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Cycling Exercise with Electrical Stimulation of Antagonist Muscles Increases Plasma Growth Hormone and IL-6

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Performing aerobics and resistance exercise at exactly the same time has not been available although combining both types of exercise in one training program has been attempted. The hybrid training system (HTS) is a resistance exercise that combines voluntary concentric muscle contractions with electrically stimulated eccentric muscle contractions. We devised an exercise technique using HTS on a cycle ergometer (HCE). Growth hormone (GH) and lactate are indicators of adequate training intensity. Interleukin-6 (IL-6) reflects enhancing lipid metabolism. The purpose of this study was to show that HCE provides sufficient exercise to stimulate the secretion of GH, lactate and IL-6. We compared an HCE test with cycle ergometer alone (CE). Ten healthy male subjects performed HCE and CE tests for 30 minutes each. The workload of both tests was set the same at 40% of each subject's peak oxygen uptake. For HCE, 2-minute HTS and 1-minute rest intervals were repeated. GH, lactate, and IL-6 were evaluated before and immediately after exercise, and at 15, 30 and 60 minutes. GH and lactate increased immediately after HCE. Moreover, the degree of the increases in GH after HCE (0 and 15 minutes) was higher than that after CE. IL-6 increased after HCE at 30 min, and the rate of change was higher than for CE. These results showed that HCE was more efficient in stimulating acute increases in GH, lactate and IL-6 than CE at the same workload. We may be able to combine electrically stimulated resistance exercise with aerobic exercise using HCE.

Keywords: cycling exercise; electrical stimulation; growth hormone; interleukin-6; lactate
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Introduction

Many ways of resistance or aerobic exercise have been developed for muscle strength or physical fitness (Taipale and Häkkinen 2013). Specifically, aerobic exercise combined with resistance exercise has been reported to not only reduce health risks and symptoms associated with physical inactivity but also to improve activities of daily living (Fyfe et al. 2014; Kang and Ratamess 2014). For instance, Sousa et al. (2014) reported that combining aerobic with resistance exercise 3 days/week for 9 months improved the distance of a 6-min walk (m) (as assessing aerobic endurance activities) in elderly people significantly greater than aerobic exercise alone even taking into account that the aerobic exercise group had more aerobic exercise volume. For metabolic syndrome patients, regular combined aerobic with resistance exercise has been recommended to decrease cardiovascular risk factors (Kang and Ratamess 2014). Tibana et al. (2014) showed that combining aerobic with resistance exercise for ten weeks reduced blood markers of inflammation such as nitric oxide, interleukin-10, osteoprotegerin, blood pressure and metabolic syndrome Z scores in women with metabolic syndrome. They reported that the improvement in the quality of life (physical function domain) and functional capacity (leg, chest and handgrip strength) reinforced the clinical relevance of combining aerobic with resistance exercise (Tibana et al. 2014). In this way, the advantages of combining aerobic exercise and resistance exercise have been reported for metabolic syndrome patients and elderly people with physical inactivity.

Cycling exercise is widely used as an aerobic exercise method, and improves exercise capacity and physical fitness (Metcalfe et al. 2012). In addition, cycling exercise is used as a resistance exercise (Macaluso et al. 2003;

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Koninckx et al. 2010). However, conventional exercise intensity, volume, frequency, repetition, velocity, and rest intervals were commonly different for aerobic and resistance exercise. Drummond et al. (2005) found that the aerobic-first sequence was more effective in augmenting excess post-exercise oxygen consumption. In their study, the aerobic exercise involved continuous running at a pace that corresponded to 70% $\dot{V}O_{2peak}$ for 25 min, whereas resistance exercise consisted of three sets of 10 repetitions at 70% of 1-repetition maximum (RM). However, Kang and Ratamess (2014) reported that the preceding aerobic exercise of high intensity or long duration might compromise the quality of subsequent resistance exercise in cases where there is not enough recovery time. Thus, people who seek to train for muscle hypertrophy have to choose to start the sequence with resistance exercise (Kang and Ratamess 2014). Performing both types of exercise at exactly the same time has not been available although combining aerobics exercise and resistance exercise in one training program has been attempted (Takeshima et al. 2004).

The combined application of electrical stimulation (ES) and volitional contraction (VC) has been said to be more effective than ES or VC alone (Dehail et al. 2008; Paillard 2008). We have developed the hybrid training system (HTS) as a type of resistance exercise that resists the motion of a volitionally contracting agonist muscle with force generated by its electrically stimulated antagonist (Shiba 2002) (Fig. 1). This method is capable of increasing torque production and muscle mass in the upper and lower extremities by amounts that are comparable to that of conventional weight training (WT) (Yanagi et al. 2003; Iwasaki et al. 2006; Matsuse et al. 2006). HTS is a beneficial technique in producing both muscle hypertrophy and strengthening in spite of using low ES intensity. Additionally, the system of HTS is compact and able to be combined with various conventional exercise methods. Recently, we have devised a new exercise technique combining aerobic and resistance exercise simultaneously using HTS on a cycle ergometer (HCE) (Masayuki et al. 2013). After testing the aerobic effect of this method, Masayuki et al. (2013) reported that HCE exercise resulted in a higher oxygen uptake than cycle ergometer alone (CE) linearly at all workload levels. However neither the long term training effects nor acute responses to training with HCE have been reported. Training effects of the acute phase include hormone and metabolism response.

Growth hormone (GH) has been shown to stimulate muscle protein synthesis and promote muscle mass growth in humans (Fryburg et al. 1991; Fryburg and Barrett 1993; Godfrey et al. 2003). It was also reported that the acute response of GH correlates well with changes in muscle size and strength (Wideman et al. 2002; Velloso 2008). Therefore, GH responses after resistance exercise show adequate exercise stimulus (McCall et al. 1999). Blood lactate indicates adequate training intensity (Bishop 2001). There is substantial evidence that the anaerobic metabolism

