Original Article

Measurement of changes in the oxygenation of quadriceps muscles during the voluntary and involuntary fatigue test in normal healthy sedentary subjects

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ABSTRACT

Rehma, A,R., Siddiqui, M., & Darain, H. (2015) Measurement of changes in the oxygenation of quadriceps muscles during the voluntary and involuntary fatigue test in normal healthy sedentary subjects. *J. Hum. Sport Exerc., 10*(4), pp.867-882. The Purpose of this study is to investigate the changes in muscle oxygen consumption in response to the different fatigue protocol cycle ergometry and electrical stimulation (voluntary and involuntary) in human quadriceps muscle using near infrared spectroscopy (NIRS). Fifteen healthy sedentary voluntary University students between ages 20-60 were invited to participate in the study. Three minutes stimulation was performed to fatigue the muscle. Changes in muscle oxygenation were measured by near infrared spectroscopy. The present resistance was calculated as the estimated maximal power output. The data were analysed using the Kolmogorov-Smirnov (K-S) test to determine the distribution. Descriptive statistics are used to characterize the shape, central tendency, and variability within a set of data. Differences were tested by utilizing the Friedman test the level of statistical significance was set at $P<0.05$. There was no significant difference ($p>0.05$) was found between right leg oxygenated (ΔHbO2), deoxygenated (ΔHHb), and total haemoglobin (ΔCHb) as compared to left leg during cycle ergometry fatigue test. On the other hand, significant difference ($p<0.05$) was found in oxygenated haemoglobin of right leg when two (cycle ergometry and electrical stimulation) fatigue results were compared. However, no significant difference ($p>0.05$) was found in deoxygenated (ΔHHb) and total haemoglobin (ΔCHb) of right leg when two (cycle ergometry and electrical stimulation) fatigue results were compared. There was no significant difference ($p>0.05$) found in oxygenated, deoxygenated and total haemoglobin between right and left leg cycle ergometry fatigue indices. Similarly, no significant difference ($p>0.05$) was found in oxygenated, deoxygenated and total haemoglobin of right leg when two (cycle ergometry and electrical stimulation) fatigue indices were compared. The significant difference ($p<0.001$) were found between two (cycle ergometry and electrical stimulation) fatigue results. This study reveals that the oxygen consumption was more in the electrical stimulation as compared to the cycle ergometry during the fatigue test. Significant difference was observed between the oxygenated haemoglobin when comparing the electrical stimulation with cycle ergometry. Similarly, significant differences were found between the legs in cycle ergometry fatigue test. influences, together with exercise-induced-effects, should be considered as causes. Results show a functioning preparation-system within the DRV for better prepared-junior-athletes to commence the IPCP. **Key words** MUSCLE OXYGENATION, MUSCLE FATIGUE, ELECTRICAL STIMULATION, CYCLE ERGOMETRY, NEAR INFRARED SPECTROSCOPY.

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INTRODUCTION

Muscle fatigue presents as a reduced ability of person to exert force. It is the consequence of motor task performed over a long period of time (Lorist et al., 2002). The causes of muscle fatigue are wide-ranging with complex interaction of peripheral and central mechanism. The mechanism of fatigue development depends upon the type of activity, duration of activity as well as the type of muscle fibres activated during an activity. These in turn deter-mine the amount of oxygen consumed and the metabolic waste products produced after an activity (Behm & St-Pierre, 1997).

Developing a protocol that can identify a possible relationship between the changes in local muscle oxygenation and de-oxygenation as well as development of fatigue in muscle (voluntary and involuntary) may provide a basis from which we can investigate these relationship in populations with impaired local muscle oxidative metabolism. Investigations of local tissue changes regarding oxygenation are often invasive and complicated with limited clinical application (Hamaoka et al., 2000; McCully & Hamaoka, 2000). The development of non-invasive monitoring technique such as Near Infrared Spectroscopy (NIRS), which is reliable and cost-effective tool, allows investigation of changes in local tissue oxygenation (Boushel, et al., 2001; Mancini et al., 1994; Ferrari et al., 2004; Bhambhani, 2004). The use of NIRS shows the differences in muscle oxygenation in individuals with different level of exercise tolerance Ferrari et al., 2004) and abnormal tissue oxygenation (Boushel, et al., 2001; Mancini et al., 1994; Ferrari et al., 2004; Bhambhani, 2004; McCully & Natelson, 1999). NIRS works by delivering near infrared light to tissues and measuring the light that is not absorbed to give a relative measure of change in tissue oxygenation (McCully & Hamaoka, 2000; Boushel, et al., 2001; Mancini et al., 1994; Ferrari et al., 2004; Bhambhani, 2004). The chromophores are compounds, which absorb light at a particular wavelength or spectrum. The wavelengths used by NIRS are within the range of 700-1000nm (Boushel & Piantadosi, 2000). The light of NIRS can easily penetrate into the biological tissue and allows detection of changes in specific chromophore concentrations in human tissue. Changes in the NIRS signals attributed to haemoglobin saturation emerge primarily from the absorption of light in arterioles, capillaries, and venules (Boushel et al., 2005).

The contractile properties of the muscle and their relationship with muscle fibre are one of another important issue making differences in the oxygen consumption in different muscles with different exercise protocols. Many studies have reported that the relationship between muscle oxygenation and fatigue during exercise and tasks specific work affects the muscles oxygenation (Bhambhani, 2004; Murthy et al., 2011; Yamada et al., 2004; Tachi et al., 2004; De Ruiter et al., 2004; Blangsted et al., 2005; McNeil et al., 2006; Theurel et al., 2007). Consequently, it is difficult to attain a clear picture of muscle oxygen consumption in a fatigued muscle. A number of studies utilized electrical stimulation and cycle ergometry exercise protocols to find the oxygen consumption during the fatigue and the recovery phase.

