

THE DEVELOPMENT OF BLOOD FLOW
RESTRICTION TRAINING AS A
REHABILITATION ADJUNCT FOLLOWING
MUSCULOSKELETAL LOWER-LIMB INJURY

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Declaration

No portion of this work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institution of learning.

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Thesis Abstract

Blood flow Restriction Training [BfRT] involves the intentional and temporary reduction of blood flow through a limb to induce short periods of mild limb ischaemia, often during periods of low-intensity exercise. A systematic review into the use of BfRT to attenuate losses to lower-limb muscle strength and size during periods of injury-related impaired weight bearing revealed wide variations in the equipment, methodology and outcomes described. No evidence specifically investigated the acute physiological effect of BfRT during un-resisted, or 'no-load' rehabilitation exercises. This doctoral research project aimed to address these issues, by developing externally-valid BfRT methodologies utilising relatively inexpensive BfR equipment, and determining the acute physiological effects of combining BfRT with 'no-load' lower-limb exercise.

Phase I recruited sixty-one healthy participants across 3 subgroups; 21 males, 19 females and 21 elite male rugby players. Short periods of lower-limb blood flow restriction [BfR] were applied to participants at 40, 60, 80, 100 or 120mmHg via a thigh blood-pressure cuff. Ultrasound imaging was used to quantify the degree of popliteal arterial blood-flow remaining [%PBfR] at each cuff pressure. Subgroups were statistically different to each other across nine physical characteristics ($p \leq 0.05$). %PBfR decreased as cuff pressure increased ($p < 0.0001$), but with no between-subgroup differences ($p = 0.122$). Only weak bivariate correlations existed between physical characteristics and %PBfR across tested cuff pressures. A polynomial equation was created to indicate %PBfR based only upon the amount of thigh-cuff pressure applied.

Phase II recruited sixteen participants ($n=9$ male, $n=7$ female) who undertook four un-resisted, seated, unilateral knee extension exercise sessions with 0, 40, 60 or 80mmHg of continuous thigh-cuff pressure applied. A near-infra red spectroscopy [NIRS] device measured tissue oxygen saturation [SmO_2] of the vastus lateralis muscle before and during exercise sessions. Compared to 0mmHg, greater cuff pressures resulted in greater drops in vastus lateralis SmO_2 during exercise sessions ($p < 0.05$). Bivariate correlations existed between physical characteristics and the mean magnitude of change in SmO_2 during BfRT sessions, including Body Mass Index (Pearson $r = 0.791$, $p < 0.001$).

Phase III recruited three injured professional rugby players to undertake lower-limb BfRT 4-5 times per week over periods of 4 to 12 weeks. BfRT sessions were as per Phase II, but delivered at higher cuff pressures (100 and 120mmHg). No adverse events or pain occurred during any BfRT session. NIRS data indicated that greater cuff pressure resulted in greater drops in vastus lateralis SmO_2 during exercise sessions. Thigh girth (recorded via tape measurement) was maintained longitudinally in all players. MRI evidence suggested that BfRT did not hinder healing from a tibial and femoral osseous stress injury.

This doctoral project has expanded the evidence base available to healthcare professionals wishing to use BfRT during lower-limb injury rehabilitation. In particular, findings support the use of relatively inexpensive blood-pressure cuffs as a method of delivering BfRT, and in the ability of BfR to amplify the acute, local metabolic demand of an un-resisted 'no-load' exercise suitable for use in rehabilitation.

List of Abbreviations

%PBfR	Percentage of Popliteal [Arterial] Blood-flow Remaining
1RM	One Repetition Maximum
6MWT	Six-Minute Walk Test
2D	Two-dimensional
ASIS	Anterior Superior Iliac Spine
BALP	Bone Alkaline Phosphatase
BfR	Blood-flow Restriction
BfRT	Blood-flow Restriction Training
BMI	Body Mass Index
CSA	Cross-sectional Area
CTX	C-terminal Cross-linking Telopeptide of Type 1 Collagen
DXA	Dual X-ray Absorptiometry
FOXO	Forkhead Box Class O
HAQ	Health Assessment Questionnaire
HCP	Healthcare Professional
HHb	Deoxygenated Haemoglobin
HiF-1	Hypoxia Inducible Factor-1
HiBfRT	High-intensity ¹ Blood-flow Restricted Resistance Training
HiRT	High-intensity ¹ Resistance Training
HR	Heart Rate
IKD	Isokinetic Dynamometry
KOOS	Knee Injury and Osteoarthritis Score

¹ High-intensity is defined as $\geq 60\%$ of an individual's 1RM or MVC

LiBfRT	Low-intensity ² Blood-flow Restricted Resistance Training
LiRT	Low-intensity ² Resistance Training
MAFbx	Muscle Atrophy F-box
MDC	Minimum Detectable Change
MSK	Musculoskeletal
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MuRF-1	Muscle RING-finger Protein-1
MVC	Maximum Voluntary Contraction
NIRS	Near Infrared Spectroscopy
NMES	Neuromuscular Electrical Stimulation
OMNI-RES	Omnibus Perceived Exertion Scale for Resistance Exercise
PWD	Pulsed-wave Doppler
SF-36	36-item Short Form Survey
SmO₂	Muscle Tissue Oxygen Saturation
PI	Pulsatility Index
RCT	Randomised Controlled Trial
RI	Resistive Index
RPE	Rate of Perceived Exertion
TAMV	Time Averaged Mean Velocity
tHb	Total Haemoglobin
TUG	Time 'Up-and-Go' Test
ULLS	Unilateral Lower-limb Suspension
VEGF	Vascular Endothelial Growth Factor

² Low-intensity is defined as $\leq 30\%$ of an individual's 1RM or MVC.

List of Figures

Figure 2.3.1. A PRISMA flowchart documenting the selection of studies included within the systematic literature review for this project.

Figure 3.3.1. The initial positioning of the thigh cuff in standing.

Figure 3.3.2. A sagittal view of the seated testing position used throughout the project.

Figure 3.3.3. A sample 2-dimensional ultrasound image of the popliteal region.

Figure 3.3.4. A typical pulsed-wave Doppler ultrasound image taken from the popliteal artery.

Figure 3.3.5. A 2-dimensional ultrasound image showing the alignment and path of the Doppler beam during blood-flow measurements.

Figure 3.3.6. A flow chart depicting the collection sequence of 2-dimensional and pulsed-wave Doppler ultrasound images within one 'image block'.

Figure 3.3.7. A 2-dimensional ultrasound image demonstrating arterial diameter measurement.

Figure 3.3.8. A Doppler image demonstrating peak blood-flow velocity measurement.

Figure 3.3.9. A Doppler image demonstrating mean blood-flow velocity measurement.

Figure 3.3.10. A Doppler image demonstrating Pulsatility Index and Resistive Index measurements.

Figure 3.3.11. A flowchart depicting the process by which the degree of blood-flow restriction occurring in the popliteal artery was determined.

Figure 3.3.12. A graph displaying the absorption spectra of oxygenated and de-oxygenated haemoglobin across near infrared light wavelengths.

Figure 3.3.13. The Moxy Monitor device.

Figure 3.3.14. Placement of the Moxy Monitor in a position of full active knee extension.

Figure 3.3.15. Wrapping of the Moxy Monitor and thigh with black cohesive bandage.

Figure 3.3.16. A Doppler image showing the measurement of RR intervals and corresponding heart rate.

Figure 3.5.1. The start position of the seated knee extension exercise.

Figure 3.5.2. The start position of the seated knee extension exercise.

Figure 4.4.1. A bar chart displaying the baseline blood flow readings taken over the course

of the Phase I pilot protocol.

Figure 4.4.2. A bar chart displaying the percentage of superficial femoral artery blood flow remaining at different thigh-cuff pressures during the Phase I pilot study.

Figure 4.5.1. A box plot displaying median value and distribution of %PBfR within each Phase I subgroup at each of the five cuff inflation pressures.

Figure 4.5.2. Phase I cohort %PBfR values fitted to a second-order polynomial trend line.

Figure 4.5.3. Acute haemodynamic responses to lower-limb blood-flow restriction, measured at the popliteal artery.

Figure 5.4.1. A flowchart describing the time-course and data collection points of a one testing session within Phase II.

Figure 5.4.2. A graphical representation of in-session changes to vastus lateralis SmO₂ during the Phase II pilot study.

Figure 5.4.3. A graphical representation of in-session changes to heart rate during the Phase II pilot study.

Figure 5.4.4. Set-by-set OMNI-RES scores recorded during exercise sessions of the Phase II pilot study.

Figure 5.5.2. Changes in cohort vastus lateralis SmO₂ values during the Phase II study exercise protocol performed at different thigh-cuff inflation pressures.

Figure 5.5.3. Changes in cohort vastus lateralis tHb across the Phase II study exercise protocol performed at different thigh-cuff inflation pressures.

Figure 5.5.4. Changes in cohort vastus lateralis HHb across the Phase II study exercise protocol performed at different thigh-cuff pressures.

Figure 5.5.5 Changes in cohort heart rate across the Phase II study exercise protocol performed at different thigh-cuff inflation pressures.

Figure 5.5.6. A scatterplot showing the bivariate association between Body Mass Index and mean %PBfR experienced over the three BfRT sessions.

Figure 6.3.1. A graphical representation of in-session changes to vastus lateralis SmO₂ during the Phase III pilot study.