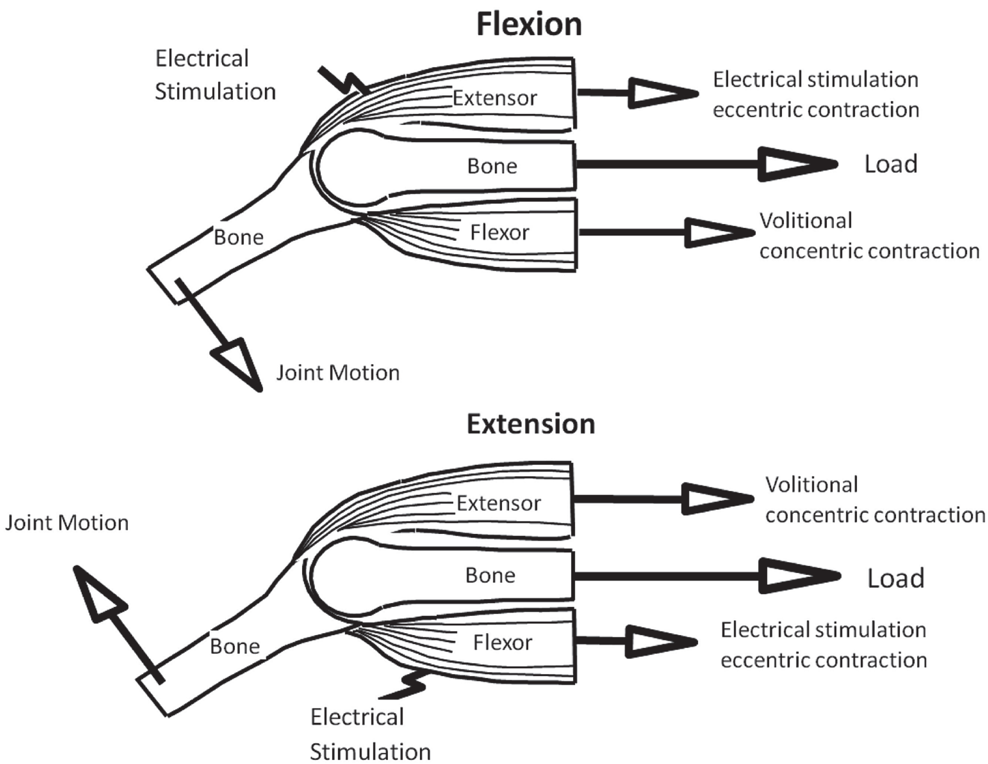

Fig. 1. Schematic model of HTS.
Both the volitionally activated agonist and the electrically stimulated antagonist contract during joint motion. The result is that both muscles are trained and a longitudinal compressive load is placed on the bone.

in the glycolytic pathway with the formation of lactate is more pronounced in ES than in voluntary exercise at identical low intensity (Kim et al.1995; Hamada et al. 2004). Gladden (2004) reported that lactate indicates local accumulation of metabolic byproducts of fast-twitch muscles as a result of ES and/or the utilization of glucose or glycogen as a fuel. It has been suggested that local accumulation of metabolic byproducts (lactate, proton) would stimulate secretion of GH through the hypothalamic pituitary axis (Takarada et al. 2000). We can estimate whether resistance exercise using ES was performed adequately by measuring lactate. Interleukin-6 (IL-6) released from contracting muscles into the circulation enhances lipolysis and gene transcription in abdominal subcutaneous fat via its effect on the adipose tissue (Pedersen et al. 2007). Furthermore, muscle-derived IL-6 is likely to inhibit low-grade TNF-α production and thereby enhance TNF-α-induced insulin resistance (Pedersen et al. 2007). IL-6 can be an index as to whether HCE enhances lipid metabolism.

We have hypothesized that HCE significantly stimulates GH, lactate and IL-6 secretion because HTS was shown to improve muscle mass and strength (Yanagi et al. 2003; Iwasaki et al. 2006; Matsuse et al. 2006). The purpose of this study was to show that HCE was sufficient exercise to stimulate GH, lactate and IL-6 secretion.

Subjects and Methods

Setting and subjects

It was designed in accordance with the ethical standards of the Helsinki Declaration of 1975 and received the approval of the Ethics Committee of Kurume University and the Japan Aerospace Exploration Agency. All procedures were fully explained to the subjects who gave their written informed consent to participate. Ten healthy young men [mean ± standard deviation (SD): age 23.8 ± 3.2 years; height 172.7 ± 4.6 cm; and weight 67.3 ± 5.9 kg, body mass index 22.6 ± 2.2 kg/m²] agreed to participate. Subjects were examined after giving consent by an orthopedic specialist who was not involved in this study. They were excluded if they clearly deviated from the inclusion criteria. This included the requirements that they had no adverse medical history, for example, fracture in upper or lower extremity, congenital heart disease, pulmonary disease or pituitary disease. They had passed an examination for normal activity of daily life, strength, sensation, and range of motion according to the criteria of the Japanese Orthopedic Association. Five of them had a history of sports activities, however we had required that they did not play any sports one week before the experiment. Environmental conditions were similar for all exercise tests (21 to 24 degrees centigrade, 45 to 55 % relative humidity).

Intervention

All subjects were measured for height and body weight, and performed the ramp exercise test to determine their $\dot{V}O_{2peak}$ and two cycle ergometer exercise tests. A ramp exercise test protocol was performed on an electronically braked cycle ergometer (75XL III, Konami, Tokyo, Japan) until subjects were not able to maintain their cadence above 60 revolutions/min. The knee joint range of motion was set at a nearly 90° arc that extended from 20° to 110° (0° indicating full knee extension) by adjusting the height of the saddle. We carried out the tests on different days at intervals of more than 1 week. Blood sampling was conducted on all subjects after both the CE test and the HCE test (Fig. 2). The subjects were asked to refrain from ingesting alcohol and caffeine for 12 hours prior to testing. The exercise test began at the same time of day (between 8:00 and 9:00 AM) for each subject to avoid diurnal variations in metabolism and hormonal responses.

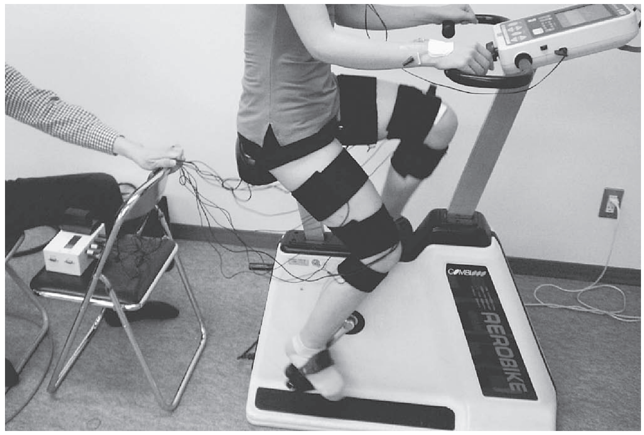

Fig. 2. The HCE method.
The subject sat on a cycle ergometer, with his hamstrings electrically stimulated as he volitionally extended his knee with his quadriceps electrically stimulated as he volitionally flexed his knee.

