Markers of inflammation and myofibrillar proteins following eccentric exercise in humans

Abstract The purpose of this study was to examine the time-course and relationships of technetium-99m ($^{99m}$Tc) neutrophils in muscle, interleukin-6 (IL-6), myosin heavy chain fragments (MHC), eccentric torque, and delayed onset muscle soreness (DOMS) following eccentric exercise in humans. Twelve male subjects completed a pre-test DOMS questionnaire, performed a strength test and had 100 ml blood withdrawn for analysis of plasma IL-6 and MHC content. The neutrophils were separated, labelled with $^{99m}$Tc, and reinfused into the subjects immediately before the exercise.

Following 300 eccentric repetitions of the right quadriceps muscles on an isokinetic dynamometer, the subjects had 10 ml of blood withdrawn and repeated the eccentric torque exercise tests and DOMS questionnaire at 0, 2, 4, 6, 20, 24, 48, 72 h, and 6 and 9 days. Bilateral images of the quadriceps muscles were taken at 2, 4, and 6 h. Computer analysis of regions of interest was used to determine the average count per pixel. The $^{99m}$Tc neutrophils and IL-6 increased up to 6 h post-exercise ($P < 0.05$). The neutrophils were greater in the exercised muscle than the non-exercised muscle ($P < 0.01$).

The DOMS was increased from 0 to 48 h, eccentric torque decreased from 2 to 24 h, and MHC peaked at 72 h post-exercise ($P < 0.001$). Significant relationships were found between IL-6 at 2 h and DOMS at 24 h post-exercise ($r = 0.68$) and assessment of the magnitude of change between IL-6 and MHC ($r = 0.66$). These findings suggest a relationship between damage to the contractile proteins and inflammation, and that DOMS is associated with inflammation but not with muscle damage.

Key words Neutrophils · Interleukin-6 · Myosin heavy chain fragments · Delayed onset muscle soreness

Introduction

Exercise-induced muscle injury has been associated with structural damage of the sarcomeres (Fridén et al. 1983; Newham 1988), protein leakage from the injured muscle fibres (Sorichter et al. 1999), an acute inflammatory reaction (Fielding et al. 1993; MacIntyre et al. 1996), loss of muscle force (Faulkner et al. 1993; MacIntyre et al. 1996) and delayed onset muscle soreness (DOMS) (Armstrong 1984; Staubes 1989). Although the structural damage, an acute inflammatory response, and the presence of DOMS have been established, the identification of markers of specific skeletal muscle injury and inflammation is an important and ongoing process.
Sorichter et al. (1999) recently summarized the literature on the identification and usefulness of markers of muscle injury and muscle membrane integrity. They reported that myoglobin, lactate dehydrogenase and creatine kinase have been amongst the most commonly reported indicators; however, these have shown considerable variability, may be related to membrane integrity only, or are not specific to skeletal muscle. In the last decade, assays using myofibrillar-bound proteins, such as myosin heavy chain (MHC) fragments of slow-twitch skeletal muscle myosin and calcium regulatory proteins such as the troponins, have been investigated as markers of the disruption that occurs within the contractile unit (for complete review see Sorichter et al. 1999). MHC fragments appear in the circulating blood 1–3 days following eccentric exercise and the content remains increased for up to 10 days (Mair et al. 1992, 1995; Sorichter et al. 1997).

Acute inflammation in tissue is an early post-injury response. It is characterized by a rapid increase in local blood flow and vascular permeability, and an influx of leucocytes, the first of which are neutrophils (Kent and Hart 1993). Neutrophils migrate towards chemoatractants, such as cytokines and complement fragments, and accumulate at the site of the injury for 6–12 h (Walker and Fantone 1993). Their lifespan in the tissue is usually to be 1–2 days (Walker and Fantone 1993). Neutrophils and macrophages are phagocytes, which release oxygen radicals and proteases (Roitt 1991), potentially causing further damage to the muscle tissue.

