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Neuromuscular adaptation after repeated exposure to whole-body cryotherapy (−110 °C)

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ABSTRACT

The aim of the study was to evaluate the effects of single and repeated whole-body cryotherapy (WBC, air −110 °C) on the neuromuscular performance in healthy subjects ($n = 14$). The flight times in a drop-jump exercise decreased after a single WBC exposure, but these changes almost vanished after repeated WBC for 3 months. This adaptation was accompanied by a decreased co-contraction of lower leg muscles during the drop-jump. In conclusion, in dynamic exercise, neuromuscular functioning may be able to adapt to repeated WBC, which might enhance the effects of therapeutic exercises in patients after the WBC.

1. Introduction

It has been shown that body cooling decreases the performance of muscles, particularly during dynamic exercise (Rintamäki et al., 1991; Ferretti, 1992; Oksa et al., 1995). Even a slightly lowered muscle temperature is able to deteriorate performance (Oksa et al., 1997). Some studies (Bawa et al., 1987; Oksa et al., 1995, 1997) have found that after cooling, the co-ordination of muscle contractions changes. Bawa et al. (1987) found that, in subjects who exercised and shivered at the same time, the antagonist muscle co-contracted together with the agonist muscle. Thus, these antagonistic muscles were "fighting" against each other. This only occurred when the subjects were shivering, and not when the subjects were in a thermoneutral state. Oksa et al. (1995, 1997) have reported that during a concentric muscle contraction in a drop-jump (shortening phase), the activity of the agonist muscle decreases and the activity of the antagonist muscle increases at the same time. This phenomenon, called the 'braking effect of the muscles, due to cooling', leads to a decrease in muscular performance. Static exercise, however, is less sensitive to cooling. It seems that muscle temperatures below 27 °C are required before significant changes may be observed (Barnes, 1983; Davies et al., 1982; Oliver et al., 1979).

Whole-body cryotherapy (WBC) is one mode of cold therapy, during which patients are repeatedly exposed to very cold air (−110 °C) while dressed in minimal clothing. The duration of WBC usually varies from 1 to 3 min and it is predominantly used to treat the pain and inflammation of different rheumatic diseases, so that the patients can do therapeutic exercises after WBC (Wichmann and Fricke, 1997; Samborski et al., 1992). In clinics, WBC has been used to reduce spasticity in some neurological diseases. WBC has been used in sports medicine to treat injuries and syndromes of overuse (Savalli et al., 2005), and to enhance recovery in athletes during heavy periods of training (Papenfuß, 2006). No information is available on the effects of long-term WBC treatment on neuromuscular performance and function, which might be important from the therapeutic point of view. In contrast to previous cold stress studies, Fricke et al. (1999) found that the isokinetic torque of the knee flexors and extensors improved after a single WBC (2 min) in untrained healthy subjects.

We know that body cooling usually decreases muscular performance (Rintamäki et al., 1991; Ferretti, 1992; Oksa et al., 1995), but very limited data exists on the adaptation of neuromuscular performance to the cold. Geurts et al. (2005, 2006) repeatedly exposed the hands of subjects to cold water for several weeks, but did not observe any signs of neuromuscular adaptation. Knowledge on the acute and long-term effects of the WBC treatment on neuromuscular function and performance is practically nonexistent. Therefore, the aims of the present study were to find out (1) what the effects of a single exposure to WBC are on neuromuscular performance,

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and (2) is repeated exposure to WBC able to induce neuromuscular adaptation.

2. Methods

2.1. Subjects

After the approval of the Ethical Committee of the Hospital District, 10 males and four females, signed informed consents and volunteered to participate in this study. Their mean age (mean ± SD) was 33 ± 9 years, height was 174 ± 9 cm, weight was 73 ± 12 kg and BMI was 24 ± 2. All subjects were sedentary and healthy, whilst none of the participants were under medication.

