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Research article

Alteration of muscle function after electrical stimulation bout of knee extensors and flexors

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Abstract
The purpose was to study the effects on muscle function of an electrical stimulation bout applied unilaterally on thigh muscles in healthy male volunteers. One group (ES group, n = 10) received consecutively 100 isometric contractions of quadriceps and 100 isometric contractions of hamstrings (on-off ratio 6-6 s) induced by neuromuscular electrical stimulations (NMES). Changes in muscle torque, muscle soreness (0-10 VAS), muscle stiffness and serum creatine kinase (CK) activity were assessed before the NMES exercise (pre-ex) as well as 24h (d+1), 48h (d+2) and 120h (d+5) after the bout. A second group (control group, n = 10) were submitted to the same test battery than the ES group and with the same time-frame. The between-group comparison indicated a significant increase in VAS scores and in serum levels of CK only in the ES group. In the ES group, changes were more pronounced in hamstrings than in quadriceps and peaked at d+2 (quadriceps VAS scores = 2.20 ± 1.55 a.u. (0 at pre-ex); hamstrings VAS scores = 3.15 ± 2.14 a.u. (0 at pre-ex); hip flexion angle = 62 ± 5° (75 ± 6° at pre-ex); CK activity = 3021 ± 2693 $IU \cdot l^{-1}$ (136 ± 50 $IU \cdot l^{-1}$ at pre-ex)). The results of the present study suggested the occurrence of muscle damage that could have been induced by the peculiar muscle recruitment in NMES and the resulting overrated mechanical stress. The sensitivity to the damaging effects of NMES appeared higher in the hamstrings than in quadriceps muscles.

Key words: Electrical stimulation, DOMS, muscle contraction, muscle damage.

Introduction

Transcutaneous neuromuscular electrical stimulation (NMES) has been used for several years to prevent muscular atrophy following immobilization (Paillard et al., 2005). It is generally acknowledged that NMES has to be applied specifically in the early phase of rehabilitation when programs based on voluntary contractions are not applicable (Bax et al., 2005). Moreover NMES is considered as a technique for improving muscle strength in athletes and able-bodied individuals (Vanderthommen and Duchateau, 2007). According to the literature, the gains observed at the end of a NMES program are proportional to the intensity of the contractions electrically evoked during the training sessions (Lai et al., 1988). Therefore it is recommended to motivate the subject to use the highest current intensity he can tolerate. Optimal conditions are generally achieved when isometric muscle contractions are evoked by a multichannel stimulation (Maffiuletti, 2010). Therefore, quadriceps NMES should be applied with at least two stimulation channels (Aldayel et al., 2010a).

However, recent studies evidenced structural muscle damage following a single NMES session (Crameri at al., 2007; Mackey at al., 2008). Crameri at al. (2007) showed that NMES of vastus lateralis provokes a significant disruption of cytoskeletal proteins (desmin) and of Z-lines as well as an increase in satellite cell markers. After a single electrical stimulation bout of gastrocnemius medialis, Mackey at al. (2008) demonstrated macrophage infiltration and Z-line disruption that was proportional to the force produced by stimulation. Even more recently, Aldayel et al. (2010b) and Jubeau et al. (2012) observed that indirect markers of muscle damage (muscle weakness, delayed onset of muscle soreness (DOMS) and plasma CK activity) were increased after a NMES bout applied on knee extensors and elbow flexors, respectively. Obviously, such morphological damages and the induced symptoms can impede rehabilitation programs of patients or training and competition of athletes.

To our knowledge, no previous study has investigated yet the damaging effects of a NMES bout on hamstrings muscles although they are regularly stimulated in rehabilitation or training programs (particularly in combination with quadriceps stimulation after anterior cruciate ligament surgery) (Snyder-Mackler at al., 1991). It has also been documented that hamstrings are more responsive than knee extensors to the damaging effects of an exercise bout composed of maximal voluntary eccentric contractions (Croisier at al., 2000). Therefore, the aim of the present work was to investigate whether or not a single bout of unilateral electrical stimulations of hamstrings and quadriceps muscles provokes an increase in markers suggesting muscle damage and to compare the quadriceps and hamstrings muscles regarding the damage symptoms resulting from the NMES session.

