

Effect of Hyperventilation During Resistance Exercise on Hormonal Response in Humans

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Abstract

TAKEMASA, T., MIYAKAWA, S., NAGATA, S., ESAKI, K., HIROKAWA, M., MACHIDA, M., KOSAKA, Y., HITOMI, Y., KIZAKI, T., OHNO, H. and HAGA, S., Effect of Hyperventilation During Resistance Exercise on Hormonal Response in Humans. *Adv. Exerc. Sports Physiol.*, Vol.10, No.2 pp.55-61, 2004. A Japanese body builder, Mr. Shigemura, recently proposed a new resistance-training protocol designed to produce muscle hypertrophy. This 'ECE' (extension-contraction-extension) protocol involves, a long period of exhalation during the muscle extension-contraction-extension phase, followed by deep inhalation at rest. We investigated the acute effects of ECE training on the hormonal response. Nine healthy male subjects each performed 5 sets of elbow flexion exercises using three protocols: 'usual' (no breathing instructions), 'occlusion' (occlusion of upper arm with a belt; no breathing instructions), and ECE. Examination of muscle oxygenation levels by near-infrared spectroscopy during exercise revealed that oxygenation level and amplitude of oxygenation were significantly higher with the ECE protocol than with the other two protocols. We concluded that use of the ECE protocol induces relatively higher oxygenation at a skeletal muscle level. Examination of the subjects' anabolic and catabolic hormonal responses revealed that although serum lactate and growth hormone levels were almost equal with the three protocols, the plasma cortisol level was significantly lower with the ECE system.

Keywords: Resistance training, Hyperventilation, Growth Hormone, Cortisol, Hypertrophy

Introduction

Muscle resistance exercise is a potent stimulus for acute increases in the concentrations of circulating hormones such as growth hormone (GH), testosterone, and cortisol (COR). These responses may expand the possibility of hormone-receptor interactions within muscle cells,

and the increased number of receptors after training may enhance the muscle protein turnover observed after resistance exercise (12, 20). Administration of GH and testosterone increases muscle protein synthesis and promotes muscle mass growth in humans (7, 8), whereas data from animal studies reveal that hypophysectomy or administration of androgen receptor antagonist suppresses the hypertrophy induced by exercise (2, 13). COR administration has a catabolic effect on myofibrillar proteins and suppresses protein synthesis (6, 15). Training studies have also shown that acute responses of GH are well correlated with changes in muscle size and strength (19).

Several resistance-training protocols have been developed to improve different muscular aspects, such as maximum strength and hypertrophy (1). These protocols, which differ in the components of acute program variables such as intensity, total work, and rest intervals, cause different hormonal responses. GH and COR concentrations were found to be higher after a muscular hypertrophy protocol than with a maximum strength protocol, but change in testosterone concentrations seemed to controversial (5, 10, 17, 18).

Among exercise parameters, there is no established breathing method that has been proven best for muscular hypertrophy. Although continuous breathing during exercise seems to be of importance, many trainees stop breathing during training sets. This increases intraabdominal pressure, drives up blood pressure, and reduces oxygen supply to the muscle and brain, and results in lightheadedness, dizziness or even passing out. Therefore, the following breathing pattern is usually recommended. "Always breathe in deeply through the nose during the eccentric phase of the lift. Hold the breath momentarily during the eccentric isometric phase, breathe out through the mouth at the same rate of movement during the concentric phase, continue to exhale during the concentric isometric phase, then repeat" (1). It is also important to be aware not to completely exhale all air from the lungs, because this will

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affect the positioning of the rib cage and produce loss of stability.

Recently, Mr. Shigemura, a Japanese body builder, proposed a training method that uses a specific breathing pattern during resistance exercise and that might be effective for producing muscular hypertrophy (22, 29). According to this protocol, the trainee starts the movement from the extended muscle position and then inhales deeply. A short time after starting the exhalation, he or she performs the contraction and subsequent extension movement, without stopping the exhalation throughout the motion. The trainee continues exhaling little by little, and only when the muscle has returned to its original extended position can he or she inhale deeply. Mr. Shigemura reports that trainee feels a burning sensation in the muscle, as if they have lifted a heavy weight to exhaustion, even when the weight is much lighter (about 60% of 1RM) than that usually used for hypertrophy exercises. He named this method the "ECE" (extension-contraction-extension) training system.

