pain sensation. In contrast to previous reports (Herbaczynska *et al.*, 1976; Nowak & Wennmalm, 1978; Young & Sparks, 1979; Demers *et al.*, 1981; Rotto *et al.*, 1989; Symons *et al.*, 1991), there was no change in the plasma levels of this compound following isokinetic testing. Furthermore, there was no significant difference between mean [PGE₂] values measured in the two exercise protocols. On the basis of these results, it is tempting to conclude that this substance was not involved in DOMS following eccentrically biased exercise. It should be kept in mind, however, that plasma levels of PGE₂ were measured in blood samples drawn from a forearm vein. For ethical and technical reasons, it was not possible to measure the concentration of this substance in arterial blood or in the venous effluent from the muscles involved. The extent to which local production of PGE₂ could be detected by measuring the plasma level of this compound in venous blood samples taken at a site distant from active muscles is unknown. With this in mind, the possible involvement of this inflammatory mediator in the generation of DOMS cannot be definitively ruled out. Further studies based on specific cyclooxygenase inhibition, could help to verify the extent to which prostaglandins are involved in DOMS following damaging exercise. It should be pointed out, however, that prophylactic (and therapeutic) administration of piroxicam – a potent non-steroidal anti-inflammatory agent – failed to reduce subjective DOMS sensations and myocellular enzyme leakage in subjects submitted to maximal isokinetic eccentric contractions according to the present protocol (unpublished results).

A significant decrease of peak torque developed by the hamstrings was observed after eccentric exercise. This finding suggests that this muscle group is more sensitive to the damaging effects of eccentric contractions than the knee extensors. Consistent with this proposal are the results of histochemical studies showing that the hamstring muscles have a relatively high proportion of fast type II fibers (Garrett *et al.*, 1984). These fibers have been found to be most severely damaged by strenuous eccentric exercise (Friden *et al.* 1983; Jones *et al.*, 1986).

The exercise-induced PMN activation occurring following eccentric exercise is in agreement with the results of previous studies (Camus *et al.*, 1992,

1993, 1994a & b) and lends further support to the hypothesis that muscle and/or connective tissue damage associated with the eccentric component of muscle contraction is involved in PMN degranulation.

Although myeloperoxidase and elastase are stored in the azurophilic granules of PMN, the pattern of change of the plasma levels of these enzymes over time were different. While the immediate postexercise increase of [MPO] was followed by a progressive decrease of this variable towards baseline values, mean [EL] significantly increased after exercise. High plasma elastase levels persisted later than MPO after exercise. Furthermore, this variable reached its highest value on day two after eccentric test. This difference in the pattern of changes of [EL] and [MPO] is surprising, given their common origin, and remains unexplained. Interestingly, different time courses of [EL] and [MPO] have been reported previously by Faymonville *et al.* (1991) in patients undergoing surgery with cardiopulmonary bypass. Among the possible reasons for this apparently unusual finding is the fact that the plasma levels of these enzymes reflect not only their release from PMN but also their elimination from plasma. From this point of view, it is worth remembering that the clearance of elastase involves the formation of elastase-antiprotease complexes which are metabolized by the reticuloendothelial system (Deby-Dupont *et al.*, 1991). In contrast, the elimination of MPO does not depend on active transport or clearance mechanisms involving antiproteases (Deby-Dupont *et al.*, 1991). On the other hand, while the radioimmunological technique used to measure [MPO] detects free MPO only, both free elastase and elastase combined with alpha-1-protease inhibitor are detected by the enzyme immunoactivation method used in this study. Accordingly, it seems reasonable to assume that the differences in the kinetics of MPO and elastase following exercise could be due, at least in part, to the processes involved in the removal of these enzymes from blood.

In conclusion, the results of the present study confirm that maximal isokinetic contractions in the eccentric mode cause extensive muscle damage, as indicated by the increase in serum CK activity and Mb concentrations, persistent activation of PMN, and acute DOMS. On the basis of the unchanged plasma PGE₂ levels over time and the absence of significant differences between mean plasma PGE₂ concentrations measured after concentric and eccentric exercises, it is tempting to speculate that this inflamma-

tory mediator is not involved in the painful sensations associated with DOMS nor in the decrease of maximal eccentric peak torque produced by the hamstring muscle group. Due to the limited amount of experimental research aimed at determining the factors brought into play in the generation of delayed muscle pain, a firm scientific rationale to define adequate strategies to reduce DOMS is still lacking. Further studies are therefore needed to elucidate the underlying mechanisms of DOMS and impaired muscle function after damaging exercise.

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