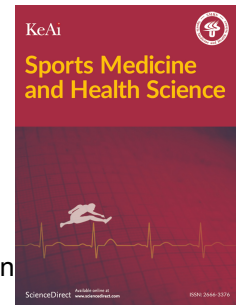# Journal Pre-proof

Change of bio-electric interferential currents of acute fatigue and recovery in male sprinters

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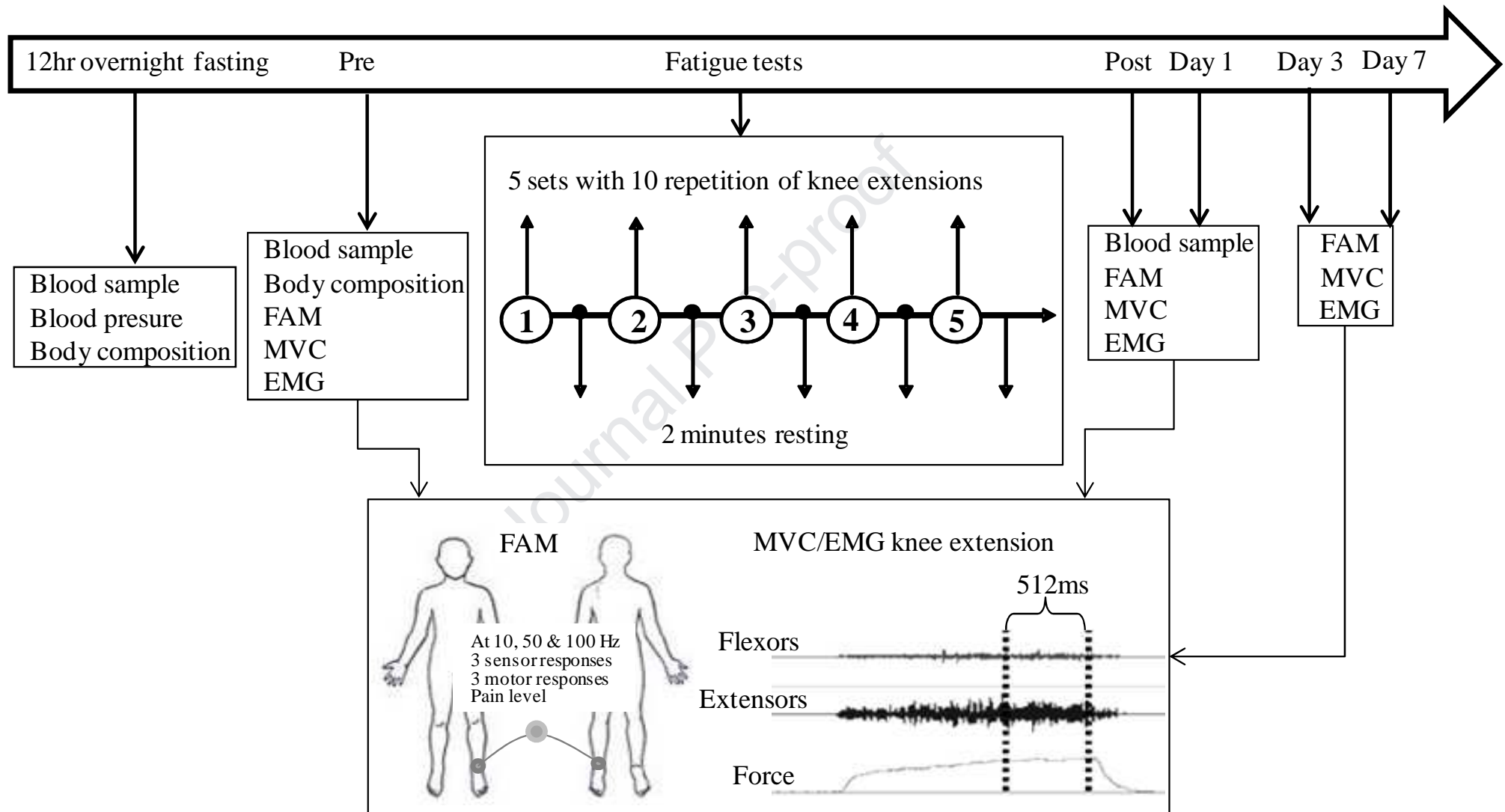
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Conflict of interest

The authors declare that there is no conflict interest regarding this study.