We wanted to find out why the ECE training system was effective in producing muscular hypertrophy, so we investigated muscle oxygenation levels and anabolic and catabolic hormone response in a single bout of exercise. We compared the ECE protocol with the 'usual method' and the 'occlusion method' (see below); the latter is also reported to be effective in creating muscular hypertrophy (24, 25).

Methods

Subjects

With the approval of the Ethics Committee of the Institute of Medical Sciences, University of Tsukuba, Japan, 9 apparently healthy male subjects (university water polo players) took part in our study. They had the following physical characteristics: mean age: 20.2 ± 0.4 years; height: 173.1 ± 1.7 cm; and body weight: 71.8 ± 1.6 kg (values are means \pm SE). After explanation of the study protocol and requirements, written informed consent was obtained before the testing began. All subjects had some weight-training experience but none of them was a competitive lifter. There was no history of any endocrine disorders, glucose intolerance, or chronic disease that might have influenced their responsiveness to strenuous exercise. Subjects were instructed to maintain their normal diet and to avoid physical activity for 24 h before the experimental trials.

Exercise conditions

On an initial visit to the laboratory, each subject was tested for maximum strength by a single-arm dumbbell curl exercise in the sitting position using the right (dominant) arm. Throughout the exercise, subjects kept the upper body upright and the upper arm declined at about 45° in front of them with the aid of an armrest. One repetition maximum

(1 RM) was determined as the maximum weight that the individual could move through the full range of motion only once without a change in body position. No less than 1 week later, the 9 subjects returned to the laboratory for the first exercise session. On the day of the experiment, following a 4 h fast, the subjects completed the arm curl routine, performing 5 sets of exercises beginning at 60% of their 1 RM. For each set of exercises, the subjects repeated the lifting movement until they failed. A 15 s rest was allowed between sets. In the successive 4 sets, the operator set the maximum weight so that the subject could complete no more than 10 reps (Fig. 1). Subjects were instructed to lift and lower the dumbbell in an approximately constant cycle (2 s for the concentric phase, 2 s for the eccentric phase, and 2 s for resting in the bottom position). Subjects performed the exercise using the three different protocols (see below) in a random sequence, each of them separated by 1 week.

The three exercise protocols

All subjects followed the three protocols with a single-arm dumbbell curl exercise in the sitting position. Subjects began the single-arm dumbbell curl movement with the biceps muscle in an extended position. They then contracted the biceps muscle and successive extension without stopping movement at the most contracted position, and repeat. The three protocols were as follows: 1) usual protocol: the subject's arm was not occluded and the subject was not instructed to breathe in any particular manner; 2) occlusion protocol: the subject's arm was occluded with a specially designed elastic belt (M.P.S.-700 Sato Sports Plaza, Tokyo, Japan; width 33 mm, length 980 mm, or KXH4100 Phenix, Tokyo, Japan; width 30 mm, length 470 mm) during the entire session (26); the belt was released immediately after the end of the session. The elastic belt was attached to the proximal end of the upper arm and tightened in accordance with the manufacturer's instructions (~ 100 mmHg). The subject was not instructed to breathe in any particular way; 3) ECE protocol: the subject was instructed to breathe as follows: continue exhaling little by little during the ECE process, and inhale deeply only when the muscle has been returned to its original extended position.

Measurement of muscle oxygenation level

Muscle oxygenation levels were measured with a NIRS (near-infrared spectroscopy; Model HEO-200, Omron, Japan), which had a flexible probe containing a light source and an optical detector, with a distance of 30 mm between them (11). A pair of two-wavelength (760 and 840 nm) light-emitting diodes was used as the light source. A silicon photodiode was used as the photodetector. When the probe was attached to the belly of the biceps muscle, the light was able to penetrate the soft tissue by 15 to 20 mm. Changes in oxyhemoglobin (ΔHbO_2), deoxyhemoglo-

bin (ΔHb), and total hemoglobin ($\Delta\text{T-Hb}$) were calculated by the following equations:

