

Research article

EFFECT OF MODERATE ALTITUDE ON PERIPHERAL MUSCLE OXYGENATION DURING LEG RESISTANCE EXERCISE IN YOUNG MALES

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ABSTRACT

Training at moderate altitude (~1800m) is often used by athletes to stimulate muscle hypoxia. However, limited data is available on peripheral muscle oxidative metabolism at this altitude (1800AL). The purpose of this study was to determine whether acute exposure to 1800AL alters muscle oxygenation in the vastus lateralis muscle during resistance exercise. Twenty young active male subjects (aged 16 – 21 yr) performed up to 50 repetitions of the parallel squat at 1800AL and near sea level (SL). They performed the exercise protocol within 3 h after arrival at 1800 AL. During the exercise, the changes in oxygenated hemoglobin (OxyHb) in the vastus lateralis muscle, arterial oxygen saturation (SpO₂), and heart rate were measured using near infrared continuous wave spectroscopy (NIRcws) and pulse oximetry, respectively. Changes in OxyHb were expressed by D_{eff} defined as the relative index of the maximum change ratio (%) from the resting level. OxyHb in the vastus lateralis muscle decreased dramatically from the resting level immediately after the start of exercise at both altitudes. The D_{eff} during exercise was significantly ($p < 0.001$) lower at 1800AL (60.4 ± 6.2 %) than at near SL (74.4 ± 7.6 %). SpO₂ during exercise was significantly ($p < 0.001$) lower at 1800AL (92.0 ± 1.7 %) than at near SL (96.7 ± 1.2 %). Differences (SL – 1800AL) in D_{eff} during exercise correlated fairly strongly with differences in SpO₂ during exercise ($r = 0.660$). These results suggested that acute exposure to moderate altitude caused a more dramatic decrease in peripheral muscle oxygenation during leg resistance exercise. It is salient to note, therefore, that peripheral muscle oxygenation status at moderate altitude could be evaluated using NIRcws and that moderate altitudes might be effectively used to apply hypoxic stress on peripheral muscles.

KEY WORDS: NIRcws, muscle oxygenation, moderate altitude, parallel squat, SpO₂

INTRODUCTION

Acute exposure to certain altitudes reduces alveolar and arterial oxygen partial pressure, and enhances the relative exercise intensities (Kuno et al., 1994). Therefore, coaches and researchers in the field of sports medicine pay particular attention to the altitude environment to take advantages of it (Kuno et al., 1994). To evaluate the effects of altitude on exercise intensities, it is extremely important to

observe the skeletal muscle oxidative function directly and evaluate the changes quantitatively.

Near infrared continuous wave spectroscopy (NIRcws) has been used to evaluate the kinetics of skeletal muscle oxygenation during various types of exercise non-invasively and continuously (Ozyener, 2002; Quaresima et al., 2003). And, the NIRcws measurement gives an overall measure of the oxygenation in the illuminated area, a decrease in

oxygen supply or an increase in oxygen consumption would cause the decrease in muscle oxygenation (Kawaguchi et al., 2001). Several studies have investigated the effects of hypoxic environments on peripheral muscle oxygenation during steady-state cycling exercise (Costes et al., 1996), arm cranking exercise (Jensen-Urstad et al., 1995), isometric contractions of the forearm (Hicks et al., 1999) and leg-kicking exercise (MacDonald et al., 1999) using NIRcws. These studies have suggested that this noninvasive approach of muscle oxygenation could help to provide a better understanding of exercising muscle metabolism when oxygen delivery is reduced in hypoxia (Costes et al., 1996).

However, most of these studies were conducted on subjects breathing hypoxic gas mixtures: 10.5% (Costes et al., 1996) to 14.0% (Hicks et al., 1999; MacDonald et al., 1999) O₂ in N₂, which corresponds to an altitude of 3000 to 5000 m. Consequently, there is limited data available on muscle oxygenation and moderate altitude, especially during acute exposure to moderate altitude environments, in which athletes actually train. The limitation of data prompted us to investigate whether acute exposure within 3 h after arrival at the moderate altitude of 1800 m (1800AL) enhances the decrease in peripheral muscle oxygenation during exercise.

