# Comparison of acute physiological effects between alternating current and pulsed current electrical muscle stimulation

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# Comparison of Acute Physiological Effects between Alternating Current and Pulsed Current Electrical Muscle Stimulation

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This thesis is submitted in fulfillment of the requirements for a degree of
Doctor of Philosophy

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18th November 2010

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# ACKNOWLEDGMENTS

# ABSTRACT

Electrical muscle stimulation (EMS) is widely used in rehabilitation and sport training, and alternating current and pulsed current EMS are commonly used. However, no systematic comparison between alternating and pulsed current EMS has been made in the previous studies. The main aim of this research was to compare acute physiological responses between the alternating and pulsed current EMS. The secondary purpose of the research was to investigate further muscle damage induced by EMS-evoked isometric contractions. Three experimental studies were conducted in the thesis project together with literature review about EMS.

The first study compared alternating current and pulsed current electrical muscle stimulation (EMS) for torque output, skin temperature ($T_{sk}$), blood lactate and hormonal responses, and skeletal muscle damage markers. Twelve healthy men (23-48 y) received alternating current EMS (2.5 kHz delivered at 75 Hz, 400 μs) for the knee extensors of one leg and pulsed current (75 Hz, 400 μs) for the other leg to induce 40 isometric contractions (on-off ratio 5-15 s) at the knee joint angle of 100° (0°: full extension). The use of the legs for each condition was counterbalanced among subjects and the two EMS bouts were separated by 2 weeks. The current amplitude was consistently increased to maximally tolerable level, and the torque and perceived intensity were recorded over 40 isometric contractions. $T_{sk}$ of the stimulated and contralateral knee extensors were measured before, during and for 30 min after EMS. Blood lactate, growth hormone, testosterone, insulin-like growth factor 1, testosterone and cortisol were measured before, during, and for 45 min following EMS. Muscle damage markers included maximal voluntary isometric contraction torque, muscle soreness with a 100-

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mm visual analogue scale and plasma creatine kinase (CK) activity, which were measured before and 1, 24, 48, 72 and 96 h after EMS. No significant differences in the torque induced during stimulation (~30% MVC) and perceived intensity were found, and changes in $T_{sk}$, blood lactate, and hormones were not significantly different between conditions. However, all of the measures showed significant (P<0.05) changes from baseline values. Skeletal muscle damage was evidenced by prolonged strength loss, development of muscle soreness, and increases in plasma CK activity, however the changes in the variables were not significantly different between conditions. It is concluded that acute effects of alternating and pulsed current EMS on the stimulated muscles are similar.

The second study compared between alternating and pulsed current electrical muscle stimulation (EMS) for muscle oxygenation and haemodynamics during isometric contractions. Nine healthy men (23-48 y) received alternating current EMS (2.5 kHz delivered at 75 Hz, 400 µs) on the knee extensors of one leg and pulsed current EMS (75 Hz, 400 µs) for the other leg at maximally tolerable intensity to induce 30 isometric contractions (on-off ratio 5-15 s) at the knee joint angle of 100° (0°: full extension) separated by 2 weeks. Using near-infrared spectroscopy, changes in vastus lateralis tissue oxygenation index (ΔTOI) and total hemoglobin volume (ΔtHb) from baseline, and torque over 30 contractions were compared between the waveforms by a two-way repeated measures ANOVA. Peak torque relative to the baseline maximal voluntary isometric knee extension torque at 100° increased over 30 contractions in response to the increase in the stimulation intensity for pulsed current but not for alternating current. The torque during isometric contractions was less stable in alternating than pulsed current EMS. No significant differences in the changes in ΔTOI

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amplitude during relaxation phases and ΔtHb amplitude during contraction and relaxation phases were evident between waveforms. However, ΔTOI amplitude during the contraction phases was significantly ($P<0.05$) greater for the pulsed current than alternating current from the 18th contraction onwards. This indicates that muscle $O_2$ consumption relative to $O_2$ supply was significantly smaller for the alternating than pulsed current EMS. This could be due to less motor unit recruitment in alternating current EMS than pulsed current EMS.

The last study compared the first and second exercise bouts consisting of electrically evoked isometric contractions for muscle damage profile. Nine healthy men (23-48 y) had two EMS bouts separated by 2 weeks. The knee extensors of one leg were stimulated by biphasic rectangular pulses (75 Hz, 400 μs, on-off ratio 5-15 s) at the knee joint angle of 100° (0°: full extension) to induce 40 isometric contractions, while the current amplitude was increased to maintain maximal force generation. Maximal voluntary isometric contraction torque (MVC) of the knee extensors at 100°, muscle soreness, pressure pain threshold, and plasma creatine kinase (CK) activity were used as indirect markers of muscle damage, and measured before and 1, 24, 48, 72 and 96 h after EMS bout, and the changes over time were compared between bouts. The torque produced during exercise was approximately 30% of MVC, and no significant difference between bouts was evident for the changes in peak and average torque over 40 contractions. MVC decreased significantly ($P<0.05$) by 26% immediately and 1 h after both bouts, but the recovery was significantly ($P<0.05$) faster after the second bout (100% at 96 h) compared with the first bout (81% at 96 h). Development of muscle soreness and tenderness, and increases in plasma CK activity were significantly ($P<0.05$) smaller after the second than the first bout. These results show that changes in

muscle damage markers were attenuated in the second EMS bout compared with the initial EMS bout.

From the studies, no large differences in acute physiological responses were evident between alternating and pulsed current EMS; however, pulsed current was shown to consume greater $O_2$ than alternating current. It was assumed that alternating current EMS results in greater muscle fatigue than pulsed current EMS, since the alternating current delivers higher number of pulses per each stimulus. It seems likely that muscle damage is attenuated after the second EMS bout, regardless of the type of current used in EMS. Thus, possible muscle damage should not limit the use of EMS in sport training and rehabilitation. More investigation is necessary to compare between alternating and pulsed current EMS for chronic responses such as the effects of EMS training on muscle function and hypertrophy.

# DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief:

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# CHAPTER ONE: INTRODUCTION

## 1.1 BACKGROUND

Electrical stimulation is used to stimulate tissues, and when it is applied to muscle specifically, it is often referred to as electrical muscle stimulation or neuromuscular electrical stimulation (97). Physiological application of electrical current was developed by an Italian physician Luigi Galvani who showed that an electrical spark produced muscle twitching of a dead frog's legs in the late 18th century (60). Currently, electrical stimulation is extensively used in rehabilitation and sport training, and some of the applications include acute and chronic pain control (255), edema reduction (17, 226), spasticity and muscle spasm management (7, 192), treatment of contractures (191), healing of skin tissue (53) and minimising disuse muscle atrophy (62, 127). Furthermore, electrical stimulation can be used as an alternative to exercise in patients with cardiac and respiratory diseases, such as chronic obstructive pulmonary disease (81, 201), severe chronic obstructive pulmonary disease (237, 257) and chronic heart failure (81, 182). Although electrical stimulation cannot replace voluntary resistance training but rather it can be considered as a complementary (186) and substitute (108) in early rehabilitation.

In addition to the various applications of electrical stimulation for rehabilitation, the use of electrical muscle stimulation [henceforth referred to as EMS] in sport training has been shown to be effective for muscle strengthening (15, 113, 144, 187, 220), power performance (105, 156, 159), increasing muscle mass (207), enhancing cardiorespiratory endurance (12) and as a complementary method for voluntary exercise training (186). For example, Stefanovska and Vodovnik (220) showed 25% increase in maximal isometric strength contraction of quadriceps muscle following 3 weeks (10

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min daily) of EMS training (25 Hz) in healthy individuals. Maffiuletti et al. (156) reported that 4-week EMS training (115-120 Hz) combined with a plyometric training program increased maximal voluntary strength by 28%, squat jump by 21%, and counter movement jump by 8% for volleyball players. Banerjee et al. (12) showed that not only muscle strength (24%) but also peak oxygen consumption (10%) of healthy sedentary adults increased following non-tetanic EMS training in which leg muscles were stimulated at 4 Hz to induce rhythmical contractions for 40 min a day, 5 days a week for 6 weeks. It appears that inclusion of EMS in sport training is effective for maximising improvement of muscle function in a short period of time (12, 113, 156). However, it is still not clear how EMS should be used in training, and how EMS parameters such as frequency and pulse duration should be set (90). Bax et al. (15) concluded in their systematic review based on meta-analysis that further research is necessary to understand the optimal EMS parameters, such as frequency, amplitude, phase/pulse duration, duty cycle, waveform and current type. These EMS parameters affect force generation in EMS.

The frequency of EMS is determined by the number of pulses per second. The most efficient frequency for strength training was reported to be within the 50 to 120 Hz range (97). The amplitude is the intensity or the magnitude of the current (58), and it must reach the threshold of nerve fibre excitation (6). The intensity of EMS determines force output (71, 232); however, it is limited by the subject's tolerance (57), and is often set according to the maximal tolerance of a subject. Pulse duration is the sum of bidirectional waveform including positive and negative phases (200). Pulse duration and amplitude determine the phase (or pulse) charge or electrical current dose delivered by each stimulus, and it is represented by the current time integral of each pulse (pulse duration × stimulation amplitude). Pulse duration plays an important role in force

production (89, 90). For example, muscle force was decreased when pulsed duration was shortened from 450 to 150 μs (90). Duty cycle is the percentage of the electrical current flow such that the duration of stimulation (on-time) and no-stimulation (off-time). Duty cycle determines the rate of muscle fatigue, and generally a shorter off-time induces greater fatigue.

The waveform and current type of EMS are dependent on the choice of stimulator (152). *Current* is the flow of electrons and *waveform* is the behavioral modulation of electron flow. In EMS, three types of waveforms are generally used: monophasic (one direction), biphasic (two directions) and polyphasic (uninterrupted bidirectional). The shape of EMS waveforms can be rectangular, square, triangular, sinusoidal or spike, and it can be symmetric (positive and negative phase are equal in shape) or asymmetrical (positive and negative phase are not equal in shape). Frequency and pulsed duration of EMS are affected by the shape of waveforms, thus affect force generation as explained above. There are two types of currents commonly used in EMS: alternating current and pulsed current.

Alternating current is a continuous series of alternating pulse that forms one burst consisting of connected biphasic medium or high frequency pulses (polyphasic) of the unit of kilohertz (kHz) (199, 240). Alternating current can be delivered in the range of 1-25 kHz (pulse per second) modulated at 1-150 Hz (burst per second) (245). In contrast, pulsed current delivers separated pulses at generally 1-150 pulses per second (1-150 Hz) with an interval between pulses (245). Since some portable or battery-operated stimulators can deliver only pulsed current, pulsed current has been more widely used in muscle training (152). The use of alternating current in muscle strengthening increased since a Russian scholar Kots had reported advantages of

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alternating current EMS (2.5 kHz), so called a Russian current (240, 249). For a better understanding of the optimal parameters of EMS to produce best possible outcomes after training with EMS, it is necessary to understand acute physiological responses of muscles stimulated by EMS. It is possible that differences between alternating current and pulsed current exist for acute effects on different physiological variables such as muscle force generation, discomfort, skin temperature, hormonal responses, muscle metabolism, and muscle fatigue. However, these have not been systematically investigated in the previous studies, although several studies (96, 109, 148, 152, 216, 239) investigated muscle force generation during alternating and pulsed current EMS.

Snyder-Mackler et al. (216) compared the torque output of the knee extensors between alternating current and pulsed current, and found that the torque output was significantly lower for 4-kHz alternating current compared with 2.5-kHz alternating current and 50-Hz pulsed current, but no significant difference in torque output was evident between the last two currents. In another study that compared the quadriceps femoris torque generation between currents, the authors reported that the torque was significantly higher for the pulsed current (50-Hz) than 2.5-kHz alternating current (147). On the contrary, several studies reported no significant difference in isometric torque of the knee extensors between alternating current and pulsed current (96, 109, 146, 152). The majority of these studies used different stimulators for the comparison between alternating current EMS and pulsed current EMS; they did not match the other parameters, such as pulse duration and frequency. Thus, the ideal comparison between currents can be achieved when all parameters are kept identical except the waveform, and when the same stimulator is used. This has not been done in the previous studies.

Several studies have compared the level of discomfort between alternating current and pulsed current EMS (25, 96, 146, 152, 243). For example, Lyons et al. (152) reported that discomfort was similar between alternating current (2.5 kHz delivered at 75 Hz) and pulsed current (75 Hz) when they were applied to quadriceps. Laufer and Elboim (146) compared three alternating currents (2.5 kHz delivered at 50, 50 and 20 Hz with burst duty cycle of 1:1, 1:4 and 1:4, respectively) and pulsed current (50 Hz) EMS applied to the forearm. They showed higher degree of discomfort during alternating current with lower modulated frequency (20 Hz) compared with others. In contrast, Ward et al. (243) showed that subjects felt more uncomfortable with pulsed current EMS (50 Hz with pulse duration of 200 or 500 μs) compared with alternating current EMS (2.5 and 1 kHz with pulse duration of 200 and 500 μs, respectively). It is necessary to clarify whether a difference in discomfort exists between alternating current and pulsed current EMS in relation to muscle force level when muscles are stimulated at maximally tolerable intensity.

Working muscles generate heat that increases the body temperature (83). In a cooling process of the body temperature, heat is transferred to peripheral regions to maintain the homeostatic balance and the thermal neutrality (83, 132). Skin temperature provides an indication of cutaneous vascular responses to exercise (115, 123), and it is affected by the vasodilation and vasoconstriction of skin blood vessels (92, 221). Although the effect of voluntary isometric exercise on cutaneous vascular responses has been documented (76, 78, 124, 125), little information is available about the changes in skin temperature during and after EMS (16). No previous study has compared skin temperature responses between alternating current and pulsed current EMS at the maximally tolerated intensity.

Little is known about the effect of EMS on hormonal secretion. To date, only two studies investigated the effect of EMS on growth hormone (GH) and cortisol responses (121, 204). Jubeau et al. (121) reported that increases in GH were significantly greater for EMS compared with the voluntary isometric contractions of the same force output. Sartorio et al. (204) compared the first and second EMS bouts (75 Hz with pulse duration of 400µs), and showed significant increases in GH following EMS, and a significant decrease in cortisol at 60 min after the first EMS with no significant difference between bouts. Testosterone and insulin-like growth factor (IGF-I) have been shown to increase in response to resistance exercise (3, 206); however, the effect of EMS on these hormones has not been investigated in the previous studies. No previous studies have yet compared alternating current and pulsed current EMS for hormonal responses.

Several studies have reported an increase in blood lactate concentration after pulsed current EMS exercise (99, 107, 121). For example, blood lactate concentration significantly increased during and after 40 isometric EMS contractions (75 Hz) for quadriceps muscles (121). However, the influence of alternating current on blood lactate changes is not clear.

During exercise, oxidative metabolism in muscle is considered the main energy sources (72), thus the assessment of oxygen ($O_2$) consumption during EMS is important. Several studies compared $O_2$ consumption between EMS-evoked and voluntary isometric contractions. Theurel et al. (225), for example, found greater $O_2$ consumption during 40 isometric contractions of the knee extensors evoked by EMS (75 Hz) compared with voluntary contractions at the same force output. Near-infrared spectroscopy (NIRS) can continuously measure local muscle oxidative metabolism (19,

73, 172), and NIRS have been used to compare between voluntary contractions and EMS (18, 170, 171). However, it is not known if alternating current and pulsed current differ in muscle oxygenation. Some studies have documented that alternating current EMS causes greater muscle fatigue than pulsed current EMS (146, 147, 220). It could be speculated that when muscle fatigue occurs in EMS, the number of muscle fibers activated is reduced, which may be detected by a decrease in muscle oxygenation.

It has been documented that muscle contractions evoked by EMS result in muscle damage (27, 121, 154, 179). For example, Jubeau et al. (121) showed that isometric contractions of the knee extensors evoked by EMS (75 Hz, pulse duration: 400μs) induced greater muscle soreness and increases in serum creatine kinase (CK) activity compared with voluntary isometric contractions of the same muscle that generated the same force as the EMS protocol. Mackey et al. (154) reported histological evidence of muscle damage, increased plasma CK activity and muscle soreness after 180 isometric contractions of the gastrocnemius muscle evoked by EMS. However, no previous study has investigated muscle damage induced by alternating current. It is well known that the magnitude of muscle damage is attenuated and recovery is enhanced when the same or similar eccentric exercise is performed again in several weeks (165, 177, 179). This protective effect induced by a single bout of eccentric exercise is often referred to as the repeated bout effect (37). It may be that the initial EMS bout results in muscle damage, but the second one induces lower or no muscle damage. However, no previous study has compared between the first and second bouts of isometric contraction induced electrically for changes in markers of muscle damage.

Therefore, the present thesis project was designed to compare alternating current and pulsed current EMS for their acute effects on varied physiological responses by

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focusing on muscle strengthening aspects of EMS for healthy individuals. The investigation of the acute effect of EMS on muscle and systemic changes is fundamental for understanding the training effect.

## 1.2 PURPOSE OF THE STUDY

The main purpose of this thesis was to compare between alternating current and pulsed current EMS for their acute effects on torque generation, discomfort, skin temperature, hormonal responses, blood lactate, muscle oxygenation, muscle fatigue, and muscle damage. To achieve the purpose, two studies were conducted. In the first study, changes in force generation, skin temperature, blood lactate concentration, hormone concentrations (GH, IGF-I, cortisol, testosterone) were compared between alternating and pulsed current EMS. The second study was designed to compare between alternating current and pulsed current for its effects on muscle oxidative metabolism, blood lactate and muscle fatigue. In addition, a third study was conducted to examine the repeated bout effect following a second bout of EMS performed two weeks after the first bout. The changes in muscle damage markers were compared between the first and second bouts separated by 2 weeks.

## 1.3 SIGNIFICANCE OF THE STUDY

EMS has several applications in either treatment or training, and the use of EMS in sport training or in exercise has been increasing in the last two decades (15, 166). However, the optimal parameters and currents of EMS to maximise the output have not been confirmed (16, 248). The EMS currents are most important parameters since they determine which type of stimulator can be used for either clinical, training or research

settings. Outcomes of this research thesis would contribute to the existing body of knowledge about the effect of alternating current and pulsed current EMS on torque generation, muscle damage, hormonal responses, skin temperature and muscle oxygenation, which provides better understanding of the theory and application of EMS in sport training.

## 1.4 RESEARCH QUESTIONS AND HYPOTHESES

The studies above were expected to answer the following research questions:

1. Is there a difference in force generation between alternating current and pulsed current EMS?

   No significant difference is evident between alternating current and pulsed current.

2. Is there a difference in the level of muscle soreness and tenderness between alternating current and pulsed current EMS?

   Alternating current is expected to cause more muscle soreness and tenderness than pulsed current.

3. Is there a significant difference in skin temperature changes between alternating current and pulsed current EMS?

   The changes in skin temperature are presumed to be higher during alternating current compared with pulsed current.

4. Is there a significant difference in hormonal responses between alternating current and pulsed current EMS?

   It is liklely that both alternating current and pulsed current have similar hormonal responses.

5. Is there a significant difference in blood lactate responses between alternating current and pulsed current EMS?

Higher blood lactate is expected during and following alternating current than pulsed current.

6.  Is there a significant difference in muscle oxygenation between alternating current and pulsed current EMS?

    Greater changes in muscle oxygenation are expected during alternating current compared with pulsed current.

7.  Is there a significant difference in muscle fatigue between alternating current and pulsed current EMS?

    Muscle fatigue is assumed to be higher during alternating current compared with pulsed current.

8.  Dose muscle damage induced by EMS differ in the case of alternating current than in the case of pulsed current?

    Alternating current is thought to induce greater muscle damage than pulsed current.

9.  Is the magnitude of muscle damage after the second bout of EMS less than that after the first EMS bout?

    No or less muscle damage is expected after the second bout of EMS compared with the first bout.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1 OVERVIEW

The current knowledge about the difference between the currents of neuromuscular electrical stimulation or electrical muscle stimulation (EMS), particularly alternating current and pulsed current, will be discussed in this chapter. Initially, the general principles of EMS are briefly explained including physiology and parameters. Subsequently, the effects of EMS on skin temperature, hormones, blood lactate, muscle oxygenation, fatigue and muscle damage are reviewed.

## 2.2 INTRODUCTION

### 2.2.1 Basic aspects of electricity

The simple definition of electricity is the flow of electric charge, which is mainly affected by four main properties: energy, potential difference, resistance and current. Energy is the power to move the charge, and it is measured by the work done per unit time. Potential difference or voltage is the pressure to bring charges between the two ends of any charge system, and it is measured by Volte. Impedance that is applied to the charge flow refers to the resistance, which is measured in Ohms. Current describes the behavior of the charge flow, and it generally comes in two forms: alternating current and direct current. During alternating current, the flow of charges or electrons is in both directions (positive and negative) to form a back-and-forth motion, while the flow of charges during direct current is in only one direction. Alternating current is widely used at home and in factories while direct current is used for small devices in a stored form,

such as batteries. The electrical current that is used for a clinical or training purpose is different than normal electricity. The current that is generated by electro-medical appliances should be intermittent for a period of micro- or millisecond.

There are three types of currents mainly used in clinical and training applications (199): direct, pulsed and alternating. The charge during direct current flows in one direction and sustains for at least one second. This type of current is usually used for iontophoresis through which ions are expelled via the skin. Alternating and pulsed current are usually used to induce muscle contractions. These types of current are extensively explained later in this chapter.

### 2.2.2 Electrical muscle stimulation

EMS or transcutaneous EMS is an application through which an electrical current, which is delivered by a stimulator, travels through the skin to initiate action potentials in sensory and motor nerves and then generate muscle contractions. Its application to neuromuscular tissue to evoke muscle contractions has been used since the eighteenth century (80), and the modern techniques and parameters of EMS were developed during the last century (199). EMS has a wide range of uses in rehabilitation (186) including injury treatments (145), preventing atrophy (8) and strength improvement (15, 209). However, the optimal parameters for EMS to achieve a desirable output are still controversial.

### 2.3 MUSCLE CONTRACTION DURING EMS

Although the general use of EMS in sport science is to imitate voluntary contractions, the mechanism and activation of skeletal muscle contractions during EMS

and voluntary actions are different. The main differences between EMS and voluntary contraction are the stimulus origin and the recruitment patterns of motor units (MUs).

**2.3.1 Contribution of the peripheral and central nervous systems**

It is obvious that the stimulus is generated from the central nerve system during voluntary contraction. During EMS, however, the muscle contraction is initiated peripherally and the peripheral activation is mainly donated to the contracting muscles, even some contributions of central nerve system were reported (44, 55). During voluntary contraction, a nerve stimulus that resides in the brain or spinal cord travels through long threadlike extensions, known as axons, to the targeted muscle cells. Indeed, the origins of all voluntary and involuntary contractions in the human body, including unconscious, automatic and reflex, are the central nervous system (the brain and the spinal cord). During EMS action, however, the depolarization of motor axons is directly evoked through a peripheral mechanism so that the stimuli, which come from the electrodes adjusted to skin, activate muscle excitation. Thus, the activation processes occur at the level of the peripheral rather than the central nervous system. In fact, the stimulation using EMS does not bypass the central nervous system since the sensory fibres are involved in the stimulation actions although the stimuli in EMS are triggered peripherally. It was thought that the activation of sensory fibres, which occurs during more units" activation in EMS, involves more motor units from the central nervous system by exciting synaptic input in spinal neurons (44, 54). The contributions of the central mechanism to contractions during EMS were reported to be mainly affected by long pulse duration, high frequency and at a relatively low intensity (54, 55). Moreover, the central mechanism was thought to contribute largely to force production (up to 42% MVC) during EMS at a wide pulse duration of 1-ms (45). Though the activation of

muscle fibres during EMS is assumed to be in a non-selective order (94), central contribution was hypothesized to be preferentially with the most fatigue-resistant muscle fibres (44, 55) than it tends towards the natural order of recruitment.

### 2.3.2 Fibre recruitment in voluntary and EMS contractions

The recruitment of voluntary MUs is suggested to follow the Henneman size principle, where small and slow motor units are recruited first and followed by the larger and fast motor units in an increasing size order (104). In view of that, the size principle was believed to be reversible in EMS since it is generally accepted that the fast fibres are thought to locate more peripherally and slow fibres more deeply toward bones so that there is more distance between electrodes and small motor units (65). In addition, fast fibres have lower action potential threshold than slow fibres, which initiates the depolarization in fast fibres before slow fibres in EMS (65). However, Feiereisen et al. (69) investigated the recruitment thresholds of motor units in the tibialis anterior muscle during voluntary and EMS contraction, and found that 94% of trails followed the size principle in voluntary contraction while only about 30% did so in EMS contractions. Thus, the phenomenon of the revised size principle may not be the case during EMS. In agreement with that, Knaflitz et al (131) found the principle size order in voluntary contraction was similar to about 72% of trials that followed EMS contraction. Based on these findings, it was suggested that motor units are recruited non-selectively and spectrally during EMS with no emphasis on the size or type of motor units (94, 120) and predominantly MUs that locate peripherally and close to the electrodes (235). The spatially fixed/non-selective pattern of motor units during EMS may lead to muscle fatigue since all fibres are simultaneity recruited (94) while in voluntary contraction the MUs are recruited asynchronously. Hence, all motor units are

stimulated at the same time due to the constant discharge frequency, during which muscle exhaust energy and accelerate fatigue. However, more motor units alternately facilitate voluntary muscle contraction, which lessen muscle fatigue.

Figure 1 shows two different motor units that stimulate contiguous and imbricate muscle fibres. It is possible during voluntary contraction to only activate motor unit 1 while motor unit 2 is resting but in EMS stimulation, both motor units are activated synchronously.
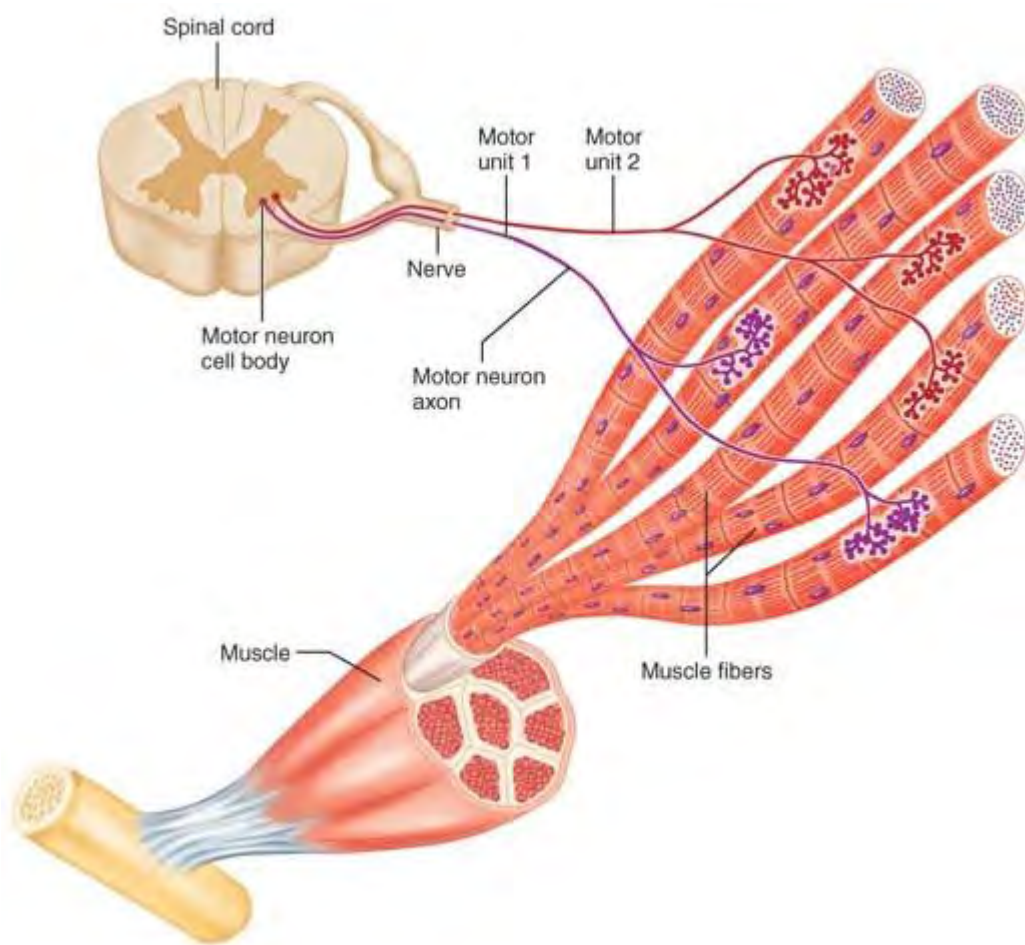


Figure 1: Schematic view of two motor units that activate different contiguous muscle fibres (Reproduced from [Marieb & Hoehn 2006]).

This difference in muscle activation between EMS and voluntary contraction raises an argument with respect to the difference in excitability level and in the amount

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of force production among MUs. The large-diameter axons are thought to have lower activation threshold and are typically located superficially (217), which can be more easily excited by EMS than by small-diameter axons. Also, the large-diameter axons are generally associated with large MUs and typically activate fast muscle fibres (94). Hence, it can be theoretically speculated that EMS mainly stimulates large MUs, which yields more force output, to provoke fast muscle fibres that cause more muscle fatigue. This may be the case for muscle fatigue where less muscle fatigue was reported at a comparable level of torque output with voluntary contraction (225) but voluntary contraction was shown to induce greater force than EMS at the maximal intensity (171). However, the muscles can respond differently when the EMS parameters are altered.

## 2.4 EMS PARAMETERS

Although EMS has been widely used in rehabilitation and strength training, the optimal parameters of EMS are still unidentified. There are several parameters of EMS that can characterize the EMS waveforms and optimize the physiological effect. These parameters include: amplitude, phase duration (Figure 2), frequency, duty cycle, ramping time and current types.
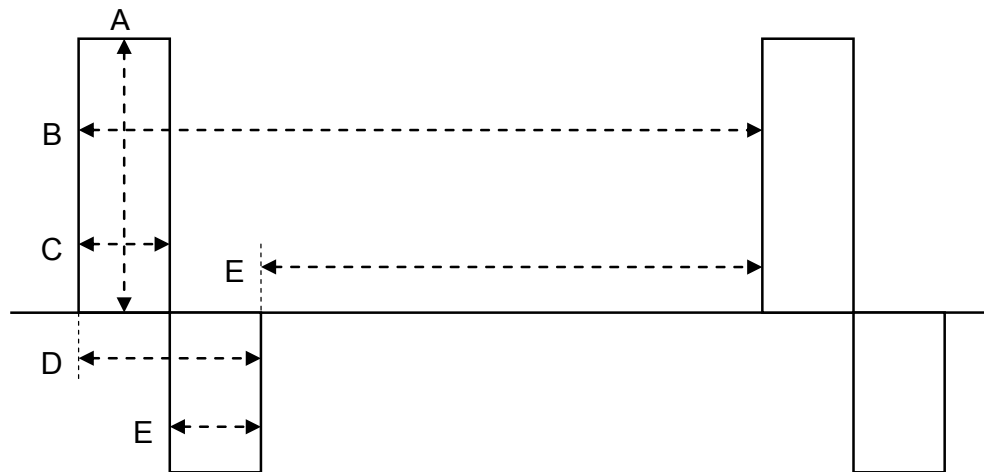
Figure 2: An illustrated diagram of a typical pulsed current waveform. **A**: Pulse amplitude, **B**: Pulse period, **C**: Phase duration, **D**: Pulse duration, **E**: Inter-pulse interval.

### 2.4.1 Amplitude

The amplitude or intensity is the magnitude of current and it is typically expressed as milliampere (mA) for constant current and millivoltage (mV) for constant voltage. When a constant current generator is used, the voltage will vary to maintain a constant current between electrodes while constant voltage generator will maintain a constant voltage irrespective of the variation in current flow due to the impedance of electrodes and tissues. An adequate level of the intensity (hereafter referred to as amplitude) is needed to exceed the motor units" threshold and to induce muscle contraction. However, high intensity applied transcutaneously through surface electrodes that may cause burn injuries to the skin (195). The intensity is so inversely proportional to the phase or pulse duration that high intensity requires short pulse duration to achieve action potential of nerve fibres and vice versa (Figure 3). The intensity is usually adjusted according to the level of subjects" comfort or tolerance. Several studies have reported a significant effect

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of current intensity on the induced torque output and muscle activation (2, 22, 90). For example, Adams and others (2) reported an increase in the knee extensor muscle fibres activated from 18% to 54% when the intensity was increased to increase MVC from 25% to 75%. In the present thesis, the intensity was set at the maximal tolerance for achieving high torque output.

**2.4.2 Phase duration**

Phase or pulse duration is the measured time for the entire period that the line of waveform is above zero in monophasic waveforms or above and below zero in biphasic waveforms, respectively. This duration is expressed as millisecond (ms) or microsecond (μs). As mentioned in the intensity, sufficient time is required to overcome the nerve fibre threshold potential (see Figure 3). It should be noted that the "stimulation duration" is not "phase" or "pulse" duration. Stimulation duration refers to the whole session of stimulating, while "phase" or "pulse" duration represents the time for a single phase or pulse. Pulse duration can play an important role in torque production (89, 90). Although the stimulated motor units can be activated and maintained with a long stimulation duration, pulse duration was reported to maximize torque output more than stimulation duration (89). In addition, it is thought that skin impedance is greatly influenced by pulse duration (240), where longer duration can lower the skin capacitive impedance, and then the current can penetrate deeper. However, some controversies exist among studies about the effect of pulse duration on force generation. Pulse duration of 500 μs was reported to produce 40% greater torque compared to 150 μs (111), while other studies showed 100 μs generated the largest torque among varied pulse durations that ranged between 5 and 1000 μs (6).

Some evidence showed that pulsed duration of 300 µs is more comfortable than 50 µs for pulsed current (31) and burst duration between 1-4 ms is best for sensory, motor and pain threshold during alternating current (241). The optimal pulse duration for torque output is still indistinct due to the integration between the pulse duration, the intensity and the variation in excitatory responses as shown in Figure 3. This figure shows the difference in the behaviour of the thresholds of four different fibres and responses: (A) sensory threshold, (B) motor threshold, (C) pain threshold and (D) maximal tolerable pain response, based on their strength-duration properties. The curves show how long pulse duration (in µs) and how much stimulus intensity (in mA) are needed to reach the activation thresholds of the fibres and responses. It is obvious that the sensory fibres require lowest intensity to be activated while the maximal tolerable pain response has the highest needed intensity in the figure. However, the difference between the thresholds becomes smaller as the pulse duration gets bigger, which indicates the great influence of pulse duration on the fibre activation.
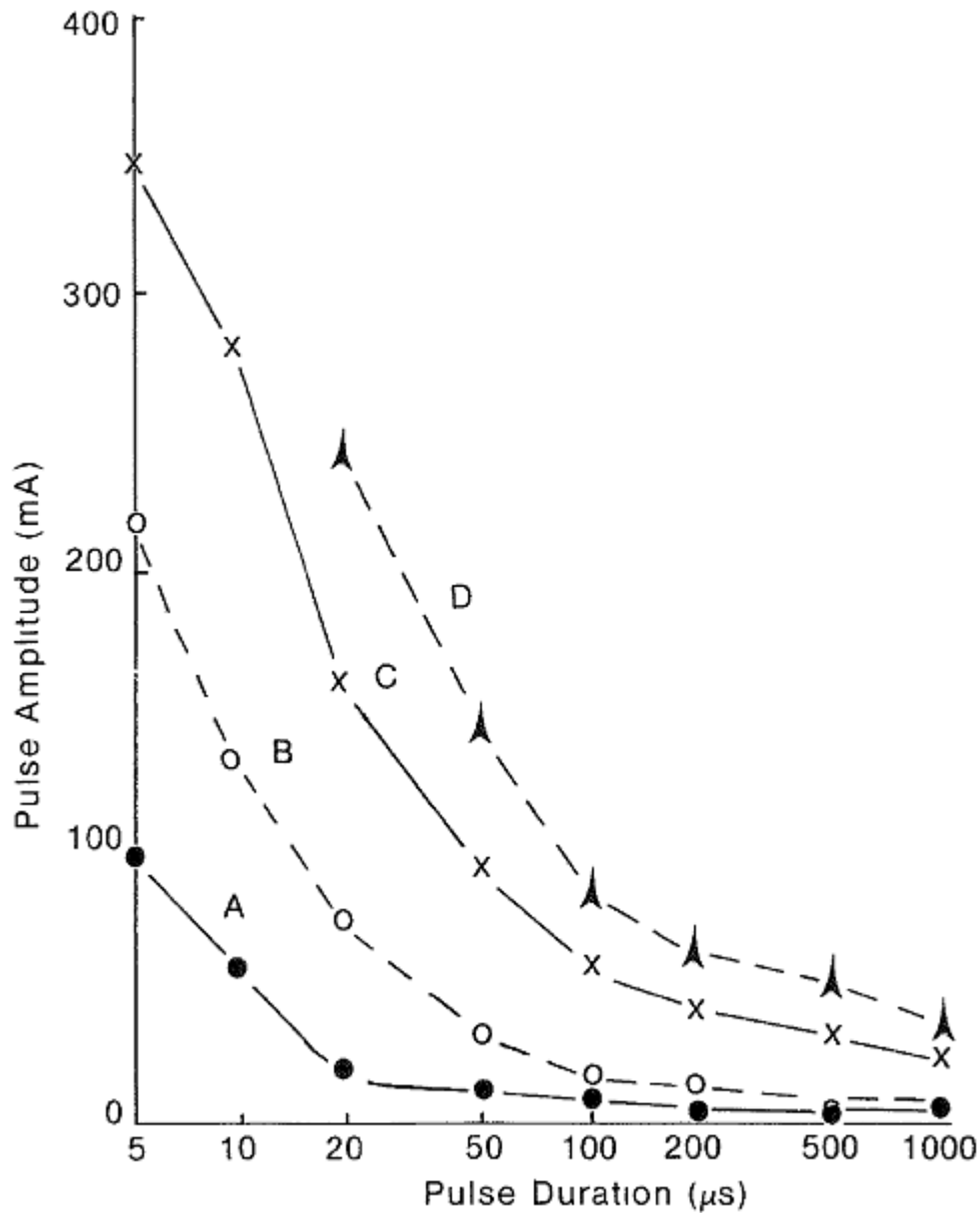
Figure 3: Changes in strength-duration curves of (A) sensory threshold, (B) motor threshold, (C) pain threshold and (D) maximal tolerable pain response. Reproduced from Alon et al (6).

A linear relationship between the intensity and pulse duration can be represented by phase charge, which is defined as the current time integration of each pulse or the area under entire pulse waveform including positive and negative phases, and it is represented by the current time integration of each pulse (pulse duration × stimulation

amplitude). Phase or pulse charge is electrically expressed as the number of electrons transmitted in each phase or pulse (58) and expressed in micro-Coulomb (μC). Depending on the characteristics of each nerve's fibres (size and the formation of the myelin), a certain level of phase charge is needed to reach or exceed the nerve threshold levels (either sensory or motor) and generate action potentials (6, 122).

Phase charge was found to affect the degree of motor unit activation (29) and the torque output during EMS contraction (122, 147), and it is affected by the shape of the waveforms (122). For instance, rectangular waveforms that are generated by constant current, have higher charge than triangular or sinusoidal waveforms at the same intensity level and phase duration due to the larger area-time integral.

### 2.4.3 Frequency

Frequency refers to the number of phases (in monophasic waveform), pulses (in biphasic waveform) or bursts (in alternating current) delivered per second, and it is specified by hertz (Hz) or kilohertz (kHz). It is common to modulate kHz frequencies, as trains or bursts per second, to be cycled within the physiological range 1-150Hz (199, 245) so that the nerve fibres can respond to stimuli. The frequency rate should be increased to achieve a summation of stimulations where nerve fibres are recruited and muscle contraction is evoked (i.e. < 20 Hz). Muscle tension is mainly influenced by motor unit firing frequency, which is controlled by EMS frequency during stimulation (64). Hence, all the stimulated nerve fibres are fired at the same frequency rate so that the motor units are activated synchronously as mentioned previously. It is generally accepted that the stimulation frequency is a major factor that affects the force induced by muscle contraction including the number of muscle fibres and muscle length during

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stimulation (161). Stimulators have different capacity to adjust the frequency and it is named as "rate" or "frequency rate" in some devices.

The frequency varies according to the purpose of the stimulation. For example, a firing rate of 50-120 Hz is usually effective for enhancing muscle strength and power (56, 159, 214). Force generation and discomfort have been shown to be influenced by the frequency rate (246). For instance, the frequency of 20 Hz was reported to produce lower torque output of knee extensors compared with 50 and 100 Hz (142). However, no difference was found between 50 and 100 Hz in torque output (142). In an early study by Edwards et al. (64), they found an increase in torque output of knee extensors as the frequency rate was increased from 3 to about 50 Hz, and then no further increase in the force was found up to 100 Hz. Moreover, stimulation at 100 Hz showed greater force generation of both tibialis anterior and triceps surae group than 200 Hz (45), which may occur because of some blockage in neuromuscular activities during high frequency. Figure 4 represents the maximum force (% of MVC) that can be obtained by a range of frequencies from 3 to 100 Hz for two difference muscles (64). These studies showed evidence that the frequency rate of 50-100 Hz can be the optimal rate for force generation. However, the limitation of using these levels of frequency rates in muscle fatigue where frequency rates exceed 50 Hz is characterized as high-frequency fatigue that may be caused by a neurotransmitter depletion and/or propagation failure (117). Since the recovery of high-frequency fatigue is rapid (117), long durations between contractions (off-time) may reduce the influence of this type of fatigue on muscle performance. The modulation of frequency by a gradual decrease during stimulation was speculated to limit the effect of fatigue and maintain the force output throughout the stimulation period (118). However, increasing frequency during fatiguing trains showed better performance for both peak forces than constant frequency (126). However, the

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modulation of EMS parameters, particularly frequency, is not a common feature among stimulators.
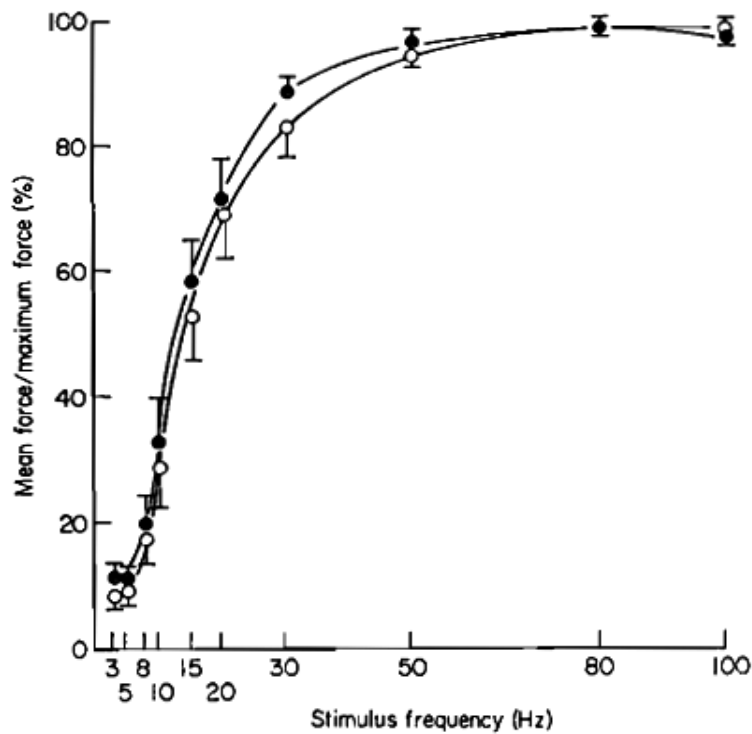


Figure 4: The changes in force-frequency curve over different frequencies of quadriceps (●) and adductor pollicis (○). Reproduced from Man et al. (64).

### 2.4.4 Duty cycle

The duty cycle is the proportion of time where the stimulation is present and absent and is defined as the ratio of on-time to off-time. The stimulus duration is a requirement for determining the duty cycle of stimulus. Without knowing off-time stimulus, it is impossible to determine the duty cycle. Thus, two contractions or more with known and fixed off-time are necessary to calculate the duty cycle of EMS exercise. One of the aforementioned studies (109) used only one evoked contraction to compare between currents, while the other one (216) used two contractions but with

unfixed off-time between contractions (minimum of 2 min). For these studies, the duty cycle could not be measured.

Duty cycle calculation can be made for pulses, bursts or stimulation by dividing the on-time on the total time as $\frac{on-time}{on-+off-time}$ (199). For example, if the pulse duration is 1 ms delivered at 100 Hz so that the off-time should be 9 ms and the calculation of pulses duty cycle would be $\frac{1}{10} \times 100$, where 10 is the pulse period (on + off-time). This means that the time of current flow equals 10% of the entire period of stimulation. The duty cycle can be also calculated for the stimulation based on the on-off time, so that if the on-off time ratio is 10:30 (10 s „on" followed by 30 s „off"), the duty cycle of stimulation will be $\frac{10}{10+30} \times 100 = 25\%$.

Ward et al. (247) reported that the maximal torque output can be achieved at burst duty cycle between 10-20% of alternating current at different burst frequencies. A direct relationship has been reported between stimulation on-time and muscle fatigue (185). Laufer and Elboim (146), for example, found that during alternating current (2500 Hz), greater muscle fatigue was induced by burst duty cycle of 50% by 20% at similar torque output. Obviously, longer off-time with respect to the on-time of stimulation will result in less muscle fatigue over time (145). This suggests that with lower duty cycle (i.e. 20%) less fatigue can be obtained.

### 2.4.5 Ramping time

Ramping (or modulations) is a gradual increase or decrease in each on-time stimulation period. In practical terms, it is a manipulation in EMS parameters to raise the current from zero to the peak of intensity and to drop the current from the peak to zero over a specific time period. While the ramping time is usually used to describe the

changes in several pulses or bursts, rise time is used to describe the changes in a single pulse. Stimulation can be ramped by modulating frequency, intensity or pulse duration depending on the objectives of the stimulation and the stimulator"s capabilities. Nerve fibres threshold may accommodate to a long ramping time period based on the general law of the nerves excitation (Du Bois Reymond law). Thus, a short rise time is necessary to avoid membrane accommodation.

The logical assumption behind using ramp time in muscle stimulation is to decrease the  habituation rate (218) that may reduce the pain perception in the sensory fibres. Ramping may help to avoid the massive current that abruptly provokes sensory fibres as well as to mimic voluntary contraction by a gradual increase in contractile force. When the level of stimulation parameters (i.e. intensity) is above the sensory fibre threshold and the aim of stimulation is to activate motor fibres, a shock response may take place and vary the subjects" comfort and tolerance of current. Nonetheless, some evidence shows no effect of ramp time on the sensory, motor and pain thresholds and muscle soreness (9, 10).

## 2.4.6 Currents and waveforms

Current is the flow of electricity or charge per time unit while waveforms determine the behaviour of this flow. In EMS, current and waveform are used alternatively to describe the current of stimulation. The most common currents of EMS are alternating current and pulsed current. In general, the therapeutic use of electrical stimulation had not started until the Faradic stimulator was discovered in the 1800s, which could deliver pulses or short duration of electrical current (80). This was known later as a pulsed current. Few decades later, a new current was demonstrated by a French physician (d'Arsonval) who was able to magnetically stimulate excitable tissue

using eddy currents (79), which become the base for using alternating current in biological tissues. Since that date, pulsed current has been more common in muscle training because it can be delivered using handy and inexpensive portable or battery-operated stimulators (152) while alternating current can only be generated by clinical stimulators. Nonetheless, Kots a Russian researcher, was the first who used alternating current in sport training, claimed an increase in muscle force (up to 40%) following alternating current training (143). His findings were reviewed in the English language literature by Selkowitz (208) who found little evidence to compare force gain using alternating current with voluntary exercise or a combination of voluntary and EMS exercise.

As shown in Figure 5, alternating current is delivered as continuous or uninterrupted pulses while pulsed current is delivered as pulsatile or interrupted pulses. Alternating current and pulsed current frequency is expressed as hertz (Hz). However, since muscle fatigue occurs rapidly and during kilohertz frequencies (23), the frequency of alternating current is modulated to cycles or bursts per second within the physiological range, 1-150 Hz (199, 240). The waveforms of alternating current must be reciprocal or bidirectional and comprise three pulses and more (polyphasic), while pulsed current can be unidirectional (monophasic) or bidirectional (biphasic).

EMS stimulators can be determined by the choice of EMS currents and other factors. Generally, there are two types of stimulators: mains and battery powered (199). The major advantages and disadvantages of these stimulators are summarised in Table 1. The easy accessibility to the portable stimulators would make pulsed current more common than alternating current.

Table 1: Advantages and disadvantages of mains- and battery-powered stimulators.

| Stimulators | Advantages | Disadvantages |
|---|---|---|
| Mains-powered | • Multi-stimulator<br>• Generate a large range of parameters | • Potentially not safe (output intensity is high)<br>• Expensive and not easy to be used<br>• Relatively large |
| Battery-powered | • Portable<br>• Easier<br>• Safer<br>• Cheaper | • Limited adjustment of currents parameters<br>• Limited treatment duration |

Pulsed current (monophasic)

Pulsed current (biphasic)

Alternating current (polyphasic)
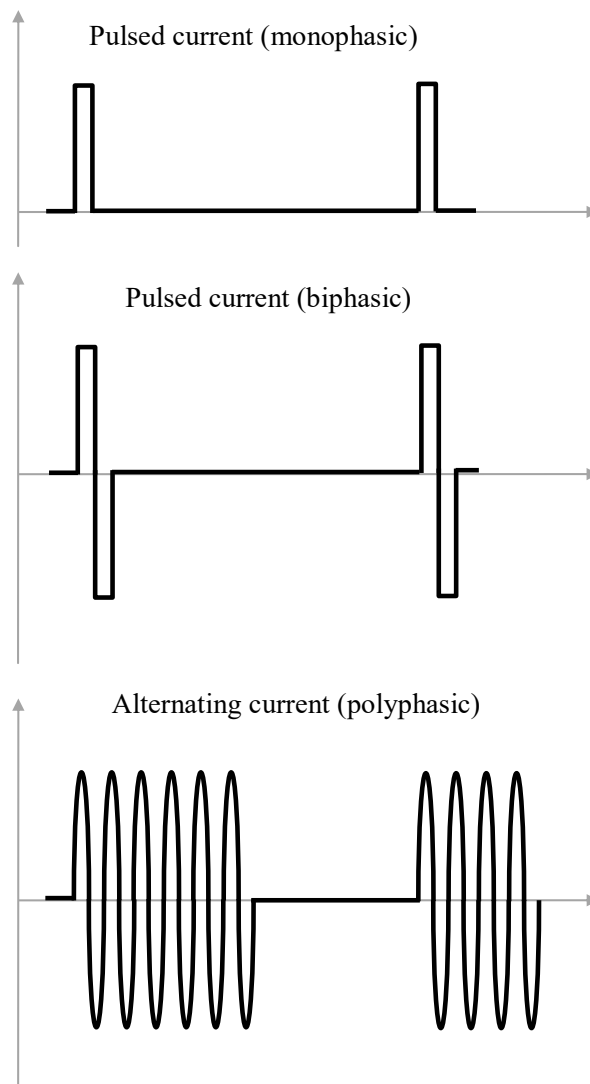
Figure 5: A schematic diagram illustrates the difference between alternating current and pulsed current.

It was assumed that alternating current can overcome skin impedance in transcutaneous peripheral nerve stimulation that the skin impedance is thought to be very low at kilohertz frequency of current (196). However, this is probably not correct because the skin impedance is largely affected by pulse duration more than by frequency (240). Thus, the skin impedance would be similar to alternating current and pulsed current when they have the same phase or pulse duration (240). It can be speculated that less current is dissipated in the superficial epidermis and consequently more current is available to stimulate underlying tissues (248). However, the advantage of alternating current over pulsed current for achieving desired physiological responses is yet to be established.

Several studies have compared between pulsed current EMS and alternating current EMS for acute (96, 109, 146, 147, 152, 216, 239, 243) and training effects (25, 220). These studies reported the difference between currents for torque output and increasing strength. However, the difference between alternating current and pulsed current is not clear for other physiological variables, such as muscle damage and hormonal responses. These responses may play an important role in sport performance and chronic adaptation. Moreover, most previous studies that compared between currents for their acute effects used different stimulators. Stimulators may be a factor and may have an influence on output even when other parameters were held identical. Therefore, the investigations of this thesis compared the acute effects of pulsed current and alternating current on several physiological aspects such as muscle damage, hormonal responses and muscle oxygenation, using the same stimulator to minimize the manufacturing variation.

## 2.5 EMS AND TORQUE GENERATION

The capability of EMS to generate torque is well documented and it is beyond the scope of the present thesis. The main focus will be on how alternating current differs from pulsed current for torque generation. From the literature, several studies have compared force generation following alternating current and pulsed current (Table 2).

