Neuromuscular response to varying pressures created by tightness of restriction cuff

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1. Introduction

The neuromuscular system adapts to acute and chronic physical stress. The level of activation and adaptations can be altered by several factors, such as the number of motor units activated and amount of blood flow to active muscle (Enoka et al., 1992). Changes in blood flow to skeletal muscle can alter the level of supply of substrates, the removal of metabolites, and fatigue, which significantly affects the recruitment and activation of muscles fibers needed to perform under specific conditions. In general, slow oxidative fibers are responsible for force production when performing low-intensity exercise and all fibers are active when performing high-intensity exercise (Laughlin and Rosegini, 2008). The increase in skeletal muscle blood flow during exercise is associated with muscle fiber type and (Laughlin et al., 1996, 1997), but reducing blood flow to the active skeletal muscle may affect the pattern and the number of skeletal muscle fiber recruitment during exercise and therefore the level of neuromuscular activation and adaptation. Blood flow restriction (BFR) is a training technique that restricts muscular blood flow during low-intensity exercise training and it has been shown to induce changes in neuromuscular activity (Karabulut et al., 2010; Moritani et al., 1992; Takarada et al., 2000). This may be a result of oxygen deprivation and metabolic production and accumulation. These changes may allow a shift in skeletal muscle activation from slow oxidative fibers to fast oxidative–glycolytic fibers, mimicking the effects of high intensity exercise. Several of the studies published (Abe et al., 2006, 2010; Ozaki et al., 2011) have reported the final pressure used (FP; highest restrictive pressure reached after inflation with air) and have not mentioned details about the importance of setting the tightness of cuffs prior to inflation. The external pressure created by the tightness of cuffs before inflation (also known as initial restrictive pressure, IRP) can change the level of venous return and metabolic byproduct such as lactate and H+ accumulation and clearance, which may alter the level of neural activity in skeletal muscles. In addition, differences in body composition between individuals may result in varying levels of blood flow restriction. The inconsistencies in the current literature on the experimental results of BFR training may be a result of the use of different equipment and/or the lack of information about the procedure. Teramoto & Golding (2006) reported no difference in muscle mass and endurance between occluded and non-occluded subjects while using blood pressure cuffs. Loenneke et al. (2012a) found no increase in metabolite accumulation when using elastic knee wraps. Laurentino et al. (2008) reported no change in strength or hypertrophy when using traditional blood pressure cuffs. Since the equipment used by the studies mentioned above cannot set IRP and most of the published BFR studies have not even mentioned IRP, it is obvious that the details about BFR training protocol were not well defined by the previous studies and were not well known by the researchers performing BFR studies. Changes in IRP may create variations in the amount of pressure applied to the thighs and in the amount of blood flow restriction. In addition, changes in skin and subcutaneous fat thickness may also change the level of external pressure created by IRP and the pressure applied to the...
of one set of 30 reps and three sets of 15 reps of knee extension exercises 1.2 s each for the concentric and the eccentric muscle actions with a 1-min inter-set rest period. The right knee extensors’ strength was measured by post-exercise MVC immediately after the knee extension exercise. The cuff remained inflated throughout the entire session, including pre-exercise MVC, rest periods, and post-exercise MVC.

2.3. Circumference and ultrasound

The right mid thigh circumference was measured with a tape measure at the midpoint of the right upper leg between the lateral epicondyle of the femur and greater trochanter. Skin and subcutaneous fat thickness was measured by using ultrasound with a 5-MHz transducer (Fukuda Denshi UF-4500). The probe head was coated with transmission gel before being positioned perpendicular to the tissue at central region over the quadriceps at the same level as the circumference measurement. The image was taken after the depth gain compensation was adjusted to improve image quality.