12hr overnight fasting   Pre   Fatigue tests   Post   Day 1   Day 3   Day 7

5 sets with 10 repetition of knee extensions

1   2   3   4   5

2 minutes resting

Blood sample
Blood presure
Body composition

Blood sample
Body composition
FAM
MVC
EMG

Blood sample
FAM
MVC
EMG

FAM
MVC
EMG

FAM

At 10, 50 & 100 Hz
3 sensor responses
3 motor responses
Pain level

MVC/EMG knee extension

512ms

Flexors

Extensors

Force

Frequency at 10Hz

P=0.022   P=0.019   Pain

P=0.033   P=0.040   Motor

P=0.004   P=0.008   Sensor

Frequency at 50Hz

Pain

Motor

P=0.004 P=0.002   Sensor

Frequency at 100Hz

P=0.031   Pain

P=0.010   Motor

P=0.054 P=0.001   Sensor

aEMG and MVC

P=0.040   aEMG/MVC

P=0.001 P=0.042 P=0.020   MVC

aEMG
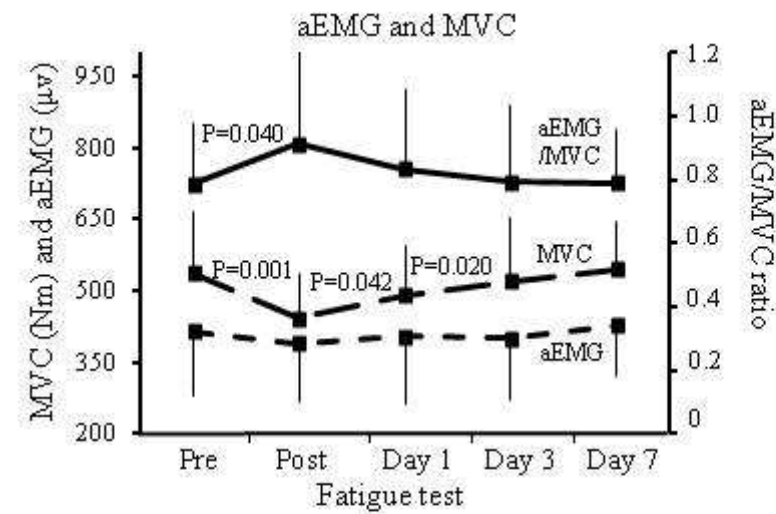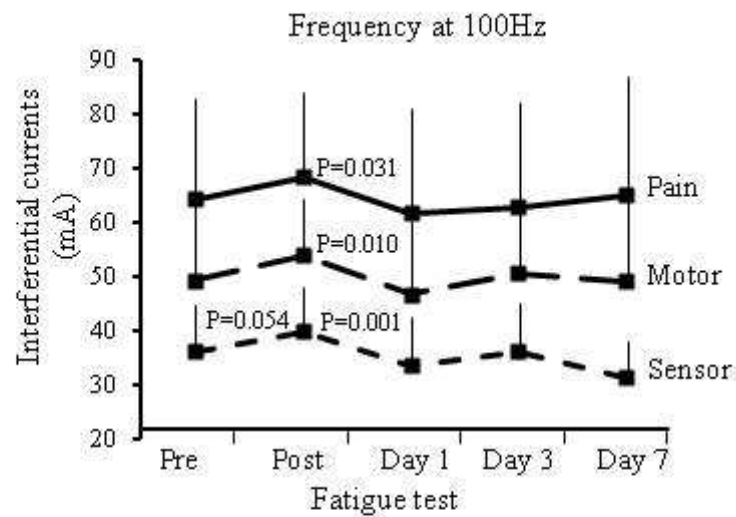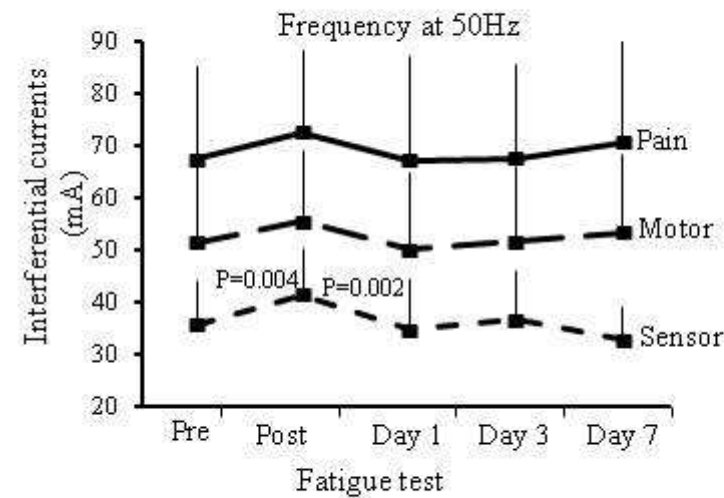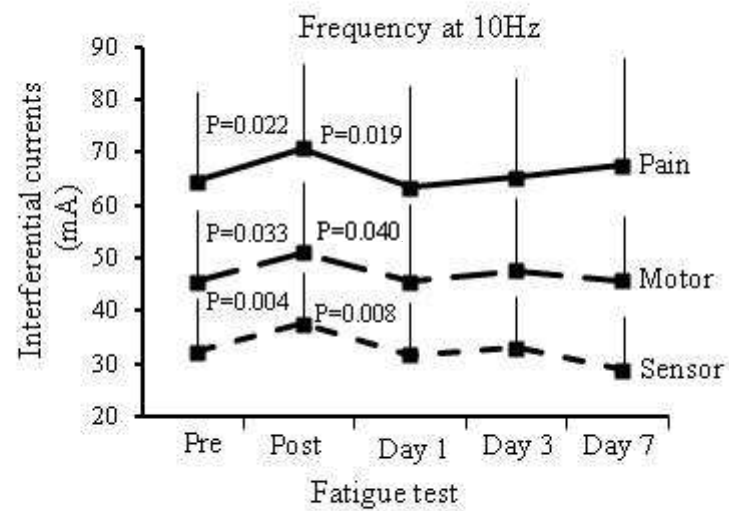
Change of bio-electric interferential currents of acute fatigue and recovery in male sprinters

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Running title: Interferential currents of fatigue and recovery

Abstract

We studied the muscle fatigue and recovery of thirty male sprinters (aged 18-22 years) using the Frequency Analysis Method (FAM). The interferential currents (ICs) with different thresholds for sensory, motor and pain responses, the maximal voluntary contraction (MVC), and the amplitude of the surface EMG (aEMG, sEMG) were assessed prior to and immediately after an acute explosive fatigue training session, and during one-week recovery. We found that IC increased on average from 32.3 ± 8.9 mA to 37.5 ± 7.5 mA in sensory response at 10 Hz immediately post training ($p = 0.004$) but decreased at 24-hr post training ($p = 0.008$) and returned to pre-levels thereafter. Motor and pain response patterns at 10 Hz were similar (motor: $p = 0.033$ and 0.040; pain: $p = 0.022$ and 0.019, respectively). The change patterns of ICs were similar to but prior to the changes of sEMG. The agreement between IC assessment and amplitude of sEMG (aEMG)/MVC ratio was good ($> 95\%$). The present study suggested that the changes in ICs were prior to the changes in both the aEMG and force during fatigue. These changes may reflect the physiological sensory change due to peripheral fatigue. FAM may be useful as an effective early detection and simple tool for monitoring muscle fatigue during training and recovery in athletes.

