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The effects of different initial restrictive pressures used to reduce blood flow and thigh composition on tissue oxygenation of the quadriceps

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Abstract

Blood flow restriction training technique can be affected by several factors resulting in changes in responses to training; therefore it is necessary to investigate and reveal detailed information about this novel training technique. Participants had their thigh size, thickness of subcutaneous fat, and regional bone free muscle mass measured prior to testing. A Near-Infrared Spectrometer was used to measure tissue oxygenation and a cardiovascular profiling system was utilised to measure stroke volume and heart rate. Initial restrictive pressure of 30, 50, and 70 mmHg were set in random order on three separate days, and then six target pressures were sequentially applied. Tissue oxygenation decreased significantly as both initial restrictive pressure and target pressures increased, but the magnitude of decreases was stronger with higher initial restrictive pressure. There were significant negative correlations between tissue oxygenation and leg lean body mass, total lean body mass, and thigh circumference when initial restrictive pressure was set at 30 mmHg. The findings indicated that changes in initial restrictive pressure affected the amount of venous return verified by the decreases in tissue oxygenation and stroke volume. In addition, thigh composition and size had a significant impact on the effects of initial restrictive pressure.

Keywords: *Blood flow restriction, tissue oxygenation, initial and target restrictive pressures, thigh composition*

Introduction

It is well established that to elicit the preferred training response i.e. muscle hypertrophy, muscle power, muscle endurance etc, the training intensity has to be specific and the suitable training principles dealing with progression and overload have to be followed. Training intensity of resistance exercise at 67–85% one repetition maximum with a moderate to high number of repetitions (6–12) per set has been recommended to induce increases in skeletal muscle strength and size (Baechle & Earle, 2000), however recently developed blood flow restriction training has greatly reduced the resistance training exercise intensity needed to potentiate physiological improvements in skeletal muscle strength (Karabulut, Abe, Sato, & Bemben, 2009; Shinohara, Kouzaki, Yoshihisa, & Fukunaga, 1998; Takarada et al., 2000b; Takarada & Ishii 2002; Takarada, Sato, & Ishii, 2002) and size (Abe, Kearns, & Sato, 2006; Beekley,

Sato, & Abe, 2005; Takarada et al., 2002; Takarada & Ishii 2002; Yasuda et al., 2005). Since this new training technique may be useful or even provide an alternative training method for the general public or special populations, such as older adults or people with health risks related with high resistance training loads, it has attracted a lot of attention.

Even though substantial evidence has been published to show the efficacy of blood flow restriction training for improving muscle strength and size, previous studies using different types of equipments such as blood pressure cuffs to restrict blood flow failed to report similar changes in muscular strength (Burgomaster et al., 2003) and size (Teramoto & Golding, 2006). Little is known about the protocol regarding restrictive pressure setting and none of the manuscripts published have defined or explained the importance of the initial restrictive pressure and target restrictive pressure to obtain desired training-related physiological adaptations. Therefore, it is

quite likely that studies using different equipments or protocols to restrict blood flow were not able to set the initial restrictive pressure and target restrictive pressure at a consistent level and monitor. The initial restrictive pressure can be defined as the amount of pressure applied to the limbs due to the tightness of cuffs before inflation with air, therefore there will always be some pressure applied even when the cuffs are deflated, which will affect the amount of blood accumulated and venous return. The target restrictive pressure refers to the highest restrictive pressure reached after inflation with air and utilised during training (refer to the method section for detailed information about the procedure).

A study by Fullana and his colleagues (2005) reported that only a pressure greater than 40 mmHg was able to reduce the venous diameter at thigh-level; therefore, it is essential to set the initial restrictive pressure at an appropriate level and increase progressively to adjust the amount of blood accumulation and venous return. Previous studies have revealed that accumulation of lactate and hydrogen ions might cause increased afferent signals from intramuscular metaboreceptors resulting in an augmented growth hormone release (Goto, Ishii, Kizuka, Takamatsu, 2005; Takarada et al., 2000a; Victor and Seals 1989). Similarly, another experiment revealed findings supporting this hypothesis (Gordon, Kraemer, Vos, Lynch, & Knuttgen, 1994) that sodium bicarbonate ingestion resulted in lower blood hydrogen ion concentration (greater pH) and thus less exercise-induced growth hormone release following 90 s cycle ergometer sprinting compared with the responses observed after placebo ingestion.

