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Original Article

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Acute effects of superimposed electromyostimulation during cycling on myokines and markers of muscle damage

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Abstract

Objectives: The purpose of the present study was to evaluate the effects of superimposed electromyostimulation (E) during cycling on myokines and markers of muscle damage, as E might be a useful tool to induce a high local stimulus to skeletal muscle during endurance training without performing high external workloads. **Methods:** 13 subjects participated in three experimental trials each lasting 60 min in a randomized order. 1) Cycling (C), 2) Cycling with superimposed E (C+E) and 3) E. Interleukin-6 (IL-6), brain-derived neurotrophic factor (BDNF), creatine kinase (CK) and myoglobin were determined before (pre) and 0', 30', 60', 240' and 24h after each intervention. **Results:** Only C+E caused significant increases in levels of CK and myoglobin. BDNF and IL-6 significantly increased after C and C+E, however increases for IL-6 were significantly higher after C+E compared to C. **Conclusion:** The present study showed that superimposed E during cycling might be a useful tool to induce a high local stimulus to skeletal muscle even when performing low to moderate external workloads. This effect might be due the activation of additional muscle fibers and mild eccentric work due to the concomitant activation of agonist and antagonist. However the higher load to skeletal muscle has to be taken into account.

Keywords: IL-6, BDNF, Creatine Kinase, Myoglobin, Perceived Physical Pain

Introduction

The endocrine system (the exercise-induced hormonal response) is important for mediating signaling pathways for both short-term homeostatic control and long-term cellular adaptations to exercise training[1]. Previous studies have shown that especially high-intensity training (HIT) induces high acute hormonal responses, which are known to be critical for adaptation processes[2] and that HIT is a time-saving strategy to improve endurance performance. However, HIT might not be practical for all kinds of subjects such as patients with certain (cardiovascular) diseases or athletes in certain situations (i.e., injuries), as HIT is very demanding for different kind of tissues and organs[3]. Therefore, it seems to be promising to look for methods that allow an intensification of endurance training without performing high external loads.

Electromyostimulation (E) is an alternative training method mainly performed in strength training and is used for its intensification. Only a few studies used isolated E in matters of improvements of endurance performance[4]. However, two main limitations of E are the strong discomfort associated with the peripheral stimulation[5] and the limited spatial recruitment of muscle fibers, which is quite superficial[6] and largely incomplete. Despite this limited spatial recruitment, we were able to show significantly greater metabolic changes (lactate, respiratory exchange ratio, base excess (BE), HCO_3^-, pCO_2) during cycling with superimposed E compared with normal cycling[7]. Also Paillard[8] described that isolated E is highly demanding on muscle metabolism and can enhance energy consumption and carbohydrate oxidation more than voluntary contraction (VC) can. Hamada et al. showed that E strongly activates anaerobic glycolysis for energy production with lactate formation and acidifies more cytoplasm than VC leading to early fatigue[9]. Furthermore, even at lower exercise intensities,

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additional E may allow one to induce a high local stimulus on skeletal muscle. The non-selective recruitment (activation of a mix of both fast and slow twitch fibers located within the "range" of the electrodes[10]) may provide (clinical) advantages in that all fibers, regardless of type, have the potential to be activated at relatively low exercise intensities, which might lead to greater skeletal muscle adaptations and improvements of endurance performance, especially in fast twitch fibers[11].

In order to gauge the additional load of E during cycling on skeletal muscle, the present study aimed to investigate the acute response of myokines and muscle damage markers. Skeletal muscle has been identified as an endocrine organ, which releases myokines like IL-6 and BDNF[12]. The increase in the plasma concentration of IL-6 during exercise has been a consistent finding, shown to be dependent on the intensity and duration of exercise[13]. Thereby the expression of IL-6 in skeletal muscle is regulated via calcium (Ca_2^+) and the intracellular glycogen content and it has been demonstrated to be released by muscle tissue in response to inflammatory action[13].