1. Introduction

Muscle fatigue is an important phenomenon in exercise and sport since it can directly result in decreases in physical performance and can even lead to a risk of musculoskeletal disorders and injuries.[1, 2] In addition, monitoring recovery from muscle fatigue is crucial in planning training and can help prevent overtraining and injuries. An important issue in sports training is how to detect and predict muscle fatigue, as well as to monitor the recovery of muscle fatigue, in an objective, yet easy-to-implement way.

There are several approaches to diagnose muscle fatigue and recovery including both subjective and objective methods.[3] The subjective methods usually employ questionnaires to evaluate the state of athletes.[4] Surface electromyography (sEMG) is one of the most common non-invasive objective measures.[5] It has been found that muscle fatigue is generally accompanied by increases in the amplitude of sEMG (aEMG) [6, 7] and decreases in the mean power frequency and/or median power frequency.[6-8] Due to the fact that the aEMG increases and maximal voluntary contraction (MVC) decreases during the muscle fatigue, the ratio of aEMG and MVC (aEMG/MVC) thus increases compared with the aEMG/MVC ratio prior to muscle fatigue. Therefore, the ratio of aEMG/MVC can be used as a simplified indicator of muscle fatigue and recovery.[9] Although sEMG has been widely used in assessing muscle activity and fatigue, the measurements are difficult to implement in out-of-laboratory settings due to the fact that the electrode placement with skin preparation require careful handling and that measurement system contains a lot of wires to be secured to avoid movement artefacts. In addition, the measurement devices are also quite heavy and clumsy to be carried with during training exercises and competition seasons.[10] Currently, a new development in sEMG

measurement technology has got more attention. For instance, wireless sEMG using textile electrodes embedded into clothing has been developed for use outside the laboratory setting.[10] However, the analysis of results can still be time consuming and the validation of the measurement needs further study. To date, professional athletes still do not have suitable devices, which are easy to use in practise, to optimise their training and avoid injury due to overly exerting themselves.

The Frequency Analysis Method (FAM) based on how electric interferential current induces when it is sent through human body (a method to assess the physiological sensory, motor and pain responses to certain stimulations) has provided a new and simple tool to assess neuromuscular conditions and effect of therapy.[11, 12] FAM is about electrically stimulating nerves and muscles using non-invasive surface electrodes. It is rather neuromuscular electrical stimulation (NMES) than EMG, since it actively forces electricity into body and does not just measure the endogenous electrical events.[13] The electric stimulation wave of FAM devices is comprised of two parts. There is a high frequency carrier wave, which helps the electricity to penetrate skin (thousands of Hz), and there is a low frequency modulation wave, which activates the nerves (tens of Hz). The low frequency wave interferes with the nerves in a sense of making an electric field. The field opens the voltage sensitive sodium channels and depolarizes the nerve. Using the rapidly alternating carrier wave decreases skin impedance. The resistance remains, but reactance becomes lower with increasing frequency.[14] Therefore, the stimulation can be given at varying modulation frequencies, e.g. 10-100Hz.

We hypothesize that the registration of the changes in the motor (and/or sensory and

pain) thresholds induced by the ICs may help to monitor muscle fatigue and recovery. Our hypothesis was based on two facts. Firstly, larger ionic changes are one of possible mechanisms of skeletal muscle fatigue[15], which would lead to lower propagation velocity of the muscular membrane. Interferential currents may induce changes in the ionic interaction on the muscular membrane, and – vice versa – disturbed ionic interaction due to fatigue may influence the interferential currents. Secondly, maximum muscle torque can be produced by properly applied stimulation by interferential therapy to improve neuromuscular conditions.[16] An application of the IC/FAM technology in sports is totally novel. In particular, there is no study that has examined if IC/FAM can be used to assess neuromuscular conditions of fatigue and recovery during sport training. Since early detection of fatigue is essential for prevention of sport injuries, if the assessments by IC/FAM can be proven, it will benefit professional athletes significantly. Therefore, the purpose of this study was to evaluate if bio-electric interferential current, through FAM, can be used to monitor the training and recovery of athletes.

2. Methods

2.1. Participants

Thirty male short-distance runners, Chinese national athlete rating level 2 (The 3rd grade in Chinese athlete rating system), were recruited from Chengdu Sport University as the participants of this study. It should be pointed out that, for male sprinter, the level 2 rating granted only if his 100-meter dash time is less than 11.5 seconds, or 200-meter dash time is less than 23.6 second. Lifestyle and behavioural characteristics as well as their training and

medical histories were collected via a self-administered questionnaire.

Written informed consent was obtained from the participants prior to the measurements, and the study was conducted according to the Declaration of Helsinki. Approval for the project was obtained before its initiation from the Ethics Committee of the Chengdu Sport Injury Hospital, Chengdu Sport University, China and was registered to the Chinese Clinical Trial Registration: ChiCTR-TNRC-10000959. All the protocol and procedures of the study met the ethical standards in sport and exercise science research.[17]

2.2. Protocol and Experimental Procedures

The measurements protocol is summarized in Figure 1. The participants were instructed not to have strenuous exercise on the previous day and to have fasted for 12 hours. The blood samples and body composition were measured during 7:00-8:00 am. Body mass index (BMI) was calculated as weight (kg) per height (m^2). Body composition was assessed by a bioimpedance device (Inbody 720 Biospace, Korea).