Table 2: Previous studies comparing between alternating current and pulsed current for peak torque generated during isometric contraction evoked by EMS in healthy subjects.

| Study (year) | Muscle, application effect | EMS characteristics | | | | | | Torque % of MVIC | Comparison for force production |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | Current | Waveform shape | Frequency | Pulse/phase duration | Stimulus amplitude | Stimulator | | |
| Walmsley et al., 1984 (239) | Knee extensors | AC1* | Sinusoidal | 4000, 4100 Hz at 75 bps | | S. tolerance | Nemectrodyn 8 | ~ 65 | AC1 = PC1 |
| | | PC1* | Spike waveform | 60 Hz | 200 µs | S. tolerance | Multitone | ~ 45 | |
| | | AC2 | Sinusoidal | 2200 Hz at 50 bps | 450 µs | S. tolerance | Electrostim 180-2 | ~ 82 | AC2 = PC2 |
| | | PC2 | | 50 Hz | 200 µs | S. tolerance | Ultra-pulsator 5 | ~ 85 | |
| Snyder-Mackler et al., 1989 (216) | Knee extensors | AC1 | Sinusoidal polyphasic | 2500 Hz at 50 bps | 200 µs | S. tolerance | Electrostim 180-2 | 61 ± 32 | AC2 < AC1 and PC; AC1 = PC |
| | | PC | Symmetrical biphasic square | 50 Hz | 200 µs | S. tolerance | Chattanooga VMS | 54 ± 28 | |
| | | AC2 | Sinusoidal | 4000, 4050 Hz at 50 bps | 125 µs | S. tolerance | Nemectrodyn 7 | 40.1 ± 21.8 | |
| Grimby and Wigerstad-Lossing, 1989 (96) | Knee extensors | AC | Sinusoidal | 2500 Hz at 50 bps | 10 µs | S. tolerance | Electro-Stim 180 | 26 ± 3 | AC = PC |
| | | PC | Monophasic rectangular | 30 Hz | 300 µs | S. tolerance | Respond II | 21 ± 4 | |
| Holcomb et al., 2000 (109) | Knee extensors | AC | Sinusoidal polyphasic | 2500 Hz at 95 bps | | S. comfort | Forte 400 Combo Chattanooga | 24 ± 4 | AC = PC |
| | | PC | Symmetrical biphasic square | 95 Hz | 200 µs | S. comfort | | 21 ± 4 | |

| Study | Muscle | Group | Waveform | Frequency | Pulse duration | Outcome measure | Device | Force | Comparison |
|---|---|---|---|---|---|---|---|---|---|
| Laufer et al., 2001 (147) | Quadriceps femoris | PC1 | Monophasic rectangular | 50 Hz | 200 µs | S. tolerance | Staodyn EMS | $37 \pm 17$ | AC < PC1 and PC2 |
| | | PC2 | Symmetrical biphasic rectangular | 50 Hz | 200 µs | S. tolerance | Staodyn EMS | $38 \pm 17$ | |
| | | AC | Sinusoidal polyphasic | 2500 Hz at 50 bps | 200 µs | S. tolerance | Dynatron 650 | $31 \pm 13$ | |
| Lyons et al., 2005 (152) | Quadriceps femoris | AC | Symmetrical biphasic triangular | 2500 Hz at 75 bps | 400 µs | S. tolerance | VersaStim 380 | $56 \pm 2$ | AC = PC |
| | | PC | Symmetrical biphasic square | 75 Hz | 250 µs | S. tolerance | Empi 300PV | $61 \pm 2$ | |
| Ward et al., 2006 (243) | Wrist extensors | AC1 | Sinusoidal polyphasic | 2500 Hz at 50 Hz | 200 µs | Number of reports of discomfort | a purpose-built device | 2.4 N·m | AC1 < AC2, PC1 and PC2; AC2 = PC1; AC2 = PC2; PC1 = PC2 |
| | | AC2 | Sinusoidal polyphasic | 1000 Hz at 50 Hz | 500 µs | | a purpose-built device | 2.9 N·m | |
| | | PC1 | Monophasic rectangular | 50 Hz | 200 µs | Number of reports of discomfort | a purpose-built device | 2.8 N·m | |
| | | PC2 | Monophasic rectangular | 50 Hz | 500 µs | | a purpose-built device | 2.6 N·m | |
| Laufer and Elboim 2008 (146) | Wrist extensors | AC1 (1:1)# | Sinusoidal polyphasic | 2500 Hz at 50 bps | 200 µs | S. tolerance | Myomed 932 | $33 \pm 1$ | AC = PC |
| | | AC2 (1:4)# | | 2500 Hz at 50 bps | 200 µs | S. tolerance | | $32 \pm 2$ | |
| | | AC3 (1:4)# | | 2500 Hz at 20 bps | 200 µs | S. tolerance | | $30 \pm 9$ | |
| | | PC | | 50 Hz | 200 µs | S. tolerance | | $33 \pm 8$ | |

\* PC = Pulsed current, AC = Alternating current. # 1:1 = burst duty cycle of 50%, 1:4 = burst duty cycle of 20%; S. tolerance: subject tolerance; S. comfort: subject comfort.

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Walmsley et al. (239) performed two experiments to compare torque values generated voluntarily and electrically, using different stimulators. From both experiments they did not find a significant difference between alternating currents (4000-4100 Hz at 75 Hz and 2200 Hz at 50 Hz) and pulsed currents (50 Hz) in torque values of knee extensors regardless of the stimulator types. Snyder-Mackler et al. (215) also compared the torque generation of three different stimulators generating three different currents as follows: 2500 Hz alternating current with 200 μs phase duration and 50 Hz bursts frequency delivered, 4000 – 4050 Hz alternating current with 125 μs phase duration and 50 Hz bursts frequency, and the last current was 50 Hz pulsed current symmetrical biphasic square waveform with 200 μs phase duration. Even though all stimulators were shown to be sufficient to improve muscle strength, they found from stimulating the quadriceps femoris muscles of 20 healthy participants that 4000 – 4050 Hz alternating current produces significantly less torque (40.05 ± 21.84% of MVC) than other currents (215). This could be attributable to the phase charge of this current which was less (125 μs phase duration) than others (200 μs phase duration) (122). However, no significant difference was found between 2500 Hz alternating current (61.60 ± 32.04% of MVIT) and 50 Hz pulsed current (54.10 ± 27.85% of MVIT), which had similar phase duration (200 μs) (215). Consistently, Grimby and Wigerstad-Lossing (96) reported similar findings whereas no significant difference in torque production between alternating current (2500 Hz at 50 Hz) and pulsed current (30 Hz) for quadriceps muscles of 15 healthy individuals at the maximal tolerance. Holcomb et al. (109) also reported no difference between pulsed current (95 Hz) and alternating current (2500 Hz at 95) for generating forceful muscle contractions (knee extensors) of 10 healthy subjects at the maximal tolerance.

Laufer et al. (147) compared the quadriceps femoris torques generated by three different waveforms and demonstrated different results. In this study, 30 subjects underwent three EMS currents as follows: 2500 Hz alternating current with 50 Hz bursts frequency delivered from a clinical stimulator and two pulsed currents (50 Hz) with different waveforms (monophasic and biphasic) delivered from a portable stimulator, where the phase duration was kept in 200 µs for all waveforms. Pulsed currents (either monophasic or biphasic) generated greater torque ($36.6 \pm 17.1$ and $38.0 \pm 16.6\%$ of MVC, respectively) than alternating current ($30.9 \pm 12.6\%$ of MVC) (147).

On the other hand, Lyons et al (152) studied the peak isometric torque of quadriceps femoris induced by two different stimulators: clinical and portable. Forty healthy subjects were tested and two EMS current protocols were used as follows: 2500 Hz alternating current with 400 µs pulse duration and 75 Hz bursts frequency delivered from the clinical stimulator and 75 Hz pulsed current with 250 µs pulse duration delivered from the portable stimulator. Findings indicated no significant difference between alternating current ($56.0 \pm 16.2\%$ of MVC) and pulsed current ($60.5 \pm 18.7\%$ of MVC) in the torque production (152), which is consistent with previous findings (96, 109, 216, 239). In addition, Ward et al. (243) also reported similar torque output of wrist extensors between two alternating currents (2500 Hz and 1000 Hz at 50 Hz) and two pulsed currents (50 Hz) with phase duration of 200 and 500 µs, respectively.

These results have been confirmed by a recent study by Laufer and Elboim (146) who compared between four different currents: three alternating currents (2.5 kHz at 50 Hz at a burst duty cycle of 1:1; 2.5 kHz at 50 Hz at a burst duty cycle of 1:4 and 2.5 kHz at 20 Hz at a burst duty cycle of 1:4) and pulsed current delivered at 50 Hz, and the phase duration was fixed for all currents at 200 µs. They found, after stimulating the

forearm muscles of healthy subjects, no effect of current types on the contraction force, which is in consistent with other studies that used the same stimuli frequency (50 Hz) for comparing between currents. They concluded that the number of stimuli per second may have more influence on the torque production than the number of pulses or bursts per second.

Parker et al. (188) compared the effect of three alternating current frequencies (2500, 3570 and 5000 Hz at 50 bursts per second with cycle duration of 400, 266 and 200 μs respectively) on quadriceps torque responses of 23 healthy subjects. The frequency at 2500 Hz was found to generate greater torque (69.4 ± 18.6% of MVC) than 3570 and 5000 Hz (38.9 ± 15.7 and 26.8 ± 14.1% of MVC, respectively). According to the previous studies that reported similar torque output between alternating current and pulsed current (96, 109, 146, 152, 216), all of them used a carrier frequency of 2500 Hz for their comparison, which was driven from Russian EMS (249). Thus, this frequency of alternating current would be the preferable option to compare with pulsed current.

The difference between alternating current and pulsed current for the training effect has been investigated in two studies (Table 3). An early study was conducted by Stefanovska and Vodovnik (220) who compared 3-week EMS training (10 min daily) of alternating current (2500 Hz at 25 Hz) and pulsed current (25 Hz) of quadriceps muscle in 5 healthy individuals for each current type; they found a higher increase in the maximal voluntary torque after pulsed current (25% of MVC) than after alternating current (13% of MVC). A training study also conducted by Bircan et al (25) compared alternating current (2500 Hz at 80 Hz) and pulsed current (80 Hz), and reported no significant difference between the two protocols for the increase in isokinetic strength after a 3-week EMS training (15 min per session, 5 days a week). These contradictory

results are possibly due to the burst cycles that were used for alternating current. In the study by Stefanovska and Vodovnik (220), the alternating current was modulated at 25 burst per second, which is close to 20 Hz that was shown to result in a higher discomfort degree than those modulated at 50 Hz (146).

Table 3: Previous studies comparing alternating current and pulsed current for changes in torque following EMS in healthy subjects.

| Study (year) | Muscle, application effect | EMS characteristics | | | | | | Torque changes (%) | Comparison for force production |
|---|---|---|---|---|---|---|---|---|---|
| | | Current | Waveform shape | Frequency | Pulse/phase duration | Stimulus amplitude | Stimulator | | |
| Stefanovska A, and Vodovnik L., 1985 (220) | Quadriceps femoris, training effect | AC | Sinusoidal | 2500 Hz at 25 bps | | Subject comfort | | ↑ 13% | AC < PC |
| | | PC | Rectangular | 30 Hz | 300 µs | Subject comfort | | ↑ 25% | |
| Bircan et al., 2002 (25) | Quadriceps femoris, training effect | AC | Bipolar interferential | 2500 Hz at 80 bps | | Subject tolerance | Myomed 932 | ↑ 14 at 60°/s, 19 at 120°/s | AC = PC |
| | | PC | Symmetrical biphasic | 80 Hz | 100 µs | Subject tolerance | Myomed 932 | ↑ 21 at 60°/s, 23 at 120°/s | |

The differences between these studies could be attributable to the variation in the current characteristics (parameters) and/or the type of stimulators used. A comparison between alternating current and pulsed current using the same stimulator and identifying the other parameters, such as pulse duration, duty cycle and intensity, may clearly demonstrate the difference between currents. Moreover, no data is available for the difference between pulsed current and alternating current in other physiological variables, such as muscle damage, skin temperature, hormonal responses and muscle oxygenation, which can expose further differences between these currents.

## 2.6 EMS AND DISCOMFORT

Discomfort is one of the major limitations of using EMS as an alternative to voluntary contraction. During EMS, the nerve axons are stimulated externally by electrical current, which include all types of peripheral nerve fibres (e.g. motor neurons and sensory receptors) in non-selective and spatial recruitment patterns (94). This behavior of untypical activation may cause the discomfort during EMS. The difference in the level of discomfort between alternating current and pulsed current has been investigated by several studies (25, 96, 146, 152, 243). Lyons et al. (152) reported similar discomfort between alternating current (2.5 kHz delivered at 75 Hz) and pulsed current (75 Hz). In consistence, Laufer and Elboim (146) reported no significant differences in the degree of discomfort between alternating current (2.5 kHz delivered at 50 burst and duty cycle of 1:1) and pulsed current (50 Hz). However, in a recent study by Ward et al. (243), pulsed current (50 Hz with pulse duration of 200 or 500 µs) showed more discomfort compared with alternating current EMS (2.5 and 1 kHz with pulse duration of 200 and 500 µs, respectively). The inconsistent results warrant further investigation for the difference between EMS currents for comfortableness.

## 2.7 EMS AND SKIN TEMPERATURE

Regulation of body temperature and blood flow distribution during exercise is associated with a reflex control of specific local circulations that determine the development of vasoconstriction or vasodilation through cutaneous vascular conductance mechanism (116). Vasoconstriction is the process by which skin vessels in the extremities are constricted to reduce blood flow to certain tissues, while during vasodilation the subcutaneous skin vessels are dilated to allow a greater volume of blood to flow to these tissues. Skin temperature is largely affected by these processes where it decreases during vasoconstriction and increases during vasodilation (115). This mechanism increases skin circulation, which is influenced by two factors: core and local skin temperature so that the increase in core temperature activates the sympathetic cholinergic nerves that lead to a vasodilation in skin blood vessels (92), and thus more blood flow under the skin. An indication of cutaneous vascular responses during and following exercise can be estimated by the changes in skin temperature (115, 123), and it is affected by the vasodilation and vasoconstriction of skin blood vessels (92, 115, 221). Several studies reported the changes of blood flow during and after voluntary contraction (13, 14, 114, 183, 197). However, the effect of EMS on blood flow in human muscle contraction is not well documented.

Currier et al. (50) investigated the effect of EMS 2500 Hz alternating current at 50 Hz burst frequency on blood flow and skin temperature on plantar flexor muscles for 28 subjects. Findings showed that EMS significantly altered blood flow while there was no effect on skin temperature. In addition, the effect of selected pulse frequencies on blood flow in the quadriceps femoris muscles of 30 healthy subjects was investigated, and it showed that the blood flow significantly increased with EMS using 10, 20, and 50 Hz pulsed current compared with 1 Hz pulsed current (229). In another study, several

frequencies were carried by pulsed galvanic stimulation to investigate the changes in blood flow and skin temperature using the vascular channels of the upper extremity of ten healthy subjects (103). Blood flow was shown to increase with increasing frequency rate and at negative polarity but no significant change in skin temperature was noted (103). Nonetheless, the galvanic stimulation is applied by a continuous direct current while pulsed and alternating stimulation are applied through an intermittent direct and alternating current, respectively. Thus, different results could be presumed because of the interrupted pulses, which allow more blood flow during inter-pulse intervals.

The time of EMS session has also an influence on the blood flow rate. The effect of EMS 30 Hz pulsed current with 75 μs phase duration (symmetric rectangular waveform) stimulating time on blood flow in quadriceps femoris muscles of ten healthy subjects has been investigated by Vanderthommen and others (233). The results of this study showed that the rate of blood flow close to EMS electrodes increases as the duration of EMS is increased (233). Conversely, another study showed that EMS 30 Hz pulsed current with 75 μs phase duration did not increase blood flow in plantar flexor muscles of 38 healthy participants (238).

As a result of the increase in metabolic activities during exercise, the body temperature increases (83). Thermoregulation plays an important role in maintaining the body‟s homeostatic balance and the thermal neutrality by several mechanisms, such as transferring heat to the periphery (shell) so the warm blood is expelled from the internal to superficial areas for cooling processes (83, 132). Although the effect of voluntary isometric exercise on cutaneous vascular responses has been documented (76, 78, 124, 125), little data is available about the changes in skin temperature during and after EMS in healthy individuals. Bennie et al. (16) reported a significant increase in skin

temperature (0.02 °C) during the initial 3 min of one EMS contraction sustained 4-min at 10% MVC using different EMS waveforms. The small changes in the skin temperature could be due to the lower force production (10% MVC) induced by EMS. However, the difference between alternating current and pulsed current for the changes in skin temperature at the maximum tolerated intensity is still unknown. Less blood flow could be expected during alternating current compared with pulsed current due to the higher number of stimuli (pulses) during alternating current. Thus, the local skin temperature would be assumed lower during alternating current.

## 2.8 EMS AND HORMONAL RESPONSES

It is known that resistance training significantly affects the secretion of anabolic (e.g. testosterone, growth hormone, insulin-like growth factors) and catabolic (e.g. cortisol, catecholamines) hormones (4, 140). Despite the fact that EMS training produces similar effects to resistance training (e.g. increases in muscle strength and size) or minimises muscle atrophy caused by disuse (66, 82), hormonal responses to EMS are not well documented. Although hormonal responses of spinal cord-injured (SCI) patients to EMS have been reported (254), little information about healthy individuals is available.

### 2.8.1 Growth hormone

Growth hormone is essential to the growth process and plays an important role in carbohydrate and lipid metabolism (51). It is secreted by the pituitary gland in response to several activities, such as exercise, and it has been shown to be greatly affected by resistance exercise (135). Jubeau et al. (121) reported that increases in blood lactate and growth hormone were significantly greater for EMS (75 Hz) compared with the

voluntary isometric contractions at the same level of torque output and at the same intensity. Sartorio et al. (204) also reported significant increases in GH following EMS (75 Hz), which were induced by 20 contractions at the maximal tolerance of quadriceps muscle. However, no data is available regarding hormonal responses to EMS for alternating current.

### 2.8.2 Testosterone

Testosterone is an anabolic-androgenic steroid, which is mainly secreted by the adrenal glands and testes in men and the adrenal glands and ovaries in women. Weight training has been widely used to increase muscle mass (256). Several studies have reported an increase in testosterone following resistance exercise (98, 106, 136) but the effect of EMS on testosterone is yet to be established in healthy individuals.

### 2.8.3 Cortisol

Cortisol is a corticosteroid hormone that is released by the adrenal cortex of the adrenal gland and its level is subject to change during the daily cycle (253). Resistance exercise has been shown to elevate the secretion of cortisol (136, 138) and it is largely effected by the intensity and volume of exercise (212). In an early study, a low frequency of transcutaneous electrical nerve stimulation (2 Hz) of elbow flexor muscle was unable to increase the serum cortisol concentration 63.(59). It is obvious that 2 pulses per second cannot generate muscle tension and thus no alteration in cortisol level would be expected. In a recent study by Sartorio et al. (204), a significant decrease in cortisol was reported at 60 min after 20 isometric contractions of the quadriceps muscle induced electrically (75 Hz). The acute effect of alternating current on cortisol

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concentration is not known, and neither is the difference between alternating current and pulsed current.

### 2.8.4 Insulin-like growth factor

Insulin-like growth factor-1 (IGF-1) is a polypeptide hormone that is released predominantly by liver and other cells (198). IGF-1 has been shown to intrinsically mediate skeletal muscle repair and adaptation in an autocrine/paracrine mode by inducing cell proliferation and subsequently enhance hypertrophy (189, 190). Hence, Froesch et al. (77) noted a significant increase in the IGF-1 plasma concentration when injecting growth hormone in animals, which demonstrates a possible role of growth hormone in regulating IGF-1 synthesis (1). The acute effect of resistance exercise on IGF-1 is not agreed upon among studies whereas some showed an increase during and immediately following exercise (137, 139) while others found no significant changes (134, 141). Nonetheless, no previous study has reported changes in IGF-1 in response to EMS, and the comparison between alternating current and pulsed current for changes in IGF-1 in healthy individuals has not yet been elucidated.

### 2.9 EMS AND ANAEROBIC METABOLISM

It is generally accepted that the formation of blood lactate starts when the energy system shifts to anaerobic metabolic pathway where the conversion of pyruvate to lactate or anaerobic glycolysis occurs. Blood lactate can rise to a very high level during intense exercise and it is affected by depleted glycogen stores (68). Several studies have investigated the influence of EMS on metabolic activation. Hultman and Sjöholm (110), for example, investigated the accumulation of blood lactate and the depreciation of adenosine triphosphate (ATP) by calculating the degradation of creatine phosphate

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(PCr) using twitch interpolation 20 Hz pulsed current with 500 µs pulse duration to stimulate the quadriceps femoris muscle of 9 healthy subjects. They found a significant increase in blood lactate accumulation within 5 s of the beginning of stimulation until 20 s then it remained relatively constant. However, the ATP content was maintained at initial levels for the first 20 s then started decreasing to 76% after 50 s. In addition, they provided evidence of the occurrence of glycogenolysis and glycolysis based on the glucose-6-phosphate changing rate (110).

Another study investigated how the glycolytic system contributes to the production of ATP. The quadriceps femoris muscles of 7 men were electrically stimulated using 20 Hz pulsed current with 500 µs pulse duration and the total exercise time was 100 s (219). They found that, at 25s of exercise, 58% of ATP production was formed by glycolysis pathway while 40% was produced by phosphocreatine metabolic pathway. However, the contribution of the glycolytic system to produce ATP increased significantly to cover 90% of energy demands beyond 25 s of the exercise. In contrast, a recent study reported a slight increase (insignificant) in serum lactate (an indicator of glycolytic reactions) after applying EMS (63/ 3 Hz pulsed current, 400 µs pulse duration) to both knee extensor muscles of five well trained subjects for 30 min (258).

Vanderthommen et al. (235) compared the changing rate of ATP and myoglobin oxygenation activated by voluntary contraction and EMS by monitoring intracellular $O_2$ and energy metabolism using NMR spectrometer technique. They reported a greater contribution of glycolysis during EMS (50 Hz pulsed current with 250 µs pulse duration) compared with voluntary contraction (235). Another study (107) also showed that EMS of the rectus femoris and vastus lateralis with 20 Hz pulsed current (200 µs pulse duration) increased blood lactate concentrations greater than $14.6 \pm 5.0$ mg/dL and

enhanced muscle hypoxia and glycolytic metabolism assessed by near-infrared spectroscopy (NIRS) measurements. Furthermore, it was reported that continuous submaximal contractions (40 s) of EMS (50 Hz pulsed current with 200 μs pulse duration) at 75% MVC for the gastrocnemius and soleus muscles (5 healthy subjects) in ischaemic condition spends more anaerobic ATP than voluntary contraction (194).

The increase in blood lactate concentration during and after EMS exercise was reported by several studies (99, 107, 121). Jubeau et al. (121) found a significant increase in blood lactate concentration during and after 40 isometric EMS contractions (75 Hz) for quadriceps muscles. These studies have demonstrated significant effects of pulsed current EMS on anaerobic metabolism and lactate; however, the effects of alternating current or the difference of pulsed current and alternating current on blood lactate concentration is unknown.

## 2.10 EMS AND OXYGEN CONSUMPTION

The energy source for skeletal muscle tends to be aerobic when exercise lasts more than 2 min at middle or high intensity and more oxidative metabolism is synthesized (32). When skeletal muscles are electrically stimulated, maximum EMS frequency and intensity are used to achieve greater muscle tension (150). A recent study that compared the changes in oxygen consumption, ventilation and respiratory exchange ratio during EMS (75 Hz pulsed current with 400 μs) and voluntary contractions of the quadriceps muscle (12 healthy men) at same force output found that the average of these variables during EMS was significantly higher in comparing with voluntary contraction (225). The phosphate/phosphocreatine (Pi/PCr) ratio also was found different between EMS and voluntary exercise where 64 isometric contractions induced electrically

increased Pi/PCr ratio greater than voluntarily at the same (20% MVC) work load (236). This finding suggests a difference in muscle activation pattern between EMS and voluntary contraction. However, these results showed the effect of EMS on the global muscle oxygenation rather than the local changes.

Near-infrared spectroscopy (NIRS) is an optical method used to monitor muscle oxidative metabolism non-invasively (30). Muscle oxygenation and blood flow have been assessed using NIRS during EMS by several studies (18, 167, 170, 171, 225). McNeil et al (167), for example, reported a high metabolic demand and more blood volume following EMS (25 Hz pulsed current, pulse width of 50 µs, 400 V) comparing with voluntary contractions of dorsiflexor muscles at equal work (50% MVC). Similarly, greater average oxygen consumption, ventilation and respiratory exchange ratio were reported during EMS (75 Hz, pulse duration of 400) compared with voluntary contractions of the quadriceps muscle at the same force output (46 ± 10% of MVC) in healthy men (225). They attributed this difference to the variance in the motor unit recruitment between EMS and voluntary contraction. On the contrary, Muthalib et al. (171) have recently reported a comparable level of oxygen demand between 30 isometric contractions induced by EMS (30Hz pulsed current, pulse duration of 200 µs) at the maximum tolerated current and 30 voluntary isometric contractions of biceps brachii muscle. These inconsistent results could be due to the level of force output where the maximal torque produced by EMS in the latter study (171) was about 30-40% MVC but the voluntary contraction was at 100% MVC. However, the differences in muscle oxygenation using NIRS between alternating current and pulsed current are currently unknown.

## 2.11 EMS AND MUSCLE FATIGUE

The failure to generate or maintain the force output is described as muscle fatigue, which may occur due to the reduction in the number of recruited muscle fibres (63). Although the origin of stimulus is peripheral during EMS, a contribution of both central and peripheral fatigue was reported during EMS (28). Interestingly, greater muscle fatigue has been shown during EMS compared with voluntary contraction at the same force output (225). The authors of this study explained their findings by the difference in the patterns of motor unit recruitment between EMS and voluntary contraction regardless of the level of force. This held true when considering the recruitment patterns during EMS where it is non-selective to fibre types (94) so that more fast (fatigable) motor units are activated at the initial stage of exercise. Thus, it would be expected that fatigue will be accelerated during EMS compared with voluntary contraction.

Muscle fatigue during EMS was reported to be greatly influenced by the parameters of EMS (24, 85), particularly EMS frequency (88). Muscle fatigue can be greatly attenuated by reducing frequency to 20 Hz or lower (118). However, higher frequency of 50-100 Hz is required to produce a maximum force as discussed earlier and reported by several studies (21, 64). This is also the case when comparing between low-frequency EMS (pulsed current) and kilohertz-frequency EMS (alternating current). Since fatigue is known as a decrease in the muscle capacity to produce maximum force generation (20), several studies have reported a comparable level of torque output between alternating current and pulsed current (96, 109, 146, 216, 220). It can be assumed that the absolute force output may not be a valid indicator of muscle fatigue during EMS contractions regardless of the EMS current types. Nonetheless, several studies have reported that pulsed current can cause less muscle fatigue than alternating current at the same force output (146) or at different force output (147, 220). Muscle

fatigue could be determined when force output is different between variables but the challenge when no significant differences in strength are detected. Grimby and Wigerstad-Lossing (96) found similar torque output between alternating and pulsed current but larger variability within the torque curve during alternating current compared with pulsed current. This could be an indication of high-frequency fatigue, where the force loss occurs extensively at a high frequency stimulation (e.g. 50 Hz and higher) and recovers rapidly due to the reduction in frequency (117). The variability in toque curve during alternating current could be due to the current incapability to activate additional motor units as a result of muscle fatigue. The reduction in the number of recruited fibres during contraction may reduce the amount of muscle volume involved in the contraction, which may be reflected in muscle oxygen demand. However, this assumption has not been confirmed yet.

## 2.12 EMS AND MUSCLE DAMAGE

The major limitation of using EMS in training is the pain during stimulation (244) and muscle damage (36). The occurrence of muscle damage after EMS has been well investigated (34, 40, 48, 121, 154, 179). However, limited research has been conducted on muscle damage and recovery of muscle function after EMS-evoked isometric contractions.

It has been documented that repetitive eccentric muscle contractions cause a significant increase in circulating creatine kinase (CK) and a decrease in muscle strength (43, 49, 160, 178). Although voluntary isometric exercise seems unlikely to induce muscle damage when performed at the same level of torque generated by EMS at

maximal intensity (121), muscle damage has been reported in several studies following isometric exercise induced electrically (36, 121, 155, 169, 179, 225, 258).

Muscle soreness and damage have been assessed before and after an EMS (70 Hz pulsed current with 40 µs) session and concentric exercise of the quadriceps muscle for 12 male athletes (169). A significant increase in plasma CK activity and muscular soreness after EMS compared with concentric exercise was reported (169). A recent study reported a significant decrease in maximal voluntary force after EMS (75 Hz pulsed current with 400 µs pulse duration) compared with voluntary contraction of the quadriceps muscle of 12 healthy men (225). These findings have been supported by a recent study that showed that maximal voluntary strength decreased significantly after EMS (75 Hz pulsed current with 400 µs pulse duration), and it also showed a significant increase in serum CK activity and muscle soreness compared with the voluntary contraction (121). Delayed-onset muscle soreness (DOMS) was higher after EMS (125 Hz pulsed current with 40 µs pulse duration) and was associated with muscular soreness and strength loss (36).

In contrast, Zorn et al (258) reported a non-significant increase in muscle soreness with no DOMS after EMS (63, 3 Hz pulsed current with 400 µs pulse duration) in both knee extensor muscles of five trained subjects even though a significant increase was shown in serum CK. This result was expected to be seen in trained subjects who may have experienced a repeated bout effect (discussed later) in the early stages of a training programme (67, 164).

Nosaka et al (179) showed that eccentric contractions induced more muscle damage than isometric contractions that were electrically evoked (100 Hz pulsed current with 400 µs pulse duration) in the elbow flexor muscles for 17 participants. Another

study reported that 30 isometric contractions of the knee extensors evoked by EMS (70 Hz, duration 40 μs, on-off ratio 6–20 s) resulted in small increases in plasma CK activity and muscle soreness after EMS (169). Myofibre damage has been investigated after applying EMS (35 Hz pulsed current with 300 μs pulse duration) and eccentric contractions on quadriceps muscle of eight healthy sedentary male subjects, and muscle fibre necrosis and subsequent regeneration was observed with the EMS condition (48). Mackey et al. (154) have also recently shown histological evidence of muscle damage such as macrophage infiltration, desmin negative fibres (Figure 6), and z-line disruption (Figure 7) after 180 isometric contractions of the gastrocnemius muscle evoked by EMS (60 Hz, duration 300 μs, on-off ratio 4–6 s) lasted 30-min, together with increased plasma creatine kinase (CK) activity and muscle soreness. However, muscle strength measures, which have been proposed as the best indicator of muscle damage (251) as a sensible evidence that affects performance, were not included in the study. The previous studies demonstrated that EMS pulsed current induced a significant muscle damage compared with voluntary contractions. However, little is known about the effects of alternating current on muscle damage or how the EMS currents (alternating current and pulsed current) are different.
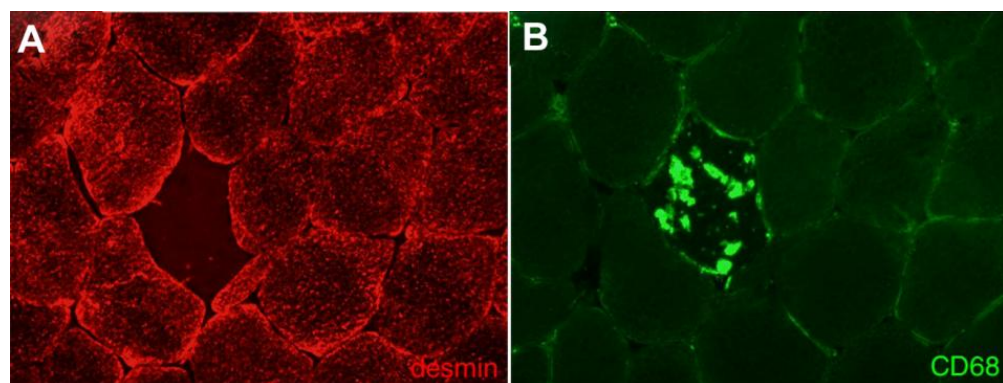


Figure 6: Desmin staining loss (A) and inflammatory cell infiltration (B) show evidence of muscle damage following 48 h of isomteric exercise induced electrically. The images were obtained using immunohistochemistry process. Reproduced from Mackey et al (154).
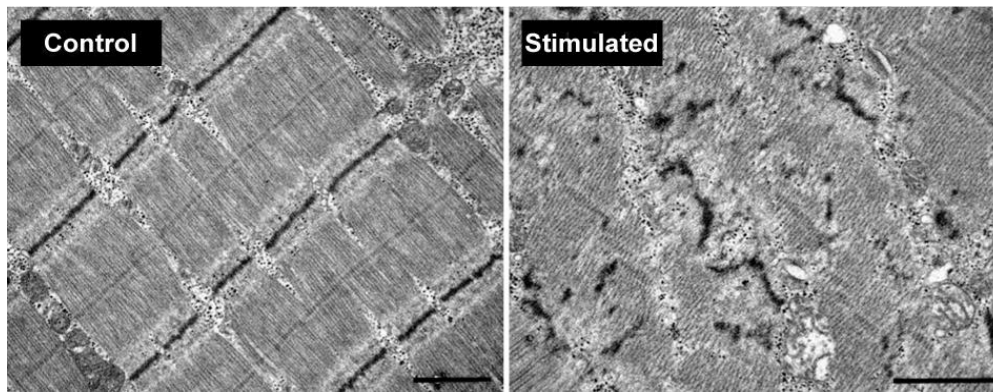
Figure 7: Myofibrillar damage of z-line disruption following 48 h of isometric exercise induced electrically compared with control sample. The images were taken using transmission electron microscopy. Reproduced from Mackey et al (154).

## 2.13 EMS AND REPEATED BOUT EFFECT

It is well documented that the magnitude of muscle damage developed after exercise is attenuated and that the recovery of muscle function is enhanced when the same or similar eccentric exercise is performed within several weeks (158, 173). This protective effect induced by a single bout of eccentric exercise is referred to as the repeated bout effect (164). The repeated bout effect has received considerable attention from researchers (27, 33, 74, 75, 163, 164, 173-175, 181, 222, 230). However, little is known about the repeated bouts of EMS on muscle damage. Nosaka et al. (179) reported that the second bout of eccentric exercise in which the elbow flexors were forcibly stretched while being stimulated by EMS (100 Hz pulsed current, 400 μs pulse duration) resulted in smaller changes in maximal voluntary isometric contraction strength, range of motion, upper arm circumference and muscle thickness, muscle soreness, plasma CK and aspartate aminotransferase levels compared with the initial bout of the same exercise performed 2 weeks before. Black et al. (27) have also recently shown that changes in T2 relaxation time of magnetic resonance images and muscle soreness following 80 lengthening contractions of the quadriceps femoris of 16 subjects

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stimulated by EMS (100 Hz pulsed current, 450 µs pulse duration) were significantly smaller after the second bout compared with the first bout that was performed 7 weeks before. However, it should be noted that these studies compared the first and second EMS bouts consisting of lengthening contractions. No previous study has investigated the repeated bout effect of EMS bouts consisting of isometric contractions that are generally performed in EMS training for changes in markers of muscle damage.

## 2.14 SUMMARY AND CONCLUSION

EMS can be delivered using different currents to excite sensory and motor nerves. Alternating current and pulsed current are most commonly used to induce muscle contractions. Pulsed current is used more frequently because almost all stimulators can produce it. However, the use of alternating current has been increased with some evidence of its benefits in strength training. The physiological responses to alternating current and pulsed current are not well known. A small number of studies have compared pulsed current and alternating current for torque output, muscle fatigue and discomfort and have shown varying results.

For other physiological aspects, such as hormonal responses, muscle oxygenation and muscle damage, little data is available for the difference between currents. In healthy populations, the effect of EMS on some different hormonal secretions is not known. Only one study reported an increase in growth hormone and cortisol after pulsed current but no studies have investigated other hormones, such as testosterone or IGF-1 for both currents. Many studies have demonstrated high muscle oxidative metabolism following pulsed current but none for alternating current. Several studies reported

muscle damage following pulsed current but little is known about alternating current or how pulsed current and alternating current differ. There is currently a clear gap in the body of knowledge regarding differences between alternating current and pulsed current.

# CHAPTER THREE: STUDY ONE

## COMPARISON BETWEEN ALTERNATING AND PULSED CURRENT ELECTRICAL MUSCLE STIMULATION FOR MUSCLE AND SYSTEMIC ACUTE RESPONSES

### 3.1 INTRODUCTION

Electrical muscle stimulation (EMS) applies electrical current transcutaneously to muscles through electrodes to induce involuntary contractions. There are two types of currents commonly used in EMS: alternating current and pulsed current. Pulsed current EMS delivers intermittent pulses at generally 1-150 Hz (245). In contrast, alternating current EMS consists of a continuous series of alternating biphasic high frequency pulses (e.g. 1-25 kHz) generally modulated and delivered within the biological range, normally between 10 and 150 Hz (245). Clinical use of alternating current EMS includes pain control, spasticity management, prevention of atrophy and edema control (242). However, it is also used in muscle training since a Russian scientist Kots advocated so called "Russian current" (2.5 kHz sinusoidal pulse stimulation) for its efficacy in increasing muscle strength in 1977 (for review see 249). Although the use of alternating current EMS has been increasing (249), pulsed current EMS is more widely used in sports training and rehabilitation, since most of the portable or battery-operated stimulators can deliver only pulsed current (152).

In alternating current EMS, the stimulus is delivered in bursts, where each burst consists of many pulses. As shown in Figure 11, the number of bursts per second in the modulated alternating current EMS is the same as the number of pulses per second in the pulsed current EMS; however, each stimulus consists of greater number of pulses in the alternating current than the pulsed current EMS. It is possible that the distinct difference in the number of pulses delivered to muscle in stimulation produces different physiological responses between alternating and pulsed current EMS. In fact, some studies (147, 220) reported a difference in muscle force generation between alternating and pulsed current EMS. However, systematical comparisons between the two waveforms for acute muscle and systemic responses are lacking.

Snyder-Mackler et al. (216) compared the torque generation of the knee extensors among three different waveforms; 2.5-kHz alternating current and 4-kHz alternating current delivered at 50 Hz, and 50-Hz pulsed current. They reported that the torque generation was significantly lower in the 4-kHz alternating current compared with others; however, no significant difference in torque output was evident between the 2.5-kHz alternating current and the 50-Hz pulsed current. Another study compared the quadriceps femoris torque generation during 2.5-kHz alternating current delivered at 50-Hz and 50-Hz pulsed current, and showed that the torque was significantly higher for the pulsed current than alternating current (147). In contrast, no difference was found in isometric torque of the knee extensors between 2.5-kHz alternating current delivered at 50-Hz and 30-Hz pulsed current (96) and between 2.5-kHz alternating current delivered at 95 burst per second and 95-Hz pulsed current (109). Other studies also reported similar force generation by 2.5-kHz alternating current delivered at 75-Hz and 75-Hz pulsed current (152), or by 2.5-kHz alternating current delivered at 50-Hz and 50-Hz pulsed current (146). It does not appear that the information regarding the difference in

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the muscle force generation between alternating and pulsed current EMS is consistent. To the best of our knowledge, no previous study has compared the torque output between pulsed current and alternating current by keeping all parameters as similarly as possible except the waveform, and using the same stimulator. It is possible that a difference exists between the two current types for perceived exertion during EMS. It may be that the effect of EMS on skin temperature is associated with discomfort, and changes in skin temperature and discomfort are different between alternating current and pulsed current EMS. However, these have not been investigated in the previous studies.

Several studies have demonstrated that skeletal muscle damage is induced by isometric contractions evoked by EMS (121, 154, 169, 179). For example, Jubeau et al. (121) showed that EMS (75 Hz pulsed current) resulted in decreases in maximal voluntary isometric contraction torque, delayed onset muscle soreness (DOMS), and increases in serum creatine kinase (CK) activity. Mackey et al. (154) have reported histological damage in muscle fibers after EMS (60 Hz pulsed current). It should be noted that all of these studies used pulsed current EMS, and no previous studies have investigated skeletal muscle damage induced by alternating current EMS.

In the study by Jubeau et al. (121), they also reported that increases in blood lactate and growth hormone (GH) were significantly greater for EMS compared with the voluntary isometric contractions of the same force output. Sartorio et al. (204) compared the first and second EMS bouts (75 Hz pulsed current with pulse duration of 400µs) consisting of 20 isometric contractions of the quadriceps femoris, and showed significant increases in GH following EMS, and a significant decrease in cortisol at 60 min after the first EMS. To understand the effect of EMS on hormonal responses better, other anabolic hormones (e.g. testosterone, insulin-like growth factor-1: IGF-1) that are

often investigated in resistance exercise (140) should be investigated. No previous study has compared between alternating current and pulsed current EMS for changes in hormones (GH, testosterone, IGF-1, and cortisol) and blood lactate.

Therefore, the purpose of this study was to compare between alternating current and pulsed current during a typical EMS strength training session (145, 234) for torque generation, perception and skin temperature, symptoms of skeletal muscle damage, and hormonal responses. To compare between pulsed current and alternating EMS, the present study set the stimulation parameters similarly between the two stimulation conditions. Based on the previous studies (95, 168, 223), 75 Hz was chosen for the frequency of the pulsed current EMS, and 2.5 kHz was chosen for alternating current (188, 210, 216). It was hypothesized that alternating current would trigger more changes in torque output, skin temperature, skeletal muscle damage and hormonal responses than pulsed current due to the greater number of pulses delivered by alternating current.

## 3.2 METHODS

### 3.2.1 Study Design

Twelve volunteers participated in two EMS sessions separated by 2 weeks; one for pulsed current session and the other for alternating current session in a randomized, counterbalanced order. The 2-week interval between bouts was set to provide a time for elevated plasma CK activity after the first EMS session to return to baseline value. The subjects did not receive any information which current they were receiving in the EMS sessions. A familiarization session was conducted before the study, which included maximal voluntary isometric contraction strength (MVC) measures at different knee joint angles (40°, 70°, 100°), and EMS that included 3-5 electrically evoked isometric

contractions at submaximal intensity. For each EMS session, the subjects were asked to report to the laboratory for 6 days; baseline measure session held 1-3 days before EMS session, EMS session day, and 4 consecutive days following the EMS session (Figure 8). Forty isometric contractions of the knee extensors of one leg were evoked in each EMS exercise, and one leg received pulsed current EMS, and the other leg had alternating current EMS. Both EMS sessions were performed at the same time of the day for each subject between 8 am and 10 am to eliminate the effects of possible diurnal variations on hormonal responses and other measures. The independent variable in this study was the waveform used in EMS (alternating current versus pulsed current), and the dependent variables consisted of knee extensors" torque and rate of perceived exertion during EMS, skin temperature, blood lactate concentration, growth hormone, testosterone, IGF-1, and cortisol concentrations, and indirect markers of muscle damage such as MVC of the knee extensors, muscle soreness, pressure pain threshold, and plasma CK activity. Figure 9 shows the experiment setting. The study was approved by the Edith Cowan University Human Research Ethics Committee.
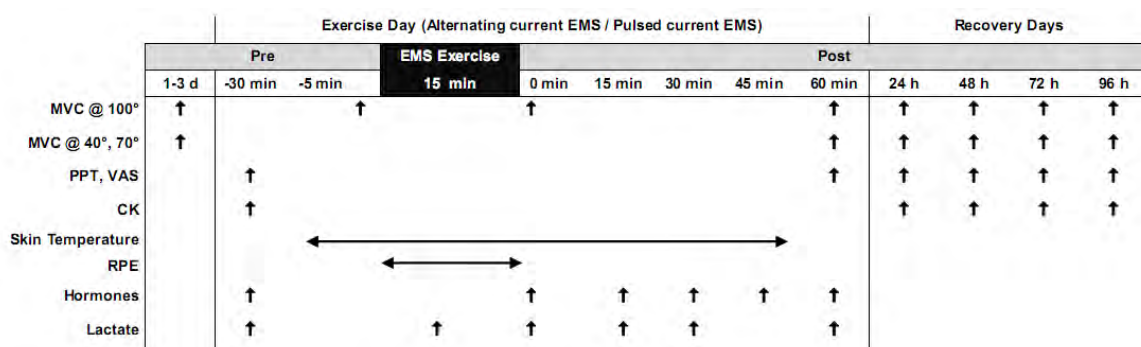


Figure 8: A diagram showing the measurement time line. MVC: maximal voluntary contraction at different knee joint angles (40°, 70° and 100°),PPT: pressure pain threshold, VAS: visual analogue scale for muscle soreness assessment, CK: plasma creatine kinase activity, RPE: rate of perceived exertion.
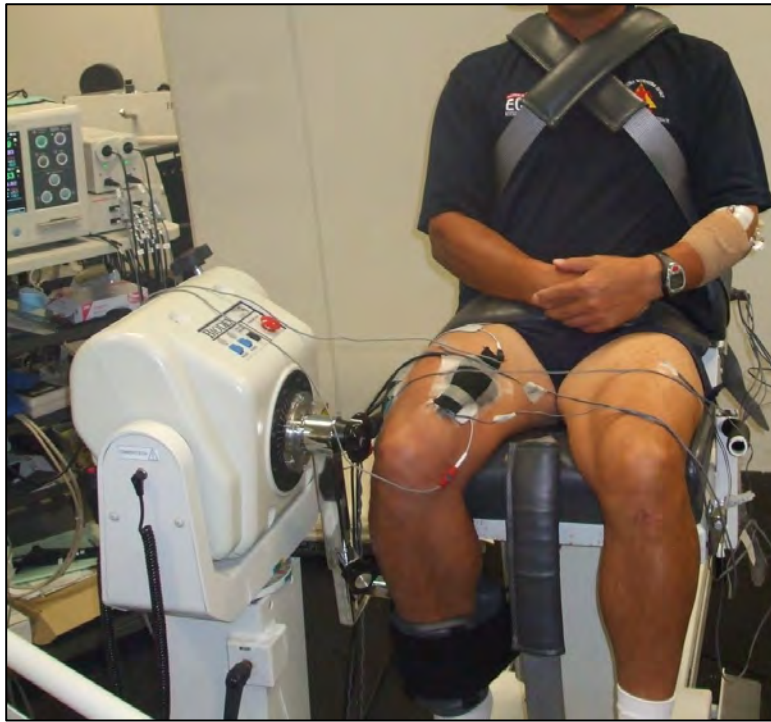
Figure 9: Experiment setting.
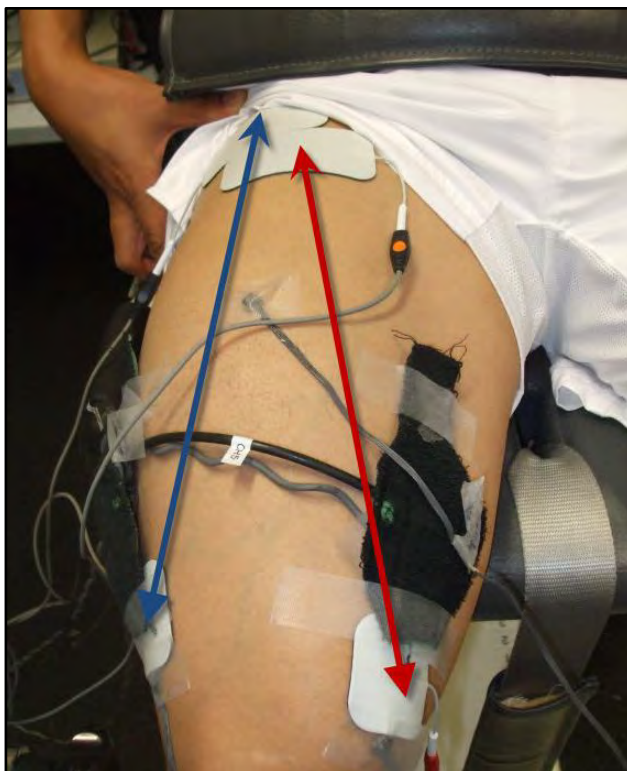


Figure 10: Electrodes placements. Electrodes between the blue arrow (left) for vastus latteralis, and the red arrow (right) for vastus medialis muscles.

### 3.2.2 Subjects

Twelve healthy men (mean ± SD age: 31.2 ± 5.5 y, body mass: 81.4 ± 15.2 kg, height: 174.3 ± 4.8 cm), who had not been involved in resistance training program for at least 6 months prior to the study and not had an injury in their knee joints, participated in this study after signing the informed consent form. During the experimental period, subjects were asked not to change their diet habits, and not to take any medicines nor have any interventions other than those given in the study. Subjects were requested to avoid consuming caffeine and alcohol one day before the EMS session and not to undertake any physical activity during the experimental period. Female subjects were excluded to avoid possible gender difference in hormonal responses (35, 137, 252) and muscle damage (42, 228), and to make the variability of the criterion measures as small as possible to increase the statistical power.

### 3.2.3 Electrical muscle stimulation (EMS)

Each subject seated on a Biodex isokinetic dynamometer chair (Biodex Medical Systems, Inc., USA) with his knee joint angle of 100° (0° corresponding to the full extension) and the trunk angle of 110°. Straps secured the pelvis and chest to minimize the movements of the hip and trunk during contractions. The type of EMS current was kept blind to the subjects for each EMS session. An Intelect Advanced®Colour Stim (Chattanooga Group, TN, USA) was used to stimulate the quadriceps femoris muscles. The skin surface was cleaned with alcohol pads, and then four self-adhesive electrodes were placed on the anterior surface of one thigh as follows: two positive electrodes (50 × 50 mm) over the motor point of the vastus lateralis and vastus medialis muscles, and two negative electrodes (50 × 100 mm) placed on the proximal portion of the

quadriceps femoris muscle based on a previous study (121). The placement of electrodes was similar between the two EMS sessions.

As shown in Figure 11, the waveform of pulsed current was biphasic symmetrical rectangular, and balanced stimulus pulses were delivered with frequency of 75 Hz, and pulse duration of 400 µs (157, 225). The EMS parameters for alternating current were adjusted at 2.5 kHz alternating sinusoidal current (pulse duration = 400 µs) and delivered in bursts with a carrier frequency of 75 Hz and the bursts duration of 6.5 ms based on previous studies (152, 202, 249). The other stimulation parameters were the same between the alternating and pulsed current EMS (Table 4). For both currents, the ratio was 5 s stimulus on time and 15 s stimulus off time, so the duty cycle time was 25%. The ramping time was included in the stimulation time (on-time) such as 1 s for the rise time and 1 s for the fall time. Current intensity started from 0 mA and was increased rapidly to muscle contraction threshold of each subject every 4-5 contraction by a 3-6 mA increment (Figure 12A). The settings were to achieve the maximum possible force output of each subject in the EMS. The EMS session consisted of a total of 45 isometric contractions of the extensor muscles for 15 min; however, only the last 40 contractions were used for further analysis, since the intensity of the first five contractions was generally very low. The isometric torque of each contraction was recorded by the isokinetic dynamometer.
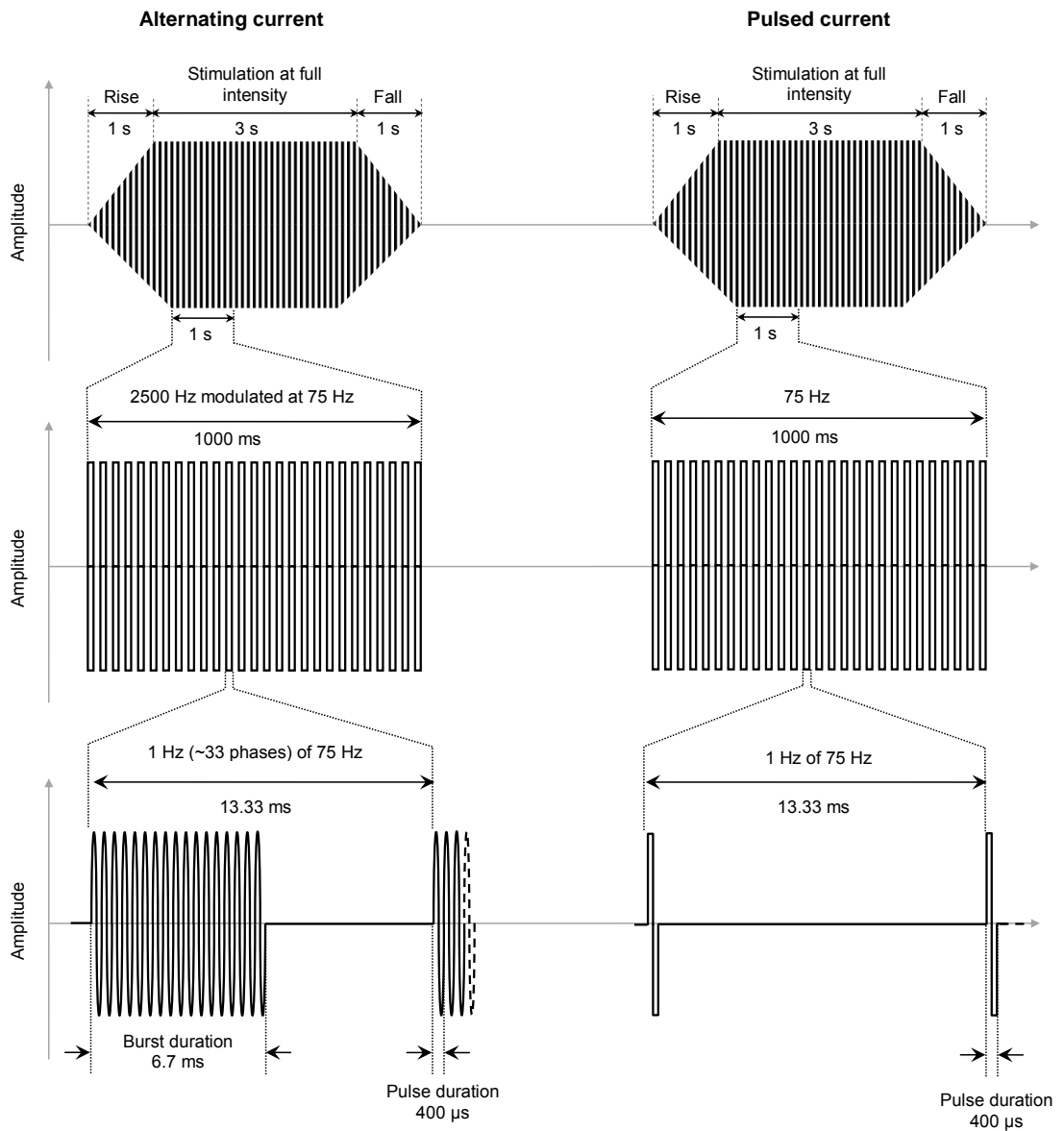
Figure 11: A schematic diagram of alternating current and pulsed current EMS for two waveforms used in the present study. The alternating current was delivered at 2500 Hz and modulated at 75 burst per second, which results in 33 pulses in each burst, while the pulsed current was delivered at 75 Hz, therefore only one pulse in each cycle. The pulse duration was 400μs for both currents.

Table 4: Stimulation parameters for alternating current and pulsed current electrical muscle stimulation.

|  | **Alternating current** | **Pulsed current** |
|---|---|---|
| Pulse frequency | 2.5 kHz delivered at 75 Hz | 75 Hz |
| Pulse duration | 400 µs | 400 µs |
| Burst duration | 6.7 ms | Not applicable |
| Stimulus on time | 5 s (rise time 1 s / fall time 1 s) | 5 s (rise time 1 s / fall time 1 s) |
| Stimulus off time | 15 s | 15 s |
| Cycle time | 13.3 ms | 13.3 ms |
| Duty cycle (on-off time) | 25 % (5 s / 15 s) | 25 % (5 s / 15 s) |
| Pulse duty cycle | Not applicable | 3 % |
| Burst duty cycle | 50 % | Not applicable |
| Waveform | Alternating sinusoidal | Biphasic, symmetrical rectangular |
| Generating current | Constant current | Constant current |
| Stimulus intensity | Maximal tolerance | Maximal tolerance |

## 3.2.4 Dependent variables

### 3.2.4.1 Knee flexion torque during EMS

Each contraction torque induced by EMS was measured and recorded over 40 isometric contractions using a Biodex isokinetic dynamometer software and saved for later analysis to determine torque (peak torque, average torque) and torque time integral of each contraction. The average torque during the EMS-evoked contraction excluding the ramp time and torque time integral of each contraction were calculated using Chart data analysis software (ADInstruments, Bella Vista, Australia).

### 3.2.4.2 Rate of perceived exertion (RPE)

The RPE during the EMS was assessed by a Borg's standard 6–20 scale at every 6 contractions.

### 3.2.4.3 Skin temperature ($T_{sk}$)

Skin temperature ($T_{sk}$) was recorded continuously before, during and for 30 min after EMS. Four skin thermistors (YSI Temperature, 400 series; Dayton, OH, USA) were placed on the skin using fixomull tape near the positive electrodes (vastus medialis and vastus lateralis), mid thigh of the stimulated leg, and the mid thigh of non-stimulated leg. Temperature was recorded by a data-logger set (Squirrel SQ series, Grant Instruments Ltd, UK) at 1 Hz with 1-min intervals starting from 5 min pre exercise to 15 min post EMS. The temperature was obtained at 5 min intervals from the recorded data for further analysis.