2.2. Procedures

WBC exposures were administered in a specially built, temperature-controlled unit (Zimmer Elektromedizin, Germany), which consists of three rooms (−10, −60 and −110 °C). The temperature of the therapy-room remained at a constant level (ca. −110 °C), and the air in the room was dry and clear. During the first visit, the subjects were exposed to a temperature of −10 °C, and during the second visit to −60 °C, and during the third visit to −110 °C. After this, the subjects only visited the therapy-room (−110 °C), three times a week for 3 months. Each period of exposure was 2 min. During the exposures, the subjects wore a bathing suit, surgical mask, cap, gloves, socks and shoes. Whilst in the therapy-room, the subjects were instructed to slightly move their fingers and legs to avoid tension. The measurements were performed before and after WBC at the beginning (a single WBC) and at the end of the 3-month study period (repeated WBC). The subjects were requested not to practice any other forms of extreme cold exposure (e.g. winter swimming) for 3 months before and during the study period. They were also advised to maintain the same physical activity level during the study as before. One male (did not want to continue) and one female (respiratory tract infection) discontinued the study after 1 month.

2.3. The measurement of neuromuscular performance and functioning

The measurements of neuromuscular performance included a drop-jump exercise and the maximal voluntary contraction (MVC) force of the wrist flexors, accompanied with electromyography (EMG) recordings in both. The measurements before and after the single WBC were carried out at the fourth exposure (at the second exposure to −110 °C), and the long-term measurements (repeated WBC) were carried out before and after the last exposure after 3 months.

2.3.1. Drop-jump exercise (stretch-shortening cycle)

The drop-jump exercise was performed from a 0.4 m bench. The subjects dropped from the bench two times onto a contact mat (Powertimer, Newtest Inc, Oulu, Finland) and performed an instantaneous maximal rebound jump whilst keeping the hands on their hips. During contact with the ground, this stretch-shortening cycle performance consists of a stretching phase (downward movement), when the m. gastrocnemius medialis is stretched and the m. tibialis anterior is shortened and a shortening phase (upward movement), when the m. gastrocnemius medialis is shortened and the m. tibialis anterior is stretched. The physically active subjects were instructed to perform the jump whilst keeping their legs as straight as possible. Beforehand, the subjects thoroughly practiced the drop-jumps with an instructor in order to avoid a learning effect during the measurements. This mode of exercise was chosen, since it has been found to be very sensitive to body cooling (Oksa et al., 1997).

2.3.2. Maximal voluntary contraction (MVC)

The wrist flexion MVC of the right arm of the subjects was measured in an upright seated position with their hips and elbow angles adjusted to 90°. The armrest of the seat supported their forearm. The subject held a static handle so that the palm of the hand was in a vertical position. The subjects were requested to try to flex (isometric) their wrist maximally, and as fast as they could, to hold the force for about 2 s and then to relax their lower arm muscles as rapidly as possible. The subjects performed two trials and the highest MVC value was used in the analysis. The maximal force level, TPT (the rise in force from the resting level to 80% MVC), and RT (restoring the force from 80% MVC to the resting level) were analyzed from the MVC data.

2.3.3. Stretch reflex

To evaluate whether the possible changes in muscle force were peripherally (e.g. changes in muscle spindle response) or centrally (changes in the motor input from central nervous system) mediated, stretch reflex responses from the forearm flexors were measured before and after the last exposure. For technical reasons, the stretch reflex responses were only measured before and after the last exposure.

The subjects were seated with their hip and elbow angles adjusted to 90° at a normal living room temperature (19–21 °C). The armrest of the seat supported the right forearm similarly as in the MVC test and the subject held the handle. In a similar position as the right hand, the left hand (palm upwards) supported a 2 kg weight. A stretch reflex was evoked by inducing a sudden movement of the right palm (a pull on the handle that the subject was holding, which induced a changing angle of the wrist joint) by a linear motor (LINMOT E1000, Sulzer Electronics AG, Switzerland). During the test, the eyes were closed and hearing protectors were worn in order to avoid excess sensory information. The reflex was elicited 12 times during both measurements. Every time the linear motor began the displacement, a signal was simultaneously sent to the EMG device to mark the starting point of the pull for the determination of the short-latency (SL), medium-latency (ML) and long-latency (LL) components of the reflex response. In addition to reflex latencies, the maximum amplitudes of the SL, ML and LL responses were analyzed. The parameter used was the ratio (r) of after WBC to before WBC.