<Insert Figure 1 here>

Participants then had a meal, and 1-2 hours later underwent the following measurement procedure. The participants were familiarized with the measurement apparatus prior to proceeding with the measurements.

Interferential currents measurement

FAM device (Juno Ltd, Oulu, Finland) was used in the measurement of interferential currents, which is operated with battery. And the device can show the output peak current of 1mA stepping from 0-100 mA for both channels. The stimulation electrodes used in this study

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were AXELGAARD STIMTRODE® ELECTRODES: ST75D BG (stainless steel knit fabric conductive media with white polycoated top) with 70mm in diameter. The electrical stimulation of FAM device causes several different effects in peripheral tissue in increasing order of interferential current: 1) it activates sensory nerves causing intensifying sensations (thresholds S); 2) it activates motoric neurons causing muscle movement of intensifying proportions (thresholds M); 3) it causes sensation of pain (threshold P).

During the measurement, participants lay on a bed with feet were hanging loosely. Two electrodes were placed on the proximal side of the talocrural joint. The stimulation electrode was on the anterior position and the reference electrode was placed on the posterior position in order to obtain lower limb physiological sensory, motor and pain responses to certain stimulations which was commonly used in the clinical settings (Figure 1, lower panel ICs measurement). During the measurement, the sensibility, motor and pain tests were done subjectively, of which the participants were requested to report their feelings of a tingling, a clear sensation, a strong sensation but still no pain, and finally pain. Specifically, the currents of 7 responses from the stimulation electrode were recorded,   including the currents of 3 sensor responses (first sensation (tingling); clear sensation; and strong sensation), the currents of 3 motor responses (weak motor contraction (the first motoric movement e.g. in the small finger); clear motor contraction (repeated muscle contraction); and strong motor contraction such as bending of the ankle), and the current of pain level (painful sensation such as unpleasant feeling). Three frequencies, 10Hz, 50Hz, and 100Hz, were used separately in the measurement. Under each frequency, the current was increased constantly by the operator manually until seven different measurable thresholds mentioned above have been reached and

detected. There was a break between each different frequency of ICs measurement to allow the nerve ion storages to recharge. The stimulations of interferential currents (ICs, mA) of sensor and motor responses were repeated 3 times automatically as 1S, 2S and 3S for sensor (threshold S) and 1M, 2M and 3M for motor responses (threshold M), respectively. The stimulation of pain response (threshold P) was named as 7P. The mean stimulation level (output) to obtain the threshold of responses (ICs, mA) of these three measurements was used as the final results.

MVC measurements

MVC and sEMG of the quadriceps femoris muscle groups of both legs were performed at baseline, followed by an acute explosive exercise until exhausting with bilateral dynamic knee extension consisting of five sets of ten repetitions at maximum load.[18] The measurements were repeated immediately after the fatigue, and on recovery days 1, 3 and 7 the same time of the day as the baseline. During the recovery phase, the athletes did not perform any training but maintained their normal daily activities.

Before the measurements, the participants carried out a 10-min warm-up with stretch body arms, legs and trunk as well as stepping and knee bending. During the measurements, participants sat on a knee extension ergometer chair which used the pneumatic resistance principle (Ab Hur Oy, Kokkola, Finland). Extraneous movement of the upper body of the participant was limited by two belts across the chest and abdomen respectively, with hip joint fixed about 110°. Peak MVC strength at a knee angle of 120° during isometric knee extensions was measured simultaneously with the measurements of interferential currents and sEMG for same side leg and the measurements have been done for left and right leg

separately (Figure 1 lower panel). The participants were asked to exert maximum force as rapidly as possible and to maintain that force for at least 3 seconds. Two trials were performed by each participant and the best record was used as final result. The values of both legs were combined for analyses.

sEMG measurement and analysis

During MVC measurement, the participants wore trousers with textile embedded into clothing (Myontec Ltd, Kuopio, Finland) to measure the average rectified value of surface electromyography signals (aEMG) of both knee extensors with muscle strength simultaneously.[18] The textile electrodes are recording electrodes, sewn onto the internal surface of shorts, and consist of conductive yarns including silver fibres and non-conductive synthetic yarns woven together to form a fabric band. The recording electrodes are located such that the bipolar electrode pair lies on the distal part of the thigh perpendicular to the femur, with 39 cm^2 electrodes conductive area, and reference electrodes parallel to the femur at lateral sides, with 39 cm^2 electrodes conductive area as well. Shorts measure EMG from the area of quadriceps femoris (vastus lateralis, medialis, intermedius and rectus femoris). To ensure proper signal conduction the electrodes were moisturized with body lotion before putting on the shorts.

aEMG was calculated using the rectified signal with a 512 ms window with a sampling frequency of 1000 Hz and a frequency band 50 Hz – 200 Hz (-3 dB). The detail information regarding analysis method has been described elsewhere.[19] Briefly, the raw EMG signal was first rectified and then averaged over 100 ms intervals and stored in a module. As module was storing only averaged rectified EMG, the aEMG was reported as the average value of both

legs.

Fatigue protocol

The fatigue protocol was the same as used in early studies.[18, 20] Briefly after warm-up, the participants were required to perform concentric knee extension (isokinetic mode) from a 90° starting position to full extension (= 180°) at MVC force level. Thereafter they lowered the load back to the starting position. Each participant performed 5 sets of 10 repetitions each, with two minutes between sets. Verbal encouragement was given, and it was ensured that with every repetition a minimum target angular position of 170° was reached. If the load could not be lifted voluntarily up to the target angle the participant was assisted slightly during the last 1-3 repetitions of the set while he maintained his maximum performance.

