**SHORT COMMUNICATION**

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**Muscle enzyme adaptation to training and tapering-off in spinal-cord-injured humans**

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**Abstract** The activity of muscle metabolic enzymes depends on the amount and type of physical training. We examined muscle enzyme adaptation to prolonged training followed by a period of lowered activity in spinal-cord-injured individuals (SCI). Ten SCI [mean age 35 (SEM 2) years, mean body mass 78 (SEM 4) kg, mean time post-injury 12 (SEM 2) years and range of lesion C5–T4] were given 12 months of functional electrical stimulation of an upright cycling motion for 30 min a day, three times a week, followed by 6 months of training once a week. Activities of glycolytic (hexokinase HK, lactate dehydrogenase LDH) and oxidative (citrate synthase CS, 3-hydroxyacyl-CoA dehydrogenase HAD) enzymes were determined in biopsies of the vastus lateralis muscle taken at 0, 3, 6, 12, and 18 months of training. The degree of sympathoadrenergic activity was evaluated from arterial concentrations of catecholamines in response to acute exercise. Training threetimes a week induced increases ($P<0.05$) in HK (150%), LDH (40%), CS (100%), and HAD (70%) activities that reached a plateau after 3 months. Peak oxygen uptake and power output during exercise by electrical stimulation rose continuously over the first 12 months. After reducing the amount of training by two-thirds, HK, LDH and CS activities remained elevated above basal levels ($P<0.05$), whereas HAD, power output and maximal oxygen uptake returned to pretraining levels ($P>0.05$). It is concluded that most improvements in glycolytic and mitochondrial oxidative enzyme activities induced by long-term training can be maintained in spinal-cord-injured individuals despite a marked reduction in training frequency unrelated to performance or to the degree of sympathoadrenergic impairment.

**Keywords** Electrical stimulation of exercise and detraining · Tetra- and paraplegia · Glycolytic and oxidative enzymes · Adrenaline · Myosin heavy chain

**Introduction**

Physical endurance training causes both central and peripheral adaptations, that depend on both amount and intensity of the training carried out (Blomqvist and Saltin 1983; Holloszy and Booth 1976; Saltin et al. 1968; Saltin and Gollnick 1983). The time pattern of adaptations in muscle metabolic capacity to physical training differs from those of improved cardiovascular performance and thus maximal oxygen uptake ($\bar{V}O_{2\text{max}}$). In general, more rapid changes are seen with respect to oxidative enzymes, as are found to be the case for $\bar{V}O_{2\text{max}}$, and similarly with detraining, muscle enzymatic parameters return much faster to their original basal values than $\bar{V}O_{2\text{max}}$ (Henriksson and Reitman 1977). Training reduction in well-trained individuals has been shown to reduce oxidative capacity in skeletal muscle and submaximal endurance performance without affecting whole body $\bar{V}O_{2\text{max}}$ (Houston et al. 1979) indicating that an association between muscle enzyme activity and performance exists. In line with a fall in oxidative enzyme activity levels being important for submaximal endurance, it has been shown that a two-thirds reduction in the amount of training in able-bodied individuals caused a drop in long-term muscle endur-
ance even though \( \dot{V}O_{2\text{max}} \) was maintained elevated (Hickson et al. 1982). However, previous studies in healthy individuals have been limited in their ability to draw strict conclusions, in that training periods were often too short to cause any plateau to be reached in muscle enzyme activity prior to any further intervention with detraining or tapering-off the amount of exercise. Spinal-cord-injured individuals have both a lowered cardiac capacity, with a varying degree of sympathetic activity depending on the spinal level of injury, and skeletal muscles with reduced amounts and activity of both oxidative and glycolytic enzymes (Castro et al. 1999; Grimby et al. 1976; Martin et al. 1992; Mohr et al. 1997; Rochester et al. 1995). Electrical stimulation of paralysed muscle has been shown to improve electrically stimulated muscle performance and to cause an increase in the concentrations of oxidative enzymes (Stein et al. 1992), but it is unknown what extent training induced muscle adaptations can be maintained in these individuals despite a marked reduction in the total amount of training. With this background the aim of the present study was to study the training induced responses in skeletal muscle enzyme activity during physical training as well as during a tapering-off period in which training was markedly reduced, and to compare these findings with concomitant changes in muscle performance, peak oxygen uptake and sympathoadrenergic activity. It was hypothesized that changes in muscle oxidative and glycolytic enzymes with training reduction would dissociate from peak oxygen uptake and muscle performance.

**Methods**

**Subjects**

Ten spinal-cord-injured (SCI) individuals [eight men and two women: mean age 35 (range 27–45) years, mean body mass 78.0 (SEM 3.8) kg] gave oral and written informed consent before participating in the training study that had been approved by the Municipal Ethics Committee of Copenhagen. Subjects were studied on average 12 (range 2–24) years after they had become injured and all were neurologically stable. Six were tetraplegic (injury level C6) and four paraplegic (injury level T4). They all had complete motor lesions in the lower extremities, and full passive ranges of motion of the hip and knee.