Figure 6.3.2. A graphical representation of in-session changes to heart rate during the Phase III pilot study.

Figure 6.3.3. Set-by-set OMNI-RES scores recorded during the exercise sessions of the Phase III pilot study.

Figure 6.4.1. Changes in vastus lateralis tissue oxygen saturation of Phase III case studies

(n=3), relative to baseline, during unweighted knee extension exercise sessions performed under different modalities of blood flow restriction.

Figure 6.4.2. Changes in vastus lateralis total haemoglobin levels of Phase III case studies, relative to baseline, during unweighted knee-extension exercise sessions performed under different modalities of blood flow restriction.

Figure 6.4.3. Changes in vastus lateralis deoxygenated haemoglobin levels of Phase III case studies, relative to baseline, during unweighted knee extension exercise sessions performed under different modalities of blood flow restriction.

Figure 6.4.4. Changes in the heart rate of Phase III case studies, relative to baseline, during an unweighted knee extension exercise performed under different modalities of blood flow restriction.

Figure 6.4.5. Mean rate of perceived Exertion of the Phase III case studies (n=3), during unweighted knee extension exercise sessions performed under different modalities of blood flow restriction.

Figure 7.5.1. A visual representation of how a thigh-cuff 'sweet-spot' pressure could be determined for use during LiBfRT, performed with a wide cuff at 40% MVC.

Figure 7.5.2. A visual representation of how a thigh-cuff 'sweet-spot' pressure could be determined for use during LiBfRT, performed with a narrow cuff at 20% MVC.

List of Tables

Table 3.1. *The prevalence of health-related exclusion criteria within lower-limb BfRT experimental studies from 1995-2014.*

Table 3.2. *A tabulated reference of the objective and subjective measures collected within each phase of this doctoral research.*

Table 4.4.1. *Participant demographics of the Phase I pilot study.*

Table 4.4.2. *Cohort values for heart rate, arterial cross-sectional area and perceptual responses during the Phase I pilot study.*

Table 4.5.1. *Phase I cohort and subgroup physical characteristics.*

Table 4.5.2. *Participant-reported values for discomfort during thigh-cuff inflations*

Table 4.5.3. *Participant-reported values for pain during thigh-cuff inflations*

Table 4.5.4. *Median popliteal artery blood flow of the Phase I cohort prior to each thigh-cuff inflation pressure.*

Table 4.5.5. *Median popliteal artery blood flow of the Phase I cohort during each thigh-cuff inflation pressure.*

Table 4.5.6. *Mean and median %PBfR values of the Phase I cohort during each thigh-cuff inflation pressure.*

Table 4.5.7. *Mean heart rate values of the Phase I cohort recorded during each thigh-cuff pressure.*

Table 4.5.8. *Median values for three haemodynamic variables recorded for the Phase I cohort during each thigh-cuff pressure.*

Table 4.5.7. *Bivariate correlations between participant-mean %PBfR and physical characteristics of the Phase I cohort.*

Table 5.7.1. *Inter-study comparisons of reported haemodynamic values within the human popliteal artery at rest.*

Table 5.4.1. *Mean values recorded during the three-minute, pre-exercise baseline periods of the Phase II pilot study.*

Table 5.4.2. *The coefficient of variation in haemodynamic values taken over each three-minute, pre-exercise baseline period of the Phase II pilot study.*

Table 5.4.3. *The mean relative change in haemodynamic values during the Phase II pilot-study exercise sessions, compared to pre-exercise baseline values.*

Table 5.5.1. Resting haemodynamic mean values for the Phase II cohort.

Table 5.5.2. Phase II cohort changes to vastus lateralis tHb during un-resisted knee exercise at different thigh-cuff inflation pressures.

Table 5.5.3. Session OMNI-RES scores reported during un-resisted knee exercise at different thigh-cuff inflation pressures.

Table 5.5.4. A comparison of physical characteristics between the Phase I and Phase II study cohorts.

Table 6.3.1. The existing stepped blood-flow restriction training protocol in place at Warrington Wolves Rugby League Club at the commencement of the project.

Table 6.3.2. The revised BfRT protocol used within the Phase III pilot study.

Table 6.3.3. Mean values recorded during the three-minute, pre-exercise baseline periods of the Phase III pilot study.

Table 6.3.4. The coefficient of variation in values taken over each three-minute, pre-exercise baseline period during the Phase III pilot study.

Table 6.3.5. The mean change in outcome measure values over the course of each exercise session, compared to pre-exercise baseline values, during the Phase III pilot study.

Table 6.4.1. The staged BfRT protocol used within the Phase III study.

Table 6.4.2. Longitudinal thigh girth measurements and LEFS scores; Phase III, case one.

Table 6.4.3. Longitudinal thigh girth measurements and LEFS scores; Phase III, case two.

Table 6.4.4. Longitudinal thigh girth measurements and LEFS scores; Phase III, case three.

Table 6.4.5. Resting mean values for haemodynamic variables recorded during the Phase III study.

Table 6.4.6. The mean in-session change to haemodynamic variables recorded during Phase III exercise sessions, compared to resting values.

CONTENTS

Acknowledgments	4
Thesis Abstract	5
List of Abbreviations	6
List of Figures	8
List of Tables	11
Contents	13
Chapter One: The Concept of Blood-flow Restriction Training [BfRT]	
1.1 Chapter Introduction	19
1.2 The Concept of BfRT	19
1.3 The Effect of BfRT on the Mechanisms of Muscular Adaptation	22
<i>1.3.1 The Modulation of Exercise-related Signals</i>	23
1.3.1.1 Local Tissue Hypoxia	23
1.3.1.2 Lactate Accumulation	23
1.3.1.3 Neuromuscular Activation and Fatigue	25
1.3.1.4 Cellular Swelling in Response to Resistance Exercise	28
1.3.2 The Transduction of Exercise-related Signals and Gene Expression	28
1.3.2.1 Systemic Hormone Levels	28
1.3.2.2 The Akt/mTOR and Myostatin-Smad Pathways	29
1.3.2.3 The Ubiquitin-proteasome Pathway	30
1.3.3 Longitudinal Muscular Adaptations in Response to BfRT	32
1.3.3.1 Muscular Hypertrophy and Strength Development	32
1.3.3.2 LiBfRT during Injury Rehabilitation	37
1.3.3.3 Skeletal Muscle Angiogenesis	37
1.3.3.4 Longitudinal Changes in Overall Physical Function	38
1.3.4 Conclusions	42
Chapter Two: The Effect of BfRT upon Lower-limb Muscle Size and Performance among Individuals with an Acute Lower-limb Injury: A Systematic Review	
2.1 Chapter Introduction	45
2.1.1 Rationale for the Review	45
2.1.2 Research Questions	47
2.2 Methods	47
2.2.1 Protocol and Registration	47
2.2.2 Eligibility Criteria	48
2.2.3 Information Sources	50
2.2.4 Search Terms	50
2.2.5 Study Selection	51
2.2.6 Data Collection Process	51

2.2.7 <i>Data Items</i>	52
2.2.8 <i>Risk of Bias within Studies</i>	53
2.3 Results	53
2.3.1 <i>Study Selection</i>	53
2.3.2 <i>Study Characteristics</i>	54
2.3.3 <i>Risk of Bias within Controlled Trials</i>	55
2.4 Discussion	55
2.4.1 <i>Summary of Evidence</i>	55
2.4.1.1 <i>Changes in Lower-limb Muscle Size and/or Girth</i>	55
2.4.1.2 <i>Changes in Lower-limb Muscular Performance</i>	63
2.4.1.3 <i>Subjective Reporting of Physical Function</i>	67
2.4.1.4 <i>BfRT Equipment and Methodology</i>	68
2.4.2 <i>Strengths and Limitations of this Review</i>	69
2.4.2.1 <i>Strengths</i>	69
2.4.2.2 <i>Limitations</i>	70
2.5 Review Conclusions	71
2.6 Project Aims and Objectives	74
2.6.1 <i>Phase I</i>	74
2.6.1.1 <i>Aim</i>	74
2.6.1.2 <i>Hypotheses</i>	75
2.6.2 <i>Phase II</i>	75
2.6.2.1 <i>Aim</i>	75
2.6.2.2 <i>Hypotheses</i>	75
2.6.3 <i>Phase III</i>	75
2.6.3.1 <i>Aim</i>	75
 Chapter Three: Research Methodologies and Outcome Measures	
3.1 Chapter Introduction	77
3.2 Research Participants	77
3.2.1 <i>Participant Recruitment</i>	77
3.2.2 <i>Testing Locations</i>	78
3.2.3 <i>Participant Consent and Ethical Approval</i>	78
3.2.4 <i>Participant Health Screening</i>	78
3.3 Project Outcome Measures	83
3.3.1 <i>Anthropometric Outcome Measures</i>	83
3.3.1.1 <i>Height/Body Weight</i>	83
3.3.1.2 <i>Brachial Blood Pressure</i>	83
3.3.1.3 <i>Leg Dimensions</i>	84
3.3.1.4 <i>Body Composition</i>	85
3.3.2 <i>Physiological Outcome Measures</i>	86
3.3.2.1 <i>Blood-flow Measurements</i>	88
3.3.2.2 <i>Microvascular Oxygenation Saturation of Tissue [SmO₂]</i>	99
3.3.2.3 <i>Heart Rate</i>	103

