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Effects of Electrical Stimulation and Voluntary Exercise on Muscle Oxygenation Assessed by NIRS

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Abstract

In this study, we investigated the effect of low-frequency ES (electric stimulation) to muscle oxygenation level by NIRS (near infrared spectroscopy) from comparing with V-Ex (voluntary exercise). Ten subjects performed ES and V-Ex test in supine position on a bed with 90 degrees flexion of the right knee joint and fixation of the right ankle to the end of the bed with a strap. NIRS probe was placed on middle point of the vastus lateralis, and four electrodes were placed across the motor point of the rectus femoris and vastus lateralis. Stimulation voltage was started at 20 V (20 Hz, pulse duration: 200 µs, duty-cycle: 1s-1s), and then was increased at a rate of 3 V/30 s until maximal tolerance level. V-Ex (isometric knee extension) was performed with same posture as in ES, and exercise pattern was set at a 1-s contraction and 1-s relaxation cycle. Exercise intensity was started at 5% MVC (maximal voluntary contraction) and was increased at a rate of 5% MVC/30 sec until exhaustion. In ES and V-Ex, tissue oxygenation index was decreased with decrease in $O_2$Hb (oxy-hemoglobin) and increase in HHb (deoxy-hemoglobin), and muscle oxygenation levels at the end of test were very alike. Oxygen consumption, heart rate, systolic and diastolic blood pressure in ES and V-Ex increased significantly, however, the degree of change in ES was significantly lower than V-Ex. Blood lactate was significantly increased in both tests. Adrenaline and noradrenaline were significantly increased in V-Ex, even though they showed no change in ES. These results lead us to believe that ES is an effective technique activated muscle hypoxia and glycolytic pathway metabolism with low stress on respiratory, circulatory and sympathetic nervous systems.

Key Words: Electrical stimulation; Voluntary exercise; Near infrared spectroscopy; Muscle oxygenation level

Introduction

Electrical stimulation (ES) uses current percutaneously to excite nerve and muscle, and it was
possible to induce muscle contraction in any time without access to a large space. In addition, stimulation intensity can be controlled optionally. Actually, ES is used as a rehabilitation technique for the prevention of muscle atrophy after injury and muscle training for patients with spinal cord injury. Moreover, many studies have examined to elucidate an effect of ES on human skeletal muscle in healthy subjects. In addition, a few reports have used ES together with specialized training programs for swimming, basketball, and weightlifting to improve exercise performance.

In general, high-frequency ES can induce strong muscular contraction but simultaneously it induces muscle fatigue easily, while low-frequency ES enables lengthy stimulation without muscle fatigue. As muscle contractive condition is influenced by frequency like this, muscle metabolic pattern during ES is probably not the same in each stimulation model. Thus, enough examination about muscle metabolism during ES must be done to establish more effective ES way.

One procedure for confirming effects of ES on muscle is the measurement of oxygen consumption (\(\dot{V}_{O_2}\)) with a metabolic analyzer. However, local muscle metabolism not can be confirmed in this way, because \(\dot{V}_{O_2}\) reflects whole-body \(O_2\) consumption. \(^{31}\)P magnetic resonance spectroscopy \((^{31}\text{P-NMR})\) was first used for measurement of local skeletal muscle metabolism and a few investigations of muscle ATP metabolism during ES have been performed with \(^{31}\)P-NMR. However, measurement used \(^{31}\)P-NMR is not easy due to the need for large equipment and high cost.

Recently, near-infrared spectroscopy (NIRS) has been used to evaluate \(O_2\) delivery and \(O_2\) utilization in skeletal muscle. NIRS uses absorption of near-infrared light at a specific wavelength and permits qualitative and noninvasive assessment of in vivo changes in oxygenated and deoxygenated hemoglobin and myoglobin in tissue. Since the NIRS kit is very compact and measurement with it is very easy compared with \(^{31}\)P-NMR, NIRS has commonly been used to investigate muscle oxidative metabolism. Nevertheless, muscle oxygenation during ES has not been elucidated sufficiently. In fact, NIRS investigation of muscle oxygenation during ES has been reported only by Bhambhani et al in spinal cord injury subjects. Because of ES has used as rehabilitation technique in clinical field at the present, it is needed to clarify the muscle oxygenation during ES in order to establish more useful rehabilitation procedure.