METHODS

Subjects

Twenty young active male volunteers (aged 16 – 21 yr) who had participated in competitive endurance sports were enrolled in this study. Prior to the start of the study, documented informed consent to participate was obtained from either the subjects themselves or their parents in accordance with the Helsinki Declaration (WMADH, 2000). No subjects were taking medications, and all were near sea level (SL) residents who had not been to the moderate altitude for at least 3 months before testing. The physiological characteristics of the subjects are presented in Table 1.

Exercise protocol

All testing sessions were conducted within 3 days. At the first day, subjects initially rested in a sitting position for 20 minutes, secondly performed up to 50 repetitions of the parallel squat, finally rested in a sitting position for 3 minutes near SL (Gifu, Japan, 40 m above SL, barometric pressure 756 Torr). Various physiological responses were recorded during this procedure. Two days later, subjects travelled to the 1800 AL (Mt. Ontake, Japan, 1800 m above SL, 610 Torr) by cars spending 2 h. They

then performed the same protocol within 3 h after arrival at 1800 AL.

Table 1. Physiological characteristics of the subjects (n=20). Data are means (±SD) and range.

Age (years)	18.1 (1.1) 16-21
Height (m)	1.69 (0.06) 1.57-1.76
Weight (kg)	57.9 (7.7) 43.5-76.5
BMI (kg·m ⁻²)	20.3 (2.2) 17.7-24.6
Body fat (%)	12.2 (2.4) 7.9-13.6
Femoral fat thickness (mm)	2.9 (.7) 2.1-4.0

BMI=body mass index

The 50 repetitions of the parallel squat were performed in a shoulder-width stance, with no resistance load, that is, with making their own weight the load. They were performed at 1 repetition per 2 s under the guidance of a supervisor with a metronome. In the descent phase, the subjects simultaneously flexed their hip and knee joints and dorsiflexed their ankle joints until the posterior of their thighs was parallel to the floor. In the ascent phase, the subjects extended their hip and knee joints and plantar flexed their ankle joints to return to the standing position (McCaw and Melrose, 1999). The temperature and relative humidity of the laboratory during all experiments were maintained at 24 °C and 50-60%, respectively.

Near infrared continuous wave spectroscopy (NIRcws) measurement

A commercially available NIRcws monitor (BOM-L1TR, Omegawave, Inc) was used to evaluate the kinetics of peripheral muscle oxygenation during rest, exercise and recovery. The instrument can emit laser light at wavelengths of 780, 810 and 830 nm, and determine the relative values of oxygenated hemoglobin (OxyHb), deoxygenated hemoglobin (DeoxyHb) and total hemoglobin (TotalHb), based on the Beer-Lambert law (Figure 1). At these wavelengths, the absorption coefficient of hemoglobin are obtained according to Matcher et al. (1995). In the present study, OxyHb, DeoxyHb and TotalHb are expressed in terms of an arbitrary unit (a.u.), that does not represent the actual physical volume. The basic principle of this measurement has been extensively discussed by Kawaguchi et al. (2001). Since the contribution of myoglobin to the NIRcws signal is thought to be minor (about 10%), the signal is believed to reflect the muscle

hemoglobin oxygenation (Wilson et al., 1989; Mancini et al., 1994; Quaresima et al., 2003).

The optical probe of the instrument was placed on the skin over the right vastus lateralis muscle, approximately 15 cm proximal from the upper margin of patella, along the vertical axis of the thigh at 1800AL and near SL. The distance between the incident and receiving point was 30 mm, and these were fixed with a tape after shielding with a rubber sheet and vinyl. The NIRcws data were input onto a personal computer every 0.5 s at a sampling frequency of 2 Hz via an A/D transducer, and the data were then averaged every 10 s using a customized software program (Bimutas, Kissei Comtech, Nagano, Japan).