2.3.4. EMG measurements

To evaluate the level of muscle activity during the drop-jump exercise, the MVC and the stretch reflex surface EMG activity (ME3000P, Mega Electronics, Kuopio, Finland) was measured. EMG signals from the skin above the working muscles were acquired with a sample rate of 1000 Hz, with the use of pregelled bipolar surface electrodes (M-OO-S, Medicotest, Denmark). The upper filter frequency was set to 500 Hz and the lower to 20 Hz. The measured signal was full wave rectified and averaged (aEMG) with a 10 ms time constant. The electrodes were placed over the belly of the muscle, and the distance between the recording contacts was 2 cm. Ground electrodes were attached above an inactive muscle. During the drop-jump, the activity of the m. tibialis anterior and the m. gastrocnemius medialis was measured. The EMG measurement began with a signal that was given for the subjects to perform the drop-jump. An on-off connector was attached to the sole of the shoe, which gave a signal of the contact phase to the EMG measurement device. The averaged EMG level was analyzed during the pre-activity phase (100 ms before the beginning of stretch phase), and during the eccentric and concentric phase of the stretch-shortening cycle. The stretch-shortening cycle began with a signal of the contact phase and ended at the point where the activity of the m. tibialis anterior distinctly increased and the m. gastrocnemius decreased. The total contact time was halved for the eccentric (stretch) and concentric (shortening) phases. In the MVC test and in the stretch reflex measurements, the EMG activity was measured from the wrist flexor (m. flexor carpi radialis) and the wrist extensor (m. brachioradialis). The averaged EMG was calculated during the total time of the MVC (ca. 2 s). To assess the frequency component of the EMG, the power spectrum was estimated by a moving fast Fourier transform (FFT window, 512 points). From the power spectra, the mean power frequency (MPF), median frequency (MF) and zero crossing rate (ZCR) were calculated to describe changes in the frequency component. MPF was chosen to represent the frequency component of the EMG results. To ensure the accuracy of the relocation of the electrodes on the skin after 3 months, their locations for each subject were carefully drawn on plastic films with the aid of anatomic marks (e.g. moles, blood vessels) and their distances from the nearest joints were carefully measured with a tape-measure.

2.4. Statistics

To compare the differences between the measured values before and after the WBC, separately for a time (0 or 3 months), analysis of variance (ANOVA) with repeated measures was used. Statistical analyses were performed using SPSS 15.0 for Windows. In addition to this, the Student's paired *t*-test was used to test the differences between the after single and after repeated WBC. The statistical significance was accepted at the 0.05 level.

3. Results

3.1. Drop-jump exercise

After a single WBC, the flight time decreased significantly ($p<0.05$), but after repeated WBC, only a similar tendency was found (Fig. 1). However, the changes did not differ significantly from each other. During the shortening phase, the averaged EMG activity of the agonist muscle (m. gastrocnemius medialis) increased by 95±108 μV after a single WBC and the increase was 259±102 μV ($p<0.05$) after repeated WBC (Table 1). These changes differed significantly ($p<0.05$) from each other. The

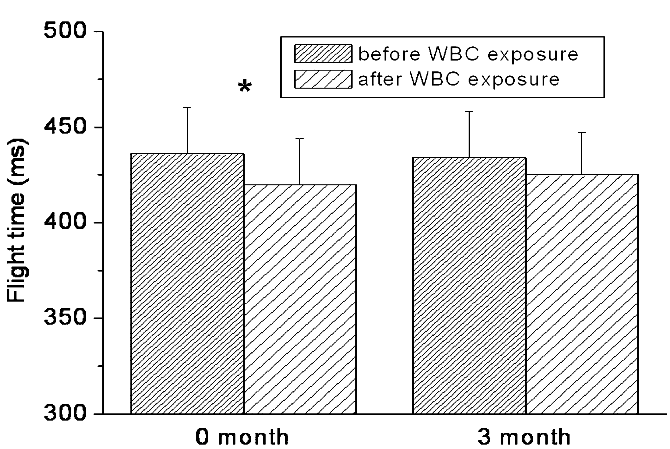

Fig. 1. The flight times of the drop-jump before and after WBC at the beginning (0 month) and at the end (3 months) of the 3-month study period. The values are means (SE) and *denotes $p<0.05$.

Table 1
The EMG activities (μv) of the agonist muscle, before and after WBC, during the preactivity (m. gastrocnemius medialis), stretch (m. tibialis anterior) and shortening (m. gastrocnemius medialis) phases of the drop-jump exercise at the first exposure and at 3 months.