Therefore, the aim of the present study was to evaluate muscle oxygenation responses during ES. In addition, we measured muscle oxygenation during voluntary exercise (V-Ex) in order to investigate about significance of muscle oxygenation during ES.

**Subjects and methods**

**Subjects**

Ten male subjects participated in this study (27.0±6.2 yr, 174.6±3.5 cm, 72.6±6.7 kg, BMI 23.8±1.6 kg/m\(^2\), and percent fat 21.6±3.9%). Percent body fat was measured by the bioelectrical impedance method (body fat analyzer TBF-102, TANITA Inc., Japan). All subjects were healthy and free of metabolic, cardiovascular, and musculoskeletal disease. Each subject was given complete information regarding the procedures, and from each written informed consent was obtained before commencement of this study.
Experimental procedure

This study consisted of two tests with ES or V-Ex. Both tests were performed after sufficient bed rest. The protocol of this study is shown in figure 1. Two tests were separated by at least 1 w and their order was randomized among subjects.

1. Electrical stimulation test

ES test was performed in supine position on a bed with 90 degrees flexion of the right knee joint and fixation of the right ankle to the end of the bed with a strap connected to a strain gauge (Fig. 1). ES started after 5 min rest in supine position on the bed. Four electrodes (HV-LLPAD, OMRON Inc., Japan) were placed across the motor point of the rectus femoris and vastus lateralis. The electrodes were connected to an electrical stimulator (Omuron Inc., Japan) that output voltage was limited to 92 V.

ES pattern was set at 20 Hz frequency, pulse current lasting 200 µs, and a 1-s on-off duty cycle, because ES with this pattern induces higher O2 uptake16). The starting stimulus voltage was 20 V, and voltage was increased 3 V/30 sec, i.e., the 1-s stimulation (muscle contraction) and 1-s relaxation cycle was repeated 15 times within 30 sec. This ES test protocol was set considered the time which sufficient muscle contraction was occurred is to be as same as possible V-Ex test without abrupt strong stimulus to muscle. Stimulation intensity was increased until maximal tolerance level (maximal voltage was 92 V). We provided subjects enough experience with muscle contraction with ES prior to performance of the experiment to remove anxiety for ES.

2. Voluntary exercise test

V-Ex test was performed in the same posture as ES (Fig. 1). In advance, maximal voluntary contraction (MVC) of isometric knee extension power was estimated with the same posture as in the test. MVC measurement was attempted two times and we adopted higher of the two.

Exercise pattern in V-Ex (isometric knee extension exercise) was set at a 1-s contraction and 1-s relaxation cycle, i.e. the same as for ES, and the 1-s muscle contraction and 1-s relaxation cycle was repeated 15 times within 30 sec. Starting exercise intensity was 5% MVC, and exercise intensity was increased 5% MVC/30 sec. Because if the exercise pattern was set smaller

Figure 1. Experimental protocol and image.
partition i.e. 3% MVC/30 sec, it was difficult to adjust a power output voluntarily, or if it was set rougher, subject get exhaustion soon. V-Ex protocol was set at 5% MVC/30 sec. The V-Ex test was carried out to exhaustion. V-Ex test was performed while monitoring power output on an oscilloscope (TEKTRONIX Inc., USA) through a force transducer (TR2105N, Sohgoheiso Inc., Japan).

**Measurement parameters**

1. VO\(_2\), VE, heart rate, and blood pressure

During the tests, ventilatory, and O\(_2\) consumption variables were measured with the AE-280S Aeromonitor (Minato Medical Science Inc., Japan). This system consisted of a microcomputer, a hot wire flow meter and a gas analyzer, which contained a sampling tube, filter, suction pump and O\(_2\) analyzer composed of a zirconium element and an infrared CO\(_2\) analyzer. The ventilatory and O\(_2\) consumption variables were calculated by the breath-by-breath method. Heart rate (HR) was monitored continuously throughout the tests by telemetry with a Lifescope 8 (Nihon-Koden Inc., Japan). These variables were averaged every 15 s. Blood pressure (BP) was measured with the STBP-780B (Nihon-CORIN Inc., Japan) every 1 min during the tests.