According to Cannon and St. Pierre (1998), cytokines function as intercellular signals. Virtually all cells with nuclei synthesize cytokines and have cytokine receptors on their cell membranes. Some cytokines, such as tumour necrosis factor (TNFz), interleukin-1 (IL-1), and IL-6, have been grouped according to their association with inflammation. When endothelial cells are stimulated by the acute inflammatory response following eccentric exercise, one of the events is the secretion of inflammatory-related cytokines by these cells. One of the cytokines released is IL-6. In addition, non-phagocytic macrophages (ED2⁺) and fibroblasts are thought to produce IL-6 later (Cannon and St. Pierre 1998), which is probably related to repair of the tissue.

Given the recent availability of assays for markers in the blood circulation of muscle injury and inflammation, and the less invasive nature of these measures, it is timely to investigate their responses following eccentric exercise and the relationships amongst them. The purpose of this study was to examine prospectively the time course and relationships of two markers of inflammation [technetium-99m (⁹⁹mTc) labelled neutrophils in muscle and the cytokine IL-6], one marker of contractile protein damage (MHC), eccentric torque, and DOMS over 9 days following strenuous eccentric exercise. It was hypothesized that there would be relationships among the estimates of acute inflammatory response (⁹⁹mTc neutrophils and IL-6), and an indicator of muscle damage (MHC), eccentric torque, and DOMS.

### Methods

#### Subjects

Twelve healthy male subjects (21–29 years) who were exercising for less than 6 h a week participated in this study. Individuals were excluded from the study if they were taking non-steroidal anti-inflammatory medication, if they were engaged in recreational exercise of more than 6 h a week, if they were jogging, running, or weight training, or if they were involved in competitive sport. The experiments complied with the current laws of Canada, the country in which the experiments were performed.

#### Experiment protocol

Approval for this study was granted by the Clinical Screening Committee for Research Involving Human Subjects at the University of British Columbia. All subjects provided written informed consent.

Prior to the eccentric exercise session, the male subjects completed a questionnaire to assess their positions on a muscle soreness scale (Descriptor Differential Scale, DDS), a test of the right eccentric quadriceps muscle strength, and had 10 ml of blood withdrawn by venipuncture for analysis of IL-6 and MHC concentrations. At 3 h before the exercise session, 100 ml of blood was withdrawn by venipuncture, and the neutrophils were separated and labelled with ⁹⁹mTc. The labelled cells were re-infused immediately before the strenuous exercise. Following 300 eccentric repetitions of the quadriceps muscles of the right lower extremity using an isokinetic dynamometer, subjects had another 10 ml of blood withdrawn, and repeated the assessment of soreness and tests of muscle strength at 0, 2, 4, 6, 20, 24, 48, 72 h, 6 and 9 days. Radiouclide images of both quadriceps muscles (lateral views) were taken at 2, 4 and 6 h post-exercise. The number of times these images were taken was limited by the availability of time in the Nuclear Medicine Department.

#### Descriptor Differential Scale

The intensity of the soreness of the quadriceps muscle (DOMS) was assessed using the sensory intensity scale of the DDS (Gracey and Kwilosz 1988). This scale contains 12 descriptor items with 21 points along the line for each descriptor. The use of this scale to evaluate muscle soreness has been reported previously (MacIntyre et al. 1995). Test-retest reliability of the sensory intensity scale has been established at r = 0.82 (Gracey and Kwilosz 1988).

#### Eccentric torque

The subjects were seated on the KinCom isokinetic dynamometer (Chattanooga Group, Hixson, Tenn., USA), and performed a strength test of the right quadriceps muscles as described previously (MacIntyre et al. 2000). Average eccentric torque normalized to body mass was calculated.

#### Laboratory assays

Blood was collected in 10-ml samples by venipuncture into EDTA tubes. The blood was centrifuged at 3,000g for 10 min. Plasma was removed, aspirated into 1-ml aliquots, and frozen at −70 °C for later analysis. Concentrations of MHC fragments were measured by an immuno-radiometric assay (ERIA Diagnostics Pasteur, Marnes la Coquette, France) according to techniques described previously (Mair et al. 1992, 1995). The IL-6 concentration was analysed using a Quantikine HS Kit (R and D Systems, Minneapolis, Minn. USA).