3.3.3 <i>Subjective Outcome Measures</i>	105
3.3.3.1 <i>Pain</i>	105
3.3.3.2 <i>Discomfort of the Testing Procedure</i>	106
3.3.3.3 <i>Rate of Perceived Exertion</i>	106
3.3.3.4 <i>Subjective Physical Function</i>	108
3.3.3.5 <i>Habitual Physical Activity</i>	110
3.4 <i>Lower-limb Blood-flow Restriction Equipment</i>	111
3.4.1 <i>Cuff Types</i>	111
3.4.2 <i>Cuff Inflation Devices</i>	111
3.5 <i>BfR Exercise Protocol</i>	112
3.6 <i>Chapter Summary</i>	113

Chapter Four: Phase I – Associations between Participant Physical Characteristics, Thigh-Cuff Pressure and the Degree of Lower-limb Blood Flow Restriction Being Delivered

4.1 <i>Chapter Introduction</i>	115
4.2 <i>Phase I Aim</i>	115
4.3 <i>Background</i>	115
4.4 <i>Phase I Pilot Study</i>	120
4.4.1 <i>Pilot Study Objectives</i>	120
4.4.2 <i>Methods</i>	121
4.4.3 <i>Results</i>	122
4.4.4 <i>Discussion</i>	125
4.4.5 <i>Conclusions and Protocol Adjustments</i>	126
4.5 <i>Phase I Main Study</i>	128
4.5.1 <i>Main Study Objectives</i>	128
4.5.2 <i>Hypotheses</i>	128
4.5.3 <i>Methods</i>	129
4.5.4 <i>Analysis</i>	130
4.5.5 <i>Results</i>	131
4.5.5.1 <i>Participant Anthropometrics</i>	131
4.5.5.2 <i>Perceptual Responses to Thigh-Cuff Inflations</i>	132
4.5.5.3 <i>Haemodynamic Measurements</i>	133
4.5.5.4 <i>Correlations between Physical Characteristics and %PBfR</i>	137
4.5.6 <i>Discussion</i>	142
4.5.6.1 <i>Reliability and Validity of Ultrasound Measurements</i>	142
4.5.6.2 <i>The Acute Haemodynamic Responses to Lower-limb BfR</i>	143
4.5.6.3 <i>Correlations between physical characteristics and %PBfR</i>	148
4.5.7 <i>Study Strengths and Limitations</i>	149
4.5.8 <i>Conclusions</i>	151

Chapter Five: Phase II – The Acute Physiological and Perceptual Effects of Adding Lower-limb BfRT to a Seated, Unweighted Knee Extension Exercise

5.1 Introduction	153
5.2 Phase II Aim	153
5.3 Background	154
5.4 Phase II Pilot Study	162
5.4.1 <i>Pilot study Objectives</i>	162
5.4.2 <i>Methods</i>	162
5.4.3 <i>Results</i>	165
5.4.4 <i>Discussion</i>	169
5.4.5 <i>Conclusions</i>	171
5.5 Phase II Main Study	171
5.5.1 <i>Main Study Objectives</i>	171
5.5.2 <i>Hypotheses</i>	171
5.5.3 <i>Methods and Statistical Analysis</i>	172
5.5.3.1 <i>Exercise Session Methodology</i>	172
5.5.3.2 <i>The Degree of Lower-Limb Blood-flow Restriction Applied</i>	173
5.5.3.3 <i>The Acute Metabolic Demand of the Knee Exercise</i>	173
5.5.3.4 <i>Subjective Responses</i>	173
5.5.3.5 <i>Associations with Physical Characteristics</i>	174
5.5.4 <i>Results</i>	174
5.5.4.1 <i>The Degree of Lower-Limb Blood-flow Restriction Applied</i>	175
5.5.4.2 <i>The Acute Metabolic Demand of the Knee Exercise</i>	175
5.5.4.3 <i>Subjective Responses</i>	182
5.5.4.3 <i>Association with Physical Characteristics</i>	183
5.5.5 <i>Discussion</i>	186
5.5.5.1 <i>The Degree of Lower-Limb Blood-flow Restriction Applied</i>	186
5.5.5.2 <i>The Acute Metabolic Demand of the Knee Exercise</i>	187
5.5.5.3 <i>Subjective Responses</i>	192
5.5.5.4 <i>Associations with Physical Characteristics</i>	193
5.5.6 <i>Study Strengths, Limitations and Future Research Directions</i>	195
5.5.7 <i>Conclusions</i>	197

Chapter Six: Phase III – The Application of Lower-limb BfRT among Injured Elite Rugby-League Players: A Real-world Case Series

6.1 Chapter Introduction	199
6.2 Phase III Aim	199
6.3 Phase III Pilot Study	199
6.3.1 <i>Pilot Study Objectives</i>	199
6.3.2 <i>Methods</i>	200
6.3.3 <i>Results</i>	203
6.3.4 <i>Discussion</i>	207
6.3.5 <i>Conclusions and Protocol Adjustments</i>	207
6.4 Phase III Main Study	208

6.4.1 Main Study Objectives	208
6.4.2 Methods	208
6.4.2.1 Lower-limb Measurements	210
6.4.2.2 Haemodynamic Variables	210
6.4.2.3 Subjective Outcome Measures	211
6.4.3 Results	211
6.4.3.1 Case One	211
6.4.3.2 Case Two	212
6.4.3.3 Case Three	214
6.4.3.4 Haemodynamic Variables	215
6.4.3.5 Perceptual Responses to Exercise	217
6.4.4 Discussion	220
6.4.4.1 Lower-limb Measurements	220
6.4.4.2 Haemodynamic Variables	221
6.4.4.3 Subjective Outcome Measures	223
6.4.5 Study Strengths and Limitations	225
6.4.6 Conclusions	225
Chapter Seven: Project Conclusions and Implications	
7.1 Chapter Introduction	227
7.2 Project Aim and Objectives	227
7.3 Key Findings	228
7.3.1 Phase I	228
7.3.2 Phase II	229
7.3.2 Phase III	230
7.4 Key Messages of This Doctoral Research Project	230
7.5 The Achievement of Project Objectives	231
7.5.1 Objective One	231
7.5.2 Objective Two	237
7.6 Recommendations for Future Research	241
7.6.1 Phase I	241
7.6.2 Phase II	242
7.6.3 Phase III	243
7.6.4 Beyond Project Phases	243
References	246
Appendices	272
I. Project Paperwork Samples	273
a. Health Screening Questionnaire (Phase II Example)	273
b. Written Consent Form (Phase I Example)	275
c. The Numerical Rating Scale for Pain	276
d. The Numerical Rating Scale for Discomfort	277

e. <i>The Omnibus Perceived Exertion Scale for Resistance Exercise</i>	278
II. A Dual-energy X-ray Absorptiometry Scan Example	279
III. Phase I Cohort Anthropometrics	280
IV. Bivariate Correlations between the In-session Changes to Haemodynamic Variables and Physical Characteristics of the Phase II Cohort	
a. <i>SmO₂ versus Physical Characteristics</i>	281
b. <i>tHb versus Physical Characteristics</i>	282
c. <i>HHb versus Physical Characteristics</i>	283
d. <i>Three-session Mean Change in Haemodynamic variables versus Physical Characteristics</i>	284
V. Systematic Review Data (Relating to chapter Two)	
a. <i>Data Synthesis Table</i>	285
b. <i>Risk of Bias Table</i>	288
VI. Research Outputs Arising from this Doctoral Research Project	
a. <i>A Conference Paper relating to The Phase I Main Study</i>	289
b. <i>A Conference Paper relating to The Phase II Main Study</i>	291

CHAPTER ONE

Blood-flow Restriction Training [BfRT]

1.1 Chapter Introduction

Blood flow Restriction Training [BfRT] involves the intentional and temporary reduction of blood flow through a limb to induce short periods of mild limb ischaemia, often during periods of low-intensity exercise. Using BfRT among populations with a lower-limb musculoskeletal injury may help to attenuate the losses in lower-limb muscle mass and strength seen during extended period of impaired weight bearing. However, there are wide variations in the equipment, methodology and outcomes described across BfRT research, with no evidence specifically investigating the acute physiological effects of BfRT during un-resisted, or 'no-load' rehabilitation exercises. In light of this, this doctoral research project aims to develop and refine the use of BfRT within the context of lower-limb injury rehabilitation.

This chapter will introduce the reader to the overall concept of BfRT. A brief overview of the concept is first given, followed by a discussion as to how BfRT may acutely modulate the exercise-related signals and cellular signalling pathways implicated in longitudinal muscular adaptation. The chapter ends with a comparison of the longitudinal effects of different resistance training modalities upon muscular adaptation and function, with and without lower-limb blood flow restriction [BfR] superimposed.

1.2 The Concept of BfRT

BfRT involves the intentional and temporary reduction of blood flow through an upper or

lower limb to induce short periods of mild limb ischaemia, often during periods of low-intensity exercise. Its inception can be largely attributed to Dr. Yoshiaki Sato, who reportedly pioneered the technique in 1966 (Sato, 2005). His theory surmised that artificially restricting blood-flow whilst completing low-intensity resistance training may replicate the physiological demands of traditional high-intensity resistance training (Kaatsu Global©, 2015a). Bicycle inner tubes, acting as rudimentary tourniquets around the proximal portions of his limbs, were used to repetitively induce blood-flow restriction [BfR], with a claimed effect of longitudinal muscle hypertrophy (Sato, 2005). In 1973, Dr. Sato reportedly combined the technique with isometric exercises to prevent lower-limb muscle atrophy after fracturing both ankles in a skiing accident. Development of more advanced restriction apparatus (Sato Sports Plaza Co. Ltd., 2014) and training protocols resulted in commercialisation and certification of the technique under the moniker of 'Kaatsu' training; a Japanese term for 'additional pressure' (Kaatsu Global©, 2015b; 2015c).