### 3.2.4.4 Blood lactate concentration

A 5 µl sample of blood was obtained by finger prick and loaded to a test strip of Lactate Pro Analyzer (Arkray, Inc. Kyoto, Japan) to determine lactate concentration. The measurement was taken before, during the middle of EMS session (after the $20^{th}$ contraction), immediately after, 15, 30 and 60 min post EMS exercise.

### 3.2.4.5 Serum hormone concentration

A 21-gauge Teflon® cannula (Becton Dickinson, Franklin Lakes, NJ) was inserted to a superficial forearm vein, and an extension tube fitted with a two-way stopcock was attached to the cannula. Blood samples was obtained into disposable syringes before, immediately after, 15, 30, 45 and 60 min following EMS, and put into a plain 3 mL Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ). The tubes were left at room temperature for 20 min to clot and centrifuged at 3,000 rpm for 10 min at 4

°C. The serum samples were frozen and stored at -80 °C for later analyses of growth-hormone (GH), total testosterone, cortisol and insulin-like growth factor-1 (IGF-1) using enzyme-linked immunosorbent assay with test kits (GH: DSL-10-1900; total testosterone: DSL-10-4000, cortisol: DSL-10-2000, IGF-1: DSL-10-9400, Diagnostic Systems Laboratories, Webster, TX, USA). Each sample was duplicated in the measures and the average value of the two values was used for further analysis. The hormones concentrations were determined using a multi-label counter set to 450 nm (VersaMax, Molecular Devices, Sunnyville, CA). The coefficient of variation (CV) for GH, total testosterone, cortisol and IGF-1 in the present study were 6.5, 4.8, 8.0 and 8.8%, respectively.

### 3.2.4.6 Maximal voluntary isometric contraction torque (MVC)

Subjects sat on the Biodex isokinetic dynamometer's chair in the same setting as the EMS protocol, and the rotation axis of the knee of the tested leg was aligned with the rotation axis of the dynamometer's armature, and the ankle cuff was joined ~1 cm proximal to the medial malleolus. Gravity corrections were made at 10° of knee flexion. Subjects were asked to keep both arms positioned across the chest with each hand clasping the opposite shoulder. MVC was measured at three different knee joint angles; 40°, 70°, and 100° at 1-3 days before, and 1, 24, 48, 72 and 96 h after EMS exercise. At immediately before and after EMS exercise, MVC was assessed at 100° only in order to minimize the influence of maximal voluntary isometric contractions on hormones, and the angle (100°) was chosen because it was the angle that the isometric contractions were evoked by EMS. No significant difference in MVC measured at 100° was found between the measures taken 1-3 days prior to and immediately before the EMS exercise. The reliability of the MVC measure at 100° indicated by CV based on the two baseline measures taken 1-3 days before and immediately before the EMS session was 5.8%.

Three maximal voluntary isometric contractions for 3 s with a 30-s rest between attempts were performed for each angle, and a 60-s rest was given between different angles. Strong verbal encouragement was given to the subjects during each trial. The peak torque from three contractions for each angle was used for later analysis. The stimulated leg was always measured first followed by the control leg, and the time lag between the legs was approximately 10 min.

### 3.2.4.7 Muscle soreness

Subjects were asked to rate their pain of the knee extensors on a 100 mm visual analogue scale (VAS) as 0 representing "no pain" and 100 as "unbearable pain" while the subjects were asked to squat. Subjects were asked to stand with their legs shoulder width apart, and bent each knee slowly to a 90° angle then return to the initial position.

### 3.2.4.8 Pressure pain threshold (PPT)

PPT was assessed using an electronic algometer (Type II, Somedic Production AB, Sollentuna, Sweden). The algometer was calibrated for each occasion, and the same investigator took all measurements. The probe head (surface area $= 1.0$ cm$^2$) of the algometer was placed perpendicular to four sites of the quadriceps femoris muscle used for the palpation soreness measures that were clearly marked by a water-proof ink pen. Force was gradually applied at a constant rate of 50-60 kPa·s$^{-1}$ until the subject reported the first feeling of noticeable pain. Three measurements were taken from each site sequentially with a minimum of 30 s interval and 1 min between different sites (151) in the following order: the middle point of the rectus femoris, the proximal points of rectus femoris, vastus medialis and vastus lateralis. The value in kilopascals (kPa) corresponding to the amount of force applied was recorded. The mean of the three

measurements for each site was used for further analysis, and the values of all sites were averaged to assess the tenderness in quadriceps muscle.

### 3.2.5 Plasma CK activity

Blood samples for CK were taken from a fingertip using a heparinized capillary (30 µl) and loaded onto a test strip. CK activity was assessed using a Reflotron spectrophotometer (Boehringer-Mannheim, Pode, Czech Republic) in duplicate, and if the difference of the two values was greater than 10%, additional measurements were taken. In this method, the normal reference ranges for adult men are 20-220 $IU \cdot L^{-1}$ according to the instruction sheet of the test kit (Reflotron® CK, Roche Diagnostics GmbH, Germany).

### 3.2.6 Statistical analysis

Changes in stimulation intensity (amplitude), RPE, and torque over 40 contractions and $T_{sk}$, blood lactate, and hormones before, during, and after EMS were compared between the current and pulsed EMS using a two-way repeated measures ANOVA. A two-way repeated measures ANOVA also compared the changes in MVC, VAS of muscle soreness, pressure pain threshold, plasma CK activity before and after EMS between the two stimulation conditions. When the ANOVA showed a significant interaction effect, a Tukey's post hoc test was followed to compare between the stimulation conditions for each time point. The statistical analyses were performed using a Statistical Package for Social Sciences (version 18.0; SPSS Inc., IL, USA). All data are expressed as mean ± SD, and a significant level was set at $P < 0.05$.

## 3.3 RESULTS

### 3.3.1 Stimulation intensity, RPE, and torque generated during EMS

Changes in stimulation intensity (Figure 12A) and RPE (Figure 12B) over 40 contractions are not significantly different between the alternating and pulsed EMS. Stimulation intensity gradually increased throughout the EMS for both currents similarly. RPE increased in the first 20 contractions and reached to a nearly maximum value after $25^{th}$ contraction, without a significant difference between the waveforms. As shown in Figure 12C, no significant difference between the waveforms was evident for the torque output over 40 contractions. The torque increased in the first 10-15 contractions in both currents, but no further increases were seen after that in spite of the increases in the intensity. The averaged torque evoked by EMS was $54 \pm 24$ Nm for alternating current and $62 \pm 28$ Nm for pulsed current with no significant difference between the currents. The level of the torque output during EMS relative to the MVC at $100°$ was $28.4 \pm 4.4\%$ for the alternating current EMS and $31.8 \pm 3.7$ % for the pulsed current EMS, without significant difference between the waveforms.
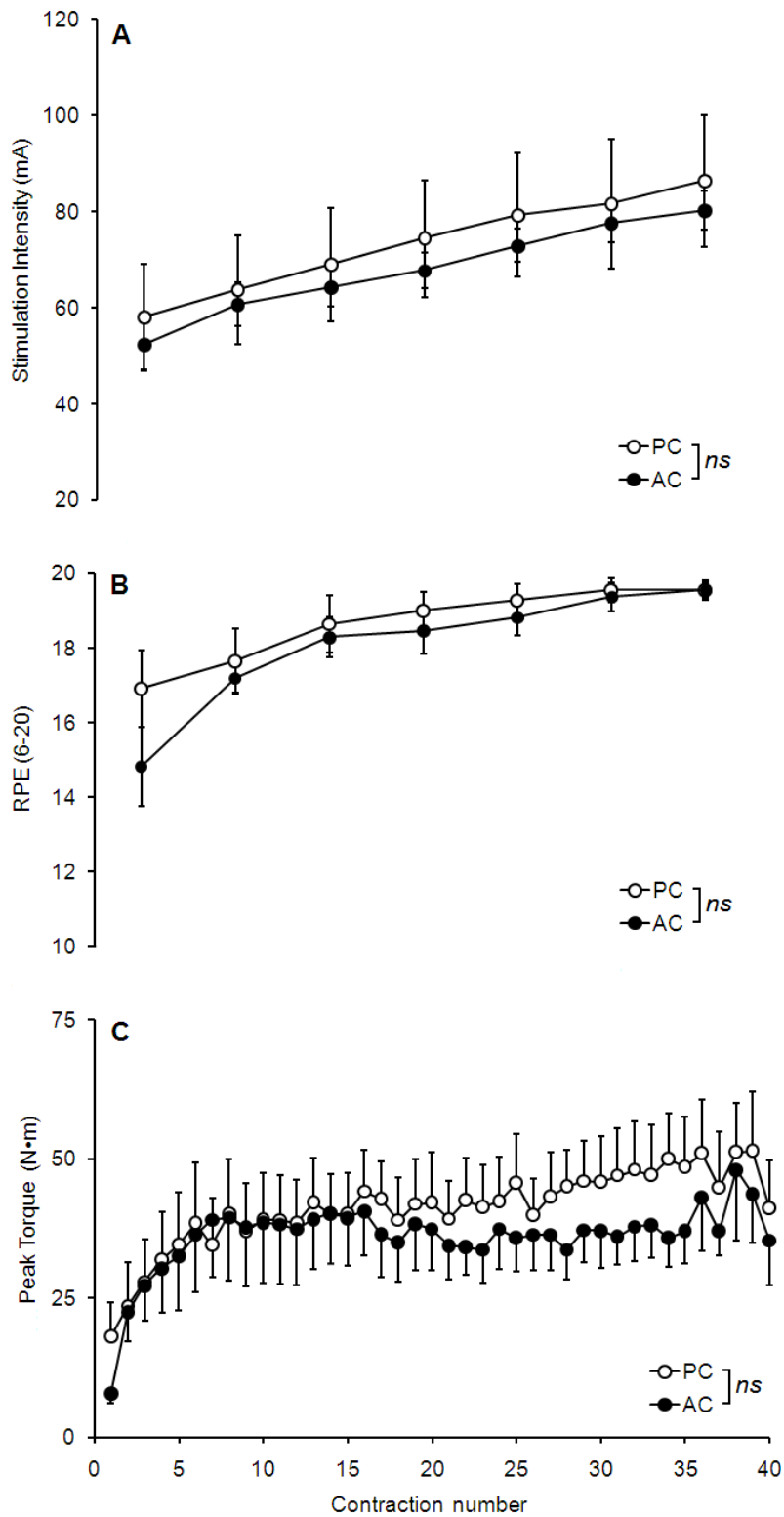
Figure 12: Changes in stimulation intensity (A), rating of perceived exertion (RPE, B), and peak torque (C) over 40 isometric contractions during alternating current (AC) and pulsed current (PC) electrical muscle stimulation. *ns*: no significant difference between currents for the changes over time.

### 3.3.2 Skin temperature

Changes in $T_{sk}$ were similar among the three sites on the stimulated leg, thus the data of the mid thigh of the simulated leg is shown in Figure 13. It also includes $T_{sk}$ of the mid thigh of the non-stimulated leg. $T_{sk}$ increased significantly from the baseline after 10 min of EMS for the stimulated leg while the control leg showed significant decreases. No significant difference was evident between the waveforms.
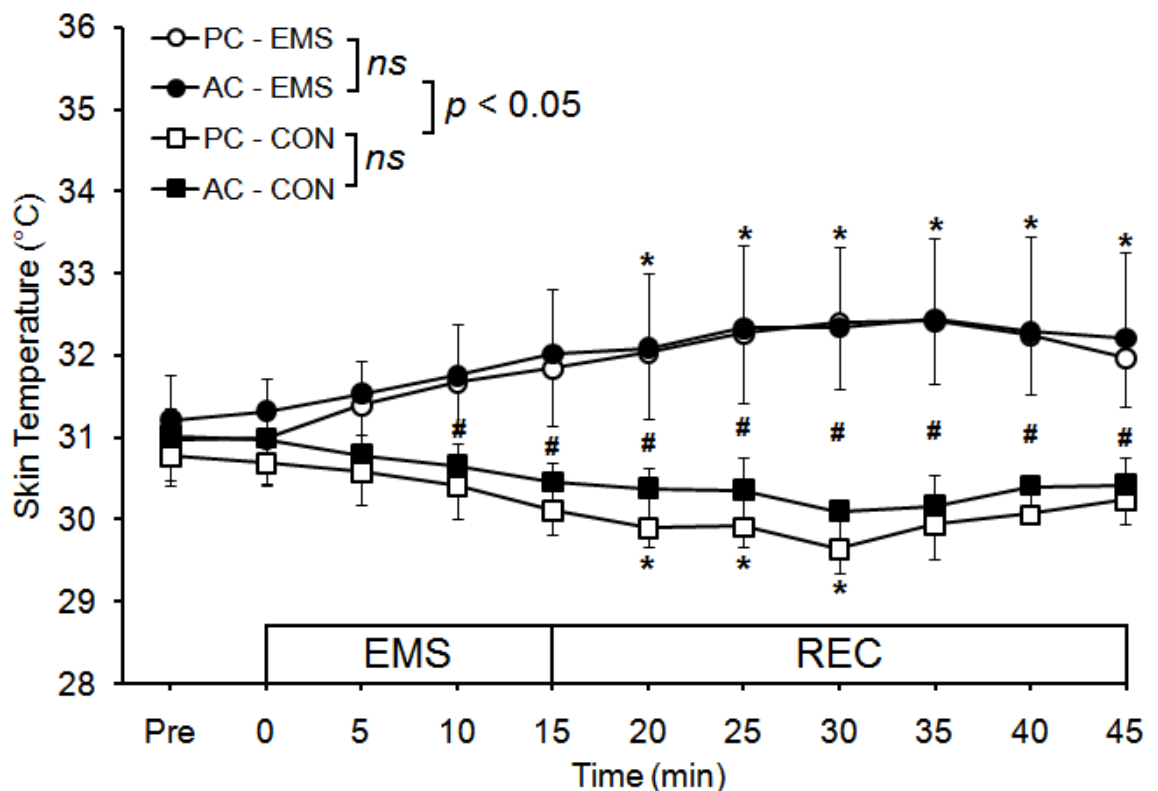


Figure 13: Changes in skin temperature of the electrically stimulated (EMS) and control (CON) leg before (Pre), during (EMS) and for 30 min after EMS (REC) for alternating current (AC) and pulsed current (PC) electrical stimulation. *ns*: no significant difference between currents for the changes over time. *: a significant ($p < 0.05$) change from the baseline, #: a significant ($p < 0.05$) difference between legs.

### 3.3.3 Blood lactate and hormones

Table 5 shows changes in blood lactate and serum hormone concentrations. Blood lactate increased significantly during and immediately after EMS, and returned to

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baseline values within 15 min post exercise. No significant difference in the changes in blood lactate concentration over time was evident between pulsed current and alternating current. No significant differences in the changes in any hormones were evident between the waveforms. Serum GH increased more than 400% after EMS and peaked at 15 min post exercise in both currents. Serum testosterone also significantly increased about 150% immediately and 15 min after EMS. Serum cortisol decreased significantly from baseline to 60 min post-EMS. Serum IGF-1 did not change significantly over time.

### 3.3.4 Muscle damage markers

MVC was not significantly different between currents before EMS for both legs for all angles (Figure 14). The MVC of the control leg did not change significantly over time following EMS. MVC decreased significantly below the baseline immediately after alternating current EMS and pulsed current EMS by 23.1 ± 4.2% and 26.1 ± 2.8 %, respectively, without significant difference between the waveforms. No significant differences in the recovery of MVC were evident between the alternating and pulsed current EMS for all angles. The recovery of MVC at 40° and 70° was significantly faster than that at 100°, and returned to the baseline values within 24 hours; however, MVC at 100° remained lower than baseline for all time points following EMS.

Muscle soreness peaked 48 h post-EMS for both currents (Figure 15A), and no significant differences in muscle soreness were evident between the waveforms. The four locations of PPT measurements showed similar values, and the changes were not significantly different among the sites, thus changes in the mean PPT value of the four sites are shown in Figure 15B. A decrease in PPT indicates that muscles became more tender after EMS, but no significant difference between the conditions was found.

Plasma CK increased significantly after both currents, and peak value was $1{,}262 \pm 339$ IU·L$^{-1}$ for alternating current, and $1{,}677 \pm 491$ for pulsed current, without significant difference in the changes between the two conditions (Figure 15C).
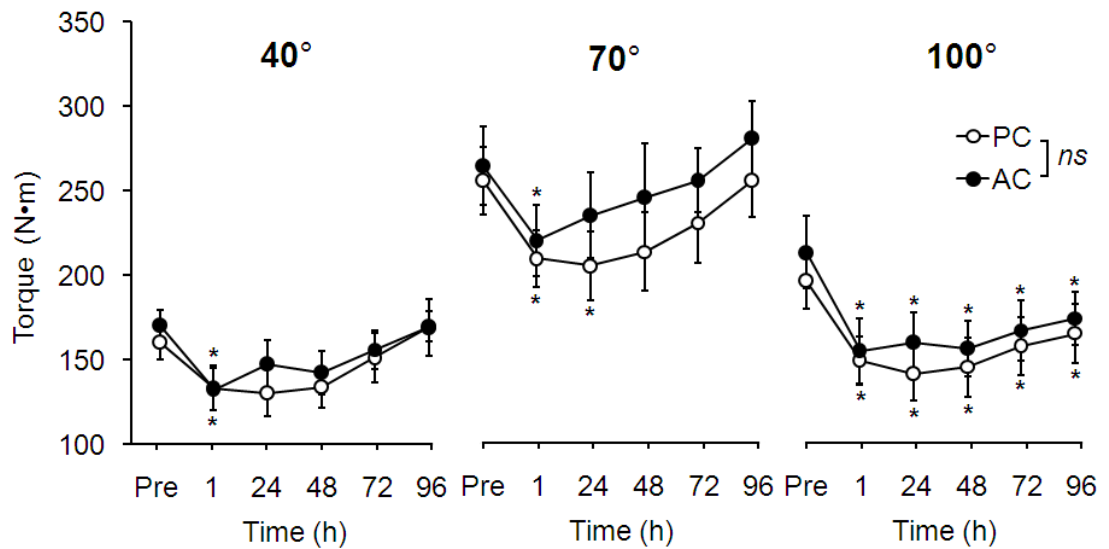


Figure 14: Changes in maximal voluntary isometric contraction torque at 40°, 70° and 100° before (Pre), immediately after (0), and 1, 24, 48, 72, and 96 hours after alternating current (AC) and pulsed current (PC) electrical stimulation. *ns*: no significant difference between currents for the changes over time. *: a significant ($p < 0.05$) difference from the baseline.

Table 5: Changes (mean ± SD) in blood lactate, and serum growth hormone (GH), testosterone, IGF-1, and cortisol concentrations before (Pre), in the middle of electrical muscle stimulation after 20th contraction (EMS), immediately after (0), and 15, 30, 45 and 60 min following alternating current (AC) and pulsed current (PC) electrical stimulation. *: significantly different from the pre value.

| Hormones | Current | Pre | EMS | 0 min | 15 min | 30 min | 45 min | 60 min |
|---|---|---|---|---|---|---|---|---|
| Lactate ($mmol·L^{-1}$) | AC | 1.5 ± 0.1 | 2.1 ± 0.1* | 2.9 ± 0.2* | 1.7 ± 0.2 | 1.4 ± 0.1 | - | 1.1 ± 0.1 |
| | PC | 1.8 ± 0.1 | 2.6 ± 0.2* | 3.4 ± 0.3* | 1.7 ± 0.1 | 1.5 ± 0.1 | - | 1.3 ± 0.1 |
| GH ($ng·mL^{-1}$) | AC | 0.8 ± 0.3 | | 3.1 ± 1.0* | 3.5 ± 0.8* | 2.9 ± 0.7* | 2.3 ± 0.5 | 1.8 ± 0.4 |
| | PC | 0.8 ± 0.2 | | 2.3 ± 0.7 | 3.1 ± 0.7* | 2.5 ± 0.7* | 1.9 ± 0.5 | 1.4 ± 0.4 |
| Testosterone ($pg·mL^{-1}$) | AC | 19.8 ± 1.6 | | 28.2 ± 3.9* | 28.1 ± 3.0* | 27.5 ± 2.8* | 25.8 ± 2.4 | 21.3 ± 2.3 |
| | PC | 18.4 ± 1.9 | | 28.4 ± 3.6* | 28.5 ± 2.9 * | 26.1 ± 3.5 | 23.3 ± 2.5 | 19.7 ± 2.1 |
| IGF-1 ($nmol·L^{-1}$) | AC | 34.4 ± 3.9 | | 33.3 ± 5.7 | 32.1 ± 4.7 | 31.2 ± 3.4 | 32.8 ± 3.5 | 28.9 ± 3.6 |
| | PC | 31.8 ± 4.8 | | 32.9 ± 4.8 | 33.2 ± 6.3 | 33.6 ± 4.7 | 34.4 ± 5.2 | 32.5 ± 5.9 |
| Cortisol ($nmol·L^{-1}$) | AC | 658.3 ± 28.4 | | 614.6 ± 29.5 | 599.6 ± 37.5 | 592.7 ± 46.4 | 542.8 ± 43.8* | 562.1 ± 5.8* |
| | PC | 692.3 ± 26.4 | | 659.6 ± 30.3 | 631.6 ± 33.9 | 591.1 ± 41.7 | 553.8 ± 44.5* | 575.2 ± 5.2* |
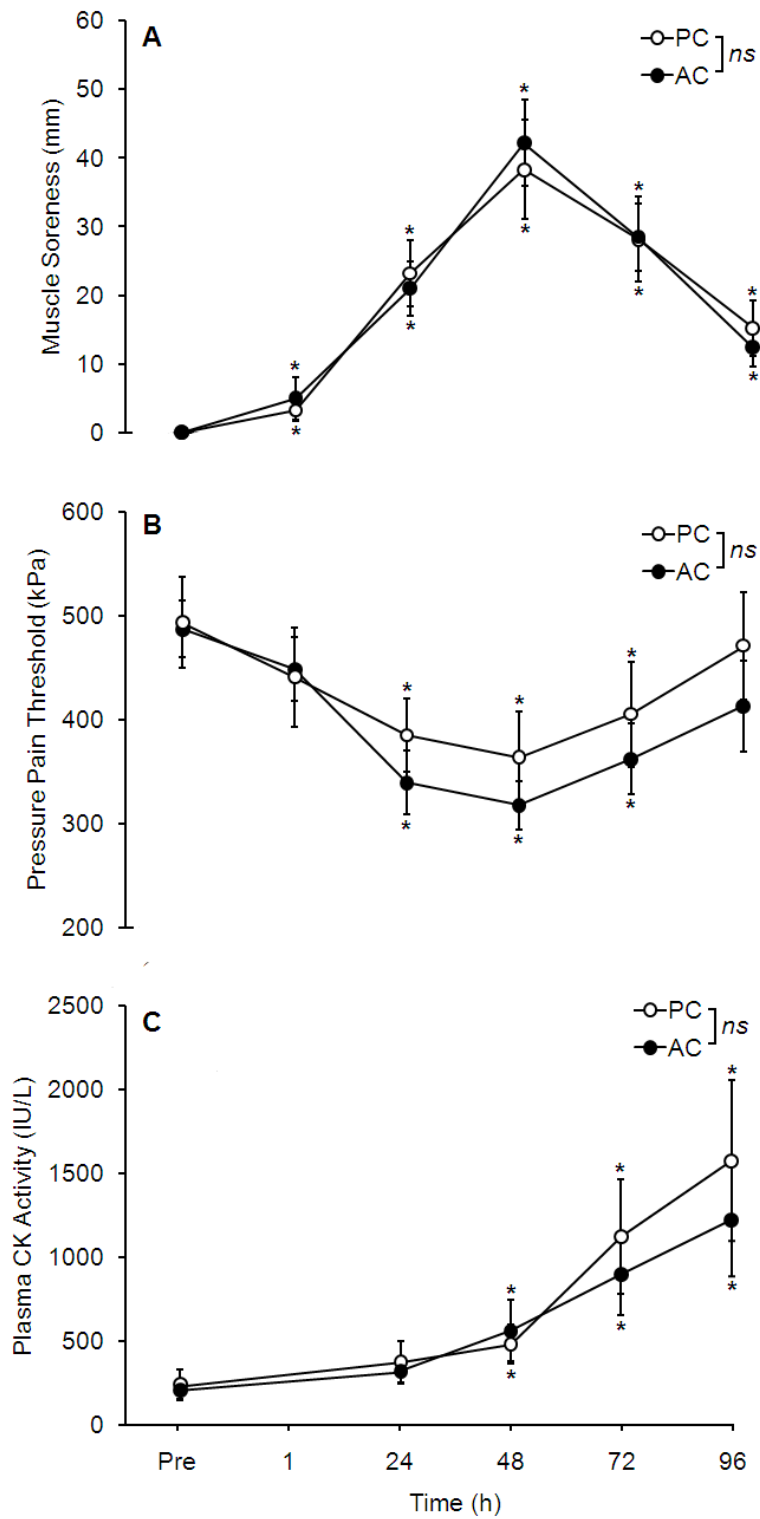
Figure 15: Changes in muscle soreness (VAS) upon squat (A), PPT (B) and plasma CK activity (C) before (Pre), and 1-96 hours after alternating current (AC) and pulsed current (PC) electrical muscle stimulation. *ns*: no significant difference between currents for the changes over time. *: a significant ($p < 0.05$) difference from the baseline value.

## 3.4. DISCUSSION

The present study showed that no significant differences existed between the alternating current and pulsed current for 1) torque generation and RPE during EMS, 2) changes in skin temperature, blood lactate, and hormones before, during, and after EMS, and 3) changes in markers of muscle damage following EMS. Additionally, this is the first study to report that testosterone increases but IGF-1 does not change significantly during and after EMS.

Our data support the previous studies (96, 109, 146, 152, 216) reporting that isometric force generation during EMS was similar between alternating and pulsed currents, in which equivalent number of stimuli per second was delivered for both current, although the number of pulses in a stimulus was different. In the present study, the currents were delivered at 75 Hz for both stimulation conditions, either bursts per second (alternating current) or pulses per second (pulsed current), and the muscles were stimulated at maximally tolerable level for both conditions (Figure 12). The other stimulation parameters were the same between the conditions (Table 5), and no significant difference in the stimulation amplitude was evident between the alternating and pulsed current EMS (Figure 12A). It seems that the number of stimuli per second determines the force generation (75 stimuli in the present study) rather than the number of pulses in each stimulus (33 stimuli for alternating current, and 1 stimulus for pulsed current in the present study). Thus, the additional pulses in the alternating current EMS do not appear to contribute to the force generation (146). Several studies have reported that greater muscle fatigue is induced during alternating current than pulsed current EMS (146, 147, 152, 220). Although the peak torque generated during EMS was not significantly different between alternating current EMS and pulsed current EMS (Figure 12C), the present study also found that the torque during alternating current EMS was

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not as stable as that during pulsed current EMS as reported in previous studies (96, 220).

The torque output increased to the level of approximately 30% MVC in the first 15 contractions and no further increases were seen afterward for both waveforms (Figure 12C), in spite of the consistent increases in the stimulation intensity (Figure 12A). The level of torque output in the present study (approximately 30% of MVC) was similar to that of a previous study (121) in which the same electrode placement and stimulation parameters (e.g. 75 Hz, pulse duration 400 μs) to those of the present study were used. The plateau of maximal torque despite the increases in stimulation intensity (Figure 12A) would indicate that no further increases in motor unit recruitment were made. It appears that muscle fatigue occurs at central and/or peripheral origins during EMS (28), which prevented further increases in force generation. It is possible that the high-frequency stimulation (75 Hz) impaired muscle excitation (46). Additionally, the plateau might be due to muscle damage, where a structural damage to the force-bearing elements and/or a failure of the excitation-contraction coupling occurred (250).

The non-linear increases in RPE appears to indicate an increase in subjects" tolerance to EMS, probably due to an increase in threshold of pain receptors (184). Two previous studies reported that the level of discomfort or pain during stimulation was similar between alternating current and pulsed current EMS (96, 152). For example, Grimby and Wigerstad-Lossing (96) reported that discomfort of the quadriceps muscle stimulated at maximally tolerable intensity was similar between alternating and pulsed current using a Borg"s scale. Lyons et al. (152) also showed no significant difference in discomfort between alternating current EMS and pulsed current EMS using a numeric pain rating scale. Since the stimulation intensity was set at the maximum tolerance of

each subject in the present study, the similar RPE between the conditions seems reasonable. Thus, neither of alternating current or pulsed current can be preferably chosen to the other in term of discomfort especially for muscle strengthening purpose.

As shown in Figure 13, $T_{sk}$ did not change during exercise but increased significantly in the stimulated leg (~ 2 C°) and decreased in the control leg (~ 1 C°) during recovery period for both stimulation conditions. It does not appear that the increases in $T_{sk}$ are associated with the discomfort of EMS. $T_{sk}$ reflects the skin blood flow, and it increases as a result of a vasodilation in skin blood vessels (92, 221). The delayed increase in $T_{sk}$ may suggest that little or no changes in skin blood flow were induced during isometric contractions, but increases in skin blood flow occurred after EMS. The decrease in $T_{sk}$ in the control leg was unexpected. However, Bishop et al. (26) reported that blood flow decreased in the hand skin during a leg exercise, and Cotzias and Marshall (47) also reported a vasoconstrictor response of the cutaneous circulation occurred in the contralateral forearm when isometric handgrip exercise was performed with the other arm. The similar changes in $T_{sk}$ between the alternating and pulsed current EMS reflect a similar muscle usage in the two conditions.

Blood lactate increased similarly in alternating current and pulsed current EMS (Table 5). The magnitude of increase in blood lactate was approximately 20% less than that reported after EMS (121) in which 40 isometric contractions of the knee extensors were evoked electrically in two legs simultaneously. It is likely the muscle volume involved in exercise has an effect on the blood lactate response. The hormonal responses to resistance exercise have been well documented (140); however, little information is available on the effects of EMS on hormonal responses. Two studies (121, 204) reported changes in GH after EMS in which pulsed current was used to stimulate the knee extensors of both legs. The present study showed that EMS increased

GH by 400% and testosterone by 150% (Table 5). The magnitude of increase in GH in the present study was less than a half of that reported in the previous studies (121, 204). This could be due to the difference in the volume of stimulated muscles that the knee extensors of both legs were stimulated in the previous studies, but only one leg was stimulated in the present study. The magnitude of increase in testosterone after EMS was similar to that reported following four sets of slow concentric exercise of knee extensors at 50% 1RM to failure (91). It is interesting that stimulation of only knee extensors of one leg increased testosterone to a similar level as resistance exercise in which more muscles mass are involved. Jubeau et al. (121) reported that the increase in GH was higher after EMS compared with voluntary contractions of the knee extensors when both were performed at the same torque output, and speculated that pain during EMS might be associated with the greater GH release in EMS than voluntary contractions. It might be that EMS is effective for stimulating anabolic hormone release to a greater extent than voluntary contractions.

It was expected to see increases in IGF-1 after EMS because GH stimulates IGF-1 secretion (231). However, no significant changes in IGF-1 were evident up to 45 min post-EMS in the present study. It is important to note that the time course of changes in IGF-1 is different from that of GH or testosterone, such that IGF-1 increases 3-9 h following resistance exercise (106, 135). Thus, it is possible that changes in IGF-1 would have been missed. Further investigation is necessary to track the IGF-1 changes following EMS for an extended period. The constant decrease in cortisol is likely due to the circadian rhythm (227), since all EMS sessions in the present study were performed in the morning, and the time between the pre-EMS blood sample and the last blood sample was approximately 3 h. It is reported that cortisol decreases approximately 300 $nmol·L^{-1}$ over the 3 h period from 8 to 11am (227), whereas the magnitude of the

decrease was approximately half ($\approx 150$ nmol$\cdot$L$^{-1}$) in a similar time frame in the present study. Sartorio et al. (204) reported that cortisol decreased at 1 h after the first EMS bout consisting of 20 isometric contractions started between 8 and 8:30am, but increased significantly for 30 min following the second EMS bout that was performed 2 h after the first bout. Thus, it is possible that EMS increased or at least attenuated the decrease in cortisol in the circadian rhythm, which would suggest catabolic aspects of EMS.

The changes in MVC, muscle soreness, and plasma CK activity after EMS exercise suggest the occurrence of muscle damage following EMS. However, no significant difference between alternating current EMS and pulsed current EMS were evident for any of the parameters (Figure 14 & Figure 15). Since the baseline MVC was similar, and the torque output during EMS was also similar between the bouts (Figure 12C), it seems reasonable to assume that mechanical stress to the muscles was similar between the conditions. It is known that the level of force produced during lengthening contractions is a strong predictor of muscle damage (251). Thus, it appears that the waveform itself does not affect the magnitude of muscle damage (146).

As shown in Figure 14, the decreases in MVC at the angle of stimulation (100°) were significantly greater and longer lasting compared with other angles (40° and 70°). It should be noted that the isometric contractions were performed at 100º in the present study, and the magnitude of MVC decrement appears to be angle specific. The overlap of myosin and actin in isometric contractions at a long length is less compared with that at a short muscle length (41), which may give more mechanical stress over sarcomeres, and cause a disruption to cross-bridge (61). Changes in MVC and plasma CK activity have been reported to be greater following eccentric exercise of elbow flexors at long muscle lengths compared with short muscle length (180). Several studies (180, 205)

have reported that isometric contractions at a long muscle length induce muscle damage but not at a short muscle length. Nosaka et al (179) found that muscle damage following intermittent isometric contractions of the elbow flexor induced by EMS was minimal when the biceps brachii was stimulated at a short muscle length (90°). However, our recent study (unpublished) found that when the biceps brachii was stimulated at a long muscle length (160°), EMS-evoked isometric contractions resulted in appreciable changes in muscle damage markers such as MVC, muscle soreness and plasma CK activity. Thus, it seems likely that the cause of muscle damage was not EMS itself, but repeated isometric contractions at a long muscle length (5).

Changes in muscle soreness following EMS were similar to those reported in previous studies in which isometric contractions of the knee extensors were evoked by EMS (5, 121). The magnitude of increase in plasma CK activity in the present study was approximately half of that reported in the previous study (121). The amount of stimulated muscles was different between the previous study (two legs) and the present study (one leg), and this could explain the difference. Our previous study (5) showed that the magnitude of muscle damage was significantly attenuated in the second EMS bouts performed 2 weeks after the initial bout using pulsed current. It seems likely that this is also the case for alternating current EMS; however, this should be investigated in future studies.

In conclusion, alternating and pulsed current have a similar effect on force production, RPE, skin temperature, hormonal responses, and muscle damage, when the stimulation parameters except the waveform were matched between the two. These findings suggest that acute effects are similar between alternating and pulsed current EMS, thus the waveform itself is less important for EMS. For example, if a EMS machine has an capacity to maximally stimulate a muscle when other parameters such

as stimulation intensity, frequency and pulse duration are the same. Considering the fact that a pulsed current stimulator is less expensive and more portable than an electrical stimulator that can deliver alternating current, pulsed current EMS may be more advantageous over alternating current EMS, especially when it is used for muscle training. It is important to note that the present study focused on the use of EMS in muscle training of healthy men; therefore it is necessary to examine women, elderly individuals, and clinical populations in future studies. Chronic effects of the use of different current in a treatment or a training program cannot be speculated from the present study. Thus, further study is warranted to compare the effects of chronic use of EMS on muscle adaptation between alternating current and pulsed current EMS for healthy and clinical populations.

# CHAPTER FOUR: STUDY TWO

## MUSCLE OXYGENATION OF VASTUS LATERALIS AND MEDIALIS MUSCLES DURING ALTERNATING AND PULSED CURRENT ELECTRICAL STIMULATION

### 4.1 INTRODUCTION

Electrical muscle stimulation (EMS) is widely used in rehabilitation and sport training, and has been shown to be effective for improving muscle strength (209, 214). There are many types of EMS, which are basically determined by waveform, frequency, intensity and pulse duration (22, 86, 90). The most commonly used EMS waveforms are alternating current and pulsed current. Pulsed current EMS has been more commonly used in sport training than alternating current EMS, because of easier access to a pulsed current stimulator (152, 240, 249). The use of alternating current in sport training increased after a Russian scientist Kots had claimed that the muscle strength increased up to 40% with alternating current (2500-Hz) modulated at 50-Hz, so called Russian current (249). If both waveforms are possible to use, the question is which is better for strength training. However, physiological differences between alternating current and pulsed current EMS are not necessarily clear, and controversy exists among the findings of previous studies.

Some studies showed that alternating current EMS induced more muscle fatigue than pulsed current EMS (146, 147, 220). For example, Stefanovska and Vodovnik (1985) reported that decreases in isometric torque of the knee extensors over 10

contractions (10 s stimulation, 50 s rest between stimulations) were significantly greater by 25% for alternating current EMS (2500-Hz delivered at 25-Hz) compared with pulsed current EMS (25-Hz), and this was also the case for 60-s continuous stimulation (the magnitude of decrease in the torque was 43% greater for the alternating current than pulsed current). If muscle fatigue develops at early stage in EMS, the mechanical stimulus to the muscle would be less, which may not be ideal for strength training. However, the greater muscle fatigue indicated by lower force generation in alternating current than pulsed current EMS reported in the previous studies (146, 147, 220) was not necessarily observed in other studies. For example, several studies (96, 109, 152, 216) reported no significant difference in isometric contraction torque between alternating and pulsed current EMS, when muscles were stimulated at the maximal tolerable intensity over intermittent isometric contractions. Thus, further study is necessary to compare muscle fatigue in EMS between alternating current and pulsed current by using other measures that could monitor changes in motor unit recruitment during EMS.

Near infrared spectroscopy (NIRS) can monitor muscle oxidative metabolism during exercise (101, 193). Felici et al. (70) reported that biceps brachii myoelectric activity assessed by surface electromyography (EMG) was correlated ($r = 0.72$) with muscle oxygenation assessed by NIRS during sustained and repeated isometric contractions. Since EMS interferes with EMG measurements, EMG cannot assess a decrease in muscle activity during EMS exercise. However, a decrease in muscle activity should be reflected in a reduction in muscle oxygen ($O_2$) consumption, thus NIRS could provide information relating to muscle fatigue (e.g. reduction in muscle activity) during muscle contractions evoked by EMS. No previous studies have investigated muscle oxygenation during alternating current EMS nor compared between

alternating and pulsed current EMS for changes in muscle oxygenation during intermittent isometric contractions of the knee extensors.

The purpose of the present study therefore was to compare between alternating current EMS and pulsed current EMS for changes in vastus lateralis and vastus medialis muscle oxygenation and change in blood volume using NIRS during intermittent isometric contractions. It was hypothesized that the muscle oxygenation during the isometric contractions would be less for alternating current EMS compared with pulsed current EMS, if the former results in greater muscle fatigue.

## 4.2 METHODS

### 4.2.1 Subjects

Nine men (mean ± SD age: 34.0 ± 7.0 y, body weight: 85.4 ± 14.1 kg, height: 174.0 ± 5.1 cm) who had not been involved in resistance training programs for at least 6 months prior to the present study were recruited. Subjects with any health problems (e.g. hypertension, epilepsy, neurological and neuromuscular disorders) or who had any muscle or joint injuries of knee were excluded. All subjects were informed about the study, and a medical questionnaire and an informed consent written form were obtained prior to participation from each subject. The subjects were requested not to involve in any physical activity before reporting to the laboratory. This study was approved by the Edith Cowan University Human Research Ethics Committee and conducted in accordance with the Declaration of Helsinki.

## 4.2.2 Electrical muscle stimulation (EMS)

All subjects received alternating current EMS for one leg, and pulsed current EMS for the other leg in different days separated by 2 weeks in a randomised, counterbalanced order with no emphasis on the limb dominance. For each EMS session, the subjects were not informed which current was applied, thus the use of the waveform was blind to the subjects. The knee extensors were stimulated by an Intelect Advanced®Colour Stim (Chattanooga Group, TN, USA) that could deliver both alternating current and pulsed current. After cleaning the skin surface with alcohol pads, two positive electrodes (50 × 50 mm) were placed over the motor point of vastus lateralis and vastus medialis, which were identified as the sites on the skin where the smallest current amplitude was required to produce muscle contraction (199). Two negative electrodes (50 × 100 mm) were placed on the proximal portion of rectus femoris muscle (121). The subjects were seated on a Biodex isokinetic dynamometer"s chair (Biodex Medical Systems, Inc., USA), and the knee joint angle was set at 100° (0° corresponding to the full extension), and the trunk, pelvis, and thigh were firmly secured to the seat by straps at trunk angle of 110°.

Figure 16 shows the diagram of the waveforms that were used in the present study for alternating and pulsed current EMS. The waveform of pulsed current was biphasic, symmetrical and rectangular, and the pulses were delivered at a frequency of 75-Hz (pulses per second) and pulse duration was 400 μs, which was used in a previous study (121). The waveform of alternating current was polyphasic, sinusoidal and balanced, and adjusted at 2500-Hz alternating current (400 μs pulse duration), delivered in bursts with a carrier frequency of 75-Hz and the burst duration was 6.5 ms. These alternating current parameter settings were similar to those used in previous studies (152, 202, 249). The stimulation protocols were adjusted to match other parameters than

waveform such as pulse duration and duty cycle as identical as possible between the alternating and pulsed current EMS. The stimulation time was 5 s followed by a 15-s rest, so the duty cycle time was 25%, and the ramping time was included in the stimulation (1 s for the rise time and 1 s for the fall time) based on previous studies (121, 152).

The stimulation amplitude was started with 0 mA, and gradually increased to a maximally tolerable intensity in the first 10 contractions to reach a plateau force, thereafter current amplitude was consistently increased every 5 contractions to complete 30 isometric contractions at maximally tolerable stimulation intensity. The magnitude of increase in the stimulation amplitude was depending on the feedback from each subject, and the increment was between 0 and 5 mA for each time point. The torque of each contraction was recorded by the Biodex software and analysed using Chart data analysis software (ADInstruments, Bella Vista, Australia).

**Alternating current**



**Pulsed current**
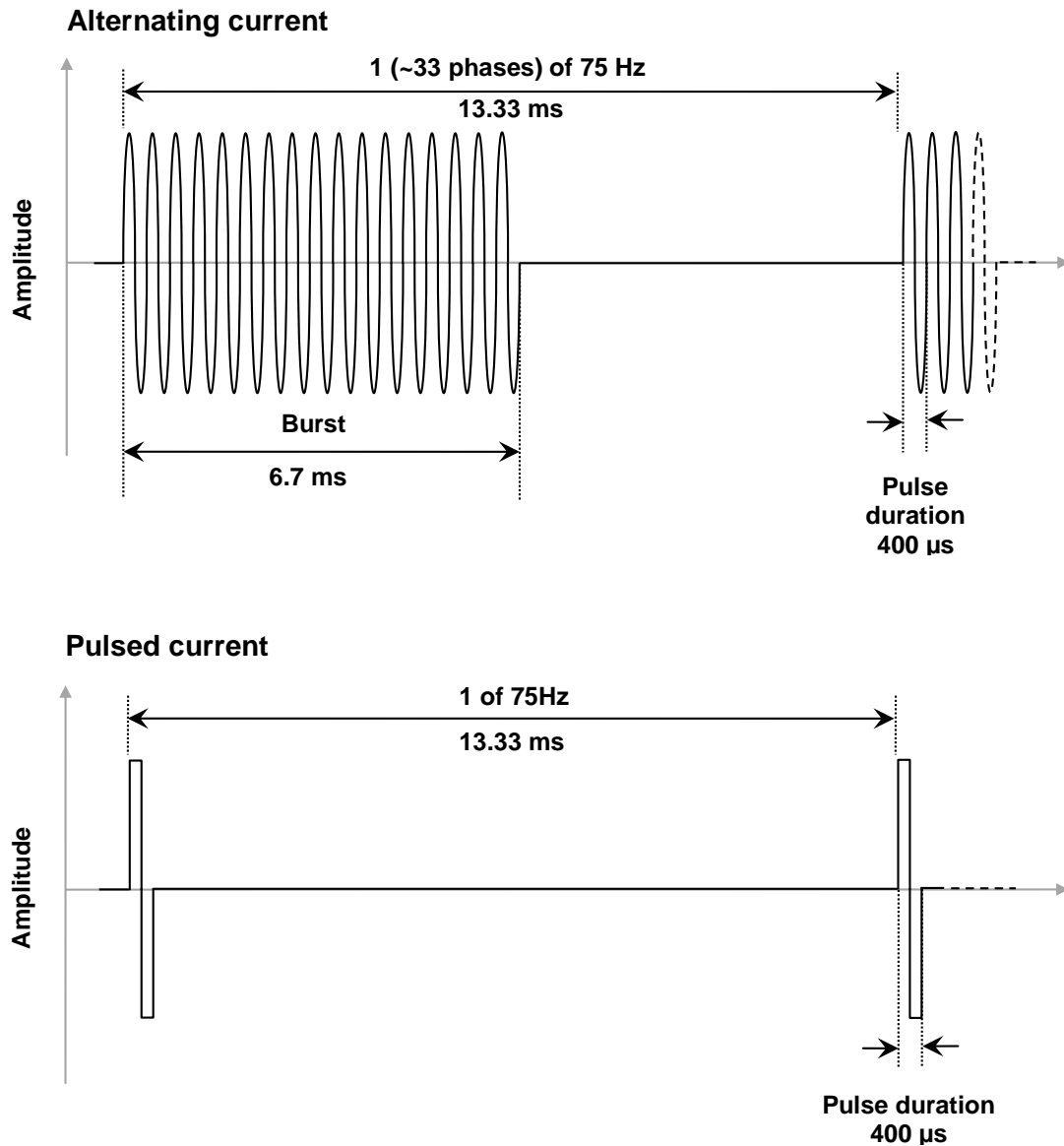


Figure 16: Schematic diagrams of alternating and pulsed waveforms used in the study.

### 4.2.3 Maximal voluntary isometric contraction torque (MVC)

Subjects sat on the Biodex isokinetic dynamometer‟s chair and the setting was the same as that of the EMS protocol described above. The rotation axis of the tested knee was aligned with the rotation axis of the dynamometer‟s armature, and the ankle cuff was joined ~1 cm proximal to the medial malleolus. Gravity corrections were made at 10° of knee flexion from a full-extension position. Subjects were asked to keep both

arms positioned across the chest with each hand clasping the opposite shoulder. MVC was measured at 100° of the knee joint, because it was the angle that the isometric contractions were evoked by EMS. Each subject performed two maximal contractions with a 30-s rest between contractions, before and immediately (within 5 s) after EMS session. The contraction lasted for 5 s and the MVC was determined by the peak of the torque curve of each contraction. The higher peak value of the two attempts was used for further analysis.

### 4.2.4 Blood lactate concentration

A 5 µl of blood was taken by finger prick and loaded to a test strip of a Lactate Pro Analyzer (Arkray, Inc. Kyoto, Japan). The samples were obtained before, immediately after the $20^{th}$ isometric contraction during the EMS session, and immediately and 15 min after EMS exercise.

### 4.2.5 Near-infrared spectroscopy (NIRS)

A NIRO-200 oximeter (Hamamatsu Photonics K.K, Hamamatsu, Japan) was used to measure the concentration changes in oxygenated haemoglobin ($\Delta O_2Hb$), deoxygenated haemoglobin ($\Delta HHb$), and total hemoglobin volume ($\Delta tHb = \Delta O_2Hb + \Delta HHb$) from an arbitrary baseline of zero. The NIRO-200 is a spatially resolved spectroscopy (224) and it provides an absolute measure of $O_2Hb$ saturation as tissue oxygenation index ($TOI = O_2Hb / tHb \times 100$). The NIRS probe secured in a rubber holder consisted of a laser diode that delivers three wavelengths (775, 810, and 850 nm) and an optical photodetector with two photodiodes, and the light emitter and detector were separated by a distance of 4 cm. The NIRS light has a penetration depth of ~2 cm (i.e., half of the distance between the emitter and the detector) from the skin surface

(39), which was shown to be sufficient for attaining a reflected light signal from the targeted muscle (162). Two NIRS probes were placed proximally adjacent to each of the EMS positive electrodes and parallel to the longitudinal axis of the mid-belly of the vastus lateralis (one probe) and vastus medialis (the other probe). The NIRS probes were covered with a black fabric cloth to minimise the interference of stray light and loss of NIR light from the interrogation field. The NIRO-200 sample rate was set at 6 Hz and the signals were recorded at 200 Hz and saved in a data acquisition system (PowerLab, ADInstruments, Bella Vista, Australia) for later analysis.

The concentration changes in $\Delta O_2Hb$, $\Delta HHb$ and $\Delta tHb$ are expressed as $\mu M \cdot cm$, and TOI is expressed as a percentage. The resting baseline values for $\Delta O_2Hb$, $\Delta HHb$, $\Delta tHb$ and $\Delta TOI$ were determined as the average of 30 s before exercise, and were represented as the magnitude of change in these parameters with respect to their respective baseline that was set to zero. As shown in Figure 17, the NIRS parameters used in the present study included minimum $\Delta TOI$ amplitude ($\Delta TOI_{min}$), maximum $\Delta TOI$ amplitude ($\Delta TOI_{max}$), mean $\Delta tHb$ amplitude ($\Delta tHb_{mean}$) and maximum $\Delta tHb$ amplitude ($\Delta tHb_{max}$) that were used in previous studies (38, 70). $\Delta TOI_{min}$ is the difference between the baseline and the lowest TOI at the end of contraction, which has been shown to be inversely proportional to $O_2$ consumption relative to $O_2$ supply; while $\Delta TOI_{max}$ is the difference between the baseline and the highest value during the relaxation phase, and indicates the $O_2$ supply relative to $O_2$ consumption. $\Delta tHb_{mean}$ was the difference between the baseline and the average $\Delta tHb$ value during contraction, and it is an indication of the blood volume/flow changes. $\Delta tHb_{max}$ was the difference between the baseline and the highest value during the relaxation phase, and reflects blood volume/flow changes during relaxation.

### 4.2.6 Statistical analyses

The normality of the data distribution was checked by a Shapiro-Wilk test for the torque and NIRS parameters. Changes in average peak torque, torque time integral, $\Delta TOI_{min}$, $\Delta TOI_{max}$, $\Delta tHb_{mean}$ and $\Delta tHb_{max}$ over 30 contractions were analysed using a one-way repeated measures ANOVA, and compared between alternating and pulsed current EMS by a two-way repeated measures ANOVA. Changes in MVC from pre to post EMS exercise, and blood lactate before, during, and after EMS were compared between waveforms using a two-way repeated measures ANOVA. When a significant interaction effect was found, a Tukey's post hoc test was followed to compare between bouts for each time point. The significance level was set at $P < 0.05$. All data are expressed as mean $\pm$ SEM.

Figure 17: Typical changes in knee extensor torque, and vastus lateralis tissue oxygenation index ($\Delta$TOI) and total haemoglobin volume ($\Delta$tHb) of a representative subject for alternating current EMS (A) and pulsed current EMS (B). The dashed grey vertical lines correspond to the onset and the end of the first 5-s contraction (on-time) and the end of the 15-s relaxation (off-time), respectively. $\Delta TOI_{min}$: minimum $\Delta$TOI amplitude, $\Delta TOI_{max}$: maximum $\Delta$TOI mplitude, $\Delta tHb_{mean}$: mean $\Delta$tHb amplitude, $\Delta tHb_{max}$: maximum $\Delta$tHb amplitude.

## 4.3 RESULTS

### 4.3.1 MVC torque and Torque during EMS

No significant difference in the baseline MVC was evident between the alternating current ($190 \pm 23$ N·m) and pulsed current ($207 \pm 22$ N·m). When comparing to pre-EMS values, MVC decreased significantly immediately after EMS by $20.8 \pm 5.1\%$ for alternating current and $24.4 \pm 8.3\%$ for pulsed current, without a significant difference between them. The torque generated during contractions was more stable in the pulsed current than alternating current EMS, especially from the half way of the EMS session, although the peak torque values were similar between the waveforms in the first 10 contractions at least (Figure 18).