2. NIRS

NIRS (NIRO-300, Hamamatsu Photonics Inc., Japan) measurements were continuously recorded during the tests. NIRS probe was placed on middle point between electrodes that placed on the vastus lateralis with an optode separation distance of 5 cm. Cloth was placed around the probe and the skin to prevent contamination from ambient light. The average of adipose tissue thickness at NIRS probe placed point assessed by caliper was 4.3 ± 0.6 mm. Adipose tissue thickness affects NIRS measurement\(^\text{17}\). However, as the difference among subjects of this study was very little, we did not adjust for adipose tissue thickness in the NIRS variables. Oxy-hemoglobin (O\(_2\)Hb), deoxy-hemoglobin (HHb), total hemoglobin (cHB), and tissue oxygenation index (TOI) were measured with NIRS. Data sample time of NIRS kit was set 2 s and each NIRS variables were averaged every 10 s to analyze.

Changes in O\(_2\)Hb and HHb were determined by measuring light attenuation at 775, 810, 850, and 905 nm wavelengths, analyzed with an algorithm incorporating the modified Beer-Lambert law: \(A = \alpha c d B + G\), where \(A\) is the measured light attenuation, \(\alpha\) is the specific extinction coefficient of the absorbing compound (measured in mol/cm), \(c\) is the concentration of the absorbing compound (measured in mol), \(d\) is the distance between the optodes over the tissue surface, \(B\) is a differential pathlength factor of 4.94\(^\text{18}\), and \(G\) is a constant reflecting the scattering of light of the tissue. From these measurements, cHB (cHB = O\(_2\)Hb + HHb) was also derived. TOI (percentage O\(_2\) saturation × 100 O\(_2\)Hb/cHB) is based on an accurate determination of the relative proportions of HHb and O\(_2\)Hb in tissue, derived from the relative absorption coefficients obtained from the slope of light attenuation at four wavelengths over a distance measured at three focal points from the light emission. The relative absorption coefficients are converted to relative concentrations of the chromophores, and thus, the absolute ratio of O\(_2\)Hb to HHb can be determined. O\(_2\) saturation was calculated by performing multilinear regression on the scattering coefficient estimates obtained at each wavelength using reference spectra of pure HHb and O\(_2\)Hb.

3. Blood sample

Blood samples were drawn from the median cubital vein at rest and immediately post-test.

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The blood samples were distributed to individual tubes, and each sample was taken after centrifugation at 3000 rpm for 10 min at 4°C. All samples were immediately frozen and submitted to measurement (Otsuka assay Inc., Osaka, Japan). Blood samples were analyzed for catecholamines (adrenaline [Ad] and noradrenaline [NAd]) using a HPLC-DPE method (HLC-8030, Tosoh Inc., Tokyo, Japan), for lactate using an enzyme method (Determiner LA, Kyowa Medics Inc., Tokyo, Japan), and for creatine phosphokinase (CPK) using an electrophoresis method (Epalyzer, Helena Laboratories Inc., Saitama, Japan).

4. Data analysis

Differences between pre- and post-test values in VO₂, heart rate, blood pressure, and blood samples were analyzed by the paired t-test. Comparison of the value at the end of ES with V-Ex was analyzed by repeated two-way ANOVA test. The probability level accepted for statistical significance was p < 0.05.

Results

Muscle oxygenation measured by NIRS

In ES, the maximal tolerance level was 89.0 ± 6.9 V, and maximal exercise intensity in V-Ex was 63.5 ± 7.5% MVC. The time required of ES test was 11.5 ± 1.2 min and V-Ex was 6.4 ± 0.7 min. No side effects were recognized until maximal ES and maximal voluntary contraction of this study. ES-induced power output was 13.6 ± 5.3% MVC, V-Ex showed about five fold higher power output compared with ES. The reason for finishing the ES test in each subject is the muscle fatigue mainly.

One case of TOI in ES and V-Ex is shown in figure 2. TOI, an index of muscle oxygenation level, was decreased with increasing load intensity in ES and V-Ex. TOI in ES was decreased with increase in stimulation intensity from 56 V, and the average of the stimulation intensity at which TOI began to decrease in this study was 60.7 ± 5.9 V. On the other hand, TOI in V-Ex was changed just after the test was started and decreased with increasing exercise intensity.

