A Pilot Study on Pain and the Upregulation of Myoglobin through Low-frequency and High-amplitude Electrical Stimulation-induced Muscle Contraction

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Abstract. [Purpose] It is well known that, in both in vivo and in vitro tests, muscle fatigue is produced by severe exercise, electrical stimulation, and so on. However, it is not clear whether or not low-frequency and high-amplitude modulation specifically affects serum myoglobin or urine myoglobin. The purpose of the present study was to determine the effect of low-frequency and high-amplitude modulation on serum myoglobin and urine myoglobin. [Methods] The study used whole blood samples and urine produced over 24 hours from the thirteen healthy subjects. [Results] There was a significant increase in serum myoglobin following electrical stimulation at a frequency of 10 Hz compared with the control group. Furthermore, within 24 hours, urine myoglobin also showed a significant increase for the test volunteers subjected to electrical stimulation at the 10 Hz frequency compared with the control group. However, there were no significant differences in the concentrations of hematologic results in subjects treated with electrical stimulation. [Conclusion] These results suggest that increased myoglobin related to muscle fatigue from electrical stimulation, particularly with a current of 10 Hz combined with a high-amplitude, may be partially related to increased muscle damage.

Key words: Myoglobin, Pain, High-amplitude electrical stimulation

INTRODUCTION

Myoglobin, which was discovered in 1897, consists of 153 amino acids and 17,500 dalton of monomeric heme protein1). Structurally, it consists of a single chain protein and a heme group2). Unlike hemoglobin, which is a tetramer, a molecule of myoglobin only has one iron atom. It has a high bonding affinity for oxygen, carbon monoxide, and nitric oxide3, 4). Myoglobin is found in muscle tissue including within muscle fiber cytoplasm and is described as an oxygen storage protein1, 5). When the oxygen supply becomes limited, myoglobin dissociates the combined oxygen and thus facilitates access to oxygen for the mitochondria. Finally, it promotes energy creation through oxidative phosphorylation3, 4). It is well known that inhibiting the function of myoglobin induces decreased oxygen uptake, decreased work output, and the production of adenosine triphosphate3, 4). However, pain and the excretion of myoglobin at the muscle are important biomarkers of muscle damage5–8). Myoglobin has a small molecular mass unlike creatine kinase, which leaks into blood (half-life: 5.5 ± 3.2 h)9, 10). It is also reported that myoglobin is useful for early diagnosis of myocardial infarction because it is rapidly excreted in urine9, 10). Some studies on pain control through limiting of sympathetic nerves by electric and hot stimuli have been carried out11, 12). It is known that applying improper electrotherapy can induce adverse effects, such as pain and muscle...
damage7, 13). However, no study has examined the effect of electric stimuli on myoglobin itself. Therefore, this study produced muscle contraction in subjects, confirmed visually, by applying frequencies of 1 Hz, 3 Hz, and 10 Hz, which were tolerable for participants, and examined any resulting pain response and changes in the volume of myoglobin in blood and urine.

SUBJECTS AND METHODS

A total of thirteen men who were healthy (age, 21–27; weight, 63.3 ± 3.3 kg; height, 172.1 ± 2.7 cm) were randomly selected. Before taking part in this study, no participants had been involved in similar experiments. The temperature was maintained at 24 ± 1 °C, and all measurements were taken with the participant in the sitting position. To reduce variable use of mechanisms of the body, the participants were housed together for 24 hours and provided limited food and drink. The participants were divided into a study group, to whom the electric stimuli were applied, and a control group. The stimulus applied was a low-frequency semisinusoidal wave, and measurements were taken (Neuromed III STT-100, Stratek Co., Ltd., Anyang, Republic of Korea) after an one-week interval. The first set of stimuli, electrical stimulation at 1 Hz, 3 Hz, and 10 Hz, respectively, was applied for 15 minutes. After 6 hours, the second set of stimuli, electrical stimulation at 1 Hz, 3 Hz, and 10 Hz, was applied for 30 minutes (Fig. 1). To induce dorsiflexion of the hand, the disperse electrode was placed on the dorsal hand, and the activated electrode set on the lateral region of the elbow joint and epicondyle of the humerus. During the test, all participants were in the sitting position with the humerus sustained comfortably on a table. The stimulation range was 16 to 30 mA, which was endurable for the participants (Fig. 1). Muscle contraction was confirmed visually. The visual analogue scale (VAS), faces pain rating scale (FRS), and Iowa pain thermometer (IPT) were used to measure the pain response and changes in serum and in urine. The values were tolerable for participants, and examined any resulting pain response and changes in the volume of myoglobin in blood and urine.

