

EMG, MUSCLE CONTRACTILE FORCE AND CREATINE KINASE OF EXERCISE INDUCED STIFFENED MUSCLE IN RABBITS

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Exercise induced muscle stiffness are often found following heavy exercise. The objective of this study was to examine the changes of EMG, maximal muscle contraction force (MMCF), and creatine kinase (CK) activity in stiff muscle. The model of exercise-induced muscle stiffness was established by muscle contraction caused by electrical pulse. MMCF and EMG of the gastrocnemius were recorded for 15 sec. by the end and before the beginning of every section of muscle contraction. CK activity in gastrocnemius homogenate was analysed. Results showed spontaneous EMG signals at the condition of no electrical stimulation, a decrease of over 60 % of control maximal muscle contraction force (cMMCF), and a loss of CK activity in stiffened muscle. It indicated that exercise-induced muscle stiffness is accompanied by structure injury.

KEY WORDS: electromyography, muscle contraction force, creatine kinase, exercise, muscle stiffness.

INTRODUCTION: Heavy exercise can produce muscle stiffness and soreness. It often occurs immediately after exercise or even during training and competition, limiting exercise performance and being one of the factors of sports injury. Studies about the mechanism of exercise-induced muscle stiffness are limited. Therefore, the prevention and treatment of exercise-induced muscle stiffness are restricted due to the unclear aetiology.

Two main hypothesis about the mechanisms of exercise-induced muscle stiffness were presented: 'muscle spasm hypothesis' (De Vries, 1961) and 'muscle oedema hypothesis' (Jacobsson, 1964). The former hypothesis was based on EMG studies that found spontaneous EMG signals in the condition where no external stimulation was put on muscle. The changed EMG patterns were found in stiff muscles in the later studies (Gollhofer, Komi, Fujitsuka, & Miyashita, 1987; Guo, 1984), but the spontaneous EMG signals could not be detected again. The results from these studies suggested that the stiff muscle might be in the condition of spasm. This hypothesis was supported by morphological examination in which a shortened length of sarcomere was found (Zhang and Guo, 1988).

The muscle oedema hypothesis was based on studies by Jacobsson (1964). It was found that exercise-induced stiff muscle showed increased weight, water contents, and enlarged volume. Muscle oedema, complained by many athletes who suffer from muscle stiffness, is also a consideration for the oedema hypothesis.

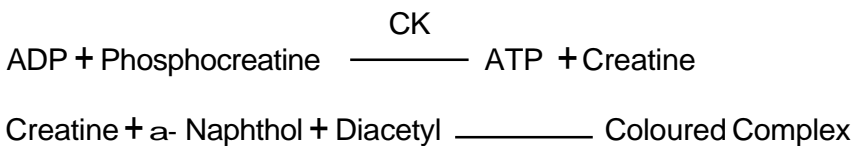
A few studies about the mechanism of exercise-induced muscle stiffness have been concentrated on an examination of the connection **between** the changes of muscle function activity and the muscular biochemical metabolism. Little information on the metabolism of exercise-induced muscle stiffness is provided. The objective of this study was to examine the changes of stiffed muscle on function and metabolism by observation of CK activity, EMG, and MMCF. It was expected to provide experimental evidence of the changes of muscular function and metabolism in stiffed muscle so as to add the understanding to the mechanism of exercise-induced muscle stiffness.

METHODS: A total of 13 male adult New Zealand white rabbits (weight = 2.0 - 2.5 kg) were used in this study. The model of exercise-induced muscle stiffness was based on the work of Zhang and Guo (1988). The animals were intravenously anaesthetised with pentobarbital (30 mg.kg⁻¹). The animals were prepared surgically. The tendon and innervation of the muscles were left intact. Two standard electrodes used for paediatric surface electrocardiogram were placed on the surface of gastrocnemius (O' Hagan, Anderson, Bell, Mittelstadt, & Clifford, 1995). The force transducer (National RM-6000) was placed on the distal tendon of the muscle. The knee joints were fixed along the sagittal axis by a metal

form with accessories. The muscle was held at the length that produced the greatest twitch force. The electrical pulses (10 - 30 V, 66 Hz, with 3 msx1000, 2 TPS) were put on the sciatic nerve. A section of muscle contraction caused by an electrical pulse consisted of three bouts, each 55 min. with an interval of 5 min. There was 1 h rest between the two sections of muscle contraction. After four sections of muscle contraction, muscle stiffness occurred (Zhang et al. 1988), likewise the MMCF showed 60% less than the cMMCF measured at the beginning of experiment.

Procedures: First, cMMCF was measured by changing the intensity of electrical stimulation before and the first section of exercise firstly and served as the control. Then, the muscle contraction was induced by the electrical pulse according to the protocol designed. EMG signals were measured for 15 sec by the end of each section of muscle contraction and before the next section. The MMCF was measured before each section of muscle contraction. The EMG signals were collected by a multi-channel electromyographicsystem (National RM-6000). The EMG signals were filtered at 5 Hz, sampled via an A/D converter at 2000 Hz and printed serially. After four sections of muscle contraction, muscle stiffness occurred. Likewise the MMCF was less 60 % of MMFC compared to cMMCF. The stiffened muscle samples were taken immediately from gastrocnemius. The samples were put in liquid nitrogen at once and then kept in refrigerator at -25 °C until CK activity of muscle were analysed.