Previous studies (Iida et al., 2005; Takano et al., 2005) reported that the blood flow restriction elicited the pooling of blood into the legs and prevention of venous return, however variability in target restrictive pressure and especially in initial restrictive pressure when the cuffs are deflated between sets may result in changes in oxygen availability, local accumulation and clearance rate of metabolic subproduct (lactic acid, hydrogen ions) leading to inconsistency in anabolic hormone secretion, especially growth hormone, and thus the magnitude of skeletal muscle adaptation. Quantifying the changes in tissue oxygenation, stoke volume, and heart rate with varying initial restrictive pressure can provide some insights to the level of oxygen availability and venous return in supine participants. Since the blood flow restriction training technique is a novel method to improve skeletal muscle strength and size and yet so little is known about the blood flow restriction training protocol used, it is essential to understand the impact of restrictive pressures used and the thigh composition on the level of oxygenation and venous return. Therefore, the purpose of the study was to examine the tissue oxygenation and venous return responses to the varying initial restrictive pressure, target restrictive pressure, and thigh composition in supine participants.

Methods

Study design

After determining each participant's right thigh size with a tape measure, subcutaneous fat by ultrasound, and body composition by using Dual Energy X-Ray Absorptiometry, participants returned to the lab on three separate days to investigate the effects of subcutaneous fat and lean muscle mass on tissue oxygenation of the quadriceps muscles while using three different initial restriction pressures. Following completion of skin preparation by shaving and cleaning with alcohol pads, an InSpectra^{TM} Tissue Spectrometer System – Model 325 was used during each bout of pressure from the blood flow restriction device to observe the amount of tissue oxygenation of the thigh. A small sensor was attached to a soft foam pad to sheild the pad from the light. The foam pad was then placed on the participant's left quadricep and the sensor remained in this position throughout the testings. Simultaneous measurements of stroke volume and heart rate were performed by an HDI/Pulswave™ CR-2000 Cardiovascular Profiling System (Hypertension Diagnostic, Inc., Eagan, Minnesota, USA) during the last 30 seconds of the 4 min for which each pressure was applied. The blood flow restriction cuffs were placed on the upper most portion of the thigh, and initial pressures of 30, 50, and 70 mmHg were applied in random order during the three separate visits. While participants were lying down, each of the following six pressures (120, 140, 160, 180, 200, and 220 mmHg) was reached in sequential order for 4 min to restrict blood flow followed by 2 min of rest.

Participants

Six young healthy males (30.0 ± 4.6 years) signed an informed consent and completed a physical activity readiness questionnaire before participating in this research study. The study protocol was approved by the University of Oklahoma Institutional Review Board for Human Participants.

Dual energy x-ray absorptiometry

Regional body compositions were determined by a trained technician using Dual Energy X-Ray Absorptiometry (GE Medical Systems, Lunar Prodigy enCORE software version 10.50.086, Madison, WI). Participants with an abdominal thickness at the umbilicus of ≤25 cm were scanned at the Standard

speed and participants with an abdominal thickness of > 25 cm were scanned at the slower Thick speed.

Circumference

Analyses of regional body composition for both legs revealed no significant differences in composition. The circumference values for the right leg were used for the analysis. The right mid thigh size was measured with a tape measure and a skinfold was taken on the front and back of the thigh at the mid point of the right upper leg between the lateral condyle of the femur and greater trochanter.

Ultrasound

The subcutaneous fat thickness of each participant was determined by using an ultrasound machine with a 5 MHz transducer (Fukuda Denshi UF-4500). Transmission gel was applied on the scanning head and the scanning head was positioned perpendicular to the tissue at the same level as the circumference measures. Subcutaneous tissue thickness was measured according to the manufacturer's guidelines.

Near-infrared spectrometer

Tissue haemoglobin oxygen saturation during each bout of pressure was monitored with an Inspectra™ Tissue Spectrometer System – Model 325. The probe pad on the quadriceps muscle was placed to a mark that was made at 50% on the line from the anterior superior iliac spine to the superior part of the patella. The skin was shaved and cleaned before placing the pad during the first trial. During the other two trials, a tape measure was used to measure and mark the same point and the skin was prepared by following the same procedure used during the first trial, then the probe pad was placed at the exact same place. This is a non-invasive method with a 25 mm reflectance probe measuring the absorption of light photons in the 680–800mm spectrum. The absorption spectrum of light remitted from a tissue sample varies mainly with oxyhaemoglobin (HbO$_2$) and deoxyhaemoglobin (HHb) level. The optical attenuation at 720 nm is responsive to oxyhaemoglobin and the attenuation at 760 nm is responsive to oxyhaemoglobin and deoxyhaemoglobin absorption (Skarda, Mulier, Myers, Taylor, & Beilman, 2007).