BDNF is a neurotrophic growth factor which is expressed in the brain and in skeletal muscle, mainly regulating neuronal development, but also regulating metabolism in skeletal muscle. Physical activity was shown to increase circulating BDNF levels 2-3-fold, whereupon the brain contributes 70-80% of the circulating BDNF during exercise[12,14].

The serum level of creatine-kinase (CK) is routinely measured as an index of muscle damage. An increase in CK may be an index of cellular necrosis and tissue damage following acute or chronic muscle injuries[15]. Thereby post-exercise levels of CK are related to the intensity of exercise and muscular strain. Also myoglobin, the oxygen binding protein in muscle, is released into the bloodstream in increasing amounts upon muscle damage[15].

The aim of the present study was to investigate the myokine responses (IL-6, BDNF) and to quantify the muscular strain (CK, myoglobin), to cycling (C), to cycling with superimposed E (C+E) and to E (E). It is hypothesized that C+E leads to higher increases in myokines and muscle damage markers than C or E alone.

Materials and methods

Subjects

Thirteen healthy, nonsmoking sport students (mean±SD, age: 24.8±3.7 years, weight: 79.4±7.5 kg, height: 186.3±4.1 cm, relative VO_2max: 51.6±5.4 $ml \cdot min^{-1} \cdot kg^{-1}$) volunteered and gave written informed consent to participate in this study. The study protocol was approved by the University's ethics review board and is in accordance with the declaration of Helsinki. All subjects were inexperienced with E.

Exercise study protocol

Before the participation, subjects performed an incremental cycling test to determine VO_2max (Zan 600, Zan Messgeräte, Oberthulba, Germany) and maximal performance in order to determine the proper intensities for each subject for the main experiments. The incremental cycling test consisted of cycling at a cadence ≥80 rpm with an initial workload of 100 W for 5 minutes and incremental 40-W increases every 5 minutes until volitional exhaustion was reached. Afterwards, subjects were familiarized with E during C for 15 min.

Subjects participated in three experimental trials each lasting 60 min and each separated by one week in a randomized order. 1) Cycling (C), 2) Cycling with superimposed E (C+E) and 3) E (E). C and C+E were carried out on a bicycle ergometer (Schoberer Rad Meßtechnik SRM GmbH, Jülich, Germany) both times adjusted to 70% peak power output (PPO) and a cadence between 80-85 rpm. E was carried out in a sitting position on a chair. For the 2 interventions with E, two circular electrodes were placed all around the thigh (44 x 4 cm; half of the distance between spina iliaca anterior superior and the proximal patellar pole) and two circular electrodes were placed all around the calf (27 x 4 cm; 1/3 of the distance between fossa poplitea and the calcaneus) stimulating the major muscles of the thigh and the calf. Two laminar electrodes (13 x 10 cm) were centrally placed at the buttocks. A bipolar rectangular pulse waveform with an impulse width of 400 µs was continuously applied with 60 Hz. The settings of the EMS device (miha bodytec, Emersacker, Germany) were kept constant, including the intensity, for C+E and E. The intensity (current) was set individually for each subject and each muscle group at the maximum tolerated intensity[16,19] (thigh: 18.5±2.3 mA; calf: 14.9±1.4 mA; buttocks: 14.9±1.8 mA) according to each athlete's discomfort threshold and at an intensity at which proper pedaling was still possible. However, due to the resistance of different tissue structures it is not possible to precisely determine the impulse intensity (mA)[18] that ultimately reaches the muscle. In order to maximize the spatial recruitment, subjects were allowed to increase the intensity of E every 10 min. Electrical stimulation was continuous and independent from cycling.

A biphasic impulse type (in comparison to monophasic current) was chosen, as it was applied in several other studies[16,17], and because it offers advantages for applying high stimulation intensities. In regard to stimulation frequency, authors recommend a wide range between 2-200 Hz. Comprehensive recommendations for high stimulation intensities range between 50-100 Hz[20]. Furthermore, in a previous study we found no differences in the metabolic response between 30 and 85 Hz[7]. For the level of impulse width, a compromise should be found to activate deeper motor units without being unpleasant for the athlete. Impulse durations between 300-400 microseconds were recommended, as long impulse durations result in deeper and more intensive muscle stimulation and thus more motor units will be recruited[20].