$$\Delta\text{HbO}_2 = K \cdot [\Delta\text{OD} (840 \text{ nm}) - 0.66\Delta\text{OD} (760 \text{ nm})]$$

$$\Delta\text{Hb} = K \cdot [0.80\Delta\text{OD} (760 \text{ nm}) - 0.59\Delta\text{OD} (840 \text{ nm})]$$

$$\Delta\text{T-Hb} = K \cdot [0.41\Delta\text{OD} (840 \text{ nm}) + 0.14\Delta\text{OD} (760 \text{ nm})]$$

where ΔOD is the change in optical density, and $K=0.146$ when the detector is 30 mm from the radiation source (21). Because the HbO_2 signal measured by NIRS indicated the relative values of tissue oxygenation, we occluded the brachial artery to establish a percentage scale for muscle oxygenation level. A pneumatic cuff was inflated to over 300 mmHg to occlude arterial blood flow for 4 to 7 min until the muscle oxygenation level had bottomed out. The resting value of muscle oxygenation was defined as 100%, and the lowest value during the occlusion was defined as 0% (Fig. 1).

Measurement of GH, lactate, COR

Blood samples were collected from an antecubital vein on the non-dominant (left) arm. Blood samples (6 mL each) were obtained 30 min before exercise and 15 min after the finish of each exercise protocol. The values taken 15 min post-exercise were used to assess GH and COR response (3, 4, 24). The blood samples were incubated for 20 min at room temperature to permit them to clot, and then centrifuged for 10 min at 3000 rpm, and 4°C to obtain serum. The serum samples were then preserved at -80°C until analysis. Lactate (LA), GH, and COR levels in the obtained sera were then measured. The increase in values of LA, GH, and COR concentration between pre- and post-exercise were defined as ΔLA , ΔGH , and ΔCOR , respectively. GH and COR concentrations were measured by radioimmunoassay, and LA concentration was measured with an automatic LA analyzer (YSI 2300 Stat Plus, Yellow Springs Instruments, OH, USA).

Statistical analyses

All data were expressed as means \pm SE. Examinations of statistical significance of the data among the exercises were based on one-way analysis of variance (ANOVA) with a Fisher's Protected Least Significant Difference post-hoc test. Statistical significance was taken as $p < 0.05$.

Results

Differences in muscle oxygenation level among the three exercise protocols

Changes in HbO_2 , Hb, T-Hb during the entire experimental process was monitored by NIRS, and Figure 1 illustrates a typical pattern. One minute after monitoring began, cuff inflation was completed to interrupt the oxygen supply to the monitored muscle area and determine the lowest oxygenation value. The cuff was then released. A few minutes' rest was then allowed to relieve any paralysis, and a warm up lift was then performed several times. When the

HbO_2 level had returned to the stabilizing point, the 5 sets of exercises with 15 s inter-set intervals was began. Figure 2 shows typical changes in the HbO_2 and T-Hb during the three exercise protocols. In the usual and occlusion protocols, breath stopping was frequently observed at the end of each set. From the obtained data, we calculated muscle oxygenation levels in each set of exercise protocols (Fig. 3A). We also examined the amplitude of muscle oxygenation change during exercise under the three protocols (Fig. 3B).

Changes in hormonal response after three exercise protocols

Figure 4 shows ΔLA , ΔGH , and ΔCOR with each exercise protocol performed by 9 subjects. Although we found no significant differences among protocols in ΔLA and ΔGH (Fig. 4A and 4B), ΔCOR under the ECE protocol tended to be less and was significantly less than under the usual protocol (Fig. 4C).

Discussion

This is the first analytical report for ECE training system, which has been thought to be an effective resistance training for muscle hypertrophy. This training method was first introduced with the Japanese local body-builder community, and the news of its effectiveness soon spread by way of electronic mailing lists. Some volunteers had tried this protocol, and we confirmed its effectiveness in a preliminary study (unpublished data). Here we gathered some physiological data on this new training method.