Whilst Kaatsu training was only recently patented, the concept of BfRT has been studied academically since 1987, with BfR induced through a variety of different restriction apparatus. This includes the use of pressurised air chambers (Eiken and Bjurstedt, 1987; Sundberg, 1993), elastic knee wraps (Head et al, 2015; Loenneke et al, 2010) and inflatable thigh-cuff devices (Cayot et al, 2015; Iida et al, 2007). Whilst training protocols and restriction apparatus differ greatly across academic texts, BfRT can be grouped into three broad categories:

- BfR combined with low-intensity or high intensity resistance training.
- BfR combined with cardiovascular exercise.
- BfR in isolation, without simultaneous exercise.

BfR combined with low-intensity or high intensity resistance training

Participants complete weighted resistance exercise using free-weights or an exercise machine. Typically, the maximum voluntary contraction [MVC] or one repetition maximum [1RM] for a given exercise is first determined. BfR is then applied via an inflatable cuff placed around the proximal part of a limb. The cuff then remains inflated throughout a multiple-set exercise session performed at a low-intensity (20-30% 1RM/MVC) before being deflated upon completion of the final exercise set (Clark et al, 2011; Ellefsen et al, 2015; Laurentino et al, 2012). Other variations exist, including the use of BfR intermittently across an exercise session, or the use of BfR during high-intensity resistance training at $\geq 60\%$ 1RM/MVC (Laurentino et al, 2008; Neto et al, 2014; Teixeira et al, 2017).

BfR combined with cardiovascular exercise

Participants complete an aerobic exercise programme such as treadmill walking (Abe et al, 2006; Abe et al, 2010a; Salvador et al, 2016), stationary cycling (Abe et al, 2010b; Corvino et al, 2014) or water-based exercise (Araújo et al, 2015). BfR is applied to both limbs either continuously or intermittently across the exercise session.

BfR in isolation, without simultaneous exercise

Participants are given repetitive bouts of lower-limb BfR at rest, without the completion of any simultaneous exercise. This application is typically given to participants who possess a lower-limb injury (Takarada et al 2000b), or who are simulating lower-limb immobilisation (Kubota et al, 2008; Kubota et al, 2011) or weightlessness (Nakajima et al, 2008), with a view to preventing or limiting muscle disuse atrophy.

Before the proposed mechanisms of action of BfRT can be discussed, the basic process by which traditional exercise (without BfR) is thought to generate muscular adaptation will first be described. Wackerhage (2014) splits this process into three stages. Firstly, exercise-related signals that indicate a perturbation to cell homeostasis are detected by sensor proteins. These signals include increased ATP turnover, a reduction in the partial pressure of oxygen and an increased production of reactive oxygen species within tissues (Camera et al, 2016; Egan and Zierath, 2013). Secondly, these signals are transduced through cellular signalling pathways, reaching effector proteins within the cell nucleus. Ultimately, these effector proteins regulate a myriad of cellular functions including muscle protein synthesis and degradation (Wackerhage, 2014). Whilst effector proteins are responsible for producing muscular adaptation, how a muscle adapts can be modulated by the frequency, intensity, duration and modality of the exercise(s) completed (Egan and Zierath, 2013). Age, gender, and environmental factors may further influence this process. (Lemmer et al, 2000; Roth et al, 2002; Zempo et al, 2016).

1.3 The Effect of BfRT on the Mechanisms of Muscular Adaptation

The precise mechanisms by which BfRT produces favourable muscular adaptations are not yet fully understood (Pearson and Hussain, 2015; Scott et al, 2015). However, existing work demonstrates that the use of BfR can affect each of the stages of muscular adaptation noted by Wackerhage (2014). This BfRT evidence base is considerable in size, consisting of over two-hundred and sixty experimental studies and 38 narrative reviews on various aspects of the topic. Therefore, the majority of literature discussed in the remainder of this chapter relates to the effects of BfR modalities most relevant to this doctoral research project; the use of lower-limb BfR in isolation, or the use of lower-limb BfR combined with low-intensity lower-limb exercise.

1.3.1 The Modulation of Acute Exercise-related Signals

1.3.1.1 Local Tissue Hypoxia

Oxygen consumption within active muscle tissue increases during exercise and can exceed the rate at which oxygen can be delivered via the cardiovascular system. This can produce an acute, hypoxic intramuscular environment during lower-limb resistance exercise (Tanimoto et al, 2006). Evidence has demonstrated the ability of BfRT to amplify the degree of acute muscle tissue deoxygenation occurring during lower-limb resistance exercise performed at lower intensities ($\leq 30\%$ 1RM) (Downs et al, 2014; Tanimoto et al, 2005).

Reduced oxygen levels within skeletal muscle are known to activate hypoxia-sensing transcription factor [HIF-1], whose target genes are involved in increasing oxygen transport to improve tissue function during low oxygen availability (Lindholm and Rundqvist, 2016). Activation of HIF-1 may regulate the expression of Vascular Endothelial Growth Factor [VEGF], an angiogenic signal protein that can induce an increase in muscle capillarisation (Hoier and Hellsten, 2014; Semenza, 2000). Work by Ameln et al (2005) has demonstrated that both HIF-1 α and VEGF levels increase acutely in response to the demands of a lower-limb knee-extension exercise. In this study, VEGF levels of nine healthy males were significantly higher when lower-limb BfR was superimposed over the exercise protocol. Subsequent studies have also shown acute VEGF mRNA expression is significantly amplified by the addition of lower-limb BfR to resistance exercise, compared to the exercise alone (Item et al, 2013; Larkin et al, 2012 Takano et al, 2005).

1.3.1.2 Lactate Accumulation

The current theories surrounding lactate have developed considerably from the

traditional viewpoint of the molecule as simply being a waste product of glycolysis and responsible for exercise-induced fatigue (Philp et al, 2005). Growing evidence supports the potential role of lactate as an anabolic signalling molecule, implicated in the mediation of exercise-induced adaptations (Nalbandian and Takeda, 2016; Philp et al, 2005; Todd, 2014). Speculatively therefore, BfRT may be of benefit if it could be utilised to create (or amplify) acute lactate accumulation within muscle tissue.

Applying lower-limb BfR does appear to amplify lactate accumulation during low-intensity resistance exercise (20-30% 1RM). For example, Fujita et al (2007) documented changes in the blood lactate of six young males (mean age; 32 ± 2 years) immediately following four sets of bilateral knee extension exercise completed at a low-intensity (20% 1RM). Exercise sessions were performed with or without BfR superimposed. Whilst blood lactate was notably elevated following both exercise conditions, levels remained significantly higher for a period of 40 minutes where BfR had been superimposed ($p < 0.05$). Fry et al (2010) later replicated these findings within a group of seven older males (mean age; 70 ± 2 years), albeit with lower magnitudes of lactate change. Low sample sizes and unreported effect sizes in the studies by Fujita et al (2007) and Fry et al (2010) may indicate a need for caution when interpreting these findings in isolation. However, recent studies have utilised greater participant numbers and more extensive reporting of statistical methods (Loenneke et al, 2016; Teixeira et al, 2017). These studies reported that adding BfR to low-intensity knee-extension exercises achieved a level of lactate accumulation which matched or exceeded that seen during high exercise intensity (70% 1RM) performed without BfR.

The capacity of BfRT to match the lactate accumulation of traditional high-intensity

resistance training does not appear universal, however. Within a group of eighteen hypertensive women, Pinto et al (2016) demonstrated that BfR combined with a session of low-intensity (20% 1RM) knee extensions did not increase blood lactate to the level seen during the completion of the same exercise at 65% 1RM without BfR. Whilst this contradicts the findings of Loenneke et al (2016), the exercise volume delivered within Pinto et al (2016) was notably lower (30 repetitions vs. 75 repetitions) with longer inter-set rest periods (60 seconds vs. 30 seconds). It is possible that, even with BfR applied, the parameters of the exercise protocol in Pinto et al (2016) were of insufficient intensity to generate the lactate levels seen in the traditional high-intensity exercise.

It should also be noted that favourable muscular adaptations can occur in the apparent absence of lactate accumulation. For example, Loenneke et al (2012e) found that repetitive bouts of treadmill walking at 4.5km/h did not significantly increase the post-session blood lactate levels of a small cohort of healthy men and women (n = 9), whether BfR was applied or not. Yet earlier longitudinal studies with both similar and older cohorts (Abe et al, 2006; Abe et al, 2010a; Ozaki et al, 2011; Sakamaki et al, 2011) demonstrated that this type of exercise, performed with BfR at a slower pace (3-4km/h), significantly increased thigh muscle size and strength compared to non-restricted control groups.

