exercise session, the angle of the device was recorded by an electrogoniometer (Goniometer System, Biometrics Ltd., Ladysmith, VA, USA) with sensors placed on the lateral aspect of the apparatus.

Heart rate: In order to confirm whether central circulation is enhanced by imposing the passive leg movement, heart rate was continuously measured by using an integrated telemetric monitor (Heart rate meter, Polar, Vantage, Finland) in two SCI patients and two normal subjects.

Data analysis

During the experiment, all data were continuously monitored by PowerLab software (Chart ver. 4, AD Instruments Inc., Milford, MA, USA) and were digitized at 1kHz for later analysis. For NIRS data, the average value in the last 30 sec at each stage and those at 1, 3, and 5 min post-exercise were evaluated for each parameter. The EMG signals were full-wave rectified after subtraction of the DC component. The magnitude of the EMG activity was quantified by the mean amplitude and integrated area of the EMG activity during the last 1 min of each stage.

Statistical analysis

Values are given as means ± SD. Statistical differences in the size of EMG value and each Hb value were tested by one-way analysis of variance (ANOVA) with repeated measures. Tukey's post hoc test was applied to identify differences between the conditions. The statistical software SPSS 11.0 was used to carry out all analyses. Significance was accepted at $p<0.05$.

RESULTS

Figure 2 shows a typical example of the EMG activity, NIRS values, and leg motion during an experiment in a SCI patient (A) and a normal subject (B). As clearly shown in this figure, there are remarkable differences in both EMG activity and NIRS parameters between the two groups. It was confirmed that the leg motion was maintained within similar range throughout the exercise in both SCI and normal subjects.

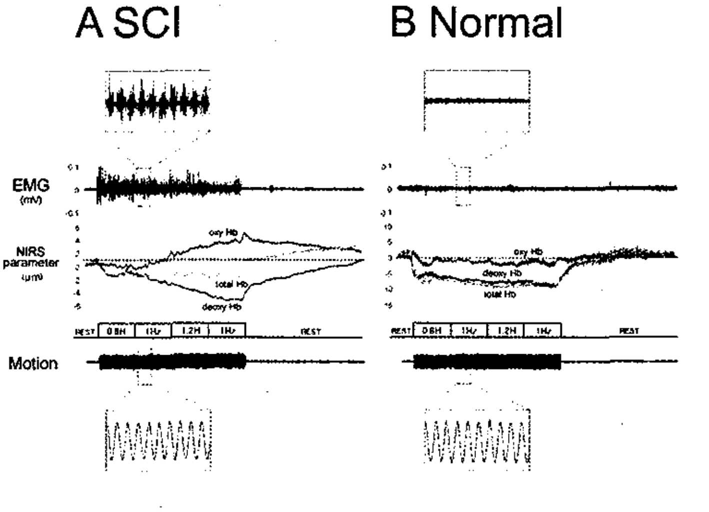

Figure 2 Typical example of the EMG activity and concentration changes in each hemoglobin parameter in a patient with motor-complete SCI (A) and a neurologically normal subject (B). Note that there are remarkable differences in both EMG activity and NIRS parameters between the two subjects. The motion of the apparatus was maintained within a similar range throughout the exercise for both subject groups.

EMG activity

During passive movement, all SCI patients showed EMG activity in the gastrocnemius muscle. The active phase of the EMG activity corresponded to the backward phase of leg movement. Despite the fact that the total (integrated) response area increased with the frequency of the movement, there was no remarkable change in amplitude (Fig. 3). Although the movement frequency in both the 2nd and 4th stages was set at 1Hz, the EMG amplitude in the 4th stage was significantly lower than that in the 2nd stage (2nd vs. 4th: 42.08 ± 3.73 vs. 29.66 ± 6.19 μV, p<0.05). In contrast to the SCI patients, normal subjects showed no visible EMG activity in the gastrocnemius muscle at any time during the exercise.