Before each experimental trial, subjects warmed up (WU) for 5 min at an intensity of 50% PPO on a cycle ergometer.

Before each experiment subjects had a standardized resting-phase of 1 hour in which 0.5 mL of water was ingested in order to assure a well-hydrated status and to provide a good conductance of the skin where the electrodes are placed. During each session environmental conditions (temperature and humidity) were kept constant and all three tests were carried out at the

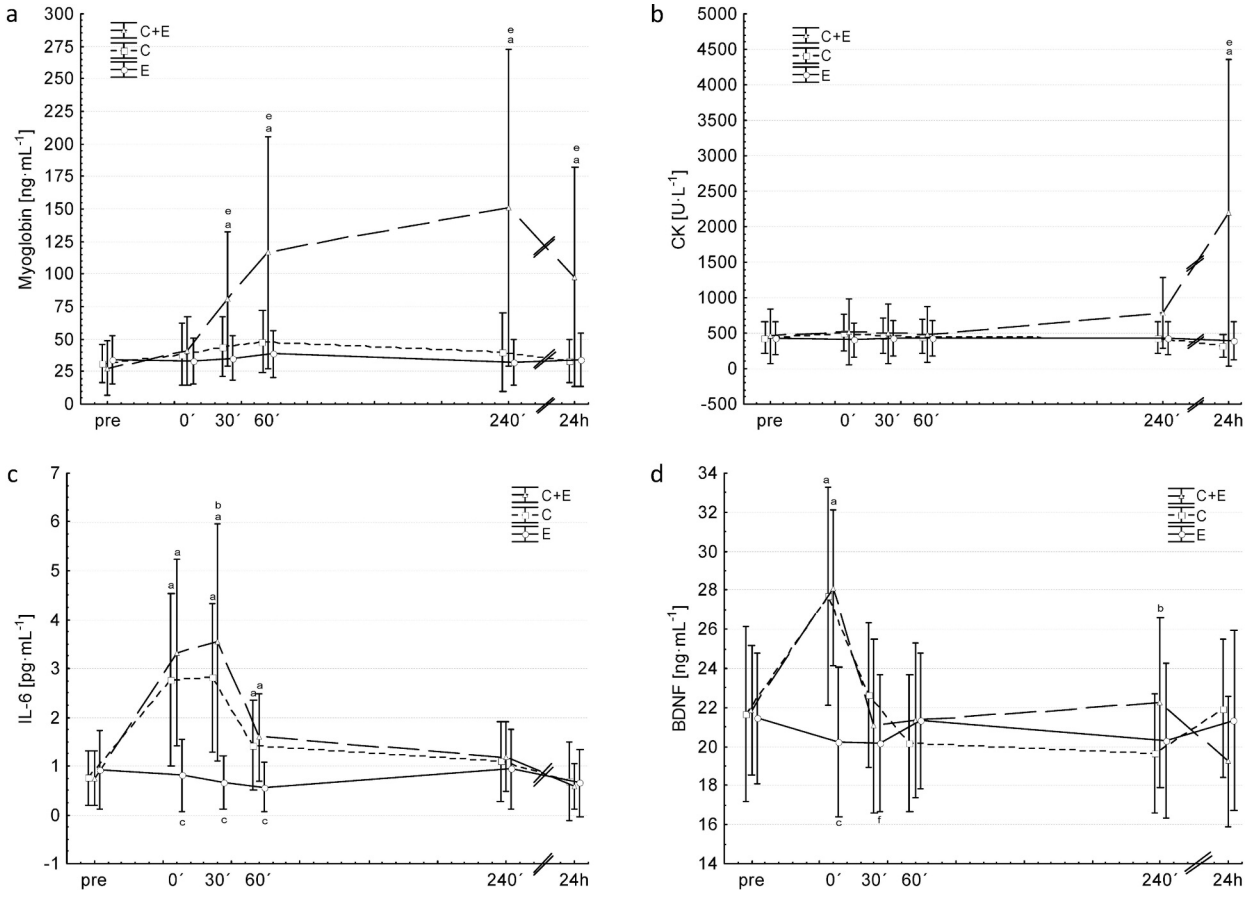

Figure 1. Changes in circulating myoglobin (a), creatine kinase (b), IL-6 (c) and BDNF (d) before (pre) and after (0´, 30´, 60´, 240´, 24h) each intervention: C+E (triangles, broken line), C (Squares, dotted line) and E (circles, solid line). [a]Significant different to pre of the same condition; [b]significant difference between C and C+E at respective time points; [c]significant difference between E and C+E/C at respective time points; [e]significant difference between C+E and C/E at respective time points; [f]significant difference between C and E at respective time points. Values are presented as means±SD.

same time of day in order to prevent diurnal variations in performance and the hormonal status.

The food intake before the tests was standardized to the extent that subjects recorded their food intake on the day before the first test, and then were advised to reproduce their diet before each test day. In addition carbohydrate-rich foods were recommended to the subjects. A last snack was allowed 2 hours before the test. The subjects were not allowed to perform strenuous exercise 24 hours before testing. 30 minutes after each of the three tests, subjects received 500 ml of a low fat chocolate milk and additional energy-bars. Food intake was adjusted so that energy intake matched the calculated energy expenditure of each trial. After the ingestion, subjects were only allowed to drink water until the last blood sample was withdrawn.

Measurements