1.3.1.3 Neuromuscular Activation and Fatigue

It has been proposed that motor units, consisting of motor neurons and muscle fibres, are recruited in size order as the force requirement of an exercise increases (Henneman et al, 1965; Mendell 2005). In practice, motor units containing predominantly slow-twitch oxidative fibres are recruited first. By increasing the firing rate of upstream neurons synapsing with motor units, predominantly fast-twitch (Type II) glycolytic fibres are then

recruited to generate additional force. During sustained exercise, where slower-twitch oxidative fibres may become fatigued over time, faster-twitch glycolytic fibres may also be progressively recruited to allow the exercise to continue (Wackerhage, 2014). Maximal neuromuscular activation and fatigue would ultimately ensue, leading to exercise failure. This 'size principle' is still not universally accepted (Bawa et al, 2014). However, recent mathematical models of motor unit fatigue that incorporate this principle are able to predict actual motor unit endurance times with good fidelity (Potvin and Fuglevand, 2017).

It has been proposed that the activation of fast-twitch/Type II fibres during exercise, independent of the intensity of the exercise being completed, is the primary variable that affects stimulation of muscle protein synthesis and thus longitudinal muscle hypertrophy (Phillips, 2009). An acute study by Burd et al (2010) appears to support this notion within the context of traditional (non-BfR) resistance exercise. Data obtained from fifteen healthy males suggested that knee extensions performed at 30% 1RM to volitional failure produce similar, significant post-session increases in mixed and myofibrillar fractional synthetic rate as knee extension performed at 90% 1RM to volitional failure. A recent, high-quality study also reflects this longitudinally, demonstrating within a cohort of forty-nine males that training to volitional failure produces similar gains in strength and muscle size over eight weeks, regardless of whether traditional high-intensity (75-90% 1RM) or low-intensity (30-50% 1RM) resistance exercise programmes are employed (Morton et al, 2016).

Loenneke et al (2011a) acknowledged the work by Phillips et al (2009) and the need to activate fast-twitch/Type II muscle fibres to stimulate muscle protein synthesis. Loenneke

et al (2011a) hypothesised that, in situations where low-intensity exercise is completed to volitional failure, adding BfR allows failure to be reached with a comparatively lower volume of work. This was substantiated by Cook et al (2013), who found that adding BfR to a low-intensity knee extension exercise reduced the amount of repetitions needed to reach failure by approximately 33%. Wernbom et al (2012) also demonstrated that knee extensions performed to volitional failure across five exercise sets generated comparatively greater ($p < 0.01$) decrements in MVC immediately post-exercise when BfR was superimposed. In studies where volitional failure was not part of the exercise protocol, statistically greater decrements in MVC were still achieved via the addition of BfR. Karabulut et al (2010) demonstrated this within their cohort of fourteen healthy males during the delivery of a knee extension exercise at 20% 1RM across five exercise sets of 20 repetitions ($p < 0.05$), with results indicating that a mixture of central and peripheral fatigue was responsible. Loenneke et al (2013a) also demonstrated significantly greater ($p < 0.05$) torque decrements post-session due to BfR, among eight healthy males who exercised across four sets performed at 30% 1RM.

Statistically significant ($p < 0.05$) pre-post decrements in knee extensor torque/MVC following lower-limb resistance exercise can persist up to forty-eight hours post-session (Wernbom et al, 2012), but the addition of BfR does not appear to significantly amplify these decrements by a statistically significant degree (Loenneke et al, 2013a; Wernbom et al, 2012). However, both studies involved low sample sizes and power calculations were absent from their texts. Further studies which address these issues, lowering the possibility of a Type II error, may provide a clearer picture as to the effect of BfR upon neuromuscular fatigue beyond one hour post-exercise.

1.3.1.4 Cellular Swelling in Response to Resistance Exercise

Traditional resistance exercise is known to produce a hydration-mediated cellular swelling response, which itself has been linked to increases in protein synthesis, reduction in proteolysis and potential hypertrophic muscular adaptation (de Freitas et al, 2017; Schoenfeld 2010; Schoenfeld 2013). During BfRT, a limb-swelling effect has been demonstrated both with and without simultaneous exercise (Loenneke et al 2012c; Loenneke et al 2016). It has been hypothesised that a cell-swelling response during BfR training occurs from a combination of blood pooling, metabolite accumulation and reactive hyperaemia (Loenneke et al, 2012a). Interestingly, a similar limb-swelling response can occur during low-intensity resistance exercise whether BfR is applied or not, providing exercise sets are completed until exhaustion (Yasuda et al, 2015). The beneficence of applying BfR in this case was a 50% reduction in the volume of exercise required to reach exhaustion.

1.3.2 The Transduction of Exercise-related Signals and Gene Expression

1.3.2.1 Systemic Hormone Levels

Changes in systemic hormone levels occur following resistance exercise, including acute increases in growth hormone, cortisol and testosterone (Kraemer et al, 2005). The magnitude of change in hormone levels appears to be acutely influenced by the parameters and intensity of the exercise completed (Kraemer et al, 1990; Smilios et al, 2003; Bottaro et al, 2009). Early work proposed that substances such as growth hormone amplified protein synthesis in response to muscular work and determined absolute muscle size (Goldberg and Goodman, 1969). Studies have shown that the addition of BfR to low-intensity (20-30% 1RM) resistance exercise amplifies the post-session blood serum levels of systemic hormones, including cortisol (Fry et al, 2010; Fujita et al, 2007), growth

hormone (Fujita et al, 2007; Takano et al, 2005; Takarada et al, 2000a) and insulin-like growth factor 1 [IGF-1] (Takano et al, 2005). Equally, evidence suggests that low-intensity BfRT programmes create similar chronic changes to resting serum hormone levels as traditional high-intensity resistance training programmes (Ellefsen et al, 2015; Kim et al, 2014). Whilst this may support the addition of BfR to low-intensity resistance programmes, a growing evidence base proposes that both acute and chronic changes in systemic hormone levels are unrelated to changes in muscle size and strength following resistance training programmes (Mitchell et al, 2013; Morton et al, 2016; West et al, 2009, West et al, 2012). Therefore, it is possible that the amplification of hormone responses by the addition of BfRT is inconsequential.

1.3.2.2 The Akt/mTOR and Myostatin-Smad Pathways

Muscle hypertrophy occurs through a shift towards muscle protein synthesis and away from protein degradation (Egerman and Glass, 2014). Primarily, two cellular signalling pathways work in tandem to regulate muscle growth (Schiaffino et al, 2013). The Akt/mTOR pathway is geared towards anabolism, transducing upstream exercise-related signals across to the cell nucleus to increase protein synthesis and muscle fibre hypertrophy (Bodine et al, 2001). In contrast, the myostatin-Smad pathway is thought to inhibit the Akt/mTOR pathway and myoblast differentiation (Schiaffino et al, 2013; Trendelenburg et al, 2009). The capacity of BfRT to affect these pathways has previously been examined.

Drummond et al (2008) delivered two low-intensity (20% 1RM) knee extension exercise sessions to six healthy young males, one session with BfR superimposed and one without, in a random-crossover design. Myostatin mRNA expression was significantly

downregulated following both exercise sessions ($p < 0.05$), with no between-session differences. Thus, it was concluded that low-intensity resistance triggered myostatin downregulation and BfR did not amplify this effect. However, a longitudinal study by Laurentino et al (2012) suggested otherwise. Twenty-nine healthy males underwent one of three training programmes for 8 weeks. Participants completed either high-intensity (80% 1RM) resistance training, or low-intensity resistance training (20%1RM) with or without BfR. Post-intervention, myostatin expression dropped by a non-significant amount in the group performing low-intensity exercise without BfR. Yet myostatin expression dropped significantly ($p < 0.05$) in groups performing high-intensity resistance exercise or low-intensity exercise with BfR. Several other studies have also demonstrated that the addition of BfR to a low-intensity (20% 1RM) knee extension exercise can significantly amplify the post-session phosphorylation of mTOR and its upstream or downstream targets (Fry et al, 2010, Fujita et al, 2007, Gundermann et al 2014). Results showed that BfR significantly increased the post-session fractional rate of muscle protein synthesis, beyond that of the low-intensity resistance exercise alone.

1.3.2.3 The Ubiquitin-proteasome Pathway

Protein degradation within skeletal muscle is primarily dependent upon the ubiquitin-proteasome pathway (Egan and Zierath, 2012). Within this pathway, the activation of Forkhead Box Class O [FOXO] transcription factors upregulate two ubiquitin ligases; atrogin-1/MAFbx and MuRF1. This upregulation triggers protein degradation, a reduction in muscle fibre size and muscle atrophy (Egerman and Glass, 2014; Sandri, 2008). It is also suggested that these ligases are transiently upregulated following resistance exercise and are implicated in subsequent muscle remodelling (Murton et al, 2008).