The femoral subcutaneous fat thickness was determined using B-mode ultrasonography (Toshiba Sonolazer- α , SSA-270A, Toshiba Medical Systems, Japan), at a frequency of 5MHz near SL. For the ultrasonography measurement, the skin at the same site as the NIRcws probe was precisely identified and marked. One experienced technician performed all the ultrasonographic measurements.

Change in peripheral muscle oxygenation (D_{eff})

In this study, the particular attention was paid to the change in peripheral muscle oxygenation (OxyHb) observed during exercise, and this change was defined as D_{eff} in a manner similar to that described by Ding et al. (2001). The mean value of OxyHb for the last 2 min of the 20 min rest was defined as the resting value of OxyHb for each subject. The D_{eff} values were expressed in percentages as the maximum change in OxyHb during exercise per the resting value of OxyHb (Figure 1).

The day-to-day reproducibility of D_{eff}

To examine the day-to-day reproducibility of D_{eff} obtained by our NIRcws method, another group of 12 healthy subjects (age: 16-21yr, height: 173.6 \pm 5.2 cm, weight: 61.4 \pm 6.4 kg, femoral fat thickness: 2.0-3.8 mm) completed the same protocol as experimental subjects on 2 different days near SL. The interval between tests was 7 days. The error and the correlation coefficient between the first and second tests were calculated.

Measurements of SpO_2 and HR

Arterial oxygen saturation (SpO_2) was measured non-invasively using a forefinger probe connected to a pulse oximetry (NPB-40, Nellcor Puritan Bennett Inc, U.S.A.) during exercise at 15 s intervals. Martin et al. (1992) and Benoit et al. (1997) have shown that SpO_2 measured non-invasively using a pulse oximetry is an accurate and valid predictor of SaO_2 during ergometer cycling in comparison with invasive methods. In order to examine the

relationship between peripheral muscle oxygenation and arterial oxygen content during exercise at 1800AL, the correlation coefficient of differences in D_{eff} and SpO_2 between SL and 1800AL during the parallel squat were calculated. Heart rate (HR) was measured at 5 s intervals during the exercise using a Heart Rate Monitor (S610, Polar Electro Corporation, Finland).

Statistical analysis

All data are expressed as means \pm SD. Two-way analysis of variance (ANOVA) of repeated measurements was used for a comparison of D_{eff} , SpO_2 and HR readings at 1800AL and SL. When a significant F ratio was obtained, Tukey's post hoc test was used to determine significant differences in the various pairwise comparisons. Pearson's correlation coefficients were used between the first and second test in examining the day-to-day reproducibility of D_{eff} , and between the differences (SL - 1800AL) in D_{eff} and in SpO_2 during exercise. These statistical analyses were performed by using the SPSS 11.0 statistical software. The level of statistical significance was set at $p < 0.05$.

RESULTS

The day-to-day reproducibility of D_{eff}

D_{eff} was 70.3 \pm 7.8% for the first test and 68.5 \pm 9.2% for the second test. The error between the two tests ranged from 0.6% to 6.0% (mean \pm SD: 2.5 \pm 1.6%). A strong relationship (correlation coefficient: 0.974) was observed between the two tests.

Peripheral muscle oxygenation responses

Figure 1 illustrates a typical example of the variation in OxyHb, DeoxyHb, and TotalHb during the course of the squat exercise. In all subjects, a dramatic decrease in OxyHb and increase in DeoxyHb (Figure 1) was observed at 1800AL and SL, immediately after the start of exercise following a plateau phase in each case. TotalHb gradually increased as exercise advanced towards a plateau phase in each case (Figure 1). These values returned to their respective resting levels after the exercise.

