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Hypoxia Increases Muscle Hypertrophy Induced by Resistance Training

Akinobu Nishimura, Masaaki Sugita, Ko Kato, Aki Fukuda, Akihiro Sudo, and Atsumasa Uchida

Purpose: Recent studies have shown that low-intensity resistance training with vascular occlusion (kaatsu training) induces muscle hypertrophy. A local hypoxic environment facilitates muscle hypertrophy during kaatsu training. We postulated that muscle hypertrophy can be more efficiently induced by placing the entire body in a hypoxic environment to induce muscle hypoxia followed by resistance training. ***Methods:*** Fourteen male university students were randomly assigned to hypoxia (Hyp) and normoxia (Norm) groups ($n = 7$ per group). Each training session proceeded at an exercise intensity of 70% of 1 repetition maximum (RM), and comprised four sets of 10 repetitions of elbow extension and flexion. Students exercised twice weekly for 6 wk and then muscle hypertrophy was assessed by magnetic resonance imaging and muscle strength was evaluated based on 1RM. ***Results:*** Muscle hypertrophy was significantly greater for the Hyp-Ex (exercised flexor of the hypoxia group) than for the Hyp-N (nonexercised flexor of the hypoxia group) or Norm-Ex flexor ($P < .05$, Bonferroni correction). Muscle hypertrophy was significantly greater for the Hyp-Ex than the Hyp-N extensor. Muscle strength was significantly increased early (by week 3) in the Hyp-Ex, but not in the Norm-Ex group. ***Conclusion:*** This study suggests that resistance training under hypoxic conditions improves muscle strength and induces muscle hypertrophy faster than under normoxic conditions, thus representing a promising new training technique.

Keywords: hypoxic conditions, resistance training, magnetic resonance imaging, MRI, rating of perceived exertion, RPE, muscle hypertrophy

Training under hypoxic conditions is believed to be generally useful for improving aerobic performance by increasing erythropoietin and maximum oxygen intake (VO_2max).[1] The altitude house,[2] where air inside the house is aspirated to increase the concentration of nitrogen and thus create a hypoxic state at low altitude, is more convenient for training under hypoxic conditions than at high altitude. Training under hypoxic conditions is currently under investigation using such facilities. On the other hand, chronic exposure to high altitude leads to a reduction in muscle cross-sectional area (CSA) and a decrease in the size of muscle fibers in humans.[3] Hypoxia per se might be responsible for this atrophy, but malnutrition and reduced activity levels are also possible causes of muscle loss.[4–6] We assume that such muscle loss has not been identified by studies using intermittent hypoxia in which subjects exercise under hypoxic conditions but live in a normoxic environment between training sessions.

The effects of resistance training under hypoxic conditions with recovery under normoxia are mostly unknown. Takarada et al[7] reported that low-intensity training (30–50% 1-repetition maximum [1RM]) with vascular occlusion not only increases electrophysiological activities during exercise, but also significantly increases the CSA of the biceps brachii, brachialis and triceps brachii muscles after 16 weeks of training. Their study showed that low-intensity resistance training with restricted muscular venous blood flow causes muscle hypertrophy and strength gain, and they described this process as *kaatsu training*. These findings suggest that restricted blood circulation causes an intermittent hypoxic and acidic muscular environment, which induces the recruitment of additional motor units and leads to increased muscle hypertrophy. We postulated that muscle hypertrophy could be efficiently achieved by resistance training under intermittent systemic hypoxia. Kaatsu training is useful for resistance training of the extremities, but not for those of the trunk muscles because tourniquets are required. If resistance training under hypoxic conditions leads to hypertrophy, it would be very useful because all muscles including those of the trunk could benefit.

We compared the induction of muscle hypertrophy by resistance training under hypoxic and normoxic conditions using magnetic resonance imaging (MRI). Muscle strength was also assessed based on 1RM (the maximum weight that can be lifted once over the entire range of motion). Levels of exertion were compared between training under hypoxic and normoxic conditions using the subjective rating of perceived exertion (RPE) scale.