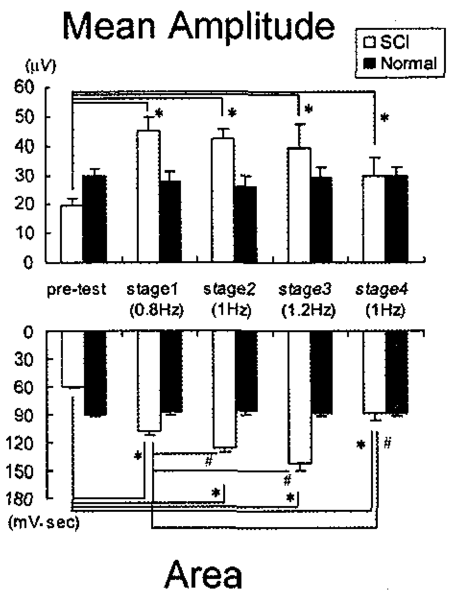

Figure 3 Mean amplitude and area of muscle EMG activity in the gastrocnemius muscle. The error bars indicate the standard error of the mean value. *: Significant difference (p<0.05) compared to the resting value. #: Significant difference to the 1[st] set value.

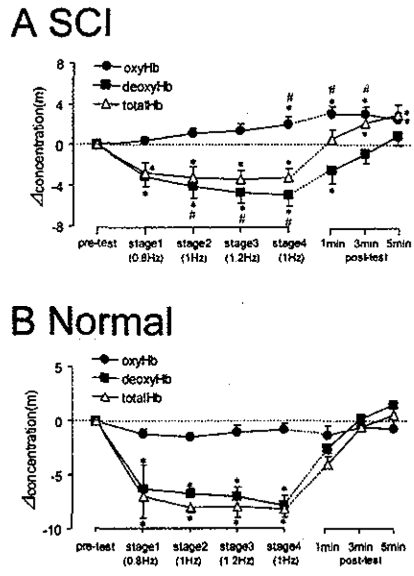

Figure 4 Concentration changes in total, oxygenated (oxy) and deoxygenated (deoxy) hemoglobin (Hb) throughout the experiment for SCI patients (A) and normal subjects (B). The error bars indicate the standard error of the mean value. *: Significant difference (p<0.05) compared to the resting value. #: Significant difference to the 1[st] set value.

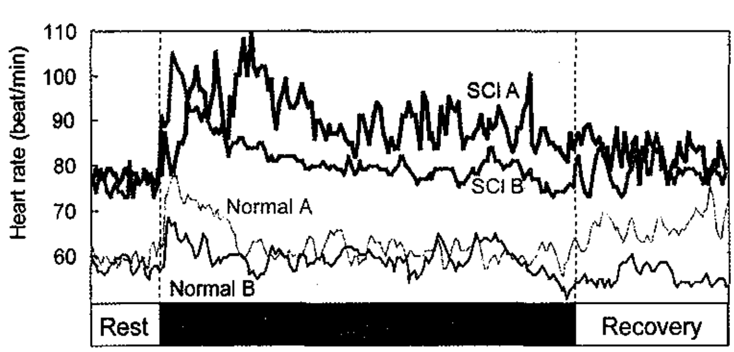

Figure 5Heart rate changes at rest, during passive leg movement, and in recovery period obtained by two SCI patients and two normal subjects.

NIRS parameters

In both the SCI and Normal groups, the concentrations of total Hb and deoxy-Hb showed rapid decrements following the onset of the exercise and remained at lower levels compared to the resting value while the legs were passively moved. The degree of the decrease of total Hb in the 1st stage was much smaller in the SCI group than in the Normal group (SCI vs. Normal: 2.79 ± 0.99 vs. 7.04 ± 2.18 μm). During the exercise period, an increase in oxy-Hb and a decrease in deoxy-Hb, which were independent of the changes in total Hb, were observed in the SCI group but not in the Normal group (Figs. 2 and 4). In the recovery stage, the total Hb level exceeded the resting value in the SCI group, while it merely recovered to the pre-test level in the Normal group.

Heart rate

Figure 5 shows the change of the heart rate at rest and during passive leg movement, and the recovery period obtained from two SCI patients and two normal subjects. As shown in this figure, heart rate increased just after the onset of passive leg movement in all subjects.

DISCUSSION

The present study was designed to examine whether or not the oxygenation level of the paralyzed muscle is altered with the EMG activity induced by imposed passive leg movement. Our primary observations are the following: (i) during passive movement, all SCI patients showed EMG activity in the gastrocnemius muscle, while none of the normal subjects showed such activity; (ii) during the exercise period, an increase in oxy-Hb and a decrease in deoxy-Hb, both of which were independent of changes in total Hb, were observed in the SCI group; (iii) in the recovery stage, total Hb exceeded the pre-exercise value in the SCI group. A possible mechanism for these changes in oxygenation level in the SCI patients and its implications for rehabilitation will be discussed below.