Methods

The Medical Ethics Committee of the University of Liege (Belgium) approved the protocol (#B70720073045). All subjects gave written informed consent before admission to the study.

Subjects and study design

Twenty sedentary or moderately active (less than 3hrs/week of leisure sports activities) healthy male (24.0 ± 3.3 years; 75.8 ± 10.3 kg) volunteered to participate in this study. No subject had been previously exposed to NMES before this study. None were involved in lower

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body resistance or endurance training at the time of the study. During the whole study period, the subjects were instructed to abstain from consumption of any form of medication and to refrain from strenuous exercise. They were also requested to abstain from the use of any technique that could influence the process of muscle recovery (stretching, hydrotherapy, massage, etc.).

Subjects were pseudo-randomly assigned to an electrical stimulation (ES) group and a control group. Both groups did not differ significantly in terms of age, weight and sports activities. In the ES group (n = 10), electrical stimulations were administered to the subjects; that was followed by several measurements of parameters which can reflect muscle damage. In the second group (control group, n = 10), only the measurements were conducted.

The time course of electrical stimulation exercise and measurements is summarized in Figure 1.

Electrical stimulation exercise

In the ES group a bout of 100 electrically elicited isometric quadriceps contractions and another bout of 100 electrically elicited isometric hamstrings contractions were administered successively to the right leg (on-off ratio 6-6 s). The order of the two NMES bouts (each lasting 20 min) was randomized, ensuring that 5 subjects started with the quadriceps stimulation and the others started with the hamstrings stimulation. A portable electrical stimulator (Compex II, Medicompex, Ecublens, Switzerland) delivered biphasic symmetric rectangular pulses (frequency 80 Hz, pulse duration 0.35 ms). During both stimulation bouts, subjects were seated on a Biodex III dynamometer (Biodex Medical Systems, Shirley, New-York, USA). Quadriceps and hamstrings were stimulated at 60º and 30º of knee flexion, respectively due to the peak torque at these lengths. The evoked torque was measured every 2 min (10 contractions). The subjects were instructed to fully rest during stimulation to avoid any superimposing with voluntary contraction.

Quadriceps stimulation

The subject was seated with the knee positioned at 60° of knee flexion (0° corresponding to the full extension) and the trunk in the vertical position. Three "stimulating" (positive) electrodes (5x5 cm) were placed over the motor points of the vastus medialis, vastus lateralis and rectus femoris. The location of the motor points was carefully determined by moving a pen electrode on the skin overlying the target muscle until the best mechanical response was found; a 1 Hz stimulation at a given stimulation intensity (~10 mA) was used. The "dispersive" (negative) electrodes (9x5 cm) were placed transversally on the proximal portion of the thigh. The investigators adjusted the current intensity to get the maximal tolerable contraction from the beginning until the end of the bout.

Hamstrings stimulation

The subject was seated with the knee positioned at 30° of knee flexion (0° corresponding to the full extension) and the trunk in the vertical position. Four "stimulating" (positive) electrodes were placed on the motor points of the semitendinosus, semimembranosus, long head and short head of the biceps femoris (to locate the motor points, the method previously described for the quadriceps was used). The "dispersive" (negative) electrodes were placed transversally on the proximal and distal portion of the thigh. The same procedure as for the quadriceps stimulation was used for the current adjustment.

Torque measurements

After a standardized warm-up (5-min cycling at 75-100 W on a bicycle ergometer (60-70 rpm)), isometric maximal voluntary torque (IMVT) of the quadriceps and hamstring muscles were assessed using the same dynamometer as the one used for the electrical stimulation exercise. The subject was placed in a sitting position with the trunk in the vertical position and was secured by means of belts placed around the chest, hips and thigh.