Researchers use NIRS to study the peripheral response to exercise by utilizing the variety of dynamic exercise especially cycling, treadmill walking and running, arm cranking and in-line skating. The purpose of this literature review is to summarize the important findings from the previous studies for the further research direction. This project aims to find the changes in muscle oxygenation and de-oxygenation in response to the electrical stimulated fatigue and cycle ergometry fatigue tests of the human quadriceps muscle. The aims of study are as follows:

• Compare the changes in oxy-haemoglobin, deoxyhaemoglobin, and total- haemoglobin in the both legs occurring during a cycle ergometry fatigue test.

• Compare the changes in oxy-haemoglobin, deoxy-haemoglobin and total- haemoglo-bin in the dominant leg during cycle ergometry fatigue test and an electrical stimulation fatigue test
• Compare the fatigue indices of the two different fatigue protocols.

METHODS

Fifteen healthy sedentary voluntary University students were invited to participate in the study. Prior to testing, subjects were asked to refrain from exhaustive exercise 48 hours prior to the session, and to maintain their normal dietary habits and come to the laboratory prior to half hour of testing. All the subjects recorded were right leg dominant asking by which leg they use to kick the football. The study was conducted in a temperature con-trolled laboratory environment (21±2°C) by the air conditioned throughout the sessions. Prior to the commencement of testing, each participant completed a health-screening questionnaire. An ethical approval was obtained from the University of East London, School of Health, and Bioscience Ethics Committee. A written informed consent was obtained from the participants prior to their testing session. The study was conducted in the university Human motor performance laboratory UH-208.

Exclusion and inclusion criteria
Age criteria were 21-60 years. Participants were required to be in good general health and were not participating in regular training or physical activity with International physical activity questionnaire (IPAQ-2002) score of low. Participants were excluded from the study if they had any lower limb neuromuscular condition or pre-existing disease which would preclude them from the exercise, or any musculoskeletal injury past one year which affecting on his normal functional activity. People with joint disease (Rheumatoid arthritis, Osteoarthritis) were also excluded from the study. No exclusion was made on the ground of gender or ethnic background. In addition, the participants could leave the study at any point.

Resting measures
The subjects were asked to stop the eating or smoking before the 2 hours of the exercise were started. Prior to testing, each subject rested for a 30 min period in a seated position. During this time, Heart Rate (HR) and Blood Pressure (BP) were obtained. BP and HR was measured by using the Omron automatic oscillometric digital blood pressure model HEM-705C (Omron Corporation, Japan). If there BP is normal (120/80) then the participants were ok for the test if it is little high or low the will record again after a 15 minutes after this if it is not normal then the person excluded from the study.

Anthropometric measures
The muscle mass of the quadriceps femoris muscle was calculated from anthropometric measurement and mathematical model of (Bangsbo, 1996), and thigh volume was recorded by using formula for the thigh volume (Jones & Pearson, 1969). The method involves measuring the circumference of the thigh and the skin fold thickness at predetermined sites. The mid-point be-tween the anterior superior iliac crest and pubic tubercle was found and marked. The distance from the mid-point and the upper edge of patella were measure as a thigh length. The points at ¼, ½ and ¾ of the thigh length are marked and used as a measuring point where the circumference of the thigh (O1, O2 and O3) and the Triplicate skin fold measurements (S1, S2 and S3) were taken on the lateral side of the thigh in standing position and relaxed position of each subject by using Harpenden skinfold callipers (Holtain Ltd, Crymych, UK). The mean value of the measurements recorded at each site was used for statistical analysis. Body mass and height were measured and recorded using a balanced scale and stadiometer (Seca, Cranlea, UK). Body mass was measured to the nearest 0.1 kg, and height to the nearest 0.1 cm. All measurements, including skin fold were recorded in cm.

Nirs (niro 200)
The NIRO 200 (Hamamatsu Photonics K K, Japan) includes the spectrometer and this spectrometer connected to the monitor. The monitor was further connected to a laptop having software for NIRS and the purpose of this laptop was to store the data (McNeil, et al., 2006). Two probes, two optodes, two holders, double-sided tape, and two-stretched self-adherent tape for the light blocking.

Experimental protocol for cycle ergometry
A dynamic voluntary cycle exercise protocol performed on the Corival V2 (Lode BV Gro-ningen, The Netherlands), a bicycle ergometry. The seat height was adjusted for each subject in alignment with the greater trochanter in standing with their feet flat on the floor a change in the seat height will affect the range and length of the muscle which would subsequently affect the muscle activity pattern. Once seated on the ergometer, the foot straps were secured.

Participants were asked to stay seated and maintain the same cycling position throughout the whole test to minimize recruitment of other muscle and reduce error. Standing out of the saddle and postural changes of the hip and knee angle can affect the recruitment pattern of muscle during cycling and may compromise blood flow affecting the NIRS re-sult (Neary, 2004).

Experimental protocol for electrical stimulation
Quadriceps muscle fatigue was measured using a specially designed isometric strength-testing chair and a four strain-gauge bridge torque transducer. Electrical signals from the torque transducer were amplified (Digitimer Neurolog NL107 Recorder Amplifier) and digitised (Cambridge Electronic Design, micro1401). Torque from maximal voluntary isometric contractions (MVIC) and electrically elicited contractions of the quadriceps fem-oris muscle were recorded on a PC for subsequent analysis using Spike data analysis software (Spike 2 Version 5.0 for Windows).

Electrical stimuli were delivered to the muscle with a Digitimer DS7 constant current stimulator via two carbon rubber electrodes (EMS), one placed proximally over the muscle belly of the rectus femoris and the second placed distally over the vastus medialis. Electrical stimuli was set at an appropriate intensity to elicit force outputs of approximately 20% of the subjects' MVIC at 40Hz. Muscle fatigue was then assessed using a modified Burke protocol (Burke, 1973).