A study by Drummond et al (2008) found that a one-off session of low-intensity (20% 1RM) resistance training upregulated MuRF1 mRNA expression post-session. The addition of BfR further amplified this response, but not by a statistically significant degree. Pre-post MAFbx mRNA expression remained unchanged in both groups. Conversely, Manini et al (2011) found that both MuRF1 and atrogin-1 expression decreased post-session, despite participants performing the same BfRT protocol as Drummond et al (2008). This mismatch may be explained by Manini et al (2011) taking post-session biopsies at eight hours, whereas Drummond et al (2008) took biopsies at 3 hours. This would suggest a time-dependent change in the expression of these ligases. A third study by Ellefsen et al (2015) appears to support this notion. At 1 hour post exercise (low-intensity knee extensions at 30% 1RM with BfR), MuRF1 expression had increased significantly ($p < 0.05$). This remained the case when the exercise and biopsy was repeated after the completion of the last exercise session of the twelve-week BfRT programme. However, in a rested state, MuRF-1 levels were numerically (but not statistically) lower after the twelve-week training programme than before the programme commenced. Atrogin-1 expression remained unchanged at all measured time points. Comparing these three studies to a collated review of other ubiquitin-proteasome evidence (Murton et al, 2008), suggests that similar modulation of these ligases occurs following traditional high-intensity resistance training. Explicitly, an acute post-session increase in MuRF-1 and MAFbx/atrogin-1 mRNA expression, followed by a return to basal levels sub-acutely, or chronic downregulation. It is possible that low-intensity BfRT may acutely amplify MuRF-1 expression and thus the activity of the ubiquitin-proteasome pathway, leading to muscular adaptation via remodelling rather than atrophy. The work by Ellefsen et al (2015) seems to support this notion, demonstrating similar pre-post changes in thigh cross-sectional area and muscle fibre transition (from Type IIx to Type IIa) between the

BfRT group and the traditional high-intensity resistance training group after their twelve-week programmes.

1.3.3 Longitudinal Muscular Adaptations in Response to BfRT

1.3.3.1 Muscular Hypertrophy and Strength Development

When attempting to elucidate the longitudinal effect of lower-limb BfRT programmes upon muscle size and strength, existing evidence typically compares a form of BfRT against a non-exercising control group, or participants completing similar exercise programmes without BfR applied. A head-to-head comparison of these studies is presented below, grouped by the type of exercise programmes that were applied.

Low-intensity Blood-flow Restricted Resistance Training [LiBfRT] vs. High-intensity Resistance Training [HiRT]

Evidence points towards both LiBfRT and HiRT being able to significantly increase post-intervention 1RM for knee extension (Laurentino et al, 2012; Martín-Hernández et al, 2013) and leg press (Libardi et al, 2015; Vechin et al, 2015) over training periods of four to 12 weeks. Across studies, between-group improvements in 1RM were either similar between modalities, or of a greater magnitude with HiRT. All four studies demonstrated statistically significant within-group increases in quadriceps cross-sectional area or muscle thickness, with no significant between-group differences.

LiBfRT vs. Low-intensity Resistance Training [LiRT]

Over an eight-week programme, Laurentino et al (2012) has shown that cohort knee-extensor 1RM can increase significantly following either LiRT or LiBfRT within healthy males ($p < 0.001$). Participants completing LiBfRT twice-weekly experienced a larger

percentage increase in pre-post 1RM than those performing LiRT twice-weekly at the same exercise intensity (mean 20.7% vs. 40.2%), but this between-group difference was not statistically significant. Conclusions were similar in respect of maximum knee extensor torque following a five-week, thrice weekly, training programme conducted by Fitschen et al (2014). However, the mean percentage increase in knee extensor torque was modest ($5.2 \pm 10.41\%$, $p = 0.033$) across the study's thirty participants ($n=25$ female) compared to the magnitude of 1RM changes seen in Laurentino et al (2012).

In contrast to Laurentino et al (2012) a superiority of LiBfRT over LiRT at increasing pre-post maximal knee extensor torque was reported by Takarada et al (2004). This study, whilst delivering the same programme duration and training frequency to Laurentino et al (2012), produced a mean increase in maximum knee extensor torque of $9.9 \pm 2.2\%$ with LiBfRT, versus $3.1 \pm 1.4\%$ with LiRT; a significant between-group difference ($p < 0.05$). The exercise dose was greater in Takarada et al (2004) compared to Laurentino et al (2012) (five sets at 20% 1RM (mean 16.8 repetitions per set) vs. 3 sets of 15 repetitions at 20% 1RM). Other factors, such as differing cuff widths, cuff pressures and the differences in group sample sizes (6 vs. 10) may have contributed to the contrasting results between studies. It is worth noting, however, that within Takarada et al (2004) the LiBfRT group completed their five exercise sets to failure, with the LiRT group being work-matched for repetitions against this. It is wholly possible that LiBfRT participants experienced significantly more physiological stress and/or muscle fibre activation than LiRT participants through exercising to failure, which expressed itself longitudinally as the comparatively greater increase in the percentage of maximum knee torque.

In terms of longitudinal changes to thigh mass, only LiBfRT increased thigh cross-sectional

area (Takarada et al, 2004) and quadriceps cross-sectional area (Laurentino et al, 2012) by a statistically significant degree. Within Fitschen et al (2014), thigh lean mass measured via Dual X-ray Absorptiometry [DXA] showed no significant pre-post intervention change across either LiRT or LiBfRT. Again, the discrepancy between study results may relate to methodological differences, with a bias towards longer training programmes producing more favourable results for LiBfRT. In summary, existing evidence suggests that superimposing BfR over LiRT does appear to amplify gains in longitudinal muscle strength within the lower-limb.

LiBfRT to volitional failure vs. LiRT to volitional failure or HiRT to volitional failure

Findings from a study by Barcelos et al (2015) suggest that similar, significant gains in knee-extensor muscle hypertrophy and strength occur across LiRT, LiBfRT and HiRT programmes lasting eight weeks, providing exercise sets are completed to volitional failure. Barcelos et al (2015) acknowledged that this may have occurred due to significant recruitment of motor units and activation of fast-twitch/Type II muscles fibres across all exercise modalities via the universal attainment of volitional failure. Thus, promoting muscular development as discussed by Burd et al (2010) and Loenneke et al (2011a). Whilst Barcelos et al (2015) had studied previously untrained males aged 18-30 years, a separate study of eighteen middle-aged participants (mean 55 ±7 years, n= 6 female) also found that both LiRT or LiBfRT performed to volitional failure produced similar within-subject, between-leg improvements in knee extensor strength over a 6-week period (Fahs et al, 2015). Quadricep muscle thickness increased within both legs, with larger effect sizes in the limb that underwent LiBfRT. Further evidence by Clark et al (2011) also demonstrated statistically significant gains in isometric knee extensor strength could occur over shorter, four-week, thrice-weekly training programmes among a cohort of

seventeen healthy participants (n=2 female) who were allocated to LiBfRT at 30% 1RM or HiRT at 80% 1RM (both to volitional failure). The HiRT group increased their strength by a higher percentage than the LiBfRT group (13% vs. 8%), but the difference was not statistically significant ($p= 0.28$, effect size 0.09).

In summary, the findings of Barcelos et al (2015) and Fahs et al (2015) give strength to the suggestion that adding BfR to LiRT already performed to volitional failure may provide no additional benefit, in terms of amplifying longitudinal increases to lower-limb muscle size and strength. However, Fahs et al (2015) did demonstrate that the volume of exercise required to reach volitional failure during LiRT was reduced by approximately 36% by the addition of BfR. Other studies have also shown a similar, volume-reducing effect (Loenneke et al, 2011b; Loenneke et al, 2012f, Wernbom et al, 2009). Whilst the participants of each of these studies were all healthy, it could be argued that the volume-reducing effect of adding BfR to LiRT could have some clinical beneficence. BfR could be used as a variable by which to control or modulate training load to increase or maximise the intensity and/or acute metabolic stress of a LiRT exercise, whilst mediating the risk of re-injury.

High-intensity Blood-flow Restricted Resistance Training [HiBfRT] vs. HiRT

Few studies have examined the effect of adding BfR to HiRT in regards to muscular development. Laurentino et al (2008) studied sixteen males over a period of 8 weeks, delivering resistance training at 60% 1RM or 80% 1RM, with or without BfR. All four modalities significantly increased knee extensor 1RM and quadriceps cross-sectional area, with no modality demonstrating superiority over the others. Over the same length of training programme, Mueller et al (2014) also demonstrated that superimposing whole-

body vibration over HiBfRT consisting of a 70% 1RM squat exercise produced the same, significant increases in knee-extensor 1RM as completing HiRT alone.

The lack of any additive effect of BfR within these studies may have a haemodynamic explanation. It is known that high-intensity muscular contractions can generate sufficient extravascular pressure to occlude the vascular network, and thus blood flow, within a muscle during its contraction. Evidence suggests that this occurs at a range of 50-64% MVC (Sadamoto et al, 1983). At this intensity of exercise, particularly where muscular relaxation time is absent or minimal, the superimposing of extravascular pressure via a restriction device may not further restrict muscular blood flow. In effect, it is not possible to restrict the blood-flow within a muscle that is already fully restricted via its own contraction. Evidence by Cayot et al (2015) appears to support this notion. In this study, BfR was applied immediately before and during knee extensions performed at 20% to 80% MVC. During the exercise performed at 20% and 40% MVC, deoxygenated haemoglobin levels within the vastus lateralis muscle grew significantly higher with BfR superimposed than without. However, this effect was negated when the exercise was completed at 60% and 80% MVC. This could explain why Laurentino et al (2008) found no additive longitudinal effect of applying BfR to exercise performed at these higher intensities. Arguably, exercise at this intensity alone was already sufficient to maximally limit blood flow through exercising lower-limb musculature and thus maximise the role that reduced blood-flow plays within the creation of acute exercise-induced metabolic stress and longitudinal muscular adaptation.