Materials and methods

Subjects
Fourteen untrained male university students (age [mean ± standard deviation], 21.4 ± 1.1 y; height, 173.0 ± 5.4 cm; body mass, 65.9 ± 8.1 kg; body fat, 12.6 ± 3.7%) provided written, informed consent to participate in the study after reviewing a detailed explanation of its purposes and procedures. Body mass and percentage fat were measured using a body composition analyzer (BC-118E, Tanita, Tokyo, Japan). The students were randomly assigned to hypoxia (Hyp) and normoxia (Norm) groups ($n = 7$ per group). Table 1 summarizes the physical characteristics of the participants. Training intensity was established based on the maximum weight that could be lifted over the entire range of motion (1RM) under normoxia. We referred to a published protocol[8] to determine 1RM from the 10RM test. Exercise intensity was set before the

first training session and then revised 3 wk later. The participants continued with their normal lives and were instructed not to start any new exercise during the course of the study. The ethics review board of Mie University approved the study protocol.

\ insert Table 1 \

Constant Hypoxic Room

The constant hypoxic room (Alticube, USK, Nara, Japan) consists of a compressor, a hypoxia generator with a polymer membrane and a hypoxic chamber covered with a membrane separation-type vinyl tent to block air (width × length × height: 2900 × 2000 × 2200 mm). Ambient air was compressed using the hypoxia generator and hyperoxic air separated by the polymer membrane from hypoxic air was sent to the hypoxic chamber to create a hypoxic environment. The oxygen concentration was maintained at 16.0% (normal oxygen concentration, approx. 21%) during training and room temperature was maintained at 27°C using an air conditioner for both groups.

Training

Each participant stood holding a dumbbell in the nondominant hand and performed standing French presses and arm curls. The Hyp and Norm groups sat inside the hypoxic chamber under hypoxic condition or outside the chamber under normoxic conditions, respectively, for 30 min before training. Both groups warmed up by performing 10 repetitions each of standing French presses and arm curls at 30% 1RM before the 30-min acclimation period. The exercise intensity during training was 70% 1RM for both standing French presses and arm curls. Each training session consisted of four sets of 10 repetitions each, with a 1-min break between sets. If 10 repetitions could not be finished, the set was ended at that point. A 3-min break was provided between sets of both exercises. The Hyp group rested in the hypoxic chamber for 30 min after the end of each training session and then left the chamber for the next bout of exercise. At 3 weeks after starting the training regime, muscle strength was measured to adjust the intensity level of exercise as needed. The groups trained twice each week for 6 consecutive weeks and all 14 participants completed the total of 12 sessions. The dumbbell was raised or lowered at comparable rates within about 3 s (1 and 2 s for concentric and eccentric movement). The mean numbers of standing French press repetitions performed by the exercised upper arms of the Hyp (Hyp-Ex) and Norm (Norm-Ex) groups were 36.2 ± 3.3 and 36.1 ± 2.9, and the mean numbers of arm curl repetitions performed by the Hyp-Ex and Norm-Ex group were 35.6 ± 4.0 and 35.4 ± 3.2, respectively. The numbers of repetitions did not significantly differ between the Hyp-Ex and Norm-Ex groups. Table 2 summarizes the exercise intensity and repetitions actually applied by the two groups. The mean duration of each training session was about 13 min.

\ insert Table 2 \

Magnetic Resonance Imaging

The cross-sectional areas of the Norm-Ex and Hyp-Ex groups, and of the nonexercised arm of the Hyp (Hyp-N) group were measured using a 1.5-T super

coiling system (Excel ART, Toshiba, Tokyo, Japan), of which the coil covered the whole upper arm. The center of the upper arm was defined as the point equidistant between the head of the humerus and the olecranon. Multislice sequences at 6-mm intervals were obtained under the following conditions: 1.0 mm interception gap, 20×20 cm field of view (FOV), spin echo T1-weighted pulse sequence, 500-ms repetition time and 15-ms echo time. The scan matrix was 208×320. Scanning was immediately initiated after supine participants relaxed while extending the arms to minimize possible effects of a gravitational fluid shift. The entire sequence was completed within 3–5 min. Images were captured along the long axis of the upper arm and the range of acceptable slices was carefully determined. Of 11 slices, three images at the center of the upper arm were selected to measure muscle CSA. Tissue outlines and the CSA of muscles and other tissues were determined using Image J (version 1.37v) software. The CSA value was the mean of three determinations. The standard deviation of these three sets of measurements was 1.0%. The percent increase in CSA relative to that before training was then calculated.