As shown in Figure 19A, the stimulation amplitude increased significantly over 30 contractions, and no significant difference in the changes in amplitude was evident between waveforms. Figure 19B shows changes in peak torque of each contraction relative to the pre-EMS MVC over 30 contractions. The peak torque during EMS ($69.1 \pm 5.6$ N·m) was approximately 30% of the pre-EMS MVC at $10^{th} - 30^{th}$ contractions for both waveforms. Changes in the peak torque over 30 contractions were significantly different between waveforms such that the torque increased significantly for the pulsed current, but no significant increases were found for alternating current.
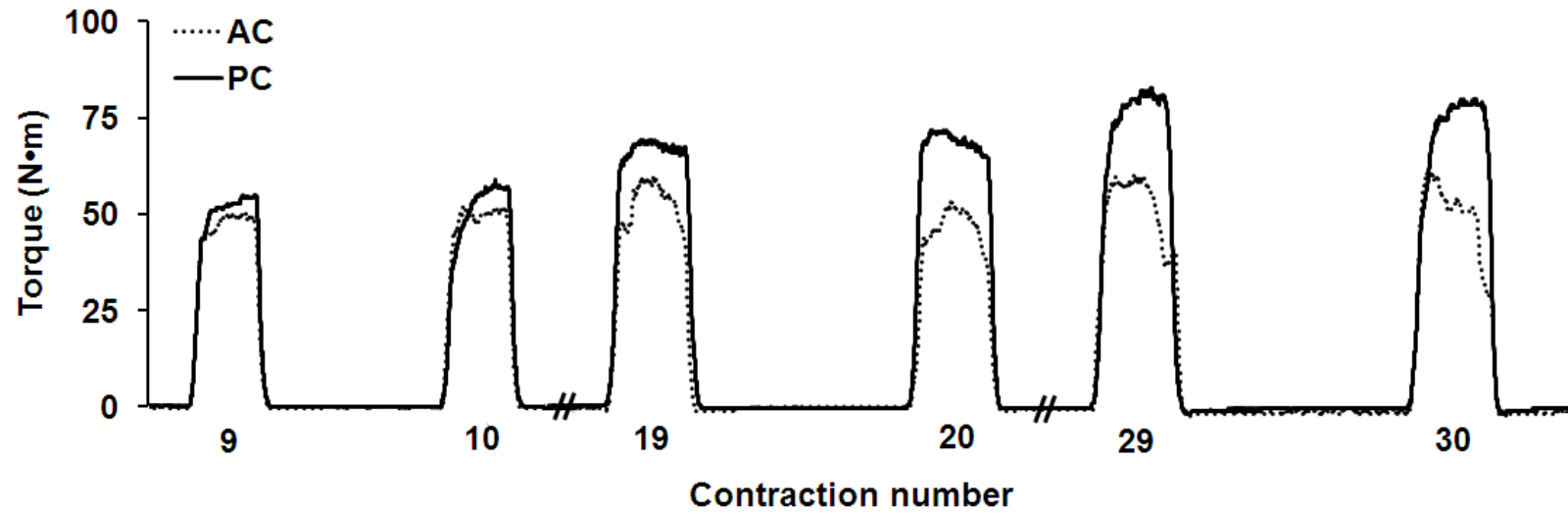
Figure 18: Typical torque curve obtained during 5-s stimulation in alternating current (dotted line) and pulsed current (solid line) EMS sessions for the 9th and 10th, 19th and 20th, and 29th and 30th isometric contraction.
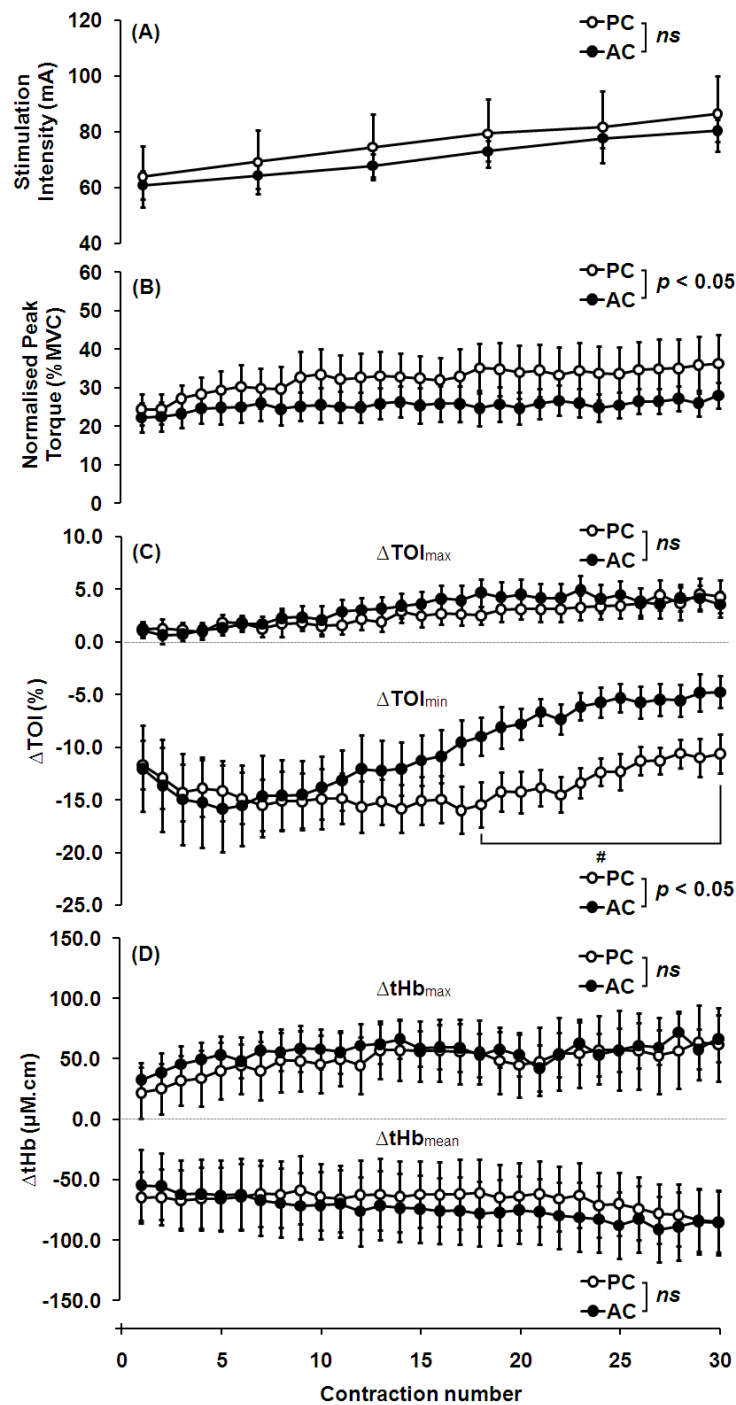
Figure 19: Changes (mean values ± SEM; N = 9) in stimulation intensity (A), peak knee extensor torque during EMS relative to the baseline maximal voluntary isometric torque (B), maximum $\Delta TOI$ amplitude ($\Delta TOI_{max}$) and minimum $\Delta TOI$ amplitude ($\Delta TOI_{min}$) (C), and maximum $\Delta tHb$ amplitude ($\Delta tHb_{max}$) and mean $\Delta tHb$ amplitude ($\Delta tHb_{mean}$) (D) for vastus lateralis muscle over 30 isometric contractions evoked by alternating current (AC) and pulsed current (PC) EMS. Interaction effect was indicated (*ns*: no significant effect, $p<0.05$: significant effect). #: significant ($P < 0.05$) difference between waveforms based on post-hoc test.

### 4.3.2 Blood lactate concentration

No significant difference between alternating current and pulsed current EMS was evident for the changes in blood lactate concentration. Blood lactate concentration increased significantly from pre ($1.5 \pm 0.1$ mM·L$^{-1}$, $1.8 \pm 0.1$ mM·L$^{-1}$) to the $20^{th}$ contraction ($2.0 \pm 0.1$ mM·L$^{-1}$, $2.6 \pm 0.2$ mM·L$^{-1}$) and immediately post-EMS ($2.7 \pm 0.2$ mM·L$^{-1}$, $3.1 \pm 0.1$ mM·L$^{-1}$) for alternating current and pulsed current, respectively, but returned to the pre-exercise value by 15 min following exercise.

### 4.3.3 NIRS parameters

No significant differences in the changes in any of the NIRS parameters over 30 contractions were evident between vastus lateralis and vastus medialis muscles. The mean TOI baseline value was approximately 60% for both waveforms with no significant difference between the conditions. Figure 17 shows typical torque, $\Delta$TOI and $\Delta$tHb traces obtained from vastus lateralis muscle for two consecutive isometric contractions (the $20^{th}$ and $21^{st}$) for the alternating current and pulsed current EMS. $\Delta$TOI decreased during contraction and reached a minimum value ($\Delta$TOI$_{min}$) at the end of each contraction, then increased toward baseline during the relaxation phase to reach the maximum level ($\Delta$TOI$_{max}$) before the next contraction. $\Delta$tHb decreased rapidly at the onset of contraction and did not significantly change during contraction ($\Delta$tHb$_{mean}$), and reached the maximum level ($\Delta$tHb$_{max}$) soon after the end of stimulation. However, the great fluctuation can be due to the electrical current since the EMS electrodes were adjacent to NIRS probes.

Figure 19C shows mean changes in $\Delta TOI_{max}$ and $\Delta TOI_{min}$ of vastus lateralis muscle over 30 isometric contractions for both waveform conditions. $\Delta TOI_{max}$ increased significantly from the first contraction to the 30th contraction for both waveforms, and no significant difference between the conditions was found. Changes in $\Delta TOI_{min}$ from the baseline were significantly smaller for the alternating current compared with pulsed current after the 18th contraction.

Mean changes in $\Delta tHb_{mean}$ and $\Delta tHb_{max}$ over 30 isometric contractions in both waveforms are shown in Figure 19D. $\Delta tHb_{mean}$ decreased significantly over 30 contractions, and no significant difference was found between conditions. No significant difference between the waveforms was evident for the changes in $\Delta tHb_{max}$, which showed significant increases over 30 contractions for both waveforms.

## 4.4 DISCUSSION

The results of this study showed that EMS-evoked peak isometric contraction torque significantly increased over the 30 contractions in response to the increases in the stimulation intensity only for the pulsed current EMS, and the decreases in $\Delta TOI$ during the contraction phases from baseline ($\Delta TOI_{min}$) were significantly greater for the pulsed current than alternating current from the 18th contraction onwards. However, no significant differences between the alternating current and pulsed current EMS were evident for the changes in MVC from pre to post EMS, the changes in $\Delta TOI_{max}$, $\Delta tHb_{max}$, and $\Delta tHb_{mean}$ over 30 isometric contractions, and the changes in blood lactate before, during and after EMS.

The torque produced during EMS-induced isometric contractions was approximately 30% of pre-EMS exercise MVC measured at the same knee joint angle

as the stimulation (i.e. 100°) for both waveforms at least for the first 10 contractions. The level of torque output was comparable to that reported in a previous study (26 ± 14% MVC) in which the knee flexors were stimulated at the same angle as that of the present study (i.e. 100º) using pulsed current (75-Hz) at maximum tolerable current intensity (121). When looking at the torque curves, the torque was less steady for the alternating than pulsed current EMS, and greater decreases in the torque were seen toward the end of each contraction in the alternating current EMS (Figure 18). This was also reported in previous studies (96, 220). Grimby and Wigerstad-Lossing (96) stated that pulsed current EMS (30-Hz) was less fatiguing than alternating current EMS (2500-Hz delivered at 50-Hz). Stefanovska and Vodovnik (220) documented that high-frequency stimuli (alternating current EMS: 2500-Hz delivered at 25-Hz) caused rapid fatigure of the stimulated muscle compared with pulsed current EMS (25-Hz).  It appears that the decrease in force toward the end of contraction in the alternating current EMS indicates that "fatigue" induced in alternating current was greater than that in pulsed current EMS.

Jones (117) described that action potential propagation along the surface membrane was affected in "high-frequency" stimulation, and this was referred to as "high-frequency fatigue" that occurs at a frequency higher than 50-Hz, resulting in force loss, but the force quickly recovers in the cessation of the stimulation. The force generation recovered rapidly in the interval between contractions in the present study (Figure 18), therefore it may be that "high-frequency fatigue" was the cause of the force loss during isometric contraction evoked by EMS.  If this was the case, it seems possible that the higher frequency of the alternating current (2500-Hz) was responsible for the decreases in the torque toward the end of each 5-s contraction.

Stefanovska & Vodovnik (220) compared between alternating current (2500-Hz modulated at 25-Hz) and pulsed current (25-Hz) for the torque generation of the knee extensors over 10 isometric contractions (10 s contraction, 50 s rest), and found greater decreases in torque during the alternating current than pulsed current EMS. However in the present study, no significant decreases in isometric torque were observed over 30 isometric contractions for both waveforms (Figure 19B). Laufer and Elboim (146) compared an alterneting current delivered at 2500-Hz (modulated either at 20-Hz or 50-Hz) and pulsed current delivered at 50-Hz for force integral of the wrist extensors over 21 consecutive isometric contractions (3 s ramp up, 7 s on, 3 s off). They reported that decreases in the force integral were significantly greater for alternating current compared with the pulsed current, and stated that this was related to a failure of the action potential to propagate within muscle fibers due to "high-frequency fatigue" as mentioned above. In the present study, it is possible that the long interval (15-s off-time) between contracions might have provided a sufficient time for recovery of high-frequency fatigue, thus the torque did not decrease over 30 contractions (Figure 19B). It should be noted that the EMS-evoked peak torque significantly increased over 30 isometric contractions only for the pulsed current EMS session in response to the increase in the stimulation intensity (Figure 19A, B). This may indicate that some muscle fibres were unable to be recruited in response to the increase in the stimulation intensity in alternating current EMS. It is also important to note that the subjects were able to tolerate higher stimulation intensity during both EMS protocols, and the stimulation intensity increased about 65% over the 30 contractions. It appears that the pain tolerance increased during EMS, which could be due to the increase in threshold of pain receptors (153, 184).

To the best of our knowledge, only one study (133) investigated vastus lateralis muscle oxidative metabolism during pulsed current EMS in healthy subjects using NIRS. Kooistra et al. (133) found no difference in muscle $O_2$ consumption when compared 10 s of EMS isometric contraction (50-70 Hz, pulsed duration: 100 µs at the highest tolerated intensity) and 15 s of a maximal voluntary isometric contraction at different angles of the knee extensors. However, they recorded the changes in $O_2$ consumption during single sustained isometric contractions, which may differ from intermittent isometric contractions performed repeatedly in the present study. As shown in Figure 19C, the changes in $\Delta TOI_{max}$ were not different between alternating current and pulsed current (Figure 19C), which may indicate a similar $O_2$ supply relative to $O_2$ consumption during the relaxation phase between waveforms. $\Delta TOI_{max}$ increased significantly over 30 isometric contractions, which is likely to indicate that $O_2$ supply during the relaxation phase increased. In contrast, $\Delta TOI_{min}$ was significantly lower in pulsed current from the $18^{th}$ contraction onwards compared with alternating current. The dynamic balance between $O_2$ demand and $O_2$ supply is reflected in the changes in $\Delta TOI$, and the changes are affected by $O_2$ extraction and blood flow/volume (100). Since blood flow/volume did not appear to be different between the waveform conditions as shown in the similar changes in $\Delta tHb$ between conditions (Figure 19D), the difference in the $\Delta TOI_{min}$ during the last 13 isometric contractions could be attributed to a difference in $O_2$ consumption between conditions.

The gradual increases in $\Delta TOI_{min}$ during the alternating current EMS may indicate decreases in $O_2$ consumption due to a smaller muscle volume being recruited. As discussed above, the torque data showed possible incapability of recruiting additional motor units in alternating current EMS. The decrease in peak torque during the

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alternating current EMS-evoked isometric contractions (Figure 17) may explain the smaller decrease in $\Delta$TOI during the contraction phase, which results in gradual increases in $\Delta\text{TOI}_{min}$ for the alternating current EMS compared with pulsed current EMS from the 18th contraction onwards (Figure 19C). It appears that the difference in $\Delta\text{TOI}_{min}$ observed between the waveforms is mainly due to the difference in evoked torque. Since the evoked torque toward the end of the EMS session was greater for pulsed current than alternating current EMS, the former required more $O_2$. It seems possible that the higher frequency stimulation in the alternating current EMS decreased acetylcholine release at the neuromuscular junction, inadequately depolarised the sarcolemma resulting in a failure of action potential propagation along the sarcolemma and/or impaired excitation-contraction coupling (211, 245), thus inducing a lower evoked torque during this condition.

The changes in $\Delta$tHb were similar between the waveform conditions (Figure 19D). As shown in Figure 17, $\Delta$tHb decreased and sustained during contractions ($\Delta\text{tHb}_{mean}$) but rapidly recovered and increased above baseline ($\Delta\text{tHb}_{max}$) during relaxation, which would indicate that blood flow was occluded during contractions but resumed between contractions. It has been shown that blood flow to the biceps brachii was completely impeded during sustained isometric contractions at 50% MVC, due to increased intramuscular pressure compressing blood vessels (203). If this is also the case for vastus lateralis and vastus medialis muscles during knee extension isometric contractions, it seems reasonable to assume that muscle blood flow was completely occluded during isometric contractions evoked by EMS in the present study. In fact, $\Delta$tHb decreased rapidly at the onset of contraction and did not change during contraction (Figure 17). However, the blood flow was restored during the interval between contractions for both stimulation conditions similarly. The gradual increases in

$\Delta tHb_{max}$ over 30 contractions appear to correspond the gradual decreases in $\Delta tHb_{mean}$ (Figure 19D), indicating greater blood flow in the region of NIRS investigation, particularly in the relaxation phases.

The difference in the muscle force characteristics between alternating and pulsed current EMS should be considered by therapists or trainers when designing a treatment or training program using EMS. It appears that muscle contractions in alternating current EMS are not as strong as those in pulsed current EMS, since muscle force produced during the alternating current EMS-evoked isometric contractions is not as stable as that in pulsed current EMS. Thus, if it is necessary to maximise mechanical stimulus to the stimulated muscles, it is better to use pulsed current EMS than alternating current EMS. In fact, several authors (96, 146, 147, 152, 220) have already documented that pulsed current is preferable to alternating current EMS, since the pulsed current is less fatiguing and induces more constant contraction in comparison to alternating current. Further studies are necessary to investigate whether a long-term use of pulsed current EMS is more beneficial for increasing muscle strength and muscle volume compared with alternating current.

In summary, the present study showed that decreases in muscle oxygenation during the contractions phases were greater for pulsed current EMS than alternating current EMS using NIRS. This was likely associated with no increases in torque in the alternating current EMS despite the increases in stimulation intensity, and less stable force output in the alternating current EMS compared with the pulsed current EMS. Further study is necessary to investigate the underlying mechanisms of the difference in the muscle force output characteristics between alternating current EMS and pulsed current EMS.

# CHAPTER FIVE: STUDY THREE

## LESS INDICATION OF MUSCLE DAMAGE IN THE SECOND THAN INITIAL ELECTRICAL MUSCLE STIMULATION BOUT CONSISTING OF ISOMETRIC CONTRACTIONS OF THE KNEE EXTENSORS

## 5.1 INTRODUCTION

Electrical muscle stimulation (EMS) has been used clinically and in sport training to improve muscle function (97, 145). EMS training has been shown to be effective for enhancing muscle strength (11, 87), power (156, 159) and muscle endurance (129), and minimising muscle atrophy caused by disuse (66, 82). While EMS provides these benefits, it is known that EMS is limited by pain during stimulation (244) and could result in delayed onset muscle soreness (36). However, limited research has been conducted concerning delayed onset muscle soreness and recovery of muscle function after EMS-evoked isometric contractions.

Mackey et al. (154) have recently shown histological evidence of muscle damage such as macrophage infiltration, desmin negative fibres, and z-line disruption after 180 isometric contractions of the gastrocnemius muscle evoked by EMS (60 Hz, duration 300 μs, on-off ratio 4–6 s), together with increased plasma creatine kinase (CK) activity and muscle soreness. However, muscle strength measures, which have been proposed as the best indicator of muscle damage (251), were not included in the study. Jubeau et al. (121) compared between 40 isometric contractions of the knee extensors evoked by

EMS (75 Hz, duration 400 µs, on-off ratio 6.25–20 s) and 40 voluntary isometric contractions of the same muscles performed at the same intensity.  They showed that muscle soreness peaked 48 h after EMS and serum CK activity exceeded 3,000 IU·L$^{-1}$ at 72 h post-EMS; however, no such changes were evident after voluntary isometric contractions.  They also reported a significantly greater decrease in maximal voluntary isometric contraction torque after EMS (-22%) compared with voluntary contractions  (-9%) at 45 min post-exercise; however, no muscle strength measures were taken after that time point to follow the recovery process.

It is well documented that the magnitude of muscle soreness developed after exercise is attenuated and recovery of muscle function is enhanced when the same or similar eccentric exercise is performed within several weeks (158, 173). This protective effect induced by a single bout of eccentric exercise is referred to as the repeated bout effect (164). It is possible that the second EMS bout does not induce as severe muscle damage as the initial EMS bout. In fact, Nosaka et al. (179) reported that the second bout of eccentric exercise in which the elbow flexors were forcibly stretched while being stimulated by EMS resulted in smaller changes in maximal voluntary isometric contraction strength, range of motion, upper arm circumference and muscle thickness, muscle soreness, plasma CK and aspartate aminotransferase activities compared with the initial bout of the same exercise performed 2 weeks before. Black and McCully (27) have recently shown that changes in T2 relaxation time of magnetic resonance images and muscle soreness following 80 lengthening contractions of the quadriceps femoris stimulated by EMS were significantly smaller after the second bout compared with the first bout that was performed 7 weeks before. However, it should be noted that these studies compared the first and second EMS bouts consisting of lengthening contractions. No previous study has investigated the repeated bout effect for EMS bouts

consisting of isometric contractions that are generally performed in EMS training, for changes in indirect markers of muscle damage.

Therefore, the present study tested the hypothesis that the second bout of EMS exercise consisting of 40 electrically evoked isometric contractions of the knee extensors performed 2 weeks after the initial EMS would result in smaller changes in maximal voluntary isometric contraction torque of the knee extensors, muscle soreness and tenderness, and plasma CK activity following EMS, and faster recovery of muscle function. Although the main aim of the current thesis was to compare between alternating current and pulsed current, only pulsed current was investigated in the this study since no significant difference was detected between alternating current and pulsed current for changes in muscle damage markers after EMS-evoked isometric contractions in the first study.

## 5.2 METHODS

### 5.2.1 Study design

Nine volunteers participated in two EMS sessions separated by 2 weeks. The two-week interval between bouts was chosen, because it appeared that the duration was long enough for the muscles to recover from the initial EMS bout, but maximal protective effect if any could be observed, based on previous studies in which maximal voluntary eccentric exercise of the elbow flexors was investigated (173, 181). For each session, the subjects were asked to report to the laboratory for 6 occasions (1-3 days before EMS, EMS day, and 4 consecutive days after EMS). Each EMS session consisted of 40 electrically evoked isometric contractions of the knee extensors of one leg, which was randomly chosen but counterbalanced among subjects, and the same leg was used for

the second bout. Each subject performed the two EMS sessions at the same time of the day, and all EMS sessions were performed between 8 am and 10 am. The intensity of EMS and torque induced by each stimulation were monitored and recorded, and rating of perceived exertion (RPE) was assessed every 2 min (6 contractions) during EMS that took 15 min to be completed. The independent variable in this study was the first versus second EMS bouts, and the dependent variables to assess muscle damage consisted of maximal voluntary isometric contraction torque (MVC) of the knee extensors, muscle soreness and tenderness, and plasma CK activity. These measures were taken 1-3 days before, immediately before, and 1, 24, 48, 72 and 96 h following exercise. The MVC was also assessed immediately after EMS. The subjects were asked to remain in the seat of the dynamometer until the 1 h post-EMS measurements for the EMS session day. The study was approved by the University Human Research Ethics Committee, and the study was conducted in conformity with the Declaration of Helsinki.

### 5.2.2 Subjects

Nine healthy men (mean ± SD age: 31.3 ± 4.7 y, body mass: 76.3 ± 11.2 kg, height: 173.2 ± 4.2 cm), who had not been involved in any resistance training program for at least 6 months prior to the study, were recruited for this study. During the experimental period, subjects were asked not to change their regular physical activities and diet habits. All subjects were informed about the study, and a written informed consent form was obtained prior to participation. Subjects were requested to avoid consuming caffeine and alcohol one day before the EMS session and not to complete any extra physical activity during the experimental period. A familiarization session was completed by the subjects at least 4 days before the data collection, which included two

MVC measures at 100° and EMS to evoke 3-5 isometric contractions at submaximal intensity.

**5.2.3 Electrical muscle stimulation (EMS)**

EMS was applied while each subject seated on a on a Biodex isokinetic dynamometer chair (Biodex Medical Systems, Inc., USA) with the knee joint angle of 100° (0° corresponding to the full extension) and the trunk angle of 110°. This knee joint angle was chosen because a previous study (Jubeau et al. 2008) showed indications of muscle damage following EMS performed at this angle (100°). For minimizing any possible movement of hip and thigh during the contractions, straps were bound across the pelvis and chest.

The quadriceps femoris muscles were stimulated by an Intelect Advanced®Colour Stim (Chattanooga Group, TN, USA). Fours self-adhesive electrodes were placed on the anterior surface of one thigh based on a previous study (121). Prior to fix the electrodes, the skin surface was cleaned with alcohol pads. Two positive electrodes (50 × 50 mm) were placed over the motor points of the vastus lateralis and vastus medialis muscles, which were identified as the sites on the skin where the smallest current amplitude was required to produce muscle contraction (199). Two negative electrodes (50 × 100 mm) were placed on the proximal portion of the quadriceps femoris muscle. The placement of the electrodes was kept as consistent as possible between bouts by the same investigator identifying the sites with the same procedures between bouts.

A biphasic symmetrical rectangular stimulus pulses were delivered with frequency of 75Hz, and pulse duration of 400 µs (121). The ratio was 5 s stimulus on time and 15 s stimulus off time, thus the duty cycle time was 25%. The ramping time was included in 'on-time' so that 1 s for the rise time and 1 s for the fall time, and only 3 s were at

dosage stimulus intensity based on the previous studies (121, 152). In this way, an isometric contraction was evoked every 20 s, and the whole EMS session lasted for 15 min to induce 45 contractions. The stimulation amplitude (intensity) was started with 0 mA, and gradually increased for the first 3-5 contractions, thereafter consistently increased every 1-3 contractions toward maximally tolerable level of each subject to induce maximal force generation. The first 5 contractions were excluded from the analysis, since the stimulation intensity did not reach the level of maximally tolerable intensity, and force produced in these contractions was low (e.g. less than 10% MVC). The amplitude of each contraction was recorded, and the subjective intensity of the stimulated contractions was assessed by a Borg's standard RPE 6-20 scale (52) at 1, 3, 5, 7, 9, 11, 13 and 15 min during the EMS. The torque produced in each contraction was recorded using the Biodex software and saved for later analysis for the determination of torque (peak torque, average torque) and torque time integral of each contraction. The average torque during the EMS-evoked contraction excluding the ramp time and torque time integral of each contraction were calculated using Chart data analysis software (ADInstruments, Bella Vista, Australia).

### 5.2.4 Dependent variables

#### *5.2.4.1 Maximal voluntary isometric contraction torque (MVC)*

MVC measures for both legs were performed on the Biodex isokinetic dynamometer. Each subject sat on the dynamometer‟s chair in the same setting as the EMS protocol such that the backrest angle was set at 110° of posterior incline, and the rotation axis of the knee was aligned with the rotation axis of the dynamometer‟s armature. MVC was measured at the knee joint angle of 100°, which was the angle used in EMS to stimulate the knee extensors. During the measurements, both arms were

positioned across the chest with each hand clasping the opposite shoulder. Subjects performed three maximal voluntary isometric contractions for 3 s with a 30-s rest between attempts. The stimulated leg was always measured first followed by the control leg, and the time lag between the legs was approximately 10 min. Because of the time constraint, the MVC measurements of the control leg were not performed immediately after EMS. The peak torque of each contraction was calculated, and the highest value of the three attempts was used for further analysis.

### 5.2.4.2 Muscle soreness

A 100 mm visual analogue scale (VAS) was used to assess muscle soreness. Subjects were asked to rate their pain on the VAS as 0 representing "no pain" and 100 as "unbearable pain" while the muscles being palpated and upon squatting motion. For palpation, four sites on the quadriceps femoris (the midpoint between apex patellae and the anterior superior iliac spin, 5 cm proximal and 5 cm distal to this point, vastus medialis and vastus lateralis) were checked by the investigator placing his three fingers at each site and applying pressure for 3 s. The pressure and palpation regions were standardized throughout the experiment such that the same investigator gave a consistent pressure between days and among subjects. The average values of the four sites were used for further analysis. For the muscle soreness upon squatting, subjects were asked to stand with their legs shoulder width apart, and bend their knees slowly to 90° and then return to the standing position. The pain level of the knee extensors having the EMS was reported immediately after the movement on the VAS scale.

### 5.2.4.3 Pressure pain threshold (PPT)

PPT was assessed using an electronic algometer (Type II, Somedic Production AB, Sollentuna, Sweden). The algometer was calibrated for each occasion, and the same

investigator took all measurements using a standardised protocol. The probe head (surface area = 1.0 cm$^2$) of the algometer was placed perpendicular to the four sites of the quadriceps femoris muscle that were used for the palpation soreness measures (the middle point of the rectus femoris, the proximal of rectus femoris, vastus medialis and vastus lateralis that were clearly marked by a water-proof ink pen).  Subjects were familiarised with 5 s of pressure in no-painful ranges (0 – 5 kPa·s$^{-1}$) before the assessments.  Force was gradually applied at a constant rate of 50-60 kPa·s$^{-1}$ until the subject reported the first feeling of noticeable pain. Three measurements were taken from each site sequentially with a 30 s interval between measures in the following order: the middle point of the rectus femoris, the proximal of rectus femoris, vastus medialis and vastus lateralis.  The value in kilopascals (kPa) corresponding to the amount of force applied to elicit pain sensation was recorded. The mean of the three measurements for each site was used for further analysis, and the values of the four sites were averaged to assess the tenderness in quadriceps muscle.

### 5.2.4.4 Plasma CK activity

A small amount of blood sample was taken from a fingertip that had been cleaned with an alcohol swab using a spring-loaded lancet. A heparinised capillary tube was used to collect a 30 µl of whole blood and loaded onto a test strip (Reflotron® Creatinine Test Tabs) of a Reflotron spectrophotometer (Boehringer-Mannheim, Pode, Czech Republic) to assess CK activity. The measurements were duplicated, and if the difference of the two values was greater than 10%, additional measurements were taken. In this method, the normal reference ranges for adult men are 20-220 IU·L$^{-1}$ according to the instruction sheet of the test kit.

### 5.2.5 Statistical analysis

The normality of the data distribution was checked by a Shapiro-Wilk test, and all variables except for CK passed the test. Changes in the MVC, VAS of muscle soreness, and pressure pain threshold over time were compared between the first and second bouts by a two-way repeated measures ANOVA. Changes in stimulation intensity (amplitude), RPE, peak and average torque, and torque time integral over 40 isometric contractions were also compared between bouts using a two-way repeated measures ANOVA. When a significant interaction effect was found, a Tukey's post hoc test was followed to compare between bouts for each time point. For plasma CK activity, because of the normality was not established, a Mann-Whitney test was used to compare between bouts for the changes over time. A Student t-test was also used to compare peak plasma CK activity and peak muscle soreness between bouts. The significance level was set at $P < 0.05$. All data are expressed as mean ± SD.

### 5.3 RESULTS

### 5.3.1 Stimulation intensity, RPE and torque during EMS

Changes in stimulation intensity and RPE over the last 40 contractions were shown in Figure 20. The stimulation intensity gradually increased throughout the EMS without significant difference between bouts. RPE increased in the first 9 min and was close to the maximum after 10 min of EMS, with no significant difference between bouts. No significant difference between bouts was evident for changes in peak torque, average torque (Figure 21), and torque time integral over the last 40 contractions. The peak torque of each contraction was approximately 15-25% higher than the average torque shown in Figure 21; however, the changes over 40 contractions were similar

between the two. The pattern of changes in the torque time integral was also similar to that shown in the average torque. The peak torque evoked by EMS was approximately 25-30% of baseline MVC torque ($185 \pm 12$ Nm). The average torque increased in the first 10-15 contractions, but no further increases were seen thereafter in spite of further increases in the stimulation intensity.
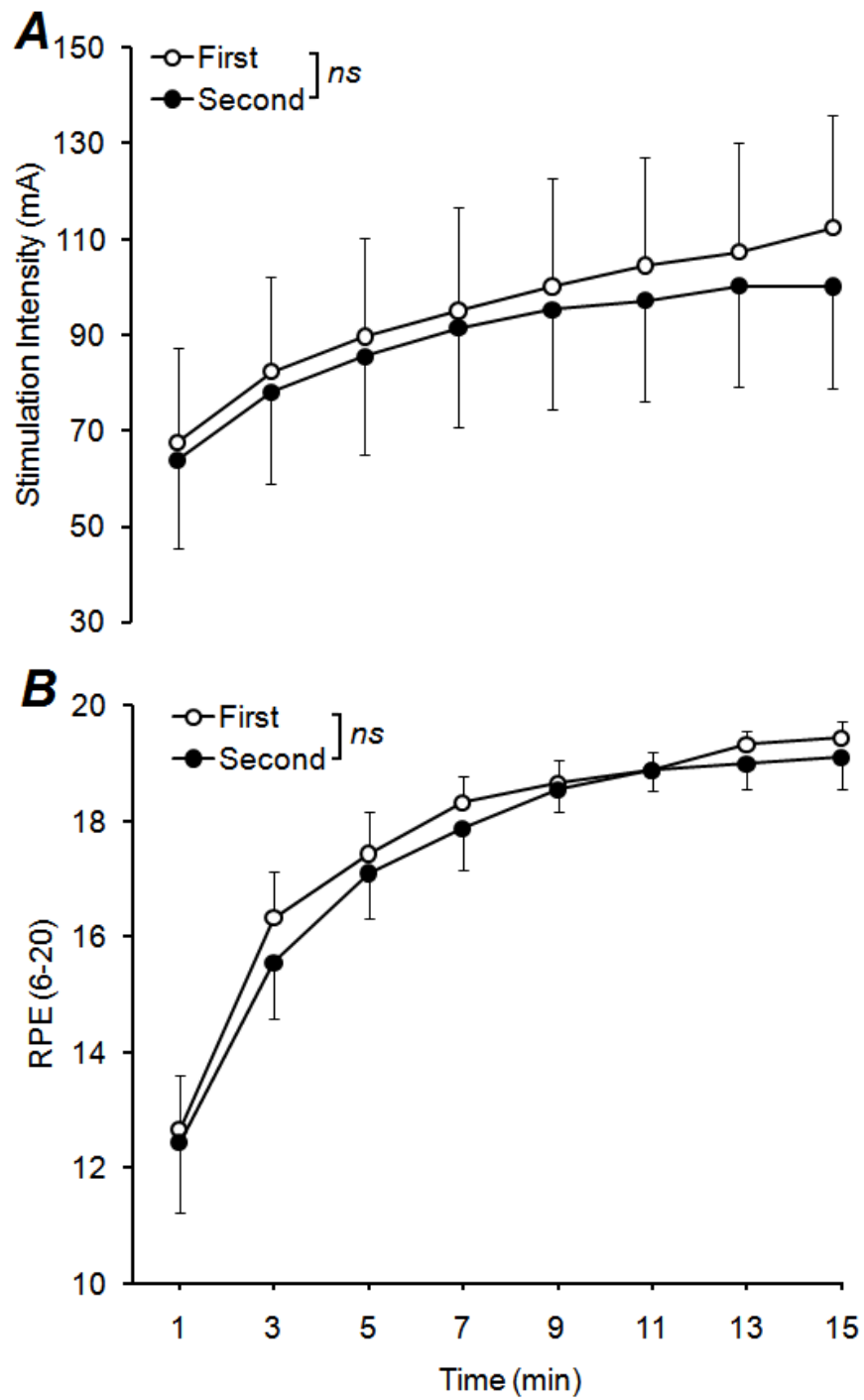
Figure 20: Stimulation intensity (*A*) and the rating of perceived exertion (*B*) during the first and second EMS bouts in which isometric contraction was evoked every 20 s for 15 min. The measurements were taken every 2 min starting at 1 min (after 3 contractions). *ns*: no significant difference between bouts for the changes over time.
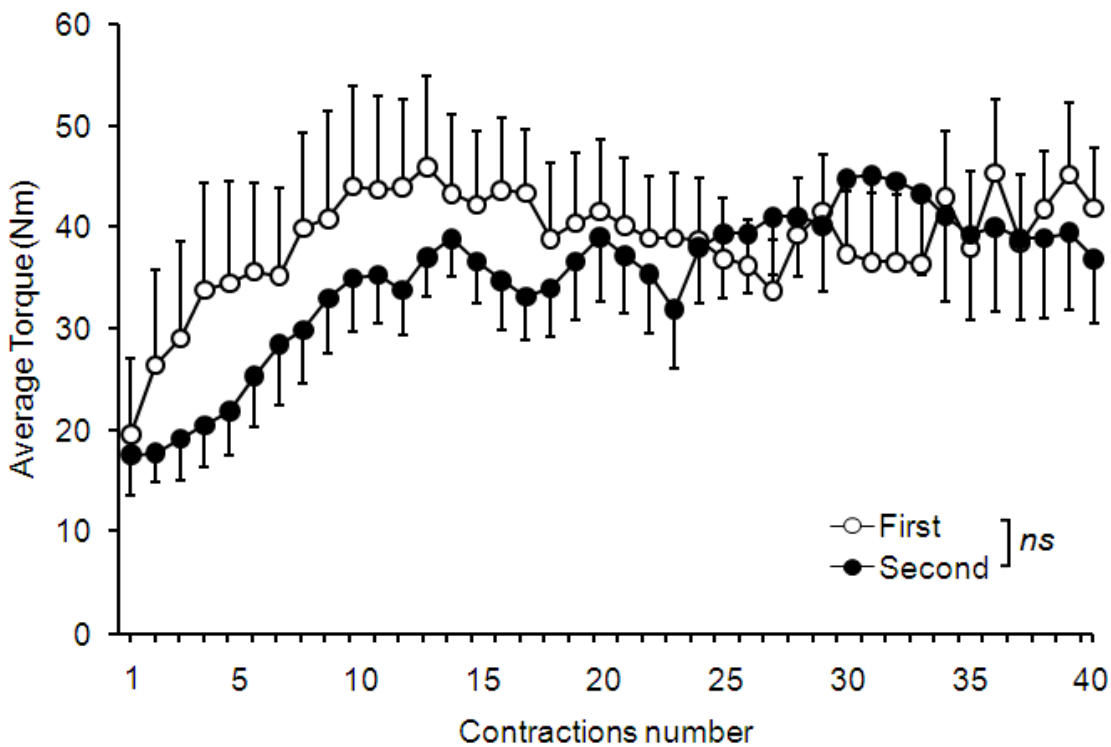
Figure 21: Average torque of 40 isometric contractions during the first and second EMS bouts. ns: no significant difference between bouts for the changes over time.

### 5.3.2 MVC

MVC was not significantly different between bouts before EMS for both legs. The MVC of the control leg did not change significantly over time following the first and second bouts. As shown in Figure 22, MVC of the stimulated leg decreased significantly below the baseline after EMS by approximately 26% immediately and 1 h after EMS for both bouts, without a significant difference between bouts. However, the recovery of MVC was significantly faster after the second bout compared with the first bout such that the MVC returned to the baseline by 72 h post-EMS for the second bout, but it was still significantly lower than the baseline at 96 h post-EMS for the first bout (81%).
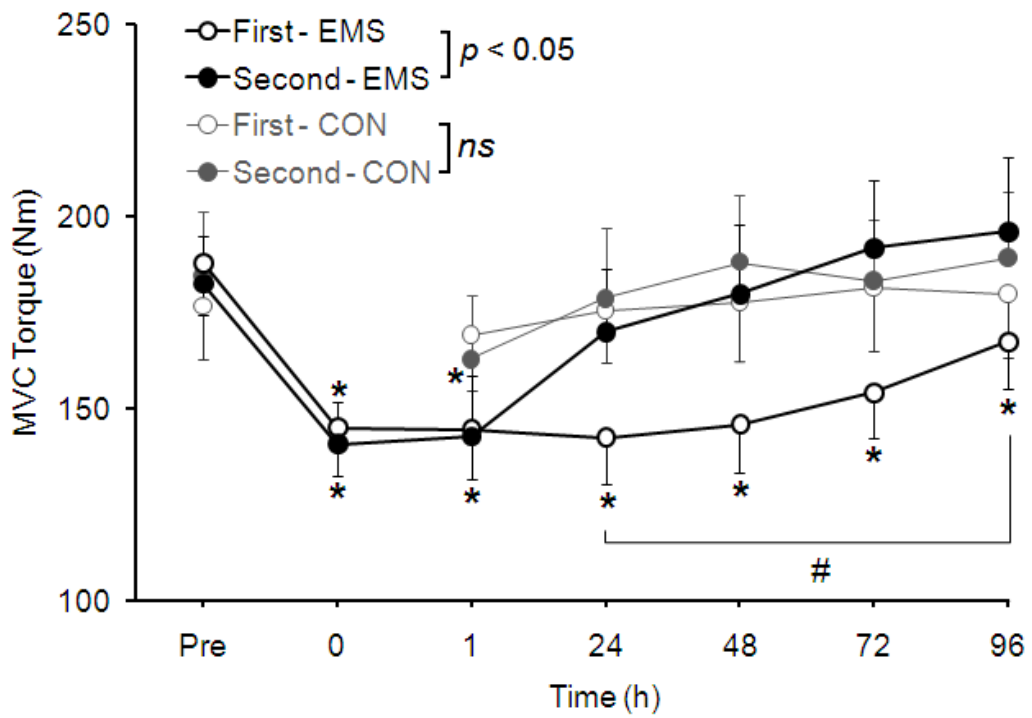
Figure 22: Maximal voluntary contraction isometric torque before (Pre), immediately after (0), and 1, 24, 48, 72, and 96 h after the first and second EMS bouts for the stimulated leg (EMS) and control leg (CON). *: significantly ($p < 0.05$) different from the baseline, #: significantly ($p < 0.05$) different between the first and second bouts for the stimulated leg.

### 5.3.3 Muscle soreness

Figure 23 shows changes in muscle soreness upon palpation and in squatting before and for 4 days after the first and second EMS bouts. No significant difference in the magnitude of muscle soreness was found among the four palpation sites. The time course of changes in muscle soreness upon palpation and in squatting was similar, and muscle soreness peaked 48 h after EMS.  The magnitude of muscle soreness was significantly less after the second bout compared with the first bout.
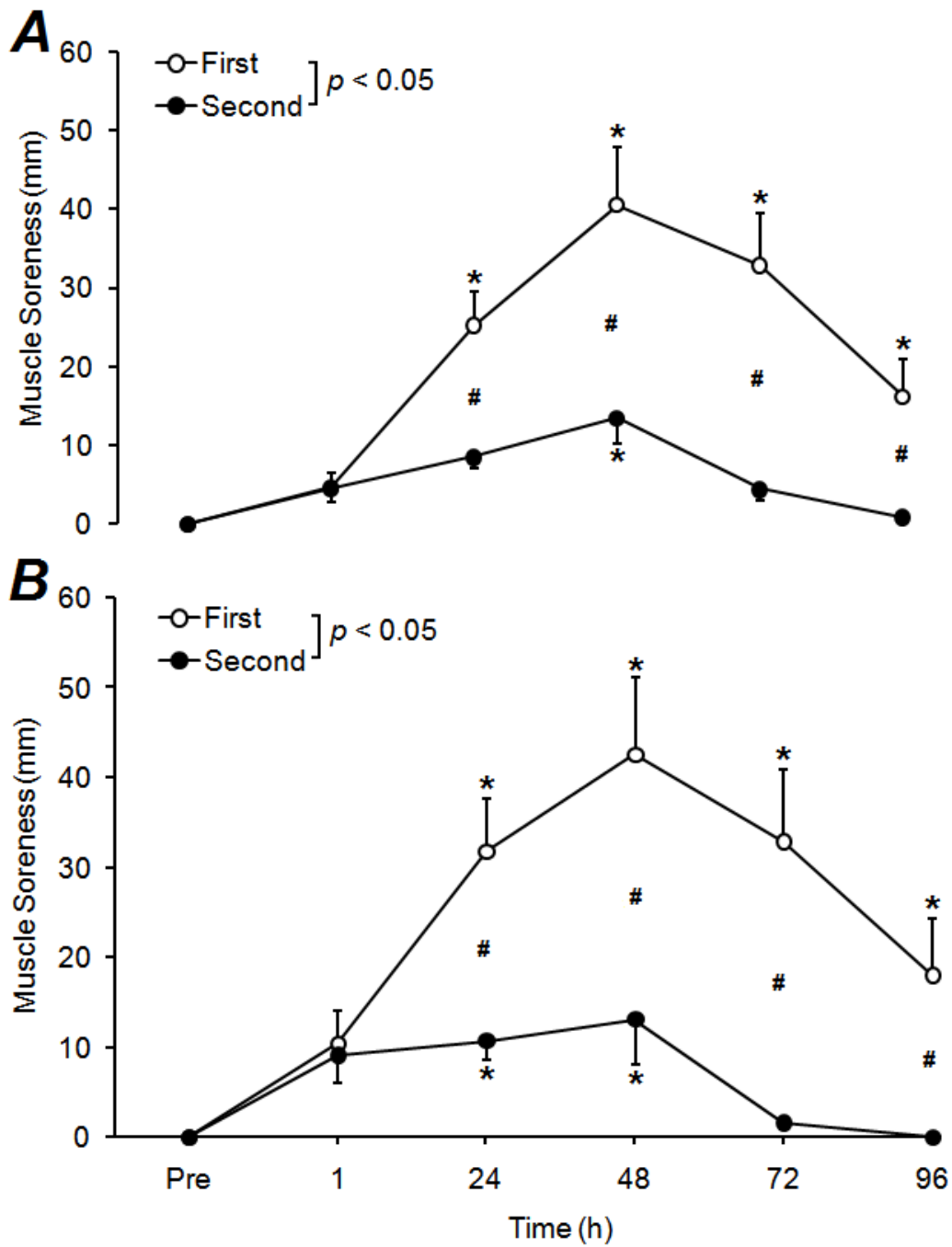
Figure 23: Muscle soreness (VAS) before (Pre), and 1-96 h after the first and second EMS bouts for the soreness upon palpation (*A*) and in squatting (*B*). *: significantly ($p < 0.05$) different from baseline, #: significantly ($p < 0.05$) different between bouts.

### 5.3.4 PPT

Muscles became more tender only after the first EMS bout, and the decrease in PPT was significantly smaller after the second bout compared with the first bout (Figure 24).
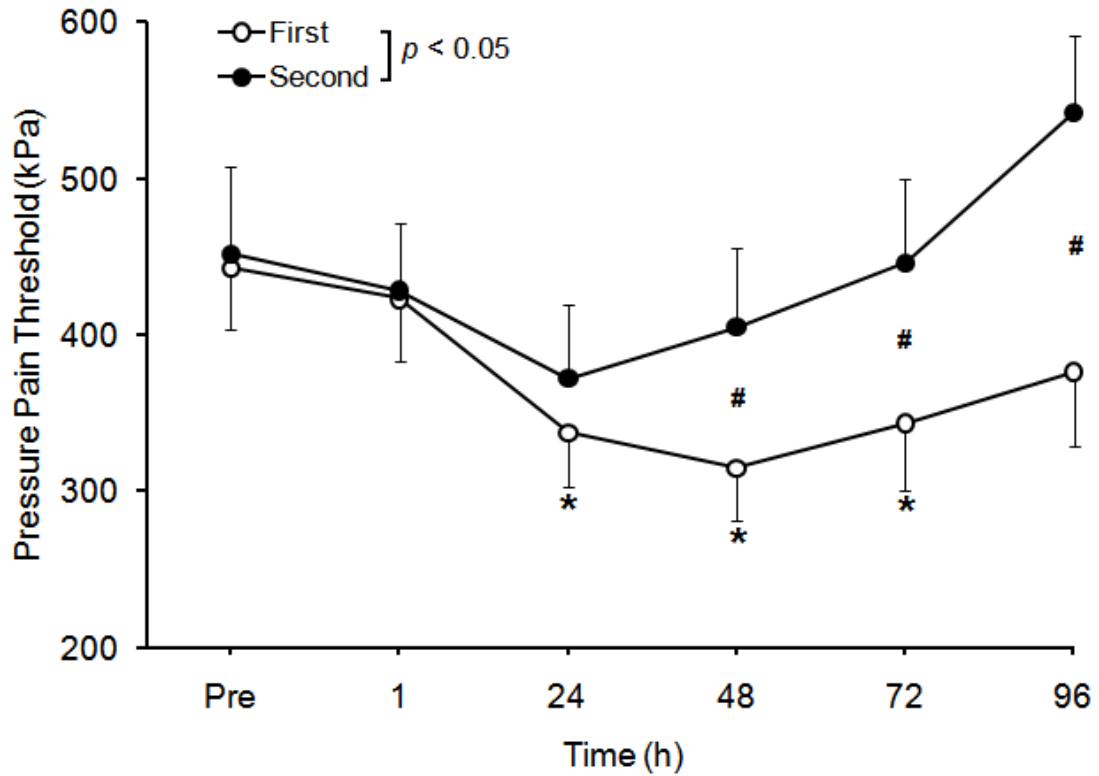


Figure 24: Pressure pain threshold before (Pre), and 1-96 h after the first and second EMS bouts. *: significantly ($p < 0.05$) different from baseline, #: significantly ($p < 0.05$) different between bouts.

### 5.3.5 Plasma CK activity

No significant difference was found for the pre-exercise CK activity between bouts. Plasma CK activity increased significantly only after the first bout and the highest value was observed at 96 h after EMS (Figure 25). The peak CK activity was significantly higher after the first bout ($1{,}431 \pm 612$ IU·L$^{-1}$) compared with the second bout ($208 \pm 35$ IU·L$^{-1}$).
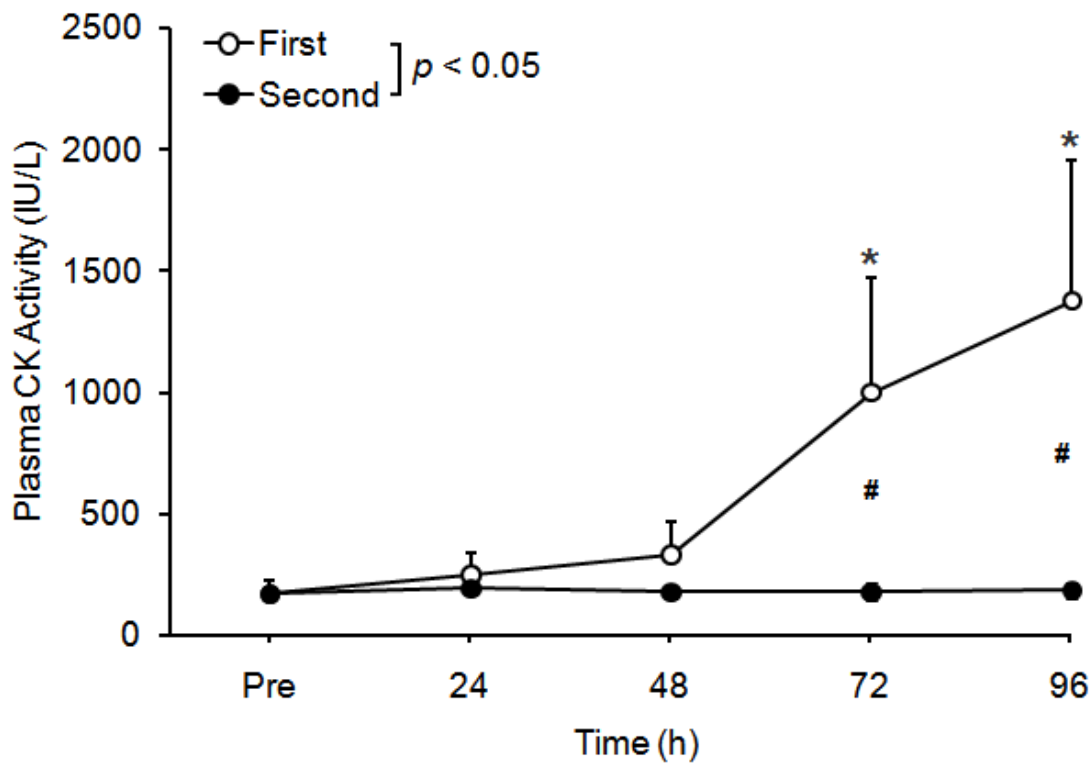
Figure 25: Plasma CK activity before (Pre), and 24-96 h after the first and second EMS bouts. *: significantly ($p < 0.05$) different from baseline, #: significantly ($p < 0.05$) different between bouts.

## 5.4 DISCUSSION

This was the first study to compare between the first and second bouts of EMS-evoked isometric contractions of the knee extensors for muscle damage profile, and to report that the repeated bout effect occurs for isometric contractions induced by EMS. The results showed that decreases in MVC lasting more than 4 days, development of muscle soreness, and increases in plasma CK activity occurred after the first EMS bout. Although the same magnitude of decrease in MVC was induced immediately and 1 h after the second EMS bout, the recovery of MVC was significantly faster, and the development of muscle soreness and increases in plasma CK activity were significantly smaller following the second bout compared with the first bout. These results supported

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the hypothesis that less indication of muscle damage would be evident in the second EMS bout compared with the first bout, and clarified the time course of changes in MVC following EMS.

A previous study (121) reported that MVC decreased 22% at 45 min after EMS consisting of 40 isometric contractions at maximally tolerable intensity. The magnitude of decrease in MVC in the present study immediately and 1 h after EMS was 26% (Figure 22), which is comparable to that shown in the previous study. The time course of changes in muscle soreness and peak muscle soreness found in the present study was similar to those reported in the previous study by Jubeau et al. (121). However, the increase in CK activity in the blood in the previous study (peak: ~3,500 IU·L$^{-1}$) was as more than 2-fold as that of the present study (peak: 1,372 IU·L$^{-1}$). This could be due to the difference in the amount of stimulated muscles between the previous study (two legs) and the present study (one leg), and the difference in other muscles involved in the exercise between studies due to a different posture (lying versus sitting). However, it appears that the magnitude of muscle damage to one leg induced by the EMS in the present study was comparable to that in the previous study (121), although there were some differences in the experimental setup. In contrast, Zorn et al. (258) reported that no delayed onset muscle soreness was induced after 30 min of EMS (63.3 Hz, duration 400 μs, on-off ratio 3.5–4.5 s) to provoke strong tetanic contractions of the knee extensors at 90° for trained cyclists, although plasma CK activity showed a small increase at 24 h post-EMS. It is possible that the difference in the subjects between the present study (untrained) and the study by Zorn et al. (trained cyclists) attributed to the different muscle soreness responses.

It is important to note that the muscle damage in the present study was induced by isometric contractions evoked by EMS. It has been documented that isometric contractions do not result in as severe muscle damage as lengthening contractions (179). Black and McCully (27) have recently reported that MVC decreased approximately 20% on 1-2 days after 80 lengthening contractions evoked by EMS, and resulted in muscle soreness (peak: approximately 60 mm in VAS). These values appear to be comparable to those in the present study, although the number of contractions (40 versus 80) and the contraction type (isometric versus eccentric) were different between the studies. Jubeau et al. (121) showed that the magnitude of muscle damage induced by isometric contractions evoked by EMS was significantly greater than that by voluntary isometric contractions at the same intensity. However, it is not known whether maximal voluntary isometric contractions result in the same magnitude of muscle damage as the isometric contractions by EMS. It should be noted that the isometric contractions were performed at a long muscle length in the present study. Several studies (119, 205) have reported that isometric contractions at a short muscle length do not induce muscle damage, but do at a longer muscle length. It may be that maximal voluntary isometric contractions of the knee extensors at a long muscle length (e.g. 100º) induce similar changes in muscle damage markers to those shown in the present study. If so, the cause of muscle damage is not EMS itself, but isometric contractions at a long muscle length. This should be investigated in future studies.

The torque evoked by EMS was estimated approximately 25-30% of the baseline MVC torque ($185 \pm 12$ Nm) and post-EMS MVC torque ($126 \pm 9$ Nm). Adam et al. (2) reported using a magnetic resonance imaging technique that the cross sectional area of the vastus lateralis activated by EMS was associated with the intensity of EMS, and approximately 25% of the cross sectional area was activated when the stimulation

induced the torque of 25% MVC. Thus, it is assumed that even when the muscles were stimulated at maximally tolerable intensity in the present study, the number of muscle fibres that contributed to the force production was less than one-third of that during maximal voluntary contractions. It is known that muscle fibre recruitment in EMS is different from voluntary contractions such that muscle fibres are recruited non-selectively, synchronously and spatially fixed during EMS (94). It is possible that the synchronous muscle fibre activation in EMS induces more mechanical stress to muscle fibres (128, 154). Hansen et al. (102) have recently shown greater myofibrillar proteolysis after lengthening contractions with EMS than maximal voluntary lengthening contractions, and stated that electrically stimulated muscle contractions are likely to result in greater intracellular strain and higher stress forces due to a highly uniform contraction of all muscle fibres within the stimulated region. This may be also the case for isometric contractions evoked by EMS.

When comparing between the first and second bouts, no significant differences in stimulation intensity and RPE (Figure 20) and peak torque produced over 40 contractions (Figure 21) were evident. It is interesting that no improvement in the tolerance to EMS was seen in the second bout. It may be that the interval between the two EMS bouts was too long to see any improvement in tolerance. It is important to note that the second EMS bout was performed after complete recovery from the first EMS bout, since there were no significant differences in any of the pre-EMS measures between bouts. Moreover, the decreases in MVC immediately and 1 h post-EMS (26%) were similar between the bouts (Figure 22). Zory et al. (259) reported that MVC decreased approximately 20% immediately after 30 isometric contractions of the knee extensors evoked by EMS (75 Hz, on-off ratio 6.25-20 s), and neuromuscular propagation failure was mainly responsible for the decrease. It seems that excitation-

contraction coupling failure was at least partially responsible for the decreases in MVC (112); however, ultrastructual changes in myofibrils as shown in the study by Mackey et al. (154) may also account for the long-lasting decreases in MVC after EMS. Therefore, the deceases in MVC immediately and 1 h following EMS in the present study are likely to reflect the combination of muscle fatigue and muscle damage, and it seems reasonable to assume that the two EMS bouts affected muscles similarly during the exercise, resulting in the similar extent of decrease in MVC within 1 h following EMS. Thus, the differences between the bouts for the changes in the dependent variables between 24 and 96 h following EMS were likely due to protective effect conferred after the first EMS bout.