A matrix of the wavelength specific attenuation values for the isolated blood circuit HbO$_2$ and HHb measurements along with a third ignored dummy variable having near zero and spectrally flat attenuation was inverted to obtain wavelength specific multiplying coefficients for predicting relative changes in oxyhaemoglobin and deoxyhaemoglobin (Matcher, Elwell, Cooper, Cope, & Delpy, 1995).

Change in total haemoglobin (Hbt) is the sum of the oxyhaemoglobin and deoxyhaemoglobin signals. The isolated blood circuit was utilised to control changes in blood %SO$_2$ and Hbt in order to verify that the oxyhaemoglobin and deoxyhaemoglobin signals produced equal but opposite changes with %SO$_2$ at constant haemoglobin. Equations which relate measured light attenuation change (A) to haemoglobin change are shown below (Myers et al. 2005; Skarda et al. 2007).

$$\Delta HbO_2 = (-0.679)A_{680} - (2.891)A_{720} + (3.570)A_{760}$$
$$\Delta HHb = (1.143)A_{680} - (1.328)A_{720} + (0.185)A_{760}$$
$$\Delta Hbt = HbO_2 + HHb$$

Stroke volume and heart rate measurements

Participants were asked to fast for at least eight hours and not to perform any strenuous physical activity prior to testing. An HDI/Pulswave™ CR-2000 Cardiovascular Profiling System (Hypertension Diagnostic, Inc., Eagan, Minnesota, USA) was used to measure stroke volume and heart rate. An appropriate adult size blood pressure cuff was placed around the upper left arm and a rigid plastic wrist stabiliser was placed on the right wrist to stabilise the radial artery during the measurement. After finding the strongest radial pulse, an Arterial Pulsewave™ Sensor was placed directly perpendicular to the skin over the radial artery for blood pressure waveform signal collection, while the arm rested in a supine position. The non-invasive sensor was adjusted to the highest relative signal strength and arterial waveforms were obtained during the last 30 seconds of each 4 min period for each pressure applied.

Blood flow restriction protocol

An elastic belt 50 mm in width was positioned around the most proximal portion of each thigh to restrict blood flow. The device (KAATSU-Master, Sato Sports Plaza, Tokyo, Japan) has a pneumatic bag along the inner surface of the elastic cuffs that are connected to an electronic air pressure control system that monitors the restriction pressures set by the investigator. Prior to progressive increases in pressure to reach the predetermined target pressure, the cuff was initially set at one of the three initial restrictive pressures (30 mmHg, 50 mmHg, or 70 mmHg) determined randomly for 4 min and then pressure was released for 2 min. The pressure was incrementally increased to 120, 140, 160, 180, 200, and 220 mmHg for 4 min and the cuffs were deflated for 2 min between pressures.

The initial restrictive pressure and target restrictive pressure protocols for legs utilised by previous studies were as follows: The cuff pressures are set between 35 and 45 mmHg for the initial restrictive pressure and between 140 and 160 mmHg for the target restrictive pressure during initial stages of training depending on the participants' age, fitness level, and training status. The cuff pressures are increased for the next training session when the whole sets and reps are completed and when the level of exertion such as rated perceived exertion is below 16 or decreased when the whole sets or reps are not completed during the previous training session. Following successful training sessions, the cuff pressures are increased progressively to pressures between 45 and 55 mmHg for the initial restrictive pressure and between 160 and180 mmHg for the target restrictive pressure. Generally, the final pressures used and kept unchanging for the rest of the training period are between 55 and 65 mmHg for the initial restrictive pressure and between 180 and 220 mmHg for the target restrictive pressure. The progression of the pressures utilised is needed in order to make exercises challenging enough and adjust the amount of blood pooling and clearance rate of metabolic subproduct in order to generate desired training-induced adaptations.