2.3. Statistical Analysis

All data were checked for normality using the Shapiro-Wilk test in PASW Statistics 18.0 for Windows. Descriptive statistics were used to present the anthropometric data as mean ± SD. Comparisons of different time points pre- and post- fatigue tests were analysed using ANOVA with repeated measures followed by Sidak for adjustment of multiple comparisons. A *p* value of < 0.05 was considered statistically significant. Bland and Altman plot analysis [22] was used to evaluate the agreement between the two measurements, IC thresholds and sEMG, on detecting fatigue and recovery. The results of IC thresholds included the mean outputs off interferential currents (ICs, mA) for seven thresholds (1S, 2S, 3S, 1M, 2M, 3M and 1P) at three frequencies (10Hz, 50Hz and 100Hz), which were compared to aEMG/MVC ratios.[21] Because results of IC thresholds and aEMG were in different units, a ratio was calculated for each variable, such as

the ratio of IC thresholds for two adjacent measures (Post/Pre or Day1/Post) and the ratio of aEMG/MVC in the same way as for IC thresholds. The computed ratios indicated the changes in the measured variables regardless their initial units. In the Bland and Altman plots, the Y axis represents the difference between the two ratios which can be written as:

$$Y = X1-X2$$

Here, X1 = ICs_Post / ICs_Pre, or ICs_Day1 / ICs_Post and

X2 = (EMG/MVC_Post)/(EMG/MVC_Pre), or (EMG/MVC_Day1)/(EMG/MVC_Post). The X axis represents the mean of the ratios of ICs and EMG/MVC in two adjacent measures, which can be written as:

$$X = (X1+X2)/2$$

Here X1 = ICs_Post / ICs_Pre, or ICs_Day1 / ICs_Post and

X2 = (EMG/MVC_Post)/(EMG/MVC_Pre), or (EMG/MVC_Day1)/(EMG/MVC_Post).

3. Results

Background characteristics of the participants are given in Table 1. On average, the athletes had 5.7 years of specific training. They practiced 4 times a week with a typical session length of two or more hours.

<Insert Table 1 here>

The mean values of IC thresholds measurements at different measurement time points are presented in Table 2. At 10 Hz frequency, IC thresholds increased significantly in sensory, motor and pain responses immediately post-fatigue ($p < 0.05$-0.01) but decreased at day 1 (p

< 0.05-0.01) and returned to pre-fatigue levels thereafter. At 50 Hz frequency, IC thresholds increased significantly in sensory and motor responses, but not in pain response, immediately post-fatigue ($p < 0.05$) and decrease at day 1 ($p < 0.05$-0.01). However, at 100 Hz frequency, there were no statistically significant differences in IC thresholds between pre- and post-fatigue.

<Insert Table 2 here>

Significant changes in MVC were found between pre- and post- fatigue tests (Figure 2 lower right panel). The MVC decreased ($p < 0.001$). No significant change was observed in average aEMG after the fatigue test, while the aEMG/MVC ratio increased significantly ($p < 0.01$) after the fatigue test (Figure 2). The patterns of average IC thresholds change from pre- to post- fatigue test in sensory, motor and pain responses were similar to the change pattern of the aEMG/MVC ratio (Figure 2).

<Insert Figure 2 here>

Good agreements between the ratio of IC responses and the ratio aEMG/MVC were found (Figure 3). The analyses showed that for all comparison, the agreements ranged from 93.1% to 96.6%. There were either one or two individuals with values out of the 2SD range; these represented less than 5% of the cases. In addition, the mean value in the Bland–Altman plots was close to zero, indicating these two methods did not differ in assessing fatigue status.

<Insert Figure 3 here>

4. Discussion

The main finding of the present study is that the thresholds of the sensory, motor and pain responses, as elicited by ICs, increased immediately post-fatigue test but decreased at 24-hr post-fatigue test and returned to the pre-level thereafter. These changes in the thresholds concurred with the decreased muscle strength and increased aEMG/MVC ratio. A decrease in muscle strength and an increase in aEMG (or in an aEMG/MVC ratio) can be assumed as an objective evidence of an acute muscle fatigue occurring during the fatigue test.

The changing patterns of aEMG/MVC ratio are in line with early studies of muscle fatigue,[13, 23, 24] with aEMG/MVC ratio increasing immediately after fatigue testing. The different change patterns of the aEMG/MVC ratio can reveal two types of fatigue: an increased aEMG/MVC ratio is classified as 'peripheral' fatigue, and a constant aEMG/MVC ratio along with a force decrease is classified as 'central' fatigue.[9] In our study, the aEMG/MVC ratio increased significantly immediately post-fatigue testing suggests that acute fatigue induced by the high-load dynamic knee extension in sprinters is mainly attributable to impaired peripheral muscle function and is not the central fatigue.

Similar patterns of change were observed in IC thresholds as well. Due to the fact that different measurement units were used for ICs and aEMG/MVC ratio, and hence direct comparisons between these two assessments was not possible, we computed a new variable reflecting the ratio between two adjacent measures of IC thresholds or aEMG/MVC ratio. This variable enables the calculation of the agreements between IC thresholds and aEMG/MVC ratio to be examined by Bland-Altman plots. The change ratio also represented the trend of the measures from pre- to post- fatigue test and from post to one day after fatigue test. We found that FAM assessment can reflect bio-signal changes after fatigue and recovery in athletes

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similarly to that of changes found in aEMG/MVC ratio, thus it can be used as a valid indicator of fatigue and recovery during sports training. It should be point out that the evaluation of IC thresholds measurements for fatigue were mainly the reflection of peripheral fatigue based on the change pattern of aEMG/MVC ratio observed in present study, although there is a possibility that the central and peripheral fatigue may simultaneously occurred during MVC measurement.