Venous blood samples were collected for the determination of interleukin-6 (IL-6), brain-derived neurotrophic factor (BDNF), creatine kinase (CK) and myoglobin. One venous blood sample was taken before exercise (pre), and five post-exercise samples were taken at 0 min (0´), 30 min (30´), 60 min (60´), 240 min (240´) and 24 h (24h) after cessation of each of the three tests. 8.5 ml of blood was collected by the Vacutainer blood withdrawal system (Becton Dickinson). After storage at 7°C for ~30 min for deactivation of coagulation factors, the blood samples were centrifuged for 10 min at 1861 g and 4°C (Rotixa 50, Hettich Zentrifugen, Mühlheim, Germany). The serum was stored at -80°C till analysis. Serum levels of Myoglobin ($ng \cdot ml^{-1}$), BDNF, ($pg \cdot ml^{-1}$) and IL-6 ($pg \cdot ml^{-1}$) were determined using commercial ELISA kits (Myoglobin ELISA EIA-3955, DRG Instruments GmbH Germany; human BDNF Immunoassay, Quantikine ELISA-DBD00; human IL-6 Immunoassay, Quantikine HS ELISA-HS600B, R&D Systems USA). CK was analyzed with an autoanalyser (ADVIA, Siemens healthcare, USA) by using an enzymatic-photometric method. All results were adjusted for changes in plasma volume (PV): PV changes in percentage of pre values= $[(Hb_{pre}/Hb_{post}) \cdot (100\text{-}Hct_{post})/[(100\text{-}Hct_{pre})\text{-}1]] \cdot 100$.

The rating of muscle soreness was assessed by sitting down on a chair from an upright posture and standing up again from this position without using the arms. The subjects were then asked to rate their perceived physical pain using a 0-10 visual analog scale (VAS) pre, directly after (0´), 240 min after (240´) and 24 h (24 h) after each intervention.

Statistics

Statistical analyses of the data were performed by using a statistics software package (Statistica for Windows, 7.0, Statsoft, Tulsa, OK). Descriptive statistics of the data are presented as means±SD. To assess the effect of the three different interventions on circulating IL-6, BDNF, CK and myoglobin, a 2-factor [intervention (C, C+E, E); time (pre, 0´, 30´, 60´, 240´, 24h)] repeated-measures ANOVA with Fisher post-hoc test was used. For each growth factor we reported the *p*-value corresponding to the main intervention effect and time effect. Statistical differences were considered to be significant for $p<0.05$. Furthermore, the effect size "partial η^2" was calculated according to the main effect over time for each intervention and parameter. It has been suggested that for ANOVA, an effect size of 0.1 represents a small effect size; 0.25, a medium effect; and 0.4, a large effect. Power (1-β) was calculated post-hoc for ANOVA repeated measures using α, sample size and effect size.