**Protocol**

The target training programme consisted of functional electrical stimulation of the legs for 30 min, three times a week for 12 months (a mean of 2.3 sessions were completed each week), followed by a target of exercise once a week for 6 months (0.9 times a week completed). The training was carried out on an upright seated computer-controlled functional electrical stimulation cycle ergometer (REGYS I Clinical Rehabilitation System, Tampa, Fla.) which has previously been described in detail (Kjaer et al. 1996). In short, the quadriceps, hamstrings and glutal muscle groups were stimulated through the skin in sequence to cause pedalling of a work-load-adjustable cycle-ergometer. The work-load was as high as possible for all subjects throughout the study. This was accomplished by adding resistance to the ergometer (0.125 kPa) after the subjects had been able to accomplish 30 min of training using a given work-load for more than 2–3 weeks. The \( \dot{V}O_{2\text{max}} \) and power output were measured every 3rd month to evaluate exercise induced changes in performance. The first measurement of \( \dot{V}O_{2\text{max}} \) was made when subjects had become habituated to exercise and were able to cycle continuously for at least 5–10 min (which occurred within a range of 0–6 weeks). The test was performed using an incremental load test (steps of 2 min, increasing the resistance stepwise by 1 kJ) until the subjects became fatigued. This was signified when the rate of pedalling had become reduced to less than 35 revolutions a minute and was achieved within a 10 min period of exercise (range 7.4–9.8 min). Expired gases were sampled during exercise for measurement of oxygen consumption and carbon dioxide production using an Oxycon chamber system (Jaeger Instruments, Heidelberg, Germany). Needle biopsies were taken from the midportion of vastus lateralis muscle (Bergstrom 1962) before and after 3, 6, 12 and 18 months of training. Local anaesthetic (2 ml, 1% lidocain, subcutaneously) was administered before cutting the skin and the biopsy procedure to avoid local acute reactions that could have generated spasm during the procedure. The biopsy was frozen immediately in liquid nitrogen for analysis of the oxidative and glycolytic enzymes. Samples were stored at –80°C until analysed. Before fluorometric measurements of citrate synthase (CS), hydroxacyl-3-dehydrogenase (HAD), lactate dehydrogenase (LDH) and hexokinase (HK) (Lowry and Passonneau 1972) biopsies were freeze dried, and connective tissue and fat tissue were removed. Samples were randomised in the different assays, but it was ensured that all samples of a given enzyme from a given individual were analysed in the same assay. A teflon catheter was inserted in one femoral artery to take blood samples at rest and after 15 and 30 min of exercise (9–12 months into the training period). Concentrations of noradrenaline and adrenaline were determined using a single isotope radioenzymatic method used previously (Kjaer et al. 1996).

**Statistics**

Statistical evaluation was made using non-parametric Wilcoxon rank sum tests and Spearman rank-sum correlation analyses. Tests showed that the data from this population were not normally distributed, and thus non-parametric tests were used. A level of 5% was considered significant (two-tailed testing).

**Results**

Mean work output per training session and \( \dot{V}O_{2\text{max}} \) initially were 5 (SEM 1) kJ and 1.20 (SEM 0.08) \( \dot{V}O_{2\text{min}}^{-1} \), respectively, in the group as measured within the first 4–6 weeks of training. In response to training three times a week these values gradually increased, being means of 10 (SEM 2) kJ and 1.31 (SEM 0.09) \( \dot{V}O_{2\text{min}}^{-1} \) at 3 months (\( P < 0.05 \)), 13 (SEM 2) kJ and 1.38 (SEM 0.10) \( \dot{V}O_{2\text{min}}^{-1} \) at 6 months (\( P < 0.05 \) compared to the value at 3 months) and 18 (SEM 2) kJ and 1.43 (SEM 0.09) \( \dot{V}O_{2\text{min}}^{-1} \) after 12 months (\( P < 0.05 \) compared to the value at 6 months). After completing 6 months of reduced training (months 12–18), mean work output per session and \( \dot{V}O_{2\text{max}} \) were lower (\( P < 0.05 \)) as compared with the measurements at 12 months [10 (SEM 2) kJ and 1.26 (SEM 0.11) \( \dot{V}O_{2\text{min}}^{-1} \), respectively]. The activities of HK (by 150%), LDH (40%), CS (100%) and HAD (70%) were higher (\( P < 0.05 \)) after 3 months of chronic electrical stimulation (Table 1), and no further increase was seen in any of the enzymes from 3 to 12 months of training. No change in the relative contribution of LDH 1 + 2 of the total LDH pool was observed in response to