Fatigue protocol (cycle ergometry and electrical stimulation)
Relative changes (Δ) for HbO2 ($\Delta\mu$M*cm) and HHb ($\Delta\mu$M*cm) were recorded at 0.5 s intervals for both legs in cycle fatigue test and the right leg for the electrical stimulation fatigue test. Fatigue indices for the NIRS variables were calculated as for the contraction fatigue indices e.g. Fatigue index (HbO2) = HbO2 (initial) – HbO2 (final)/ HbO2 (initial). This was also calculated for values of HHb and total Hb. The HbO2, HHb and total Hb initial values were selected as the first 2.5 seconds and final values as the last 2.5 sec of the fatiguing contractions for both cycle ergometry and electrical stimulation.

Statistical analysis
Statistical Package for the Social Sciences (IBM SPSS Statistics 20.0) was used to analyse the data. The data were analysed using the Kolmogorov-Smirnov (K-S) test to deter-mine the distribution. Results indicated the spread of the data were not significantly different from normal or uniform distributions. Owing to the small (n=15), non-random sample used in this study and the inconclusive results regarding distribution of data, non-parametric tests were applied and consequently median values and the total ranges of values for each variable have also been reported. All data were expressed as means and

standard deviation (±SD in Table 1). Descriptive statistics were used to characterize the shape, central tendency, and variability within a set of data22. Differences were tested by utilizing the Friedman test the level of statistical significance was set at $p<0.05$.

RESULTS

Fifteen healthy university students (12 male and 3 female) volunteered to participate in this study. Demographic characteristics of the participants can be found in (Table1).

Table 1. Characteristics of the participants.

Variables	Mean	SD	Max	Min
Age (years)	28	3.4	33	19
BMI (Kg/m^2)	24.78	3.27	32.21	18.73
Weight (Kg)_	72.77	14.95	113	51
Height (m)	1.7	0.079	1.87	1.514
Systolic BP (mmHg)	106	7.79	97	129
Diastolic BP (mmHg)	82.5	0.5	91	63
Heart rate (bpm)	73.13	11	92	52
Upper thigh circumference (cm)	60.87	5.98	75	72
Middle thigh circumference (cm)	54.75	5.32	65	46
Lower thigh circumference (cm)	46.24	4.49	53	38
Upper Skin fold of thigh (cm)	2.642	0.86	3.8	1.1
Middle Skin fold of thigh (cm)	2.37	0.99	3.9	0.84
Lower Skin fold of thigh (cm)	2.06	1.09	4.3	0.44
Thigh length (cm)	42.53	2.39	46	37
Calculated thigh mass (Kg)	3.58	0.66	4.28	1.79
Calculated thigh volume (dm^3)	7.72	1.81	12.82	4.7

CHANGES IN ΔHBO2, ΔHHB AND ΔCHB BETWEEN RIGHT AND LEFT LEG CYCLE ERGOMETRY FATIGUE PROTOCOL.

Result shows no significant difference ($p>0.05$) between right and left legs HbO_2, HHb and CHb after the 30 seconds of fatigue test (Table 2, Fig.1). The changes in oxygen saturation between the right and left leg were recorded during the cycle ergometry fatigue test and the mean value of left leg HbO_2, HHb and CHb was higher as compared to the right leg.

Table 2. Changes in HbO_2, HHb and CHb between right and left leg cycle ergometry fatigue protocol.

µM*cm	N	Mean	SD	Max	Min	25th	50th	75th	Sig
Initial	15	-30.1	29.2	18.3	-72.1	-51	-27.3	-37.6	0.197
Final	15	-12.91	13.16	6.5	-34.6	-34.6	-7.4	-33.8	
CyR HbO2	15	-17.22	16.04	48.9	-78.6	-16.4	-19.9	-3.8	
Initial	15	-39.26	39.87	0.7	-108.5	-58	-40.1	-43.1	
Final	15	-11.6	8.776	-1	-27.4	-24.8	-11.5	-15.4	
CyL HbO2	15	-27.66	27.1	12.1	-103	-33.2	-28.6	-27.7	

Initial	15	-6.28	21.04	44	-30.5	-24.6	-7.5	-12.2	0.439
Final	15	3.72	26.41	51.8	-37.3	13.6	5.9	10.2	
CyR HHb	15	-10	-5.33	81.3	-55.1	-38.2	-13.4	-22.4	
Initial	15	-13.21	20.76	14.6	-60	0	-12.7	-38.1	
Final	15	15.53	18.58	24.4	-41.9	2.1	3.5	-41.9	
CyL HHb	15	-14.78	2.18	29.1	-77.8	-2.1	-16.2	3.8	
Initial	15	-36.4	31.83	15	-92.2	-75.6	-34.8	-49.8	0.439
Final	15	-9.19	19.07	27	-34	-23.6	-1.5	-21	
CyR CHb	15	-27.22	31.36	9.6	-84.4	-21.6	-21.3	2	
Initial	15	-52.48	51.05	-1	-168.5	-58	-52.8	-81.2	
Final	15	-10.02	19.72	12.3	-57.3	-22.7	-8	-57.3	
CyL CHb	15	-42.45	31.33	12.2	-180.8	-35.3	-44.8	-23.9	