Results

Heart rate showed no significant differences between C (151±11 bpm) and C+E (153±11 bpm), but both interventions showed significantly higher levels compared to E (56±12 bpm).

Power output during C and C+E was 212±30 W (70% PPO).

Creatine Kinase (CK)

Over-all ANOVA showed a significant time effect ($p=0.008$; partial $\eta^2=0.28$; power=0.4), no intervention effect ($p=0.07$; partial $\eta^2=0.25$; power=0.51) and a significant interaction effect (intervention * time) ($p<0.001$; partial $\eta^2=0.38$; power=0.57). Post-hoc analysis revealed that C+E significantly increased CK levels 24h after exercise compared to pre, whereas C and E did not induce any significant changes (Figure 1b).

Myoglobin

Over-all ANOVA showed a significant time effect (partial $\eta^2=0.46$; power=0.97), intervention effect (partial $\eta^2=0.48$; power=0.9) and a significant interaction effect (intervention * time) (partial $\eta^2=0.4$; power=0.9) (all $p<0.001$). Post-hoc analysis revealed that C+E significantly increased myoglobin levels 30´, 60´, 240´and 24h after exercise compared to pre, whereas E and C induced no significant changes. C+E showed significantly higher values 30´, 60´, 240´and 24h compared to C and E (Figure 1a).

IL-6

Over-all ANOVA showed a significant time effect (partial $\eta^2=0.74$; power=1.0), intervention effect (partial $\eta^2=0.56$; power=0.98) and a significant interaction effect (intervention * time) (partial $\eta^2=0.55$; power=0.99) (all $p<0.001$). Post-hoc analysis revealed that C+E and C significantly increased IL-6 levels 0´, 30´, and 60´after exercise. 30´ after C+E IL-6 values were significantly higher compared to C. IL-6 levels were significantly lower 0´, 30´ and 60´ after E compared to C+E and C (Figure 1c).

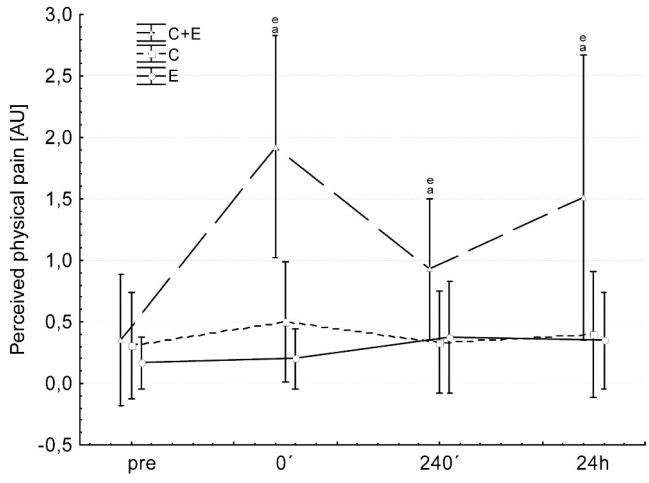

Figure 2. Changes in perceived physical pain before (pre) and after (0´, 240´, 24h) each intervention: C+E (triangles, broken line), C (Squares, dotted line) and E (circles, solid line). [a]significant different to pre of the same condition; [e]significant difference between C+E and C/E at respective time points. Values are presented as means±SD.