Ramp exercise test protocol

After a two-min rest sitting on the cycle ergometer, the test started at 20 watts (W), and the workload was increased by 20 or 30 W/min according to the physiological profile of each subject (Myers and Bellin 2000). Pedaling cadence was kept constant at 60 to 80 rev/min using a pedal frequency meter depending on the subject's preference. The exercise test was terminated when the pedal cadence could not be maintained at 60 rev/min, and $\dot{V}O_{2peak}$ was determined. Verbal encouragement was given during the ramp exercise test. $\dot{V}O_{2peak}$ of all subjects were 43.5 ± 9.8 ml/kg/min (mean ± SD).

CE test protocol

After a 2-min warm-up at 30 W, the subjects began the actual exercise protocol. The workload was set at 40% of each subject's $\dot{V}O_{2peak}$ for 30 min (Fig. 3A). Pedaling cadence was kept constant at 60-80 rev/min with the aid of a pedal frequency meter. We adjusted the workload for all subjects to reach each target heart rate during the test.

HCE test protocol

HTS was performed simultaneously during volitional cycle ergometer with the subject's hamstrings electrically stimulated as he volitionally extended his knee, and his quadriceps electrically stimulated as he volitionally flexed his knee to provide motion resistance. The pedaling cadence was kept at 60-80 rev/min. During HCE both lower extremities were stimulated corresponding to the bending motions of the knee using HTS. After a 2-min warm-up at 30 W, the subjects began the actual exercise protocol. The workload was set at 40% of each subject's $\dot{V}O_{2peak}$. 2-min HTS and 1-min rest intervals were repeated during the ergometer exercise for 30 min (Fig. 3B).

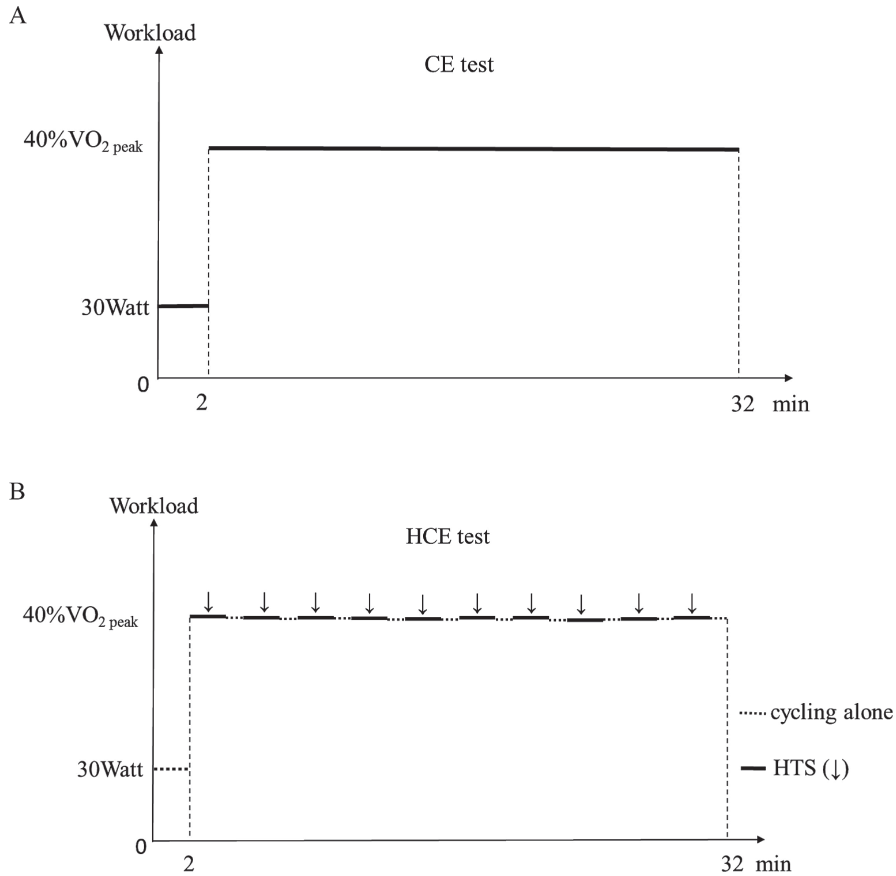

Fig. 3. The protocols of the CE test and the HCE test.
(A) The CE test. The workload was 40% of $\dot{V}O_{2peak}$ throughout the CE test. (B) The HCE test. 2-minute HTS and 1-minute rest intervals were repeated during ergometer exercise for 30 minutes in the HCE test. We calculated the workload by the linear regression equation and adjusted it as being the same oxygen uptake as CE for HCE. In addition, we adjusted the load for all subjects to reach their individual target heart rate during both tests.

Masayuki et al. (2013) reported that HCE exercise resulted in a higher oxygen uptake than CE linearly at all workload levels. They also noted that oxygen uptake during HCE was significantly higher than during CE at an average of about 21.1% (Masayuki et al. 2013). Based on the information, we calculated the workload using the linear regression equation and adjusted it to be the same oxygen uptake during HCE. In addition, we adjusted the load for all subjects to reach their individual target heart rate during the HCE test.

Electrical stimulation protocol

The ES device has been described previously (Yanagi et al. 2003; Matsuse et al. 2006) and consists of a custom designed waveform generator capable of delivering stimulating signals with unique frequencies and waveforms to as many as 4 pairs of electrodes and a joint motion sensor (Mutoh Engineering Inc., Tokyo, Japan) that triggers stimulation of the antagonist once it senses the initiation of an agonist's VC (Yoshimitsu et al. 2010). Pairs of 3 × 6-cm low impedance gel-coated carbon electrodes (Sekisui Chemical Co., LTD, Tokyo, Japan) were placed over each motor point on the quadriceps and the hamstrings and a detector was also attached (Fig. 4).