Statistical analyses

A two-way repeated measures ANOVA [initial pressures (3) x restriction pressures (5)] was used to analyse the effects of different initial pressure on tissue oxygenation. Relationships between variables were assessed by Pearson Correlation Coefficients. Data were expressed as means $\pm$ SE. An alpha of 0.05 was used to determine statistical significance and data were analyzed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL).

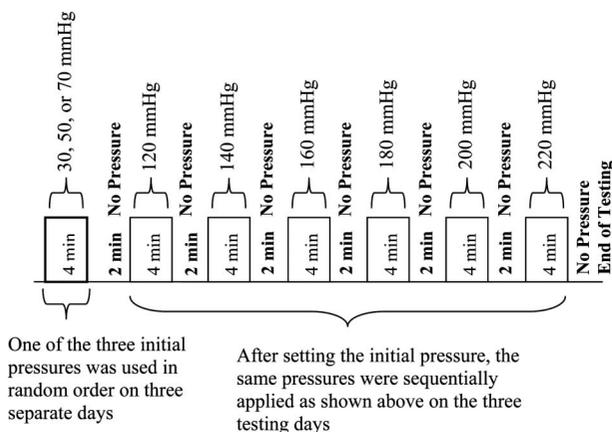## Results

Even though six different pressures were set following the initial 30, 50, or 70 mmHg pressures, most of the participants felt nausea and only one participant completed the test for the pressures over 180 mmHg when the initial pressure of 70 mmHg was used. Therefore, no statistical anlysis was performed for tissue oxygenation values for the pressures greater than 180 mmHg, when initial pressure was set at 70 mmHg. The observed power, a post-hoc power calculation that has been performed after the completion of the study, was 0.99 for tissue oxygenation. The physical characteristics of the participants are presented in Table I.

Tissue oxygenation values were significantly lower during the session using initial pressure of 70 mmHg compared with the session using initial pressure of 30 and 50 mmHg ($P < 0.01$) and the session using initial pressure of 50 mmHg compared with the session using initial pressure of 30 mmHg ($P < 0.01$) (Figure 2). For each condition, when the resticitve

Table I. Physical characteristics of participants.

Age (years)	28 ± 2.0
Height (cm)	179.3 ± 1.6
Weight (kg)	83.2 ± 7.2
Thigh Circumference (cm)	55.3 ± 2.5
Subcutaneous Fat Thickness (mm)	6.5 ± 0.2
Leg LBM (kg)	10.8 ± 0.5
Total LBM (kg)	63.8 ± 2.9

Note: Leg Lean Body Mass (leg LBM), and Total Lean Body Mass (Total LBM). Values are means $\pm$ SE.
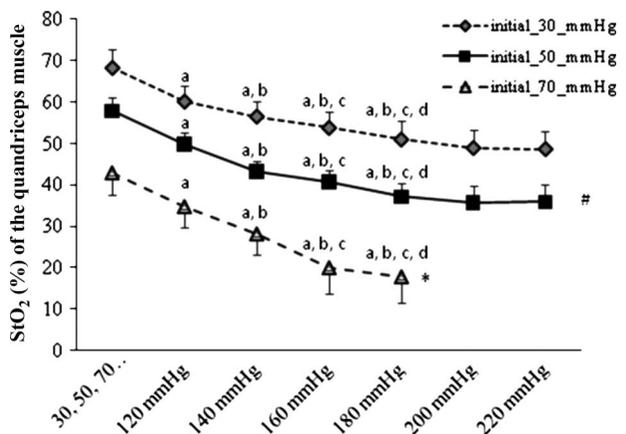

Figure 2. Tissue hemoglobin oxygenation saturation (StO$_2$) of the quads when different initial pressures were applied to restrict blood flow. [#]Significantly different ($P < 0.05$) from initial pressure 30 mmHg.[*]Significantly different ($P < 0.05$) from initial pressure 30 and 50 mmHg. [a]Significantly different ($P < 0.05$) from baseline; [b]significantly different ($P < 0.05$) from 120 mmHg; [c]significantly different ($P < 0.05$) from 140 mmHg. [d]significantly different ($P < 0.05$) from 160 mmHg. Values reported as Mean $\pm$ SE (N = 6).