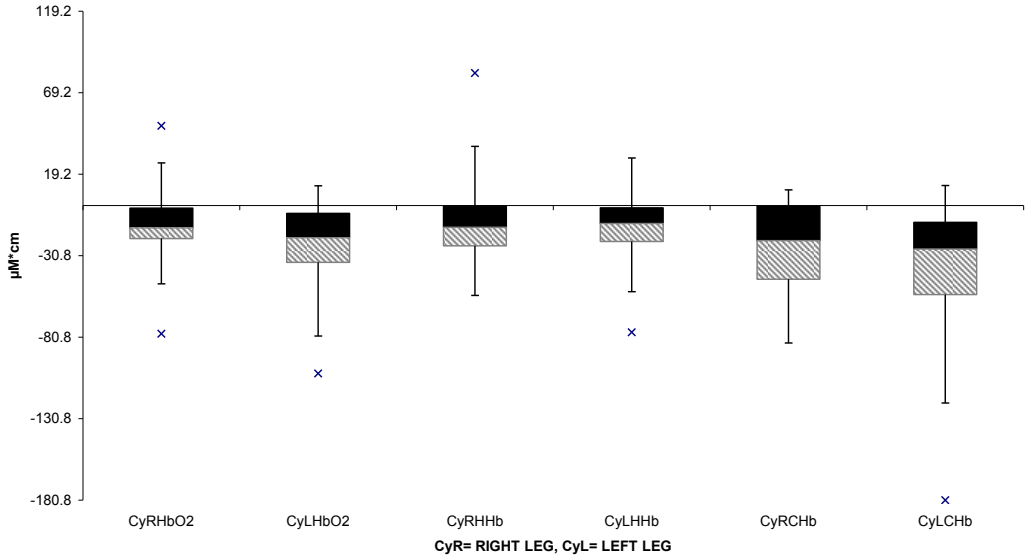

Figure 1. Shows the comparison between the right and left leg of HbO_2, HHb and CHb during cycle ergometry fatigue test.

CHANGES BETWEEN RIGHT LEGS HBO2, HHB AND CHB DURING THE CYCLE ERGOMETRY AND ELECTRICAL STIMULATION FATIGUE RESULTS

Result showed significant difference (p<0.05) between right leg HbO_2 haemoglobin as the mean value of right leg HbO_2 haemoglobin during stimulation was double (-36.44) than the mean value of right leg oxygenated haemoglobin HbO_2 during cycling (-17.22). There was no significant difference (p>0.05) between HHb and CHb, when comparing two different fatigue (cycle ergometry and electrical stimulation) results. The mean value of HHb (-10) was one third in cycle fatigue than the stimulation fatigue (-28.32).

Similarly the mean value of CHb (-27.22) was one third in cycle fatigue than the stimulation fatigue (-64.76) (Table 3, Fig. 2).

Table 3. Mean and standard deviation values of HbO_2, HHb and CHb between right legs (Cycle ergometry and electrical stimulation) results.

µM*cm	N	Mean	Sd	Max	Min	25th	50th	75th	Sig
Initial	15	-30.1	29.2	18.3	-72.1	-51	-27.3	-37.6	0.02
Final	15	-12.91	13.16	6.5	-34.6	-34.6	-7.4	-33.8	
CyR HbO_2	15	-17.22	16.04	48.9	-78.6	-16.4	-19.9	-3.8	
Initial	15	-43.48	37.06	37.06	-163.3	-52.5	-36.3	-61.98	
Final	15	-7.04	31.26	45.5	-105.6	-15.7	-9.7	0.31	
StR HbO_2	15	-36.44	5.79	96.5	-208.8	-36.6	-26.6	-62.3	
Initial	15	-6.28	21.04	44	-30.5	-24.6	-7.5	-12.2	0.439
Final	15	3.72	26.41	51.8	-37.3	13.6	5.9	10.2	
CyR HHb	15	-10	-5.33	81.3	-55.1	-38.2	-13.4	-22.4	
Initial	15	-24.09	20.7	20.7	-65.7	2.5	-22.3	-33.43	
Final	15	4.22	31.62	110	-42.2	-1.6	-5.1	-1.36	
StR HHb	15	-28.32	-10.92	33.6	-128.8	4.1	-17.2	-32.06	
Initial	15	-36.4	31.83	15	-92.2	-75.6	-34.8	-49.8	0.197
Final	15	-9.19	19.07	27	-34	-23.6	-1.5	-21	
CyR CHb	15	-27.22	31.36	9.6	-84.4	-21.6	-21.3	2	
Initial	15	-67.58	51.63	51.63	-218.7	-50	-58.6	-95.41	
Final	15	-2.81	26.65	58.6	-43.9	-17.3	-14.8	-1.05	
StR CHb	15	-64.76	24.97	24.97	-277.3	-32.7	-43.8	-94.36	

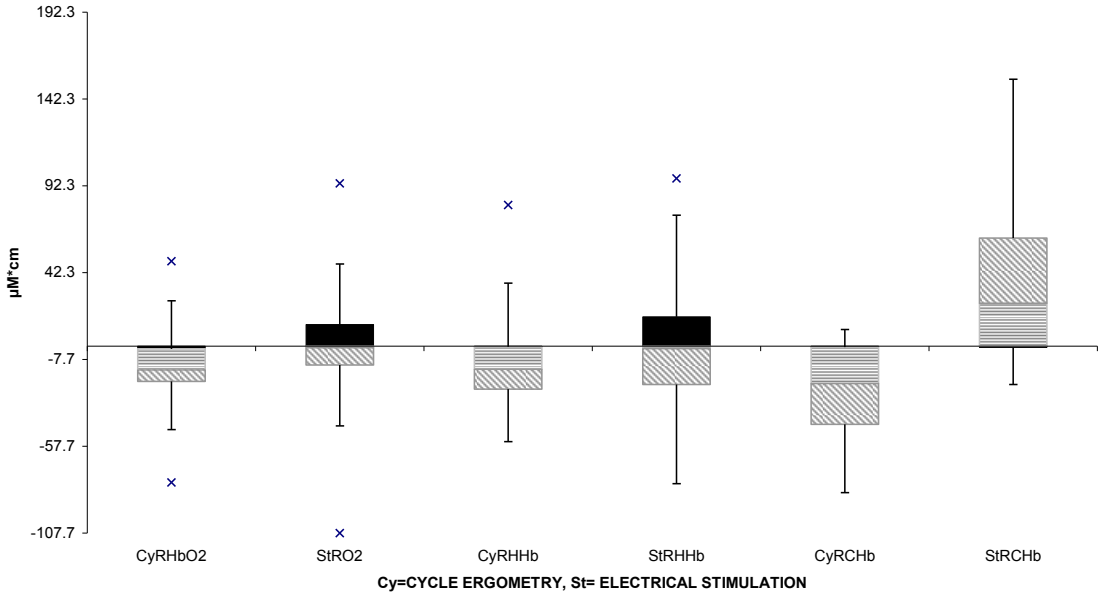

Figure 2. Comparison between right legs HbO_2, HHb and CHb during cycle ergometry and electrical stimulation fatigue test.