2.4. Blood flow restriction

During both testing sessions, the elastic blood pressure cuff (KAATSU Master, Sato Sports Plaza, Tokyo, Japan) was placed proximal to the inguinal area of the right thigh. The cuff was manually tightened to a randomly determined IRP (40–45 mmHg or 60–65 mmHg) and then was inflated to reach 120 mmHg. The pressure was held at 120 mmHg for 30 s and released for 10 s. The cuff pressure was progressively increased by 20 mmHg while holding for 30 s at each pressure and releasing for 10 s between increments until reaching the final pressure used during exercise. The final pressure was determined by using the following formula:

\[
\text{Final Pressure} = \text{Arm Systolic Blood Pressure} \times 1.44
\]

2.5. EMG

A DE-2.3 sensor (Delsys Inc., Boston, MA) that contains two electrodes consisting of parallel bars spaced 10 mm apart was used to collect muscle activity and record on an EMG Myomonitor-4 (Delsys Inc., Boston, MA). Before placing the electrodes, skin was shaved with a hand razor, slightly abraded with sandpaper to remove superficial epithelial cells, and cleaned with alcohol. A single differential surface EMG electrode (Delsys Inc., Boston, MA) was placed parallel over the long axis of the muscle belly of the VL at 33.3% from the lateral femoral epicondyde to the greater trochanter. A ground electrode was placed over the patella. Surface EMG signals were collected during MVCs and repetitive dynamic knee extension exercises for both sessions.

2.6. Signal processing

The EMG signals were amplified by a gain of 1000 using a BagnoliTM 8-channel system (Delsys Inc., Boston, MA) and band-pass filtered (15 Hz to 500 Hz) by using EMGWorks Datalogger software (Delsys Inc., Boston, MA). The raw EMG signals were then transmitted to a computer at a sampling rate of 2000 Hz and stored in digital format using EMGWorks Acquisition software (Delsys Inc., Boston, MA).

2.7. Biodex

Pre- and post-exercise seated knee extension muscle strength was measured with the Biodex System 4 Pro isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY). The participants were seated on the dynamometer chair with the chair's backrest

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circulatory pathways. Thicker skin and subcutaneous fat in the thighs may act as a barrier before the BFR cuffs and circulatory pathways resulting in low or no change in the diameter of the targeted blood vessels minimizing overall effect of external pressure created by IRP. Since these questions have not been investigated and changes in blood flow and venous return may affect the number and the level of skeletal muscle fibers activation, it is necessary to examine the effects of IRP and skin and subcutaneous fat thickness on neuromuscular activity to effectively use BFR training technique.

The purpose of the study was to examine the effect of different IRPs on neuromuscular activation while determining the strongest individual variable (leg circumference vs. skin and subcutaneous fat thickness) for the changes in EMG activity with varying IRPs. We hypothesized that variation in IRPs would result in significant changes in EMG RMS and MDF during exercise and during post-exercise MVC. We also hypothesized that the level of neuromuscular activity would be affected by thigh ST due to the variation in pressure created by IRP.

2. Methods

2.1. Participants

The characteristics of the subjects are presented in Table 1. Thirty-four young males and females (23.7 ± 0.7 years) took part in this study. The study protocol was approved by the University of Texas at Brownsville Institutional Review Board for Human Participants prior to initiation of the study. Participants read and signed an informed consent document before participation in the study and all participants were screened by questionnaires (health status questionnaire and a physical activity readiness questionnaire) prior to participation.

2.2. Study design

A randomized, counterbalanced, within-subjects experimental design was used to examine the effects of IRP and skin and subcutaneous fat thickness on neuromuscular function. Participants reported to the research laboratory on three separate days to complete this study (one familiarization and 2 testing sessions). Participants had their upper leg length, thigh circumference, and skin and subcutaneous fat thickness determined. A single pre-amplified EMG electrode was placed over the longitudinal axis of the Vastus Lateralis. The BFR belt was placed on the highest point to the inguinal area of the right thigh. The cuff was manually tightened to a randomly determined IRP (40–45 mmHg or 60–65 mmHg) and then was inflated to reach 120 mmHg. The pressure was held at 120 mmHg for 30 s and released for 10 s. The cuff pressure was progressively increased by 20 mmHg while holding for 30 s at each pressure and releasing for 10 s between increments until reaching the final pressure used during exercise. The final pressure was determined by using the following formula:

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inclined to 85 and secured to the chair in accordance with the Bio- 
Biodex Medical Systems, Inc., Shirley, NY). Tibia-femoral joint 
placement (rotation of axis) was determined by aligning the right 
kleen joint with the axis of rotation of the attachment arm. The le-
ver arm pad was secured to the subject’s lower leg just ~1 in. su-
perior to the malleoli. The subjects were secured to the Biodex chair 
by two diagonal straps that crossed over the chest and a seatbelt 
over their hips to prevent extraneous movement. For all pre- and 
post-exercise isometric MVC force measurements, the knee joint 
angle was set at 60° below the horizontal plane.