The muscle samples were weighted and cut into very small pieces for homogenisation. The protein content of muscle homogenate was determined by the method of Lowry et al using bovine serum albumin as a standard (Georg-Burkhard, 1983). CK activity was determined using Sigma 520 kit (Hughes 1962). The reactions used to measure CK are as follows:



The change of colour is proportional to CK activity. The absorption of coloured complex was read (520 nm) at 25 °C. Each sample was assayed at least in duplicate. Activities were calculated as the mean of two values that differed by no more than 10 % of the lower value. The CK activity was presented with Sigma u.ml⁻¹.

The EMG signals recorded and printed out serially on papers were analysed by reading absolute EMG signal intensity approach. Signal intensity from peak to peak in millimetres was used for comparison. The measurements of MMCF were compared to the cMMCF and presented as the percentage of cMMCF (% cMMCF). CK activity was shown as Mean ± SD. Statistical significance was set at p<0.05.

RESULTS: MMCF in stiff muscle decreased 60 % compared to cMMCF. The muscle contraction force curve did not return to the baseline, showing no fully relaxation. In the stiff muscle EMG signal intensity significantly increased when the muscle contracted (p<0.05), 25 mm for the first bout of muscle contraction and 36 mm for the last bout of muscle contraction. Studies demonstrated that increased intensity of EMG signal is found in fatiguing muscle. Additionally, in eight rabbits the spontaneous EMG signals were detected when the stiff muscle was not stimulated by electrical pulses. The CK activity in stiffed muscle was significantly lower than control muscle, 15.37 ± 10.00 Sigma u.ml⁻¹ for stiffened muscle and 48.68 ± 4.81 Sigma u.ml⁻¹ for control muscle.

DISCUSSION: The spontaneous EMG signals were detected in the stiff muscle in 8 rabbits. This result was consistent with De Vries' s study (1961). The evidences of the spontaneous EMG signals indicated that the muscle was in the condition of spasm. Therefore, De Vries assumed that muscle stiffness followed by exercise resulted from the muscle spasm. In this study, the contraction force curve of muscle did not return to the baseline in stiff muscle, which was the evidence that muscle did not fully relaxed and was

in the condition of spasm. One reason inducing muscle spasm may be related to the changes of muscular metabolism, i.e. lower pH, high concentration of Ca^{++} , and energy deficiency (Fitts, 1994). Another reason leading to muscle spasm may be involved in damage of muscular structure. Based on the knowledge and experiences of neurophysiology and clinical medicine, the spontaneous EMG activity was regarded as pathological evidence. It can also be found in the conditions of the rupture of myofibers or the changes of the motor end-plate. The changes of sarcolemma characteristics and rupture of myofibers had been detected in several studies (e.g., Armstrong, 1984). Avela and Komi (1998) investigated the interaction between muscle stiffness and stretch reflex sensitivity after long-term stretch-shortening cycle exercise. Their results suggested that the decreased muscle performance is not simply a direct effect of central or peripheral fatigue, but is partly due to impairment of the ability to utilise stiffness-related elastic energy. Therefore, exercise-induced muscle stiffness may be an evidence of muscle fatigue or injury.

The results of this study showed that there was a loss of CK activity in the stiff muscle. CK mainly distributes in myocardium, brain and skeletal muscle. When these tissues are damaged or the membrane permeability of the cells increased, CK is released into blood circulation. Therefore, elevated levels of serum CK has been used to estimate tissue damage such as skeletal muscle (Clarkson, Cymes, McCarmick, Turcotte, & White, 1986). The significantly decreased CK activity in stiff muscle might be related to muscle injury (Armstrong, 1984 and 1990; Clarkson, et al. 1986). Two main reasons are thought to lower enzymes activity in muscle: damage of muscular structure and alteration of sarcolemma permeability (Armstrong, 1984). Altland (1961) found evidence of local muscle necrosis and elevated serum enzyme activity following 16 h exercise in rats. The duration and intensity of exercise in this study was closed to the duration and intensity of exercise in Altland's experiment. Therefore, the local injury of muscle might occur in the stiffened muscle. One limitation in this study was that the microstructure or ultrastructure of the stiff muscle could not be examined, so the muscle injury was estimated by the change of CK activity in muscle only rather than direct observation by morphological examination.

Exercise-induced muscle stiffness was thought to be the result of muscle spasm or oedema (De Vries, 1961; Jacobsson, 1964). Results obtained from the current study demonstrated that exercise-induced muscle stiffness was accompanied by muscle spasm indicated by spontaneous EMG signals recorded and muscle injury showed by a loss of CK activity in the muscle. Therefore, exercise-induced muscle stiffness should be an evidence of muscle fatigue or injury.

CONCLUSION: This study demonstrated that exercise-induced muscle stiffness was accompanied by muscle spasm and injury, which were indicated by recorded spontaneous EMG signals and decreased CK activity in stiff muscle. Therefore exercise-induced muscle stiffness is related to muscle fatigue and injury. The delay or relief of muscle fatigue and prevention of muscle injury should be the strategies of prevention and treatment of exercise-induced muscle stiffness.

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