As for the electrical stimulation bouts, quadriceps and hamstrings were tested with the knee positioned at 60° and 30° of knee flexion, respectively. For each muscle group, subjects were familiarized with the test by performing 5 graded submaximal isometric contractions whereby subjects built up to a near-maximum effort

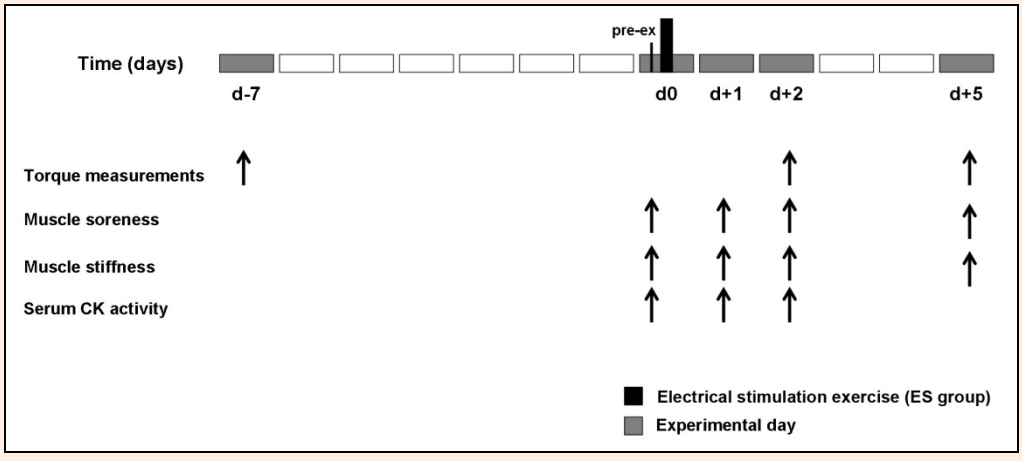

Figure 1. Time course of the electrical stimulation exercise (ES group) and the measurements for the dependent variables (ES group and control group). Pre-ex = before the electrical stimulation exercise; ES = electrical stimulation.

(~95%) on the last repetition. After a rest period of 2 min subjects exerted three 6-seconds isometric maximal voluntary contractions at one-minute intervals. The best result of the 3 contractions was selected to be the true IMVT value. A rest period of 2 min was respected between testing of both muscle groups.

IMVT was measured one week (d-7) before the electrical stimulation exercise as well as 48h (d+2) and 120h (d+5) after the electrical stimulation exercise. The baseline isometric torque was measured one week before performing the electrical stimulation exercise rather than at day zero in order to avoid any interference with the NMES bout regarding the appearance of muscle damage symptoms. We also avoided to measure maximal torque at d+1 for the same reason.

Muscle soreness perception

The subjective presence and intensity of DOMS were evaluated using a visual analogue pain scale (VAS) ranging from 0 (no pain) to 10 (worst imaginable pain) arbitrary units (a.u.). Quadriceps and hamstrings soreness perception was assessed successively, in a free standing and motionless position. This evaluation was realized before the electrical stimulation exercise (pre-ex) and 24h (d+1), 48h (d+2) and 120h (d+5) after the electrical stimulation exercise.

Muscle stiffness

The flexibility of the right quadriceps was tested using the prone quadriceps flexibility test: with the subject prone, the examiner passively flexed the right knee until the subject perceived painful sensations; then, the distance from heel to buttock was measured (Witvrouw at al., 2003).

The flexibility of the right hamstring muscles was tested with the straight leg raising test: with the subject supine, the examiner passively raised the leg with the knee fully extended until the subject perceived painful sensations; then, the range of hip flexion was measured. Concerning the goniometer, its axis was placed over the major trochanter, the stationary arm was placed horizontally (parallel to the table) and the moving arm pointing to the lateral epicondyle of the femur (Witvrouw at al., 2003).

Muscle stiffness was measured before the electrical stimulation exercise (pre-ex) as well as 24h (d+1), 48h (d+2) and 120h (d+5) after the electrical stimulation exercise.

Serum creatine kinase (CK) activity

Increased serum activity of CK was used as an indirect index of exercise-induced muscle damage. A 4-ml blood sample was taken by venipuncture one hour before the electrical stimulation exercise (pre-ex). Two additional blood samples were drawn 24h (d+1) and 48h (d+2) after the electrical stimulation exercise. Each venous blood sample was allowed to clot at room temperature; the activity of CK was measured spectrophotometrically (Szasz et al., 1976).

Statistical analysis

Values are expressed throughout this study as mean ± SD. Normal distribution of torque and muscle stiffness measurements was checked using the Shapiro-Wilk test. Outcome changes over time were assessed by using a two-way ANOVA with repeated measures. When the analysis of variance revealed a significant interaction effect, it was then determined if the time effect and/or the group effect were significant. The scheffe post-hoc test was applied to determine between-means differences if significant effect was found. A P-value ≤ 0.05 was considered to represent statistical significance.