Muscle Strength

We determined muscle strength of each participant as 1RM from 10 RM tests[8] at 1 wk before, and at 3 and 6 wk after starting training. We referred to a published protocol[8] to determine 1RM from the 10RM test (for example, 10 repetition maximum equals 75% 1RM). The intensity of subsequent training sessions was adjusted based on 1RM measured 3 weeks after start of training.

Saturation (SpO$_2$)

We measured SpO_2 at 10 min before, and at 0 and 30 min and 24 h after each training session using a PULSOX-3i (Minolta, Osaka, Japan) placed on the index finger of the dominant hand (nonexercise side). The SpO_2 after 24 h was measured in both groups under normoxic conditions.

Rating of Perceived Exertion

The level of perceived exertion was assessed after training using the Borg 6-20 RPE scale.[9] After each training session, the examiner showed the board with RPE scores to the participant. The RPE scores of the participants were determined based on their descriptions of difficulty in lifting the weight ranging from "very, very light" (6 points) to "very, very heavy" (20 points).

Statistical Analysis

Unless otherwise stated, standard deviations were calculated for variables. The statistical significance of differences among the degrees of change in the CSA of Hyp-Ex, Hyp-N and Norm-Ex was determined using the one-way analysis of variance (ANOVA). The statistical significance of differences among the CSA of Hyp-Ex, Hyp-N, and Norm-Ex was determined using a two-way (group × time) ANOVA. The statistical significance of differences between the muscle strength of Hyp-Ex and Norm-Ex was determined using a two-way (group × time) repeated-measures ANOVA. The significance of individual differences was evaluated using the Bonferroni post hoc test. Differences between two variables within the same individual were examined using Student's paired t test. Differences in physical data, characterization of exercise training, SpO_2 and RPE between Hyp and Norm were

examined using Student's unpaired t test. A value of $P < .05$ with the Bonferroni correction was regarded as significant for all statistical analyses.

Results

Changes in CSA After Training
Table 3 shows the CSAs of all groups. The percentage increases of the elbow extensor and flexor of the Hyp-Ex group were significantly larger ($P < .05$) compared with before training (7.3 and 9.6%, respectively at week 6; Figure 1). In contrast, the CSA values of the Norm-Ex and Hyp-N groups did not significantly increase.

\ insert Table 3 \

Muscle Strength (1RM)
Figure 2 shows the 1RM for all groups. This value significantly increased in both the Hyp-Ex and Norm-Ex groups. Muscle strength following the standing French presses and arm curls in the Hyp-Ex group increased by 71.1 and 61.6%, respectively, and in the Norm-Ex group by 56.4 and 38.9%, respectively, with no significant differences between the groups. Muscle strength significantly increased after 6 wk and between 3 and 6 wk of training in both groups, but at 3 wk of training only in the Hyp-Ex group.

\ insert Figure 1 thru 4 \

Saturation—SpO$_2$
Figure 3 shows the effect of training under hypoxic and normoxic conditions on SpO$_2$. The SpO$_2$ value of the Hyp group was significantly lower at 10 min before and at 0 and 30 min after training. At 24 h after training, no significant intergroup differences were apparent, because SpO$_2$ was measured in both groups under normoxic conditions.

Rating of Perceived Exertion
Figure 4 shows that the RPE did not significantly differ after either the standing French presses or arm curls.

Discussion

Compared with normoxic conditions, resistance training under hypoxic conditions induced more muscle hypertrophy without differences in RPE during training. Muscle strength (defined as 1RM) was significantly increased at week 6 for both groups, but only for the Hyp-Ex group at week 3, suggesting that exposure to hypoxia accelerates increases in muscle strength. Muscle hypertrophy was not significant in the Hyp-N group (non-exercised-arm under hypoxic conditions), indicating that hypoxia alone is insufficient to increase muscle strength (1RM) or induce muscle hypertrophy and that it must be combined with exercise to achieve these results.