The mechanism of using IC thresholds to detect muscle fatigue is complex and has not been studied thoroughly. The ICs were firstly used in electrotherapy because the electrical impedance of skin decreases as the frequency increases.[11, 14, 25, 26] A high-frequency current can pass the skin without much energy dissipation in the skin and consequent adverse effects. In practical using, the effective frequency in the tissue is the modulating frequency (1-250Hz).[27, 28] And the carrier frequency is higher (2.5 kHz to 10 kHz) than the maximum firing rate of the neurons or muscle fibres. With amplitude modulated at low frequencies, the signal can reach deep muscles and nerves[29, 30] to stimulate voluntary muscles, promote an increase in peripheral blood flow and reduce pain without any adverse effects.[14] Thus, in measurement of IC thresholds, asymmetrical threshold intervals can indicate a musculoskeletal status and/or disorder. However, IC threshold is relatively new measurements in terms of monitoring fatigue status thus it needs to be validated.

With regard to the current study, it should be pointed out that the places of IC thresholds measurements were taken place (talocrural joint) at a distance from fatigue (knee extensor) muscles. The locations of the electrodes of FAM device were chosen according to the manual protocol, and the easier place to measure was used because a. we wanted validation for an

easily applicable method for everyday use (and it's easier to measure from ankle and look at the toes than do the measurement more proximally); and b. it was done because it works is this way as well as needed in practice. A possible explanation for the changes in the thresholds we found in the peripheral parts of the legs during fatigue of the thigh muscles can be that the metabolic and inflammatory factors induced by fatigue may also affect more peripheral tissue. It should be pointed out that inhibition of sensory, motor and pain system may play a role in the changing thresholds of their responses. The IC measurements were taken at a different place than the muscles which were highest stressed during the effort. It might actually have been even more informative to measure closer to the affected muscles. Thus, more proximal measurement should be encouraged for further study.

There are some limitations in our study. First, the mechanism underlying IC changes during the fatigue tests is still unknown. We can only ascribe that the increase in ICs post-fatigue and decrease thereafter is connected with the fatigue-induced changes taking place in muscle. Second, the comparison of sensor and pain thresholds between days on the same participant may be dependent on the habituation of the participant. For example, the sensations ('tingling sensation'; 'clear sensation'; 'strong sensation') is still subjective measures. However, the IC thresholds results were similar to the valid EMG results and day after day measurements, participants are able to better detect the IC thresholds activation and better endure pain at higher activation level. Thirdly, the sample size of current study is relatively small and only male sprinters were studied. Studies with larger populations, including females and different sporting activities, are needed to verify the wider applicability of IC thresholds.

5. Conclusions

This study has shown that measurement of bio-electric interferential currents thresholds is equivalent to the aEMG/MVC ratio in identifying fatigue status and recovery of elite sprinters. These changes after acute fatigue tests and during recovery may reflect the physiological sensory change due to peripheral fatigue. However, further investigations based on IC thresholds are needed to verify the results in other sports events and explore the underlying mechanisms. Nerveless, FAM may be useful as an effective early detection and simple tool for monitoring muscle fatigue during training and recovery in athletes.

Abbreviation List

aEMG   amplitude of surface electromyography

BMI   body mass index

EMG   electromyography

FAM   frequency analysis method

ICs   interferential currents

MVC   maximal voluntary contraction

sEMG   surface electromyography

Conflict of interest

The authors declare that there is no conflict interest regarding this study.

Submission statement

This manuscript has not been published and is not under consideration for publication

elsewhere.

Author's contribution

Y.T. and Y.L. designed the study and wrote the manuscript. R.Y., H.X., W.N., B.Z. and C.W. conducted the experiment and analysed the data. J.L. and B.H. commented and edited the final document.

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Captions and Legends for Tables and Figures

Table 1. Background information of participants (n = 30)

Abbreviations: SD = standard deviation; BMI = body mass index.

Table 2. Descriptive statistics for FAM data before and after fatigue tests (Mean with SD given in brackets)

Abbreviations: FAM = Frequency Analysis Method; SD = standard deviation; 1S = 1st threshold of sensor response; 2S = 2nd threshold of sensor response; 3S = 3rd threshold of sensor response; 4M = 1st threshold of motor response; 5M = 2nd threshold of motor response; 6M = 3rd threshold of motor response; 7P = threshold of pain response.

[a]Indicates difference ($p < 0.05$) compared to the pre-fatigue test

[b]Indicates difference ($p < 0.01$) compared to the pre-fatigue test

[c]Indicates difference ($p < 0.05$) compared to the post-fatigue test

[d]Indicates difference ($p < 0.01$) compared to the post-fatigue test

[e]Indicates difference ($p < 0.05$) compared to one day after the fatigue test

[f]Indicates difference ($p < 0.01$) compared to one day after the fatigue test

[g]Indicates difference ($p < 0.05$) compared to three days after the fatigue test

[h]Indicates difference ($p < 0.01$) compared to three days after the fatigue test

Figure 1 Measurements protocol

Abbreviations: FAM = Frequency Analysis Method; MVC = maximal voluntary contraction; EMG = electromyography.

Figure 2 Changes of the thresholds of interferential currents at different frequencies and aEMG/MVC after fatigue test and during recovery in male sprinters

Abbreviations: MVC = maximal voluntary contraction; aEMG = amplitude of surface electromyography.

Figure 3 Bland-Altman analysis plotted from the ratio of ICs and the ratio of aEMG/MVC.