Results

Electrical stimulation exercise

Stimulation intensity increased significantly ($p \leq 0.05$) and similarly throughout both bouts (Figure 2A). At the end of quadriceps and hamstrings stimulation, the mean current intensity reached 77 ± 21 and 73 ± 19 mA, respectively.

Despite the increase in stimulation intensity, the torque output remained stable or decreased slowly during both bouts (Figure 2B) ($p = 0.45$). Evoked torques were significantly ($p \leq 0.05$) higher during quadriceps stimulation (29 ± 12 % of pre-exercise IMVT) than during hamstrings stimulation (16 ± 10 % of pre-exercise IMVT).

Torque measurements

At baseline (d-7), the mean IMVT reached 254 ± 62 N·m (ES group) and 275 ± 69 N·m (control group) for the quadriceps and 122 ± 21 N·m (ES group) and 143 ± 30 N·m (control group) for the hamstrings muscles. Those baseline values did not differ significantly between groups.

Regarding the quadriceps, except for a modest torque decrease at d+2 in the ES group (- 4.4%), values remained stable over time in both groups ($p \geq 0.05$). Regarding the hamstrings, the torque decrease was more pronounced at d+2 in the ES group (- 9%) (Figure 3). Analysis of variance confirmed a significant “time” effect ($p = 0.022$).

Perception of muscle soreness (Figure 4A and 4B)

After the stimulation bout, pain sensations increased significantly in both muscles and peaked at d+2 (quadriceps and hamstrings VAS scores = 2.20 ± 1.55 a.u. and 3.15 ± 2.14 a.u., respectively). Afterwards VAS scores decreased significantly and d+5 VAS scores (quadriceps VAS = 0.45 ± 1.09 a.u. and hamstrings VAS = 0.55 ± 0.96 a.u.) did not differ significantly from baseline scores. Analysis of variance revealed a significant “group” effect ($p < 0.001$) but also a significant “time” effect ($p < 0.001$).

Muscle stiffness

Changes in quadriceps stiffness are illustrated in Figure 4C. Before the electrical stimulation exercise (pre-ex), the mean distance from heel to buttock reached 6.7± 6.1 cm and 8.2 ± 5.2 cm in the ES group and the control group, respectively ($p > 0.05$). Analysis of variance indicated that there was no interaction effect ($p = 0.82$).

Changes in hamstrings stiffness are illustrated in Figure 4D. Before the NMES bout (pre-ex), the mean hip