	First time	3 months
Preactivity		
Before WBC	451 (231)	458 (200)
After WBC	517 (195)	557 (180)*
Stretch		
Before WBC	605 (209)	481 (194)
After WBC	715 (273)	526 (348)
Shortening		
Before WBC	446 (126)	580 (196)
After WBC	541 (324)	839 (411)*

The values are means (SD). The significance in relation to before the WBC is denoted by *$p<0.05$

Table 2
The EMG activities (μv) of the antagonist muscle before and after WBC during the preactivity (m. tibialis anterior), stretch (m. gastrocnemius medialis) and shortening (m. tibialis anterior) phases of the drop-jump exercise at the first exposure and at 3 months.

	First time	3 months
Preactivity		
Before WBC	165 (77)	199 (91)
After WBC	177 (84)	207 (100)
Stretch		
Before WBC	165 (53)	180 (39)
After WBC	167 (59)	184 (65)
Shortening		
Before WBC	95 (47)	171 (91)
After WBC	121 (69)	169 (81)

The values are means (SD).

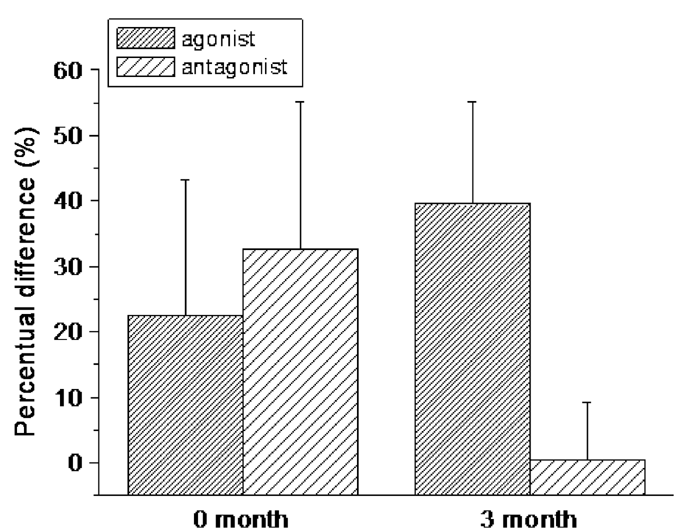

Fig. 2. The percentual difference of the averaged EMG activity of the agonist (m. gastrocnemius medialis) and the antagonist (m. tibialis anterior) muscle before and after WBC, during the shortening phase of the drop-jump in the beginning (0 month) and at the end (3 months) of the 3-month study period. The values are means (SE).

averaged EMG activity of the antagonist muscle (m. tibialis anterior) after a single WBC increased by 26±25 μV, but after repeated WBC, it decreased by 2±14 μV (Table 2), however, these changes did not differ significantly from each other. Fig. 2 shows the percentile difference of these activities after single and repeated WBC. During the stretch phase, no significant changes were found in the averaged EMG of the agonist (m. tibialis anterior) (Table 1) and antagonist (m. gastrocnemius medialis) (Table 2) muscles, neither after a single or repeated WBC. During the pre-activity phase after a single WBC, the averaged EMG activity of the agonist muscle increased by $66 \pm 57\,\mu V$ and by $99 \pm 27\,\mu V$ ($p < 0.05$) after repeated WBC (Table 1), but these changes did not differ significantly from each other. During the different phases of the drop-jump, no significant changes were found in the MPF of the agonist and antagonist muscles, neither after a single nor repeated WBC.

3.2. MVC

The maximal force level, TPT and RT did not change significantly, either after a single or after repeated WBC. The averaged EMG activity of the agonist after a single WBC, however, only increased by $84 \pm 37\,\mu V$, but after repeated WBC, it increased by $188 \pm 60\,\mu V$ ($p < 0.05$). The averaged EMG activity of the antagonist increased by $50 \pm 24\,\mu V$ and $41 \pm 17\,\mu V$ ($p < 0.05$) (Table 3), respectively. These changes, however, did not differ significantly from each other. The percentual differences of these activities after single and repeated WBC are shown in Fig. 3. After a single WBC, the MPF of the agonist decreased by 18 ± 3 Hz ($p < 0.05$) and after repeated WBC, it decreased by 14 ± 4 Hz ($p < 0.05$). There were no significant differences between the changes of MPF at the beginning and at 3 months. The MPF of the antagonist showed no significant change.

Table 3
The EMG activities (μv) of the agonist (m. flexor carpi radialis) and antagonist (m. brachioradialis) in MVC at the first exposure and at 3 months.