2.8. Statistical analyses

All data were expressed as means ± SE. Repeated measures AN-
OVAs (time [pre- vs. post-exercise MVC] × condition [40 vs. 
60 mmHg]) were used to analyze the EMG RMS and EMG MDF dur-
ing MVC. In addition, two separate two-way repeated measures 
ANOVAs [condition [40 vs. 60 mmHg] × repetitions [average of 5 
contractions]] were used to analyze the EMG RMS and MDF data 
during the knee extension exercises. Bonferroni’s post hoc tests 
were performed to determine further differences when significant 
main effects were found. A multivariate stepwise regression anal-
ysis was used to identify the relation between independent vari-
ables (leg circumference and skin and subcutaneous fat 
thickness) and the changes in EMG activity. An alpha level of 
0.05 was used to determine statistical significance and the statisti-
cal analyses were undertaken by using SPSS 19.0 for Windows 
(SPSS Inc., Chicago, IL).

3. Results

Males were significantly taller (p < 0.001) and had higher body 
mass (p < 0.001), however females had significantly higher skin 
and subcutaneous fat thickness values compared to males 
(p < 0.001). Thigh circumference did not differ significantly be-
tween males and females. Pre-exercise MVC values were signifi-
cantly lower when IRP of 60 mmHg was used compared to pre-
exercise MVC values observed when IRP of 40 mmHg was used.

The average EMG RMS values for every 5 repetitions were plot-
ted for each set with IRP of 40 mmHg and with IRP of 60 mmHg 
(Fig. 1). A repeated measures ANOVA (gender as between-subject 
factor and skin and subcutaneous fat as covariate) detected a time 
main effect (p < 0.001) for EMG RMS during knee extension 
exercises and there were interactions for time × condition 
(p = 0.01) for EMG RMS. A multivariate stepwise regression 
analysis confirmed that skin and subcutaneous fat thickness was 
the only factor independently associated with the changes in 
EMG RMS during exercises with IRP-40 (β = ranged from −0.482 
to −0.608, p < 0.01) and IRP-60 (β = ranged from −0.575 to 
−0.604, p < 0.01). There was a trend for condition main effect 
(p = 0.09) for EMG RMS during MVC (Fig. 2).

The average EMG MDF values for every 5 repetitions were plotted 
for each set with IRP of 40 mmHg and with IRP of 60 mmHg 
(Fig. 3). Repeated measures ANOVA (gender as between-subject 
factor and skin and subcutaneous fat thickness as covariate) also 
showed that there was a significant condition (p < 0.01) and time 
main effect (p < 0.01) and an interaction for condition × skin and 
subcutaneous fat thickness (p < 0.04) and time × gender (p < 0.05) 
for EMG MDF during knee extension exercises. EMG MDF values 
during exercises were higher when IRP of 60 mmHg was used com-
pared to the session used IRP of 40 mmHg. Skin and subcutaneous 
fat thickness was also found to be the strongest individual variable 
for the changes in MDF values for the set 1 reps 21–30, the set 3 
reps 11–15, and set 4 reps 11–15 when IRP-40 was used and set 1 
reps 6–15 when IRP-60 was used (β = ranged from −0.357 to −0.504, 
< 0.04). Leg circumference was a statistically significant predictor 
only for the changes in MDF values (β = ranged from −0.401 to 
−0.498, p < 0.02) just for the set 4 reps 1–5 when IRP-40 was used 
and set 1 reps 1–5, set 2 reps 6–10, set 3 reps 1–5, set 4 reps 1–15 
when IRP-60 was used. There was a condition main effect (p < 0.04) 
and a trend for time main effect (p = 0.08) and a trend for an inter-
action for time × skin and subcutaneous fat thickness (p = 0.05) for 
EMG MDF during MVC (Fig. 2).