As shown in Figure 2-A, the average D_{eff} at 1800AL (60.4 \pm 6.2%) was significantly ($p < 0.001$) lower than that at SL (74.4 \pm 7.6%). In sixteen of the subjects, there were larger differences in D_{eff} between 1800AL and SL than the maximal error (6.0%) between the two tests for the day-to-day reproducibility measurement (Figure 2-B).

SpO_2 and HR responses

SpO_2 during rest, exercise and recovery was significantly lower at 1800AL than near SL ($p < 0.001$, Figure 3). In particular, SpO_2 decreased more

dramatically at 1800AL ($92.0 \pm 1.7\%$) than near SL during exercise ($96.7 \pm 1.2\%$). HR during rest, exercise and recovery was significantly higher at 1800AL than near SL ($p < 0.001$, Figure 4).

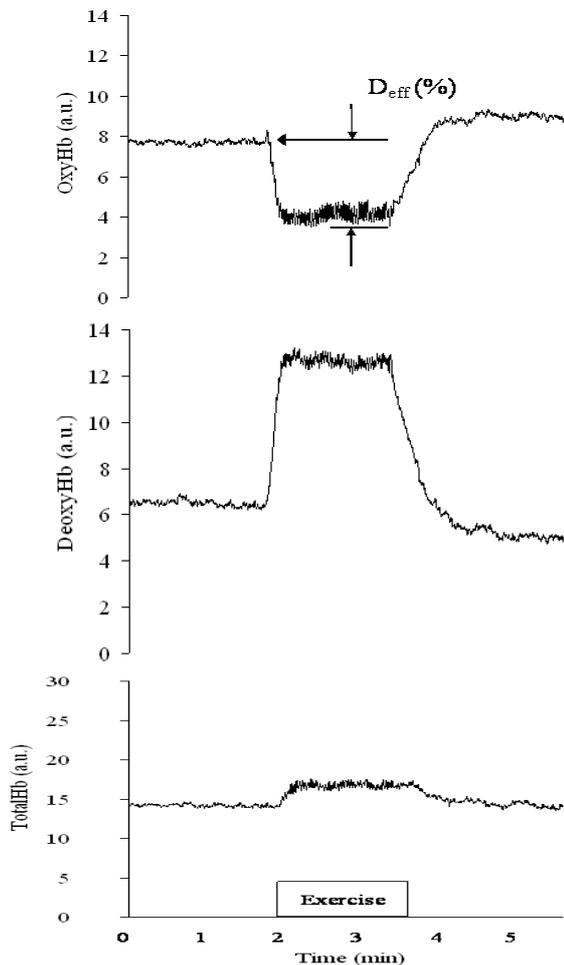


Figure 1. Typical changes in OxyHb, DeoxyHb and TotalHb during rest, the parallel squat and recovery. a.u. =arbitrary unit

Correlation between peripheral muscle oxygenation and arterial oxygen content

As shown in Figure 5, the differences between SL and 1800AL in D_{eff} during exercise correlated strongly with those in SpO_2 during exercise ($r = 0.660$).

DISCUSSION

Several investigators have confirmed that NIRcws is a more viable predictor of peripheral muscle oxygenation compared to other reliable muscle metabolic measurements. McCully et al. (1994), Hamaoka et al. (1996) and Sako et al. (2001) have reported a parallel decrease in NIRcws signal and in substrates measured by magnetic nuclear resonance spectroscopy, particularly the Pi-to-phosphocreatine ratio. Additionally, a strong relationship between the kinetics of muscle oxygenation during exercise and the ventilatory threshold (Bhambhani et al., 1997),

the lactate threshold (Belardinelli et al., 1995; Grassi et al., 1999) and muscle effluent venous oxygen saturation (Wilson et al., 1989; Mancini et al., 1994) have also been demonstrated. Thus, at present, there seems to be general agreement on NIRcws as a non-invasive and reliable method for measuring peripheral muscle oxidative metabolism that avoids the inconvenience and the potential risks associated with the insertion of a radial artery catheter. The present data corroborates these findings and supports the reliability of day-to-day measurements using NIRcws ($r=0.974$).