The present study was the first to compare between the first and second EMS bouts consisting of isometric contractions; however, two previous studies (27, 179) had compared between the first and second EMS bouts with lengthening contractions. Black and McCully (27) compared between the first and second bouts consisting of 80 lengthening contractions of the knee extensors with EMS separated by 7 weeks, and reported that the changes in T2 and muscle soreness were significantly smaller for the second bout than the first bout. Nosaka et al. (179) examined the elbow flexors for their responses to first and second bouts of lengthening contractions with EMS separated by 2 weeks. They showed faster recovery of MVC, smaller decrease in range of motion, less swelling of the muscle, smaller increases in muscle soreness and blood markers of muscle damage (e.g. CK) following the second bout compared with the first bout, which was similar to the repeated bout effect reported in the studies comparing two bouts of voluntary eccentric exercise (33, 174). It appears that the characteristics of the repeated bout effect found in the present study were similar to those found in the previous EMS (27, 179) and voluntary eccentric exercise studies (33, 174).

The underlying mechanisms of the repeated bout effect have not been elucidated; however, a combination of neural, mechanical, and cellular adaptations has been documented (164). It seems that neural adaptations (e.g. more efficient recruitment of motor units, increased synchrony of motor unit firing, better distribution of the workload among muscle fibres, improved usage of synergist muscles, increased slow-twitch fibre recruitment) had little or minor contribution to the repeated bout effect in EMS found in the present study, since it is unlikely that the listed neural adaptations were induced by the initial EMS. However, it should be noted that EMS does not actually bypass the peripheral nervous system, because of the bilateral propagation of action potentials along the stimulated axons (44), and would even activate selected brain regions in a dose-response manner (213). Thus other neural adaptations might be involved in the protective effect conferred by the initial EMS. The repeated bout effect following EMS may be attributed more to mechanical adaptations (e.g. increases in passive or dynamic muscle stiffness, remodeling of intermediate filament system, increased intramuscular connective tissue) and/or cellular adaptations (e.g. longitudinal addition of sarcomeres, adaptation in inflammatory response, adaptation to maintain E-C coupling, strengthened plasma membrane, increased protein synthesis, increased stress proteins, removal of stress-susceptible fibres). Further muscle histological and molecular investigations are necessary to investigate the underlying mechanisms of the repeated bout effect.

The findings of the present study and previous studies (121, 154) show that muscle soreness and other symptoms of muscle damage (e.g. prolonged strength loss) are caused by EMS-evoked isometric contractions, but changes in the markers of muscle damage induced in the subsequent EMS bout are not as large as that after the initial bout. Thus, practitioners do not need to be concerned so much about muscle

damage in EMS when it is repeatedly used, and muscle damage caused by EMS should not limit the utilisation of EMS in training and/or rehabilitation. If it is necessary to avoid muscle damage in the first EMS bout, the strategies that have been used to minimise eccentric exercise-induced muscle damage such as pre-conditioning muscle by increasing the intensity, number of contractions, muscle length gradually (119, 176, 205) may be effective. However this warrants further investigations to confirm this. It is also important to investigate further muscle damage in EMS of other modes, intensities, and duration, and whether the effect of EMS on muscles is similar among different muscles. In conclusion, EMS induces symptoms of muscle damage, but a protective adaptation is conferred after the initial EMS bout, resulting in less indication of muscle damage following the second EMS bout.

# CHAPTER SIX: OVERALL DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

The main aim of this thesis project is to compare between alternating current and pulsed current electrical muscle stimulation (EMS) for torque output, discomfort skin temperature, hormonal responses, blood lactate, muscle oxygenation, muscle fatigue and muscle damage. The difference between alternating current and pulsed current EMS for torque output, discomfort, skin temperature, hormonal responses, blood lactate and muscle damage were investigated in Study 1. In Study 2, muscle oxygenation during alternating current and pulsed current EMS was compared, and muscle fatigue was assessed. In the third study, since no significant difference in muscle damage between alternating current and pulsed current EMS was found in Study 1, the incidence of repeated bout effect was examined only for pulsed current EMS. As shown in Chapter 1, there were nine research questions in the present project. The answers to the questions are shown below, and a brief discussion for each question is made. More detailed discussion can be found in the discussion in the Chapters 3-5. Based on the answers found in the present project, this chapter discusses which would be better for muscle strengthening purpose in sport training, alternating current EMS or pulsed current EMS. This chapter also provides future research directions based on the findings and limitations of the studies conducted in the thesis, followed by conclusion.

## 6.1 ANSWERS TO THE RESEARCH QUESTIONS

*Q1: Is there a difference in force generation between alternating current and pulsed current EMS?*

No. Torque output during EMS was approximately 30% of MVC with no significant difference between alternating current and pulsed current. This finding supports the previous studies which reported the similarity of generated muscle force in both alternating current and pulsed current EMS (96, 109, 152, 216). The number of pulses per second does not appear to affect force output. The frequency of alternating current was modulated to 75 bursts per second, which was matched with pulsed current delivered at 75 pulses per second in the present study. Although the peak and average torque were not significantly different between alternating and pulsed current EMS in Study 1, Study 2 showed that the average torque of the only 30 contractions relative to MVC was significantly greater for pulsed current EMS compared with alternating current EMS when the first ten contractions before the plateau were ignored. It should be noted that the torque did not increase with the increase in the stimulation intensity for alternating current EMS, but steady increases in torque were observed for pulsed current EMS. The force output during alternating current was less steady and decreased earlier within a contraction compared with pulsed current EMS, especially toward the end of the EMS session. As seen in Study 3 (Figure 18), the toque traces seem to be more consistent during pulsed current compared with alternating current, particularly during the last ten contractions. Previous studies (96, 220) also reported greater fluctuations of force during alternating current EMS compared with pulsed current EMS, and suggested greater muscle fatigue during alternating current than pulsed current EMS.

*Q2: Is there a difference in the level of discomfort between alternating current and pulsed current EMS?*

No. The results of Study 1 and 2 showed a comparable level of perceived discomfort between alternating current and pulsed current EMS-evoked isometric contractions. This finding is consistent with previous studies reporting similar

discomfort between alternating current and pulsed current in which muscles were stimulated at maximally tolerable levels (25, 96, 146, 152). Since intensity of EMS was set at the maximal tolerance, it seems reasonable that no difference in the perceived discomfort existed between the current types. It is also important to note that no significant difference between the current types existed for the torque level during the stimulation at maximal tolerable level.

*Q3: Is there a significant difference in skin temperature changes between alternating current and pulsed current EMS?*

No. The change in skin temperature was seen only during the recovery time (peak at 15 min post-EMS) where it increased ~ 2°C in the stimulated muscles. The increases in skin temperature at the end of exercise and during recovery indicate more activation in the cutaneous vessels to allow more blood flow peripherally for the heat exchange process. No significant difference in the increase in skin temperature between alternating and pulsed current EMS suggests that both conditions similarly increased the skin blood flow.

*Q4: Is there a significant difference in hormonal responses between alternating current and pulsed current EMS?*

No. The effects of EMS on growth hormone (GH), testosterone, cortisol and insulin-like growth factor-1 (IGF-1) were similar between alternating and pulsed current EMS. Since GH and testosterone responses are affected by the intensity of muscle contractions (3, 206), it seems reasonable that both resulted in similar increases in GH and testosterone because of the similar force level. Cortisol showed no increase immediately after EMS, but it decreased following EMS without significant difference between alternating current and pulsed current EMS. No changes in IGF-1 was evident

between alternating current and pulsed current EMS, which may be related to the delayed increases in IGF-1 (106, 135).

*Q5: Is there a significant difference in blood lactate responses between alternating current and pulsed current EMS?*

No. Blood lactate increased significantly during EMS and reached the peak at the end of the session for both current conditions similarly. This suggests that energy source (e.g. glycolysis) for the EMS-evoked contractions was not different in both alternating current and pulsed current EMS.

*Q6: Is there a significant difference in muscle oxygenation between alternating current and pulsed current EMS?*

Yes. Although there was no significant differences in $\Delta TOI_{max}$, $\Delta tHb_{mean}$ and $\Delta tHb_{max}$ over 30 contractions were observed, $\Delta TOI_{min}$ was significantly higher for alternating current during the last 18 contractions. This indicates lower $O_2$ demand during alternating current compared with pulsed current EMS-evoked isometric contractions. The difference may be attributed to the incapability of alternating current EMS to recruit additional motor units, which reduced the muscle volume involved in the contractions. This could rebut the thought that high frequency stimulation can lower the skin impedance and thus have analgesic effects (84, 130) and thus more current penetrates tissue and stimulates more fibres.

*Q7: Is there a significant difference in muscle fatigue between alternating current and pulsed current EMS?*

Yes. The torque data in Study 2 showed a possibility that muscle fatigue during alternating current is greater compared with pulsed current EMS. In addition, as shown above, $O_2$ consumption during contractions was lower during alternating current

compared with pulsed current EMS. Muscle fatigue is thought to be related to the number of pulses (2.5 kHz vs 75 Hz) more than bursts (146).

*Q8: Dose muscle damage induced by EMS differ in the case of alternating current than in the case of pulsed current?*

No. The indirect markers of muscle damage such as loss of muscle force, muscle soreness and tenderness, and increases in plasma CK activities showed significant changes after EMS with no difference between current conditions. The torque output and the amplitude of the intensity during EMS session were similar in both alternating and pulsed current, which suggests that mechanical stress applied to the muscles was similar between the conditions. The magnitude of muscle damage is unlikely affected by the waveform of stimulation current.

*Q9: Is the magnitude of muscle damage after the second bout of EMS less than that after the first EMS bout?*

Yes. It was shown that the magnitude of change in maximal isometric contraction strength, muscle soreness and plasma CK activity was significantly smaller after the second EMS bout compared with first bout. This suggests that the first EMS session induced greater muscle damage than the second session, and muscle damage was attenuated in the subsequent EMS session. This represents a typical protective effect induced by the previous EMS session, which is similar to the repeated bout effect reported in eccentric exercise (27, 179). Since no difference in muscle damage was evident between alternating current and pulsed current EMS, it seems reasonable to assume that a similar repeated bout effect would be observed for alternating current EMS.

## 6.2 INTEGRATED DISCUSSION – WHICH IS BETTER: ALTERNATING CURRENT EMS OR PULSED CURRENT EMS?

As shown above, differences between alternating and pulsed current EMS were limited to some aspects of force generation and muscle oxygenation during contraction phases. The less steady force generation in a contraction and smaller decreases in muscle oxygenation during the contraction phases in alternating current than pulsed current EMS are likely due to greater muscle fatigue occurring during alternating current EMS. Muscle fatigue is considered a major factor that affects performance during exercise (149). Avoiding or limiting muscle fatigue would enhance the benefits of any strength training program (93).

Since other parameters (discomfort, skin temperature, hormonal responses, muscle damage) examined in the present study did not show significant differences between alternating current and pulsed current EMS, the choice of current cannot be made based on these. However, if it is necessary to maximise mechanical stimulus to muscles, it is important to minimise muscle fatigue during the EMS unless the training for fatigue is desirable. Moreover, a stimulator that can produce alternating current is generally more expensive than the one that produces only pulsed current. In fact, the most of currently available portable stimulators cannot produce alternating current. Thus, it seems reasonable to state that pulsed current EMS is more advantageous than alternating current EMS, at least for muscle strengthening purposes for healthy individuals. However, it is not possible to speculate from the present study whether pulsed current EMS actually results in greater strength gains and muscle hypertrophy. Further studies are warranted.

The use of EMS in sport training or exercise has increased in the last two decades (15, 166), and it is expected that the use of EMS would further increase in healthy and

clinical populations. As shown in the present study, muscle damage is one of the issues that practitioners should be concerned about; however, as shown in the present study, muscle damage may not be a big issue when EMS is repeatedly performed, if care is taken for the first EMS session. Moreover, it appears that muscle length rather than EMS itself is a factor to determine the magnitude of muscle damage. Thus, muscle damage should not discourage the use of EMS in strength training.

Although the present study focused on the current of EMS, there are other parameters such as frequency, amplitude, phase/pulse duration, duty cycle and waveform to be concerned to set up optimum EMS procedures for muscle training. It should be noted that the present study compared alternating and pulsed current EMS by matching the other parameters rather than current as closely as possible. Thus, many other comparisons can be made for alternating and pulsed current EMS by modulating the parameters. This thesis project is only a step toward better understanding of the theory and application of EMS in sport training.

## 6.3   LIMITATIONS   OF   THE   PRESENT   PROJECT   AND RECOMMENDATIONS

One of the limitations of the present thesis project was the number of subjects used in the studies. In the first study, 12 subjects were recruited, which met the statistical power of the required sample size estimation. The sample size of the subsequent studies (n=9) was also considered to be adequate to find a possible difference between alternating current and pulsed current EMS (Study 2) or between the first and second bout EMS (Study 3). Because of the difficulty of recruiting subjects who met the criteria, the number of subjects was minimal.

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In the present study, only young healthy men were used as subjects. Young healthy men could have endured the discomfort of EMS of alternating and pulsed current. Thus, it is not clear if the findings of the present study are reproducible for other populations such as women, elderly or clinical individuals.

As mentioned previously, the comparisons between alternating and pulsed current EMS were made by settings of the other EMS parameters such as stimulus frequency (75 Hz), pulse duration (400 µs) and duty cycle (25%) that were the same between the conditions. The variation of these parameters may produce different results in terms of the difference between alternating current and pulsed current. Further comparisons between alternating and pulsed current using different settings of EMS parameters are necessary.

The responses of leg muscles to EMS were reported to be different from those of arm muscles (dorsal forearm muscle) where legs (dorsiflexor muscle) have higher thresholds of motor nerve excitation than forearm (122). EMS is used not only for the knee extensors but also other muscles. It is important to investigate other muscles to compare between alternating current and pulsed current EMS.

The present study focused on the acute effects of EMS; however, it is not possible to speculate the effects of chronic use of EMS (i.e. EMS training) on muscle function and muscle hypertrophy from the results of the present study. Only two studies (25, 220) compared between alternating and pulsed current for the training effect on muscle strength in healthy subjects. Although both reported a significant increase in the strength, one study (220) showed that the increase in strength following pulsed current was greater than that following alternating current, while the other (25) showed no difference between the two conditions. From the acute responses found in the present

study, it is assumed that pulsed current EMS training might result in greater muscle strength and muscle mass gains compared with alternating current EMS training, since mechanical stress during pulsed current stimulation seemed to be greater than in alternating current stimulation. This hypothesis should be investigated in future studies.

Muscle damage was assessed in this study using indirect markers such as force loss, muscle soreness and plasma CK activity. It cannot be denied that morphological changes differ between alternating current EMS and pulsed current EMS. It is interesting to compare between alternating current EMS and pulsed current EMS for histological changes in myofibre contractile components and sarcolemma following acute bout as well as repeated bouts of EMS session.

## 6.4 CONCLUSION

In conclusion, no significant difference was evident between alternating current and pulsed current for their acute effects on force output, muscle damage, hormonal response, skin temperature and blood lactate, which could be due to the comparable level of torque output during EMS and the number of stimuli per second (75 Hz). The difference that was shown in muscle oxygenation during the second half of EMS session could be due to muscle fatigue where alternating current probably caused more fatigue of the stimulated muscles as a result of high-frequency fatigue. It can be suggested that pulsed current conditions are preferable to alternating current for muscle strengthening of healthy individuals because of its accessibility. However, more investigation is necessary to substantiate this conclusion for different populations, and EMS training effects on muscle function and muscle hypertrophy should be investigated in future studies. In addition, investigating different dosages of EMS parameters may

bridge the gap for their effect on different physiological variables. Thus, further studies related to dose-responsiveness of alternating and pulsed current are needed.

# REFERENCES

1.  **Adams GR**. Role of insulin-like growth factor-I in the regulation of skeletal muscle adaptation to increased loading. *Exerc Sport Sci Rev* 26: 31-60, 1998.
2.  **Adams GR, Harris RT, Woodard D, and Dudley GA**. Mapping of electrical muscle stimulation using MRI. *J Appl Physiol* 74: 532-537, 1993.
3.  **Ahtiainen JP, Pakarinen A, Kraemer WJ, and Hakkinen K**. Acute hormonal and neuromuscular responses and recovery to forced vs maximum repetitions multiple resistance exercises. *Int J Sports Med* 24: 410-418, 2003.
4.  **Ahtiainen JP, Pakarinen A, Kraemer WJ, and Hakkinen K**. Acute hormonal responses to heavy resistance exercise in strength athletes versus nonathletes. *Can J Appl Physiol* 29: 527-543, 2004.
5.  **Aldayel A, Jubeau M, McGuigan MR, and Nosaka K**. Less indication of muscle damage in the second than initial electrical muscle stimulation bout consisting of isometric contractions of the knee extensors. *Eur J Appl Physiol* 108: 709-717, 2010.
6.  **Alon G, Allin J, and Inbar GF**. Optimization of pulse duration and pulse charge during transcutaneous electrical nerve stimulation. *Aust J Physiother* 19: 196–210, 1983.
7.  **Bajd T, Gregoric M, Vodovnik L, and Benko H**. Electrical stimulation in treating spasticity resulting from spinal cord injury. *Arch Phys Med Rehabil* 66: 515-517, 1985.
8.  **Baldi JC, Jackson RD, Moraille R, and Mysiw WJ**. Muscle atrophy is prevented in patients with acute spinal cord injury using functional electrical stimulation. *Spinal Cord* 36: 463-469, 1998.
9.  **Balogun JA**. Effects of ramp time on sensory, motor and tolerance thresholds during exogenous electrical stimulation. *J Sports Med Phys Fitness* 31: 521-526, 1991.
10. **Balogun JA**. Pain complaint and muscle soreness associated with high-voltage electrical stimulation: effect of ramp time. *Percept Mot Skills* 62: 799-810, 1986.
11. **Balogun JA, Onilari OO, Akeju OA, and Marzouk DK**. High voltage electrical stimulation in the augmentation of muscle strength: effects of pulse frequency. *Arch Phys Med Rehabil* 74: 910-916, 1993.
12. **Banerjee P, Caulfield B, Crowe L, and Clark A**. Prolonged electrical muscle stimulation exercise improves strength and aerobic capacity in healthy sedentary adults. *J Appl Physiol* 99: 2307-2311, 2005.
13. **Barcroft H, and Dornhorst AC**. The blood flow through the human calf during rhythmic exercise. *J Physiol* 109: 402-411, pl, 1949.
14. **Barcroft H, and Millen JL**. The blood flow through muscle during sustained contraction. *J Physiol* 97: 17-31, 1939.
15. **Bax L, Staes F, and Verhagen A**. Does neuromuscular electrical stimulation strengthen the quadriceps femoris? A systematic review of randomised controlled trials. *Sports Med* 35: 191-212, 2005.
16. **Bennie SD, Petrofsky JS, Nisperos J, Tsurudome M, and Laymon M**. Toward the optimal waveform for electrical stimulation of human muscle. (Vers une forme d ' onde optimale pour la stimulation electrique des muscles humains.). *Eur J Appl Physiol* 88: 13-197, 2002.

17.     **Bettany JA, Fish DR, and Mendel FC**. Influence of high voltage pulsed direct current on edema formation following impact injury. *Phys Ther* 70: 219-224, 1990.

18.     **Bhambhani Y, Tuchak C, Burnham R, Jeon J, and Maikala R**. Quadriceps muscle deoxygenation during functional electrical stimulation in adults with spinal cord injury. *Spinal Cord* 38: 630-638, 2000.

19.     **Bhambhani YN**. Muscle oxygenation trends during dynamic exercise measured by near infrared spectroscopy. *Can J Appl Physiol* 29: 504-523, 2004.

20.     **Bigland-Ritchie B**. Muscle fatigue and the influence of changing neural drive. *Clin Chest Med* 5: 21-34, 1984.

21.     **Binder-Macleod SA, and Guerin T**. Preservation of force output through progressive reduction of stimulation frequency in human quadriceps femoris muscle. *Phys Ther* 70: 619-625, 1990.

22.     **Binder-Macleod SA, Halden EE, and Jungles KA**. Effects of stimulation intensity on the physiological responses of human motor units. *Med Sci Sports Exerc* 27: 556-565, 1995.

23.     **Binder-Macleod SA, Lee SC, Russ DW, and Kucharski LJ**. Effects of activation pattern on human skeletal muscle fatigue. *Muscle Nerve* 21: 1145-1152, 1998.

24.     **Binder-Macleod SA, and Snyder-Mackler L**. Muscle fatigue: clinical implications for fatigue assessment and neuromuscular electrical stimulation. *Phys Ther* 73: 902-910, 1993.

25.     **Bircan C, Senocak O, Peker O, Kaya A, Tamci SA, Gulbahar S, and Akalin E**. Efficacy of two forms of electrical stimulation in increasing quadriceps strength: a randomized controlled trial. *Clin Rehabil* 16: 194-199, 2002.

26.     **Bishop JM, Donald KW, Taylor SH, and Wormald PN**. The blood flow in the human arm during supine leg exercise. *J Physiol* 137: 294-308, 1957.

27.     **Black CD, and McCully KK**. Muscle injury after repeated bouts of voluntary and electrically stimulated exercise. *Med Sci Sports Exerc* 40: 1605-1615, 2008.

28.     **Boerio D, Jubeau M, Zory R, and Maffiuletti NA**. Central and peripheral fatigue after electrostimulation-induced resistance exercise. *Med Sci Sports Exerc* 37: 973-978, 2005.

29.     **Botter A, Merletti R, and Minetto MA**. Pulse charge and not waveform affects M-wave properties during progressive motor unit activation. *J Electromyogr Kinesiol* 19: 564-573, 2009.

30.     **Boushel R, and Piantadosi CA**. Near-infrared spectroscopy for monitoring muscle oxygenation. *Acta Physiol Scand* 168: 615-622, 2000.

31.     **Bowman BR, and Baker LL**. Effects of waveform parameters on comfort during transcutaneous neuromuscular electrical stimulation. *Ann Biomed Eng* 13: 59-74, 1985.

32.     **Brooks GA, Fahey TD, and Baldwin KM**. *Exercise physiology: human bioenergetics and its applications*. New York: McGraw-Hill, (4th). 2004.

33.     **Brown SJ, Child RB, Day SH, and Donnelly AE**. Exercise-induced skeletal muscle damage and adaptation following repeated bouts of eccentric muscle contractions. *J Sports Sci* 15: 215-222, 1997.

34.     **Brown SJ, Child RB, Donnelly AE, Saxton JM, and Day SH**. Changes in human skeletal muscle contractile function following stimulated eccentric exercise. *Eur J Appl Physiol Occup Physiol* 72: 515-521, 1996.

35. **Bunt JC, Bahr JM, and Bemben DA**. Comparison of estradiol and testosterone levels during and immediately following prolonged exercise in moderately active and trained males and females. *Endocr Res* 13: 157-172, 1987.

36. **Butterfield DL, Draper DO, Ricard MD, Myrer JW, Schulthies SS, and Durrant E**. The effects of high-volt pulsed current electrical stimulation on delayed-onset muscle soreness. *J Athl Train* 32: 15-20, 1997.

37. **Byrnes WC, Clarkson PM, White JS, Hsieh SS, Frykman PN, and Maughan RJ**. Delayed onset muscle soreness following repeated bouts of downhill running. *J Appl Physiol* 59: 710-715, 1985.

38. **Cettolo V, Ferrari M, Biasini V, and Quaresima V**. Vastus lateralis O2 desaturation in response to fast and short maximal contraction. *Med Sci Sports Exerc* 39: 1949-1959, 2007.

39. **Chance B, Nioka S, Kent J, McCully K, Fountain M, Greenfeld R, and Holtom G**. Time-resolved spectroscopy of hemoglobin and myoglobin in resting and ischemic muscle. *Anal Biochem* 174: 698-707, 1988.

40. **Child RB, Brown SJ, Day SH, Saxton JM, and Donnelly AE**. Manipulation of knee extensor force using percutaneous electrical myostimulation during eccentric actions: effects on indices of muscle damage in humans. *Int J Sports Med* 19: 468-473, 1998.

41. **Child RB, Saxton JM, and Donnelly AE**. Comparison of eccentric knee extensor muscle actions at two muscle lengths on indices of damage and angle-specific force production in humans. *J Sports Sci* 16: 301-308, 1998.

42. **Clarkson PM, and Hubal MJ**. Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil* 81: S52-69, 2002.

43. **Clarkson PM, Kearns AK, Rouzier P, Rubin R, and Thompson PD**. Serum creatine kinase levels and renal function measures in exertional muscle damage. *Med Sci Sports Exerc* 38: 623-627, 2006.

44. **Collins DF**. Central contributions to contractions evoked by tetanic neuromuscular electrical stimulation. *Exerc Sport Sci Rev* 35: 102-109, 2007.

45. **Collins DF, Burke D, and Gandevia SC**. Sustained contractions produced by plateau-like behaviour in human motoneurones. *J Physiol* 538: 289-301, 2002.

46. **Cooper RG, Edwards RH, Gibson H, and Stokes MJ**. Human muscle fatigue: frequency dependence of excitation and force generation. *J Physiol* 397: 585-599, 1988.

47. **Cotzias C, and Marshall JM**. Differential effects of isometric exercise on the cutaneous circulation of different regions. *Clin Auton Res* 2: 235-241, 1992.

48. **Crameri RM, Aagaard P, Qvortrup K, Langberg H, Olesen J, and Kjaer M**. Myofibre damage in human skeletal muscle: effects of electrical stimulation versus voluntary contraction. *J Physiol* 583: 365-380, 2007.

49. **Crameri RM, Langberg H, Magnusson P, Jensen CH, Schroder HD, Olesen JL, Suetta C, Teisner B, and Kjaer M**. Changes in satellite cells in human skeletal muscle after a single bout of high intensity exercise. *J Physiol* 558: 333-340, 2004.

50. **Currier DP, Petrilli CR, and Threlkeld AJ**. Effect of graded electrical stimulation on blood flow to healthy muscle. *Phys Ther* 66: 937-943, 1986.

51. **Davidson MB**. Effect of growth hormone on carbohydrate and lipid metabolism. *Endocr Rev* 8: 115-131, 1987.

52. **Day ML, McGuigan MR, Brice G, and Foster C**. Monitoring exercise intensity during resistance training using the session RPE scale. *J Strength Cond Res* 18: 353-358, 2004.

53. **Dayton PD, and Palladino SJ**. Electrical stimulation of cutaneous ulcerations. A literature review. *J Am Podiatr Med Assoc* 79: 318-321, 1989.

54. **Dean JC, Yates LM, and Collins DF**. Turning off the central contribution to contractions evoked by neuromuscular electrical stimulation. *Muscle Nerve* 38: 978-986, 2008.

55. **Dean JC, Yates LM, and Collins DF**. Turning on the central contribution to contractions evoked by neuromuscular electrical stimulation. *J Appl Physiol* 103: 170-176, 2007.

56. **Delitto A, Brown M, Strube MJ, Rose SJ, and Lehman RC**. Electrical stimulation of quadriceps femoris in an elite weight lifter: a single subject experiment. *Int J Sports Med* 10: 187-191, 1989.

57. **Delitto A, Strube MJ, Shulman AD, and Minor SD**. A study of discomfort with electrical stimulation. *Phys Ther* 72: 410-421; discussion on 421-414, 1992.

58. **Denegar CR**. *Therapeutic modalities for athletic injuries*. Champaign, IL: Human Kinetics, 2000, p. xv, 247 p.

59. **Denegar CR, Perrin DH, Rogol AD, and Rutt RA**. Influence of transcutaneous electrical nerve stimulation on pain, range of motion, and serum cortisol concentration in females experiencing delayed onset muscle soreness. *J Orthop Sports Phys Ther* 11: 100-103, 1989.

60. **Denny-Brown D**. On the nature of postural reflexes. *Proceedings of the Royal Society of London Series B, Containing Papers of a Biological Character* 104: 252-301, 1929.

61. **Desbrosses K, Babault N, Scaglioni G, Meyer JP, and Pousson M**. Neural activation after maximal isometric contractions at different muscle lengths. *Med Sci Sports Exerc* 38: 937-944, 2006.

62. **Dupont Salter AC, Richmond FJ, and Loeb GE**. Prevention of muscle disuse atrophy by low-frequency electrical stimulation in rats. *IEEE Trans Neural Syst Rehabil Eng* 11: 218-226, 2003.

63. **Edwards RH**. Human muscle function and fatigue. *Ciba Found Symp* 82: 1-18, 1981.

64. **Edwards RH, Young A, Hosking GP, and Jones DA**. Human skeletal muscle function: description of tests and normal values. *Clin Sci Mol Med* 52: 283-290, 1977.

65. **Enoka RM**. Activation order of motor axons in electrically evoked contractions. *Muscle Nerve* 25: 763-764, 2002.

66. **Eriksson E, and Haggmark T**. Comparison of isometric muscle training and electrical stimulation supplementing isometric muscle training in the recovery after major knee ligament surgery. A preliminary report. *Am J Sports Med* 7: 169-171, 1979.

67. **Falvo MJ, Schilling BK, Bloomer RJ, and Smith WA**. Repeated bout effect is absent in resistance trained men: An electromyographic analysis. *J Electromyogr Kinesiol* 2008.

68. **Faude O, Kindermann W, and Meyer T**. Lactate threshold concepts: how valid are they? *Sports Med* 39: 469-490, 2009.

69. **Feiereisen P, Duchateau J, and Hainaut K**. Motor unit recruitment order during voluntary and electrically induced contractions in the tibialis anterior. *Exp Brain Res* 114: 117-123, 1997.

70. **Felici F, Quaresima V, Fattorini L, Sbriccoli P, Filligoi GC, and Ferrari M**. Biceps brachii myoelectric and oxygenation changes during static and sinusoidal isometric exercises. *J Electromyogr Kinesiol* 19: e1-11, 2009.

71. **Ferguson JP, Blackley MW, Knight RD, Sutlive TG, Underwood FB, and Greathouse DG**. Effects of varying electrode site placements on the torque output of an electrically stimulated involuntary quadriceps femoris muscle contraction. *J Orthop Sports Phys Ther* 11: 24-29, 1989.

72. **Ferrari M, Binzoni T, and Quaresima V**. Oxidative metabolism in muscle. *Philos Trans R Soc Lond B Biol Sci* 352: 677-683, 1997.

73. **Ferrari M, Mottola L, and Quaresima V**. Principles, techniques, and limitations of near infrared spectroscopy. *Can J Appl Physiol* 29: 463-487, 2004.

74. **Foley JM, Jayaraman RC, Prior BM, Pivarnik JM, and Meyer RA**. MR measurements of muscle damage and adaptation after eccentric exercise. *J Appl Physiol* 87: 2311-2318, 1999.

75. **Forbes SC, Raymer GH, Kowalchuk JM, Thompson RT, and Marsh GD**. Effects of recovery time on phosphocreatine kinetics during repeated bouts of heavy-intensity exercise. *Eur J Appl Physiol* 103: 665-675, 2008.

76. **Franklin PJ, Green DJ, and Cable NT**. The influence of thermoregulatory mechanisms on post-exercise hypotension in humans. *J Physiol* 470: 231-241, 1993.

77. **Froesch ER, Schmid C, Schwander J, and Zapf J**. Actions of insulin-like growth factors. *Annu Rev Physiol* 47: 443-467, 1985.

78. **Gaffney FA, Sjogaard G, and Saltin B**. Cardiovascular and metabolic responses to static contraction in man. *Acta Physiol Scand* 138: 249-258, 1990.

79. **Geddes LA**. Retrospectroscope. d'Arsonval, physician and inventor. *IEEE Eng Med Biol Mag* 18: 118-122, 1999.

80. **Geddes LA**. A short history of the electrical stimulation of excitable tissue. Including electrotherapeutic applications. *Physiologist* 27: S1-47, 1984.

81. **Gerovasili V, Stefanidis K, Vitzilaios K, Karatzanos E, Politis P, Koroneos A, Chatzimichail A, Routsi C, Roussos C, and Nanas S**. Electrical muscle stimulation preserves the muscle mass of critically ill patients: a randomized study. *Crit Care* 13: R161, 2009.

82. **Gibson JN, Morrison WL, Scrimgeour CM, Smith K, Stoward PJ, and Rennie MJ**. Effects of therapeutic percutaneous electrical stimulation of atrophic human quadriceps on muscle composition, protein synthesis and contractile properties. *Eur J Clin Invest* 19: 206-212, 1989.

83. **Gleeson M**. Temperature regulation during exercise. *Int J Sports Med* 19 Suppl 2: S96-99, 1998.

84. **Goats GC**. Interferential current therapy. *Br J Sports Med* 24: 87-92, 1990.

85. **Gondin J, Giannesini B, Vilmen C, Dalmasso C, le Fur Y, Cozzone PJ, and Bendahan D**. Effects of stimulation frequency and pulse duration on fatigue and metabolic cost during a single bout of neuromuscular electrical stimulation. *Muscle Nerve* 2010.

86. **Gondin J, Giannesini B, Vilmen C, Dalmasso C, le Fur Y, Cozzone PJ, and Bendahan D**. Effects of stimulation frequency and pulse duration on fatigue and

metabolic cost during a single bout of neuromuscular electrical stimulation. *Muscle Nerve* 41: 667-678, 2010.

87. **Gondin J, Guette M, Ballay Y, and Martin A**. Electromyostimulation training effects on neural drive and muscle architecture. *Med Sci Sports Exerc* 37: 1291-1299, 2005.

88. **Gorgey AS, Black CD, Elder CP, and Dudley GA**. Effects of electrical stimulation parameters on fatigue in skeletal muscle. *J Orthop Sports Phys Ther* 39: 684-692, 2009.

89. **Gorgey AS, and Dudley GA**. The role of pulse duration and stimulation duration in maximizing the normalized torque during neuromuscular electrical stimulation. *J Orthop Sports Phys Ther* 38: 508-516, 2008.

90. **Gorgey AS, Mahoney E, Kendall T, and Dudley GA**. Effects of neuromuscular electrical stimulation parameters on specific tension. *Eur J Appl Physiol* 97: 737-744, 2006.

91. **Goto K, Ishii N, Kizuka T, Kraemer RR, Honda Y, and Takamatsu K**. Hormonal and metabolic responses to slow movement resistance exercise with different durations of concentric and eccentric actions. *Eur J Appl Physiol* 106: 731-739, 2009.

92. **Grant RT, and Holling HE**. Further observations on the vascular responses of the human limb to body warming; evidence for sympathetic vasodilator nerves in the normal subject. *Clin Sci* 3: 273-285, 1936.

93. **Green HJ**. Mechanisms of muscle fatigue in intense exercise. *J Sports Sci* 15: 247-256, 1997.

94. **Gregory CM, and Bickel CS**. Recruitment patterns in human skeletal muscle during electrical stimulation. *Phys Ther* 85: 358-364, 2005.

95. **Gregory CM, Dixon W, and Bickel CS**. Impact of varying pulse frequency and duration on muscle torque production and fatigue. *Muscle Nerve* 35: 504-509, 2007.

96. **Grimby G, and Wigerstad-Lossing I**. Comparison of high- and low-frequency muscle stimulators. *Arch Phys Med Rehabil* 70: 835-838, 1989.

97. **Hainaut K, and Duchateau J**. Neuromuscular electrical stimulation and voluntary exercise. *Sports Med* 14: 100-113, 1992.

98. **Hakkinen K, and Pakarinen A**. Acute hormonal responses to heavy resistance exercise in men and women at different ages. *Int J Sports Med* 16: 507-513, 1995.

99. **Hamada T, Hayashi T, Kimura T, Nakao K, and Moritani T**. Electrical stimulation of human lower extremities enhances energy consumption, carbohydrate oxidation, and whole body glucose uptake. *J Appl Physiol* 96: 911-916, 2004.

100. **Hamaoka T, McCully KK, Quaresima V, Yamamoto K, and Chance B**. Near-infrared spectroscopy/imaging for monitoring muscle oxygenation and oxidative metabolism in healthy and diseased humans. *J Biomed Opt* 12: 062105, 2007.

101. **Hampson NB, and Piantadosi CA**. Near infrared monitoring of human skeletal muscle oxygenation during forearm ischemia. *J Appl Physiol* 64: 2449-2457, 1988.

102. **Hansen M, Trappe T, Crameri RM, Qvortrup K, Kjaer M, and Langberg H**. Myofibrillar proteolysis in response to voluntary or electrically stimulated muscle contractions in humans. *Scand J Med Sci Sports* 19: 75-82, 2009.

103. **Hecker B, Carron H, and Schwartz DP**. Pulsed galvanic stimulation: effects of current frequency and polarity on blood flow in healthy subjects. *Arch Phys Med Rehabil* 66: 369-371, 1985.

104. **Henneman E, Somjen G, and Carpenter DO**. Functional Significance of Cell Size in Spinal Motoneurons. *J Neurophysiol* 28: 560-580, 1965.

105. **Herrero JA, Izquierdo M, Maffiuletti NA, and Garcia-Lopez J**. Electromyostimulation and plyometric training effects on jumping and sprint time. *Int J Sports Med* 27: 533-539, 2006.

106. **Hickson RC, Hidaka K, Foster C, Falduto MT, and Chatterton RT, Jr.** Successive time courses of strength development and steroid hormone responses to heavy-resistance training. *J Appl Physiol* 76: 663-670, 1994.

107. **Hirata K, Hara T, Oshima Y, Yoshikawa T, and Fujimoto S**. Effects of electrical stimulation and voluntary exercise on muscle oxygenation assessed by NIRS. *Osaka City Med J* 52: 67-78, 2006.

108. **Holcomb WR**. Is neuromuscular electrical stimulation an effective alternative to resistance training? *Strength Cond J* 27: 76-79, 2005.

109. **Holcomb WR, Golestani S, and Hill S**. A comparison of knee-extension torque production with biphasic versus Russian current. *J Sport Rehabil* 9: 229-239, 2000.

110. **Hultman E, and Sjoholm H**. Energy metabolism and contraction force of human skeletal muscle in situ during electrical stimulation. *J Physiol* 345: 525-532, 1983.

111. **Hultman E, Sjoholm H, Jaderholm-Ek I, and Krynicki J**. Evaluation of methods for electrical stimulation of human skeletal muscle in situ. *Pflugers Arch* 398: 139-141, 1983.

112. **Ingalls CP, Wenke JC, Nofal T, and Armstrong RB**. Adaptation to lengthening contraction-induced injury in mouse muscle. *J Appl Physiol* 97: 1067-1076, 2004.

113. **Iwasaki T, Shiba N, Matsuse H, Nago T, Umezu Y, Tagawa Y, Nagata K, and Basford JR**. Improvement in knee extension strength through training by means of combined electrical stimulation and voluntary muscle contraction. *Tohoku J Exp Med* 209: 33-40, 2006.

114. **Jasperse JL, Seals DR, and Callister R**. Active forearm blood flow adjustments to handgrip exercise in young and older healthy men. *J Physiol* 474: 353-360, 1994.

115. **Johnson JM**. Exercise and the cutaneous circulation. *Exerc Sport Sci Rev* 20: 59-97, 1992.

116. **Johnson JM**. Nonthermoregulatory control of human skin blood flow. *J Appl Physiol* 61: 1613-1622, 1986.

117. **Jones DA**. High-and low-frequency fatigue revisited. *Acta Physiol Scand* 156: 265-270, 1996.

118. **Jones DA, Bigland-Ritchie B, and Edwards RH**. Excitation frequency and muscle fatigue: mechanical responses during voluntary and stimulated contractions. *Exp Neurol* 64: 401-413, 1979.

119. **Jones DA, Newham DJ, and Torgan C**. Mechanical influences on long-lasting human muscle fatigue and delayed-onset pain. *J Physiol* 412: 415-427, 1989.

120. **Jubeau M, Gondin J, Martin A, Sartorio A, and Maffiuletti NA**. Random motor unit activation by electrostimulation. *Int J Sports Med* 28: 901-904, 2007.

121. **Jubeau M, Sartorio A, Marinone PG, Agosti F, Van Hoecke J, Nosaka K, and Maffiuletti NA**. Comparison between voluntary and stimulated contractions of the quadriceps femoris for growth hormone response and muscle damage. *J Appl Physiol* 104: 75-81, 2008.

122. **Kantor G, Alon G, and Ho HS**. The effects of selected stimulus waveforms on pulse and phase characteristics at sensory and motor thresholds. *Phys Ther* 74: 951-962, 1994.

123. **Kenney WL, and Johnson JM**. Control of skin blood flow during exercise. *Med Sci Sports Exerc* 24: 303-312, 1992.

124. **Kenny GP, Chen AA, Nurbakhsh BA, Denis PM, Proulx CE, and Giesbrecht GG**. Moderate exercise increases postexercise thresholds for vasoconstriction and shivering. *J Appl Physiol* 85: 1357-1361, 1998.

125. **Kenny GP, Proulx CE, Denis PM, and Giesbrecht GG**. Moderate exercise increases the post exercise resting warm thermoregulatory response thresholds. *Aviat Space Environ Med* 71: 914-919, 2000.

126. **Kesar T, Chou LW, and Binder-Macleod SA**. Effects of stimulation frequency versus pulse duration modulation on muscle fatigue. *J Electromyogr Kinesiol* 18: 662-671, 2008.

127. **Kidd GL**. Electrical stimulation for disuse muscle atrophy. *Lancet* 2: 1025, 1988.

128. **Kim CK, Bangsbo J, Strange S, Karpakka J, and Saltin B**. Metabolic response and muscle glycogen depletion pattern during prolonged electrically induced dynamic exercise in man. *Scand J Rehabil Med* 27: 51-58, 1995.

129. **Kim CK, Takala TE, Seger J, and Karpakka J**. Training effects of electrically induced dynamic contractions in human quadriceps muscle. *Aviat Space Environ Med* 66: 251-255, 1995.

130. **Kloth LC**. *Interference current*. East Norwalk, CT: Appleton & Lange, electrotherapy C (2nd). 1991.

131. **Knaflitz M, Merletti R, and De Luca CJ**. Inference of motor unit recruitment order in voluntary and electrically elicited contractions. *J Appl Physiol* 68: 1657-1667, 1990.

132. **Kondo N, Takano S, Aoki K, Shibasaki M, Tominaga H, and Inoue Y**. Regional differences in the effect of exercise intensity on thermoregulatory sweating and cutaneous vasodilation. *Acta Physiol Scand* 164: 71-78, 1998.

133. **Kooistra RD, de Ruiter CJ, and de Haan A**. Knee angle-dependent oxygen consumption of human quadriceps muscles during maximal voluntary and electrically evoked contractions. *Eur J Appl Physiol* 102: 233-242, 2008.

134. **Kraemer WJ, Aguilera BA, Terada M, Newton RU, Lynch JM, Rosendaal G, McBride JM, Gordon SE, and Hakkinen K**. Responses of IGF-I to endogenous increases in growth hormone after heavy-resistance exercise. *J Appl Physiol* 79: 1310-1315, 1995.

135. **Kraemer WJ, Fleck SJ, Dziados JE, Harman EA, Marchitelli LJ, Gordon SE, Mello R, Frykman PN, Koziris LP, and Triplett NT**. Changes in hormonal concentrations after different heavy-resistance exercise protocols in women. *J Appl Physiol* 75: 594-604, 1993.

136. **Kraemer WJ, Fleck SJ, Maresh CM, Ratamess NA, Gordon SE, Goetz KL, Harman EA, Frykman PN, Volek JS, Mazzetti SA, Fry AC, Marchitelli LJ, and Patton JF**. Acute hormonal responses to a single bout of heavy resistance

121. **Jubeau M, Sartorio A, Marinone PG, Agosti F, Van Hoecke J, Nosaka K, and Maffiuletti NA**. Comparison between voluntary and stimulated contractions of the quadriceps femoris for growth hormone response and muscle damage. *J Appl Physiol* 104: 75-81, 2008.

122. **Kantor G, Alon G, and Ho HS**. The effects of selected stimulus waveforms on pulse and phase characteristics at sensory and motor thresholds. *Phys Ther* 74: 951-962, 1994.

123. **Kenney WL, and Johnson JM**. Control of skin blood flow during exercise. *Med Sci Sports Exerc* 24: 303-312, 1992.

124. **Kenny GP, Chen AA, Nurbakhsh BA, Denis PM, Proulx CE, and Giesbrecht GG**. Moderate exercise increases postexercise thresholds for vasoconstriction and shivering. *J Appl Physiol* 85: 1357-1361, 1998.

125. **Kenny GP, Proulx CE, Denis PM, and Giesbrecht GG**. Moderate exercise increases the post exercise resting warm thermoregulatory response thresholds. *Aviat Space Environ Med* 71: 914-919, 2000.

126. **Kesar T, Chou LW, and Binder-Macleod SA**. Effects of stimulation frequency versus pulse duration modulation on muscle fatigue. *J Electromyogr Kinesiol* 18: 662-671, 2008.

127. **Kidd GL**. Electrical stimulation for disuse muscle atrophy. *Lancet* 2: 1025, 1988.

128. **Kim CK, Bangsbo J, Strange S, Karpakka J, and Saltin B**. Metabolic response and muscle glycogen depletion pattern during prolonged electrically induced dynamic exercise in man. *Scand J Rehabil Med* 27: 51-58, 1995.

129. **Kim CK, Takala TE, Seger J, and Karpakka J**. Training effects of electrically induced dynamic contractions in human quadriceps muscle. *Aviat Space Environ Med* 66: 251-255, 1995.

130. **Kloth LC**. *Interference current*. East Norwalk, CT: Appleton & Lange, electrotherapy C (2nd). 1991.

131. **Knaflitz M, Merletti R, and De Luca CJ**. Inference of motor unit recruitment order in voluntary and electrically elicited contractions. *J Appl Physiol* 68: 1657-1667, 1990.

132. **Kondo N, Takano S, Aoki K, Shibasaki M, Tominaga H, and Inoue Y**. Regional differences in the effect of exercise intensity on thermoregulatory sweating and cutaneous vasodilation. *Acta Physiol Scand* 164: 71-78, 1998.

133. **Kooistra RD, de Ruiter CJ, and de Haan A**. Knee angle-dependent oxygen consumption of human quadriceps muscles during maximal voluntary and electrically evoked contractions. *Eur J Appl Physiol* 102: 233-242, 2008.

134. **Kraemer WJ, Aguilera BA, Terada M, Newton RU, Lynch JM, Rosendaal G, McBride JM, Gordon SE, and Hakkinen K**. Responses of IGF-I to endogenous increases in growth hormone after heavy-resistance exercise. *J Appl Physiol* 79: 1310-1315, 1995.

135. **Kraemer WJ, Fleck SJ, Dziados JE, Harman EA, Marchitelli LJ, Gordon SE, Mello R, Frykman PN, Koziris LP, and Triplett NT**. Changes in hormonal concentrations after different heavy-resistance exercise protocols in women. *J Appl Physiol* 75: 594-604, 1993.

136. **Kraemer WJ, Fleck SJ, Maresh CM, Ratamess NA, Gordon SE, Goetz KL, Harman EA, Frykman PN, Volek JS, Mazzetti SA, Fry AC, Marchitelli LJ, and Patton JF**. Acute hormonal responses to a single bout of heavy resistance

exercise in trained power lifters and untrained men. *Can J Appl Physiol* 24: 524-537, 1999.

137. **Kraemer WJ, Gordon SE, Fleck SJ, Marchitelli LJ, Mello R, Dziados JE, Friedl K, Harman E, Maresh C, and Fry AC**. Endogenous anabolic hormonal and growth factor responses to heavy resistance exercise in males and females. *Int J Sports Med* 12: 228-235, 1991.

138. **Kraemer WJ, Hakkinen K, Newton RU, Nindl BC, Volek JS, McCormick M, Gotshalk LA, Gordon SE, Fleck SJ, Campbell WW, Putukian M, and Evans WJ**. Effects of heavy-resistance training on hormonal response patterns in younger vs. older men. *J Appl Physiol* 87: 982-992, 1999.

139. **Kraemer WJ, Marchitelli L, Gordon SE, Harman E, Dziados JE, Mello R, Frykman P, McCurry D, and Fleck SJ**. Hormonal and growth factor responses to heavy resistance exercise protocols. *J Appl Physiol* 69: 1442-1450, 1990.

140. **Kraemer WJ, and Ratamess NA**. Hormonal responses and adaptations to resistance exercise and training. *Sports Med* 35: 339-361, 2005.

141. **Kraemer WJ, Volek JS, Bush JA, Putukian M, and Sebastianelli WJ**. Hormonal responses to consecutive days of heavy-resistance exercise with or without nutritional supplementation. *J Appl Physiol* 85: 1544-1555, 1998.

142. **Kramer JF**. Effect of electrical stimulation current frequencies on isometric knee extension torque. *Phys Ther* 67: 31-38, 1987.

143. **Kramer JF, and Mendryk SW**. Electrical stimulation as a strength improvement technique: a review. *J Orthop Sports Phys Ther* 4: 91-98, 1982.

144. **Kubiak RJ, Kubiak RJ, Whitman KM, and Johnston RM**. Changes in quadriceps femoris muscle strength using isometric exercise versus electrical stimulation. *J Orthop Sports Phys Ther* 8: 537-541, 1987.

145. **Lake DA**. Neuromuscular electrical stimulation. An overview and its application in the treatment of sports injuries. *Sports Med* 13: 320-336, 1992.

146. **Laufer Y, and Elboim M**. Effect of burst frequency and duration of kilohertz-frequency alternating currents and of low-frequency pulsed currents on strength of contraction, muscle fatigue, and perceived discomfort. *Phys Ther* 88: 1167-1176, 2008.

147. **Laufer Y, Ries JD, Leininger PM, and Alon G**. Quadriceps femoris muscle torques and fatigue generated by neuromuscular electrical stimulation with three different waveforms. *Phys Ther* 81: 1307-1316, 2001.

148. **Laufer Y, Ries JD, Leininger PM, and Alon G**. Quadriceps femoris muscle torques and fatigue generated by neuromusuclar electrical stimulation with three different waveforms. *Physical therapy* 81: 1307-131610, 2001.

149. **Lewis SF, and Fulco CS**. A new approach to studying muscle fatigue and factors affecting performance during dynamic exercise in humans. *Exerc Sport Sci Rev* 26: 91-116, 1998.

150. **Lieber RL, and Kelly MJ**. Torque history of electrically stimulated human quadriceps: implications for stimulation therapy. *J Orthop Res* 11: 131-141, 1993.

151. **Loram LC, Mitchell D, and Fuller A**. Rofecoxib and Tramadol do not attenuate delayed-onset muscle soreness or ischaemic pain in human volunteers. *Can J Physiol Pharmacol* 83: 1137-1145, 2005.

152. **Lyons CL, Robb JB, Irrgang JJ, and Fitzgerald GK**. Differences in quadriceps femoris muscle torque when using a clinical electrical stimulator versus a portable electrical stimulator. *Phys Ther* 85: 44-51, 2005.

153. **Mackel RG, and Brink EE**. Accommodation in single human nerve fibers in vivo. *Muscle Nerve* 18: 469-471, 1995.

154. **Mackey AL, Bojsen-Moller J, Qvortrup K, Langberg H, Suetta C, Kalliokoski KK, Kjaer M, and Magnusson SP**. Evidence of skeletal muscle damage following electrically stimulated isometric muscle contractions in humans. *J Appl Physiol* 105: 1620-1627, 2008.

155. **Mackey AL, Bojsen-Moller J, Qvortrup K, Langberg H, Suetta C, Kalliokoski KK, Kjaer M, and Magnusson SP**. Evidence of skeletal muscle damage following electrically stimulated isometric muscle contractions in humans. *J Appl Physiol* 2008.

156. **Maffiuletti NA, Dugnani S, Folz M, Di Pierno E, and Mauro F**. Effect of combined electrostimulation and plyometric training on vertical jump height. *Med Sci Sports Exerc* 34: 1638-1644, 2002.

157. **Maffiuletti NA, Zory R, Miotti D, Pellegrino MA, Jubeau M, and Bottinelli R**. Neuromuscular adaptations to electrostimulation resistance training. *Am J Phys Med Rehabil* 85: 167-175, 2006.

158. **Mair J, Mayr M, Muller E, Koller A, Haid C, Artner-Dworzak E, Calzolari C, Larue C, and Puschendorf B**. Rapid adaptation to eccentric exercise-induced muscle damage. *Int J Sports Med* 16: 352-356, 1995.

159. **Malatesta D, Cattaneo F, Dugnani S, and Maffiuletti NA**. Effects of electromyostimulation training and volleyball practice on jumping ability. *J Strength Cond Res* 17: 573-579, 2003.

160. **Malm C, Sjodin TL, Sjoberg B, Lenkei R, Renstrom P, Lundberg IE, and Ekblom B**. Leukocytes, cytokines, growth factors and hormones in human skeletal muscle and blood after uphill or downhill running. *J Physiol* 556: 983-1000, 2004.

161. **Man WD, Moxham J, and Polkey MI**. Magnetic stimulation for the measurement of respiratory and skeletal muscle function. *Eur Respir J* 24: 846-860, 2004.

162. **McCully KK, and Hamaoka T**. Near-infrared spectroscopy: what can it tell us about oxygen saturation in skeletal muscle? *Exerc Sport Sci Rev* 28: 123-127, 2000.

163. **McHugh MP, Connolly DA, Eston RG, Gartman EJ, and Gleim GW**. Electromyographic analysis of repeated bouts of eccentric exercise. *J Sports Sci* 19: 163-170, 2001.

164. **McHugh MP, Connolly DA, Eston RG, and Gleim GW**. Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. *Sports Med* 27: 157-170, 1999.

165. **McHugh MP, and Tetro DT**. Changes in the relationship between joint angle and torque production associated with the repeated bout effect. *J Sports Sci* 21: 927-932, 2003.

166. **McLoda TA, and Carmack JA**. Optimal Burst Duration During a Facilitated Quadriceps Femoris Contraction. *J Athl Train* 35: 145-150, 2000.

167. **McNeil CJ, Murray BJ, and Rice CL**. Differential changes in muscle oxygenation between voluntary and stimulated isometric fatigue of human dorsiflexors. *J Appl Physiol* 100: 890-895, 2006.

168. **Meyers BM, Nguyen J, and Cafarelli E**. Obtaining force-frequency curves with a single 3-second train of stimuli. *Muscle Nerve* 24: 1332-1338, 2001.

169. **Moreau D, Dubots P, Boggio V, Guilland JC, and Cometti G**. Effects of electromyostimulation and strength training on muscle soreness, muscle damage and sympathetic activation. *J Sports Sci* 13: 95-100, 1995.

170. **Muraki S, Fornusek C, Raymond J, and Davis GM**. Muscle oxygenation during prolonged electrical stimulation-evoked cycling in paraplegics. *Appl Physiol Nutr Metab* 32: 463-472, 2007.

171. **Muthalib M, Jubeau M, Millet GY, Maffiuletti NA, and Nosaka K**. Comparison between electrically evoked and voluntary isometric contractions for biceps brachii muscle oxidative metabolism using near-infrared spectroscopy. *Eur J Appl Physiol* 107: 235-241, 2009.