4. Discussion

The findings from the present study provide evidence that even 
though the FP was higher than the IRPs and FP was kept same for 
both sessions; changes in IRP had significant impacts on pre-exer-
cise leg strength, and RMS values from pre- to post-exercise MVC. 
In addition, this is the first study proving that skin and subcutane-
ous fat thickness is the most important independent variable cre-
ating variations in the amount of pressure being applied by the 
tightness of the BFR cuff before inflation. Thigh circumference 
was only an important variable for MDF values during the first 
10 repetitions.
Depressed pre-exercise MVC values during IRP of 60 mmHg highlight how important and crucial this overlooked variable (IRP) of BFR training protocol is. The findings of the present study show that even a slight difference in IRP during BFR training studies may cause false and inconsistent findings within and between research studies. Since some of the previous studies have used equipment that cannot determine and set IRP (Loenneke et al., 2010; Teramoto and Golding, 2006), it is very logical to assume that the reason for the inconsistency in findings across studies is inappropriate and/or inconsistent IRP setting.

Even though the decreases in RMS values from pre to post-exercise during strength testing did not reach statistical significance, the trend detected for RMS values may suggest a fatigue-induced decrease in force production as a result of the differences in pressures generated by IRP. Takarada et al. (2000b) suggests that the combination of low-intensity resistance exercise and BFR may increase muscle recruitment and changes in fiber type by creating hypoxic conditions in the working muscle coupled with accumulating lactate levels. It is also stated that increasing intramuscular pressure could affect blood circulation resulting in even higher lactate build-up. EMG MDF generally decreases during fatigue (Hermens et al., 1999; Masuda et al., 1999). In the present study, the variation in pressures created by IRP also caused significant changes in MDF values highlighting the importance of IRP on neuromuscular system.

Hypoxia induced neuromuscular activation is also supported by a study conducted by Katayama et al. (2010), where it was determined that hypoxic conditions were responsible for changes in muscle fiber activation from type I to type II as well as increased motor unit recruitment. Since slow-twitch muscle fibers rely heavily on oxidative phosphorylation, they may have been unable to contribute to isometric force production due to reduced blood flow to skeletal muscles during the post-exercise MVC trials with BFR. It can also be speculated that IRP 40 mmHg does not place a high enough restrictive force upon the circulatory pathways during low-intensity exercises, which allows enough amount of oxygen delivery to the working muscles and large amount of type I muscle fibers contribution to the overall RMS values. However, the same RMS response was not present in the female population, which can be explained by ST changing the degree of pressure created by the cuff. These findings show that ST is an important independent variable affecting EMG response and indicate that higher restrictive pressures may be needed for females or for individuals with high ST.

Loenneke et al. (2012b) determined that thigh circumference, and not subcutaneous fat thickness, was the most important factor...
when setting FP for occluding blood flow. However, the data from the present study shows that skin and subcutaneous fat thickness must also be considered when complete occlusion is not desired. It was previously reported that increasing level of applied pressure directly affected the amount of venous return, which was verified by the decreases in stroke volume values (Iida et al., 2005; Karabulut et al., 2011). The greater fat accumulation in the thighs may be acting as a barrier between the BFR cuffs and circulatory pathways. This barrier of skin and subcutaneous fat thickness could be responsible for the changes in neuromuscular response by changing the level of blood flow and accumulation of metabolites.

The data in this study are indispensable for understanding the importance of IRP and skin and subcutaneous fat thickness on neuromuscular response and the findings will help researchers understand the details of BFR protocol. The present study is the first study reporting that skin and subcutaneous fat thickness directly affects the overall effectiveness of IRP by causing variations in the amount of pressure being placed on the circulatory pathways during exercises. Therefore, IRP should be taken into consideration when conducting BFR training studies and the BFR cuff may need to be placed tighter for the individuals, who have high skin and subcutaneous fat thickness to be able to induce similar BFR training-related changes. While this study shows IRP and skin and subcutaneous fat thickness as vital variables for BFR protocol, further research is required to determine proper setting for IRP based on skin and subcutaneous fat thickness and how the cuff tightness and pressures should be adjusted to follow the training principle of overload and progression for BFR training studies.

Conflict of interest

The authors declare that they have no conflict of interest.

References

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Guillermo Perez is currently working on his Master’s degree in Exercise Science at the University of Texas at Brownsville. He received his Bachelor’s degree in 2012, and plans on attending physical therapy school. His research interests include endocrinological responses to exercise, assessments of endothelial function and arterial stiffness, and neuromuscular adaptation.