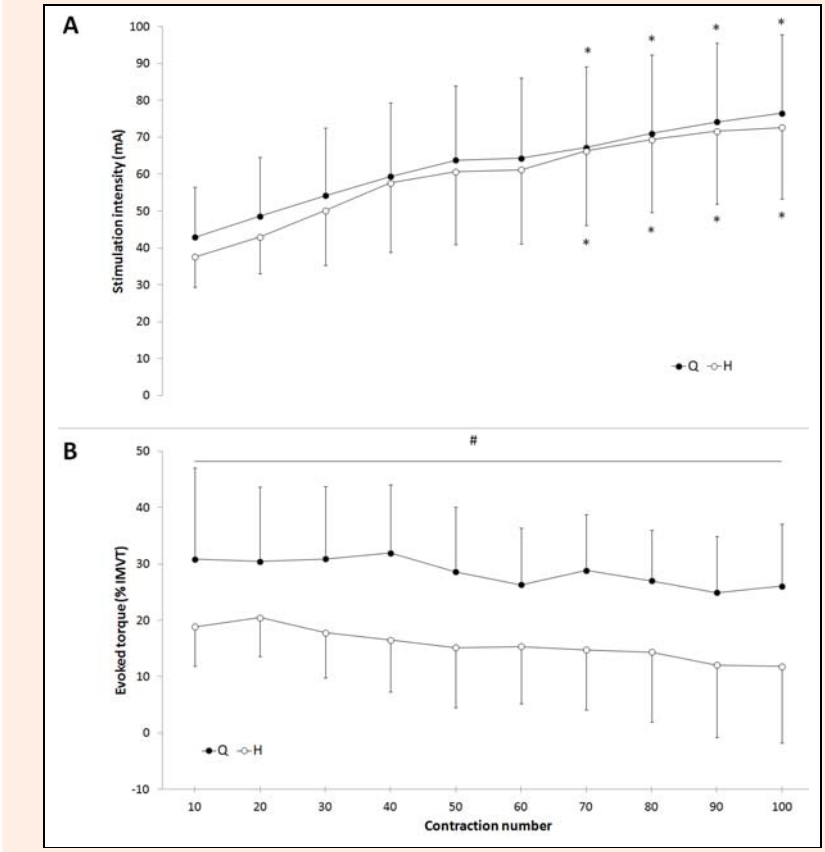

Figure 2. Changes (mean and standard deviation, n = 10) in stimulation intensity (A) and in evoked torque (B) over 100 contractions evoked electrically in quadriceps (Q) and hamstrings (H) muscles. * = significantly ($p \leq 0.05$) different from contraction 10; # = significant ($p \leq 0.05$) difference between Q and H.

flexion angle reached 75°± 6° and 77°± 11° in the ES group and the control group, respectively ($p > 0.05$). After the bout, hamstrings flexibility remained stable for the control group whereas it decreased in the ES group (with the lowest value measured at d+2: 62°± 5°). Analysis of variance confirmed a "group" effect ($p < 0.001$).

Serum CK activity (Figure 4E)

Before the electrical stimulation bout (pre-ex) mean CK activity was similar in both group (136 ± 50 $IU \cdot l^{-1}$ for the ES group and 150 ± 86 $IU \cdot l^{-1}$ for the control group, $p > 0.05$). After NMES, CK activity remained stable in the control group while it was increased at d+1 (927 ± 613 $IU \cdot l^{-1}$, $p = 0.52$) and at d+2 (3021 ± 2693 IU/l, $p \leq 0.001$) in the ES group. Analysis of variance confirmed a significant "group" effect ($p < 0.001$) as well as a significant "time" effect ($p < 0.001$).

Discussion

The purpose of the present work was to study the effects of a NMES session applied consecutively on quadriceps and hamstrings and to compare both muscle groups regarding the degree of damage symptoms. The subject was motivated to tolerate the highest current intensity as possible because evoked muscle tension constitutes the key factor for optimizing NMES effects in rehabilitation or training programs (Maffiuletti, 2010). The progressive increase in stimulation intensity and the final intensity level (~75 mA at the end of the bout) are in accordance with previous studies in which authors also stimulated quadriceps at maximal tolerance (Aldayel at al., 2010a and 2010b). The levels of torque evoked during the quadriceps stimulation (~29% of IMVT) are also in accordance with values reported in the literature (Aldayel et al., 2010a; 2010b; Grimby and Wigerstad-Lossing 1989; Jubeau at al., 2008). The NMES inability to produce a torque corresponding at 100% of maximal voluntary torque is well known; it mainly results from the difficulty to recruit all the motor units due to the discomfort associated with intense electric stimulations. To our knowledge, there is no data available in the literature concerning the torque evoked by hamstrings stimulation. The lower torque evoked during hamstrings stimulation (~16% of IMVT) in comparison with quadriceps stimulation (~29% of IMVT) could be induced by a higher discomfort associated with hamstrings stimulation and/or by differences in synergist muscle activity during assessment of maximal voluntary torque of both muscle groups. The plateau observed in the evoked torque although the current intensity was increased, probably results from central and/or peripheral fatigue occurring in the stimulated muscle. This fatigue is probably linked to the specificities of the electrically induced contraction (Vanderthommen and Duchateau, 2007).