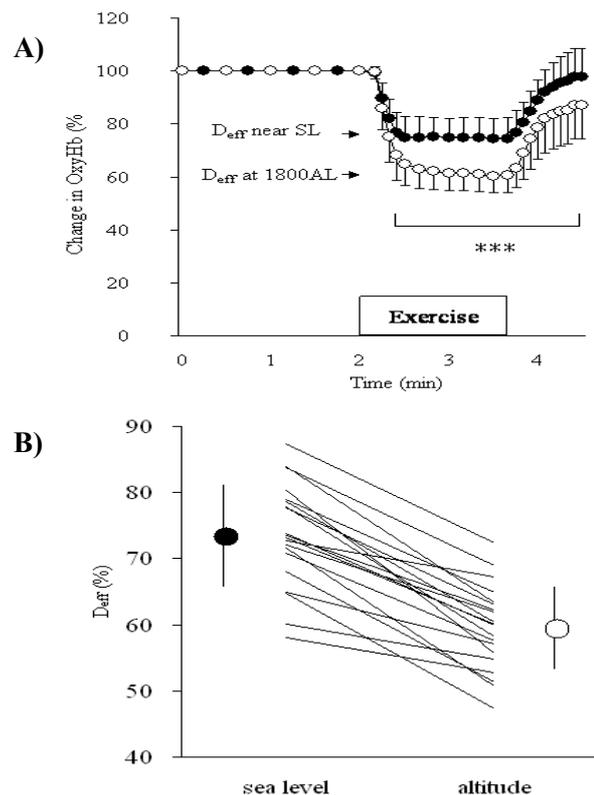


Figure 2. A) change in OxyHb during rest, the parallel squat and recovery and B) change in individual and mean values of D_{eff} during exercise between 1800AL (\circ) and SL (\bullet). SL=sea level. 1800AL=1800 m above sea level. Values are mean \pm SD. *** $p < 0.001$, Significant difference between 1800AL and near SL (two-way ANOVA for repeated measures, Tukey's post hoc test).

The present study was designed to investigate how acute 1800AL exposure influences muscle oxygenation during submaximal leg resistance exercise using NIRcws signals recorded percutaneously on the vastus lateralis. As expected, muscle oxygenation in the vastus lateralis during parallel squat at 1800AL decreased to a lower level as compared with at near SL (Figure 2). These results resemble those of Costes et al. (1996) who reported that infrared muscle oxygen saturation was

99.3 ± 3.1% at rest and decreased slightly to 94.9±6.2% during cycling exercise in normoxia, whereas, in hypoxia, infrared muscle oxygen saturation at rest (91.7 ± 3.3%) was significantly lower than in normoxia and decreased dramatically to 82.7 ± 6.6% during cycling exercise. These results demonstrate that hypoxia or moderate altitude provide a sufficient environment to increase the hypoxic stress to peripheral muscles, even if differences of exercise adopted in the two studies were taken into account.