Fig. 1. Schematic representation of the experimental protocol for the normal healthy volunteers

Serum and urine myoglobin values were determined as described in the SUBJECTS AND METHODS section. SSW, semisinusoidal wave; E, electrical stimulation; dorsiflexion, extension of wrist joint; VAS, visual analogue scale; FRS, faces pain rating scale; IPT, Iowa pain thermometer

DISCUSSION

In rehabilitation, electrical stimulation is commonly applied to stimulate nerves and muscle15. However, fatigue resistance, density of capillaries, age, and medical history differ in each patient. When electric stimuli are applied without taking this into account, the resulting muscle fatigue can induce the accumulation of lactic acid and metabolites16, 17. Muscle fatigue is the condition in which force-generating ability is decreased and power output cannot be sustained. It therefore means a decrease in performance18. It is known that the accumulation of lactic acid
and the related decrease in pH is the mechanism of muscle fatigue, as this inhibits reactions between Ca²⁺ and tropo -

nin C19). In a previous study, it was found that inhibiting Ca²⁺ uptake of the sarcoplasmic reticulum or decreases in Ca²⁺ release of the sarcoplasmic reticulum can lead to fatigue20). It has been reported that increases in extracellular K⁺ and intracellular Na⁺, caused by decreases in the function of Na⁺/K⁺-ATPase, are related to muscle fatigue21). The freeing of bradykinin and increasing activation of cy -

clooxygenase cause these elements to accumulate and can induce pain22, 23 ). The concentration of myoglobin is ap -

proximately 0.05–5 mmol/kg; it exists in skeletal muscle, cell membranes, and cytoplasm 3). The function of myoglo -

bin is storage and provision of oxygen for the tricarboxylic acid cycle of mitochondria. It is important in the production of adenosine triphosphate 4). Diffusion occurs because cytochrome has an oxygen bonding affinity 50 times higher than that of myoglobin 23). In other words, oxygen diffuses from myoglobin to mitochondria during muscle contrac -

tions, and this process is reversed during relaxation 24). The relevance of myoglobin being found in blood serum fol -

lowing exercise or muscle tissue damage is evident6, 25 ). Sabria et al. reported that serum myoglobin increased ac -

cording to exercise intensity, while there was no change during moderate exercise26). In a study by Kirwan et al., the value of both creatine kinase and myoglobin increased after unilateral isometric knee extension exercise with forty maximal voluntary contractions 27). Roti et al. measured the levels of creatine phosphokinase, lactate dehydrogenase, and myoglobin in blood in 33 football players during the 24 hours following a football game 28). The myoglobin val -

ue was greatly increased immediately after the game and then gradually decreased over time 28). Also, Paul et al. 29) reported results for measures taken from 17 weight lifters, stating that creatine kinase and myoglobin were increased after 3 sets of 6 weight lifting exercises with intensities of 70–80%. This indicates muscle damage29). Similar results were found in studies on soldiers, which showed that serum myoglobin was greatly increased after intense exercise 30). Viitasalo et al. said that serum myoglobin increased in case groups to which a water jet massage had been applied for 20–30 minutes, in contrast with control groups 31). In this study, the values of myoglobin in serum and urine were sig -

nificantly increased according to the increasing frequency of electrical stimuli. It is reported that myoglobin leaks into the blood of patients with cardiac infarction, renal failure, Duchenne type muscular dystrophy, and polymyositis, and so its presence can be used for diagnosis 9, 10, 32). Terrados et al. compared normobaric and hypobaric conditions and found that myoglobin increased in the latter condition 33).