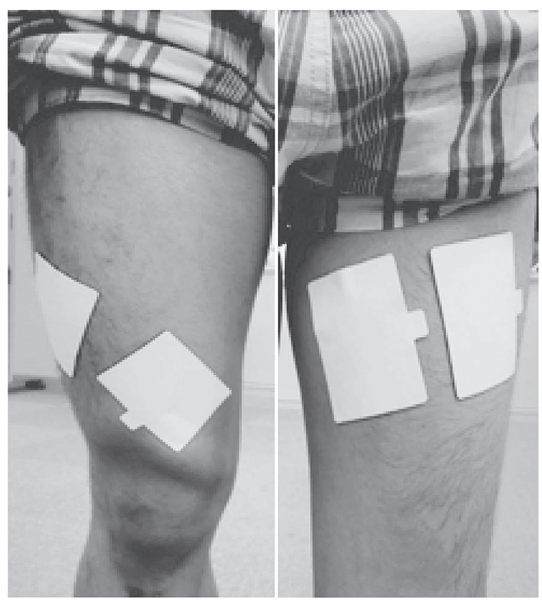

Fig. 4. Surface electrodes.
Gel-coated carbon electrodes are placed over the motor point of the quadriceps and hamstrings.

Stimulation parameters

The stimulation waveform used in this study was similar in some ways to that of "Russian stimulation" (Ward and Shkuratova

2002) and consisted of a 5,000 Hz carrier frequency modulated at 40 Hz (2.4 ms on, 22.6 ms off) to deliver a rectangular biphasic pulse (Yanagi et al. 2003). The electrical stimulator gave constant voltage stimulus to the human body (regulated voltage). It had a stimulus pattern with interlock and a limiter for safety. Therefore, the effective current was interlocked at 20 mA, and the peak voltage and current was limited to under 72 V and 90 mA. Stimulation intensities were determined before the evaluation session began. Matsuse et al. (2006) compared a group performing HTS at 25-30% maximum VC and conventional WT at 40% maximum VC 3 times per week for 8 weeks. They reported that biceps and triceps cross-sectional areas significantly increased by 10% and were larger than those in the WT group. In their study, stimulation intensities were set at the value of the threshold intensity plus two-thirds of the difference between the maximum comfortable and threshold intensities. In our study, we adjusted stimulation intensity levels to 80% of the maximum comfortable intensity which was near values of the threshold intensity plus two-thirds of the difference between the maximum comfortable and threshold intensities (Matsuse et al. 2006; Takano et al. 2010).

Blood sampling

Before the session, the subjects rested for 10 min. in the sitting position, after which a pre-exercise (Pre) blood sample was obtained. Blood samples were also obtained immediately after exercise (0 min), as well as after 15 min, 30 min and 60 min. Blood samples (20 ml for each point of measurement) were drawn through an indwelling cannula in the antecubital vein in supine position. All blood samples were centrifuged for 10 min, and the supernatant plasma stored at −20˚C until analysis. They were analyzed by SRL (Special Reference Laboratories, Tokyo, Japan). Plasma GH concentration was measured using the electrochemiluminescence immunoassay (ECLIA) method. Plasma lactate concentration was measured using the lactate oxidase method with an automatic analyzer (JCA-BM8000; Japan Electron Optics Laboratory., Tokyo, Japan) (Determiner LA; Kyowa Medex Co., Ltd., Tokyo, Japan) (Asanuma et al. 1985). Plasma IL-6 concentration was measured using the chemiluminescence enzyme immunoassay method (LUMIPULSE F; Fujirebio Inc., Tokyo, Japan).

Statistical analysis

All variables are presented as means and ± SD. Values for concentrations of GH and lactate were assessed using a paired t-test in order to compare the differences between pre-test and each time point after the test. We mainly compared the differences between pre-test and immediately after the test. In order to compare HCE with CE, a two-tailed pared t-test was used. Because the pre values were different between HCE and CE, the rate of change was evaluated for IL-6. All the statistical analyses were performed using JMP Version 11.0 statistical software (SAS Institute Inc., Cary, NC, USA), and p-values less than or equal to 0.05 were considered to be statistically significant.

Results

Plasma concentrations of GH, lactate, and IL-6

The changes in plasma concentrations of GH, IL-6, and lactate are shown in Fig. 5. The plasma concentration of GH peaked immediately after exercise in both the HCE and CE tests (Fig. 5A) (HCE: 8.0 ± 5.7 ng/ml, $P < 0.002$ and CE: 3.9 ± 4.6 ng/ml, $P < 0.029$). Also, the plasma concentration of GH at 15 min was significantly higher than that at pre-test in both the HCE and CE tests (HCE: 6.2 ± 3.5 ng/ml, $P < 0.001$ and CE: 3.5 ± 3.4 ng/ml, $P < 0.013$). The plasma concentration of GH during the HCE test was significantly higher than in the CE test immediately after exercise ($P < 0.014$) and at 15 min ($P < 0.008$).

Plasma concentrations of lactate peaked immediately after exercise in the HCE test (0 min: 19.6 ± 11.5 mg/dl, $P < 0.023$, Fig. 5B). However, there was no significant difference between HCE and CE after exercise ($P < 0.075$).

The plasma concentration of IL-6 was significantly higher at 30 min after the HCE test (Fig. 5C): pre-HCE, 1.4 ± 0.7 pg/mL vs. 1.8 ± 0.7 pg/mL after 30 min ($P < 0.003$). In contrast, the plasma concentration of IL-6 (pre-CE, 1.1± 0.5 pg/mL) was not significantly increased after 30 min of CE (1.2 ± 0.5 pg/mL, $P < 0.103$). The rate of change in IL-6 concentrations at 30 min after the HCE test was significantly higher than that with CE (HCE: 1.4 ± 0.3 times vs. CE: 1.2 ± 0.3 times, $P < 0.036$).

Discussion

The findings of this study showed that HCE could stimulate acute responses in GH, lactate, and IL-6. In particular, the increase in GH concentration after the HCE test was much greater than for CE. HCE may be a more effective exercise method for stimulating GH than conventional moderate cycling exercise (less than 60% $\dot{V}O_{2peak}$) even at the same exercise intensities.