![Figure 2](image-url)  
*Figure 2.* Changes in TOI (tissue oxygenation index) on vastus lateralis muscle induced by electrical stimulation (left) and voluntary exercise (right). TOI decreased with increase in stimulation intensity from 56 V in ES (electrical stimulation), on the other side it decreased just after the start of V-Ex (voluntary exercise) with increasing exercise intensity.
Changes in $\text{O}_2\text{Hb}$, $\text{HHb}$, and $\text{cHHb}$ after subtracting each baseline value are shown in two patterns (Fig. 3). In one case, $\text{O}_2\text{Hb}$ and $\text{HHb}$ changed like mirror image (decrease in $\text{O}_2\text{Hb}$ and increase in $\text{HHb}$) [Type-A], while in the other, though decrease in $\text{O}_2\text{Hb}$ was slight, TOI decreased with increase in $\text{HHb}$ [Type-B]. $\text{cHB}$ was increased in both types. With ES, six subjects exhibited Type-A and four subjects Type-B findings, and with V-Ex five subjects exhibited Type-A and five subjects Type-B findings. The average [±SD] of each oxygenation index ($\text{O}_2\text{Hb}$, $\text{HHb}$, $\text{cHB}$, and TOI) at the end of the tests in both types are shown in Table 1. Neither type-A nor type-B was showed significant differences between ES and V-Ex in any oxygenation index.

Figure 3. Oxy-Hemoglobin ($\text{O}_2\text{Hb}$), deoxy-Hemoglobin ($\text{HHb}$), and total Hemoglobin ($\text{cHB}$) on vastus lateralis muscle after subtracting each baseline value are shown in upper panel (electrical stimulation) and bottom panel (voluntary exercise). Type-A (left row) shows the case that $\text{O}_2\text{Hb}$ and $\text{HHb}$ changed like mirror image. Type-B (right row) shows the case that decrease in $\text{O}_2\text{Hb}$ was slight, TOI decreased with increase in $\text{HHb}$. 
Ventilatory and circulatory responses

Ventilatory gas volume (VE), VO2 and HR during ES and V-Ex of the subject indicated in figure 2 are shown in figure 4. VE, VO2, and HR during V-Ex increased with exercise intensity. 

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<tr>
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<th>Type-A</th>
<th>Type-B</th>
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<tr>
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<td>ES (n = 6)</td>
<td>V-Ex (n = 4)</td>
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<tr>
<td>O2Hb µmol/L</td>
<td>- 18.57 ± 6.19</td>
<td>- 17.83 ± 3.67</td>
</tr>
<tr>
<td>HHb µmol/L</td>
<td>31.69 ± 7.81</td>
<td>38.76 ± 10.15</td>
</tr>
<tr>
<td>cHB µmol/L</td>
<td>13.12 ± 7.11</td>
<td>20.93 ± 11.15</td>
</tr>
<tr>
<td>TOI %</td>
<td>36.47 ± 13.05</td>
<td>34.58 ± 14.25</td>
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Data are mean ± SD. P value was analyzed by repeated two way ANOVA. ES, electrical stimulation; V-Ex, voluntary exercise; O2Hb, oxy-hemoglobin; HHb, deoxy-hemoglobin; cHB, total hemoglobin; and TOI, tissue oxygenation index.

Table 1. NIRS indices on vastus lateralis muscle at end of the tests in type-A and type-B

Figure 4. Changes in VE (ventilatory gas volume: upper), VO2 (oxygen consumption: middle), and HR (heart rate: bottom) during ES (electrical stimulation) and V-Ex (voluntary exercise). VE, VO2, and HR during V-Ex increased with exercise intensity. These variables in ES increased as well, but the degree of change was not remarkable as it was for V-Ex.
In ES as well, though the degree of change was not remarkable as it was for V-Ex, these variables increased.

Significant increase in VE and VO₂ was observed in both tests (p<0.001) (Fig. 5). However, VE at the end of the test was significantly higher in V-Ex (24.92 ± 4.70 L/min; p<0.001) than in ES (13.01 ± 2.78 L/min), and VO₂ was also significantly higher in V-Ex (716.6 ± 143.5 mL/min) than in ES (335.5 ± 49.9 mL/min) (p<0.001). In HR as well, a significant increase was observed in both tests (p<0.01), and significantly higher in V-Ex (98 ± 16 beats/min) than in ES (73 ± 7 beats/min) (p<0.01).

Significant increase in diastolic and systolic BP was observed in both tests (p<0.001) (Fig. 5).