One of the three initial pressures was used in random order on three separate days

After setting the initial pressure, the same pressures were sequentially applied as shown above on the three testing days

Figure 1. Schematic representation of blood flow restriction procedure throughout the experiment.

pressures were progressively increased, the amount of tissue oxygenation decreased significantly ($P < 0.05$). The pattern of changes in stroke volume and heart rate values with increasing initial restrictive pressure are displayed in Figure 3. With increased initial restrictive pressure, the stroke volume values decreased, whereas the heart rate values increased. The data for stroke volume and heart rate were collected from five participants. Therefore, the values for statistical analyses were not reported even though there were significant differences between sessions for changes in stroke volume and heart rate when statistical analyses were performed. The pattern of changes in StO_2 is shown in Figure 4 for one participant throughout the experiment to highlight why the initial restrictive pressure is important in terms of tissue oxygenation when the cuffs are deflated.

The Pearson Correlation Coefficients between each initial pressure and the body composition parameters are displayed in Table II. When initial pressure of 30 mmHg and following 6 target pressures were used, tissue oxygenation was inversely and significantly related to leg lean body mass ($r = -0.86$ to -0.95), and total lean body mass

($r = -0.79$ to -0.95), however there was a non significant weak to moderate association with subcutaneous fat ($r = -0.17$ to -0.79). A significant positive correlation was detected between tissue oxygenation and thigh circumference ($r = -0.90$ to -0.82 for the pressures of 30 and 120 mmHg, respectively), however when the restriction pressure was increased, correlations became non-significant and moderate in strength ($r = 0.54$ to 0.74). Throughout the session when initial pressure was set at 50 mmHg, tissue oxygenation was generally inversely, but not significantly, related to leg lean body mass ($r = -0.37$ to -0.68), total lean body mass ($r = -0.38$ to -0.73), thigh circumference ($r = -0.22$ to -0.61), and subcutaneous fat ($r = -0.35$ to 0.05). Non significant weak to moderate correlations were detected between tissue oxygenation and leg lean body mass ($r = -0.41$ to 0.37), total lean body mass ($r = -0.24$ to 0.21), thigh circumference ($r = -0.18$ to 0.42), and subcutaneous fat ($r = 0.09$ to 0.79) when initial pressure was set at 70 mmHg. It should be noted that since four participants completed the whole testing when initial pressure 70 mmHg was used, statistical analyses were not performed for pressures above 180 mmHg.

Discussion

The present study is the first to highlight the significance of the initial restrictive pressure used during blood flow restriction technique and to provide further evidence that may help researchers to understand the contentious results found in the
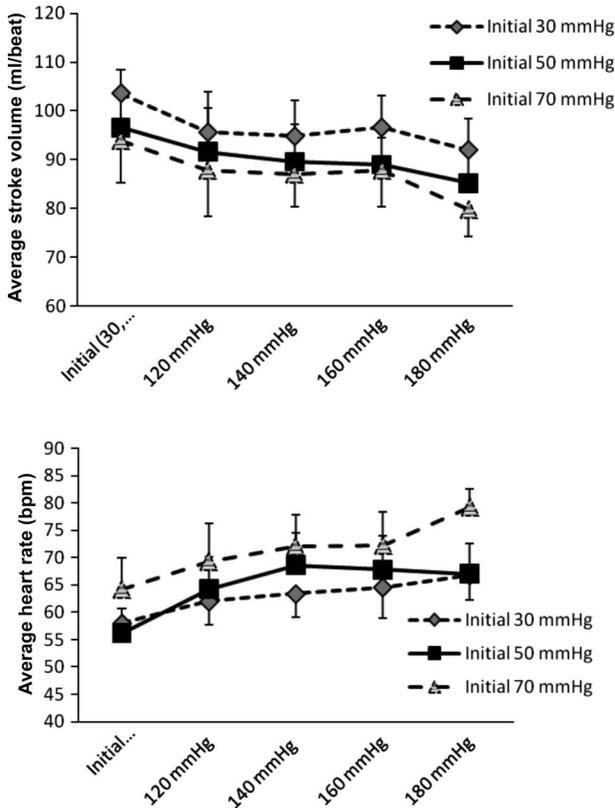