Growth hormone

A number of studies described the role of anabolic hormones associated with exercise-induced muscular hypertrophy (Kraemer et al. 1990; McCall et al. 1999; Godfrey et al. 2003; Kraemer and Ratamess 2005). Fryburg and Barrett (1993) reported that GH administration increases muscle protein synthesis and promotes muscle mass growth in humans. Also, it was reported that the acute response of GH correlates well with changes in muscle size and strength (McCall et al. 1999; Wideman et al. 2002). Moreover, GH response is stimulated by not only resistance exercise but also aerobic exercise depending on workload and duration. Luger et al. (1992) reported that GH response to exercise is obtained at a workload of 50% of $\dot{V}O_{2peak}$ for 30 minutes generated by treadmill exercise. In our study, GH concentration in the plasma increased immediately after the CE test with moderate aerobic exercise intensity of 40% of $\dot{V}O_{2peak}$. Jubeau et al. (2008) reported that a single bout of ES exercise at a frequency of (75 Hz) biphasic symmetric rectangular pulses and an intensity of (26 ± 19% of maximal voluntary contraction force) for 18 minutes was able to induce markedly higher GH responses than those obtained with voluntary contractions of the same intensity. In our study, HCE induced significantly higher GH response than CE even though the aerobic exercise intensity was adjusted to the same 40% of $\dot{V}O_{2peak}$. ES seems to be effective in stimulating GH response. Matsuse et al. (2010)

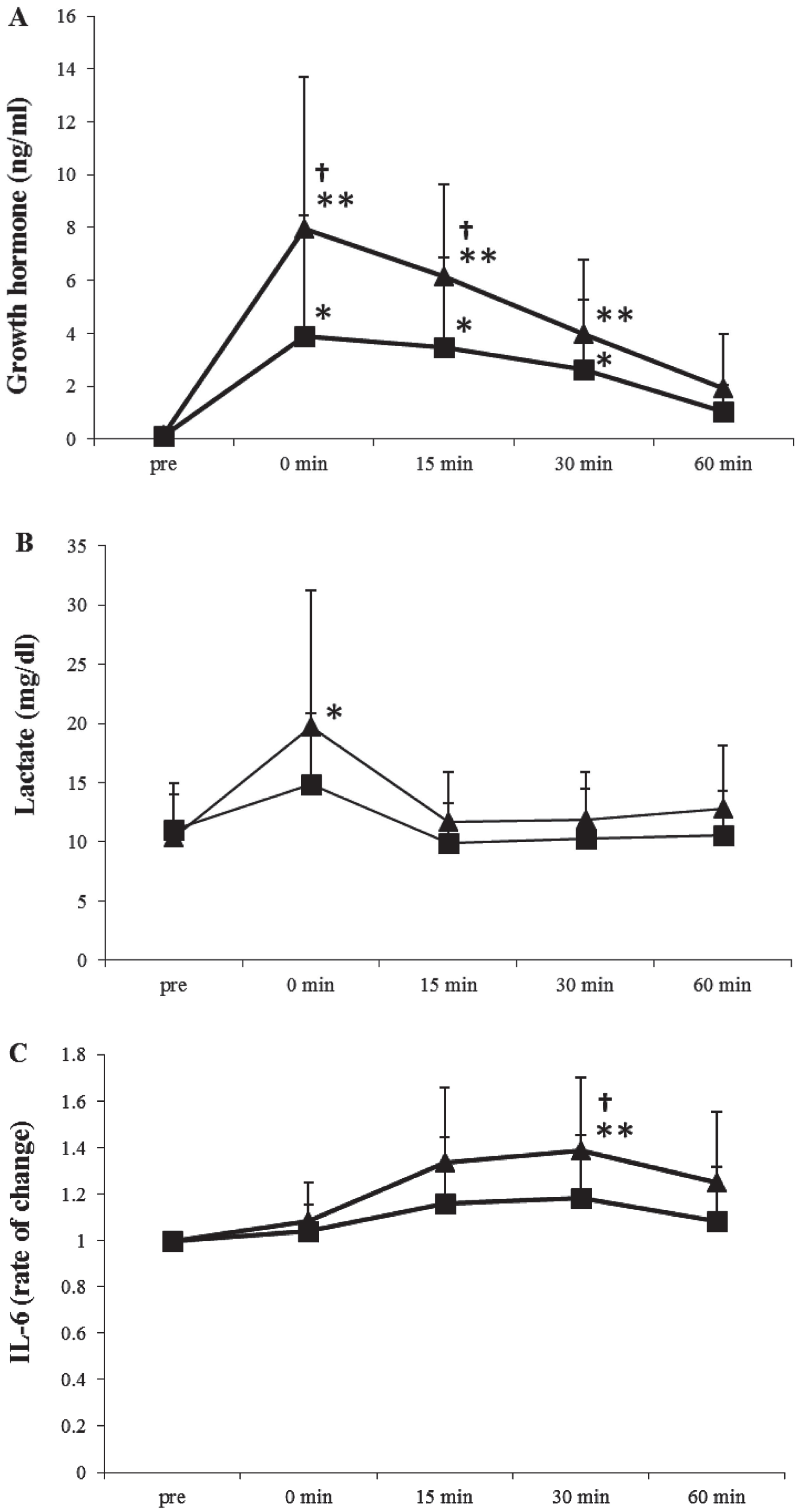

Fig. 5. Hormonal changes.
The vertical axis shows the changes in plasma concentrations of growth hormone (A), lactate (B), and the rate of change of IL-6 (C) after exercise with HCE (▲) and CE (■). Values are means ± SD (n = 10). The horizontal axis shows experiment time.
Significant difference from pre-exercise value, *P < 0.05; **P < 0.01.
Significant difference between HCE and CE, †P < 0.05.