**Figure 5.** Changes in VO₂ (oxygen consumption), VE (ventilatory gas volume), HR (heart rate), systolic blood pressure, and diastolic blood pressure in ES (electrical stimulation; closed square) and V-Ex (voluntary exercise; opened circle). Significant increase in VE, VO₂, and HR was observed in both tests and these variables at the end of the test were significantly higher in V-Ex than in ES. Diastolic and systolic blood pressure were significantly increased in both tests and systolic blood pressure at the end of the test was significantly higher in V-Ex than in ES. #: pre vs post (*p<0.01, **p<0.001). ES, electrical stimulation; V-Ex, voluntary exercise; and CPK, creatine phosphokinase.

**Table 2. Metabolic parameters in each experiment**

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<th>Metabolic Parameter</th>
<th>ES</th>
<th>V-Ex</th>
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<tr>
<td></td>
<td>pre</td>
<td>post</td>
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<tr>
<td>Adrenaline (pg/mL)</td>
<td>30.4 ± 15.6</td>
<td>37.6 ± 13.2</td>
</tr>
<tr>
<td>Noradrenaline (pg/mL)</td>
<td>273.8 ± 75.4</td>
<td>293.8 ± 60.1</td>
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<tr>
<td>Lactate (mg/dL)</td>
<td>11.5 ± 3.8</td>
<td>14.6 ± 5.0*</td>
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<tr>
<td>CPK (U/L)</td>
<td>137.6 ± 48.3</td>
<td>137.6 ± 50.2</td>
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Data are mean ± SD. Alphabets in table (a or b) express significant differences. *: significant differences between pre and post value in each test analyzed by paired t-test. #: significant differences between post ES and post V-Ex value analyzed by repeated two way ANOVA. ES, electrical stimulation; V-Ex, voluntary exercise; and CPK, creatine phosphokinase.
Systolic BP at the end of the test was significantly higher in V-Ex (162.6 ± 25.7 mm Hg) than in ES (144.9 ± 17.5 mm Hg; p < 0.05). Diastolic BP at the end of the test was 90.0 ± 14.9 mm Hg in ES and 100.6 ± 16.3 mm Hg in V-Ex with no significant difference observed between ES and V-Ex.

**Blood samples**

Ad and NAd were significantly increased in V-Ex (p < 0.001), but these variables were not changed in ES. Ad and NAd at the end of V-Ex were significantly higher than at the end of ES (p < 0.01) (Table 2). On the other hand, lactate was significantly increased in both tests (p < 0.05), and significant difference between ES and V-Ex was not observed. CPK changed in neither ES nor V-Ex.

**Discussion**

In this study, ES-induced decrease in muscle oxygenation and blood lactate accumulation at maximum level was very similar for ES and V-Ex. However, VO₂, VE, HR, BP, and catecholamine (Ad and NAd) levels were significantly lower in ES than in V-Ex.

Muscle oxygenation level measured by NIRS decreased during incremental exercise, and the reproducibility of the rate of decrease was demonstrated⁹. Many investigations about changes in muscle oxygenation during exercise have been reported, but there are few reports on muscle oxygenation during ES. Bhambhani et al.⁴ reported that muscle oxygenation level decreased in vastus lateralis muscle during ramp cycling exercise with functional electrical stimulation to the gluteus maximus and quadriceps in spinal cord injury patients. Although the exercise pattern and type of subject in present study differed from those in Bhambhani’s study, muscle oxygenation level decreased, and O₂ consumption in muscle assessed by NIRS increased with ES. Thus, it was shown that ES could induce an O₂ metabolism on local skeletal muscle despite lack of voluntary muscle contraction.

Two patterns muscle oxygenation were observed during ES (and V-Ex). When exercise was started, O₂ extraction was enhanced in activated muscle with increase in load intensity. Because of the increase in HHb reflects O₂ consumption in skeletal muscle, O₂ supply from arterial blood flow into activated muscle might be lower than O₂ extraction volume in Type-A. On the other hand, Type-B showed low decrease in O₂Hb. Therefore, enough arterial blood volume might flow into activated muscle in Type-B. In other words, sufficient peripheral blood circulation might occur in Type-B subjects.

During V-Ex, NIRS changed immediately after the start of exercise, but in ES, a threshold at which NIRS began to change was observed. This threshold differed among subjects, and at present, it is unclear why a threshold appeared. However it is important to clarify individual thresholds, because ES intensity above the threshold is needed to induce O₂ metabolism in muscle.