Figure 3. Changes in average values of stroke volume (SV) and heart rate (HR) for each initial restrictive pressure (IRP) and target restrictive pressure (TRP) used throughout the testing. Values reported as Mean ± SE (N = 5).
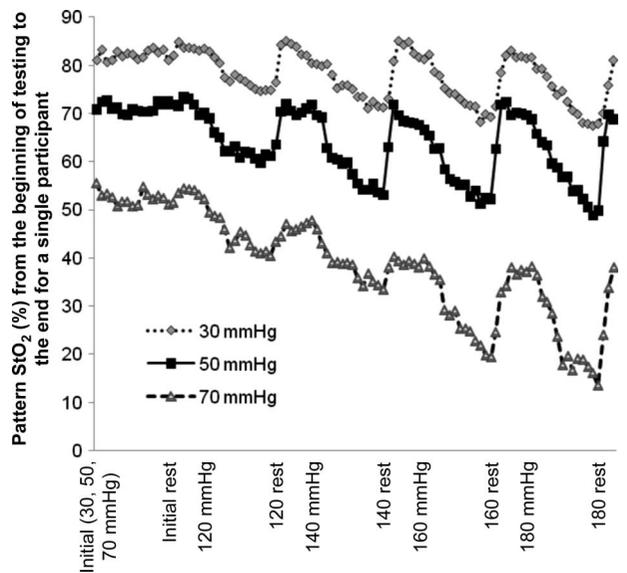

Figure 4. Pattern of changes in tissue hemoglobin oxygen saturation (StO_2) for approximately every 15 seconds in the quads for one subject throughout the experiment.

Table II. Pearson correlations between oxygenations for each pressure and thigh composition.

	Initial Pressure 30 mmHg							Initial Pressure 50 mmHg							Initial Pressure 70 mmHg				
	30 mmHg	120 mmHg	140 mmHg	160 mmHg	180 mmHg	200 mmHg	220 mmHg	50 mmHg	120 mmHg	140 mmHg	160 mmHg	180 mmHg	200 mmHg	220 mmHg	70 mmHg	120 mmHg	140 mmHg	160 mmHg	180 mmHg
Thigh circ	−0.90*	−0.82*	−0.74	−0.70	−0.69	−0.54	−0.55	−0.61	−0.57	−0.40	−0.22	−0.38	−0.25	−0.39	−0.18	−0.05	−0.05	0.32	0.32
Subcut fat	−0.79	−0.54	−0.41	−0.37	−0.36	−0.20	−0.17	−0.35	−0.13	0.06	0.19	0.11	0.22	0.05	0.09	0.30	0.39	0.74	0.75
Leg LBM	−0.88*	−0.95*	−0.91*	−0.92*	−0.93*	−0.86*	−0.87*	−0.67	−0.68	−0.55	−0.44	−0.50	−0.37	−0.53	−0.41	−0.41	−0.34	0.03	0.00
Total LBM	−0.85*	−0.95*	−0.91*	−0.91*	−0.86*	−0.79*	−0.85*	−0.50	−0.58	−0.53	−0.37	−0.61	−0.53	−0.73	−0.17	−0.24	−0.24	0.02	0.05

Note: Circumference (Thigh circ), Subcutaneous fat (Subcut fat), Leg lean body mass (leg LBM), and Total lean body mass (Total LBM)
*$p <0.05$, significance level.

literature. When initial pressure was set at 30 mmHg, the relationships between leg composition parameters and tissue oxygenation were negatively correlated and were amplified by greater amounts of leg lean body mass, total lean body mass, and larger circumferences. The importance of initial restrictive pressure is signified by Figure 4 showing that tissue oxygenation decreased with increased initial restrictive pressure and did not reach the previous level even though the cuffs were deflated between pressures used. The findings of the present study were in agreement with previous findings indicating that initial restrictive pressure over 40 mmHg might induce pooling of blood into the legs even when the cuffs are deflated (Fullana et al., 2005) and cause target restrictive pressure-dependent decreases in femoral arterial blood flow (Iida et al., 2005). The findings on the importance of initial restrictive pressure indicated that blood flow distal to the cuffs and venous return were affected and could be a factor for the amount of subproduct production and clearance resulting in variations in the findings reported by previous studies. The data signified that the low cuff pressure might be enough for the individuals who have more muscle mass during initial stages of training.