1.3.3.2 LiBfRT during Injury Rehabilitation

The potential of LiBfRT to prevent or attenuate lower-limb muscle atrophy and strength loss following a musculoskeletal injury is addressed specifically within chapter two. Aside to this, two studies have tracked the longitudinal recovery of lower-limb muscle strength and size in groups with established atrophy due to an injury. Tennent et al (2017) added three lower-limb LiBfRT exercises to a traditional knee arthroscopy rehabilitation programme of ten participants. After twelve sessions of physiotherapy, the LiBfRT group displayed increases in knee extension and flexion strength that were twice the magnitude of those seen in the traditional rehabilitation group (n=7). Thigh girth also increased to a statistically significant level ($p \leq 0.01$), but only within the group completing LiBfRT. Similar success was seen in a case series by Hylden et al (2015), who delivered a LiBfRT programme to seven males with long-standing unilateral strength deficits due to musculoskeletal injury. All cases significantly improved peak torque and average power of the knee flexors and extensors after a minimum of two weeks training. However, a lack of case-controls blurs the specific contribution that BfR may have made to these findings.

1.3.3.3 Skeletal Muscle Angiogenesis

Exercise-induced angiogenesis within skeletal muscle is thought to be modulated by number of factors, including endothelial shear stress due to increased blood flow, mechanical tissue stretch and enhanced metabolism (Hoier et al, 2014). Within Mueller et al (2014), four weeks of high-intensity (70% 1RM) squat training with simultaneous BfR and whole-body vibration produced a significant within-group increase in capillary-to-fibre ratio of the vastus lateralis ($p < 0.01$). This increase was significantly greater than the increase seen within a control group completing traditional squats at 70% 1RM ($p < 0.01$). However, due to the lack of a 'vibration only' or LiRT group it is difficult to elucidate

whether BfR or vibration amplified these angiogenic changes, or by what extent. An earlier study by Evans et al (2010) provided a clearer picture, through the delivery a four-week training protocol of bodyweight heel raises to a cohort of nine healthy male participants. Both legs were exercised, one under BfR. Post-intervention, authors noted that calf filtration capacity (a reported measure of capillarisation) within the cohort had increased by 26% within the blood-flow restricted leg ($p < 0.05$). However, filtration capacity also increased by 23% in the unrestricted leg ($p = 0.06$). This negligible between-leg difference could indicate no additive effect of BfR upon calf muscle angiogenesis, or the need for a larger sample size to improve the experiment's statistical power to detect smaller between-leg changes post intervention. In the apparent absence of further evidence, it is difficult to confirm that BfR added during resistance training specifically amplifies any angiogenic effect within skeletal muscle, particularly over time periods exceeding four weeks.

1.3.3.4 Longitudinal Changes in Overall Physical Function

Within older populations or those with a chronic disease, the application of LiBfRT appears both safe and beneficial to functional performance. For example, a randomised clinical trial by Bryk et al (2016) delivered a six-week LiBfRT programme to 17 women diagnosed with knee osteoarthritis (age; 62.3 ± 7.0 years), with a second group ($n=17$, age; 60.4 ± 6.7 years) completing conventional HiRT. The LiBfRT programme was equally effective as the HiRT programme in improving self-reported function, measured via the Lesquene Questionnaire, and objective function via the Timed 'Up-and-Go' [TUG] test. Doubts have been raised as to the internal consistency and reliability of the Lesquene questionnaire in measuring both physical function and pain (Veenhof et al, 2006). On review of other outcome data, however, the group-means for the TUG did exceed the

minimal detectable change [MDC] of this outcome measure, if compared to a similar reference population (n=65, mean age; 54.9 years, MDC; 1.1 seconds, Alghadir et al, 2015). These functional improvements were also accompanied by concomitant increases in maximal isometric quadricep strength ($p = 0.001$). Pain reduction post-intervention, as measured via Numerical Rating Scale, also exceeded the minimal clinically important difference of two points (LiBfRT; -3.3 ± 2.2 vs. HiRT; -2.5 ± 1.8). Thus, the use of LiBfRT to improve physical function whilst reducing osteoarthritic knee pain does seem justified. Particularly as LiBfRT also generated significantly less pain than HiRT during the exercise sessions (LiBfRT; 2.5 ± 1.5 vs. HiRT; 6.2 ± 2.2 , $p = 0.01$).

A randomised trial by Yokokawa et al (2008) confirmed the functional benefits of using BfR within an older, but healthy, population. Nineteen participants (mean age; 70.7 ± 4.3 years) completed eight weeks of supervised, twice-weekly sessions of body-weight resistance exercises under BfR. A second group of twenty-five participants (mean age; 70.6 ± 5.0 years) completed 8 weeks of supervised, twice-weekly dynamic balance exercises performed without BfR. Post-intervention, both groups had significantly improved their performance across a range of functional outcomes, including maximum step distance and the Functional Reach Test ($p \leq 0.01$). Performance in the TUG was significantly better in the BfR group compared to the dynamic balance group ($p < 0.001$). This finding was accompanied by improvements in isometric knee extension strength among the BfR group, but not the dynamic balance group ($p \leq 0.007$).

A study by Mattar et al (2014) delivered a twelve-week lower-limb LiBfRT programme, consisting of leg press and knee extension exercises at 20-30% 1RM, to a cohort of thirteen participants with inflammatory muscular conditions; polymyositis and

dermomyositis. Compared to baseline, participants significantly increased functional performance, measured via the TUG test and timed stands. The absence of raw TUG test values within the article's text makes it difficult to ascertain whether the group's 4.5% improvement in this outcome exceeded the MDC. However, post-intervention measures of subjective health also improved compared to pre-intervention (SF-36 and HAQ; $p < 0.05$). Longitudinal LiBfRT programmes have also been implicated in the increase of horizontal walking speed, but not the TUG test, in a 74-year old male with inclusion body myositis (Jørgensen et al, 2015), who completed LiBfRT for 12 weeks. A second male patient with the same condition experienced a six-second improvement in the TUG test over the same time period (Gualano et al, 2010). In all of these studies (Gualano et al, 2010; Jørgensen et al, 2015; Mattar et al, 2014) the lack of case-controls or participants undertaking traditional LiRT does make it impossible to draw out the specific contribution that BfR made towards these improvements.

Beyond resistance training, work by Abe et al (2010a) has delivered twenty minutes of treadmill walking to healthy older participants (aged 60-78 years), five times per week over a 6-week training programme. Participants with BfR superimposed during training significantly reduced the time needed to complete the TUG test post-intervention, with concomitant increases in sit-to-stand performance, whilst the group training without BfR saw no significant improvement. Functional improvements could be linked to statistically significant within-group increases in dynamic lower-limb strength and skeletal muscle mass ($p \leq 0.05$) in the BfR group, but with an absence of change in cardiovascular exercise capacity.

Whilst Abe et al (2010a) used a narrow-width (5cm) inflated to 180-200mmHg during training sessions, a recent study by Clarkson et al (2017) utilised a wider (10.5cm) cuff inflated to a lower pressure (mean 134.4 ± 4 mmHg) to produce favourable post-intervention functional improvements within a similarly aged, healthy population (mean 69 ± 6 years). A training volume half that of Abe et al (2010a), performed over a similar time frame (six weeks), produced improvements in all tested measures of physical function within the group completing treadmill walking with BfR (TUG test, total sit-to-stands in 30 seconds, 6MWT, Queen's College Step Test). These improvements were statistically greater than those seen in the control group (treadmill walking without BfR) when measured by percentage improvement ($p < 0.05$). When reviewing the raw values for these outcomes, however, the between-group differences were less apparent. Only the total number of sit-to-stands performed in thirty seconds displayed a between-group statistical difference post-intervention ($p < 0.05$). In the TUG, the BfR group-mean improved by approximately 0.7 seconds, whilst the control group-mean improved by approximately 0.3 seconds. The lack of an existing MDC regarding the use of the TUG among healthy populations makes it difficult to ascertain if either group experienced a real functional improvement.

Within healthy, younger adults and athletes, measures of somatic functional performance are less common within BfRT literature. Lin et al (2014) suggested that LiBfRT and LiRT were equally effective at reducing the 100-metre sprint time of twenty-one healthy college students. Manimmanakorn et al (2013) found that LiBfRT at 20% 1RM produced significantly greater changes in 5-metre sprint times, the 5-0-5 agility test and the 20-metre shuttle run test than LiRT alone. Interestingly, participants who underwent LiRT with superimposed hypoxia (via a face mask) increased their functional performance by

similar levels to the LiBfRT group. These improvements were accompanied by similar increases in VO_2 Max and dynamic knee extension, suggesting that either adjunct (BfR or hypoxia via face mask) can drive muscular and cardiovascular development during LiRT by limiting of oxygen delivery to tissues.

In summary, randomised clinical trial evidence does support the use of BfR during low-intensity exercise to generate statistically significant longitudinal improvements in subjective and objective physical function, similar to that seen in HiRT or a dynamic balance programme (Bryk et al, 2016; Yokokawa et al, 2008). Whilst this appears to be true in both clinical and healthy populations, the way in which data is presented in some other article texts makes it difficult to elucidate whether statistically-significant improvements in physical function exceed the MDC of the selected outcome measures used (often the TUG test). Further controlled trials that contain both LiRT, LiBfRT and HiRT groups are warranted to determine the specific longitudinal benefit of BfR upon physical function, particularly among clinical populations.