The time course of gains in muscle strength is affected by neural factors and by muscle hypertrophy.[10] We found here that 1RM had significantly increased in the

Hyp-Ex group by week 3, whereas 6 wk were required to elicit an increase in CSA. We also found a significant increase in 1RM of the Hyp-Ex group by week 6, although CSA in this group did not increased significantly until that time. Dissociation between increases in 1RM and in CSA would be expected since neuromuscular adaptations occur before hypertrophy.[11–13] The role of neural factors is particularly critical during the early phase of strength training. Although protein synthesis is noticeable after a single strength training session,[14] overt changes in muscle hypertrophy are not evident until 8 wk of exercise training in beginners.[13,15–17] This delay, which is concurrent with a substantial gain in muscle strength, has led some to suggest that neural factors are important.[12] Surface electromyography (SEMG) findings have revealed that strength gains during the early phase of a training regimen without noticeable muscle hypertrophy are associated with an increase in the amplitude of SEMG activity. This has been interpreted as an increase in neural drive, which denotes the magnitude of efferent neural output from the CNS to active muscle fibers. Moritani et al[12] concluded that increases in the amplitude of the SEMG signal appear well before muscle hypertrophy, and thus neural factors account for the larger proportion of the initial increase in strength. The present study found that 1RM significantly increased only in the Hyp-Ex group after 3 wk. Although neural factors were involved in both groups, the effect of early muscle hypertrophy might have increased muscle strength after 3 wk in the Hyp-Ex group.

Takarada et al[7] reported that vascular occlusion combined with low-intensity resistance training (Kaatsu training) effectively increases cross-sectional area and muscle strength. Abe et al[18] reported that muscle hypertrophy and muscle strength could be increased by walking slowly while restricting blood flow. They also reported that the combination of vascular occlusion and resistance training achieves muscle hypertrophy because of hypoxia. They suggested that restricted blood circulation causes a hypoxic and acidic muscular environment, which leads to greater muscle hypertrophy. We believe that this mechanism functioned in the present study. The effects of exercise training regimens under hypoxia are likely mediated by moderate production of reactive oxygen species (ROS) promoting tissue growth,[7] growth hormone secretion stimulated by the intramuscular accumulation of metabolic subproducts, such as lactate,[19] testosterone secretion in Leydig cells[20] and additional recruitment of type II fibers.[7] These studies suggest that the ability of resistance exercise to elicit muscle hypertrophy involves not only mechanical stress but also metabolic, hormonal and neuronal factors. However, the precise mechanism of muscle hypertrophy in resistance training under hypoxia (hypoxic training) requires further studies for clarification.

Narici et al[3] reported that subjects exposed to chronic hypoxia show less muscle hypertrophy than the same subjects exposed to normoxia and Mizuno et al[21] also reported that general chronic exposure to hypoxia leads to muscle atrophy rather than hypertrophy. However, these studies had a different design from our study in that the conditions comprised chronic hypoxia, whereas our conditions consisted of training under hypoxia with recovery under normoxia. Some authors[4–6] have reported that chronic hypoxia leads to malnutrition and reduced activity levels; thus, chronic hypoxia is considered a possible cause of muscle loss. Friedmann et al[22] reported that strength training under hypoxia with recovery under normoxia could not promote muscle hypertrophy. However, their study conditions comprised low-resistance (30% 1RM)/high repetition whereas ours comprised moderate resistance (70% 1RM)/low repetition. Considering these findings, hypoxic training cannot lead to muscle

hypertrophy simply by low resistance training such as kaatsu training. The induction of muscle hypertrophy under hypoxia requires high resistance training.

This study suggests that exercise combined with exposure to hypoxia effectively induces muscle hypertrophy and increases muscle strength. However, with kaatsu training, tourniquets can only be placed on the extremities, whereas training under hypoxic conditions can exercise all muscles, including those of the trunk. Thus, more balanced muscle training programs can be designed, as this method is applicable to the whole body.

One limitation of this study is that it was not blinded (the Norm group trained outside the chamber, so the subjects knew the group [Hyp or Norm] to which they had been assigned), so some biases may affect the results. Another is that we used only one O_2 level (16%), so how the level of O_2 affects muscle hypertrophy is unclear. Further studies (several levels of hypoxia) are required to determine a dose-response effect.

Because hypoxia leads to hypoxic pulmonary hypertension,[23,24] adaptation must be carefully controlled. Although further investigation is needed, high resistance training under hypoxia appears to offer a practical and effective method of increasing strength and inducing muscle hypertrophy.