172. **Neary JP**. Application of near infrared spectroscopy to exercise sports science. *Can J Appl Physiol* 29: 488-503, 2004.

173. **Newham DJ, Jones DA, and Clarkson PM**. Repeated high-force eccentric exercise: effects on muscle pain and damage. *J Appl Physiol* 63: 1381-1386, 1987.

174. **Nosaka K, and Clarkson PM**. Muscle damage following repeated bouts of high force eccentric exercise. *Med Sci Sports Exerc* 27: 1263-1269, 1995.

175. **Nosaka K, Clarkson PM, McGuiggin ME, and Byrne JM**. Time course of muscle adaptation after high force eccentric exercise. *Eur J Appl Physiol Occup Physiol* 63: 70-76, 1991.

176. **Nosaka K, Muthalib M, Lavender A, and Laursen PB**. Attenuation of muscle damage by preconditioning with muscle hyperthermia 1-day prior to eccentric exercise. *Eur J Appl Physiol* 99: 183-192, 2007.

177. **Nosaka K, and Newton M**. Repeated eccentric exercise bouts do not exacerbate muscle damage and repair. *J Strength Cond Res* 16: 117-122, 2002.

178. **Nosaka K, Newton M, and Sacco P**. Delayed-onset muscle soreness does not reflect the magnitude of eccentric exercise-induced muscle damage. *Scand J Med Sci Sports* 12: 337-346, 2002.

179. **Nosaka K, Newton M, and Sacco P**. Responses of human elbow flexor muscles to electrically stimulated forced lengthening exercise. *Acta Physiol Scand* 174: 137-145, 2002.

180. **Nosaka K, and Sakamoto K**. Effect of elbow joint angle on the magnitude of muscle damage to the elbow flexors. *Med Sci Sports Exerc* 33: 22-29, 2001.

181. **Nosaka K, Sakamoto K, Newton M, and Sacco P**. The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. *Eur J Appl Physiol* 85: 34-40, 2001.

182. **Nuhr MJ, Pette D, Berger R, Quittan M, Crevenna R, Huelsman M, Wiesinger GF, Moser P, Fialka-Moser V, and Pacher R**. Beneficial effects of chronic low-frequency stimulation of thigh muscles in patients with advanced chronic heart failure. *Eur Heart J* 25: 136-143, 2004.

183. **Ohmori F, Shimizu S, and Kagaya A**. Exercise-induced blood flow in relation to muscle relaxation period. *Dyn Med* 6: 5, 2007.

184. **Owens J, and Malone TR**. Treatment parameters of high frequency electrical stimulation as established on the Electro-Stim 180. *J Orthop Sports Phys Ther* 4: 162-168, 1983.

185. **Packman-Braun R**. Relationship between functional electrical stimulation duty cycle and fatigue in wrist extensor muscles of patients with hemiparesis. *Phys Ther* 68: 51-56, 1988.

186. **Paillard T**. Combined application of neuromuscular electrical stimulation and voluntary muscular contractions. *Sports Med* 38: 161-177, 2008.

187. **Parker MG, Bennett MJ, Hieb MA, Hollar AC, and Roe AA**. Strength response in human femoris muscle during 2 neuromuscular electrical stimulation programs. *J Orthop Sports Phys Ther* 33: 719-726, 2003.

188. **Parker MG, Keller L, and Evenson J**. Torque responses in human quadriceps to burst-modulated alternating current at 3 carrier frequencies. *J Orthop Sports Phys Ther* 35: 239-245, 2005.

189. **Philippou A, Halapas A, Maridaki M, and Koutsilieris M**. Type I insulin-like growth factor receptor signaling in skeletal muscle regeneration and hypertrophy. *J Musculoskelet Neuronal Interact* 7: 208-218, 2007.

190. **Philippou A, Maridaki M, Halapas A, and Koutsilieris M**. The role of the insulin-like growth factor 1 (IGF-1) in skeletal muscle physiology. *In Vivo* 21: 45-54, 2007.

191. **Postans N, Wright P, Bromwich W, Wilkinson I, Farmer SE, and Swain I**. The combined effect of Dynamic splinting and Neuromuscular electrical stimulation in reducing wrist and elbow contractures in six children with Cerebral palsy. *Prosthet Orthot Int* 2010.

192. **Potisk KP, Gregoric M, and Vodovnik L**. Effects of transcutaneous electrical nerve stimulation (TENS) on spasticity in patients with hemiplegia. *Scand J Rehabil Med* 27: 169-174, 1995.

193. **Quaresima V, Lepanto R, and Ferrari M**. The use of near infrared spectroscopy in sports medicine. *J Sports Med Phys Fitness* 43: 1-13, 2003.

194. **Ratkevicius A, Mizuno M, Povilonis E, and Quistorff B**. Energy metabolism of the gastrocnemius and soleus muscles during isometric voluntary and electrically induced contractions in man. *J Physiol* 507: 593-602, 1998.

195. **Reilly JP**. *Applied bioelectricity : from electrical stimulation to electropathology*. New York: Springer, 1998, p. xix, 563 p.

196. **Reilly JP, and Antoni H**. *Electrical stimulation and electropathology*. Cambridge England ; New York: Cambridge University Press, 1992, p. xvii, 504 p.

197. **Richardson D, and Shewchuk R**. Effects of contraction force and frequency on postexercise hyperemia in human calf muscles. *J Appl Physiol* 49: 649-654, 1980.

198. **Rinderknecht E, and Humbel RE**. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *J Biol Chem* 253: 2769-2776, 1978.

199. **Robertson V, Ward AR, Low J, and Reed A**. *Electrotherapy explained: principles and practice*. London: Butterworth-Heinemann Ltd, (4th). 2006, p. 447.

200. **Robinson AJ, and Snyder-Mackler L**. *Clinical Electrophysiology: Electrotherapy and Electrophysiologic Testing*. Lippincott Williams & Wilkins, 2007.

201. **Roig M, and Reid WD**. Electrical stimulation and peripheral muscle function in COPD: a systematic review. *Respir Med* 103: 485-495, 2009.

202. **Rooney JG, Currier DP, and Nitz AJ**. Effect of variation in the burst and carrier frequency modes of neuromuscular electrical stimulation on pain perception of healthy subjects. *Phys Ther* 72: 800-806; discussion 807-809, 1992.

203. **Sadamoto T, Bonde-Petersen F, and Suzuki Y**. Skeletal muscle tension, flow, pressure, and EMG during sustained isometric contractions in humans. *Eur J Appl Physiol Occup Physiol* 51: 395-408, 1983.

204. **Sartorio A, Jubeau M, Agosti F, De Col A, Marazzi N, Lafortuna CL, and Maffiuletti NA**. GH responses to two consecutive bouts of neuromuscular electrical stimulation in healthy adults. *Eur J Endocrinol* 158: 311-316, 2008.

205. **Saxton JM, and Donnelly AE**. Length-specific impairment of skeletal muscle contractile function after eccentric muscle actions in man. *Clin Sci (Lond)* 90: 119-125, 1996.

206. **Schwab R, Johnson GO, Housh TJ, Kinder JE, and Weir JP**. Acute effects of different intensities of weight lifting on serum testosterone. *Med Sci Sports Exerc* 25: 1381-1385, 1993.

207. **Scremin AM, Kurta L, Gentili A, Wiseman B, Perell K, Kunkel C, and Scremin OU**. Increasing muscle mass in spinal cord injured persons with a functional electrical stimulation exercise program. *Arch Phys Med Rehabil* 80: 1531-1536, 1999.

208. **Selkowitz DM**. High frequency electrical stimulation in muscle strengthening. A review and discussion. *Am J Sports Med* 17: 103-111, 1989.

209. **Selkowitz DM**. Improvement in isometric strength of the quadriceps femoris muscle after training with electrical stimulation. *Phys Ther* 65: 186-196, 1985.

210. **Selkowitz DM, Rossman EG, and Fitzpatrick S**. Effect of burst-modulated alternating current carrier frequency on current amplitude required to produce maximally tolerated electrically stimulated quadriceps femoris knee extension torque. *Am J Phys Med Rehabil* 88: 973-978, 2009.

211. **Sieck GC, and Prakash YS**. Fatigue at the neuromuscular junction. Branch point vs. presynaptic vs. postsynaptic mechanisms. *Adv Exp Med Biol* 384: 83-100, 1995.

212. **Smilios I, Pilianidis T, Karamouzis M, and Tokmakidis SP**. Hormonal responses after various resistance exercise protocols. *Med Sci Sports Exerc* 35: 644-654, 2003.

213. **Smith GV, Alon G, Roys SR, and Gullapalli RP**. Functional MRI determination of a dose-response relationship to lower extremity neuromuscular electrical stimulation in healthy subjects. *Exp Brain Res* 150: 33-39, 2003.

214. **Snyder-Mackler L, Delitto A, Stralka SW, and Bailey SL**. Use of electrical stimulation to enhance recovery of quadriceps femoris muscle force production in patients following anterior cruciate ligament reconstruction. *Phys Ther* 74: 901-907, 1994.

215. **Snyder-Mackler L, Garrett M, and Roberts M**. A comparison of torque generating capabilities of three different electrical stimulating currents. *The Journal of orthopaedic and sports physical therapy (Baltimore)* 10: 297-301, 1989.

216. **Snyder-Mackler L, Garrett M, and Roberts M**. A comparison of torque generating capabilities of three different electrical stimulating currents. *J Orthop Sports Phys Ther* 10: 297-301, 1989.

217.   **Solomonow M**. External control of the neuromuscular system. *IEEE Trans Biomed Eng* 31: 752-763, 1984.

218.   **Spielholz NI, and Nolan MF**. Conventional TENS and the phenomena of accommodation, adaptation, habituation, and electrode polarization. *J Clin Electrophysiol* 7: 16-19, 1995.

219.   **Spriet LL, Soderlund K, Bergstrom M, and Hultman E**. Anaerobic energy release in skeletal muscle during electrical stimulation in men. *J Appl Physiol* 62: 611-615, 1987.

220.   **Stefanovska A, and Vodovnik L**. Change in muscle force following electrical stimulation. Dependence on stimulation waveform and frequency. *Scand J Rehabil Med* 17: 141-146, 1985.

221.   **Stoner HB, Barker P, Riding GS, Hazlehurst DE, Taylor L, and Marcuson RW**. Relationships between skin temperature and perfusion in the arm and leg. *Clin Physiol* 11: 27-40, 1991.

222.   **Stupka N, Tarnopolsky MA, Yardley NJ, and Phillips SM**. Cellular adaptation to repeated eccentric exercise-induced muscle damage. *J Appl Physiol* 91: 1669-1678, 2001.

223.   **Sugai N, Worsley R, and Payne JP**. Tetanic force development of adductor pollicis muscle in anesthetized man. *J Appl Physiol* 39: 714-717, 1975.

224.   **Suzuki S, Takasaki S, Ozaki T, and Kobayashi Y**. Tissue oxygenation monitor using NIR spatially resolved spectroscopy. edited by Britton C, Robert RA, and Bruce JTSPIE, 1999, p. 582-592.

225.   **Theurel J, Lepers R, Pardon L, and Maffiuletti NA**. Differences in cardiorespiratory and neuromuscular responses between voluntary and stimulated contractions of the quadriceps femoris muscle. *Respir Physiol Neurobiol* 157: 341-347, 2007.

226.   **Thornton RM, Mendel FC, and Fish DR**. Effects of electrical stimulation on edema formation in different strains of rats. *Phys Ther* 78: 386-394, 1998.

227.   **Thuma JR, Gilders R, Verdun M, and Loucks AB**. Circadian rhythm of cortisol confounds cortisol responses to exercise: implications for future research. *J Appl Physiol* 78: 1657-1664, 1995.

228.   **Tiidus PM**. Estrogen and gender effects on muscle damage, inflammation, and oxidative stress. *Can J Appl Physiol* 25: 274-287, 2000.

229.   **Tracy JE, Currier DP, and Threlkeld AJ**. Comparison of selected pulse frequencies from two different electrical stimulators on blood flow in healthy subjects. *Phys Ther* 68: 1526-1532, 1988.

230.   **Triffletti P, Litchfield PE, Clarkson PM, and Byrnes WC**. Creatine kinase and muscle soreness after repeated isometric exercise. *Med Sci Sports Exerc* 20: 242-248, 1988.

231.   **Turner JD, Rotwein P, Novakofski J, and Bechtel PJ**. Induction of mRNA for IGF-I and -II during growth hormone-stimulated muscle hypertrophy. *Am J Physiol* 255: E513-517, 1988.

232.   **Underwood FB, Kremser GL, Finstuen K, and Greathouse DG**. Increasing Involuntary Torque Production by Using TENS. *J Orthop Sports Phys Ther* 12: 101-104, 1990.

233.   **Vanderthommen M, Depresseux JC, Dauchat L, Degueldre C, Croisier JL, and Crielaard JM**. Blood flow variation in human muscle during electrically stimulated exercise bouts. *Arch Phys Med Rehabil* 83: 936-941, 2002.

234. **Vanderthommen M, and Duchateau J**. Electrical stimulation as a modality to improve performance of the neuromuscular system. *Exerc Sport Sci Rev* 35: 180-185, 2007.

235. **Vanderthommen M, Duteil S, Wary C, Raynaud JS, Leroy-Willig A, Crielaard JM, and Carlier PG**. A comparison of voluntary and electrically induced contractions by interleaved 1H- and 31P-NMRS in humans. *J Appl Physiol* 94: 1012-1024, 2003.

236. **Vanderthommen M, Gilles R, Carlier P, Ciancabilla F, Zahlan O, Sluse F, and Crielaard JM**. Human muscle energetics during voluntary and electrically induced isometric contractions as measured by 31P NMR spectroscopy. *Int J Sports Med* 20: 279-283, 1999.

237. **Vivodtzev I, Pepin JL, Vottero G, Mayer V, Porsin B, Levy P, and Wuyam B**. Improvement in quadriceps strength and dyspnea in daily tasks after 1 month of electrical stimulation in severely deconditioned and malnourished COPD. *Chest* 129: 1540-1548, 2006.

238. **Walker DC, Currier DP, and Threlkeld AJ**. Effects of high voltage pulsed electrical stimulation on blood flow. *Phys Ther* 68: 481-485, 1988.

239. **Walmsley RP**. A comparison of torque generated by knee extension with a maximal voluntary muscle contraction vis-à-vis electrical stimulation *J Orthop Sports Phys Ther* 6: 10-17, 1984.

240. **Ward AR**. Electrical stimulation using kilohertz-frequency alternating current. *Phys Ther* 89: 181-190, 2009.

241. **Ward AR, and Chuen WL**. Lowering of sensory, motor, and pain-tolerance thresholds with burst duration using kilohertz-frequency alternating current electric stimulation: part II. *Arch Phys Med Rehabil* 90: 1619-1627, 2009.

242. **Ward AR, Lucas-Toumbourou S, and McCarthy B**. A comparison of the analgesic efficacy of medium-frequency alternating current and TENS. *Physiotherapy* 95: 280-288, 2009.

243. **Ward AR, Oliver WG, and Buccella D**. Wrist extensor torque production and discomfort associated with low-frequency and burst-modulated kilohertz-frequency currents. *Phys Ther* 86: 1360-1367, 2006.

244. **Ward AR, and Robertson VJ**. Sensory, motor, and pain thresholds for stimulation with medium frequency alternating current. *Arch Phys Med Rehabil* 79: 273-278, 1998.

245. **Ward AR, and Robertson VJ**. The variation in fatigue rate with frequency using kHz frequency alternating current. *Med Eng Phys* 22: 637-646, 2000.

246. **Ward AR, and Robertson VJ**. Variation in torque production with frequency using medium frequency alternating current. *Arch Phys Med Rehabil* 79: 1399-1404, 1998.

247. **Ward AR, Robertson VJ, and Ioannou H**. The effect of duty cycle and frequency on muscle torque production using kilohertz frequency range alternating current. *Med Eng Phys* 26: 569-579, 2004.

248. **Ward AR, Robertson VJ, and Makowski RJ**. Optimal frequencies for electric stimulation using medium-frequency alternating current. *Arch Phys Med Rehabil* 83: 1024-1027, 2002.

249. **Ward AR, and Shkuratova N**. Russian electrical stimulation: the early experiments. *Phys Ther* 82: 1019-1030, 2002.

250. **Warren GL, Ingalls CP, Lowe DA, and Armstrong RB**. What mechanisms contribute to the strength loss that occurs during and in the recovery from skeletal muscle injury? *J Orthop Sports Phys Ther* 32: 58-64, 2002.

251. **Warren GL, Lowe DA, and Armstrong RB**. Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med* 27: 43-59, 1999.

252. **Webb ML, Wallace JP, Hamill C, Hodgson JL, and Mashaly MM**. Serum testosterone concentration during two hours of moderate intensity treadmill running in trained men and women. *Endocr Res* 10: 27-38, 1984.

253. **Weitzman ED, Fukushima D, Nogeire C, Roffwarg H, Gallagher TF, and Hellman L**. Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *J Clin Endocrinol Metab* 33: 14-22, 1971.

254. **Wheeler GD, Ashley EA, Harber V, Laskin JJ, Olenik LM, Sloley D, Burnham R, Steadward RD, and Cumming DC**. Hormonal responses to graded-resistance, FES-assisted strength training in spinal cord-injured. *Spinal Cord* 34: 264-267, 1996.

255. **White PF, Li S, and Chiu JW**. Electroanalgesia: its role in acute and chronic pain management. *Anesth Analg* 92: 505-513, 2001.

256. **Wilson JD**. Androgen abuse by athletes. *Endocr Rev* 9: 181-199, 1988.

257. **Zanotti E, Felicetti G, Maini M, and Fracchia C**. Peripheral muscle strength training in bed-bound patients with COPD receiving mechanical ventilation: effect of electrical stimulation. *Chest* 124: 292-296, 2003.

258. **Zorn C, Szekeres T, Keilani M, Fialka-Moser V, and Crevenna R**. Effects of neuromuscular electrical stimulation of the knee extensor muscles on muscle soreness and different serum parameters in young male athletes: preliminary data. *Br J Sports Med* 41: 914-916, 2007.

259. **Zory R, Boerio D, Jubeau M, and Maffiuletti NA**. Central and peripheral fatigue of the knee extensor muscles induced by electromyostimulation. *Int J Sports Med* 26: 847-853, 2005.

# APPENDICES

## RESEARCH ETHICS

Thursday, 20 September 2007 5:50 PM

**Dear Aldayel**

**07-146 ALDAYEL**
**Comparison between pulsed current and alternating current in electrical muscle stimulation for muscle function, muscle damage and hormonal responses**

**Student Number: 10033294**

The ECU Human Research Ethics Committee (HREC) has reviewed your application and has granted ethics approval for your research project.

The approval period is from 20 September 2007 to 31 December 2008.

The Graduate School has been informed and they will issue formal notification of approval. Please note that the submission and approval of your research proposal is a separate process to obtaining ethics approval and that no recruitment of participants and/or data collection can commence until formal notification of both ethics approval and approval of your research proposal has been received.

The National Statement indicates that the HREC is required to retain on file a copy of each approved research project. Please forward one signed paper copy of your finalised application, including all attachments, to the ethics office (if this has not already been done).

Please note the following conditions of approval:
The HREC has a requirement that all approved projects are subject to monitoring conditions. This includes completion of an annual report (for projects longer than one year) and completion of a final report at the completion of the project. An outline of the monitoring conditions and an ethics report form are attached for your information. You will also be notified when a report is due.

Please feel free to contact me if you require any further information.

Regards
Kim

Kim Gifkins
Research Ethics Officer
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**INFORMATION LETTER**

## INFORMATION LETTER TO PARTICIPANTS

### Project Title

The differences between the effects of electrical pulsed current and alternating current on muscle damage and hormonal responses.

### Purpose

The aim of this project is to compare two types of electrical stimulation currents: pulsed and alternating current.

### Overview

This study will include three electrical muscle stimulation (EMS) sessions. One week will be given between first and second session, and at least one week between the second and third session. For each session, you will be asked to report to the Exercise Physiology Laboratory (JO 19.150) for 6 consecutive days (1 day before, EMS session day and 4 days after the EMS session). During the day before EMS session, muscle strength will be measured. During the EMS session, one of your legs will receive an electrical stimulation for 15 minutes, and blood sample will be collected before, immediately after, and 15, 30, 45 and 60 minutes after EMS from your vein for hormone measurements. In addition, skin temperature, heart rate, blood pressure, muscle soreness and tenderness, Creatine kinase and muscle force will be monitored and measured. During the days after EMS session, muscle force and soreness will be measured and a drop of blood will be collected from your finger. The total time commitment will be about 5 hours for each session (15 hours for the whole experiment). To start this study, you need to go through all details and then fill out the medical questionnaire and sign the informed consent. You have been

selected to participate in the study because you are healthy and untrained male. This research has been approved by the ECU Human Research Ethics Committee.

## **Background**

Electrical stimulation is a process through which a stimulator releases several electrical impulses that travel through the skin to the underlying motor units to induce involuntary muscle contractions. These contractions are similar to voluntary ones; however, you cannot control the contraction. Electrical stimulation is clinically used to help people who have a difficulty inducing voluntarily muscle contraction, such as spinal cord injury. In addition, electrical stimulation has been widely used to increase muscle strength in healthy individuals and some athlete trainers use it as a part of their training programs. Recent research shows that electrical stimulation can significantly improve muscle function. Nevertheless, the optimal setting for electrical stimulation is still unknown. Thus, the outcomes of this study will help us to specify a better choice of electrical stimulation parameters in terms of muscle damage and hormonal responses.

## **Methods**

You are asked to participate in the study because you are healthy and untrained male. Prior to beginning study activities, you will be asked to complete a general medical questionnaire with questions pertaining to lifestyle (e.g. exercise history, medications, etc). As a participant, you will attend one day before the electrical stimulation session, the day of electrical stimulation, and every day for four days after the experiment. This will be repeated three times over a 5 – 8 week period.  You will involve three separate electrical stimulation sessions. In the first session, a specific current will be applied to one leg and in the second session the same current will be applied to the same leg. One week will be allowed between these sessions. The other current will be applied to the other

leg during the third week. One week at least will be allowed after the ending of session two. This design will enable us to determine the influence of each current on muscle damage and hormonal responses. Also, it will show us the effect of a second bout on these variables.

## Measurements

You will need to attend a familiarization session several days prior the experiment commencement. During this session, you will be familiarized with electrical muscle stimulation and muscle force and soreness measurements.

As mentioned, you will be involved in three electrical stimulation sessions, for each one you will come to the laboratory six times in six continuous days as below.

**The day before (30 min):** The maximal isometric and dynamic strength will be measured. You will set on a isokinetic dynamometer (Biodex) and the maximal isometric strength will be measured at three angles (100º, 70 º and 40 º) of your full knee extension. After a few minutes rest, your isokinetic force will be measured at three angular velocities; 30°•sec-1, 180°•sec-1, 300°•sec-1. The range of motion will be 60° starting from 100° of full knee extension. It will take about 15 minutes to complete this testing.

**The day of electrical stimulation(~3 hours):** During the day prior to this session, you will be asked to avoid consuming alcohol and performing any sport training. All measurements on this day will be obtained during the morning as follows:

*Before exercise (60 minutes):*

- You will sit on the stimulation chair.

- You will have three electrodes placed in various part of your thigh.
- You will have four thermistors (temperature devices) attached to the skin on different part of your thigh.
- You will have a cannula inserted into a vein of your arm and fixed for drawing blood samples.
- 3 mL of blood (less than one teaspoon) will be obtained.
- A drop of blood will be taken from a finger via skin puncture device.
- You will rank the pain on a visual analogue scale.
- You will report your pain threshold when a pressure from an algometer is applied.
- Your maximal tolerance of the electrical stimulation intensity will be determined by 2-6 contractions.

*During exercise (15 minutes):*

- Electrical stimulation will be applied to one thigh for 17.5 minutes and 40 contractions will be performed.
- Skin temperature, heart rate and blood pressure will be monitored continuously.
- Force production of each contraction will be recoded.

*After exercise (100 minutes):*

- 3 mL blood sample from the vein and a drop blood sample from the finger will be obtained at five time points: immediately after, 15, 30, 45 and 60 minutes.
- You will rank the pain on a visual analogue scale.
- You will state your pain threshold when a pressure applied from the algometer is applied.
- Skin temperature will be monitored for 15 minutes.
- At the end, muscle force will be measured.

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***The days after exercise (40 min):*** you will come every day to the laboratory for four days after the experiment for about 40 minutes to perform the following measurements:

- A drop of blood from your finger.
- The maximal isometric and dynamic strength.
- The pain on a visual analogue scale
- The pain threshold from the algometer.

You will need to avoid any physical and sport training during the whole session (6 days) except the usual daily activities. All these measurements will be repeated three times as mentioned.

## Risks

You may experience some mild discomfort with the electrical stimulation. There may also be some discomfort with having the blood sample taken. Muscle soreness could be felt after exercise and it will disappear within 2-3 days.

## Benefits

Participation in the study will give you an opportunity to have several sessions of electrical stimulation exercise and experience this kind of training. Also, you will get an indication of your level of muscle strength. All study activities are provided at no cost to the participants.

## Confidentiality

Your results will be kept as confidential as is possible by law. All data will be kept in the possession of the investigators. If the results of the study are

published in a scientific journal, your identity will not be revealed. Participants will not be referred to by name during research reports or study discussions. All records will be stored in a locked filing cabinet with restricted access for a minimum of five years in a private office. All computer records are restricted by password.


**<u>Contacting the Investigators</u>**

We are happy to answer any questions you may have at this time. If you have any queries later, please do not hesitate to contact:

Abdulaziz Aldayel at (08) 6304 5152, email <u>a.aldayel@ecu.edu.au</u>

Dr Mike McGuigan at (08) 6304 2118, email <u>m.mcguigan@ecu.edu.au</u>

Associate Professor Ken Nosaka at (08) 6304 5655, email <u>k.nosaka@ecu.edu.au</u>


If you have any concerns or complaints about the research project and wish to talk to an independent person, you may contact:

Research Ethics Officer

Human Research Ethics Officer

Edith Cowan University

100 Joondalup Drive

JOONDALUP WA 6027

Phone: (08) 6304 2170

Email: <u>research.ethics@ecu.edu.au</u>


**<u>Feedback</u>**

All participants will be provided with test results as soon as they are available. A summary of study results will be made available to all interested participants upon completion of the trial.

## Voluntary Participation

Whether you decide to participate in the study or not, your decision will not prejudice you in any way. If you do decide to participate, you are free to withdraw your consent and discontinue your involvement at any time.

## Privacy statement

The conduct of this research involves the collection, access and / or use of your identified personal information. The information collected is confidential and will not be disclosed to third parties without your consent, except to meet government, legal or other regulatory authority requirements. A de-identified copy of this data may be used for other research purposes. However, your anonymity will at all times be safeguarded.

**INFORMED CONSENT FORM**

<u>Project Title</u>

**The differences between the effects of electrical pulsed current and alternating current on muscle damage and hormonal responses**

I have read the information letter and the consent form. I agree to participate in the study entitled 'The differences between the effects of electrical pulsed current and alternating current on muscle damage and hormonal responses' and give my consent freely. I understand that the study will be carried out as described in the information letter, a copy of which I have retained. I recognize that I will receive electrical stimulation to my muscles and that this may cause discomfort and that blood samples will be collected from my vein and fingers throughout the research. I am aware the study consists of three sessions, each session contains 6 consecutive days as described in the information letter. I realise that whether or not I decide to participate is my decision. I also realise that I can withdraw from the study at any time and that I do not have to give any reasons for withdrawing. I have had all questions answered to my satisfaction.

Date: _____

Participant: _____

# MEDICAL QUESTIONNAIRE

The following questionnaire is designed to establish a background of your medical history, and identify any injury and or illness that may influence your testing and performance.

Please answer all questions as accurately as possible and if you are unsure about anything please ask for clarification. All information provided is strictly confidential. If you answer YES to any non-exercise related question that may contraindicate you from completing this study a clearance from a qualified medical practitioner will be required prior to commencement of any exercising or testing.

**Personal Details**
Name: _____     Subject No.: _____

**Medical History**
Have you ever suffered, or do you currently suffering any of the following: Tick if YES

| | | | |
|---|---|---|---|
| Heart disease/abnormalities | ☐ | High or abnormal blood pressure | ☐ |
| Angina severe headaches | ☐ | High cholesterol | ☐ |
| Epilepsy | ☐ | Rheumatic fever | ☐ |
| Asthma or bronchitis | ☐ | Recurring back/knee or neck pain | ☐ |
| Back, knee or other joint | ☐ | Any injury/breaks in muscle or | ☐ |

problems                              joint

| | | | |
|---|---|---|---|
| Diabetes | | Severe allergies | |
| Infectious diseases | | Any neurological disorders | |
| Any infectious diseases | | Any neuromuscular disorders | |
| Are you on any medications? | | | |

| If the answer to any of these questions is YES, please give details here: |
|---|
| |

| Is there any other condition not previously mentioned which may affect your knee exercise? |
|---|
| |

## Lifestyle Habits

| Questions | Yes |
|---|---|
| Have you participated in resistance training in the last six months? | |
| Do you exercise regularly?<br><br>If YES, what do you do? | |

| | |
|---|---|
| Do you smoke tobacco? <br><br> If YES, how much per day? | |

| | |
|---|---|
| Do you consume alcohol? <br><br> If YES, how much per week? | |

| | |
|---|---|
| Do you consume tea or coffee? <br><br> If YES, how many cups per day? | |

**Declaration**

I acknowledge that the information provided on this form is to the best of my knowledge, a true and accurate indication of my current state of health.

*Participant* Name: _____

Date: _____

# PUBLICATIONS FROM THESIS

First study:

Aldayel, A., Jubeau, M., McGuigan, M., & Nosaka, K. (2010). Comparison between alternating and pulsed current electrical muscle stimulation for muscle and systemic acute responses. *J Appl Physiol, 109*(3), 735-744. doi: japplphysiol.00189.2010 [pii]10.1152/japplphysiol.00189.2010

Second study:

Aldayel, A., Muthalib, M., Jubeau, M., McGuigan, M., & Nosaka, K. (2010). Muscle oxygenation of vastus lateralis and medialis muscles during alternating and pulsed current electrical stimulation. *Eur J Appl Physiol*. doi: 10.1007/s00421-010-1699-9

Third study:

Aldayel, A., Jubeau, M., McGuigan, M. R., & Nosaka, K. (2010). Less indication of muscle damage in the second than initial electrical muscle stimulation bout consisting of isometric contractions of the knee extensors. *Eur J Appl Physiol, 108*(4), 709-717. doi: 10.1007/s00421-009-1278-0

# Chapter 3 (Study 1)

# Comparison between alternating and pulsed current electrical muscle stimulation for muscle and systemic acute responses

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[1]*Edith Cowan University, Joondalup, Australia;* [2]*King Saud University, Riyadh, Saudi Arabia;* [3]*Université de Lyon, Saint-Etienne, France, and Exercise Physiology Laboratory, Jean Monnet University, Saint-Etienne, France;* [4]*New Zealand Academy of Sport North Island, Auckland;* and [5]*Auckland University of Technology, Auckland, New Zealand*

Aldayel A, Jubeau M, McGuigan M, Nosaka K. Comparison between alternating and pulsed current electrical muscle stimulation for muscle and systemic acute responses. *J Appl Physiol* 109: 735–744, 2010. First published July 1, 2010; doi:10.1152/japplphysiol.00189.2010.—This study compared alternating and pulsed current electrical muscle stimulation (EMS) for torque output, skin temperature ($T_{sk}$), blood lactate and hormonal responses, and skeletal muscle damage markers. Twelve healthy men (23–48 yr) received alternating current EMS (2.5 kHz delivered at 75 Hz, 400 μs) for the knee extensors of one leg and pulsed current (75 Hz, 400 μs) for the other leg to induce 40 isometric contractions (on-off ratio 5–15 s) at the knee joint angle of 100° (0°: full extension). The use of the legs for each condition was counterbalanced among subjects, and the two EMS bouts were separated by 2 wk. The current amplitude was consistently increased to maximally tolerable level, and the torque and perceived intensity were recorded over 40 isometric contractions. $T_{sk}$ of the stimulated and contralateral knee extensors were measured before, during, and for 30 min after EMS. Blood lactate, growth hormone, testosterone, insulin-like growth factor 1, testosterone, and cortisol were measured before, during, and for 45 min following EMS. Muscle damage markers included maximal voluntary isometric contraction torque, muscle soreness with a 100-mm visual analog scale, and plasma creatine kinase (CK) activity, which were measured before and 1, 24, 48, 72, and 96 h after EMS. No significant differences in the torque induced during stimulation (~30% maximal voluntary isometric contraction) and perceived intensity were found, and changes in $T_{sk}$, blood lactate, and hormones were not significantly different between conditions. However, all of the measures showed significant ($P < 0.05$) changes from baseline values. Skeletal muscle damage was evidenced by prolonged strength loss, development of muscle soreness, and increases in plasma CK activity; however, the changes in the variables were not significantly different between conditions. It is concluded that acute effects of alternating and pulsed current EMS on the stimulated muscles are similar.

neuromuscular electrical stimulation; Russian current; skin temperature; blood lactate; muscle soreness; creatine kinase; maximal voluntary isometric contraction; growth hormone; IGF-1; testosterone; cortisol

ELECTRICAL MUSCLE STIMULATION (EMS) applies electrical current transcutaneously to muscles through electrodes to induce involuntary contractions. There are two types of currents commonly used in EMS: alternating current and pulsed current. Pulsed current EMS delivers intermittent pulses at generally 1–150 Hz (47). In contrast, alternating current EMS consists of a continuous series of alternating biphasic high-frequency pulses (e.g., 1–25 kHz) generally modulated and delivered

within the biological range, normally between 10 and 150 Hz (47). Clinical use of alternating current EMS includes pain control, spasticity management, prevention of atrophy, and edema control (46). However, it is also used in muscle training since a Russian scientist Kots advocated so called "Russian current" (2.5-kHz sinusoidal pulse stimulation) for its efficacy in increasing muscle strength in 1977 (for review see 48). Although the use of alternating current EMS has been increasing (48), pulsed current EMS is more widely used in sports training and rehabilitation, since most of the portable or battery-operated stimulators can deliver only pulsed current (24).

In alternating current EMS, the stimulus is delivered in bursts, where each burst consists of many pulses. As shown in Fig. 1, the number of bursts per second in the modulated alternating current EMS is the same as the number of pulses per second in the pulsed current EMS; however, each stimulus consists of greater number of pulses in the alternating current than the pulsed current EMS. It is possible that the distinct difference in the number of pulses delivered to muscle in stimulation produces different physiological responses between alternating and pulsed current EMS. In fact, some studies (22, 38) reported a difference in muscle force generation between alternating and pulsed current EMS. However, systematical comparisons between the two waveforms for acute muscle and systemic responses are lacking.

Snyder-Mackler et al. (37) compared the torque generation of the knee extensors among three different waveforms; 2.5-kHz alternating current and 4-kHz alternating current delivered at 50 Hz, and 50-Hz pulsed current. They reported that the torque generation was significantly lower in the 4-kHz alternating current compared with others; however, no significant difference in torque output was evident between the 2.5-kHz alternating current and the 50-Hz pulsed current. Another study compared the quadriceps fermoris torque generation during 2.5-kHz alternating current delivered at 50 Hz and 50-Hz pulsed current, and showed that the torque was significantly higher for the pulsed current than alternating current (22). In contrast, no difference was found in isometric torque of the knee extensors between 2.5-kHz alternating current delivered at 50 Hz and 30-Hz pulsed current (13) and between 2.5-kHz alternating current delivered at 95 bursts/s and 95-Hz pulsed current (15). Other studies also reported similar force generation by 2.5-kHz alternating current delivered at 75 Hz and 75-Hz pulsed current (24), or by 2.5-kHz alternating current delivered at 50 Hz and 50-Hz pulsed current (21). It does not appear that the information regarding the difference in the muscle force generation between alternating and pulsed current EMS is consistent. To the best of our knowledge, no previous study has compared the torque output between pulsed current
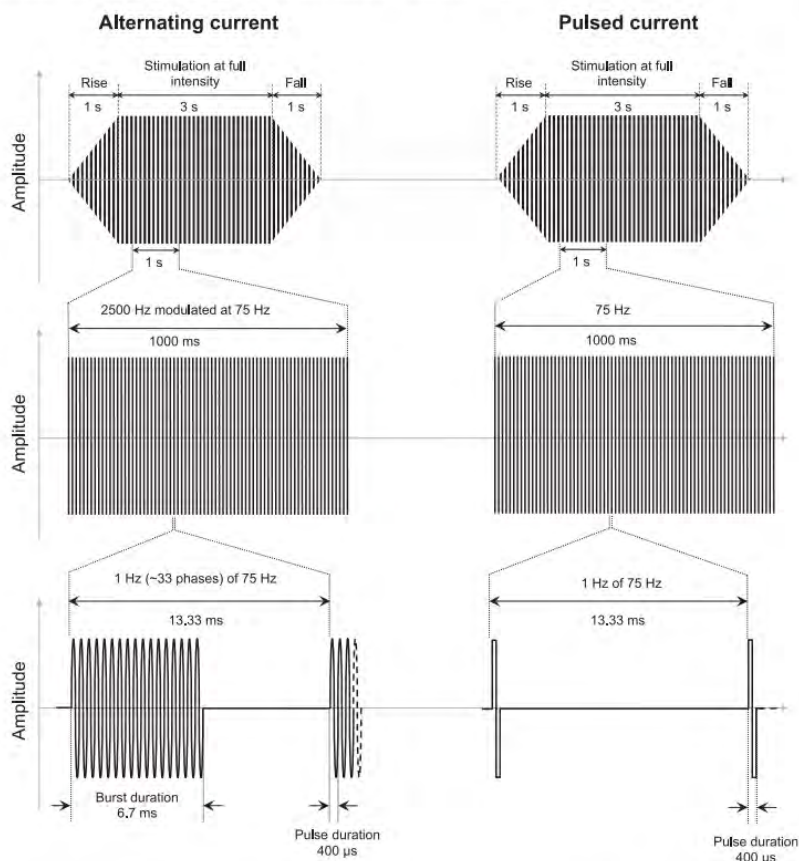
Fig. 1. Schematic diagram of alternating current and pulsed current electrical muscle stimulation (EMS) for 2 waveforms used in the present study. The alternating current was delivered at 2,500 Hz and modulated at 75 bursts/s, which results in 33 pulses in each burst, while the pulsed current was delivered at 75 Hz, and therefore only one pulse in each cycle. The pulse duration was 400 μs for both currents.

and alternating current by keeping all parameters as similar as possible except the waveform, and using the same stimulator. It is possible that a difference exists between the two current types for perceived exertion during EMS. It may be that the effect of EMS on skin temperature is associated with discomfort, and changes in skin temperature and discomfort are different between alternating current and pulsed current EMS. However, these have not been investigated in the previous studies.

Several studies have demonstrated that skeletal muscle damage is induced by isometric contractions evoked by EMS (16, 25, 28, 29). For example, Jubeau et al. (16) showed that EMS (75-Hz pulsed current) resulted in decreases in maximal voluntary isometric contraction torque, delayed onset muscle soreness (DOMS), and increases in serum creatine kinase (CK) activity. Mackey et al. (25) have reported histological damage in muscle fibers after EMS (60-Hz pulsed current). It should be noted that all of these studies used pulsed current EMS, and no previous studies have investigated skeletal muscle damage induced by alternating current EMS.

In the study by Jubeau et al. (16), they also reported that increases in blood lactate and growth hormone (GH) were significantly greater for EMS compared with the voluntary isometric contractions of the same force output. Sartorio et al.

(34) compared the first and second EMS bouts (75-Hz pulsed current with pulse duration of 400 μs) consisting of 20 isometric contractions of the quadriceps femoris and showed significant increases in GH following EMS and a significant decrease in cortisol at 60 min after the first EMS. To understand the effect of EMS on hormonal responses better, other anabolic hormones (e.g., testosterone, IGF-1) that are often investigated in resistance exercise (19) should be investigated. No previous study has compared alternating current and pulsed current EMS for changes in hormones (GH, testosterone, IGF-1, and cortisol) and blood lactate.

Therefore, the purpose of this study was to compare alternating current and pulsed current during a typical EMS strength training session (20, 45) for torque generation, perception and skin temperature, symptoms of skeletal muscle damage, and hormonal responses. To compare pulsed current and alternating EMS, the present study set the stimulation parameters similarly between the two stimulation conditions. Based on the previous studies (12, 27, 40), 75 Hz was chosen for the frequency of the pulsed current EMS, and 2.5 kHz was chosen for alternating current (32, 36, 37). It was hypothesized that significant differences would be evident between alternating and pulsed current EMS for torque output, skin temperature, skeletal muscle damage, and hormonal responses.

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## METHODS

### Study Design

Twelve volunteers participated in two EMS sessions separated by 2 wk: one for pulsed current session and the other for alternating current session in a randomized, counterbalanced order. The 2-wk interval between bouts was set to provide a time for elevated plasma CK activity after the first EMS session to return to baseline value. The subjects did not receive any information which current they were receiving in the EMS sessions. A familiarization session was conducted before the study, which included maximal voluntary isometric contraction strength (MVC) measures at different knee joint angles (40°, 70°, 100°), and EMS that included three to five electrically evoked isometric contractions at submaximal intensity. For each EMS session, the subjects were asked to report to the laboratory for 6 days: baseline measure session held 1–3 days before EMS session, EMS session day, and 4 consecutive days following the EMS session (Fig. 2). Forty isometric contractions of the knee extensors of one leg were evoked in each EMS exercise, and one leg received pulsed current EMS, and the other leg had alternating current EMS. Both EMS sessions were performed at the same time of the day for each subject between 8 AM and 10 AM to eliminate the effects of possible diurnal variations on hormonal responses and other measures. The independent variable in this study was the waveform used in EMS (alternating current vs. pulsed current), and the dependent variables consisted of knee extensors' torque and rate of perceived exertion during EMS, skin temperature, blood lactate concentration, growth hormone, testosterone, IGF-1, and cortisol concentrations, and indirect markers of muscle damage such as MVC of the knee extensors, muscle soreness, pressure pain threshold, and plasma CK activity. The study was approved by the Edith Cowan University Human Research Ethics Committee.

### Subjects

Twelve healthy men (mean ± SD age: 31.2 ± 5.5 yr, body mass: 81.4 ± 15.2 kg, height: 174.3 ± 4.8 cm), who had not been involved in resistance training program for at least 6 mo before the study and not had an injury in their knee joints, participated in this study after signing the informed consent form. During the experimental period, subjects were asked not to change their diet habits and not to take any medicines nor have any interventions other than those given in the study. Subjects were requested to avoid consuming caffeine and alcohol 1 day before the EMS session and not to undertake any physical activity during the experimental period. Female subjects were excluded to avoid possible sex difference in hormonal responses (4, 18, 51) and muscle damage (6, 43), and to make the variability of

the criterion measures as small as possible to increase the statistical power.

### EMS

Each subject seated on a Biodex isokinetic dynamometer chair (Biodex Medical Systems) with his knee joint angle of 100° (0° corresponding to the full extension) and the trunk angle of 110°. Straps secured the pelvis and chest to minimize the movements of the hip and trunk during contractions. An Intelect Advanced Colour Stim (Chattanooga Group, TN) was used to stimulate the quadriceps femoris muscles. Four self-adhesive electrodes were placed on the anterior surface of one thigh as follows: two positive electrodes (50 × 50 mm) over the motor point of the vastus lateralis and vastus medialis muscles, and two negative electrodes (50 × 100 mm) placed on the proximal portion of the quadriceps femoris muscle based on a previous study (16). The placement of electrodes was similar between the two EMS sessions.

As shown in Fig. 1, the waveform of pulsed current was biphasic symmetrical rectangular, and balanced stimulus pulses were delivered with frequency of 75 Hz and pulse duration of 400 μs (26, 41). The EMS parameters for alternating current were adjusted at 2.5-kHz alternating sinusoidal current (pulse duration = 400 μs) and delivered in bursts with a carrier frequency of 75 Hz and the bursts duration of 6.5 ms based on previous studies (24, 33, 48). The other stimulation parameters were the same between the alternating and pulsed current EMS (Table 1). For both currents, the ratio was 5 s stimulus on time and 15 s stimulus off time, so the duty cycle time was 25%. The ramping time was included in the stimulation time (on-time) such as 1 s for the rise time and 1 s for the fall time. Current intensity started from 0 mA and was increased rapidly to muscle contraction threshold of each subject every four to five contractions by a 3- to 6-mA increment (Fig. 3A). The settings were to achieve the maximum possible force output of each subject in the EMS. The EMS session consisted of a total of 45 isometric contractions of the extensor muscles for 15 min; however, only the last 40 contractions were used for further analysis, since the intensity of the first five contractions was generally very low. The isometric torque of each contraction was recorded by the isokinetic dynamometer.

### Dependent Variables

*Knee flexion torque during EMS.* Each contraction torque induced by EMS was measured and recorded over 40 isometric contractions using a Biodex isokinetic dynamometer software and saved for later analysis to determine torque (peak torque, average torque) and torque time integral of each contraction. The average torque during the EMS-evoked contraction excluding the ramp time and torque time
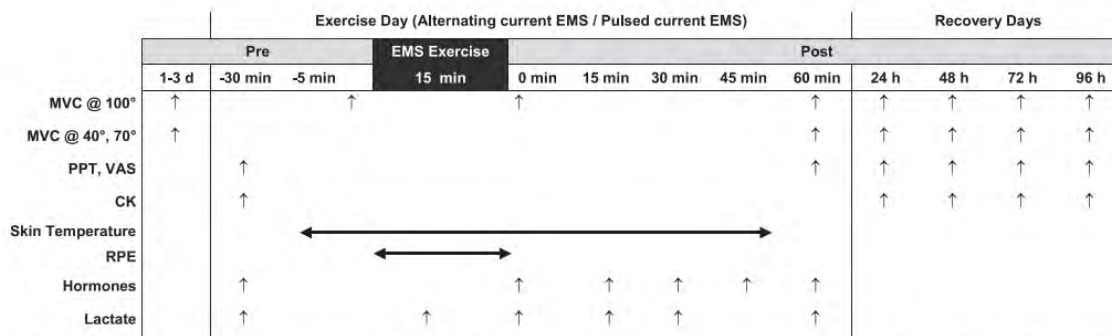


Fig. 2. Diagram showing the measurement time line. MVC, maximal voluntary contraction at different knee joint angles (40°, 70° and 100°); PPT, pressure pain threshold; VAS, visual analog scale for muscle soreness assessment; CK, plasma creatine kinase activity; RPE, rating of perceived exertion.

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Table 1. *Stimulation parameters for alternating current and pulsed current electrical muscle stimulation*

| | AC | PC |
|---|---|---|
| Pulse frequency | 2.5 kHz delivered at 75 Hz | 75 Hz |
| Pulse duration | 400 μs | 400 μs |
| Burst duration | 6.7 ms | Not applicable |
| Stimulus on time | 5 s (rise time 1 s/fall time 1 s) | 5 s (rise time 1 s/fall time 1 s) |
| Stimulus off time | 15 s | 15 s |
| Cycle time | 13.3 ms | 13.3 ms |
| Duty cycle (on-off time) | 25 % (5 s /15 s) | 25 % (5 s/15 s) |
| Pulse duty cycle | Not applicable | 3 % |
| Burst duty cycle | 50 % | Not applicable |
| Waveform | Alternating sinusoidal | Biphasic, symmetrical rectangular |
| Stimulus intensity | Maximal tolerance | Maximal tolerance |

AC, alternating current; PC, pulsed current.

integral of each contraction were calculated using Chart data analysis software (ADInstruments, Bella Vista, Australia).

*Rating of perceived exertion.* The rating of perceived exertion (RPE) during the EMS was assessed by a Borg's standard 6–20 scale at every six contractions.

*Skin temperature.* Skin temperature ($T_{sk}$) was recorded continuously before, during, and for 30 min after EMS. Four skin thermistors (YSI Temperature, 400 series; Dayton, OH) were placed on the skin using fixomull tape near the positive electrodes (vastus medialis and vastus lateralis), mid thigh of the stimulated leg, and the mid thigh of nonstimulated leg. Temperature was recorded by a data-logger set (Squirrel SQ series, Grant Instruments) at 1 Hz with 1-min intervals starting from 5 min preexercise to 15 min post-EMS. The temperature was obtained at 5-min intervals from the recorded data for further analysis.

*Blood lactate concentration.* A 5-μl sample of blood was obtained by finger prick and loaded to a test strip of Lactate Pro Analyzer (Arkray, Kyoto, Japan) to determine lactate concentration. The measurement was taken before, during the middle of EMS session (after the 20th contraction), and immediately after and 15, 30, and 60 min post-EMS exercise.

*Serum hormone concentration.* A 21-gauge Teflon cannula (Becton Dickinson, Franklin Lakes, NJ) was inserted to a superficial forearm vein, and an extension tube fitted with a two-way stopcock was attached to the cannula. Blood samples were obtained into disposable syringes before, immediately after, and 15, 30, 45, and 60 min following EMS and put into plain 3-ml Vacutainer tubes (Becton Dickinson). The tubes were left at room temperature for 20 min to clot and centrifuged at 3,000 rpm for 10 min at 4°C. The serum samples were frozen and stored at −80°C for later analyses of growth hormone (GH), total testosterone, cortisol, and IGF-1 using enzyme-linked immunosorbent assay with test kits (GH: DSL-10-1900; total testosterone: DSL-10-4000, cortisol: DSL-10-2000, IGF-1: DSL-10-9400, Diagnostic Systems Laboratories, Webster, TX). Each sample was duplicated in the measures, and the average value of the two values was used for further analysis. The hormone concentrations were determined using a multilabel counter set to 450 nm (VersaMax, Molecular Devices, Sunnyville, CA). The coefficient of variation (CV) for GH, total testosterone, cortisol, and IGF-1 in the present study were 6.5, 4.8, 8.0, and 8.8%, respectively.

*Maximal voluntary isometric contraction torque.* Subjects sat on the Biodex isokinetic dynamometer's chair in the same setting as the EMS protocol, the rotation axis of the knee of the tested leg was aligned with the rotation axis of the dynamometer's armature, and the ankle cuff was joined ~1 cm proximal to the medial malleolus. Gravity corrections were made at 10° of knee flexion. Subjects were asked to keep both arms positioned across the chest with each hand clasping the opposite shoulder. MVC was measured at three different knee joint angles, 40°, 70°, and 100°, at 1–3 days before and 1, 24, 48, 72, and 96 h after EMS exercise. At

immediately before and after EMS exercise, MVC was assessed at 100° only to minimize the influence of maximal voluntary isometric contractions on hormones, and the angle (100°) was chosen because it was the angle that the isometric contractions were evoked by EMS. No significant difference in MVC measured at 100° was found between the measures taken 1–3 days before and immediately before the EMS exercise. The reliability of the MVC measure at 100° indicated by CV based on the two baseline measures taken 1–3 days before and immediately before the EMS session was 5.8%. Three maximal voluntary isometric contractions for 3 s with a 30-s rest between attempts were performed for each angle, and a 60-s rest was given between different angles. Strong verbal encouragement was given to the subjects during each trial. The peak torque from three contractions for each angle was used for later analysis. The stimulated leg was always measured first followed by the control leg, and the time lag between the legs was ~10 min.

*Muscle soreness.* Subjects were asked to rate their pain of the knee extensors on a 100-mm visual analog scale (VAS) with 0 representing "no pain" and 100 as "unbearable pain" while the subjects were asked to squat. Subjects were asked to stand with their legs shoulder width apart and bent each knee slowly to a 90° angle, then returned to the initial position.

*Pressure pain threshold.* Pressure pain threshold (PPT) was assessed using an electronic algometer (Type II, Somedic Production AB, Sollentuna, Sweden). The algometer was calibrated for each occasion, and the same investigator took all measurements. The probe head (surface area = 1.0 cm²) of the algometer was placed perpendicular to four sites of the quadriceps femoris muscle used for the palpation soreness measures that were clearly marked by a water-proof ink pen. Force was gradually applied at a constant rate of 50–60 kPa/s until the subject reported the first feeling of noticeable pain. Three measurements were taken from each site sequentially with a minimum of 30-s interval and 1 min between different sites (23) in the following order: the middle point of the rectus femoris, the proximal points of rectus femoris, vastus medialis, and vastus lateralis. The value in kilopascals (kPa) corresponding to the amount of force applied was recorded. The mean of the three measurements for each site was used for further analysis, and the values of all sites were averaged to assess the tenderness in quadriceps muscle.

*Plasma CK activity.* Blood samples for CK were taken from a fingertip using a heparinized capillary (30 μl) and loaded onto a test strip. CK activity was assessed using a Reflotron spectrophotometer (Boehringer-Mannheim, Pode, Czech Republic) in duplicate, and if the difference of the two values was greater than 10%, additional measurements were taken. In this method, the normal reference ranges for adult men are 20–220 IU/l according to the instruction sheet of the test kit (Reflotron CK, Roche Diagnostics).
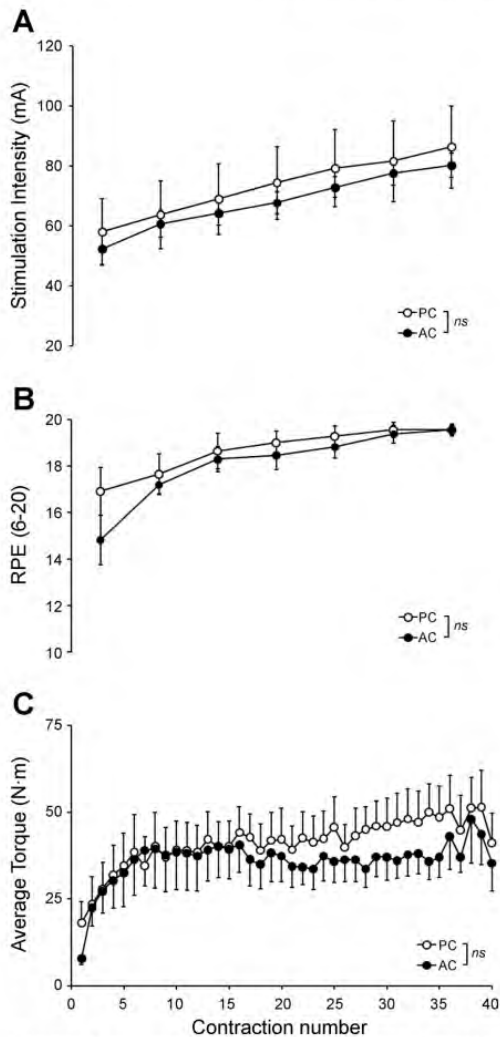
Fig. 3. Changes in stimulation intensity (*A*), RPE (*B*), and peak torque (*C*) over 40 isometric contractions during alternating current (AC) and pulsed current (PC) EMS. ns, no significant difference between currents for the changes over time.