We initially considered that ECE training system had features of hypoxia during movement, because of the long exhalation period. We assumed that this was a key factor in this training method, and therefore used the NIRS system to examine muscle oxygenation level during exercise with this and two other training protocols. The muscle oxygenation levels with the three different protocols differed from each other, but contrary to our assumption, the muscle oxygenation level during ECE training revealed higher oxygenation level than with the usual and occlusion protocols (Fig. 3A). This was surprising, but we considered that it was a result of the deep inhaling during the muscle resting period. The mean amplitude of the oxygenation level also gave evidence for the hyperventilation during exercise (Fig. 3B). Moreover, it was interesting to examine the muscle oxygenation level during exercise under the occlusion protocol, which had previously been described as a "hypoxic condition". In the first set, the oxygenation level was the lowest of the three protocols, but in the 4 successive sets, the oxygenation level was elevated and significantly higher than in the usual mode of exercise. It is true that hyperemia can be observed in the distal part of the limb when venous flow is occluded, but the occlusion pressure (~ 100 mmHg) was not high enough to stop the arterial flow during heavy resistance exercise. Therefore, from the second

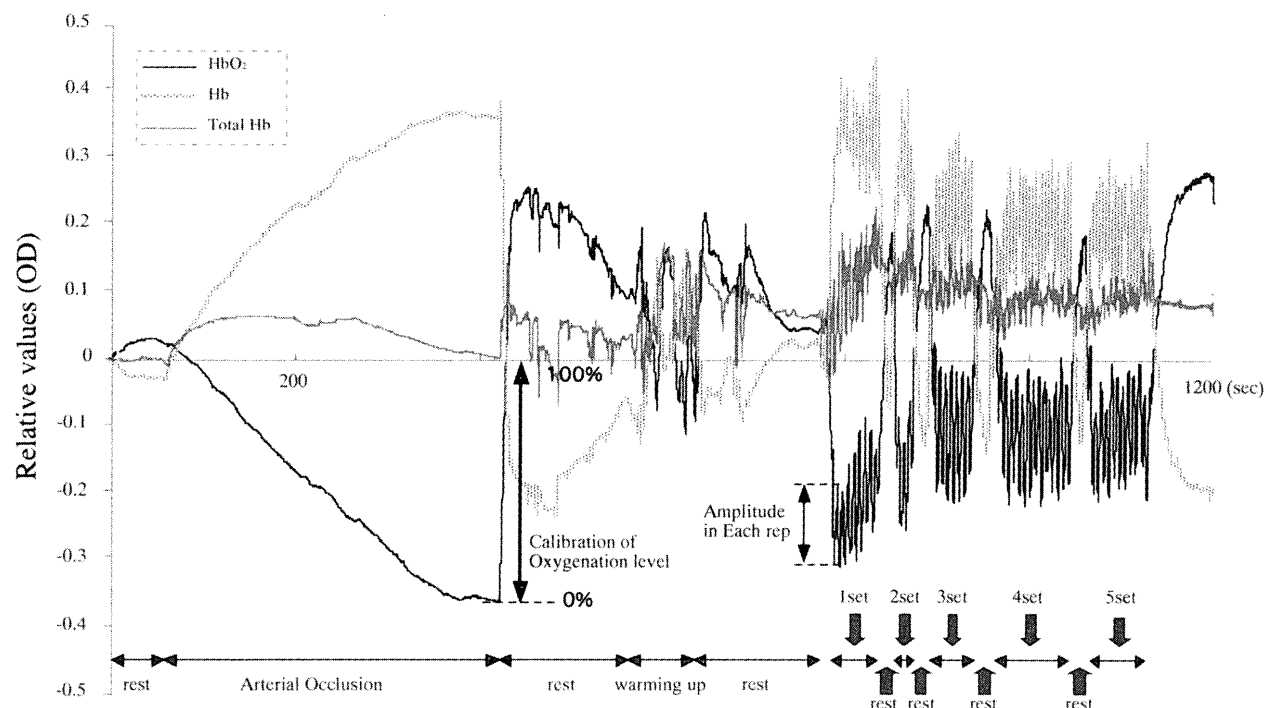


Fig. 1 Typical muscle oxygenation pattern during elbow flex exercise. First of all, calibration of muscle oxygenation level was executed as follows. After 1 min rest, brachial artery was occluded until the HbO_2 level was bottomed out. This level was defined as 0% and resting value as 100%. Several warming up lifts were allowed to the subject, and when the oxygenation level stabilized, 5 sets of exercise experiment, including 4 times 15 sec inter-set interval, began. $[\text{HbO}_2]$, $[\text{Hb}]$, and $[\text{Total Hb}]$ were monitored by NIRS (near-infrared spectroscopy). Changes in oxyhemoglobin (ΔHbO_2), deoxyhemoglobin (ΔHb), and total hemoglobin ($\Delta\text{T-Hb}$) were calculated by the following equations:

$$\Delta\text{HbO}_2 = K \cdot [\Delta\text{OD}(840 \text{ nm}) - 0.66\Delta\text{OD}(760 \text{ nm})]$$

$$\Delta\text{Hb} = K \cdot [0.80\Delta\text{OD}(760 \text{ nm}) - 0.59\Delta\text{OD}(840 \text{ nm})]$$

$$\Delta\text{T-Hb} = K \cdot [0.41\Delta\text{OD}(840 \text{ nm}) + 0.14\Delta\text{OD}(760 \text{ nm})]$$