Muscle activity during passive movement

Despite the motor paralyses in their lower legs, all six SCI subjects showed EMG activity in the paralyzed gastrocnemius muscle during passive movement. On the other hand, no normal subjects showed any EMG activity in the gastrocnemius muscle, even though the applied leg movements were identical to those applied to the SCI patients. In our previous data, it was found that the passive leg movement can also induce EMG activity in other lower leg muscles; for instance, the soleus and biceps femoris muscles (Kawashima et al. 2005). It is possible that the observed muscle activity consisted of complex spinal reflexes rather than simple stretch reflex responses induced by rhythmical stretching of the muscle-tendon (Harkema 2001, Kawashima et al. 2005), and that the lack of EMG activity in normal subjects can be partly explained by the inhibitory neural input from a higher center to the spinal motoneurons (Dietz et al. 1995). We do not discuss any further details of the neural mechanism of this EMG activity here because this paper is concerned primarily with the relationship between the magnitude of muscular activity and the degree of the Hb value. The neural mechanism underlying this EMG activity has been described in detail in the previous research (for a review see Harkema 2001).

The degree of changes in the NIRS signals should strongly depend on the muscle contraction level. It is therefore important to know how much the muscle activity occurs during passive leg movement. However, it is difficult to evaluate the muscle contraction level using the percentage of the maximal voluntary contraction (%MVC), which is commonly used to normalize and evaluate the muscle contraction level, because the SCI patients cannot accomplish voluntary contraction. When the EMG activity of the paralyzed muscle is expressed with size relative to the MVC obtained by normal subjects (average: 417.7 ± 43.24 μV), it corresponds to approximately 10% MVC. Given the muscle atrophy of the paralyzed muscle (Lotta et al. 1991, Castro et al. 1999), it can be assumed that the SCI patients have an MVC lower than that in the normal subjects. Therefore, we estimate the contraction level observed in the SCI patients as no less than 10% MVC.

Changes in hemoglobin concentration during exercise

In the present study, both the SCI and Normal groups showed a rapid decrease in the total Hb concentration following the onset of exercise, and maintained the lower value while the legs were passively moved. We considered venous blood in the calf to be complete, that is, to have reached plateau level, at the beginning of the passive leg movement, since subjects were kept in standing posture until the total Hb value stabilized. By imposing passive leg movement, the pooling venous blood might be expelled from the calf because intramusclar pressure is increased due to the imposed length changes in the muscle (Quaresima et al. 2001, Sako et al. 2001) irrespective of the appearance of EMG activity. Therefore, it seems reasonable to assume that the decreased total Hb observed in this study during movement is explained by this expulsion of the pooling venous blood in the calf.

In addition, the degree of the concentration changes in the total Hb was much larger in the Normal group than in the SCI group. This result is consistent with the report of Van Beekvelt et al. (2000) that muscle pump activity induced by imposing electrical stimulation is reduced in SCI subjects compared to healthy subjects. It has been suggested that this reduced muscle pump activity might be explained by the muscle atrophy and low venous capacity found in SCI subjects (Hopman et al. 1994). It is possible explanation for this result that SCI patients have more fat because the larger fat contains result in lower NIRS signals (Van Beekvelt et al. 2001). However, as described later, other our results which the oxy- and deoxy-Hb showed in the opposite concentration changes would not be expected from fat for the same reason.

In the present study, a gradual increase in oxy-Hb and decrease in deoxy-Hb that were independent of changes in total Hb were observed in the SCI subjects during the exercise period. On the contrary, there is no obvious concentration change of the oxy-Hb in the normal subjects who showed no EMG activity during passive leg movement. If no muscle oxygen consumption and/or supply was induced by the imposed movement, both the oxy-Hb and the deoxy-Hb should vary in a manner related to the concentration changes in the total Hb. Taken together with the occurrence of EMG activity in the SCI group, this change in the muscle oxygenation level can be attributed to the muscle activity produced by imposing passive leg movements. These results are in good agreement with a recent report by Bhambhani et al. (2000), who suggest that changes in the oxygenation level in the paralyzed rectus femoris muscle during cycling movement are generated by functional electrical stimulation.

With respect to muscle oxygenation during exercise, previous studies have reported that continuous muscle contraction at moderate intensity follows the increments of deoxy-Hb because of the oxygen consumption in the acting muscle (Boushel et al.