By means of morphological and histological

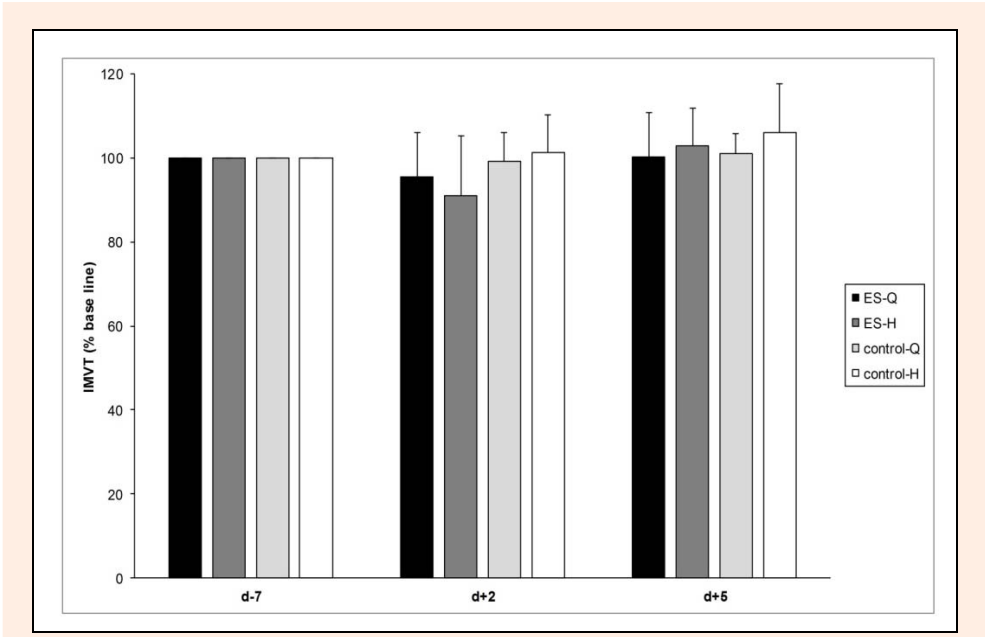

Figure 3. Changes (mean and standard deviation) in isometric maximal voluntary torque of quadriceps (Q) and hamstrings (H) expressed in percentage of baseline values in the electrostimulated group (ES, n = 10) and in the control group (control, n = 10) seven days before the electrical stimulation exercise (d-7), two days (d+2) and five days (d+5) after exercise.

invasive assessments, previous studies reported that NMES could result in disruption of muscle fibers and connective tissue (Crameri et al. 2007; Mackey at al. 2008). However, in those studies, the stimulated bout did not reflect the stimulation conditions regularly used by clinicians or trainers (isometric contractions induced by a multichannel stimulation). Indeed, Crameri at al. (2007) only stimulated the vastus lateralis and induced eccentric contractions whereas Mackey at al. (2008) also used a single channel to recruit the gastrocnemius medialis. Recently, some authors measured changes in indirect markers of muscle damage after a NMES bout applied isometrically on knee extensors (Aldayel et al. 2010a; 2010b; Jubeau at al. 2008) or elbow flexors (Jubeau at al. 2012). Those studies unanimously indicated that muscle weakness, muscle soreness and increased serum CK activity occur a few days following a stimulation session; such results confirmed that electrically evoked isometric contractions can induce muscle damage. In the present work, a unilateral stimulation of knee flexor and extensor muscles was used in order to reflect usual application of NMES in rehabilitation and sports fields and because electrically induced
hamstrings damage has not been documented yet.

The main findings of the present study were that muscle soreness and stiffness occurred in the stimulated thigh and was associated with a significant CK activity increase. These variables are considered as relevant indicators of DOMS and muscle damage (Proske et al., 2005). Generally, DOMS occurs in skeletal muscle after strenuous exercise, especially in case of intense eccentric contractions (Croisier et al., 2003). The present study shows that quadriceps and hamstrings stimulation during a single session can result in symptoms suggesting muscle damage although the isometric contractions corresponded only to ~20-30% of the IMVT. Such observation could result from the specificities of NMES in the pattern of muscle contractile activity: a) the stimulation frequency that is regularly used to ensure a maximal tetanic force (50-100 Hz) imposes on stimulated fibers a synchronous over activation that is associated with an exaggerated metabolic demand (Matheson et al., 1992); b) NMES preferentially recruits axonal branches near the electrode (Vanderthommen et al., 2000). Therefore a decrease in the mechanical response linked to fatigue of the superficial fibers can only be compensated by an increase in stimulation intensity, which depolarizes new fibers at a greater distance from electrode but continues to impose a sustained contractile activity to the superficial ones that are exhausted (Vanderthommen et al., 2003).
In summary, it can be speculated that such a limited and fixed spatial recruitment and such a synchronous and supraphysiological temporal recruitment in neighboring fibers (Maffiuletti, 2010) provoke an exaggerated mechanical stress that could induce muscle damage (Jubeau et al., 2008). In a recent review, Nosaka at al. (2011) confirmed that the peculiar recruitment in NMES and the resulting overrated mechanical stress might induce damage to cytoskeleton, myofilaments and connective tissue surrounding muscle fibers.