ΔOD is the change in optical density. Black line; $[\text{HbO}_2]$, dotted line; $[\text{Hb}]$ and gray line; $[\text{Total Hb}]$.

set onwards, the blood pressure presumably overcame the occlusion pressure and oxygenated blood entered the upper arm muscle.

To our knowledge, the effects of hyperventilation during exercise have not been investigated, and because we considered that hyperventilation was a key factor in the new training protocol, we first measured serum LA (Fig. 4A), which is usually arisen by heavy resistance exercise. Occlusion during low-intensity exercise restricted the blood circulation and the resulting hypoxic and LA-arisen acidic intramuscular environment would have induced additional motor-unit recruitment to maintain the given level of force, thereby evoking an increase in the electrical activity of the muscle (25). The ECE training method raised the LA level almost as high as that observed in resistance training (23), or in occlusion training (26). so it could be said that the volunteers accomplished heavy resistance exercise in each protocol.

High-intensity, heavy resistance exercise is well known to induce a high-level acute GH response (4). It has been suggested that an exercise-induced acute increase in

serum GH concentration may be a response to central stimulation by brain motor center activity (16) and/or to changes in acid-base balance within the loaded muscles via afferent feedback from peripheral chemoreceptors (9). We detected GH elevation with all three protocols (Fig. 4B), and the levels of GH were comparable to those reported previously in heavy resistance training (23). Significant differences in GH level between the occlusion and usual protocols were not observed, although a 290-fold elevation of GH with occlusion has been reported previously (24). This discrepancy could be related to methodological differences (arm vs. leg) between studies or short time hypoxic condition found in this study could not induce drastic hormonal response in spite of occlusion mode of exercise.

COR is a representative catabolic hormone and, generally, heavy resistance exercise has been reported to increase COR concentration (4, 19, 27, 28). Physical training may have a different impact on circulating COR concentration. Some studies have reported a decreased basal COR concentration following a period of endurance training (28) or by low volume resistance exercise (17). Increases