2001, Takaishi et al. 2002). Although we estimated muscle was "active" during passive leg movement, the present results did not show increments of the deoxy-Hb. According to the general principle, concentration changes in oxy- and deoxy-Hb are dependent on the dynamics of the equilibrium between tissue oxygen demand and supply (Belardinelli et al. 1995, Homma et al. 1996). Therefore, a possible reason for our result is that oxygen delivery far exceeds the oxygen extraction in the acting muscle. The enhancement of heart rate during passive leg motion (Fig. 5) is one of supporting evidence for this notion.

Changes in hemoglobin concentration after exercise

After the cessation of the passive leg movement, the concentration of total Hb in the SCI group exceeded the pre-exercise level, while that in the Normal group simply recovered to the pre-exercise level. Since the total Hb reflects the degree of muscle blood flow (Boushel 2001), these changes may suggest that enhancement of the muscle blood flow occurred in the SCI group, possibly resulting from the muscle contraction and oxygenation during the exercise period. It is likely that the excess total Hb following exercise resulted from the pooling of blood in the calf. Nevertheless, in this study, the subjects were kept in a standing posture on the apparatus prior to the initiation of the exercise period until the total Hb value reached a constant level; therefore, the above total Hb changes during the recovery stage cannot be explained solely by blood pooling in the calf. These total Hb changes may be due to post-exercise hyperemia (Taylor et al. 1988).

Heart rate changes by imposing passive leg movement

As shown in the Figure 5, HR shows an increase after the onset of the passive leg movement in both SCI and normal subjects. These results provide an evidence for the enhancement of central circulation by imposing passive leg motion even in the SCI patients. Most simple explanation is that increments of the venous return due to the muscle pump activity result in the central circulation (Rowland 2001). However, taken together with the results of differences in the EMG activity, there would be different mechanisms underlying the enhanced heart rate between two groups. In the case of the normal subjects, it is plausible that the enhancement of the HR is induced by the neuronal factor, which is an afferent neural signal from the mechanoreceptor by inducing muscle stretching (Gladwell and Coote 2002). On the other hand, this neuronal factor is not suitable for the explanation of SCI results because of their sensory paralysis. Rather, our results, those are an appearance of muscular activity and an alteration of the NIRS signals, imply that a metabolic changes accompany with the muscle contraction seem to play a primary role in the enhancement of the central circulation. Since we don't still have any direct evidences, further investigations are needed to clarify this point.

Implications for rehabilitation

As mentioned in the *Introduction*, chronic inactivity and hypocirculation of the paralyzed area are crucial factors in secondary impairment in SCI subjects (Noreau et al. 2000). The present results provide an indirect evidence that passive leg movement performed in a standing posture could alter the oxygenation level of the paralyzed muscle, and has the potential to facilitate circulation of the paralyzed area. Given the fact that the muscle contraction level during normal walking is about 15% MVC (Masumoto et al. 2004), it is considered that the muscle contraction level observed in this study is adequate to facilitate neural activity and circulation of the paralyzed area.

On a practical level, the subjects in the present study did not move their upper limbs and trunk voluntarily because our aim was to examine whether or not the oxygenation level of the paralyzed muscle was altered by imposing passive leg movement. In a non-experimental situation, however, patients would commonly operate the device themselves by manipulating the lever with their upper limbs. It is possible that the additional voluntary upper limb movement could enhance circulation not only in the voluntarily acting area but also in the paralyzed area.

Although muscular activity in the paralyzed area can also be induced by applying electrical stimulation, as is the case in functional electrical stimulation (FES) (Mutton et al. 1997, Sampson et al. 2000, Bélanger et al. 2000), there are essential differences between our method and the FES technique. Previous investigations pointed out as one of major disadvantages of the FES technique that it is difficult to generate FES-induced continuous muscle contractions without fatigue (for review see Stein et al. 2002). The muscle fatigue can be attributed the fact that fatigable motor unit preferentially recruitment by imposing electrical stimulation, in that, large motor nerves are more easily activated than smaller ones. In contrast, in the case of the passive leg movement produced in the present study, the motor units are presumably recruited according to the size principle (Henneman and Mendell 1981) since the afferent inputs were offered from proprioceptors by imposing muscle stretch and body load. Furthermore, passive leg movement is simpler and more practical than FES, and has a lower risk of misuse. Therefore, this type of passive leg movement might be a useful and efficient method for rehabilitation following SCI.

Conclusion

The present results demonstrate that passive leg movement can induce not only muscular activity but also alteration of the muscle oxygenation level in the paralyzed lower limb. There may be increased oxygen consumption, but this could not be ascertained from the measurements in this paper. Further study will be needed to clarify this issue.

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