Conclusions

Compared with normoxic conditions, resistance training under hypoxic conditions efficiently improves muscle strength and rapidly induces muscle hypertrophy without an obvious increase in the rate of perceived exertion (RPE) since it occurred at 6 rather than at 8 wk in this group of beginners. While further studies are currently investigating whether harmful effects can be induced during training, resistance training under hypoxic conditions might be a novel method of increasing strength and muscle size.

Acknowledgments

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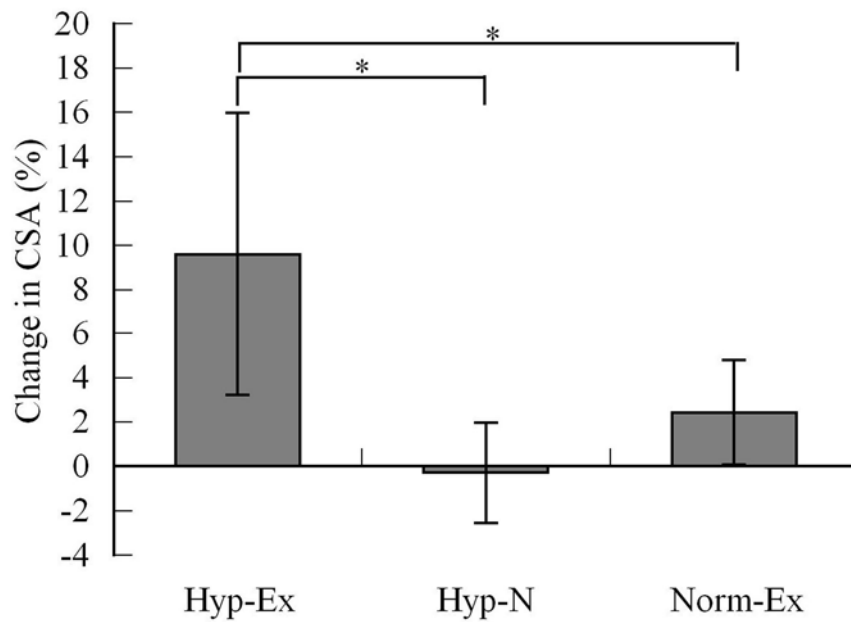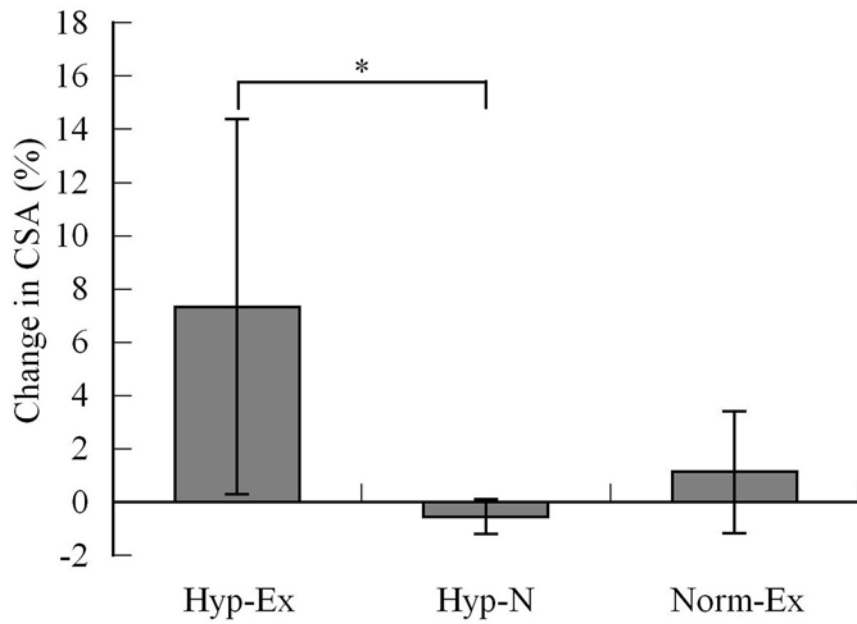