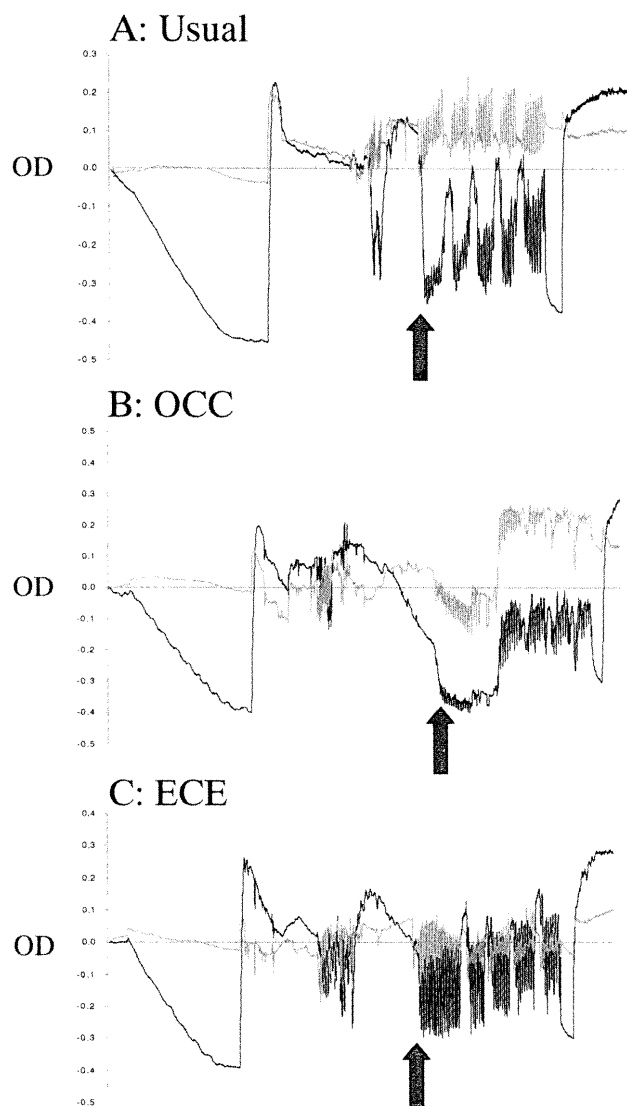


Fig. 2 Typical $[HbO_2]$ and $[Total\ Hb]$ patterns found during three modes of exercise. A: Usual mode, B: OCC (occlusion) mode and C: ECE (extension-contraction-extension) mode. Arrows indicate the starting point of exercise. Black line; $[HbO_2]$ and gray line; $[Total\ Hb]$.

in serum testosterone levels and decreases in serum COR associated with increased strength have been reported during early-phase adaptations to heavy-resistance training (14). However, excessively strenuous exercise training is likely to cause an increase in COR concentration, although it has been suggested that decreased COR concentrations provide more favorable conditions for reduced fiber protein degeneration or increased protein synthesis (17). We observed that COR production in ECE training was the lowest among the three exercise protocols (Fig. 4C), which means that this training method is an ideal protocol for producing muscle hypertrophy.