## Statistical Analysis

Changes in stimulation intensity (amplitude), RPE, and torque over 40 contractions and $T_{sk}$, blood lactate, and hormones before, during, and after EMS were compared between the current and pulsed EMS using a two-way repeated-measures ANOVA. A two-way repeated-measures ANOVA also compared the changes in MVC, VAS of muscle soreness, pressure pain threshold, and plasma CK activity before and after EMS between the two stimulation conditions. When the ANOVA showed a significant interaction effect, a Tukey's post hoc test was followed to compare between the stimulation conditions for each time point. The statistical analyses were performed using a Statistical Package for Social Sciences (version 18.0; SPSS). All data are expressed as means ± SD, and a significance level was set at $P < 0.05$.

## RESULTS

### Stimulation Intensity, RPE, and Torque Generated During EMS

Changes in stimulation intensity (Fig. 3*A*) and RPE (Fig. 3*B*) over 40 contractions are not significantly different between the alternating and pulsed EMS. Stimulation intensity gradually increased throughout the EMS for both currents similarly. RPE increased in the first 20 contractions and reached to a nearly maximum value after the 25th contraction, without a significant difference between the waveforms. As shown in Fig. 3*C*, no significant difference between the waveforms was evident for the torque output over 40 contractions. The torque increased in the first 10–15 contractions in both currents, but no further increases were seen after that despite the increases in the intensity. The averaged torque evoked by EMS was 54 ± 24 N·m for alternating current and 62 ± 28 N·m for pulsed current with no significant difference between the currents. The level of the torque output during EMS relative to the MVC at 100° was 28.4 ± 4.4% for the alternating current EMS and 31.8 ± 3.7% for the pulsed current EMS, without significant difference between the waveforms.

### Skin Temperature

Changes in $T_{sk}$ were similar among the three sites on the stimulated leg; thus the data of the midthigh of the simulated leg are shown in Fig. 4. It also includes $T_{sk}$ of the midthigh of the nonstimulated leg. $T_{sk}$ increased significantly from the baseline after 10 min of EMS for the stimulated leg while the control leg showed significant decreases. No significant difference was evident between the waveforms.

### Blood Lactate and Hormones

Table 2 shows changes in blood lactate and serum hormone concentrations. Blood lactate increased significantly during and immediately after EMS and returned to baseline values within 15 min postexercise. No significant difference in the changes in blood lactate concentration over time was evident



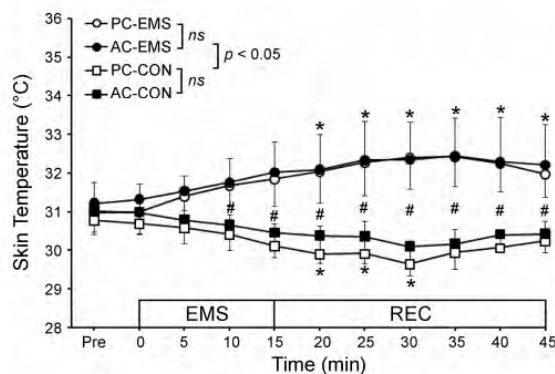Fig. 4. Changes in skin temperature of the electrically stimulated (EMS) and control (CON) leg before (Pre), during (EMS), and for 30 min after EMS (REC) for alternating current (AC) and pulsed current (PC) electrical stimulation. ns, no significant difference between currents for the changes over time. *Significant ($P < 0.05$) change from baseline. #Significant ($P < 0.05$) difference between legs.

Table 2. *Changes in blood lactate, and serum GH, testosterone, IGF-1, and cortisol concentrations before (Pre), in the middle of electrical muscle stimulation after 20th contraction (EMS), immediately after (0), and 15, 30, 45 and 60 min following alternating current and pulsed current electrical muscle stimulation*

| Hormones | Current | Pre | EMS | 0 min | 15 min | 30 min | 45 min | 60 min |
|---|---|---|---|---|---|---|---|---|
| Lactate, mmol/l | AC | 1.5 ± 0.1 | 2.1 ± 0.1* | 2.9 ± 0.2* | 1.7 ± 0.2 | 1.4 ± 0.1 | - | 1.1 ± 0.1 |
| | PC | 1.8 ± 0.1 | 2.6 ± 0.2* | 3.4 ± 0.3* | 1.7 ± 0.1 | 1.5 ± 0.1 | - | 1.3 ± 0.1 |
| GH, ng/ml | AC | 0.8 ± 0.3 | | 3.1 ± 1.0* | 3.5 ± 0.8* | 2.9 ± 0.7* | 2.3 ± 0.5 | 1.8 ± 0.4 |
| | PC | 0.8 ± 0.2 | | 2.3 ± 0.7 | 3.1 ± 0.7* | 2.5 ± 0.7* | 1.9 ± 0.5 | 1.4 ± 0.4 |
| Testosterone, pg/ml | AC | 19.8 ± 1.6 | | 28.2 ± 3.9* | 28.1 ± 3.0* | 27.5 ± 2.8* | 25.8 ± 2.4 | 21.3 ± 2.3 |
| | PC | 18.4 ± 1.9 | | 28.4 ± 3.6* | 28.5 ± 2.9* | 26.1 ± 3.5 | 23.3 ± 2.5 | 19.7 ± 2.1 |
| IGF-1, nmol/l | AC | 34.4 ± 3.9 | | 33.3 ± 5.7 | 32.1 ± 4.7 | 31.2 ± 3.4 | 32.8 ± 3.5 | 28.9 ± 3.6 |
| | PC | 31.8 ± 4.8 | | 32.9 ± 4.8 | 33.2 ± 6.3 | 33.6 ± 4.7 | 34.4 ± 5.2 | 32.5 ± 5.9 |
| Cortisol, nmol/l | AC | 658.3 ± 28.4 | | 614.6 ± 29.5 | 599.6 ± 37.5 | 592.7 ± 46.4 | 542.8 ± 43.8* | 562.1 ± 5.8* |
| | PC | 692.3 ± 26.4 | | 659.6 ± 30.3 | 631.6 ± 33.9 | 591.1 ± 41.7 | 553.8 ± 44.5* | 575.2 ± 5.2* |

Values are mean changes ± SD. GH, growth hormone. *Significantly different from Pre value.

between pulsed current and alternating current. No significant differences in the changes in any hormones were evident between the waveforms. Serum GH increased more than 400% after EMS and peaked at 15 min postexercise in both currents. Serum testosterone also significantly increased ~150% immediately and 15 min after EMS. Serum cortisol decreased significantly from baseline to 60 min post-EMS. Serum IGF-1 did not change significantly over time.

### Muscle Damage Markers

MVC was not significantly different between currents before EMS for both legs for all angles (Fig. 5). The MVC of the control leg did not change significantly over time following EMS. MVC decreased significantly below the baseline immediately after alternating current EMS and pulsed current EMS by 23.1 ± 4.2% and 26.1 ± 2.8%, respectively, without significant difference between the waveforms. No significant differences in the recovery of MVC were evident between the alternating and pulsed current EMS for all angles. The recovery of MVC at 40° and 70° was significantly faster than that at 100° and returned to the baseline values within 24 h; however, MVC at 100° remained lower than baseline for all time points following EMS.

Muscle soreness peaked 48 h post-EMS for both currents (Fig. 6A), and no significant differences in muscle soreness were evident between the waveforms. The four locations of PPT measurements showed similar values, and the changes were not significantly different among the sites; thus changes

in the mean PPT value of the four sites are shown in Fig. 6B. A decrease in PPT indicates that muscles became more tender after EMS, but no significant difference between the conditions was found. Plasma CK increased significantly after both currents, and peak value was 1,262 ± 339 IU/l for alternating current, and 1,677 ± 491 IU/l for pulsed current, without significant difference in the changes between the two conditions (Fig. 6C).

### DISCUSSION

The present study showed that no significant differences existed between the alternating current and pulsed current for *1*) torque generation and RPE during EMS, *2*) changes in skin temperature, blood lactate, and hormones before, during, and after EMS, and *3*) changes in markers of muscle damage following EMS. Additionally, this is the first study to report that testosterone increases but IGF-1 does not change significantly during and after EMS.

Our data support the previous studies (13, 15, 21, 24, 37) reporting that isometric force generation during EMS was similar between alternating and pulsed currents, in which an equivalent number of stimuli per second was delivered for both currents, although the number of pulses in a stimulus was different. In the present study, the currents were delivered at 75 Hz for both stimulation conditions, either bursts per second (alternating current) or pulses per second (pulsed current), and the muscles were stimulated at maximally tolerable level for both conditions (Fig. 1). The other stimulation parameters were



Fig. 5. Changes in MVC torque at 40°, 70°, and 100° before (Pre), and 1, 24, 48, 72, and 96 h after alternating current (AC) and pulsed current (PC) electrical stimulation. ns, no significant difference between currents for the changes over time. *Significant (P < 0.05) difference from the baseline.
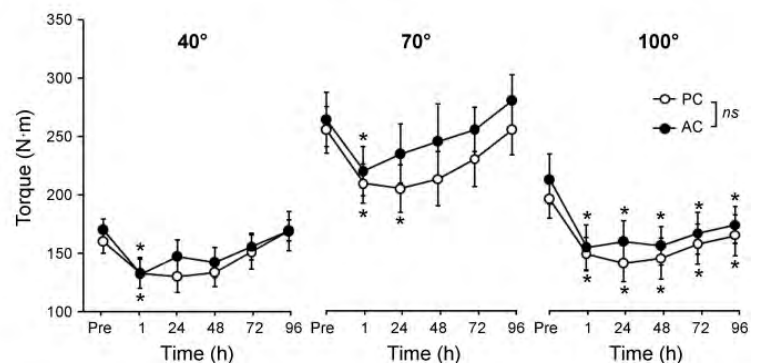
Fig. 6. Changes in muscle soreness (VAS) on squat (*A*), PPT (*B*), and plasma CK activity (*C*) before (Pre), and 1–96 h after alternating current (AC) and pulsed current (PC) electrical muscle stimulation. ns, no significant difference between currents for the changes over time. *Significant ($P < 0.05$) difference from baseline value.
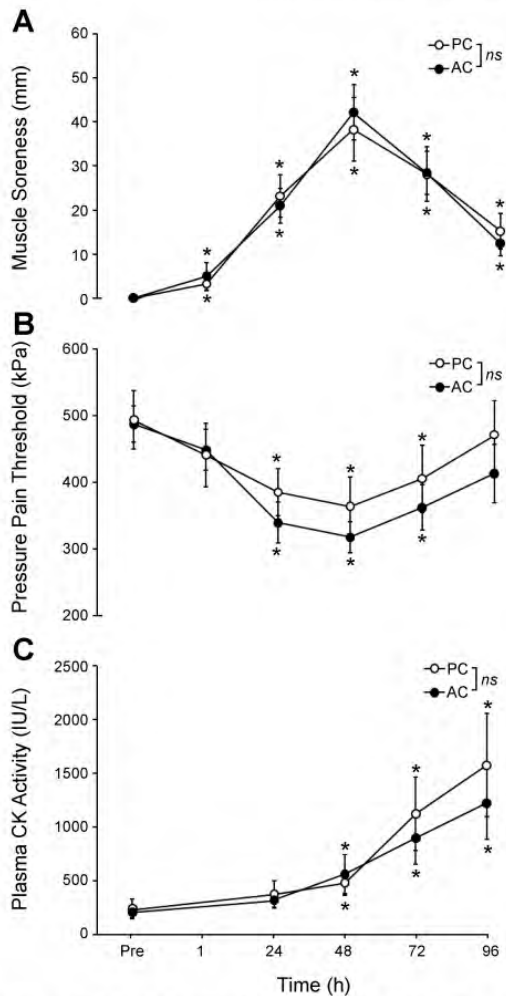
the same between the conditions (Table 1), and no significant difference in the stimulation amplitude was evident between the alternating and pulsed current EMS (Fig. 3A). It seems that the number of stimuli per second determines the force generation (75 stimuli in the present study) rather than the number of pulses in each stimulus (33 stimuli for alternating current, and 1 stimulus for pulsed current in the present study). Thus the additional pulses in the alternating current EMS do not appear to contribute to the force generation (21). Several studies have reported that greater muscle fatigue is induced during alternating current than pulsed current EMS (21, 22, 24, 38). Although the peak torque generated during EMS was not significantly different between alternating current EMS and pulsed current EMS (Fig. 3C), the present study also found that the torque

during alternating current EMS was not as stable as that during pulsed current EMS as reported in previous studies (13, 38).

The torque output increased to the level of ~30% MVC in the first 15 contractions and no further increases were seen afterward for both waveforms (Fig. 3C), despite the consistent increases in the stimulation intensity (Fig. 3A). The level of torque output in the present study (~30% of MVC) was similar to that of a previous study (16) in which the same electrode placement and stimulation parameters (e.g., 75 Hz, pulse duration 400 μs) as those of the present study were used. The plateau of maximal torque despite the increases in stimulation intensity (Fig. 3A) would indicate that no further increases in motor unit recruitment were made. It appears that muscle fatigue occurs at central and/or peripheral origins during EMS (3), which prevented further increases in force generation. It is possible that the high-frequency stimulation (75 Hz) impaired muscle excitation (7). Additionally, the plateau might be due to muscle damage, where a structural damage to the force-bearing elements and/or a failure of the excitation-contraction coupling occurred (49).

The nonlinear increases in RPE appear to indicate an increase in subjects' tolerance to EMS, probably due to an increase in threshold of pain receptors (31). Two previous studies reported that the level of discomfort or pain during stimulation was similar between alternating current and pulsed current EMS (13, 24). For example, Grimby and Wigerstad-Lossing (13) reported that discomfort of the quadriceps muscle stimulated at maximally tolerable intensity was similar between alternating and pulsed current using a Borg's scale. Lyons et al. (24) also showed no significant difference in discomfort between alternating current EMS and pulsed current EMS using a numeric pain rating scale. Since the stimulation intensity was set at the maximum tolerance of each subject in the present study, the similar RPE between the conditions seems reasonable. Thus neither of alternating current or pulsed current can be preferably chosen to the other in terms of discomfort especially for muscle strengthening purpose.

As shown in Fig. 4, $T_{sk}$ did not change during exercise but increased significantly in the stimulated leg (~2 C°) and decreased in the control leg (~1 C°) during recovery period for both stimulation conditions. It does not appear that the increases in $T_{sk}$ are associated with the discomfort of EMS. $T_{sk}$ reflects the skin blood flow, and it increases as a result of a vasodilation in skin blood vessels (11, 39). The delayed increase in $T_{sk}$ may suggest that little or no changes in skin blood flow were induced during isometric contractions, but increases in skin blood flow occurred after EMS. The decrease in $T_{sk}$ in the control leg was unexpected. However, Bishop et al. (2) reported that blood flow decreased in the hand skin during a leg exercise, and Cotzias and Marshall (8) also reported a vasoconstrictor response of the cutaneous circulation occurred in the contralateral forearm when isometric handgrip exercise was performed with the other arm. The similar changes in $T_{sk}$ between the alternating and pulsed current EMS reflect a similar muscle usage in the two conditions.

Blood lactate increased similarly in alternating current and pulsed current EMS (Table 2). The magnitude of increase in blood lactate was ~20% less than that reported after EMS (16) in which 40 isometric contractions of the knee extensors were evoked electrically in two legs simultaneously. It is likely the

muscle volume involved in exercise has an effect on the blood lactate response. The hormonal responses to resistance exercise have been well documented (19); however, little information is available on the effects of EMS on hormonal responses. Two studies (16, 34) reported changes in GH after EMS in which pulsed current was used to stimulate the knee extensors of both legs. The present study showed that EMS increased GH by 400% and testosterone by 150% (Table 2). The magnitude of increase in GH in the present study was less than half of that reported in the previous studies (16, 34). This could be due to the difference in the volume of stimulated muscles, i.e., that the knee extensors of both legs were stimulated in the previous studies, but only one leg was stimulated in the present study. The magnitude of increase in testosterone after EMS was similar to that reported following four sets of slow concentric exercise of knee extensors at 50% one repetition maximum (1RM) to failure (10). It is interesting that stimulation of only knee extensors of one leg increased testosterone to a similar level as resistance exercise in which more muscles mass are involved. Jubeau et al. (16) reported that the increase in GH was higher after EMS compared with voluntary contractions of the knee extensors when both were performed at the same torque output and speculated that pain during EMS might be associated with the greater GH release in EMS than voluntary contractions. It might be that EMS is effective for stimulating anabolic hormone release to a greater extent than voluntary contractions.

It was expected to see increases in IGF-1 after EMS because GH stimulates IGF-1 secretion (44). However, no significant changes in IGF-1 were evident up to 45 min post-EMS in the present study. It is important to note that the time course of changes in IGF-1 is different from that of GH or testosterone, such that IGF-1 increases 3–9 h following resistance exercise (14, 17). Thus it is possible that changes in IGF-1 would have been missed. Further investigation is necessary to track the IGF-1 changes following EMS for an extended period. The constant decrease in cortisol is likely due to the circadian rhythm (42), since all EMS sessions in the present study were performed in the morning, and the time between the pre-EMS blood sample and the last blood sample was ~3 h. It is reported that cortisol decreases ~300 nmol/l over the 3-h period from 8 AM to 11 AM (42), whereas the magnitude of the decrease was approximately half (~150 nmol/l) in a similar time frame in the present study. Sartorio et al. (34) reported that cortisol decreased at 1 h after the first EMS bout consisting of 20 isometric contractions started between 8 and 8:30 AM but increased significantly for 30 min following the second EMS bout that was performed 2 h after the first bout. Thus it is possible that EMS increased or at least attenuated the decrease in cortisol in the circadian rhythm, which would suggest catabolic aspects of EMS.

The changes in MVC, muscle soreness, and plasma CK activity after EMS exercise suggest the occurrence of muscle damage following EMS. However, no significant difference between alternating current EMS and pulsed current EMS were evident for any of the parameters (Figs. 5 and 6). Since the baseline MVC was similar, and the torque output during EMS was also similar between the bouts (Fig. 3C), it seems reasonable to assume that mechanical stress to the muscles was similar between the conditions. It is known that the level of force produced during lengthening contractions is a strong predictor of muscle dam-

age (50). Thus it appears that the waveform itself does not affect the magnitude of muscle damage (21), but the similar intensity of muscle contractions between the two EMS conditions was the reason for the nonsignificant differences in the muscle damage characteristics.

As shown in Fig. 5, the decreases in MVC at the angle of stimulation (100°) were significantly greater and longer lasting compared with other angles (40° and 70°). It should be noted that the isometric contractions were performed at 100° in the present study, and the magnitude of MVC decrement appears to be angle specific. The overlap of myosin and actin in isometric contractions at a long length is less compared with that at a short muscle length (5), which may give more mechanical stress over sarcomeres, and cause a disruption to cross bridge (9). Changes in MVC and plasma CK activity have been reported to be greater following eccentric exercise of elbow flexors at long muscle lengths compared with short muscle length (30). Several studies (30, 35) have reported that isometric contractions at a long muscle length induce muscle damage but not at a short muscle length. Nosaka et al. (29) found that muscle damage following intermittent isometric contractions of the elbow flexor induced by EMS was minimal when the biceps brachii was stimulated at a short muscle length (90°). However, our recent study (unpublished) found that when the biceps brachii was stimulated at a long muscle length (160°), EMS-evoked isometric contractions resulted in appreciable changes in muscle damage markers such as MVC, muscle soreness, and plasma CK activity. Thus it seems likely that the cause of muscle damage was not EMS itself but repeated isometric contractions at a long muscle length (1).

Changes in muscle soreness following EMS were similar to those reported in previous studies in which isometric contractions of the knee extensors were evoked by EMS (1, 16). The magnitude of increase in plasma CK activity in the present study was approximately half of that reported in the previous study (16). The amount of stimulated muscles was different between the previous study (two legs) and the present study (one leg), and this could explain the difference. Our previous study (1) showed that the magnitude of muscle damage was significantly attenuated in the second EMS bouts performed 2 wk after the initial bout using pulsed current. It seems likely that this is also the case for alternating current EMS; however, this should be investigated in future studies.

In conclusion, alternating and pulsed current have a similar effect on force production, RPE, skin temperature, hormonal responses, and muscle damage, when the stimulation parameters except the waveform were matched between the two. These findings suggest that acute effects are similar between alternating and pulsed current EMS; thus the waveform itself is less important for EMS, if an EMS machine has an capacity to maximally stimulate a muscle when other parameters such as stimulation intensity, frequency, and pulse duration are the same. Considering the fact that a pulsed current stimulator is less expensive and more portable than an electrical stimulator that can deliver alternating current, pulsed current EMS may be more advantageous over alternating current EMS, especially when it is used for muscle training. It is important to note that the present study focused on the use of EMS in muscle training of healthy men; therefore it is necessary to examine women, elderly individuals, and clinical populations in future studies. Chronic effects of the use of different current in a treatment or

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a training program cannot be speculated from the present study. Thus further study is warranted to compare the effects of chronic use of EMS on muscle adaptation between alternating current and pulsed current EMS for healthy and clinical populations.

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

## REFERENCES

1. **Aldayel A, Jubeau M, McGuigan MR, Nosaka K.** Less indication of muscle damage in the second than initial electrical muscle stimulation bout consisting of isometric contractions of the knee extensors. *Eur J Appl Physiol* 108: 709–717, 2010.
2. **Bishop JM, Donald KW, Taylor SH, Wormald PN.** The blood flow in the human arm during supine leg exercise. *J Physiol* 137: 294–308, 1957.
3. **Boerio D, Jubeau M, Zory R, Maffiuletti NA.** Central and peripheral fatigue after electrostimulation-induced resistance exercise. *Med Sci Sports Exerc* 37: 973–978, 2005.
4. **Bunt JC, Bahr JM, Bemben DA.** Comparison of estradiol and testosterone levels during and immediately following prolonged exercise in moderately active and trained males and females. *Endocr Res* 13: 157–172, 1987.
5. **Child RB, Saxton JM, Donnelly AE.** Comparison of eccentric knee extensor muscle actions at two muscle lengths on indices of damage and angle-specific force production in humans. *J Sports Sci* 16: 301–308, 1998.
6. **Clarkson PM, Hubal MJ.** Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil* 81: S52–S69, 2002.
7. **Cooper RG, Edwards RH, Gibson H, Stokes MJ.** Human muscle fatigue: frequency dependence of excitation and force generation. *J Physiol* 397: 585–599, 1988.
8. **Cotzias C, Marshall JM.** Differential effects of isometric exercise on the cutaneous circulation of different regions. *Clin Auton Res* 2: 235–241, 1992.
9. **Desbrosses K, Babault N, Scaglioni G, Meyer JP, Pousson M.** Neural activation after maximal isometric contractions at different muscle lengths. *Med Sci Sports Exerc* 38: 937–944, 2006.
10. **Goto K, Ishii N, Kizuka T, Kraemer RR, Honda Y, Takamatsu K.** Hormonal and metabolic responses to slow movement resistance exercise with different durations of concentric and eccentric actions. *Eur J Appl Physiol* 106: 731–739, 2009.
11. **Grant RT, Holling HE.** Further observations on the vascular responses of the human limb to body warming; evidence for sympathetic vasodilator nerves in the normal subject. *Clin Sci* 3: 273–285, 1936.
12. **Gregory CM, Dixon W, Bickel CS.** Impact of varying pulse frequency and duration on muscle torque production and fatigue. *Muscle Nerve* 35: 504–509, 2007.
13. **Grimby G, Wigerstad-Lossing I.** Comparison of high- and low-frequency muscle stimulators. *Arch Phys Med Rehabil* 70: 835–838, 1989.
14. **Hickson RC, Hidaka K, Foster C, Falduto MT, Chatterton RT Jr.** Successive time courses of strength development and steroid hormone responses to heavy-resistance training. *J Appl Physiol* 76: 663–670, 1994.
15. **Holcomb WR, Golestani S, Hill S.** A comparison of knee-extension torque production with biphasic versus Russian current. *J Sport Rehabil* 9: 229–239, 2000.
16. **Jubeau M, Sartorio A, Marinone PG, Agosti F, Van Hoecke J, Nosaka K, Maffiuletti NA.** Comparison between voluntary and stimulated contractions of the quadriceps femoris for growth hormone response and muscle damage. *J Appl Physiol* 104: 75–81, 2008.
17. **Kraemer WJ, Fleck SJ, Dziados JE, Harman EA, Marchitelli LJ, Gordon SE, Mello R, Frykman PN, Koziris LP, Triplett NT.** Changes in hormonal concentrations after different heavy-resistance exercise protocols in women. *J Appl Physiol* 75: 594–604, 1993.
18. **Kraemer WJ, Gordon SE, Fleck SJ, Marchitelli LJ, Mello R, Dziados JE, Friedl K, Harman E, Maresh C, Fry AC.** Endogenous anabolic

hormonal and growth factor responses to heavy resistance exercise in males and females. *Int J Sports Med* 12: 228–235, 1991.
19. **Kraemer WJ, Ratamess NA.** Hormonal responses and adaptations to resistance exercise and training. *Sports Med* 35: 339–361, 2005.
20. **Lake DA.** Neuromuscular electrical stimulation. An overview and its application in the treatment of sports injuries. *Sports Med* 13: 320–336, 1992.
21. **Laufer Y, Elboim M.** Effect of burst frequency and duration of kilohertz-frequency alternating currents and of low-frequency pulsed currents on strength of contraction, muscle fatigue, and perceived discomfort. *Phys Ther* 88: 1167–1176, 2008.
22. **Laufer Y, Ries JD, Leininger PM, Alon G.** Quadriceps femoris muscle torques and fatigue generated by neuromuscular electrical stimulation with three different waveforms. *Phys Ther* 81: 1307–1316, 2001.
23. **Loram LC, Mitchell D, Fuller A.** Rofecoxib and Tramadol do not attenuate delayed-onset muscle soreness or ischaemic pain in human volunteers. *Can J Physiol Pharmacol* 83: 1137–1145, 2005.
24. **Lyons CL, Robb JB, Irrgang JJ, Fitzgerald GK.** Differences in quadriceps femoris muscle torque when using a clinical electrical stimulator versus a portable electrical stimulator. *Phys Ther* 85: 44–51, 2005.
25. **Mackey AL, Bojsen-Moller J, Qvortrup K, Langberg H, Suetta C, Kalliokoski KK, Kjaer M, Magnusson SP.** Evidence of skeletal muscle damage following electrically stimulated isometric muscle contractions in humans. *J Appl Physiol* 105: 1620–1627, 2008.
26. **Maffiuletti NA, Zory R, Miotti D, Pellegrino MA, Jubeau M, Bottinelli R.** Neuromuscular adaptations to electrostimulation resistance training. *Am J Phys Med Rehabil* 85: 167–175, 2006.
27. **Meyers BM, Nguyen J, Cafarelli E.** Obtaining force-frequency curves with a single 3-second train of stimuli. *Muscle Nerve* 24: 1332–1338, 2001.
28. **Moreau D, Dubots P, Boggio V, Guilland JC, Cometti G.** Effects of electromyostimulation and strength training on muscle soreness, muscle damage and sympathetic activation. *J Sports Sci* 13: 95–100, 1995.
29. **Nosaka K, Newton M, Sacco P.** Responses of human elbow flexor muscles to electrically stimulated forced lengthening exercise. *Acta Physiol Scand* 174: 137–145, 2002.
30. **Nosaka K, Sakamoto K.** Effect of elbow joint angle on the magnitude of muscle damage to the elbow flexors. *Med Sci Sports Exerc* 33: 22–29, 2001.
31. **Owens J, Malone TR.** Treatment parameters of high frequency electrical stimulation as established on the Electro-Stim 180. *J Orthop Sports Phys Ther* 4: 162–168, 1983.
32. **Parker MG, Keller L, Evenson J.** Torque responses in human quadriceps to burst-modulated alternating current at 3 carrier frequencies. *J Orthop Sports Phys Ther* 35: 239–245, 2005.
33. **Rooney JG, Currier DP, Nitz AJ.** Effect of variation in the burst and carrier frequency modes of neuromuscular electrical stimulation on pain perception of healthy subjects. *Phys Ther* 72: 800–806; discussion 807–809, 1992.
34. **Sartorio A, Jubeau M, Agosti F, De Col A, Marazzi N, Lafortuna CL, Maffiuletti NA.** GH responses to two consecutive bouts of neuromuscular electrical stimulation in healthy adults. *Eur J Endocrinol* 158: 311–316, 2008.
35. **Saxton JM, Donnelly AE.** Length-specific impairment of skeletal muscle contractile function after eccentric muscle actions in man. *Clin Sci (Lond)* 90: 119–125, 1996.
36. **Selkowitz DM, Rossman EG, Fitzpatrick S.** Effect of burst-modulated alternating current carrier frequency on current amplitude required to produce maximally tolerated electrically stimulated quadriceps femoris knee extension torque. *Am J Phys Med Rehabil* 88: 973–978, 2009.
37. **Snyder-Mackler L, Garrett M, Roberts M.** A comparison of torque generating capabilities of three different electrical stimulating currents. *J Orthop Sports Phys Ther* 10: 297–301, 1989.
38. **Stefanovska A, Vodovnik L.** Change in muscle force following electrical stimulation. Dependence on stimulation waveform and frequency. *Scand J Rehabil Med* 17: 141–146, 1985.
39. **Stoner HB, Barker P, Riding GS, Hazlehurst DE, Taylor L, Marcuson RW.** Relationships between skin temperature and perfusion in the arm and leg. *Clin Physiol* 11: 27–40, 1991.
40. **Sugai N, Worsley R, Payne JP.** Tetanic force development of adductor pollicis muscle in anesthetized man. *J Appl Physiol* 39: 714–717, 1975.
41. **Theurel J, Lepers R, Pardon L, Maffiuletti NA.** Differences in cardio-respiratory and neuromuscular responses between voluntary and stimu-

lated contractions of the quadriceps femoris muscle. *Respir Physiol Neurobiol* 157: 341–347, 2007.

42. **Thuma JR, Gilders R, Verdun M, Loucks AB.** Circadian rhythm of cortisol confounds cortisol responses to exercise: implications for future research. *J Appl Physiol* 78: 1657–1664, 1995.

43. **Tiidus PM.** Estrogen and gender effects on muscle damage, inflammation, and oxidative stress. *Can J Appl Physiol* 25: 274–287, 2000.

44. **Turner JD, Rotwein P, Novakofski J, Bechtel PJ.** Induction of mRNA for IGF-I and -II during growth hormone-stimulated muscle hypertrophy. *Am J Physiol Endocrinol Metab* 255: E513–E517, 1988.

45. **Vanderthommen M, Duchateau J.** Electrical stimulation as a modality to improve performance of the neuromuscular system. *Exerc Sport Sci Rev* 35: 180–185, 2007.

46. **Ward AR, Lucas-Toumbourou S, McCarthy B.** A comparison of the analgesic efficacy of medium-frequency alternating current and TENS. *Physiotherapy* 95: 280–288, 2009.

47. **Ward AR, Robertson VJ.** The variation in fatigue rate with frequency using kHz frequency alternating current. *Med Eng Phys* 22: 637–646, 2000.

48. **Ward AR, Shkuratova N.** Russian electrical stimulation: the early experiments. *Phys Ther* 82: 1019–1030, 2002.

49. **Warren GL, Ingalls CP, Lowe DA, Armstrong RB.** What mechanisms contribute to the strength loss that occurs during and in the recovery from skeletal muscle injury? *J Orthop Sports Phys Ther* 32: 58–64, 2002.

50. **Warren GL, Lowe DA, Armstrong RB.** Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med* 27: 43–59, 1999.

51. **Webb ML, Wallace JP, Hamill C, Hodgson JL, Mashaly MM.** Serum testosterone concentration during two hours of moderate intensity treadmill running in trained men and women. *Endocr Res* 10: 27–38, 1984.

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# Chapter 4 (Study 2)

ORIGINAL ARTICLE

# Muscle oxygenation of vastus lateralis and medialis muscles during alternating and pulsed current electrical stimulation

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**Abstract** This study compared between alternating and pulsed current electrical muscle stimulation (EMS) for muscle oxygenation and blood volume during isometric contractions. Nine healthy men (23–48 years) received alternating current EMS (2500 Hz) modulated at 75 Hz on the knee extensors of one leg, and pulsed current EMS (75 Hz) for the other leg separated by 2 weeks in a randomised, counter-balanced order. Pulse duration (400 μs), on–off ratio (5–15 s) and other stimulation parameters were matched between conditions and 30 isometric contractions were induced at the knee joint angle of 100° (0° full extension). Changes in tissue oxygenation index ($\Delta$TOI) and total hemoglobin volume ($\Delta$tHb) of vastus lateralis and medialis muscles over 30 contractions were assessed by a near-infrared spectroscopy, and were compared between conditions by a two-way repeated measures ANOVA. Peak torque produced during EMS increased over 30 contractions in response to the increase in the stimulation intensity for pulsed current, but not for the alternating current EMS. The torque during each isometric contraction was less stable in alternating than pulsed current EMS. The changes in $\Delta$TOI amplitude during relaxation phases and $\Delta$tHb amplitude were not significantly different between conditions. However, the decreases in $\Delta$TOI amplitude during contraction phases from baseline were significantly ($P < 0.05$) greater for the pulsed current than alternating current from the 18th contraction ($-15.6 \pm 2.3$ vs. $-8.9 \pm 1.8\%$) to 30th contraction ($-10.7 \pm 1.8$ vs. $-4.8 \pm 1.5\%$). These results suggest that the muscles were less activated in the alternating current EMS when compared with the pulsed current EMS.

**Keywords** Neuromuscular electrical stimulation · Near-infrared spectroscopy · Blood flow · Fatigue · Motor unit recruitment · Isometric contractions

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## Introduction

Electrical muscle stimulation (EMS) is widely used in rehabilitation and sport training, and has been shown to be effective for improving muscle strength (Selkowitz 1985; Snyder-Mackler et al. 1994). There are many types of EMS, which are basically determined by waveform, frequency, intensity and pulse duration (Binder-Macleod et al. 1995; Gondin et al. 2010; Gorgey et al. 2006). The most commonly used EMS waveforms are alternating current and pulsed current. Pulsed current EMS has been more commonly used in sport training than alternating current

EMS, because of easier access to a pulsed current stimulator (Lyons et al. 2005; Ward 2009; Ward and Shkuratova 2002). The use of alternating current in sport training increased after a Russian scientist Kots had claimed that the muscle strength increased up to 40% with alternating current (2500 Hz) modulated at 50 Hz, so called Russian current (Ward and Shkuratova 2002). If both waveforms are possible to use, the question is which is better for strength training. However, physiological differences between alternating current and pulsed current EMS are not necessarily clear, and controversy exists among the findings of previous studies.

Some studies showed that alternating current induced more muscle fatigue than pulsed current EMS (Laufer and Elboim 2008; Laufer et al. 2001; Stefanovska and Vodovnik 1985). For example, Stefanovska and Vodovnik (1985) reported that decreases in isometric torque of the knee extensors over 10 contractions (10 s stimulation, 50 s rest between stimulations) were significantly greater by 25% for alternating current EMS (2500 Hz delivered at 25 Hz) when compared with pulsed current EMS (25 Hz), and this was also the case for 60-s continuous stimulation (the magnitude of decrease in the torque was 43% greater for the alternating current than pulsed current). If muscle fatigue develops at early stage in EMS, the mechanical stimulus to the muscle would be less, which may not be ideal for strength training. However, the greater muscle fatigue indicated by lower force generation in alternating current than pulsed current EMS reported in the previous studies (Laufer and Elboim 2008; Laufer et al. 2001; Stefanovska and Vodovnik 1985) was not necessarily observed in other studies. For example, several studies (Grimby and Wigerstad-Lossing 1989; Holcomb et al. 2000; Lyons et al. 2005; Snyder-Mackler et al. 1989) reported no significant difference in isometric contraction torque between alternating and pulsed current EMS, when muscles were stimulated at the maximal tolerable intensity over intermittent isometric contractions. Thus, further study is necessary to compare muscle fatigue in EMS between alternating current and pulsed current using other measures that could monitor changes in motor unit recruitment during EMS.

Near-infrared spectroscopy (NIRS) can monitor muscle oxidative metabolism during exercise (Hampson and Piantadosi 1988; Quaresima et al. 2003). Felici et al. (2009) reported that biceps brachii myoelectric activity assessed by surface electromyography (EMG) was correlated ($r = 0.72$) with muscle oxygenation assessed by NIRS during sustained and repeated isometric contractions. Since EMS interferes with EMG measurements, EMG cannot assess a decrease in muscle activity during EMS exercise. However, a decrease in muscle activity should be reflected in a reduction in muscle oxygen ($O_2$)

consumption, thus NIRS could provide information relating to muscle fatigue (e.g., reduction in muscle activity) during muscle contractions evoked by EMS. No previous studies have investigated muscle oxygenation during alternating current EMS nor compared between alternating and pulsed current EMS for changes in muscle oxygenation during intermittent isometric contractions of the knee extensors.

The purpose of the present study therefore was to compare between alternating current EMS and pulsed current EMS for changes in vastus lateralis and vastus medialis muscle oxygenation and change in blood volume using NIRS during intermittent isometric contractions. It was hypothesized that the muscle oxygenation during the isometric contractions would be less for alternating current EMS compared with pulsed current EMS, if the former results in greater muscle fatigue.

## Methods

### Subjects

Nine men (mean ± SD age: 34.0 ± 7.0 years, body weight: 85.4 ± 14.1 kg, height: 174.0 ± 5.1 cm) who had not been involved in resistance training programs for at least 6 months prior to the present study were recruited. Subjects with any health problems (e.g., hypertension, epilepsy, neurological and neuromuscular disorders) or who had any muscle or joint injuries of knee were excluded. All subjects were informed about the study, and a medical questionnaire and an informed consent written form were obtained prior to participation from each subject. The subjects were requested not to involve in any physical activity before reporting to the laboratory. This study was approved by the Edith Cowan University Human Research Ethics Committee and conducted in accordance with the Declaration of Helsinki.

### EMS

All subjects received alternating current EMS for one leg, and pulsed current EMS for the other leg in different days separated by 2 weeks in a randomised, counter-balanced order with no emphasis on the limb dominance. For each EMS session, the subjects were not informed which current was applied, thus the use of the waveform was blind to the subjects. The knee extensors were stimulated by an Intelect Advanced®Colour Stim (Chattanooga Group, TN, USA) that could deliver both alternating current and pulsed current. After cleaning the skin surface with alcohol pads, two positive electrodes (50 × 50 mm) were placed over the motor point of vastus lateralis and vastus medialis, which

were identified as the sites on the skin where the smallest current amplitude was required to produce muscle contraction (Robertson et al. 2006). Two negative electrodes ($50 \times 100$ mm) were placed on the proximal portion of rectus femoris muscle (Jubeau et al. 2008). The subjects were seated on a Biodex isokinetic dynamometer's chair (Biodex Medical Systems, Inc., USA), and the knee joint angle was set at $100°$ ($0°$ corresponding to the full extension), and the trunk, pelvis, and thigh were firmly secured to the seat by straps at trunk angle of $110°$.

Figure 1 shows the diagram of the waveforms that were used in the present study for alternating and pulsed current EMS. The waveform of pulsed current was biphasic, symmetrical and rectangular, and the pulses were delivered at a frequency of 75 Hz (pulses per second) and pulse duration was 400 μs, which was used in a previous study (Jubeau et al. 2008). The waveform of alternating current was polyphasic, sinusoidal and balanced, and adjusted at 2500 Hz alternating current (400 μs pulse duration), delivered in bursts with a carrier frequency of 75 Hz and the burst duration was 6.5 ms. These alternating current parameter settings were similar to those used in previous studies (Lyons et al. 2005; Rooney et al. 1992; Ward and Shkuratova 2002). The stimulation protocols were adjusted to match other parameters than waveform such as pulse duration and duty cycle as identical as possible between the alternating and pulsed current EMS. The stimulation time was 5 s followed by a 15-s rest, so the duty cycle time was 25%, and the ramping time was included in the stimulation (1 s for the rise time and 1 s for the fall time) based on previous studies (Jubeau et al. 2008; Lyons et al. 2005).

The stimulation amplitude was started with 0 mA, and gradually increased to a maximally tolerable intensity in the first 10 contractions to reach a plateau force, thereafter current amplitude was consistently increased every five contractions to complete 30 isometric contractions at maximally tolerable stimulation intensity. The magnitude of increase in the stimulation amplitude was depending on the feedback from each subject, and the increment was between 0 and 5 mA for each time point. The torque of each contraction was recorded by the Biodex software and analysed using Chart data analysis software (ADInstruments, Bella Vista, Australia).

Maximal voluntary isometric contraction torque

Subjects sat on the Biodex isokinetic dynamometer's chair and the setting was the same as that of the EMS protocol described above. The rotation axis of the tested knee was aligned with the rotation axis of the dynamometer's armature, and the ankle cuff was joined ~1 cm proximal to the medial malleolus. Gravity corrections were made at $10°$ of knee flexion from a full-extension position. Subjects

were asked to keep both arms positioned across the chest with each hand clasping the opposite shoulder. Maximal voluntary isometric contraction (MVC) was measured at $100°$ of the knee joint, because it was the angle that the isometric contractions were evoked by EMS. Each subject performed two maximal contractions with a 30-s rest between contractions, before and immediately (within 5 s) after EMS session. The contraction lasted for 5 s and the MVC was determined by the peak of the torque curve for each contraction. The higher peak value of the two attempts was used for further analysis.
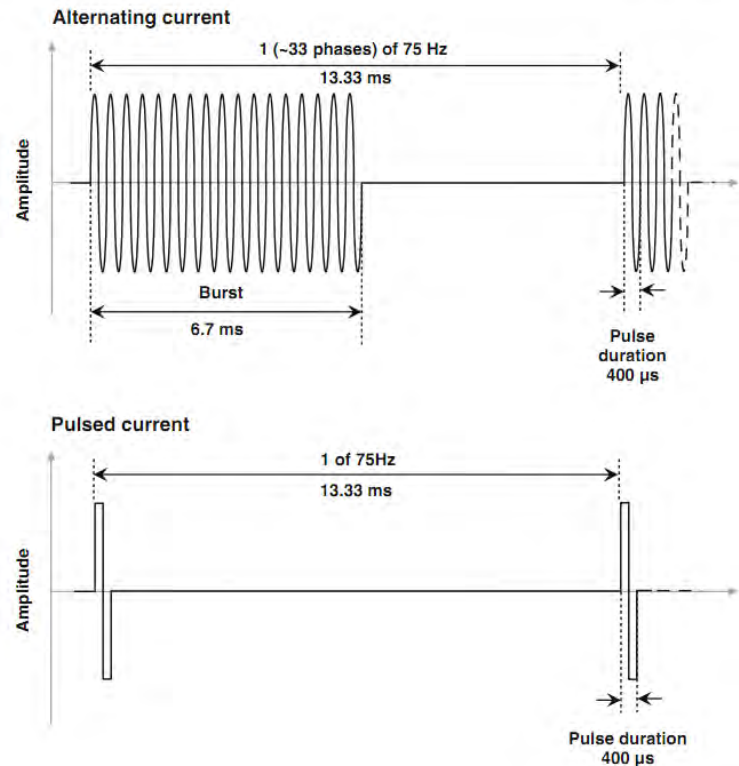
Blood lactate concentration

A 5 μl of blood was taken by finger prick and loaded to a test strip of a Lactate Pro Analyzer (Arkray, Inc. Kyoto, Japan). The samples were obtained before, immediately after the 20th isometric contraction during the EMS session, and immediately and 15 min after EMS exercise.

Near-infrared spectroscopy

A NIRO-200 oximeter (Hamamatsu Photonics K.K, Hamamatsu, Japan) was used to measure the concentration changes in oxygenated haemoglobin ($\Delta O_2Hb$), deoxygenated haemoglobin ($\Delta HHb$), and total hemoglobin volume ($\Delta tHb = \Delta O_2Hb + \Delta HHb$) from an arbitrary baseline of zero. The NIRO-200 is a spatially resolved spectroscopy (Suzuki et al. 1999) and it provides an absolute measure of $O_2Hb$ saturation as tissue oxygenation index (TOI = $O_2Hb/tHb \times 100$). The NIRS probe secured in a rubber holder consisted of a laser diode that delivers three wavelengths (775, 810, and 850 nm) and an optical photodetector with two photodiodes, and the light emitter and detector were separated by a distance of 4 cm. The NIRS light has a penetration depth of ~2 cm (i.e., half of the distance between the emitter and the detector) from the skin surface (Chance et al. 1988), which was shown to be sufficient for attaining a reflected light signal from the targeted muscle (McCully and Hamaoka 2000). Two NIRS probes were placed proximally adjacent to each of the EMS positive electrodes and parallel to the longitudinal axis of the mid-belly of the vastus lateralis (one probe) and vastus medialis (the other probe). The NIRS probes were covered with a black fabric cloth to minimise the interference of stray light and loss of NIR light from the interrogation field. The NIRO-200 sample rate was set at 6 Hz, and the signals were recorded at 200 Hz and saved in a data acquisition system (PowerLab, ADInstruments, Bella Vista, Australia) for later analysis.

The concentration changes in $\Delta O_2Hb$, $\Delta HHb$ and $\Delta tHb$ are expressed as μM cm, and TOI is expressed as a percentage. The resting baseline values for $\Delta O_2Hb$, $\Delta HHb$, $\Delta tHb$ and $\Delta TOI$ were determined as the average of 30 s

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**Fig. 1** Schematic diagrams of alternating and pulsed waveforms used in the study



Changes in average peak torque, torque time integral, $\Delta TOI_{min}$, $\Delta TOI_{max}$, $\Delta tHb_{mean}$ and $\Delta tHb_{max}$ over 30 contractions were analysed using a one-way repeated measures ANOVA, and compared between alternating and pulsed current EMS by a two-way repeated measures ANOVA. Changes in MVC from pre to post EMS exercise, and blood lactate before, during, and after EMS were compared between waveforms using a two-way repeated measures ANOVA. When a significant interaction effect was found, a Tukey's post hoc test was followed to compare between bouts for each time point. The significance level was set at $P < 0.05$. All data are expressed as mean $\pm$ SEM.

before exercise, and were represented as the magnitude of change in these parameters with respect to their respective baseline that was set to zero. As shown in Fig. 2, the NIRS parameters used in the present study included minimum $\Delta TOI$ amplitude ($\Delta TOI_{min}$), maximum $\Delta TOI$ amplitude ($\Delta TOI_{max}$), mean $\Delta tHb$ amplitude ($\Delta tHb_{mean}$) and maximum $\Delta tHb$ amplitude ($\Delta tHb_{max}$) that were used in previous studies (Cettolo et al. 2007; Felici et al. 2009). $\Delta TOI_{min}$ is the difference between the baseline and the lowest TOI at the end of contraction, which has been shown to be inversely proportional to $O_2$ consumption relative to $O_2$ supply; while $\Delta TOI_{max}$ is the difference between the baseline and the highest value during the relaxation phase, and indicates the $O_2$ supply relative to $O_2$ consumption. $\Delta tHb_{mean}$ was the difference between the baseline and the average $\Delta tHb$ value during contraction, and it is an indication of the blood volume/flow changes. $\Delta tHb_{max}$ was the difference between the baseline and the highest value during the relaxation phase, and reflects blood volume/flow changes during relaxation.

## Results

### MVC torque and torque during EMS

No significant difference in the baseline MVC was evident between the alternating current ($190 \pm 23$ Nm) and pulsed current ($207 \pm 22$ Nm). When comparing to pre-EMS values, MVC decreased significantly immediately after EMS by $20.8 \pm 5.1\%$ for alternating current and $24.4 \pm 8.3\%$ for pulsed current, without a significant difference between them. The torque generated during contractions was more

### Statistical analyses

The normality of the data distribution was checked by a Shapiro–Wilk test for the torque and NIRS parameters.

**Fig. 2** Typical changes in knee extensor torque, and vastus lateralis tissue oxygenation index ($\Delta$TOI) and total haemoglobin volume ($\Delta$tHb) of a representative subject for alternating current EMS (**a**) and pulsed current EMS (**b**). The *dashed grey vertical lines* correspond to the onset and the end of the first 5-s contraction (on-time) and the end of the 15-s relaxation (off-time), respectively. $\Delta TOI_{min}$ minimum $\Delta$TOI amplitude, $\Delta TOI_{max}$ maximum $\Delta$TOI amplitude, $\Delta tHb_{mean}$ mean $\Delta$tHb amplitude, $\Delta tHb_{max}$ maximum $\Delta$tHb amplitude
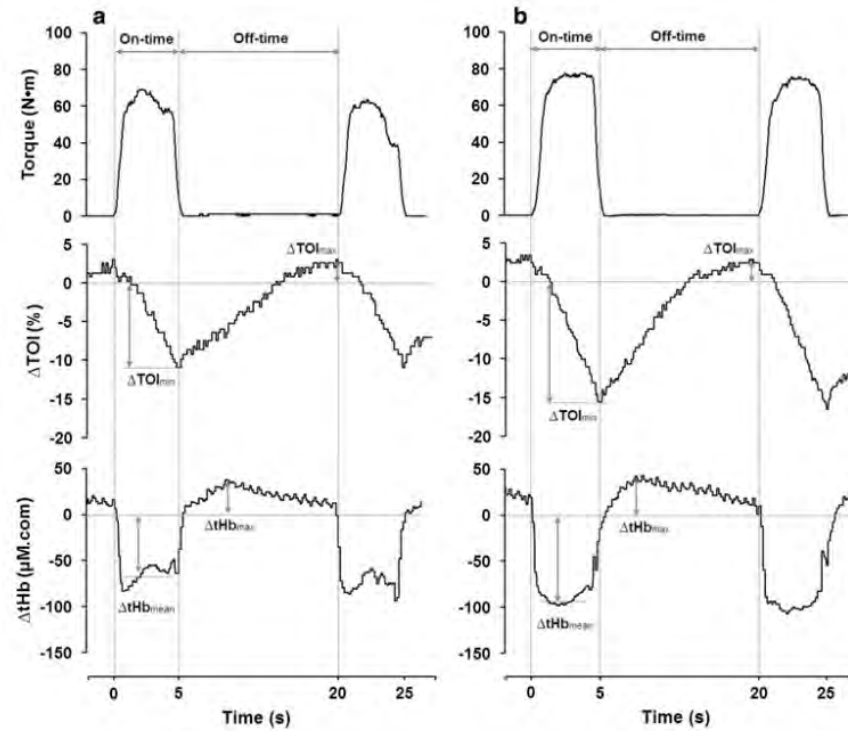


stable in the pulsed current than alternating current EMS, especially from the half way of the EMS session, although the peak torque values were similar between the waveforms in the first 10 contractions at least (Fig. 3).
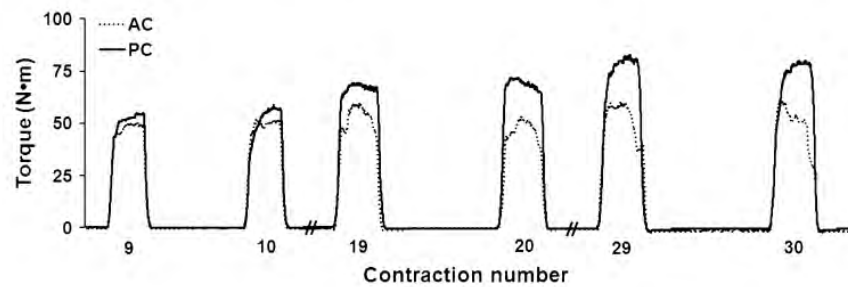
As shown in Fig. 4a, the stimulation amplitude increased significantly over 30 contractions, and no significant difference in the changes in amplitude was evident between waveforms. Figure 4b shows changes in peak torque of each contraction relative to the pre-EMS MVC over 30 contractions. The peak torque during EMS (69.1 $\pm$ 5.6 Nm) was approximately 30% of the pre-EMS MVC at 10th–30th contractions for both waveforms. Changes in the peak torque over 30 contractions were significantly different between waveforms such that the torque increased

significantly for the pulsed current, but no significant increases were found for alternating current.

Blood lactate concentration

No significant difference between alternating current and pulsed current EMS was evident for the changes in blood lactate concentration. Blood lactate concentration increased significantly from pre ($1.5 \pm 0.1$, $1.8 \pm 0.1$ mM L$^{-1}$) to the 20th contraction ($2.0 \pm 0.1$, $2.6 \pm 0.2$ mM L$^{-1}$) and immediately post-EMS ($2.7 \pm 0.2$, $3.1 \pm 0.1$ mM L$^{-1}$) for alternating current and pulsed current, respectively, but returned to the pre-exercise value by 15 min following exercise.

**Fig. 3** Typical torque curve obtained during 5-s stimulation in alternating current (*dotted lines*) and pulsed current (*solid line*) EMS sessions for the 9th and 10th, 19th and 20th, and 29th and 30th isometric contraction

**Fig. 4** Changes (mean values ± SEM; $N = 9$) in stimulation intensity (**a**), peak knee extensor torque during EMS relative to the baseline maximal voluntary isometric torque (**b**), maximum ΔTOI amplitude (ΔTOI$_{max}$) and minimum ΔTOI amplitude (ΔTOI$_{min}$) (**c**), and maximum ΔtHb amplitude (ΔtHb$_{max}$) and mean ΔtHb amplitude (ΔtHb$_{mean}$) (**d**) for vastus lateralis muscle over 30 isometric contractions evoked by alternating current (*AC*) and pulsed current (*PC*) EMS. Interaction effect was indicated (*n.s.* no significant effect, $P < 0.05$ significant effect). # significant ($P < 0.05$) difference between waveforms based on post hoc test

### NIRS parameters

No significant differences in the changes in any of the NIRS parameters over 30 contractions were evident between vastus lateralis and vastus medialis muscles. The mean TOI baseline value was approximately 60% for both

waveforms with no significant difference between the conditions. Figure 2 shows typical torque, ΔTOI and ΔtHb traces obtained from vastus lateralis muscle for two consecutive isometric contractions (20th and 21st) for the alternating current and pulsed current EMS. ΔTOI decreased during contraction and reached ΔTOI$_{min}$ at the end of each contraction, then increased toward baseline during the relaxation phase to reach ΔTOI$_{max}$ before the next contraction. ΔtHb decreased rapidly at the onset of contraction and did not significantly change during contraction (ΔtHb$_{mean}$), and reached ΔtHb$_{max}$ soon after the end of stimulation.

Figure 4c shows mean changes in ΔTOI$_{max}$ and ΔTOI$_{min}$ of vastus lateralis muscle over 30 isometric contractions for both waveform conditions. ΔTOI$_{max}$ increased significantly from the first contraction to the 30th contraction for both waveforms, and no significant difference between the conditions was found. Changes in ΔTOI$_{min}$ from the baseline were significantly smaller for the alternating current when compared with pulsed current after the 18th contraction.

Mean changes in ΔtHb$_{mean}$ and ΔtHb$_{max}$ over 30 isometric contractions in both waveforms are shown in Fig. 4d. ΔtHb$_{mean}$ decreased significantly over 30 contractions, and no significant difference was found between conditions. No significant difference between the waveforms was evident for the changes in ΔtHb$_{max}$, which showed significant increases over 30 contractions for both waveforms.

## Discussion

The results of this study showed that EMS-evoked peak isometric contraction torque significantly increased over the 30 contractions in response to the increases in the stimulation intensity only for the pulsed current EMS, and the decreases in ΔTOI during the contraction phases from baseline (ΔTOI$_{min}$) were significantly greater for the pulsed current than alternating current from the 18th contraction onwards. However, no significant differences between the alternating current and pulsed current EMS were evident for the changes in MVC from pre to post EMS, the changes in ΔTOI$_{max}$, ΔtHb$_{max}$, and ΔtHb$_{mean}$ over 30 isometric contractions, and the changes in blood lactate before, during and after EMS.