reported that the increase in the plasma concentration of GH after HTS was much greater than after typical resistance exercise. And Iwasaki et al. (2006) reported the efficacy of HTS compared to conventional WT for increasing muscle strength around the knee. Two matched groups, each of 8 healthy young men exercised 3 times per week for six weeks. Both groups showed significantly increased strength in concentric torque at 30 degrees/sec (HTS +28%, WT +33%) and at 180 degrees/sec (HTS +33%, WT +38%). They reported that HTS is comparable to WT for increasing muscle strength with the exception of high-speed contractions (Iwasaki et al. 2006). Therefore HCE may be more effective for muscle strengthening than VC alone on an ergometer. However, GH response to HCE in this study was not as great as for HTS with knee bending, which was reported by Matsuse et al. (2010). The effect of HCE resistance exercise may be smaller than for HTS. Takano et al. (2010) investigated the effect of HTS on knee extensor strength for elderly people. Twenty subjects were randomly divided into two groups: the HTS group and the WT group. All subjects performed knee flexion and extension for 19 min per session, twice a week for 12 weeks. They reported that knee extension torque significantly increased in both groups (39% in HTS group and 42% in WT group, $P < 0.05$). Also they showed that a cross-sectional area of the quadriceps significantly increased in both groups (9% in HTS group and 14% in WT group, $P < 0.05$). Butterfield et al. (1997) reported that short-term treatment with GH in elderly women has been shown to induce elevated muscle protein synthesis and net whole body protein synthesis, respectively. Also, Coiro et al. (2010) reported that prolonged physical training protects against age-dependent decline in GH secretion in women. In our study, we investigated the acute response of hormone in healthy young men. In addition to this, if HCE is applied to elderly people, it would have possibility to gain the muscle strengthening in elderly people.

Lactate

There is substantial evidence that the anaerobic metabolism in the glycolytic pathway which results in lactate formation is more pronounced in ES than in voluntary exercise at identical low intensities (Kim et al.1995; Hamada et al. 2004). Additionally, because HTS causes a simultaneous contraction of the agonist and antagonist, it is able to activate more muscle mass than a contraction of the agonist alone. Matsuse et al. (2010) reported that resistance exercise with HTS stimulated significantly more lactate response than did typical WT. Also, HCE stimulated significantly more lactate response than the value of the pre-test. This lactate increase after the HCE test suggests a local accumulation of metabolic byproducts as a result of HTS. Richter et al. (1988) reported that, during recovery from short-term exercise, or even during exercise, there is net lactate uptake from the blood by resting muscles or by other muscles that are exercising at a low to moderate intensity. Gladden (2004) also reported that lactate produced in fast-twitch muscles was oxidized in cardiac muscles and slow-twitch muscles and provides them energy. During HCE, 2-minute HTS and 1-minute rest intervals were repeated in order to avoid difficulty pedaling due to muscle fatigue. Aerobic exercise of VC was continued during 1-minute rest intervals. Lactate produced from antagonist muscles by HCE might be oxidized in agonist muscles during rest intervals and provide energy to agonist muscles. Therefore, it was not significantly higher than CE, but lactate had increased moderately after HCE exercise. It seems that ES is able to stimulate sufficient contraction to produce energy by the glycolytic pathway even at a low exercise intensity of 40% of $\dot{V}O_{2peak}$. Thus, HCE might be able to provide both aerobic exercise systemically and resistance exercise locally at the same time even at low to moderate intensities (less than 60% $\dot{V}O_{2peak}$).

Interleukin-6

Muscle-derived IL-6 should be classified as a myokine with endocrine effects (Pedersen et al. 2007). Wolsk et al. (2010) reported that recombinant human IL-6 infusion caused an increase of unidirectional fatty acid and glycerol release in the skeletal muscle, indicative of an increase in lipolysis. It was reported that IL-6 released from contracting muscles into the circulation enhances lipolysis and gene transcription in abdominal subcutaneous fat via its effect on adipose tissue (Pedersen et al. 2007). Kawaguchi et al. (2011) conducted an experiment in which twelve patients with nonalcoholic fatty liver disease (NAFLD) who were resistant to lifestyle counseling exercised with HTS in the lower extremities twice a week for 12 weeks. They reported that HTS exercise improved hepatic steatosis, insulin resistance and serum IL-6 levels in NAFLD patients (Kawaguchi et al. 2011). Febbraio et al. (2003) reported that plasma IL-6 doubled after 25 minutes of supine bicycle exercise at 60% $\dot{V}O_{2peak}$ and this significant result suggested that IL-6 production was related to the mass of muscle activated. HCE increased plasma IL-6 1.4 times significantly, but CE did not produce significant results, although aerobic exercise workload was the same. It is presumed that IL-6 response reflected the mass of muscle activated by HTS. HCE's increased stimulation of muscle-derived IL-6 has possibility to be effective for metabolic syndrome patients.

Izquierdo et al. (2005) reported that prolonged low-frequency combined resistance and cycling endurance training led to great gains in maximal dynamic strength and power-load characteristics of the leg extensors muscles. Goto et al. (2007) showed that the blood concentrations of free fatty acids, glycerol and GH during submaximal endurance cycling exercise were enhanced by prior resistance exercise. Thus, a combination of resistance and endurance training is effective. However, conventionally, both resistance and cycling endurance training were necessary to be performed separately with the proper number of repetitions. Masayuki et al. (2013) evaluated $\dot{V}O_2$ during aerobic exercise on an ergometer with HTS. 11 healthy young men exercised on a cycle ergometer starting at 20 Watts and increasing by 20 Watts every 3 minutes to 100 Watts, with CE and with HCE. They showed that $\dot{V}O_2$ during HCE was significantly higher than during CE at an average of about 21.1% ($P < 0.001$) at the same workload. They also found that HCE at moderate intensity seems to result in a linear relationship between $\dot{V}O_2$ and the work rate in the same way as conventional aerobic exercise. They concluded that HCE may be an exercise technique that could combine resistance exercise with aerobic exercise (Masayuki et al. 2013). Thus HCE has the possibility to perform both types of exercise simultaneously, and may shorten exercise time. Also, it does not require large extra equipment because it is simply combined with conventional cycle ergometer exercise. Furthermore, if we use a supine exercise bicycle, it can be used in a limited space such as a bedside or home. In the future, HCE is expected to be applied to patients with metabolic syndrome or elderly people.

Limitation

This study was limited by the small number of subjects and the fact that all of them were young men. Estrogens affect the release of GH and are inhibited by somatostatin (Kraemer et al. 2012), therefore women need to perform the exercise test in the same period of their menstrual cycle. We hope to do similar testing for women and elderly people in the near future. To evaluate an effect of aerobic exercise needs to compare with some various exercise intensities. Although the volume of muscles in lower extremities could have influenced the results, we had not evaluated them by magnetic resonance imaging. It was desirable to add to the measurements some parameters of an insulin-like growth factor-1 and cortisol to predict muscular hypertrophy, however we did not measure them. Because the same subjects performed the HCE test and CE test, we should have randomized the order of tests to avoid the possibility of influence on the results. A long-term training study is necessary to show whether HCE is an effective technique combining aerobic and resistance exercise by evaluating exercise capacity, physical fitness, muscle strength, muscle mass.