Figure 1 — Degree of change in cross-sectional area (CSA) of extensors (top) and flexors (bottom) after 6 wk of training. *Significant differences between groups (*P* < .05). Hyp-Ex, exercised arm of hypoxia group; Hyp-N, nonexercised arm of hypoxia group; Norm-Ex, exercised arm of normoxia group.
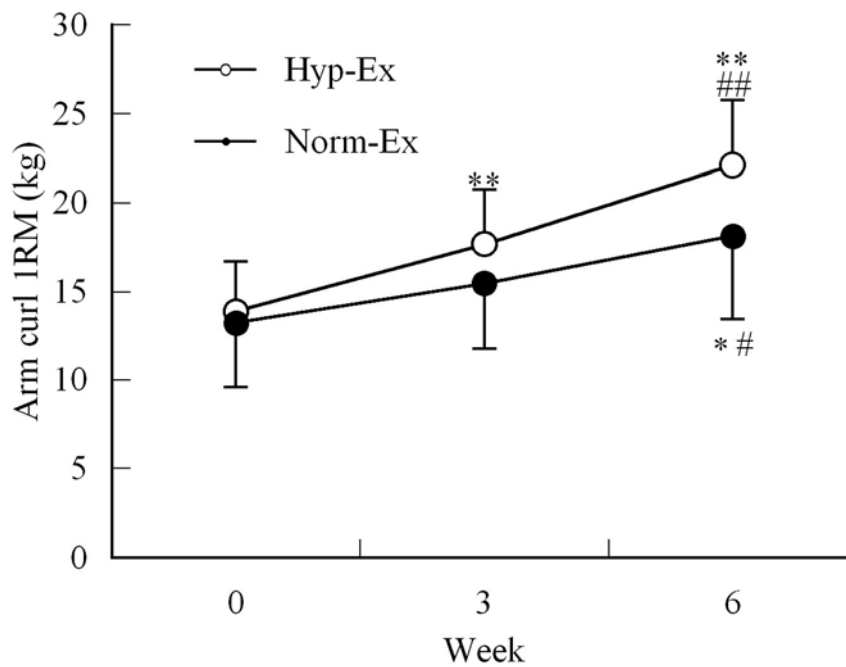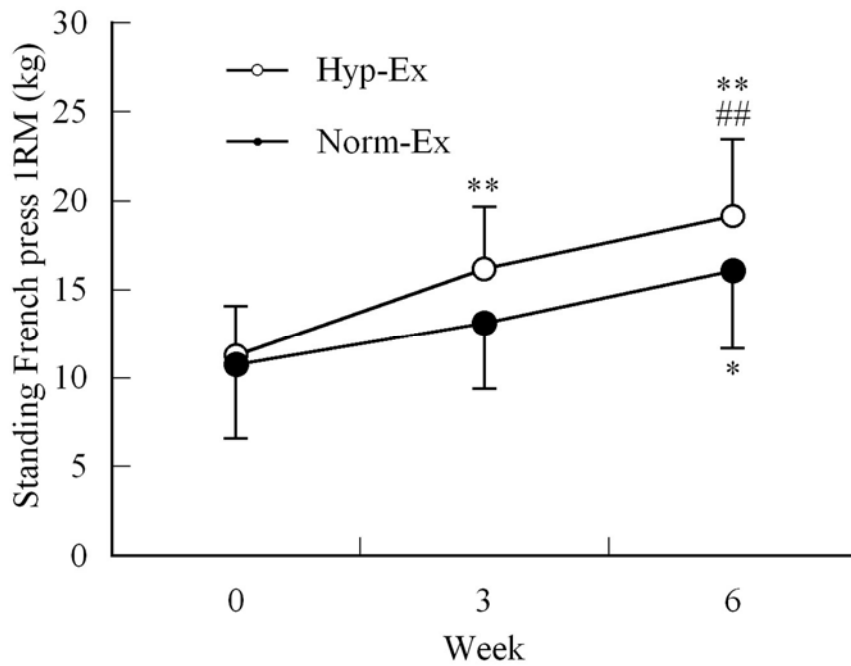
Figure 2 — Degree of change in muscle strength (1 repetition maximum [RM]) of the extensors (top) and flexor (bottom) after 6 wk of training. The Hyp-Ex and Norm-Ex groups did not significantly differ. Significant differences from pretraining values, *P < .05 and **P < .01, respectively; significant differences from 3-wk values, [#]P < .05 and [##]P < .01, respectively. Hyp-Ex, exercised arm of hypoxia group; Norm-Ex, exercised arm of normoxia group.
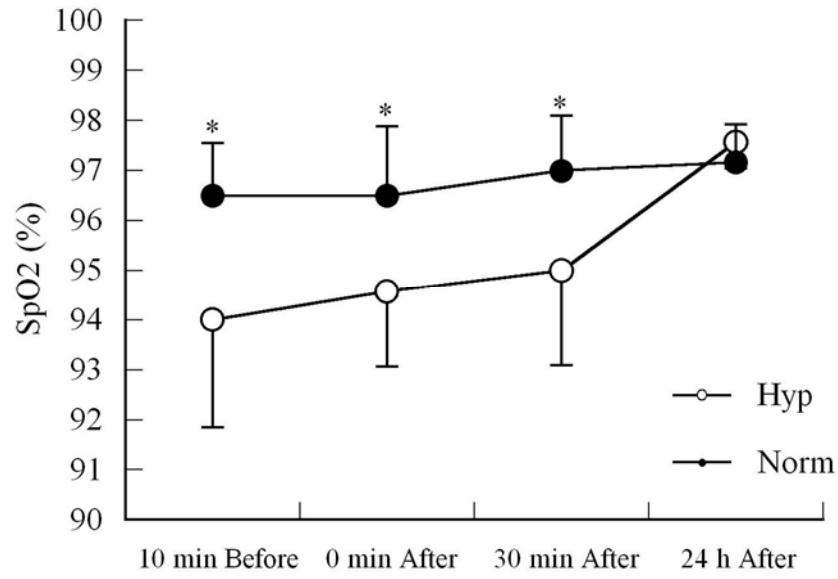
Figure 3 — Mean SpO_2 for hypoxia (Hyp) and normoxia (Norm) groups. All values of SpO_2 were measured at 24 h after training under normoxic conditions. *Significant differences between groups ($P <$ .05).
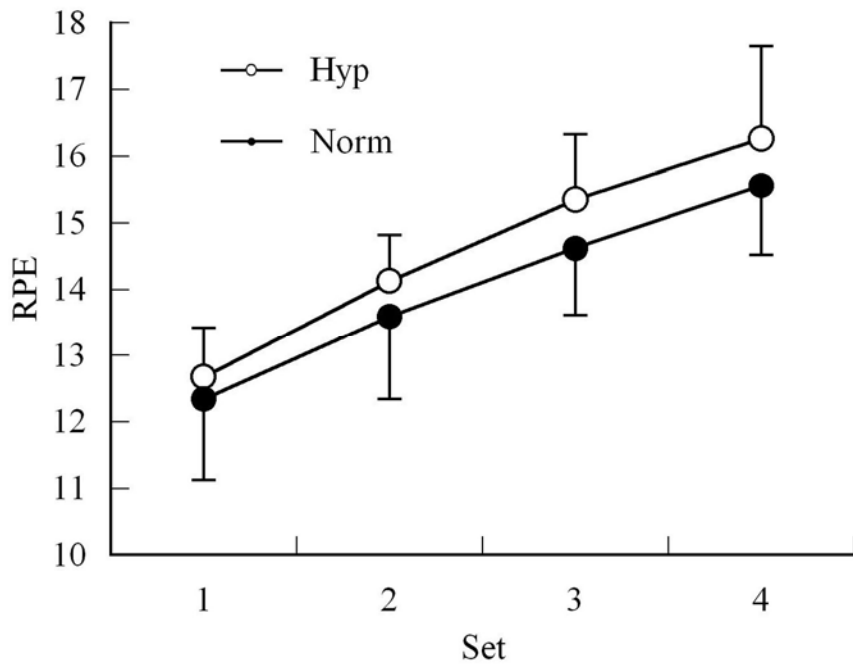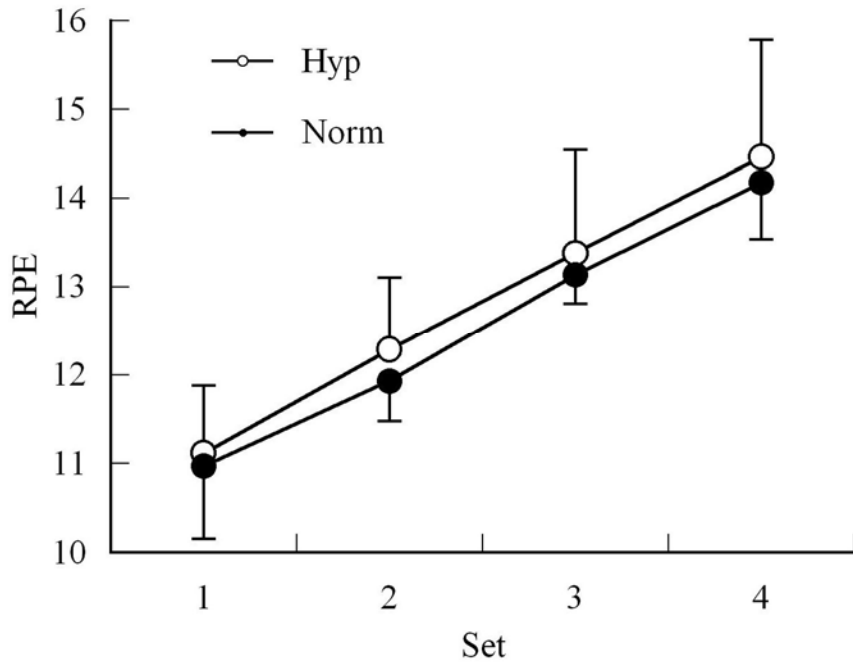
Figure 4 — Changes in rating of perceived exertion (RPE) after each set of standing French presses (top) and arm curls (bottom). Intergroup differences are not significantly different at any time point. Hyp, hypoxia group; Norm, normoxia group.