	First time	3 months
The agonist		
Before WBC	646 (207)	593 (207)
After WBC	730 (233)	781 (216)*
The antagonist		
Before WBC	111 (54)	146 (133)
After WBC	160 (122)	187 (167)*

The values are means (SD). The significance in relation to before the WBC is denoted by $^*p < 0.05$.

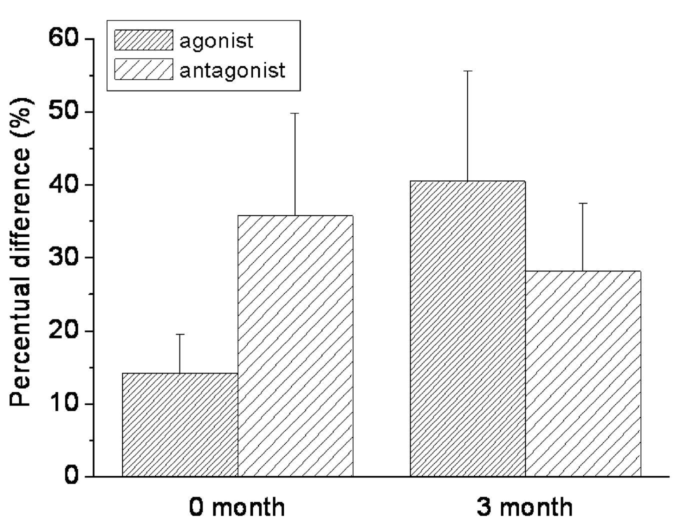

Fig. 3. The percentual difference of the averaged EMG activity of the agonist (wrist flexor) and the antagonist (wrist extensor) muscle, before and after WBC in MVC. The values are means (SE).

3.3. Stretch reflex

After a single WBC, the amplitude of the SL response ($r = 1.20$) was higher ($p < 0.05$), but the amplitude of the ML (1.09) and LL responses (0.86) were not significantly different. The latencies of the SL, ML and LL responses did not change

4. Discussion

The results of the present study showed that the single WBC decreased flight time in the drop-jump exercise, but after 3 months of repeated WBC, this change almost vanished. After repeated WBC, the activity of the agonist muscle increased more and the activity of the antagonist increased less, indicating a reduced co-contraction (Bawa et al., 1987). These results allowed us to argue that neuromuscular adaptation had taken place due to the repeated WBC, since the subjects did not practice the drop-jump exercise during the 3 months' period.

To our knowledge, the adaptation of neuromuscular performance has not been mentioned in literature, although humans can adapt physiologically to the cold. In the present study, after repeated WBC, the averaged EMG activity of the agonist muscle increased more and the EMG activity of the antagonist muscle was less during the shortening phase, indicating a diminished co-contraction. These changes in muscle activity may explain, at least partially, the smaller reduction in flight time observed in the drop-jump test after repeated WBC. The number of mechanisms inducing the observed changes in the drop-jump may be vast. One potential candidate could be the adaptation of the muscle spindle. Oksa et al. (2000) have reported that after acute cooling, decreased spindle activity was seen as decreased aEMG activity of the agonist muscle and increased aEMG activity of the antagonist muscle, during the shortening phase of the stretch-shortening cycle. If the muscle spindle is able to adapt to the cold, it should be seen as a reduced co-contraction (Bawa et al., 1987) or braking effect (Oksa et al., 1995, 1997).

It is known that temperature is an important modulator of the neuromuscular function (Davies and Young, 1983; De Ruiter et al., 1999). However, to our knowledge, no research has been conducted regarding the effect of repetitive WBC on neuromuscular function, however, there are studies using milder and different types of exposures. Geurts et al. (2005) investigated the effects of cold acclimation on the neuromuscular function of the hand (the first dorsal interosseus muscle). In their study, the subjects immersed one hand in 8 °C water for 30 min, 5 days a week for 3 weeks, which means 15 immersions. On the first and the last day, the evoked and voluntary force measurements of the FDI were performed before and after immersion. Likewise, Geurts et al. (2006) examined the effects of repeated cold exposure (2 weeks, daily) with an elevated core temperature from exercise (cycling), on the neuromuscular function of the hand. In their local cold exposure studies (Geurts et al., 2005, 2006), no adaptation in neuromuscular function was found. Geurts et al. (2005) speculated that a larger change in the temperature of the hand may be required to observe significant changes in the neuromuscular function after repeated cold exposures. It should be noted that the subjects in our study had 36 exposures, therefore, we may assume that considerably more than 15, or different types of, exposures are needed so that changes/adaptation in the neuromuscular function may be observed.