Conclusions

This study's results show that HCE could greatly induce acute GH response at moderate exercise intensity than CE in healthy young men. HCE seems to be a more effective method than conventional cycle ergometer exercise in regards to muscle training. We may be able to combine electrically stimulated resistance exercise with aerobic exercise using HCE.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

References

Asanuma, K., Ariga, T., Aida, N., Miyasaka, A., Nagai, Y., Miyagawa, T., Minowa, M., Yoshida, M., Tsuda, M. & Tatano, T. (1985) A new method for simultaneous autoanalysis of plasma levels of lactic acid and pyruvic acid by means of the oxidase system. *J. Anal. Bio-Sci.*, **8**, 16-24.

Bishop, D. (2001) Evaluation of the Accusport lactate analyser. *Int. J. Sports Med.*, **22**, 525-530.

Butterfield, G.E., Thompson, J., Rennie, M.J., Marcus, R., Hintz, R.L. & Hoffman, A.R. (1997) Effect of rhGH and rhIGF-I treatment on protein utilization in elderly women. *Am. J. Physiol.*, **272**, E94-99.

Coiro, V., Volpi, R., Gramellini, D., Maffei, M.L., Volta, E., Melani, A. & Chiodera, P. (2010) Effect of physical training on age-related reduction of GH secretion during exercise in normally cycling women. *Maturitas*, **65**, 392-395.

Dehail, P., Duclos, C. & Barat, M. (2008) Electrical stimulation and muscle strengthening. *Ann. Readapt. Med. Phys.*, **51**, 441-451.

Drummond, M.J., Vehrs, P.R., Schaalje, G.B. & Parcell, A.C. (2005) Aerobic and resistance exercise sequence affects excess postexercise oxygen consumption. *J. Strength Cond. Res.*, **19**, 332-337.

Febbraio, M.A., Steensberg, A., Starkie, R.L., McConell, G.K. & Kingwell, B.A. (2003) Skeletal muscle interleukin-6 and tumor necrosis factor-alpha release in healthy subjects and patients with type 2 diabetes at rest and during exercise. *Metabolism*, **52**, 939-944.

Fryburg, D.A. & Barrett, E.J. (1993) Growth hormone acutely stimulates skeletal muscle but not whole-body protein synthesis in humans. *Metabolism*, **42**, 1223-1227.

Fryburg, D.A., Gelfand, R.A. & Barrett, E.J. (1991) Growth hormone acutely stimulates forearm muscle protein synthesis in normal humans. *Am. J. Physiol.*, **260**, E499-504.

Fyfe, J.J., Bishop, D.J. & Stepto, N.K. (2014) Interference between concurrent resistance and endurance exercise: molecular bases and the role of individual training variables. *Sports Med.*, **44**,743-762.

Gladden, L.B. (2004) Lactate metabolism: a new paradigm for the third millennium. *J. Physiol.*, **558**, 5-30.

Godfrey, R.J., Madgwick, Z. & Whyte, G.P. (2003) The exercise-induced growth hormone response in athletes. *Sports Med.*, **33**, 599-613.

Goto, K., Ishii, N., Sugihara, S., Yoshioka, T. & Takamatsu, K. (2007) Effects of resistance exercise on lipolysis during subsequent submaximal exercise. *Med. Sci. Sports Exerc.*, **39**, 308-315.

Hamada, T., Hayashi, T., Kimura, T., Nakao, K. & Moritani, T. (2004) Electrical stimulation of human lower extremities enhances energy consumption, carbohydrate oxidation, and whole body glucose uptake. *J. Appl. Physiol.*, **96**, 911-916.

Iwasaki, T., Shiba, N., Matsuse, H., Nago, T., Umezu, Y., Tagawa, Y., Nagata, K. & Basford, J.R. (2006) Improvement in knee extension strength through training by means of combined electrical stimulation and voluntary muscle contraction. *Tohoku J. Exp. Med.*, **209**, 33-40.

Izquierdo, M., Häkkinen, K., Ibáñez, J., Kraemer, W.J. & Gorostiaga, E.M. (2005) Effects of combined resistance and cardiovascular training on strength, power, muscle cross-sectional area, and endurance markers in middle-aged men. *Eur. J. Appl. Physiol.*, **94**, 70-75.

Jubeau, M., Sartorio, A., Marinone, P,G., Agosti, F., Van Hoecke, J., Nosaka, K. & Maffiuletti, N.A. (2008) Comparison between voluntary and stimulated contractions of the quadriceps femoris for growth hormone response and muscle damage. *J. Appl. Physiol*., **104**, 75-81.

Kang, J. & Ratamess, N. (2014) Which comes first? Resistance before aerobic exercise or vice versa? *ACSMs Health Fit. J*., **18**, 9-14.

Kawaguchi, T., Shiba, N., Maeda, T., Matsugaki, T., Takano, Y., Itou, M., Sakata, M., Taniguchi, E., Nagata, K. & Sata, M. (2011) Hybrid training of voluntary and electrical muscle contractions reduces steatosis, insulin resistance, and IL-6 levels in patients with NAFLD: a pilot study. *J. Gastroenterol*., **46**, 746-757.

Kim, C.K., Bangsbo, J., Strange, S., Karpakka, J. & Saltin, B. (1995) Metabolic response and muscle glycogen depletion pattern during prolonged electrically induced dynamic exercise in man. *Scand. J. Rehabil. Med*., **27**, 51-58.

Koninckx, E., Van Leemputte, M. & Hespel, P. (2010) Effect of isokinetic cycling versus weight training on maximal power output and endurance performance in cycling. *Eur. J. Appl. Physiol*., **109**, 699-708.

Kraemer, R.R., Francois, M. & Castracane, V.D. (2012) Estrogen mediation of hormone responses to exercise. *Metabolism*, **61**, 1337-1346.

Kraemer, W.J., Marchitelli, L., Gordon, S.E., Harman, E., Dziados, J.E., Mello, R., Frykman, P., McCurry, D. & Fleck, S.J. (1990) Hormonal and growth factor responses to heavy resistance exercise protocols. *J. Appl. Physiol.*, **69**, 1442-1450.