Table 1   Physical data of hypoxia (Hyp) and normoxia (Norm) groups before and after 6 wk of training

	Hyp ($n = 7$)		Norm ($n = 7$)	
	Pretraining	Posttraining	Pretraining	Posttraining
Age (y)	22.7 ± 2.7	—	21.6 ± 1.6	—
Height (cm)	174.6 ± 5.0	—	171.4 ± 5.2	—
Body mass (kg)	66.8 ± 6.0	66.9 ± 6.8	65.0 ± 8.1	65.1 ± 8.1
Body fat (%)	12.3 ± 3.0	13.2 ± 0.7	12.8 ± 4.5	12.7 ± 4.3

Note. Body mass and percentage fat were measured using a body composition analyzer (BC-118E, Tanita, Tokyo, Japan). The Hyp and Norm groups did not significantly differ.

Table 2   Characterization of exercise training

			Hyp-Ex	Norm-Ex
French press	Intensity % 1RM (%)	0–3 wk	70.7 ± 2.1	70.6 ± 1.7
		4–6 wk	69.6 ± 1.4	71.0 ± 1.5
	Repetitions		36.2 ± 3.3	36.1 ± 2.9
Arm curl	Intensity % 1RM (%)	0–3 wk	70.3 ± 0.8	69.5 ± 1.3
		4–6 wk	70.1 ± 0.7	69.7 ± 0.5
	Repetitions		35.6 ± 4.0	35.4 ± 3.2

Note. The Hyp-Ex and Norm-Ex groups did not significantly differ. RM, repetition maximum; Hyp-Ex, exercised arm of hypoxia group; Norm-Ex, exercised arm of normoxia group.

Table 3   Cross-sectional areas (CSA) of elbow extensor and flexor before and after 6 weeks of training

	Extensors (cm^2)			Flexors (cm^2)		
	Pretraining	Posttraining	ΔChange	Pretraining	Posttraining	ΔChange
Hyp-Ex	25.6 ± 2.6	27.5 ± 3.9*	1.9 ± 1.1	12.9 ± 1.1	14.2 ± 1.5*	1.3 ± 0.9
Hyp-N	26.3 ± 3.1	26.2 ± 3.2	–0.1 ± 0.2	13.2 ± 1.5	13.1 ± 1.7	0.0 ± 0.3
Norm-Ex	25.8 ± 4.4	25.9 ± 4.4	0.3 ± 0.6	12.3 ± 1.9	12.5 ± 1.9	0.3 ± 0.3

Note. The Hyp-Ex, Hyp-N and Norm-Ex groups did not significantly differ. *$P < .05$, significant differences from pretraining values. RM, repetition maximum; Hyp-Ex, exercised arm of hypoxia group; Hyp-N, nonexercised arm of hypoxia group; Norm-Ex, exercised arm of normoxia group.