BDNF

Over-all ANOVA showed a significant time effect ($p<0.001$; partial $\eta^2=0.52$; power=1.0), intervention effect ($p=0.03$; (partial $\eta^2=0.3$; power=0.68) and interaction effect (intervention * time) ($p<0.001$; partial $\eta^2=0.33$; power=1.0)). Post-hoc analysis revealed that C+E and C significantly increased BDNF levels 0´ after exercise compared to pre, whereas E induced no significant changes. BDNF levels were significantly lower 0´and 30´ after E compared to C+E and C. 240´after exercise C+E showed significantly higher values compare to C (Figure 1d).

Perceived physical pain

Muscle soreness was highest after C+E compared to both other interventions at all time points post intervention. C and E did not differ between each other (Figure 2).

Discussion

The present study investigated the effects of C, E and C+E on markers of muscle damage (myoglobin & CK) and on two myokines (IL-6 & BDNF). The major findings of the present study are that C+E caused the highest increases in myoglobin and creatine kinase accompanied with the highest ratings of muscle soreness, whereas C and E had no major effects. Fur-

thermore, IL-6 showed the highest increases after C+E followed by C and E. Serum BDNF levels were significantly increased by C and C+E, whereas E induced no significant changes.

The major source of serum IL-6 levels during exercise is the skeletal muscle[12,13], and previous studies showed that the intensity and duration of exercise influence the increase in serum IL-6 levels[21]. This sensitivity to exercise intensity indirectly represents the muscle mass involved in the activity[13]. IL-6 is important for the maintenance of glucose homeostasis and fat oxidation. Low intracellular glycogen concentrations stimulate IL-6 production and release via an AMPK-dependent pathway[22] in order to mobilize glucose and FFA from liver and fat[13]. The higher increases of IL-6 after C+E compared to C of the present study might be due to the previously described additional recruitment of muscle fibers and MUs during (superimposed) E[11], increasing the muscle mass involved, the intensity of exercise and therefore the energy demand of the muscle. Besides the energetic regulation of IL-6, the expression in skeletal muscle is also regulated via changes in calcium (Ca_2^+) homeostasis[23]. The additional recruitment of muscle fibers during C+E might alter the Ca_2^+ homeostasis in skeletal muscle more than C and might therefore be a trigger for an increased expression/release of IL-6 as well. Furthermore, IL-6 has been demonstrated to be released by muscle tissue in response to inflammatory action and muscle damage[13]. As we were able to show significantly higher muscle damage after C+E, a higher inflammatory response might also explain the higher IL-6 release. However, this mechanism might only play a minor role, as inflammatory induced IL-6 release might occur later than directly after exercise[24].

BDNF plays a key role in regulating survival, growth and maintenance of neurons[25], however, BDNF has also been identified as a key component of central metabolic pathways and as a regulator of metabolism in skeletal muscle[12,26]. The results of BDNF of the present study are in line with previous studies, showing significant increases after exercise[27]. Although, previous studies suggested a link between BDNF elevations and exercise intensity[27,28], the intensification of endurance training with superimposed E in the present study did not lead to higher increases in BDNF levels. However, previous studies mainly compared more distinct graded exercise test to exhaustion, with low intensity, short duration exercise. This might explain the previously observed link to exercise intensity and the link to metabolic challenges imposed by strenuous exercise on the brain and BDNF production. However, up to now, the underlying molecular mechanisms driving the elevation of BDNF remain mainly unknown. Wrann et al.[29] recently showed that endurance exercise stimulates hippocampal Fndc5 gene expression (irisin) through a PGC-1α/Errα transcriptional complex. This elevated Fndc5 gene expression in turn stimulates BDNF gene expression. These findings link endurance exercise and important metabolic mediators (PGC-1a and FNDC5) with BDNF expression in the brain[29] and might explain the influence of exercise intensity. Furthermore, studies showed that BDNF is released from the cerebral vascular endothelium following hypoxic stress[30]. It can be speculated that exercise could result in cerebral hypoxic stress, since cerebral oxygen tension decreases during strenuous exercise[31], although, this might not have been the case for the chosen intensity of the present study. The fate, however, of the BDNF in the periphery remains unclear. BDNF enhances lipid oxidation in the muscles[26] and it could thus be speculated that the muscles take up BDNF.