A represents pre- to post- fatigue test and B represents post- and day1 after fatigue test. The Y axis represents the difference between the ratio of ICs and the ratio of aEMG/MVC in two adjacent measures, and the X axis represents the mean of those above mentioned two ratios. The solid line in the middle represents the mean and the other solid lines represent ±2 SD for the whole sample. Each circle represents individual and filled circles represent individuals out of the range of ±2 SD.

Abbreviations: MVC = maximal voluntary contraction; aEMG = amplitude of surface electromyography; SD = standard deviation; ICs = interferential currents; Ref = reference.

Table 1. Background information of participants (n = 30)

Variables	Mean (SD)
Age (years)	20.1 (0.8)
Height (cm)	173.1 (3.9)
Weight (kg)	66.1 (7.9)
BMI	22.1 (2.5)
Fat (%)	15.8 (4.0)
Specific sport training (years)	5.7 (2.0)
Self-estimated health status: excellent/perfect/good/fair (%)	26.7/43.3/26.7/3.3
Smoking (Yes)	60%

Drinks	(Yes %)	Amount (ml/wk) mean and range
Beer	33.3	895 (200 – 2750)
Juice	10.0	1833 (1000 -3500)
Energy drink	13.3	1350 (500 – 2500)
Milk products	36.7	1138 (500 – 2000)
Soybean	20.0	1033 (400 – 1400)
Tea	20.0	2300 (600 – 7000)

Abbreviations: SD = standard deviation; BMI = body mass index.

Table 2. **Descriptive statistics for FAM data before and after fatigue tests (Mean with SD given in brackets)**

Variables		Pre	Post	Day1	Day3	Day7
10 Hz	1S	27.1 (7.5)	31.4 (8.1)^a	26.4 (7.7)^d	28.0 (7.0)^e	24.0 (4.5)^{dh}
	2S	32.8 (8.6)	36.4(8.4)^b	31.5(8.7)^d	33.0(7.5)^f	29.2(5.4)^{dh}
	3S	37.3 (10.7)	42.3(8.9)^b	37.8(10.1)^d	38.4(9.1)^f	35.0(6.7)^{dh}
	4M	41.8 (13.5)	45.3(14.1)	43.2(12.3)	43.2(12.6)	41.1(8.5)
	5M	44.5 (16.8)	53.4(12.1)^b	47.3(14.6)^d	49.0(12.2)	46.1(11.0)^d
	6M	50.7 (17.4)	58.4(12.2)	49.8(16.9)^d	54.1(13.9)	52.1(14.0)
	7P	64.2 (17.0)	69.9(17.2)^b	64.7(18.4)^c	66.0(18.1)	69.8(18.9)
50 Hz	1S	29.2 (7.3)	34.1(9.5)^a	28.6(7.7)^d	30.8(7.8)^f	26.3(5.0)^{dh}
	2S	35.7 (8.3)	40.1(10.5)^a	34.7(8.7)^d	36.7(8.9)^f	32.9(6.0)^{dg}
	3S	42.6 (9.9)	47.7(10.4)^a	41.9(10.8)^d	43.4(11.6)	40.5(8.2)^d
	4M	46.7 (14.2)	52.3(13.8)^a	47.7(11.8)^d	48.4(12.4)	47.3(11.0)^c
	5M	51.4 (15.8)	55.2(17.1)	51.1(15.4)	53.7(13.7)	55.0(13.3)
	6M	58.4 (16.7)	60.9(16.1)	53.4(18.0)^c	58.8(15.7)	58.9(19.6)
	7P	67.3 (18.3)	71.7(17.1)	68.9(18.8)	68.4(16.8)	73.3(19.3)
100 Hz	1S	30.1 (7.6)	33.2(8.9)	28.6(7.6)^c	30.3(7.8)^e	26.5(5.3)^{dh}
	2S	36.4 (8.6)	39.4(9.1)	34.1(8.2)^{ad}	36.0(8.7)^f	32.4(6.5)^{dg}
	3S	42.1 (9.4)	45.6(10.1)	40.7(10.0)^c	42.6(10.3)^f	38.3(8.9)^{dg}
	4M	46.0 (13.7)	50.2(13.4)	45.1(10.9)	47.2(13.3)	45.6(10.5)
	5M	49.7 (16.4)	55.1(14.6)	50.9(12.3)	52.9(13.1)^e	52.1(13.1)
	6M	55.2 (18.1)	57.1(19.1)	50.9(17.0)^c	56.8(16.9)	57.5(18.4)
	7P	64.3 (19.2)	67.4(17.2)	63.9(19.4)	63.7(18.4)	68.1(21.1)

Abbreviations: FAM = Frequency Analysis Method; SD = standard deviation; 1S = 1st threshold of sensor response; 2S = 2nd threshold of sensor response; 3S = 3rd threshold of sensor response;

4M = 1st threshold of motor response; 5M = 2nd threshold of motor response; 6M = 3rd threshold of motor response; 7P = threshold of pain response.