The torque produced during EMS-induced isometric contractions was approximately 30% of pre-EMS exercise MVC measured at the same knee joint angle as the stimulation (i.e., 100°) for both waveforms at least for the first ten contractions. The level of torque output was comparable to that reported in a previous study (26 ± 14% MVC) in which the knee flexors were stimulated at the same angle

as that of the present study (i.e., 100°) using pulsed current (75 Hz) at maximum tolerable current intensity (Jubeau et al. 2008). When looking at the torque curves, the torque was less steady for the alternating than pulsed current EMS, and greater decreases in the torque were seen toward the end of each contraction in the alternating current EMS (Fig. 3). This was also reported in previous studies (Grimby and Wigerstad-Lossing 1989; Stefanovska and Vodovnik 1985). Grimby and Wigerstad-Lossing (1989) stated that pulsed current EMS (30 Hz) was less fatiguing than alternating current EMS (2500 Hz delivered at 50 Hz). Stefanovska and Vodovnik (1985) documented that high-frequency stimuli (alternating current EMS: 2500 Hz delivered at 25 Hz) caused rapid fatigue of the stimulated muscle when compared with pulsed current EMS (25 Hz). It appears that the decrease in force toward the end of contraction in the alternating current EMS indicates that "fatigue" induced in alternating current was greater than that in pulsed current EMS.

Jones (1996) described that action potential propagation along the surface membrane was affected in "high-frequency" stimulation, and this was referred to as "high-frequency fatigue" that occurs at a frequency higher than 50 Hz, resulting in force loss, but the force quickly recovers in the cessation of the stimulation. The force generation recovered rapidly in the interval between contractions in the present study (Fig. 3), therefore it may be that "high-frequency fatigue" was the cause of the force loss during isometric contraction evoked by EMS. If this was the case, it seems possible that the higher frequency of the alternating current (2500 Hz) was responsible for the decreases in the torque toward the end of each 5 s contraction.

Stefanovska and Vodovnik (1985) compared between alternating current (2500 Hz modulated at 25 Hz) and pulsed current (25 Hz) for the torque generation of the knee extensors over 10 isometric contractions (10 s contraction, 50 s rest), and found greater decreases in torque during the alternating current than pulsed current EMS. However in the present study, no significant decreases in isometric torque were observed over 30 isometric contractions for both waveforms (Fig. 4b). Laufer and Elboim (2008) compared alterneting current delivered at 2500 Hz (modulated either at 20 Hz or 50 Hz) and pulsed current delivered at 50 Hz for force integral of the wrist extensors over 21 consecutive isometric contractions (3 s ramp up, 7 s on, 3 s off). They reported that decreases in the force integral were significantly greater for alternating current compared with the pulsed current, and stated that this was related to a failure of the action potential to propagate within muscle fibers due to "high-frequency fatigue" as mentioned above. In the present study, it is possible that the long interval (15 s off-time) between contractions might

have provided a sufficient time for recovery of high-frequency fatigue, thus the torque was not decreased over 30 contractions (Fig. 4b). It should be noted that the EMS-evoked peak torque significantly increased over 30 isometric contractions only for the pulsed current EMS session in response to the increase in the stimulation intensity (Fig. 4a, b). This may indicate that some muscle fibres were unable to be recruited in response to the increase in the stimulation intensity in alternating current EMS. It is also important to note that the subjects were able to tolerate higher stimulation intensity during both EMS protocols, and the stimulation intensity increased about 65% over the 30 contractions. It appears that the pain tolerance increased during EMS, which could be due to the increase in threshold of pain receptors (Mackel and Brink 1995; Owens and Malone 1983).

To the best of our knowledge, only one study (Kooistra et al. 2008) investigated vastus lateralis muscle oxidative metabolism during pulsed current EMS in healthy subjects using NIRS. Kooistra et al. (2008) found no difference in muscle $O_2$ consumption when compared 10 s of EMS isometric contraction (50–70 Hz, pulsed duration: 100 μs at the highest tolerated intensity) and 15 s of a maximal voluntary isometric contraction at different angles of the knee extensors. However, they recorded the changes in $O_2$ consumption during single sustained isometric contractions, which may differ from intermittent isometric contractions performed repeatedly in the present study. As shown in Fig. 4c, the changes in $\Delta TOI_{max}$ were not different between alternating current and pulsed current (Fig. 4c), which may indicate a similar $O_2$ supply relative to $O_2$ consumption during the relaxation phase between waveforms. $\Delta TOI_{max}$ increased significantly over 30 isometric contractions, which is likely to indicate that $O_2$ supply during the relaxation phase increased. In contrast, $\Delta TOI_{min}$ was significantly lower in pulsed current from the 18th contraction onwards when compared with alternating current. The dynamic balance between $O_2$ demand and $O_2$ supply is reflected in the changes in $\Delta TOI$, and the changes are affected by $O_2$ extraction and blood flow/volume (Hamaoka et al. 2007). Since blood flow/volume did not appear to be different between the waveform conditions as shown in the similar changes in $\Delta tHb$ between conditions (Fig. 4d), the difference in the $\Delta TOI_{min}$ during the last 13 isometric contractions could be attributed to a difference in $O_2$ consumption between conditions.

The gradual increases in $\Delta TOI_{min}$ during the alternating current EMS may indicate decreases in $O_2$ consumption due to a smaller muscle volume being recruited. As discussed above, the torque data showed possible incapability of recruiting additional motor units in alternating current EMS. The decrease in peak torque during the alternating current EMS-evoked isometric contractions (Fig. 2) may

explain the smaller decrease in $\Delta$TOI during the contraction phase, which results in gradual increases in $\Delta$TOI$_{min}$ for the alternating current EMS compared with pulsed current EMS from the 18th contraction onwards (Fig. 4c). It appears that the difference in $\Delta$TOI$_{min}$ observed between the waveforms is mainly due to the difference in evoked torque. Since the evoked torque toward the end of the EMS session was greater for pulsed current than alternating current EMS, the former required more O$_2$. It seems possible that the higher frequency stimulation in the alternating current EMS decreased acetylcholine release at the neuromuscular junction, inadequately depolarised the sarcolemma resulting in a failure of action potential propagation along the sarcolemma and/or impaired excitation–contraction coupling (Sieck and Prakash 1995; Ward and Robertson 2000), thus inducing a lower evoked torque during this condition.

The changes in $\Delta$tHb were similar between the waveform conditions (Fig. 4d). As shown in Fig. 2, $\Delta$tHb decreased and sustained during contractions ($\Delta$tHb$_{mean}$) but rapidly recovered and increased above baseline ($\Delta$tHb$_{max}$) during relaxation, which would indicate that blood flow was occluded during contractions but resumed between contractions. It has been shown that blood flow to the biceps brachii was completely impeded during sustained isometric contractions at 50% MVC, due to increased intramuscular pressure compressing blood vessels (Sadamoto et al. 1983). If this is also the case for vastus lateralis and vastus medialis muscles during knee extension isometric contractions, it seems reasonable to assume that muscle blood flow was completely occluded during isometric contractions evoked by EMS in the present study. In fact, $\Delta$tHb decreased rapidly at the onset of contraction and did not change during contraction (Fig. 2). However, the blood flow was restored during the interval between contractions for both stimulation conditions similarly. The gradual increases in $\Delta$tHb$_{max}$ over 30 contractions appear to correspond the gradual decreases in $\Delta$tHb$_{mean}$ (Fig. 4d), indicating greater blood flow in the region of NIRS investigation, particularly in the relaxation phases.

The difference in the muscle force characteristics between alternating and pulsed current EMS should be considered by therapists or trainers when designing a treatment or training program using EMS. It appears that muscle contractions in alternating current EMS are not as strong as those in pulsed current EMS, since muscle force produced during the alternating current EMS-evoked isometric contractions is not as stable as that in pulsed current EMS. Thus, if it is necessary to maximise mechanical stimulus to the stimulated muscles, it is better to use pulsed current EMS than alternating current EMS. In fact, several authors (Grimby and Wigerstad-Lossing 1989; Laufer and Elboim 2008; Laufer et al. 2001; Lyons et al. 2005;

Stefanovska and Vodovnik 1985) have already documented that pulsed current is preferable to alternating current EMS, since the pulsed current is less fatiguing and induces more constant contraction in comparison to alternating current. Further studies are necessary to investigate whether a long-term use of pulsed current EMS is more beneficial for increasing muscle strength and muscle volume when compared with alternating current.

In summary, the present study showed that decreases in muscle oxygenation during the contractions phases were greater for pulsed current EMS than alternating current EMS using NIRS. This was likely associated with no increases in torque in the alternating current EMS despite the increases in stimulation intensity, and less stable force output in the alternating current EMS when compared with the pulsed current EMS. Further study is necessary to investigate the underlying mechanisms of the difference in the muscle force output characteristics between alternating and pulsed current EMS.

## References

Binder-Macleod SA, Halden EE, Jungles KA (1995) Effects of stimulation intensity on the physiological responses of human motor units. Med Sci Sports Exerc 27:556–565

Cettolo V, Ferrari M, Biasini V, Quaresima V (2007) Vastus lateralis O$_2$ desaturation in response to fast and short maximal contraction. Med Sci Sports Exerc 39:1949–1959

Chance B, Nioka S, Kent J, McCully K, Fountain M, Greenfeld R, Holtom G (1988) Time-resolved spectroscopy of hemoglobin and myoglobin in resting and ischemic muscle. Anal Biochem 174:698–707

Felici F, Quaresima V, Fattorini L, Sbriccoli P, Filligoi GC, Ferrari M (2009) Biceps brachii myoelectric and oxygenation changes during static and sinusoidal isometric exercises. J Electromyogr Kinesiol 19:e1–e11

Gondin J, Giannesini B, Vilmen C, Dalmasso C, le Fur Y, Cozzone PJ, Bendahan D (2010) Effects of stimulation frequency and pulse duration on fatigue and metabolic cost during a single bout of neuromuscular electrical stimulation. Muscle Nerve 41:667–678

Gorgey AS, Mahoney E, Kendall T, Dudley GA (2006) Effects of neuromuscular electrical stimulation parameters on specific tension. Eur J Appl Physiol 97:737–744

Grimby G, Wigerstad-Lossing I (1989) Comparison of high- and low-frequency muscle stimulators. Arch Phys Med Rehabil 70:835–838

Hamaoka T, McCully KK, Quaresima V, Yamamoto K, Chance B (2007) Near-infrared spectroscopy/imaging for monitoring muscle oxygenation and oxidative metabolism in healthy and diseased humans. J Biomed Opt 12:062105

Hampson NB, Piantadosi CA (1988) Near infrared monitoring of human skeletal muscle oxygenation during forearm ischemia. J Appl Physiol 64:2449–2457

Holcomb WR, Golestani S, Hill S (2000) A comparison of knee-extension torque production with biphasic versus Russian current. J Sport Rehabil 9:229–239

Jones DA (1996) High-and low-frequency fatigue revisited. Acta Physiol Scand 156:265–270

Jubeau M, Sartorio A, Marinone PG, Agosti F, Van Hoecke J, Nosaka K, Maffiuletti NA (2008) Comparison between voluntary and stimulated contractions of the quadriceps femoris for growth hormone response and muscle damage. J Appl Physiol 104:75–81

Kooistra RD, de Ruiter CJ, de Haan A (2008) Knee angle-dependent oxygen consumption of human quadriceps muscles during maximal voluntary and electrically evoked contractions. Eur J Appl Physiol 102:233–242

Laufer Y, Elboim M (2008) Effect of burst frequency and duration of kilohertz-frequency alternating currents and of low-frequency pulsed currents on strength of contraction, muscle fatigue, and perceived discomfort. Phys Ther 88:1167–1176

Laufer Y, Ries JD, Leininger PM, Alon G (2001) Quadriceps femoris muscle torques and fatigue generated by neuromuscular electrical stimulation with three different waveforms. Phys Ther 81:1307–1316

Lyons CL, Robb JB, Irrgang JJ, Fitzgerald GK (2005) Differences in quadriceps femoris muscle torque when using a clinical electrical stimulator versus a portable electrical stimulator. Phys Ther 85:44–51

Mackel RG, Brink EE (1995) Accommodation in single human nerve fibers in vivo. Muscle Nerve 18:469–471

McCully KK, Hamaoka T (2000) Near-infrared spectroscopy: what can it tell us about oxygen saturation in skeletal muscle? Exerc Sport Sci Rev 28:123–127

Owens J, Malone TR (1983) Treatment parameters of high frequency electrical stimulation as established on the Electro-Stim 180. J Orthop Sports Phys Ther 4:162–168

Quaresima V, Lepanto R, Ferrari M (2003) The use of near infrared spectroscopy in sports medicine. J Sports Med Phys Fitness 43:1–13

Robertson V, Ward AR, Low J, Reed A (2006) Electrotherapy explained: principles and practice. Butterworth-Heinemann Ltd, London

Rooney JG, Currier DP, Nitz AJ (1992) Effect of variation in the burst and carrier frequency modes of neuromuscular electrical stimulation on pain perception of healthy subjects. Phys Ther 72:800–806 discussion 807–809

Sadamoto T, Bonde-Petersen F, Suzuki Y (1983) Skeletal muscle tension, flow, pressure, and EMG during sustained isometric contractions in humans. Eur J Appl Physiol Occup Physiol 51:395–408

Selkowitz DM (1985) Improvement in isometric strength of the quadriceps femoris muscle after training with electrical stimulation. Phys Ther 65:186–196

Sieck GC, Prakash YS (1995) Fatigue at the neuromuscular junction. Branch point vs. presynaptic vs. postsynaptic mechanisms. Adv Exp Med Biol 384:83–100

Snyder-Mackler L, Garrett M, Roberts M (1989) A comparison of torque generating capabilities of three different electrical stimulating currents. J Orthop Sports Phys Ther 10:297–301

Snyder-Mackler L, Delitto A, Stralka SW, Bailey SL (1994) Use of electrical stimulation to enhance recovery of quadriceps femoris muscle force production in patients following anterior cruciate ligament reconstruction. Phys Ther 74:901–907

Stefanovska A, Vodovnik L (1985) Change in muscle force following electrical stimulation. Dependence on stimulation waveform and frequency. Scand J Rehabil Med 17:141–146

Suzuki S, Takasaki S, Ozaki T, Kobayashi Y (1999) Tissue oxygenation monitor using NIR spatially resolved spectroscopy. In: Britton C, Robert RA, Bruce JT (eds) SPIE, pp 582–592

Ward AR (2009) Electrical stimulation using kilohertz-frequency alternating current. Phys Ther 89:181–190

Ward AR, Robertson VJ (2000) The variation in fatigue rate with frequency using kHz frequency alternating current. Med Eng Phys 22:637–646

Ward AR, Shkuratova N (2002) Russian electrical stimulation: the early experiments. Phys Ther 82:1019–1030

# Chapter 5 (Study 3)

ORIGINAL ARTICLE

# Less indication of muscle damage in the second than initial electrical muscle stimulation bout consisting of isometric contractions of the knee extensors

Abdulaziz Aldayel · Marc Jubeau ·
Michael R. McGuigan · Kazunori Nosaka

**Abstract** This study compared the first and second exercise bouts consisting of electrically evoked isometric contractions for muscle damage profile. Nine healthy men (31 ± 4 years) had two electrical muscle stimulation (EMS) bouts separated by 2 weeks. The knee extensors of one leg were stimulated by biphasic rectangular pulses (75 Hz, 400 μs, on–off ratio 5–15 s) at the knee joint angle of 100° (0°, full extension) to induce 40 isometric contractions, while the current amplitude was increased to maintain maximal force generation. Maximal voluntary isometric contraction (MVC) torque of the knee extensors at 100°, muscle soreness, pressure pain threshold and plasma creatine kinase (CK) activity were used as indirect markers of muscle damage, and measured before and 1, 24, 48, 72 and 96 h after EMS bout, and the changes over time were compared between bouts. The torque produced during exercise was approximately 30% of MVC, and no significant difference between bouts was evident for the changes in peak and average torque over 40 contractions. MVC decreased significantly ($P < 0.05$) by 26% immediately and 1 h after both bouts, but the recovery was significantly ($P < 0.05$) faster after the second bout (100% at 96 h) compared with the first bout (81% at 96 h). Development of muscle soreness and tenderness, and increases in plasma CK activity were significantly ($P < 0.05$) smaller after the second than the first bout. These results show that changes in muscle damage markers were attenuated in the second EMS bout compared with the initial EMS bout.

**Keywords** Maximal voluntary contraction · Creatine kinase · Muscle soreness · Pressure pain threshold · Repeated bout effect

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## Introduction

Electrical muscle stimulation (EMS) has been used clinically and in sport training to improve muscle function (Hainaut and Duchateau 1992; Lake 1992). EMS training has been shown to be effective for enhancing muscle strength (Balogun et al. 1993; Gondin et al. 2005), power (Maffiuletti et al. 2002; Malatesta et al. 2003) and muscle endurance (Kim et al. 1995b), and minimizing muscle atrophy caused by disuse (Eriksson and Haggmark 1979; Gibson et al. 1989). While EMS provides these benefits, it is known that EMS is limited by pain during stimulation (Ward and Robertson 1998) and could result in delayed onset muscle soreness (Butterfield et al. 1997). However, limited research has been conducted concerning delayed onset muscle soreness and recovery of muscle function after EMS-evoked isometric contractions.

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Mackey et al. (2008) have recently shown histological evidence of muscle damage such as macrophage infiltration, desmin negative fibres and $z$-line disruption after 180 isometric contractions of the gastrocnemius muscle evoked by EMS (60 Hz, duration 300 μs, on–off ratio 4–6 s), together with increased plasma creatine kinase (CK) activity and muscle soreness. However, muscle strength measures, which have been proposed as the best indicator of muscle damage (Warren et al. 1999), were not included in the study. Jubeau et al. (2008) compared between 40 isometric contractions of the knee extensors evoked by EMS (75 Hz, duration 400 μs, on–off ratio 6.25–20 s) and 40 voluntary isometric contractions of the same muscles performed at the same intensity. They showed that muscle soreness peaked 48 h after EMS and serum CK activity exceeded 3,000 IU $l^{-1}$ at 72 h post-EMS; however, no such changes were evident after voluntary isometric contractions. They also reported a significantly greater decrease in maximal voluntary isometric contraction (MVC) torque after EMS (−22%) compared with voluntary contractions (−9%) at 45 min post-exercise; however, no muscle strength measures were taken after that time point to follow the recovery process.

It is well documented that the magnitude of muscle soreness developed after exercise is attenuated and recovery of muscle function is enhanced when the same or similar eccentric exercise is performed within several weeks (Mair et al. 1995; Newham et al. 1987). This protective effect induced by a single bout of eccentric exercise is referred to as the repeated bout effect (McHugh et al. 1999). It is possible that the second EMS bout does not induce as severe muscle damage as the initial EMS bout. In fact, Nosaka et al. (2002) reported that the second bout of eccentric exercise in which the elbow flexors were forcibly stretched while being stimulated by EMS resulted in smaller changes in MVC strength, range of motion, upper arm circumference and muscle thickness, muscle soreness, plasma CK and aspartate aminotransferase activities compared with the initial bout of the same exercise performed 2 weeks before. Black and McCully (2008) have recently shown that changes in T2 relaxation time of magnetic resonance images and muscle soreness following 80 lengthening contractions of the quadriceps femoris stimulated by EMS were significantly smaller after the second bout compared with the first bout that was performed 7 weeks before. However, it should be noted that these studies compared the first and second EMS bouts consisting of lengthening contractions. No previous study has investigated the repeated bout effect for EMS bouts consisting of isometric contractions that are generally performed in EMS training, for changes in indirect markers of muscle damage.

Therefore, the present study tested the hypothesis that the second bout of EMS exercise consisting of 40 electrically evoked isometric contractions of the knee extensors performed 2 weeks after the initial EMS would result in smaller changes in MVC torque of the knee extensors, muscle soreness and tenderness, and plasma CK activity following EMS, and faster recovery of muscle function, compared with the first bout.

## Methods

### Study design

Nine volunteers participated in two EMS sessions separated by 2 weeks. The 2-week interval between bouts was chosen, because it appeared that the duration was long enough for the muscles to recover from the initial EMS bout, but maximal protective effect if any could be observed, based on previous studies in which maximal voluntary eccentric exercise of the elbow flexors was investigated (Newham et al. 1987; Nosaka et al. 2001). For each session, the subjects were asked to report to the laboratory for six occasions (1–3 days before EMS, EMS day and four consecutive days after EMS). Each EMS session consisted of 40 electrically evoked isometric contractions of the knee extensors of one leg, which was randomly chosen but counterbalanced among subjects, and the same leg was used for the second bout. Each subject performed the two EMS sessions at the same time of the day, and all EMS sessions were performed between 8.00 a.m. and 10.00 a.m. The intensity of EMS and torque induced by each stimulation was monitored and recorded, and rating of perceived exertion (RPE) was assessed every 2 min (six contractions) during EMS that took 15 min to be completed. The independent variable in this study was the first versus second EMS bouts, and the dependent variables to assess muscle damage consisted of MVC torque of the knee extensors, muscle soreness and tenderness and plasma CK activity. These measures were taken 1–3 days before, immediately before and 1, 24, 48, 72 and 96 h following exercise. The MVC was also assessed immediately after EMS. The subjects were asked to remain in the seat of the dynamometer until the 1 h post-EMS measurements for the EMS session day. The study was approved by the University Human Research Ethics Committee, and the study was conducted in conformity with the Declaration of Helsinki.

### Subjects

Nine healthy men (mean ± SD age 31.3 ± 4.7 years, body mass 76.3 ± 11.2 kg, height 173.2 ± 4.2 cm), who had not been involved in any resistance training programme for

at least 6 months prior to the study, were recruited for this study. During the experimental period, subjects were asked not to change their regular physical activities and diet habits. All subjects were informed about the study, and a written informed consent form was obtained prior to participation. Subjects were requested to avoid consuming caffeine and alcohol 1 day before the EMS session and not to complete any extra physical activity during the experimental period. A familiarization session was completed by the subjects at least 4 days before the data collection, which included two MVC measures at 100° and EMS to evoke three to five isometric contractions at submaximal intensity.

Electrical muscle stimulation

Electrical muscle stimulation was applied while each subject seated on a Biodex isokinetic dynamometer chair (Biodex Medical Systems, Inc., USA) with the knee joint angle of 100° (0° corresponding to the full extension) and the trunk angle of 110°. This knee joint angle was chosen because a previous study (Jubeau et al. 2008) showed indications of muscle damage following EMS performed at this angle (100°). For minimizing any possible movement of hip and thigh during the contractions, straps were bound across the pelvis and chest.

The quadriceps femoris muscles were stimulated by an Intelect Advanced® Colour Stim (Chattanooga Group, TN, USA). Fours self-adhesive electrodes were placed on the anterior surface of one thigh based on a previous study (Jubeau et al. 2008). Prior to fix the electrodes, the skin surface was cleaned with alcohol pads. Two positive electrodes (50 mm × 50 mm) were placed over the motor points of the vastus lateralis and vastus medialis muscles, which were identified as the sites on the skin where the smallest current amplitude was required to produce muscle contraction (Robertson et al. 2006). Two negative electrodes (50 mm × 100 mm) were placed on the proximal portion of the quadriceps femoris muscle. The placement of the electrodes was kept as consistent as possible between bouts by the same investigator identifying the sites with the same procedures between bouts.

A biphasic symmetrical rectangular stimulus pulses were delivered with frequency of 75 Hz, and pulse duration of 400 µs (Jubeau et al. 2008). The ratio was 5 s stimulus on-time and 15 s stimulus off time, thus the duty cycle time was 25%. The ramping time was included in "on-time" so that 1 s for the rise time and 1 s for the fall time, and only 3 s were at dosage stimulus intensity based on the previous studies (Jubeau et al. 2008; Lyons et al. 2005). In this way, an isometric contraction was evoked every 20 s, and the whole EMS session lasted for 15 min to induce 45 contractions. The stimulation amplitude (intensity) was started with 0 mA, and gradually increased for the first three to five contractions, thereafter consistently increased every one to three contractions towards maximally tolerable level of each subject to induce maximal force generation. The first five contractions were excluded from the analysis, since the stimulation intensity did not reach the level of maximally tolerable intensity, and force produced in these contractions was low (e.g. less than 10% MVC). The amplitude of each contraction was recorded, and the subjective intensity of the stimulated contractions was assessed by a Borg's standard RPE 6–20 scale (Day et al. 2004) at 1, 3, 5, 7, 9, 11, 13 and 15 min during the EMS. The torque produced in each contraction was recorded using the Biodex software and saved for later analysis for the determination of torque (peak torque, average torque) and torque time integral of each contraction. The average torque during the EMS-evoked contraction excluding the ramp time and torque time integral of each contraction was calculated using Chart data analysis software (ADInstruments, Bella Vista, NSW, Australia).

Dependent variables

*Maximal voluntary isometric contraction torque*

Maximal voluntary isometric contraction torque measures for both legs were performed on the Biodex isokinetic dynamometer. Each subject sat on the dynamometer's chair in the same setting as the EMS protocol such that the backrest angle was set at 110° of posterior incline, and the rotation axis of the knee was aligned with the rotation axis of the dynamometer's armature. MVC was measured at the knee joint angle of 100°, which was the angle used in EMS to stimulate the knee extensors. During the measurements, both arms were positioned across the chest with each hand clasping the opposite shoulder. Subjects performed three MVC for 3 s with a 30-s rest between attempts. The stimulated leg was always measured first followed by the control leg, and the time lag between the legs was approximately 10 min. Because of the time constraint, the MVC measurements of the control leg were not performed immediately after EMS. The peak torque of each contraction was calculated, and the highest value of the three attempts was used for further analysis.

*Muscle soreness*

A 100 mm visual analogue scale (VAS) was used to assess muscle soreness. Subjects were asked to rate their pain on the VAS as 0 representing "no pain" and 100 as "unbearable pain" while the muscles being palpated and upon squatting motion. For palpation, four sites on the quadriceps femoris (the midpoint between apex patellae

and the anterior superior iliac spin, 5 cm proximal and 5 cm distal to this point, vastus medialis and vastus lateralis) were checked by the investigator placing his three fingers at each site and applying pressure for 3 s. The pressure and palpation regions were standardized throughout the experiment such that the same investigator gave a consistent pressure between days and among subjects. The average values of the four sites were used for further analysis. For the muscle soreness upon squatting, subjects were asked to stand with their legs shoulder width apart, and bend their knees slowly to 90° and then return to the standing position. The pain level of the knee extensors having the EMS was reported immediately after the movement on the VAS scale.

*Pressure pain threshold*

Pressure pain threshold (PPT) was assessed using an electronic algometer (Type II, Somedic Production AB, Sollentuna, Sweden). The algometer was calibrated for each occasion, and the same investigator took all measurements using a standardized protocol. The probe head (surface area = $1.0$ cm$^2$) of the algometer was placed perpendicular to the four sites of the quadriceps femoris muscle that were used for the palpation soreness measures (the middle point of the rectus femoris, the proximal of rectus femoris, vastus medialis and vastus lateralis that were clearly marked by a water-proof ink pen). Subjects were familiarized with 5 s of pressure in no-painful ranges (0–5 kPa s$^{-1}$) before the assessments. Force was gradually applied at a constant rate of 50–60 kPa s$^{-1}$ until the subject reported the first feeling of noticeable pain. Three measurements were taken from each site sequentially with a 30 s interval between measures in the following order: the middle point of the rectus femoris, the proximal of rectus femoris, vastus medialis and vastus lateralis. The value in kilopascals (kPa) corresponding to the amount of force applied to elicit pain sensation was recorded. The mean of the three measurements for each site was used for further analysis, and the values of the four sites were averaged to assess the tenderness in quadriceps muscle.

*Plasma CK activity*

A small amount of blood sample was taken from a fingertip that had been cleaned with an alcohol swab using a spring-loaded lancet. A heparinized capillary tube was used to collect a 30 μl of whole blood and loaded onto a test strip (Reflotron$^®$ Creatinine Test Tabs) of a Reflotron spectrophotometer (Boehringer-Mannheim, Pode, Czech Republic) to assess CK activity. The measurements were duplicated, and if the difference of the two values was greater than 10%, additional measurements were taken. In this method, the

normal reference ranges for adult men are 20–220 IU l$^{-1}$ according to the instruction sheet of the test kit.

*Statistical analysis*

The normality of the data distribution was checked by a Shapiro–Wilk test, and all variables except for CK passed the test. Changes in the MVC, VAS of muscle soreness and PPT over time were compared between the first and second bouts by two-way repeated measures ANOVA. Changes in stimulation intensity (amplitude), RPE, peak and average torque, and torque time integral over 40 isometric contractions were also compared between bouts using two-way repeated measures ANOVA. When a significant interaction effect was found, a Tukey's post hoc test was followed to compare between bouts for each time point. For plasma CK activity, because of the normality was not established, a Mann–Whitney test was used to compare between bouts for the changes over time. A Student's *t* test was also used to compare peak plasma CK activity and peak muscle soreness between bouts. The significance level was set at $P < 0.05$. All data are expressed as mean ± SD.

## Results

Stimulation intensity, RPE and torque during EMS

Changes in stimulation intensity and RPE over the last 40 contractions were shown in Fig. 1. The stimulation intensity gradually increased throughout the EMS without significant difference between bouts. RPE increased in the first 9 min and was close to the maximum after 10 min of EMS, with no significant difference between bouts. No significant difference between bouts was evident for changes in peak torque, average torque (Fig. 2) and torque time integral over the last 40 contractions. The peak torque of each contraction was approximately 15–25% higher than the average torque shown in Fig. 2; however, the changes over 40 contractions were similar between the two. The pattern of changes in the torque time integral was also similar to that shown in the average torque. The peak torque evoked by EMS was approximately 25–30% of baseline MVC torque (185 ± 12 Nm). The average torque increased in the first 10–15 contractions, but no further increases were seen thereafter in spite of further increases in the stimulation intensity.
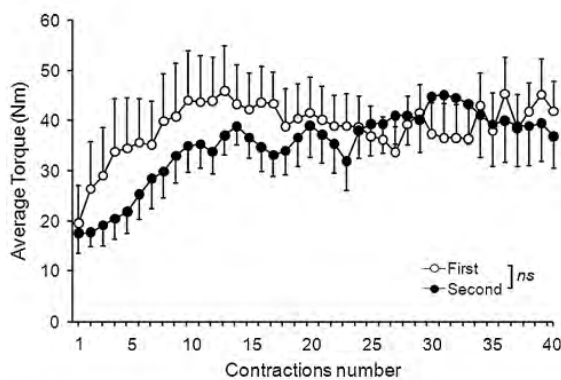
Maximal voluntary isometric contraction torque

Maximal voluntary isometric contraction torque was not significantly different between bouts before EMS for both legs. The MVC of the control leg did not change
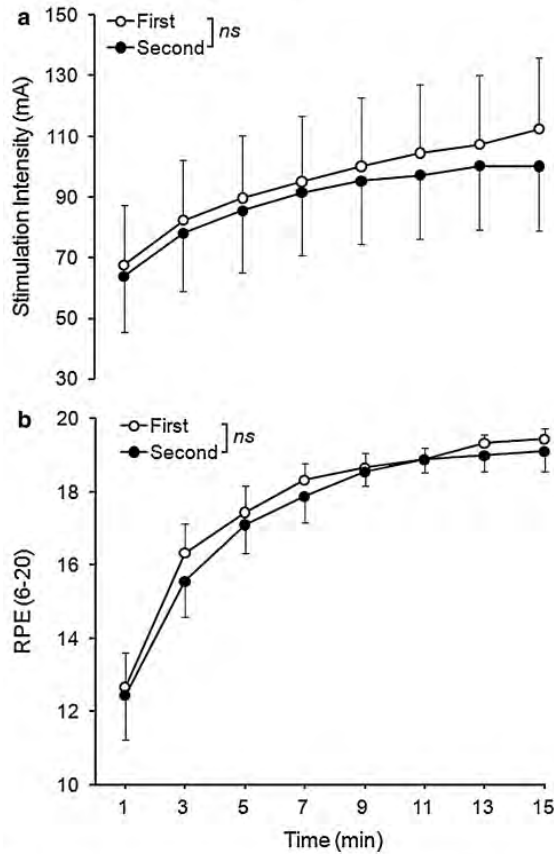
**Fig. 1** Stimulation intensity (**a**) and the rating of perceived exertion (**b**) during the first and second EMS bouts in which isometric contraction was evoked every 20 s for 15 min. The measurements were taken every 2 min starting at 1 min (after three contractions). *ns* no significant difference between bouts for the changes over time



**Fig. 2** Average torque of 40 isometric contractions during the first and second EMS bouts. *ns* no significant difference between bouts for the changes over time
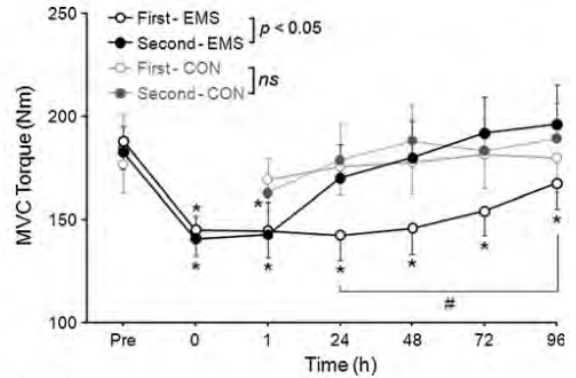


**Fig. 3** Maximal voluntary contraction isometric torque before (Pre), immediately after (0) and 1, 24, 48, 72 and 96 h after the first and second EMS bouts for the stimulated leg (*EMS*) and control leg (*CON*). *Asterisk* denotes significant ($P < 0.05$) difference from the baseline, *hash* denotes significant ($P < 0.05$) difference between the first and second bouts for the stimulated leg

significantly over time following the first and second bouts (Fig. 3). As shown in Fig. 3, MVC of the stimulated leg decreased significantly below the baseline after EMS by approximately 26% immediately and 1 h after EMS for both bouts, without a significant difference between bouts. However, the recovery of MVC was significantly faster after the second bout compared with the first bout such that the MVC returned to the baseline by 72 h post-EMS for the second bout, but it was still significantly lower than the baseline at 96 h post-EMS for the first bout (81%).
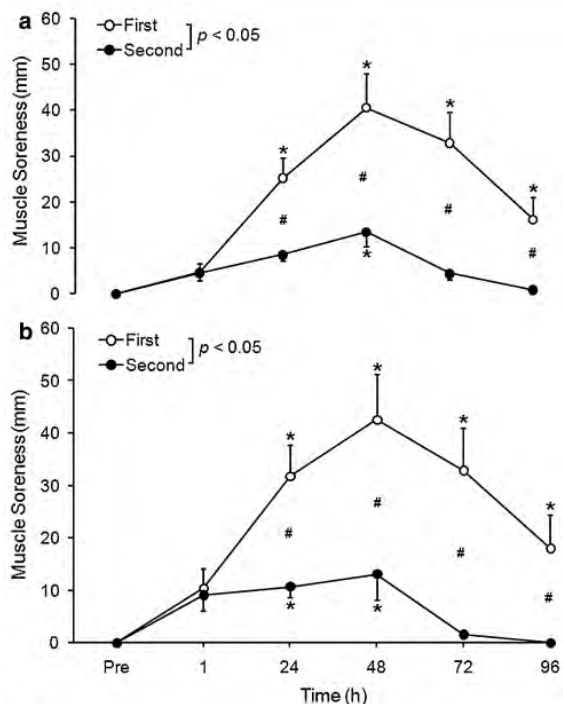
Muscle soreness

Figure 4 shows changes in muscle soreness upon palpation and in squatting before and for 4 days after the first and second EMS bouts. No significant difference in the magnitude of muscle soreness was found among the four palpation sites. The time course of changes in muscle soreness upon palpation and in squatting was similar, and muscle soreness peaked 48 h after EMS. The magnitude of muscle soreness was significantly less after the second bout compared with the first bout.
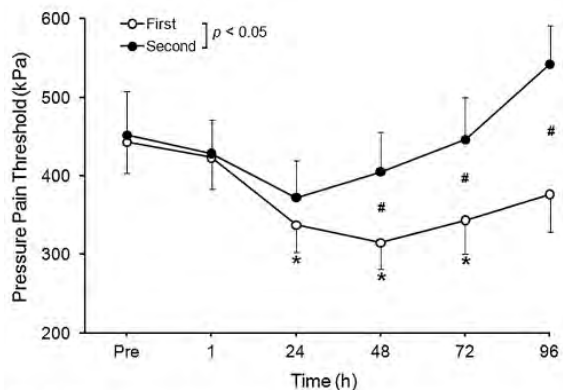
Pressure pain threshold

Muscles became more tender only after the first EMS bout, and the decrease in PPT was significantly smaller after the second bout compared with the first bout (Fig. 5).

Plasma CK activity

No significant difference was found for the pre-exercise CK activity between bouts. Plasma CK activity increased

Fig. 4 Muscle soreness (VAS) before (*Pre*), and 1–96 h after the first and second EMS bouts for the soreness upon palpation (**a**) and in squatting (**b**). *Asterisk* denotes significant ($P < 0.05$) difference from baseline, *hash* denotes significant ($P < 0.05$) difference between bouts



Fig. 5 Pressure pain threshold before (*Pre*), and 1–96 h after the first and second EMS bouts. *Asterisk* denotes significant ($P < 0.05$) difference from baseline, *hash* denotes significant ($P < 0.05$) difference between bouts

significantly only after the first bout and the highest value was observed at 96 h after EMS (Fig. 6). The peak CK activity was significantly higher after the first bout ($1{,}431 \pm 612$ IU l$^{-1}$) compared with the second bout ($208 \pm 35$ IU l$^{-1}$).
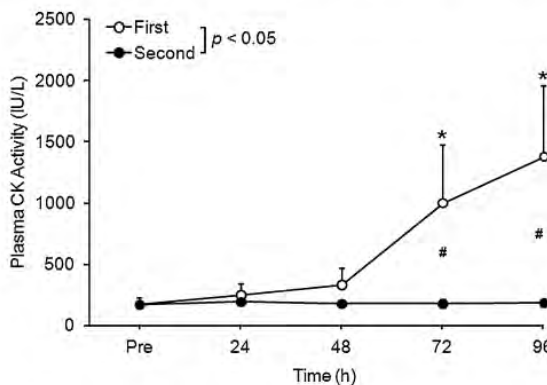


Fig. 6 Plasma CK activity before (*Pre*), and 24–96 h after the first and second EMS bouts. *Asterisk* denotes significant ($P < 0.05$) difference from baseline, *hash* denotes significant ($P < 0.05$) difference between bouts

## Discussion

This was the first study to compare between the first and second bouts of EMS-evoked isometric contractions of the knee extensors for muscle damage profile, and to report that the repeated bout effect occurs for isometric contractions induced by EMS. The results showed that decreases in MVC lasting more than 4 days, development of muscle soreness, and increases in plasma CK activity occurred after the first EMS bout. Although the same magnitude of decrease in MVC was induced immediately and 1 h after the second EMS bout, the recovery of MVC was significantly faster, and the development of muscle soreness and increases in plasma CK activity were significantly smaller following the second bout compared with the first bout. These results supported the hypothesis that less indication of muscle damage would be evident in the second EMS bout compared with the first bout, and clarified the time course of changes in MVC following EMS.

A previous study (Jubeau et al. 2008) reported that MVC decreased 22% at 45 min after EMS consisting of 40 isometric contractions at maximally tolerable intensity. The magnitude of decrease in MVC in the present study immediately and 1 h after EMS was 26% (Fig. 3), which is comparable to that shown in the previous study. The time course of changes in muscle soreness and peak muscle soreness found in the present study was similar to those reported in the previous study by Jubeau et al. (2008). However, the increase in CK activity in the blood in the previous study (peak $\sim 3{,}500$ IU l$^{-1}$) was as more than twofold as that of the present study (peak 1,372 IU l$^{-1}$). This could be due to the difference in the amount of stimulated muscles between the previous study (two legs) and the present study (one leg), and the difference in other muscles involved in the exercise between studies due to a

different posture (lying versus sitting). However, it appears that the magnitude of muscle damage to one leg induced by the EMS in the present study was comparable to that in the previous study (Jubeau et al. 2008), although there were some differences in the experimental setup. In contrast, Zorn et al. (2007) reported that no delayed onset muscle soreness was induced after 30 min of EMS (63.3 Hz, duration 400 μs, on–off ratio 3.5–4.5 s) to provoke strong tetanic contractions of the knee extensors at 90° for trained cyclists, although plasma CK activity showed a small increase at 24 h post-EMS. It is possible that the difference in the subjects between the present study (untrained) and the study by Zorn et al. (trained cyclists) attributed to the different muscle soreness responses.

It is important to note that the muscle damage in the present study was induced by isometric contractions evoked by EMS. It has been documented that isometric contractions do not result in as severe muscle damage as lengthening contractions (Nosaka et al. 2002). Black and McCully (2008) have recently reported that MVC decreased approximately 20% on 1–2 days after 80 lengthening contractions evoked by EMS, and resulted in muscle soreness (peak: approximately 60 mm in VAS). These values appear to be comparable to those in the present study, although the number of contractions (40 versus 80) and the contraction type (isometric versus eccentric) were different between the studies. Jubeau et al. (2008) showed that the magnitude of muscle damage induced by isometric contractions evoked by EMS was significantly greater than that by voluntary isometric contractions at the same intensity. However, it is not known whether maximal voluntary isometric contractions result in the same magnitude of muscle damage as the isometric contractions by EMS. It should be noted that the isometric contractions were performed at a long muscle length in the present study. Several studies (Jones et al. 1989; Saxton and Donnelly 1996) have reported that isometric contractions at a short muscle length do not induce muscle damage, but do at a longer muscle length. It may be that maximal voluntary isometric contractions of the knee extensors at a long muscle length (e.g. 100°) induce similar changes in muscle damage markers to those shown in the present study. If so, the cause of muscle damage is not EMS itself, but isometric contractions at a long muscle length. This should be investigated in future studies.

The torque evoked by EMS was estimated approximately 25–30% of the baseline MVC torque (185 ± 12 Nm) and post-EMS MVC torque (126 ± 9 Nm). Adams et al. (1993) reported using a magnetic resonance imaging technique that the cross-sectional area of the vastus lateralis activated by EMS was associated with the intensity of EMS, and approximately 25% of the cross-sectional area was activated when the stimulation induced the torque of 25% MVC. Thus, it is assumed that even

when the muscles were stimulated at maximally tolerable intensity in the present study, the number of muscle fibres that contributed to the force production was less than one-third of that during maximal voluntary contractions. It is known that muscle fibre recruitment in EMS is different from voluntary contractions such that muscle fibres are recruited non-selectively, synchronously and spatially fixed during EMS (Gregory and Bickel 2005). It is possible that the synchronous muscle fibre activation in EMS induces more mechanical stress to muscle fibres (Kim et al. 1995a; Mackey et al. 2008). Hansen et al. (2009) have recently shown greater myofibrillar proteolysis after lengthening contractions with EMS than maximal voluntary lengthening contractions, and stated that electrically stimulated muscle contractions are likely to result in greater intracellular strain and higher stress forces due to a highly uniform contraction of all muscle fibres within the stimulated region. This may be also the case for isometric contractions evoked by EMS.

When comparing between the first and second bouts, no significant differences in stimulation intensity and RPE (Fig. 1) and peak torque produced over 40 contractions (Fig. 2) were evident. It is interesting that no improvement in the tolerance to EMS was seen in the second bout. It may be that the interval between the two EMS bouts was too long to see any improvement in tolerance. It is important to note that the second EMS bout was performed after complete recovery from the first EMS bout, since there were no significant differences in any of the pre-EMS measures between bouts. Moreover, the decreases in MVC immediately and 1 h post-EMS (26%) were similar between the bouts (Fig. 3). Zory et al. (2005) reported that MVC decreased approximately 20% immediately after 30 isometric contractions of the knee extensors evoked by EMS (75 Hz, on–off ratio 6.25–20 s), and neuromuscular propagation failure was mainly responsible for the decrease. It seems that excitation–contraction coupling failure was at least partially responsible for the decreases in MVC (Ingalls et al. 2004); however, ultrastructural changes in myofibrils as shown in the study by Mackey et al. (2008) may also account for the long-lasting decreases in MVC after EMS. Therefore, the decreases in MVC immediately and 1 h following EMS in the present study are likely to reflect the combination of muscle fatigue and muscle damage, and it seems reasonable to assume that the two EMS bouts affected muscles similarly during the exercise, resulting in the similar extent of decrease in MVC within 1 h following EMS. Thus, the differences between the bouts for the changes in the dependent variables between 24 and 96 h following EMS were likely due to protective effect conferred after the first EMS bout.

The present study was the first to compare between the first and second EMS bouts consisting of isometric

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contractions; however, two previous studies (Black and McCully 2008; Nosaka et al. 2002) had compared between the first and second EMS bouts with lengthening contractions. Black and McCully (2008) compared between the first and second bouts consisting of 80 lengthening contractions of the knee extensors with EMS separated by 7 weeks, and reported that the changes in T2 and muscle soreness were significantly smaller for the second bout than the first bout. Nosaka et al. (2002) examined the elbow flexors for their responses to first and second bouts of lengthening contractions with EMS separated by 2 weeks. They showed faster recovery of MVC, smaller decrease in range of motion, less swelling of the muscle, smaller increases in muscle soreness and blood markers of muscle damage (e.g. CK) following the second bout compared with the first bout, which was similar to the repeated bout effect reported in the studies comparing two bouts of voluntary eccentric exercise (Brown et al. 1997; Nosaka and Clarkson 1995). It appears that the characteristics of the repeated bout effect found in the present study were similar to those found in the previous EMS (Black and McCully 2008; Nosaka et al. 2002) and voluntary eccentric exercise studies (Brown et al. 1997; Nosaka and Clarkson 1995).

The underlying mechanisms of the repeated bout effect have not been elucidated; however, a combination of neural, mechanical and cellular adaptations has been documented (McHugh et al. 1999). It seems that neural adaptations (e.g. more efficient recruitment of motor units, increased synchrony of motor unit firing, better distribution of the workload among muscle fibres, improved usage of synergist muscles, increased slow-twitch fibre recruitment) had little or minor contribution to the repeated bout effect in EMS found in the present study, since it is unlikely that the listed neural adaptations were induced by the initial EMS. However, it should be noted that EMS does not actually bypass the peripheral nervous system, because of the bilateral propagation of action potentials along the stimulated axons (Collins 2007), and would even activate selected brain regions in a dose–response manner (Smith et al. 2003). Thus, other neural adaptations might be involved in the protective effect conferred by the initial EMS. The repeated bout effect following EMS may be attributed more to mechanical adaptations (e.g. increases in passive or dynamic muscle stiffness, remodelling of intermediate filament system, increased intramuscular connective tissue) and/or cellular adaptations (e.g. longitudinal addition of sarcomeres, adaptation in inflammatory response, adaptation to maintain E–C coupling, strengthened plasma membrane, increased protein synthesis, increased stress proteins, removal of stress-susceptible fibres). Further muscle histological and molecular investigations are necessary to investigate the underlying mechanisms of the repeated bout effect.

The findings of the present study and previous studies (Jubeau et al. 2008; Mackey et al. 2008) show that muscle soreness and other symptoms of muscle damage (e.g. prolonged strength loss) are caused by EMS-evoked isometric contractions, but changes in the markers of muscle damage induced in the subsequent EMS bout are not as large as that after the initial bout. Thus, practitioners do not need to be concerned so much about muscle damage in EMS when it is repeatedly used, and muscle damage caused by EMS should not limit the utilization of EMS in training and/or rehabilitation. If it is necessary to avoid muscle damage in the first EMS bout, the strategies that have been used to minimize eccentric exercise-induced muscle damage such as pre-conditioning muscle by increasing the intensity, number of contractions, muscle length gradually (Jones et al. 1989; Nosaka et al. 2007; Saxton and Donnelly 1996) may be effective. However, this warrants further investigations to confirm this. It is also important to investigate further muscle damage in EMS of other modes, intensities and duration, and whether the effect of EMS on muscles is similar among different muscles. In conclusion, EMS induces symptoms of muscle damage, but a protective adaptation is conferred after the initial EMS bout, resulting in less indication of muscle damage following the second EMS bout.

## References

Adams GR, Harris RT, Woodard D, Dudley GA (1993) Mapping of electrical muscle stimulation using MRI. J Appl Physiol 74:532–537

Balogun JA, Balogun JA, Onilari OO, Akeju OA, Marzouk DK (1993) High voltage electrical stimulation in the augmentation of muscle strength: effects of pulse frequency. Arch Phys Med Rehabil 74:910–916

Black CD, McCully KK (2008) Muscle injury after repeated bouts of voluntary and electrically stimulated exercise. Med Sci Sports Exerc 40:1605–1615

Brown SJ, Child RB, Day SH, Donnelly AE (1997) Exercise-induced skeletal muscle damage and adaptation following repeated bouts of eccentric muscle contractions. J Sports Sci 15:215–222

Butterfield DL, Draper DO, Ricard MD, Myrer JW, Schulthies SS, Durrant E (1997) The effects of high-volt pulsed current electrical stimulation on delayed-onset muscle soreness. J Athl Train 32:15–20

Collins DF (2007) Central contributions to contractions evoked by tetanic neuromuscular electrical stimulation. Exerc Sport Sci Rev 35:102–109

Day ML, McGuigan MR, Brice G, Foster C (2004) Monitoring exercise intensity during resistance training using the session RPE scale. J Strength Cond Res 18:353–358

Eriksson E, Haggmark T (1979) Comparison of isometric muscle training and electrical stimulation supplementing isometric muscle training in the recovery after major knee ligament surgery: a preliminary report. Am J Sports Med 7:169–171

Gibson JN, Morrison WL, Scrimgeour CM, Smith K, Stoward PJ, Rennie MJ (1989) Effects of therapeutic percutaneous electrical stimulation of atrophic human quadriceps on muscle composition, protein synthesis and contractile properties. Eur J Clin Invest 19:206–212

Gondin J, Guette M, Ballay Y, Martin A (2005) Electromyostimulation training effects on neural drive and muscle architecture. Med Sci Sports Exerc 37:1291–1299

Gregory CM, Bickel CS (2005) Recruitment patterns in human skeletal muscle during electrical stimulation. Phys Ther 85:358–364

Hainaut K, Duchateau J (1992) Neuromuscular electrical stimulation and voluntary exercise. Sports Med 14:100–113

Hansen M, Trappe T, Crameri RM, Qvortrup K, Kjaer M, Langberg H (2009) Myofibrillar proteolysis in response to voluntary or electrically stimulated muscle contractions in humans. Scand J Med Sci Sports 19:75–82

Ingalls CP, Wenke JC, Nofal T, Armstrong RB (2004) Adaptation to lengthening contraction-induced injury in mouse muscle. J Appl Physiol 97:1067–1076

Jones DA, Newham DJ, Torgan C (1989) Mechanical influences on long-lasting human muscle fatigue and delayed-onset pain. J Physiol 412:415–427

Jubeau M, Sartorio A, Marinone PG, Agosti F, Van Hoecke J, Nosaka K, Maffiuletti NA (2008) Comparison between voluntary and stimulated contractions of the quadriceps femoris for growth hormone response and muscle damage. J Appl Physiol 104:75–81

Kim CK, Bangsbo J, Strange S, Karpakka J, Saltin B (1995a) Metabolic response and muscle glycogen depletion pattern during prolonged electrically induced dynamic exercise in man. Scand J Rehabil Med 27:51–58

Kim CK, Takala TE, Seger J, Karpakka J (1995b) Training effects of electrically induced dynamic contractions in human quadriceps muscle. Aviat Space Environ Med 66:251–255

Lake DA (1992) Neuromuscular electrical stimulation: an overview and its application in the treatment of sports injuries. Sports Med 13:320–336

Lyons CL, Robb JB, Irrgang JJ, Fitzgerald GK (2005) Differences in quadriceps femoris muscle torque when using a clinical electrical stimulator versus a portable electrical stimulator. Phys Ther 85:44–51

Mackey AL, Bojsen-Moller J, Qvortrup K, Langberg H, Suetta C, Kalliokoski KK, Kjaer M, Magnusson SP (2008) Evidence of skeletal muscle damage following electrically stimulated isometric muscle contractions in humans. J Appl Physiol 105:1620–1627

Maffiuletti NA, Dugnani S, Folz M, Di Pierno E, Mauro F (2002) Effect of combined electrostimulation and plyometric training on vertical jump height. Med Sci Sports Exerc 34:1638–1644

Mair J, Mayr M, Muller E, Koller A, Haid C, Artner-Dworzak E, Calzolari C, Larue C, Puschendorf B (1995) Rapid adaptation to eccentric exercise-induced muscle damage. Int J Sports Med 16:352–356

Malatesta D, Cattaneo F, Dugnani S, Maffiuletti NA (2003) Effects of electromyostimulation training and volleyball practice on jumping ability. J Strength Cond Res 17:573–579

McHugh MP, Connolly DA, Eston RG, Gleim GW (1999) Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. Sports Med 27:157–170

Newham DJ, Jones DA, Clarkson PM (1987) Repeated high-force eccentric exercise: effects on muscle pain and damage. J Appl Physiol 63:1381–1386

Nosaka K, Clarkson PM (1995) Muscle damage following repeated bouts of high force eccentric exercise. Med Sci Sports Exerc 27:1263–1269

Nosaka K, Sakamoto K, Newton M, Sacco P (2001) The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. Eur J Appl Physiol 85:34–40

Nosaka K, Newton M, Sacco P (2002) Responses of human elbow flexor muscles to electrically stimulated forced lengthening exercise. Acta Physiol Scand 174:137–145

Nosaka K, Muthalib M, Lavender A, Laursen PB (2007) Attenuation of muscle damage by preconditioning with muscle hyperthermia 1-day prior to eccentric exercise. Eur J Appl Physiol 99:183–192

Robertson V, Ward AR, Low J, Reed A (2006) Electrotherapy explained: principles and practice. Butterworth-Heinemann Ltd, London

Saxton JM, Donnelly AE (1996) Length-specific impairment of skeletal muscle contractile function after eccentric muscle actions in man. Clin Sci (Lond) 90:119–125

Smith GV, Alon G, Roys SR, Gullapalli RP (2003) Functional MRI determination of a dose–response relationship to lower extremity neuromuscular electrical stimulation in healthy subjects. Exp Brain Res 150:33–39

Ward AR, Robertson VJ (1998) Sensory, motor, and pain thresholds for stimulation with medium frequency alternating current. Arch Phys Med Rehabil 79:273–278

Warren GL, Lowe DA, Armstrong RB (1999) Measurement tools used in the study of eccentric contraction-induced injury. Sports Med 27:43–59

Zorn C, Szekeres T, Keilani M, Fialka-Moser V, Crevenna R (2007) Effects of neuromuscular electrical stimulation of the knee extensor muscles on muscle soreness and different serum parameters in young male athletes: preliminary data. Br J Sports Med 41:914–916

Zory R, Boerio D, Jubeau M, Maffiuletti NA (2005) Central and peripheral fatigue of the knee extensor muscles induced by electromyostimulation. Int J Sports Med 26:847–853