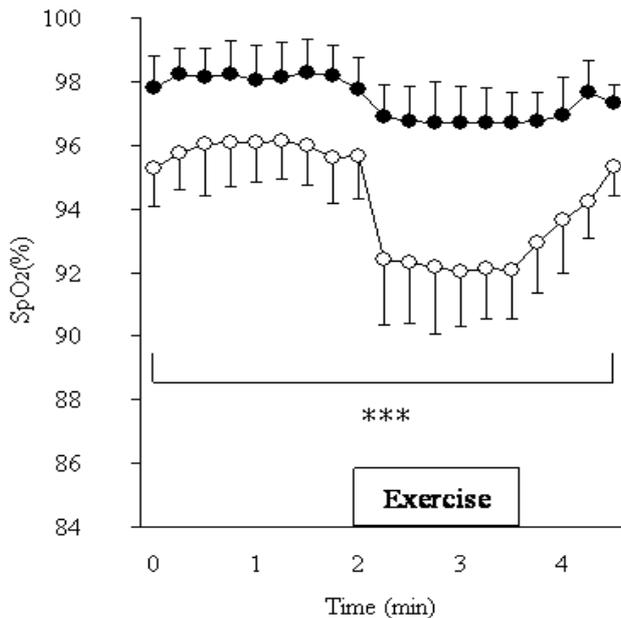


Figure 3. Change in SpO₂ during rest, the parallel squat and recovery at 1800AL (○) and near SL (●). SpO₂=arterial oxygen saturation. SL=sea level. 1800AL=1800 m above sea level. Values are mean±SD. ***p < 0.001, Significant difference between 1800AL and near SL (two-way ANOVA for repeated measures, Tukey's post hoc test).

As one possible explanation for the dramatic decrease in muscle oxygenation in hypoxia, Costes et al. (1996) reported that the reduced arterial oxygen content plus metabolic demand decreases the overall muscle oxygen content during hypoxic exercise, whereas only venous blood is deoxygenated during normoxic exercise by metabolic demand. The muscular oxygen content depends on oxygen delivery, which is determined by the arterial oxygen content and blood flow. With ascent to altitude, arterial oxygen partial pressure falls, and the resulting decrease in arterial and venous oxygen content reduce the quantity of oxygen available for extraction. Additionally, the less oxygen extraction seems to induce marked decrease in muscular oxygen content both at rest and during exercise (Raynaud et al., 1986). In the present study, the dramatic decreases in muscle oxygenation

were accompanied by dramatic decreases in SpO₂ during the parallel squat at 1800AL (Figure 5). This result agrees with those reported in the previous study (Belardinelli et al., 1995; Costes et al., 1996), and suggests that the vastus lateralis muscle oxygenation during leg resistance exercise decreased dramatically because arterial oxygen content was reduced due to the limited O₂ supply at 1800AL.

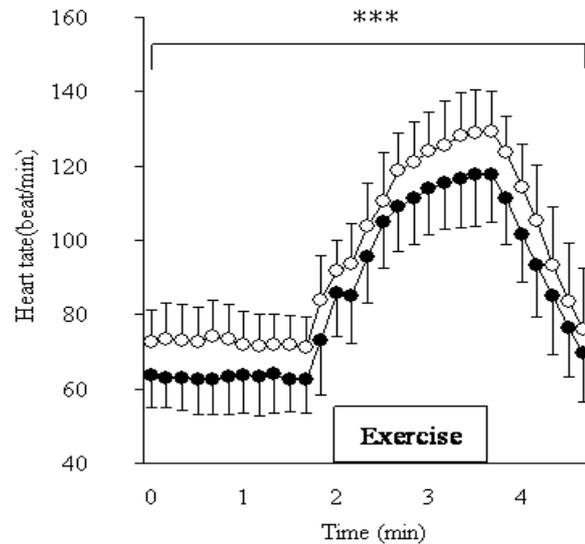


Figure 4. Change in HR during rest, the parallel squat and recovery at 1800AL (○) and near SL (●). HR=heart rate. SL=sea level. 1800AL=1800 m above sea level. Values are mean±SD. ***p < 0.001, Significant difference between 1800AL and near SL (two-way ANOVA for repeated measures, Tukey's post hoc test).

Cardiovascular adaptation thus elevated HR the may begin to compensate the reduction in arterial oxygen content immediately after arrival at altitude, but this adaptation may disappear with acclimatization occurring tens of hours later. Weston et al. (2001) reported that HR during exercise was higher at 6 h after arrival at moderate altitude (1700m) than sea level but had returned to sea level at 18 h and 47 h after arrival. In the present study, the exercise protocol was performed within 3 h after arrival at 1800AL, and such acute exposure to altitude might cause a more dramatic decrease in peripheral muscle oxygenation and SpO₂ and increase in HR during the exercise.