Some symptoms of muscle damage (torque decrease and stiffness increase) were not observed in the knee extensors following the NMES bout although our stimulated exercise (100 contractions, duty cycle = 50%) was more strenuous than the quadriceps electrical stimulation bout (40-50 contractions, duty cycle = ~ 33%) studied by the group of Nosaka (Aldayel et al., 2010a; 2010b; Jubeau at al., 2008). The difference between studies regarding the subject position (the knee was more flexed (100°) in the previous studies than in the present study (60°)) could explain this discrepancy. In fact, muscle length could have a major influence on the occurrence

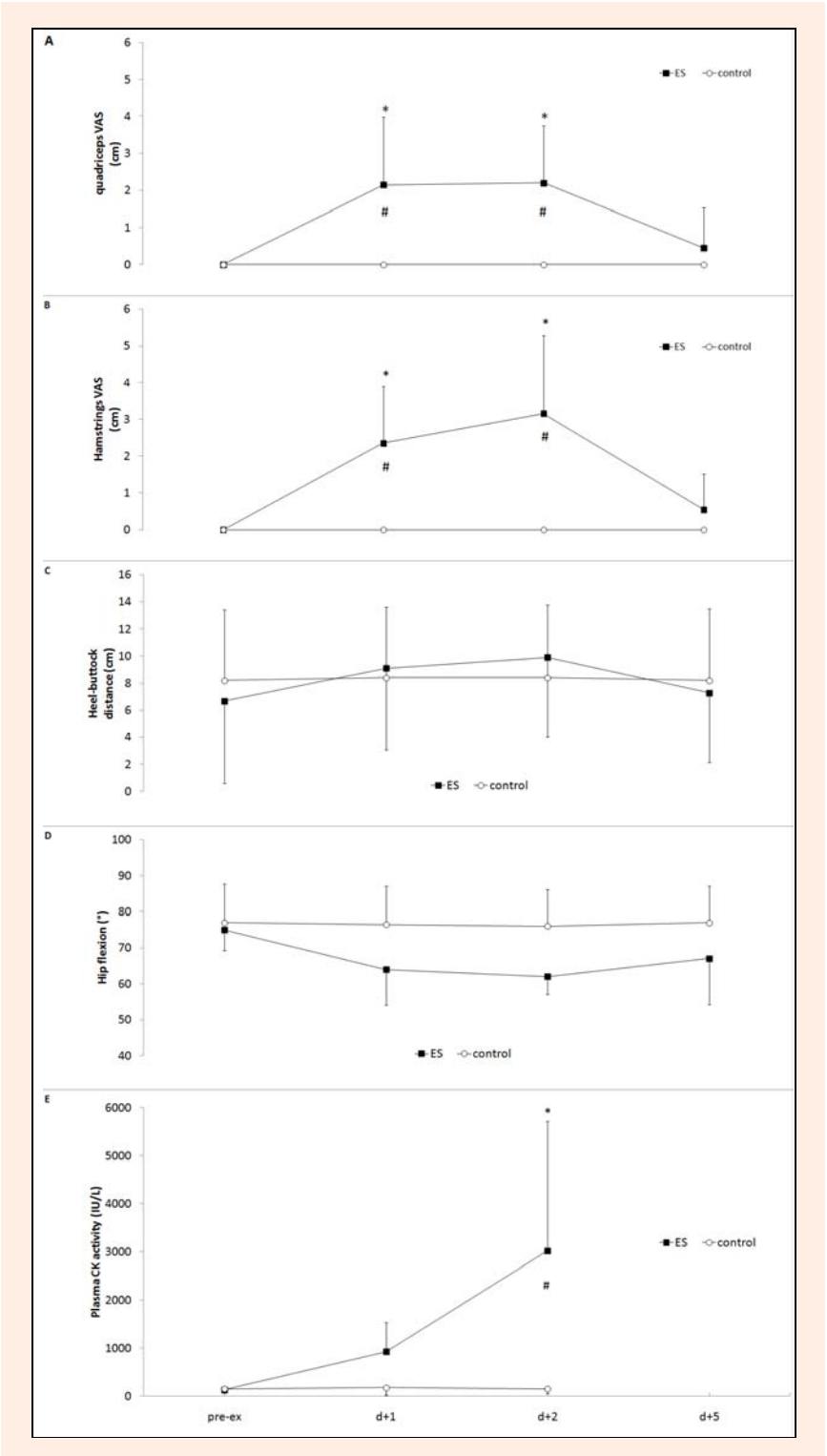

Figure 4. Changes (mean and standard deviation) in perception of quadriceps soreness (VAS) (A), in perception of hamstrings soreness (VAS) (B), in quadriceps flexibility (C), in hamstrings flexibility (D) and in serum CK activity (E) in the electrostimulated group (ES, n = 10) and in the control group (control, n = 10) before the electrical stimulation exercise (pre-ex), one day (d+1), two days (d+2) and five days (d+5) after exercise. * = significantly ($p \leq 0.05$) different from baseline value (pre-ex). # = significant ($p \leq 0.05$) difference between ES and control.

of damage induced by electrically induced contractions: the extent of muscle damage seems to occur especially when the muscle is stimulated at a greater length (Nosaka at al., 2011).

After the stimulation bout, hamstrings torque and flexibility were reduced (-9% and -13°, respectively) whereas those variables remained stable with regard to the quadriceps. This suggests a more pronounced alteration in muscle function and a higher sensitivity to the damaging effects of a NMES bout in knee flexor than in knee exten-

sor muscles. Reminding the lesser torque evoked during hamstrings stimulation (~16% of IMVT) in comparison with quadriceps stimulation (~29% of IMVT), the hypothesis of a higher sensitivity of hamstrings is reinforced. The especially high hamstrings responsiveness to the damaging effects of an exercise bout has already been reported following voluntary eccentric contractions (Croisier at al., 2000). This susceptibility might result from the high hamstrings proportion of fast type II fibers (Garrett at al., 1984). In fact, these fibers have been found to be most severely damaged by strenuous exercise (Jones et al. 1986). The superficial and nonselective pattern of