Resistance exercise with occlusion is interesting from

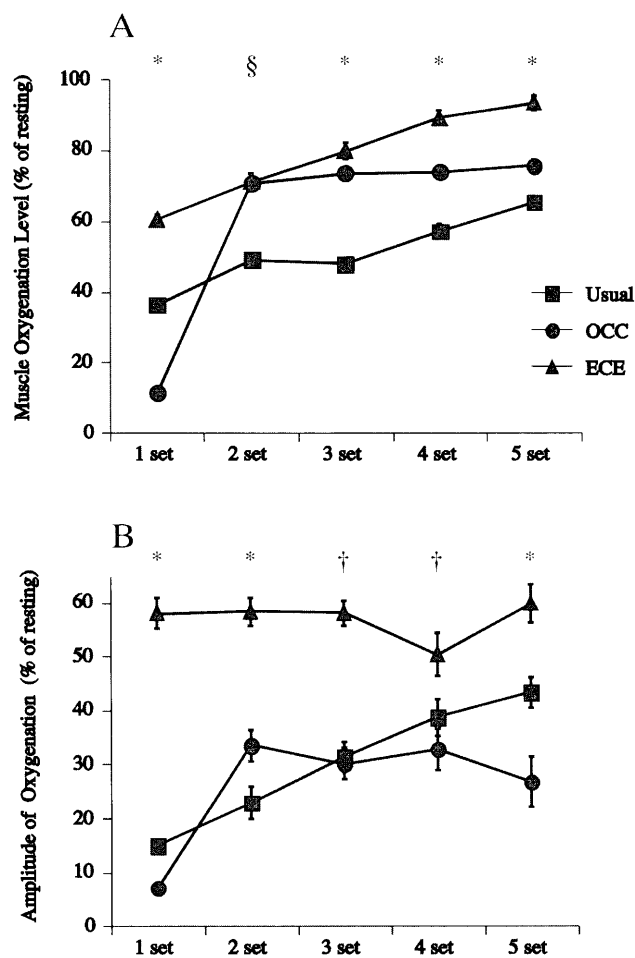


Fig. 3 Difference of muscle oxygenation level (A) and amplitude of oxygenation (B) among three modes of exercise. Data from Fig. 2 are analyzed. All values are mean \pm SE. Closed square; Usual mode, closed circle; occlusion mode, and closed triangle; ECE mode. *: Significant difference ($p < 0.05$) among each of three modes of exercise. §: Significant difference ($p < 0.05$) between both usual and OCC, usual and ECE modes of exercise. †: Significant difference ($p < 0.05$) between both ECE and usual, ECE and OCC modes of exercise.

a physiological view point, but this protocol carries limits and risks for ordinary people: that is, 1) applied only for the arms and legs because of indispensable occlusion; 2) a special device and detailed instructions are essential for proper use of this methodology; 3) during exercise with occlusion, the trainee feels relatively strenuous pain from the hyperemia, and subcutaneous bleeding is sometimes observed at distal to the occlusion; and 4) both microdamage to the blood vessels and subtle changes in blood flow possibly stimulate thrombosis and may cause thrombus formation by ischemia-reperfusion. On the other hand, the ECE training protocol has the following characteristics: 1) it can be applied to every muscle in the body; 2) no special device is needed; 3) continuous breathing prevents the tran-

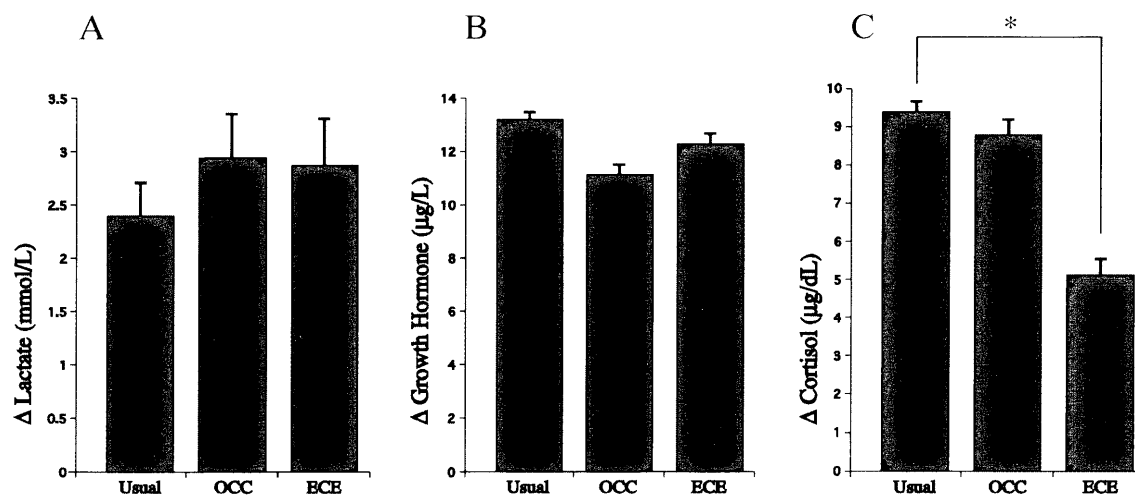


Fig. 4 Hormonal response by three mode of exercise. Increasing values in serum lactate (A), growth hormone (B), and cortisol (C) was depicted. All values are mean \pm SE ($n=9$). Asterisk (*) indicates the significant difference between usual and ECE mode of exercise ($p<0.05$).

sient hypertension caused by constrained respiration; 4) hypertrophy-oriented resistance training can be accomplished even with a relatively light weight, indicating that it enables isolation of the targeted muscle and can be performed even if the joint involved is not in good condition; 5) it induces a burning feeling very rapidly (within 4 or 5 sets), making it a time-saving form of training.

Here we show the evidences that the ECE training protocol is possibly a potent training method for inducing muscle hypertrophy. Although GH production was almost the same with other methods, it significantly reduced COR production, concomitant with higher oxygenation in skeletal muscle. In a future study we would like to elucidate the molecular events that occur during hyperventilation with exercise.

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