FATIGUE INDEXES OF HBO2, HHB AND CHB BETWEEN RIGHT AND LEFT LEGS CYCLE ERGOMETRY PROTOCOL.

There was no significant difference (p>0.05) between right and left leg HbO_2, HHb and CHb fatigue indices (Table 4, Fig. 3).

Table 4. Mean value and standard deviation between the right and left leg cycle ergometry fatigue indices.

µM*cm	N	Mean	Sd	Max	Min	25th	50th	75th	Sig
FiCyR HbO2	15	-117.55	183.41	185.51	-624.02	-118.84	-77.61	-54.4	0.071
FiCyL HbO2	15	-108.25	92.31	92.31	-414.15	-100.75	-414.15	-68.77	
FiCyR HHb	15	62.39	436.44	1566.39	-273.92	30.68	-30.1	71.16	0.796
FiCyL HHb	15	58.54	101.18	166.36	-189.9	20	9.4	14.85	
FiCyR CHb	15	-116.94	102.56	102.56	-385.75	-103.37	-100.65	-39.11	0.439
FiCyL CHb	15	-91.63	54.74	54.74	-202.75	-97.13	-113.39	-67.95	

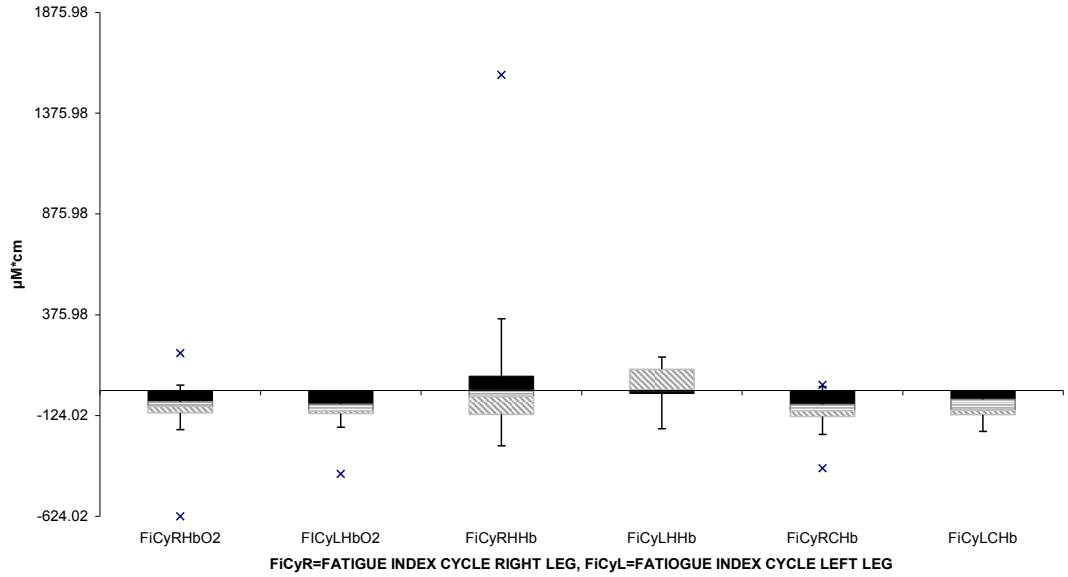

Figure 3. Differences between the right and left leg cycle ergometry fatigue indexes.

FATIGUE INDEXES OF HBO2, HHB AND CHB BETWEEN RIGHT LEG FOR CYCLE ERGOMETRY AND ELECTRICAL STIMULATION PROTOCOLS.

The result showed no significant difference (p>0.05) between right leg HbO_2, HHb, CHb fatigue indices during electrical stimulation and cycle ergometry fatigue test (Table 5, Fig.4).

Table 5. Mean values and standard deviation between the right legs fatigue index of HbO_2, HHb and CHb cycle ergometry and electrical stimulation.

µM*cm	N	Mean	SD	Max	Min	25th	50th	75th	Sig
FiCyR HbO2	15	-117.55	183.41	185.51	-624.02	-118.84	-77.61	-54.4	0.197
FiStR HbO2	15	-128.98	291.2	291.2	-1169.53	-82.4	-21.68	-63.02	
FiCyR HHb	15	62.39	436.44	1566.39	-273.92	30.68	-30.1	71.16	0.766
FiStR HHb	15	48.5	189.74	566.3	-363.66	66.5	1.66	-45.16	
FiCyR CHb	15	-116.94	102.56	102.56	-385.75	-103.37	-100.65	-39.11	0.197
FiStR CHb	15	-96.5	78.53	78.5	-279.85	-84.6	-29.53	-83.85	