The findings of the present study were consistent with those from another study (Iida et al., 2005) reporting the same changes in haemodynamic parameters including heart rate and stroke volume in supine participants. Iida and his colleagues (Iida et al., 2005) reported decreases in stroke volume with increasing levels of the applied-pressure and the reductions in stroke volume were compensated for by an increased heart rate. Changes in femoral blood flow and pooling of blood in the thighs were measured using ultrasonography and the authors indicated that application of 200 mmHg resulted in decreases in stroke volume of 31.1% due to blood pooling in the vascular and extra-cellular compartment of the legs. It is worth noting that the participants of the present study were also in supine position during testing and the same pattern of changes in stroke volume and heart rate were observed in response to the pressure applied indicating that changes in stroke volume could also be due to venous blood pooling. In addition, similar to the previously reported results (Iida et al., 2005), the present study also observed initial restrictive pressure-dependent increases in the dark-red coloration of the skin in the legs signifying blood pooling in the thighs.

Several studies using the method of vascular restriction during resistance training have reported contrasting findings. Burgomaster et al. (2003) found after an 8-week training period performing resistance training with vascular restriction compared to low intensity resistance training without

blood flow restriction, there were no differences in changes in muscular strength between arms trained with or without vascular restriction. The cuff was deflated during the 5-min recovery interval after the third set and then reinflated to complete the remaining 3 sets of elbow flexor resistance exercises. It is important to note if the initial pressure of the cuffs prior to exercise was low (<40 mmHg), deflating the target pressure in between sets would result in an 'escape' of accumulated blood and an increased amount of clearing of metabolites produced, thus reducing the augmentation of anabolic stimulating by metabolites.

It is worth mentioning that even when the target pressure was constantly applied throughout rest periods during training, the initial pressure could be responsible for some of the training-induced adaptations. One may think that when the target pressures used are greater than the initial pressures or when the target pressures were maintained throughout training sessions, the initial pressure should not matter. However, the findings from the present study indicated that the initial pressure used might be important. The participants completed the whole procedure with no blood flow restriction-related problems such as nausea or dizziness, when the initial pressure was set at 30 and 50 mmHg; but several of the participants felt nausea and could not complete the test for the target pressures over 180 mmHg when the initial restrictive pressure was set at 70 mmHg.

Conflicting findings about the association between endogenous hormone response and muscle growth have been previously reported (West et al., 2010). West et al. (2010) investigated the effects of resistance exercise-induced increases in endogenous hormones on muscle strength and hypertrophy. The participants in the low hormone group performed arm curl exercise to sustain basal hormone concentrations; the participants in the high hormone group performed identical arm exercise to the low hormone group followed immediately by a high volume of leg exercise to obtain increases in endogenous hormones. No significant differences in cross sectional area between groups were observed even though the high hormone group had a significantly greater endogenous hormone response. The exact reasons for the conflicting results remain unclear and speculative, but since increases in hormones were caused by leg resistance exercise, low amount of local metabolite production in the elbow flexors could be causing limited stimulations of autocrine and paracrine signalling pathways leading to limited receptors and cell activation. On the other hand, considerable evidence has been accumulated supporting the hypothesis that increases in certain metabolites, in particular lactic acid and hydrogen ion concentra-

tions, increases the secretion of various anabolic hormones such as growth hormone (Ebbeling & Clarkson 1989; Fujita et al., 2007; Kraemer et al., 1998; Taylor, Butler, & Gandevia, 2000) leading to the enhancement of the training stimulus. Previous studies (Fujita et al., 2007; Takano et al., 2005;) have shown that growth hormone and plasma lactate concentrations were significantly higher after resistance training with blood flow restriction than resistance training without restriction of blood flow. It can be speculated that with the limited venous return during resistance training with vascular restriction, an amplified level of metabolite accumulation may lead to a greater endogenous hormonal response upon release of pressure, thus may be contributing to greater skeletal muscle strength and size development.

In conclusion, the data from the present study suggests that the initial restrictive pressure of the restrictive cuffs affected the amount of venous return and tissue oxygenation indicating the importance of initial pressure to manipulate the amount of blood and metabolite accumulation, which may be a responsible factor for varying findings from previous blood flow restriction training studies. Thigh composition and size were also important to increase the effects of initial restrictive pressures and therefore should be taken into account when initial pressures are set. It should be noted that the participants in the present study were investigated in a supine position; however, most of the protocols require exercises to be performed in a seated or standing position. Even though a similar negative relationship between initial restrictive pressure and tissue oxygenation during exercise is expected, the response could be different. Future studies are needed to investigate the effects of different initial pressures on tissue oxygenation during exercise, the amount of metabolite produced, and anabolic hormone concentration.

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