Kraemer, W.J. & Ratamess, N.A. (2005) Hormonal responses and adaptations to resistance exercise and training. *Sports Med*., **35**, 339-361.

Luger, A., Watschinger, B., Deuster, P., Svoboda, T., Clodi, M. & Chrousos, G.P. (1992) Plasma growth hormone and prolactin responses to graded levels of acute exercise and to a lactate infusion. *Neuroendocrinology*, **56**, 112-117.

Macaluso, A., Young, A., Gibb, K.S., Rowe, D.A. & De Vito, G. (2003) Cycling as a novel approach to resistance training increases muscle strength, power, and selected functional abilities in healthy older women. *J. Appl. Physiol.*, **95**, 2544-2553.

Masayuki, O., Matsuse, H., Takano, Y., Yamada, S., Ohshima, H., Tagawa, Y. & Shiba, N. (2013) Oxygen uptake during aerobic cycling exercise simultaneously combined with neuromuscular electrical stimulation of antagonists. *J. Nov. Physiother*., **3**, 185.

Matsuse, H., Nago, T., Takano, Y. & Shiba, N. (2010) Plasma growth hormone is elevated immediately after resistance exercise with electrical stimulation and voluntary muscle contraction. *Tohoku J. Exp. Med*., **222**, 69-75.

Matsuse, H., Shiba, N., Umezu, Y., Nago, T., Tagawa, Y., Kakuma, T., Nagata, K. & Basford, J.R. (2006) Muscle training by means of combined electrical stimulation and volitional contraction. *Aviat. Space Environ. Med.*, **77**, 581-585.

McCall, G.E., Byrnes, W.C., Fleck, S.J., Dickinson, A. & Kraemer, W.J. (1999) Acute and chronic hormonal responses to resistance training designed to promote muscle hypertrophy. *Can. J. Appl. Physiol.*, **24**, 96-107.

Metcalfe, R.S., Babraj, J.A., Fawkner, S.G. & Vollaard, N.B. (2012) Towards the minimal amount of exercise for improving metabolic health: beneficial effects of reduced-exertion high-intensity interval training. *Eur. J. Appl. Physiol*., **112**, 2767-2775.

Myers, J. & Bellin, D. (2000) Ramp exercise protocols for clinical and cardiopulmonary exercise testing. *Sports Med*., **30**, 23-29.

Paillard, T. (2008) Combined application of neuromuscular electrical stimulation and voluntary muscular contractions. *Sports Med.*, **38**, 161-177.

Pedersen, B.K., Akerström, T.C., Nielsen, A.R. & Fischer, C.P. (2007) Role of myokines in exercise and metabolism. *J. Appl. Physiol*., **103**, 1093-1098.

Richter, E.A., Kiens, B., Saltin, B., Christensen, N.J. & Savard, G. (1988) Skeletal muscle glucose uptake during dynamic exercise in humans: role of muscle mass. *Am. J. Physiol*., **254**, E555-E561.

Shiba, N., inventor; Kurume University, assignee (2002) Apparatus for strengthening muscles. US patent 6,456,885. 2002 Sep 24.

Sousa, N., Mendes, R., Abrantes, C., Sampaio, J. & Oliveira, J. (2014) Effectiveness of combined exercise training to improve functional fitness in older adults: a randomized controlled trial. *Geriatr. Gerontol. Int*., **14**, 892-898.

Taipale, R.S. & Häkkinen, K. (2013) Acute hormonal and force responses to combined strength and endurance loadings in men and women: the "order effect". *PLoS One*, **8**, e55051.

Takano, Y., Haneda, Y., Maeda, T., Sakai, Y., Matsuse, H., Kawaguchi, T., Tagawa, Y. & Shiba, N. (2010) Increasing muscle strength and mass of thigh in elderly people with the hybrid-training method of electrical stimulation and volitional contraction. *Tohoku J. Exp. Med*., **221**, 77-85.

Takarada, Y., Nakamura, Y., Aruga, S., Onda, T., Miyazaki, S. & Ishii, N. (2000) Rapid increase in plasma growth hormone after low-intensity resistance exercise with vascular occlusion. *J. Appl. Physiol.*, **88**, 61-65.

Takeshima, N., Rogers, M.E., Islam, M.M., Yamauchi, T., Watanabe, E. & Okada, A. (2004) Effect of concurrent aerobic and resistance circuit exercise training on fitness in older adults. *Eur. J. Appl. Physiol*., **93**, 173-182.

Tibana, R.A., Nascimento, Dda. C., de Sousa, N.M., de Souza, V.C., Durigan, J., Vieira, A., Bottaro, M., Nóbrega, Ode. T., de Almeida, J.A., Navalta, J.W., Franco, O.L. & Prestes, J. (2014) Enhancing of women functional status with metabolic syndrome by cardioprotective and anti-inflammatory effects of combined aerobic and resistance training. *PLoS One*, **9**, e110160.

Velloso, C.P. (2008) Regulation of muscle mass by growth hormone and IGF-I. *Br. J. Pharmacol*., **154**, 557-568.

Ward, A.R. & Shkuratova, N. (2002) Russian electrical stimulation: the early experiments. *Phys. Ther*., **82**, 1019-1030.

Wideman, L., Weltman, J.Y., Hartman, M.L., Veldhuis, J.D. & Weltman, A. (2002) Growth hormone release during acute and chronic aerobic and resistance exercise: recent findings. *Sports Med.*, **32**, 987-1004.

Wolsk, E., Mygind, H., Grøndahl, T.S., Pedersen, B.K. & van Hall, G. (2010) IL-6 selectively stimulates fat metabolism in human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab*., **299**, E832-E840.

Yanagi, T., Shiba, N., Maeda, T., Iwasa, K., Umezu, Y., Tagawa, Y., Matsuo, S., Nagata, K., Yamamoto, T. & Basford, J.R. (2003) Agonist contractions against electrically stimulated antagonists. *Arch. Phys. Med. Rehabil.*, **84**, 843-848.

Yoshimitsu, K., Shiba, N., Matsuse, H., Takano, Y., Matsugaki, T., Inada, T., Tagawa, Y. & Nagata, K. (2010) Development of a training method for weightless environment using both electrical stimulation and voluntary muscle contraction. *Tohoku J. Exp. Med.*, **220**, 83-93.