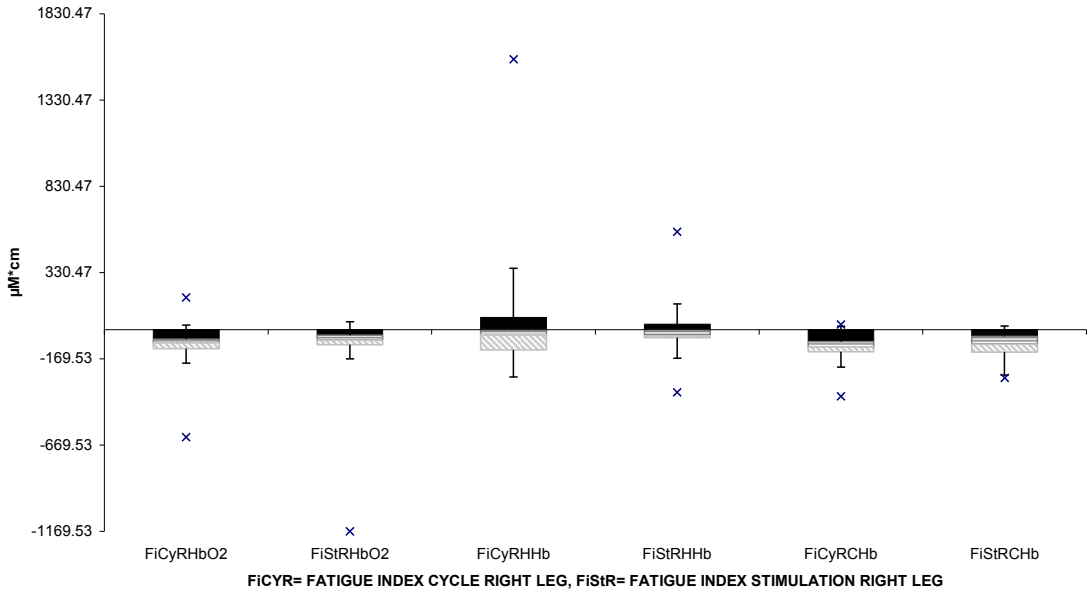

Figure 4. Comparison between the right legs fatigue index of HbO_2, HHb and CHb (cycle ergometry and electrical stimulation).

FATIGUE INDEX OF DIFFERENT FATIGUE PROTOCOLS (ELECTRICAL STIMULATION AND CYCLE ERGOME-TRY) UTILIZING THE SAME HEALTHY SEDENTARY SUBJECTS.

Result showed significant difference (p<0.001) between two different (cycle ergometry and electrical stimulation) fatigue test. This is because of the cycle ergometry fatigue result measure in both legs instead of one leg of electrical stimulation See (Table 6, Fig. 5).

Table 6. Mean values and standard deviation between the cycle ergometry and electrical stimulation fatigue tests.

µM*cm	N	Mean	Sd	Max	Min	25th	50th	75th	Sig
Fatigue cycle ergometry	15	13.6	7.34	22	-2	2	13	15	0.000
Fatigue stimulation	15	59.24	12.02	87.1	12.02	75	55.7	37.3	

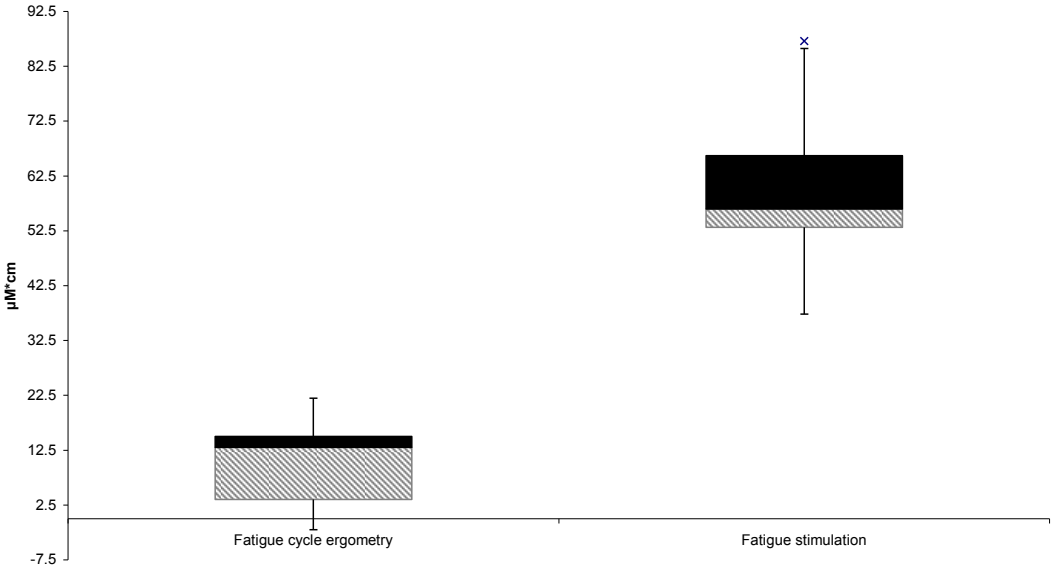

Figure 5. Comparison between the fatigue index of cycle ergometry and electrical stimulation.

DISCUSSION

The main objective of this research was to investigate the differences in changes HbO_2, HHb and CHb during voluntary and involuntary fatigue. There was no significant difference (p>0.05) found in HbO_2, HHb and CHb between right and left leg after the 30 seconds of high intensity cycling ergometry fatigue test. Significant difference (p<0.05) was seen in HbO_2 when cycle fatigue was compared with the electrical stimulation fatigue. In addition, no significant difference was found in HbO_2, HHb and CHb when two fatigues were compared; however, there was a difference (p<0.001) in the percentage fatigue achieved with each test.

Effects of muscle fatigue on tissue oxygenation level

Numerous studies have been conducted to determine the changes in HbO_2, HHb and CHb during exercises done to elicit fatigue (Murthy et al., 2001; Yamada, et al., 2004; McNeil et al., 2006; Hicks et al., 1999; Mead et al., 2006; Kawaguchi et al., 2006). The findings of these studies were compared with the findings of the present study utilizing different test conditions for muscle fatigue.