Jensen-Urstad et al. (1995) and MacDonald et al. (1999) also investigated the effects of hypoxia on peripheral muscle oxygenation during exercise using NIRCws. In their results, the recovery of muscle oxygen desaturation after or during arm exercise or leg kicking exercise was slower under hypoxia than under normoxia. Although there is little similarity between their results and ours because of the differences in the exercise types,

intensities, and experimental methods, in both cases, dramatical differences were observed in the kinetics of peripheral muscle oxygenation during exercise depending on whether the experiments were conducted under hypoxic or normoxic conditions. Thus, this noninvasive approach of monitoring muscle oxygenation by NIRcws may be able to elucidate exercising muscle metabolism not only at high altitude and under hypoxic conditions but also at moderate altitude, in which athletes actually train.

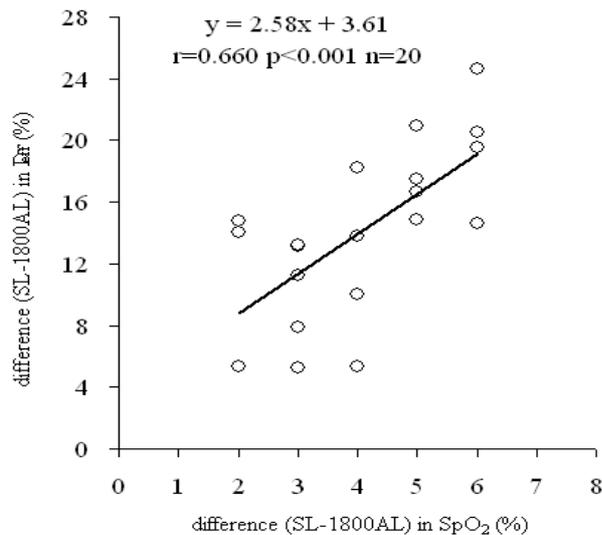


Figure 5. Pearson's correlation coefficients between the differences (SL-1800AL) in D_{eff} and those in SpO_2 during the parallel squat. SL=sea level. 1800AL=1800 m above sea level.

For our NIRS instrument, Kawaguchi et al. (2001) reported that there was a marked correlation between muscle oxygenation and systemic oxygen consumption during an incremental exercise test ($r = 0.726 - 0.978$). Additionally, the day-to-day reproducibility of D_{eff} during the parallel squat was examined and a high reproducibility ($r=0.974$) and small error between two sessions ($< 6.0\%$) were found in the present study. These data demonstrate that D_{eff} obtained by our NIRcws method is reproducible, and would hence be an appropriate variable for accurately characterizing the peripheral muscle oxidative metabolism (Ding et al., 2001).

Adipose thickness of the subjects is the main factor influencing the sensitivity and accuracy of NIRcws (Ding et al., 2001; Quaresima et al., 2003). Because all of the subjects to be evaluated were thin (fat $< 13.6\%$) and had a thin adipose (< 4.0 mm) on their vastus lateralis muscle (Table 1), for an appropriate source-detector distance (30 mm in this study), a higher sensitivity and lower error would be used (Ding et al., 2001; Wang et al., 2001).

CONCLUSIONS

In conclusion, the vastus lateralis muscle oxygenation during the parallel squat at 1800AL decreased more dramatically compared to SL. The results suggested that an acute exposure to moderate altitude causes a more dramatical decrease in peripheral muscle oxygenation during leg resistance exercise. This study demonstrated that peripheral muscle oxygenation status at moderate altitude could be evaluated easily and reliably using NIRcws and that moderate altitudes might be effectively used to apply hypoxic stress on peripheral muscles.

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**KEY POINTS**

- The change in muscle oxygenation during the parallel squat at 1800 altitude and near sea level was investigated using near infrared continuous wave spectroscopy (NIRcws)
- The muscle oxygenation during exercise at 1800 altitude decreased more dramatically compared to sea level.
- NIRcws could help to provide a better understanding of exercising muscle metabolism at moderate altitude.

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