[a]Indicates difference ($p < 0.05$) compared to the pre-fatigue test

[b]Indicates difference ($p < 0.01$) compared to the pre-fatigue test

[c]Indicates difference ($p < 0.05$) compared to the post-fatigue test

[d]Indicates difference ($p < 0.01$) compared to the post-fatigue test

[e]Indicates difference ($p < 0.05$) compared to one day after the fatigue test

[f]Indicates difference ($p < 0.01$) compared to one day after the fatigue test

[g]Indicates difference ($p < 0.05$) compared to three days after the fatigue test

[h]Indicates difference ($p < 0.01$) compared to three days after the fatigue test

A. Pre to post fatigue test
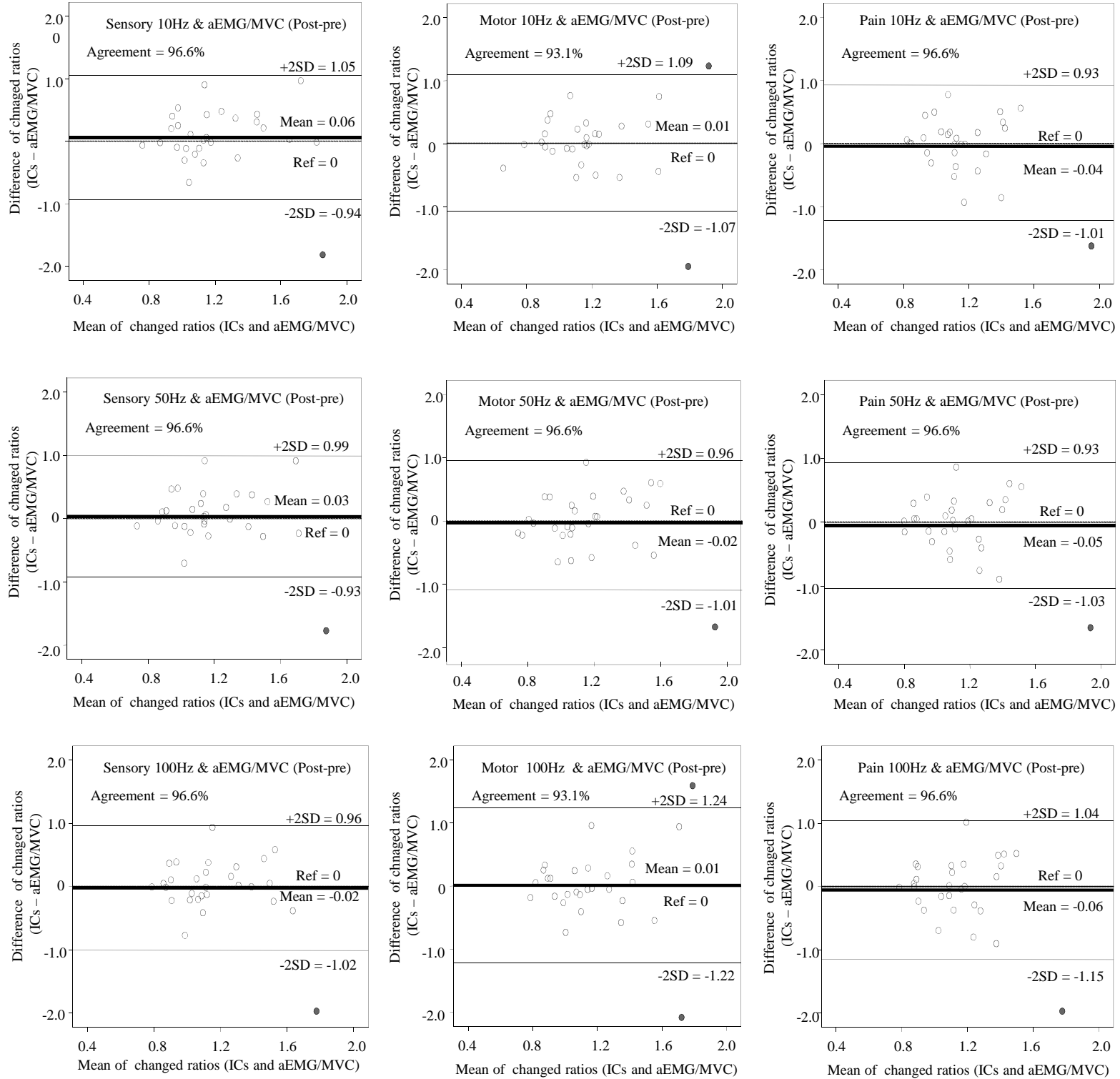
B. Post to day 1 after fatigue test

Sensory 10Hz & aEMG/MVC (Day1-post)
Agreement = 96.6%
+2SD = 0.87
Ref = 0
Mean = -0.08
-2SD = -1.02

Motor 10Hz & aEMG/MVC (Day1-post)
Agreement = 93.1%
+2SD = 0.92
Ref = 0
Mean = -0.01
-2SD = -0.95

Pain 10Hz & aEMG/MVC (Day1-post)
Agreement = 96.6%
+2SD = 0.91
Ref = 0
Mean = -0.03
-2SD = -0.98

Sensory 50Hz & aEMG/MVC (Day 1-post)
Agreement = 96.6%
+2SD = 0.83
Ref = 0
Mean = -0.09
-2SD = -1.00

Motor 50Hz & aEMG/MVC (Day 1-post)
Agreement = 96.6%
+2SD = 0.93
Ref = 0
Mean = -0.01
-2SD = -0.95

Pain 50Hz & aEMG/MVC (Day 1-post)
Agreement = 96.6%
+2SD = 1.02
Mean = 0.01
Ref = 0
-2SD = -1.01

Sensory 100Hz & aEMG/MVC (Day1-post)
Agreement = 96.6%
+2SD = 0.79
Ref = 0
Mean = -0.09
-2SD = -0.97

Motor 100Hz & aEMG/MVC (Day 1-post)
Agreement = 96.6%
+2SD = 0.90
Ref = 0
Mean = -0.06
-2SD = -1.02

Pain 10Hz & aEMG/MVC (Day 1-post)
Agreement = 96.6%
+2SD = 1.06
Ref = 0
Mean = -0.01
-2SD = -1.09

Difference of chnaged ratios (ICs – aEMG/MVC)

Mean of changed ratios (ICs and aEMG/MVC)