The results from the above-mentioned studies measured the reduced amount of oxygen in the muscle during the exercise. This reduced amount of HbO_2 depends upon the different level of activity applied in the experiments (McNeil et al., 2006; Mead et al., 2006; Kawaguchi et al., 2006). Current study involved two different exercise protocols (voluntary and involuntary). The results from our study showed significant reduction in HbO_2 during the cycle ergometry and electrical stimulation fatigue test. However, this reduction was found same between the different exercise tests. There are many reasons involved, which can reduce the amount of oxygen consumption during the fatigue test. Skeletal muscles deoxygenate to varying degrees during exercise in accordance with work intensity and level of training (Stuart et al., 1987; Rsssier, et al., 1997; Nioka et al., 1998). The imbalance amount of work performance is the most important factor differentiating between voluntary and involuntary exercise. This is illustrated by the low torque found in the electrical stimulation as compared to the cycle ergometry fatigue protocol (Stuart et al., 1987; Rsssier, et al., 1997).

High metabolic demand due to the electrical stimulation

It has been consistently reported that electrical stimulation contractions are more metabolically demanding than voluntary contractions (Hamada et al., 2004; Kim et al., 1995; Sweeney & Stull, 1986; Vanderthommen et al., 2003). The main consensus is that electrical stimulation invokes a greater energy demand because of the greater turnover of ATP and PCr as well as a decrease in pH in electrical stimulation as compared to the voluntary exercise (Hamada et al., 2004; Kim et al., 1995; Sweeney & Stull, 1986). Yamada et al. (2004) and Baker et al. (2001) found significant correlation between the amount of fatigue developed and the changes in muscle HbO2, HHb and CHb due to the high metabolic demand during the electrical stimulation. The current results showed high percentage of fatigue development 59.2% in electrical stimulation as compared to 13.6% in cycle ergometry fatigue. The most important and pervasive is the imbalance between the electrical stimulation and cycle ergometry protocol in the amount of work performance. The cycle ergometry fatigue test was of short duration (30 seconds) whereas the electrical stimulation fatigue was of long duration (3 minutes). Since, difference in timing between two fatigue protocols has affected the outcome measures, results were significantly different.

Difference the amount of oxygenation between the legs

Comparing the differences in oxygenation between the right and left leg cycle ergometry fatigue, no significant difference (p>0.05) found in HbO_2, HHb and total Hb. However, significant difference (p<0.05s) was found in oxygenated haemoglobin when comparing between the two (electrical stimulation and cycle ergometry) fatigue test. There are several reasons, which account for the increased in the level of total haemoglobin and hence the blood flows in the muscle tissue. It has been shown in the studies that vascular occlusion and increased muscle pressure during the contraction period causes pooling of blood in the small capacitance vessels as the arteries and veins are compressed due to mechanical pressure (Yamada et al., 2004). It is accepted that NIRS measures signals from the small vessels that is the arterioles, venules and capillaries (Ferrari et al., 2004). However, there is no established direct relationship between a change in blood volume and a corresponding change in blood flow during the fatigue. The increase in the total haemoglobin attributed to greater amounts of blood entering the muscle (McNeil et al., 2006). In addition, the steady increase in total HbO_2 observed was due to increase in muscle temperature during the prolonged exercise bout (Ferrari, 2004).

Despite that, the percentage differences between the legs, increased amount of deoxygenated haemoglobin and total haemoglobin recorded which was due to the anaerobic work of type II fibres, which produces the increase amount of lactic acid during the work. The lactic acid in turn dissociates in the hydrogen ions and the lactate. This causes an increased de-saturation of oxygenated haemoglobin, which

seen as an increase in the levels of deoxygenated haemoglobin and hence to compensate for this, the amount of total haemoglobin is increased (Yamada, 2004).

Relationship between muscle fatigue and tissue oxygenation level

A number of studies have been conducted to explore the relationship between the de-velopments of fatigue and the availability and utilization of oxygen during the exercise (Baker et al., 2001a-b; Vedsted et al., 2006). The exact mechanism depends on many factors such as type of contraction (Vedsted et al., 2006), the method of contraction such as cycle ergometry (voluntary muscle contraction) (Murthy et al., 2001; Blangsted et al., 2005) and electrical stimulation (involuntary muscle contraction) (McNeil et al., 2006; Mead et al., 2006). De Ruiter et al. (2004) investigated the type of muscle (single or in the form of group) which was involved during the contraction. The intensity of exercise (Baker et al., 2001) and the availability of blood supply in the contracted muscle linked to the fatigue development and oxygen consumption (Yamada et al., 2004; Tachi et al., 2004). The results of these aforementioned studies found that the rate of fatigue development is dependent upon the amount of muscle oxygenation. Blangsted et al. (2005) and Mead et al. (2006) found that the muscle oxygenation is not always related to a reduction of fatigue levels. Murthy et al. (2001) conducted the study to solve this controversy suggesting that the development of fatigue depends upon the muscle oxygenation only, if the oxygenation is reduced more than 7%. The results from some non-human studies also found controversial because of above two variables (Stainsby et al., 1990; Eiken et al., 1984; Hogan et al., 1994). Result from the present study shows de-creased amount of oxygen during the two fatigue (cycle ergometry and electrical stimulation) fatigue test, which is one of the reason to develop the fatigue in the muscle.