muscle recruitment during NMES implies that this technique can activate fast motor units even during contractions of low level intensity (Matheson at al., 1992) and could reinforce the hypothesis of the greater sensitivity of hamstrings to muscle damage induced by stimulation. This hypothesis has to be counterbalanced by the fact that both bouts were performed at a different knee angle resulting in different muscle length. As mentioned above, muscle length is one of the factors that can also affect the magnitude of muscle damage (Nosaka at al., 2011). In our experimental design we stimulated the hamstrings and the quadriceps at 30° and at 60°of knee flexion, respectively because it has been demonstrated that the torques evoked in knee flexors and extensors peaked at these angles (Hausdorff and Durfee, 1991). Actually the training effects induced by NMES sessions are maximized when stimulations induce the greatest muscle tension (Lai et al., 1988).

Conclusion

A stimulation bout administered to the quadriceps and hamstrings increased indirect markers of muscle damage (muscle soreness, hamstrings weakness and stiffness and serum CK activity). The fact that a single NMES session, similar to those used in field conditions of training or rehabilitation, can induce discomfort and impair muscle function is of particular interest for trainers and physiotherapists. However it is important to remember that the level of those indirect markers does not necessary reflect the magnitude of the structural damage. The damage symptoms (decrease in muscle torque and flexibility) resulting from the NMES session were higher in hamstrings than in quadriceps. Further studies are needed to confirm the higher sensitivity of hamstrings to the damaging effects of a stimulation bout and to investigate if preconditioning sessions could attenuate hamstrings damage induced by NMES.

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Key points

- A stimulation bout of quadriceps and hamstrings that reflects usual application of NMES, increases indirect markers of muscle damage (muscle soreness, muscle weakness and stiffness and serum CK activity).
- The occurrence of muscle damage could have been induced by the peculiar muscle recruitment in NMES and the resulting overrated mechanical stress.
- The sensitivity to the damaging effects of NMES appears higher in the hamstrings than in quadriceps muscles.

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