Previous studies showed that exercise is associated with remarkable changes in (inflammatory) cytokines and damage markers and that the exercise-related response of these markers can be used to gauge exercise load[32]. Creatine kinase (CK) and myoglobin are well known for their role in muscle disruption, where CK peaks around 24-72 hours, and myoglobin with a much shorter (hours) response[15], which is supported by the present results. Myoglobin is about half the size of CK, which makes it easier to permeate the membrane and Mb is released from damaged muscle directly into the bloodstream whereas CK is released first into the lymph[15,33]. The significant increase of both markers after C+E only, clearly showed the high muscular strain of superimposed E during cycling. These higher increases might be due to the additional recruitment of (type II) muscle fibers, but more likely due to eccentric contractions (which normally do not occur during cycling), as the agonist and antagonist were stimulated simultaneously due to the use of circular electrodes. However, Chen et al.[34] also reported that CK significantly increased by about 6-times in rats following a fatiguing swimming protocol, an activity known to lack eccentric contractions. Significant CK increases were also found in humans immediately and 24 h after an exhaustive 90 min swimming protocol[35], or 90 min cycling exercise[36], also known to lack mechanical impact and eccentric contractions. Therefore, it was suggested that the energy status (severe fatigue) of the muscle cells probably contributes to the amount of CK efflux independent from mechanically induced membrane damage[37,38], which might also have been the case in the present study.

Several previous studies reported a repeated bout effect of muscle damage (makers) and soreness induced by E[39,40]. We cannot exclude a repeated bout effect in the present study; however, we therefore randomized all three trials, so that possible repeated bout effects might have canceled each other out.

We are aware that a different approach would be to trigger stimulation to coincide with muscle EMG activity during the exercise, so that E contributes to the cycling (to volitional contractions), and does not interfere with normal contraction/relaxation cycles. However, the superimposed E during cycling of the present study might be a helpful and easy method to induce additional 'mild eccentric exercise'[41]. Previous studies indicate that muscle tissue reacts specifically and differently to the combination of mechanical and metabolic stress induced by concentric and eccentric endurance-type training[42]. Repetitive submaximal concentric exercise (i.e., shortening contractions during normal cycling) mainly leads to adaptations of muscle oxidative metabolism and endurance, while eccentric exercise (i.e., lengthening contractions like during superimposed E during cycling) results in muscle growth and gain of muscle strength[41]. As preventing the loss of muscle mass with age, of patients or of athletes during and after injuries is an im-

portant aim for maintaining health and performance, the approach of the present study might be helpful to prevent this loss due to the eccentric component. Superimposed E may provide better results than volitional training for subjects, when they are unable to sustain an adequate volitional training intensity and duration to gain benefit from the intervention[10]. Orthopedic patients who cannot perform high-intensity voluntary contractions because of injury, recent surgery or impaired activation[43,44], and also athletes requiring high levels of muscle strength and power[45,46], might benefit from the use of superimposed E exercise - even at low intensity - to (re)train at least some of the fast fibers that otherwise can only be activated using high-force voluntary efforts.

A recent study showed that 'mild eccentric exercise' can increase Hsp72 content in skeletal muscle compared to concentric exercise, which is known to provide protection to skeletal muscle[47]. However, if this is the case for superimposed E needs to be shown in future studies and it has to be considered that 'mild eccentric exercise' has a molecular signature distinctly different from intensive concentric exercise as well as from maximal eccentric exercise[41].

In summary, the higher increases of CK, myoglobin and IL-6 after C+E indirectly show that a larger muscle mass is activated and that superimposed E induces a higher strain to skeletal muscles than normal C.

The present study showed that superimposed E during cycling might be a useful method to induce a high local stimulus to skeletal muscle even when performing low to moderate external workloads and therefore to intensify endurance training. This effect might be due the activation of additional muscle fibers and due to 'mild eccentric work' induced by the concomitant activation of agonist and antagonist. However, the higher load of superimposed E and the induced damage to skeletal muscle compared to C has to be taken into account.

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