Intensity of exercise

High-intensity cycle ergometry has been widely used to measure muscular performance throughout maximal exercise (Baker et al., 2001). Blood oxygen found reduced about 15- 30% during the high intensity exercise (Richardson et al., 1993; Rowell et al., 1989). However, one important finding of these studies is that the studies did find a relationship between fatigue development and oxygenation involving high intensity exercises. This could again be attributed to the fact that intra-muscular pressure increases at high intensity exercise, which diverts the metabolism to anaerobic pathways. This promotes the fatigue via other pathways other than oxygen reduction or aerobic pathways (Yamada et al., 2004). Baker et al. (2001) results show the substantial muscular upper body contribution during the high intensity exercise in cycle ergometry. Regardless of that Blangsted et al. (2005) and McNeil et al. (2006) results failed to achieve any significant correlation between the fatigue and oxygenation level. Only because of the intensity of work activity was low (10% MVC) as compared to our study. The failure to achieve significant correlation between the level of fatigue development and the level of oxygenation could be attributed to the fact that other mechanisms might be involved in the fatigue development apart from oxygenation. The results of cycle ergometry exercise was similar to Yamada et al. (2004) as current study utilized the same high intensity exercise which also supported by the Baker et al. (2001) where they found the substantial contribution of upper body muscle involved to increase the whole body blood flow. This has increased the metabolic demand of the muscle. Ultimately, the changes in the oxygen level in the muscle were found due to the high intensity exercise. On the other hand Inbar et al. (1996) and Van Mil et al. (1996) calculated the resistive force from body composition and utilized high intensity cycle ergometry exercise. This showed the realistic performance concerning the muscle fatigue and oxygen consumption. The current study was performed on the same mode as Inbar et al. (1996) and Van Mil et al. (1996) used in their study. In addition, the same results were obtained for high intensity cycle ergometry exercise and the development of fatigue with the reduction in the amount of oxygen.

Muscle fibres type

Peripheral fatigue can occur, when the muscle activated by electrical stimulation (Vollestad, 1997). Apart from the oxidative pathway, there are other mechanisms involved during the con-tractions to elicit fatigue including the creatinine phosphate pathway and the anaerobic pathway (glycolysis). This increases the concentration of H+ ions in the muscle and renders the fatigue production. The other reasons include reduced release of calcium ions from the sarcoplasmic reticulum, reduced release of acetylcholine from end channels of nerve (Van Mil et al., 1996).

Electrical stimulation was applied transcutaneously and stimulates the nerve fibres, which present in the periphery of the muscle. McNeil et al. (2006) found type II muscle fibres of quadriceps were excited at 40Hz, which is comparable to the physiological frequency of motor unit activation. In addition, it was found that the tetanic contractions induce mechanical compression (increased intramuscular pressure) which occludes the blood sup-ply to the muscle fibres (Cole & Brown, 2000). Yamada et al. (2004) found in his study that under conditions of ischemia, the type II fibres are activated because the consumption of less amount of oxygen as they work via anaerobic metabolic pathways. The increase in oxygenated haemoglobin in the post fatigue occlusion condition could again be attributed to certain factors. Non-oxidative pathways are one of the reasons, which increase the metabolite accumulation after ATP production. This increased oxygenated haemoglobin level is used to re-synthesise the creatinine phosphate, pyruvic acid and glucose from lactic acid. Another reason for increased oxygenated haemoglobin is the secretion of epinephrine during exercise.

Effects of skin fold thickness on tissue oxygenation

Results of this study revealed negative correlation with the NIRS parameters. Moreover, the correlation was weak during the exercise phases as compared to those taken at rest. In a previous study, which have been conducted to find the effects of the width of skin fold thickness and the amount of adipose tissue on the measurements of oxygenation levels by near infrared spectroscopy (Ferrari, 2004). Matsushita et al, [46] conducted a study to deter-mine the effects of adipose tissue on the measurements obtained by near infrared spec-troscopy in the leg muscle. The results of their study revealed that the sensitivity of the NIRS instrument decreases by increasing the amount of adipose tissue. Although, the adipose tissue has a very small absorption at the wavelengths used during the experi-ment due to its scant blood flow, it increases signal-to-noise ratio of near infrared spec-troscopy. In addition, signals would be detected from adipose tissue as well as superficial parts of muscles. Another study was conducted by van Beekvelt [47] to examine the in-fluence of adipose tissue in forearm on the measurements of muscle oxygenation meas-ured by NIRS. The study involved measurements during resting state and during exer-cises at different intensities as determined by the percentage of maximum voluntary con-traction.

Problems and limitations

Despite this being a small physiological study, in the small convenience sample used and the likelihood that data were not normally distributed; it is difficult to apply the results of this study to a wider population. The inability of the participants to maintain the required workload during 30-seconds fatigue protocol of cycle erogmetry has affected the fatigue development in the muscles, which eventually has affected the results of the present study. Imbalance amount of work duration was applied between the electrical stimulation and cycle ergometry protocol one of the reason, which has affected the result. This study was performed on young male and females therefore findings should not be generalised to a population outside the age range. All participants in this study are Asians and we did not include other ethnic origins.

CONCLUSIONS

While comparing the changes in oxygenated haemoglobin during two different protocols, it is important to understand difference in response of skeletal muscle to electrical stimu-lation and cycle ergometry. This study reveals that the oxygen consumption more in the electrical stimulation as compared to the cycle ergometry during the fatigue test. Signifi-cant difference found between the oxygenated haemoglobin when comparing the elec-trical stimulation with cycle ergometry. Similarly, significant differences were found between the legs in cycle ergometry fatigue test. Changes induced by fatigue influenced by whether the muscle is contracting voluntarily or is being induced to contract. Muscles with higher percentages of fast-twitch fibres have been shown to fatigue more rapidly than the muscles with a greater percentage of slow-twitch fibres.

COMPETING OF INTEREST

“Authors have declared that no competing interests exist and no funding obtained for this study.”

AUTHORS CONTRIBUTION

“AR conceived the idea, designed the study, and wrote the first draft of the manuscript. MAS and HD managed the analysis of the study and revised the manuscript. All authors read and approved the final manuscript.”

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