Dietz et al. (1981) and Komi (1983) have reported that during the pre-activity phase, an anticipatory effect (increased EMG activity) occurs before the stretch phase of the stretch-shortening cycle. It has been reported that after cooling, increased EMG activity enhances the utilisation of the elastic components of the working muscles (Asmussen et al., 1976). In the present study, during the pre-activity phase after repeated WBC, the averaged EMG activity of the agonist muscle increased more than after a single WBC, thus suggesting that the utilisation of the elastic components could be enhanced after repeated WBC. If so, this increase in muscle activity during the pre-activity phase may also explain the smaller reduction in flight time after repeated WBC and also support the notion of an adaptation in neuromuscular performance.

In the MVC test (isometric) of the right arm after repeated WBC, the averaged EMG activity of the agonist muscle tended to increase more (ns) than after a single WBC, whereas the EMG activity of the antagonist tended to increase less (ns) than after a single WBC, indicating a reduced level of co-contraction after repeated WBC. Although there was no statistical significance in the results, there may be a physiological effect on performance. Furthermore, although there were changes in EMG activity that might be able to reduce performance, the level of the maximal force remained unaffected. This may be due to a different type (isometric) of exercise. In human studies, the maximal isometric force level has been found to be relatively stable within the muscle temperature range of 27–40 °C (Clarke and Royce, 1962). We do not know the muscle temperature after WBC, but in our earlier study (Westerlund et al., 2003), we showed that the skin temperature of the forearm decreased rapidly to 5.2 °C, but also experienced a rapid recovery after exiting the therapy-room (−110 °C). Therefore, we may assume that only superficial parts of the muscles are cooled and due to a strong vasoconstriction and short period of exposure, the 'critical' muscle temperature of 27 °C for the reduction of the maximal isometric force was not reached.

Cooling of the working muscles has been related to the increased TPT (Ranatunga et al., 1987) and RT (Wiles and Edwards, 1982) too. The reasons for these changes have been explained by a slowed ATP hydrolysis (Ferretti, 1992), slowed Ca^{2+} release and uptake from the sarcoplasmic reticulum (Kössler et al., 1987), and a decreased Ca^{2+} sensitivity of the actomyosin (Sweitzer and Moss, 1990). In parallel with the unchanged maximal force level, the TPT and RT also did not change, which may refer to the fact that the change in muscle temperature was not sufficient to induce changes in these parameters either.

Many studies have shown that cooling suppresses the stretch reflex amplitude, due to a decreased activity of the muscle spindles, which may lead to a decreased force production of a muscle (Knutsson and Mattsson, 1969; Oksa et al., 2000). Decreased muscular performance after cooling is related to the co-ordination of the muscle contraction (Oksa et al., 1995, 1997; Bawa et al., 1987). In the present study, the increase in co-contraction was observed after a single WBC, but after a repeated WBC the co-contraction was reduced. Furthermore, the amplitude of the SL component increased after a single WBC (a successful SL measurement only at 3 months) therefore, it may be considered that WBC has increased alpha-motoneuron excitability and gamma-motoneuron (muscle spindle) sensitivity (Bell and Lehmann 1987). On the other hand, there are studies, which show that working in cold conditions with thermoneutral muscle temperature is able to induce enhanced stretch reflex response. Due to technical problems therefore, concise conclusions regarding the role of the stretch reflex are difficult to make.

In conclusion, the finding of the present study was that neuromuscular adaptation may take place, especially in dynamic performance, after 3 months of repeated exposure (3 times/week) to WBC. A single WBC decreased flight times in a drop-jump exercise, but after repeated WBC, these changes almost vanished. This adaptation was confirmed by the change of the activity of the agonist muscle, which increased more and the change of the activity of the antagonist muscle, which increased less/ did not change after repeated WBC, indicating a reduced co-contraction. After a single WBC, the results of the present study were similar as in earlier cold exposure studies. If the same type of adaptation of the neuromuscular function also occurs in patients, it might then allow the patients (besides reduced pain and stiffness) to perform the therapeutic exercises more effectively after being repeatedly exposed to WBC. For this, a further study is necessary.

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