Table 1. Hematologic changes due to electrical stimulation in healthy volunteers

| Parameter              | Control     | 1 Hz        | 3 Hz        | 10 Hz       |
|------------------------|-------------|-------------|-------------|-------------|
| RBC (× 10⁹/mm³)        | 4.9 ± 0.7   | 4.9 ± 0.2   | 4.9 ± 0.2   | 4.9 ± 0.2   |
| Hb (g/dl)              | 15.5 ± 0.3  | 15.5 ± 0.5  | 15.2 ± 1.0  | 16.0 ± 1.0  |
| Hct (%)                | 44.4 ± 1.7  | 44.6 ± 2.0  | 46.3 ± 2.0  | 46.0 ± 2.3  |
| MCV (fl)               | 92.0 ± 8.0  | 93.6 ± 2.2  | 94.0 ± 9.4  | 90.7 ± 8.1  |
| MCH (pg)               | 31.9 ± 0.5  | 31.9 ± 1.1  | 32.7 ± 1.5  | 32.1 ± 1.3  |
| MCHC (g/dl)            | 34.4 ± 0.4  | 34.9 ± 0.8  | 34.1 ± 1.1  | 34.2 ± 1.0  |
| SGOT (IU/l)            | 15.8 ± 0.8  | 18.0 ± 1.8  | 18.7 ± 1.5  | 19.5 ± 1.6  |
| SGPT (IU/l)            | 9.0 ± 0.8   | 11.0 ± 1.2  | 11.2 ± 0.4  | 12.3 ± 1.8  |
| Uric Acid (mg/dl)      | 6.1 ± 2.1   | 6.1 ± 2.0   | 6.0 ± 1.9   | 6.1 ± 2.3   |
| BUN (mg/dl)            | 12.2 ± 3.1  | 11.6 ± 3.1  | 13.0 ± 2.8  | 13.2 ± 3.9  |

The data are expressed as means±SEM. RBC, red blood cell or erythrocyte; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase; BUN, blood urea nitrogen

Table 2. The effects of phase duration-dependent electrical stimulation on pain and myoglobin in healthy volunteers

| Parameter              | Control     | 1 Hz        | 3 Hz        | 10 Hz       |
|------------------------|-------------|-------------|-------------|-------------|
| Serum myoglobin (ng/ml)| 7.7 ± 1.5   | 7.7 ± 2.2   | 8.8 ± 4.0   | 17.3 ± 4.7* |
| Urine myoglobin (ng/ml)| 5.1 ± 0.4   | 4.0 ± 0.5   | 4.2 ± 0.4   | 8.3 ± 0.8*  |
| Visual analogue scale  | 2.7 ± 0.9   | 3.0 ± 1.5   | 4.3 ± 1.8   | 6.7 ± 2.3*  |
| Faces pain rating scale| 2.3 ± 0.9   | 2.7 ± 1.2   | 3.7 ± 0.9   | 6.0 ± 1.5*  |
| Iowa pain thermometer  | 2.3 ± 0.9   | 2.7 ± 0.9   | 3.3 ± 0.7   | 6.7 ± 1.9*  |
| Total pain scale       | 2.4 ± 0.4   | 2.8 ± 0.6   | 3.8 ± 0.6   | 6.4 ± 0.8*  |

The data are expressed as mean±SEM. * p < 0.05
Cole suggested that myoglobin is important in maintaining muscle function and \( O_2 \) consumption\(^3\), \(^4\). He reported that the myoglobin of the dog gastrocnemius-plantaris muscle under conditions of hypoxia increased after isometric exercise with twitch stimulation at \( 3 \text{ Hz} \), \(^3\). \(^4\). We indirectly confirmed that electric stimuli do not affect liver function either\(^3\). \(^6\).

However, the values of serum and urine myoglobin showed a statistically significant increase for the 24 hours following application of the electric stimuli. Thus, based on the results of this and previous studies, it is important that electric stimuli are applied solely within the appropriate range of pain. Also, physical therapists must carefully manage use of electric stimuli according to the subjective experience of their patient\(^7\). However, it is difficult to be more specific with these limited results; more systematic and multilateral studies are needed\(^3\).

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