Hypoxic stress contributes to muscle hypertrophy²⁰. If resistance training is practiced with vascular occlusion, muscle hypertrophy is easily induced with low load²¹,²². Since muscle oxygenation level decreased with ES in the present study, the previously reported muscle hypertrophy with ES²³,²⁷ may also participate in muscle hypoxia. In the future, detailed examination including NIRS is needed to establish a more useful ES protocol.

Significant difference in blood lactate concentration was not observed between ES and V-Ex.
This result showed that muscle contraction with ES appeared to sufficiently enhance energy metabolism in the glycolytic pathway in skeletal muscle. In previous studies, blood lactate accumulation or decrease in pH in skeletal muscle with ES have been reported\textsuperscript{12,24}. As type \( \alpha \) units which have a large capacity for glycogen utilization in humans\textsuperscript{24} are activated earlier than Type \( \alpha \) units during ES\textsuperscript{26}, it may be one reason why energy metabolism in the glycolytic pathway was enhanced and blood lactate was accumulated in ES. These results indicate that ES not only decreases muscle oxygenation level but also enhances energy metabolism in the glycolytic pathway in skeletal muscle. Hamada et al had shown the enhancement of whole body glucose uptake not only during but also after ES.

Increase in VO\textsubscript{2} reflected whole-body O\textsubscript{2} consumption was observed with compulsory muscle contraction with ES. However, changing volume of VO\textsubscript{2} in ES was significantly lower than that in V-Ex. This result reveals a difference in motor unit recruitment between ES and V-Ex. Actually, power output in ES (13.6 \( \pm \) 5.3% MVC) was very low compared with that in V-Ex (63.5 \( \pm \) 7.5% MVC). In ES, muscles related to exercise were only the stimulated by the stimulator, while V-Ex mobilized not only the stimulated muscle (rectus femoris and vastus lateralis) but also the trunk and lower limb muscle groups. The difference in HR between ES and V-Ex may also be related to the difference in whole-body demanded O\textsubscript{2} volume. Because muscle power output, i.e., motor unit recruitment, largely differed between ES and V-Ex, it was clear to differ from the effects on the circulatory system between the two tests. However, stimulation or exercise intensity increased to the maximal level of subjects in both tests, and in that situation, muscle oxygenation level and blood lactate accumulation between ES and V-Ex did not showed significant differences. It was one of the important observations in the present study.

CPK, which reflects muscle damage, was only minimally changed in ES and V-Ex. Eriksson et al\textsuperscript{24} also observed no increase in CPK with ES. On the other hand, Ad and NAd significantly increased in V-Ex, and in addition, heart rate and blood pressure significantly increased too. These findings indicate that the sympathetic nervous system was activated by the V-Ex test. Secretion of adrenocorticotropic hormone from the pituitary gland depends on centrifugal impulses from motor cortex\textsuperscript{27}. Since ES-induced muscle contraction is not voluntary exercise, blood catecholamine levels might not be changed in ES. Blood lactate concentration significantly increased in both tests, and no significantly differences between ES and V-Ex were observed. Thus, it was indicated that ES is procedure to induce a decrease of muscle oxygenation and activated energy metabolism in the glycolytic pathway with low O\textsubscript{2} consumption and restraint of sympathetic nervous system activity. These effects of ES on muscle metabolism and the circulatory system can be applied clinically. In fact, muscle strength and endurance exercise performance were improved by ES in COPD\textsuperscript{26} and heart failure patients\textsuperscript{29,30}. Therefore, ES-induced muscle contraction may be an effective procedure to enhance muscle metabolism for patients with severe obesity, diabetes mellitus, and orthopedic disorders. Furthermore, as the ES muscle stimulation has little influence on respiratory and circulatory systems, it can be used for the rehabilitation without symptoms of dyspnea in severe patients with pulmonary diseases, and without high load on the circulatory systems in patients with hypertension or heart diseases.

In conclusion, ES induced decrease in muscle oxygenation levels and lactate accumulation similar to V-Ex with little affect on the respiratory, circulatory and sympathetic nervous systems. The present findings suggest that ES can be effective muscle exercise way for subjects...
who are limited to perform activities.

Acknowledgement

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