1.3.4 Conclusions

In summary, the process of muscular adaptation in response to conventional exercise is complex and the mechanisms responsible are still not fully understood. However, a significant weight of evidence suggests that lower-limb BfR can affect these mechanisms. This is achieved through the amplification of acute exercise-induced metabolic stress, driven primarily by the impairment of oxygen delivery to exercising muscle tissue. Increased neuromuscular activation ensues, leading to enhanced neuromuscular fatigue or a reduction in the time/volume of exercise required to reach volitional failure. This alters exercise-related signals, modulating the cellular pathways involved in signal

transduction and appearing to alter gene expression in favour of muscular development (strength and hypertrophy), skeletal muscle angiogenesis and somatic physical performance.

The evidence base surrounding the effect of lower-limb BfR on muscular development and performance is extensive, with studies tending to concur as to its overall beneficence in this context. However, many studies with comparator or control groups contain a relatively small number of participants per group ($n \leq 10$) and do not report statistical power or sample size calculations. This can lead to Type II errors (a failure to detect a true effect) within or between groups, or may cause statistically significant results to poorly represent the effect that may be seen in the population(s) from which the study participants are drawn from (Button et al, 2013; VanVoorhis and Morgan, 2007). Infrequent reporting of effect sizes and confidence intervals also limits the ability of the reader to interpret the strength of the reported results. In light of this, an element of caution needs to be taken when interpreting the overall findings and implications of these studies until they can be validated by further randomised-controlled trials and large cohort studies. The conclusions drawn from this presented evidence are as follows;

- 1) Where exercise is not completed to volitional failure, adding BfR to lower-limb LiRT (typically at 20-30% 1RM or MVC) appears to increase the acute metabolic stress of the exercise and amplify subsequent cellular responses. Compared to traditional LiRT, evidence points towards LiBfRT programmes enhancing longitudinal gains in lower-limb muscle size, strength and physical function across a range of adult populations. This finding is not universal across the selected literature, however, with some studies indicating that the addition of BfR produced no additive effect.

- 2) Where resistance exercise is completed to volitional failure, LiBfRT (typically at 20-30% 1RM or MVC) does not appear to augment or amplify lower-limb muscular adaptation beyond that seen in traditional LiRT or HiRT. However, LiBfRT reduces amount of exercise volume or repetitions required to reach volitional failure by approximately 30-40% compared to LiRT. From a clinical perspective, this may allow physiotherapists additional fidelity when progressing patients through a rehabilitation programme. Exercise to volitional failure could be introduced via LiBfRT before progressing through traditional LiRT and HiRT, for example. This could minimise the risk of re-injury via mechanical overload whilst still being able to access the purported benefits of volitional failure training.
- 3) Adding BfR to lower-limb HiRT ($\geq 60\%$ 1RM or MVC) does not appear to augment the acute metabolic stress of the exercise, or longitudinal muscular adaptation beyond that seen in traditional HiRT. It is possible that lower-limb muscular blood flow is already occluded or severely limited during resistance exercise at this intensity, due to the degree of extravascular pressure generated by surrounding muscles during their contraction. In this case, attempting to superimpose artificial restriction may provide no measurable benefit.

This chapter has summarised the concept of BfRT in terms of its historical background and the proposed effects of BfR upon the mechanisms of muscular adaptation. Discussion has also been made as to the effects of BfR applied during variations of lower-limb resistance exercise. The next chapter presents a systematic review, which specifically investigates the use of lower-limb BfRT to attenuate lower-limb muscle disuse atrophy and strength loss.

CHAPTER TWO

The effect of BfRT upon Lower-limb Muscle Size and Performance among Individuals with an Acute Lower-limb Injury: A Systematic Review

2.1 Chapter Introduction

This chapter investigates and critically appraises the existing academic evidence base surrounding the use of lower-limb BfRT to prevent or attenuate loss of lower-limb muscle size and performance. More specifically, the attenuation of muscle size and performance due to extended periods of impaired weight-bearing following a musculoskeletal lower-limb injury. The rationale for this review and its objectives are first established, followed by a description of the systematic search strategy. A narrative, critical appraisal of the selected literature is then presented with discussion as to methodological quality and potential risks of bias within studies. Finally, conclusions are drawn as the effects of lower-limb BfRT within the context of the review, leading into the aims and objectives of this doctoral research project described within chapter three.

2.1.1 Rationale for the Review

Two reviews have provided concise summaries into the potential of BfRT as a rehabilitative treatment adjunct (Scott et al, 2015; Slyz et al, 2016). A narrative review by Scott et al (2015) summarised the general use of BfRT for muscular development. Three key papers were cited within this review that purported the ability of BfRT to limit losses in thigh muscle strength or size during periods of limb immobilisation (Kubota et al, 2008; Kubota et al, 2011; Takarada et al, 2000b). A systematic review and meta-analysis by Slyz et al (2016) also concluded that BfR added to low-intensity exercise could amplify

longitudinal increases in muscle size and strength, proposing the value that BfRT may have in future rehabilitative care. However, the systematic review by Slyz et al (2016) included only studies involving healthy (uninjured) participants. Their proposition was therefore speculative.

Recently, a systematic review and meta-analysis of controlled trials completed by Hughes et al (2017) reported an efficacy of low-intensity BfRT within clinical musculoskeletal populations receiving this intervention. The majority of selected studies (13 out of 20) investigated the use of BfRT within healthy, elderly participants, whilst remaining studies investigated the use of BfRT following anterior cruciate ligament [ACL] reconstruction (n=3), the presence (or risk) of knee osteoarthritis (n=3) or polymyositis/dermatomyositis (n=1). Low-intensity BfRT had a moderate effect over conventional low-intensity training in terms of increasing maximal muscle strength over periods of up to twelve weeks, measured predominantly via pre-post changes in one repetition maximum [1RM] (Hedges' $g = 0.523$, 95% CI 0.263 to 0.784, $p < 0.001$). However, conventional high-intensity training was more effective in terms of these outcomes than low-intensity BfRT (Hedges' $g = 0.674$, 95% CI 0.296 to 1.052, $p < 0.001$). Authors also suggested that low-intensity BfRT was a more tolerable alternative in regards to minimising exercise-related pain.

The systematic review and meta-analysis by Hughes et al (2017) provides useful and informative evidence-based recommendations for clinicians looking to use BfRT within the context of musculoskeletal rehabilitation. Whilst this work did review controlled-trial evidence regarding populations recovering from ACL reconstruction, the question as to whether BfRT has an ability to attenuate (rather than redress) lower-limb disuse muscle

atrophy has not been fully addressed by way of a review. A systematic review of such nature may be of considerable use to clinicians looking to prevent disuse atrophy within acutely or sub-acutely injured patients. Particularly in those restricted to extended periods of impaired weight-bearing to whom traditional resistance training is contraindicated. This review should identify the extent of existing evidence in regards to the prevention of disuse atrophy and strength loss, the methodological quality of this evidence and whether the application of BfRT appeared efficacious. Additionally, the identification of any consensus regarding the restriction equipment used, the cuff pressures applied and the exercises used, may allow a generic lower-limb BfRT protocol to be generated for use among injured patients.

2.1.2 Research Questions

This review aimed to answer two research questions;

- I. In adults with an acute lower-limb musculoskeletal injury, does the addition of low-intensity BfRT to an exercise or rehabilitation programme attenuate losses in lower-limb muscular size and/or performance?
- II. Using existing evidence, is it possible to identify or propose a valid low-intensity BfRT protocol for use within individuals with an acute lower-limb musculoskeletal injury?

2.2 Methods

2.2.1 Protocol and Registration

The search protocol for this review was registered within the International Prospective Register of Systematic Reviews [PROSEPRO] (Centre for Reviews and Dissemination, 2017) on the 10th November 2015, Registration Number CRD42015026789. Reporting is in

accordance with the PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions (Liberati et al, 2009).

2.2.2 Eligibility Criteria

Any type of study design, including controlled trials, cohort studies, case series, or single case studies were eligible for inclusion within the review. The following exclusion criteria were applied;

- Duplicate search records
- Studies not available in the English language
- Studies not from a peer-reviewed journal source
- Studies involving non-human participants
- Studies unrelated to BfR in the context of this review (for example, the effect of ablation therapy upon carotid vascular occlusion).

Further criteria for inclusion and exclusion are listed below in the Population, Intervention, Comparator/Control, Outcome [PICO] format.

Population

Inclusion Criteria:

Adults (aged ≥ 18 years) who had;

- A current lower-limb musculoskeletal injury to one lower-limb. The injury (or resultant surgery) should have be of a magnitude that prevented normal weight bearing for a period of at least fourteen consecutive days. Or;
- Were uninjured, but agreed to undertake a voluntary period of walking with crutches whilst one limb was prevented from bearing weight via a cast or boot, analogous to what would occur following a significant lower-limb injury.

Normal weight bearing must have been prevented for at least 14 consecutive days.

Exclusion Criteria:

- Studies that investigated participants with a significant underlying disease affecting the cardiovascular system, such as diabetes or congestive heart failure.
- Studies that investigated participants with multiple musculoskeletal injuries of the chest, head or upper limbs.
- Studies that investigated participants with generally accepted contraindications to BfRT (such as a medical history of deep vein thrombosis).

Intervention

Eligible studies must have applied BfRT to at least one individual's lower-limb over a programme of multiple sessions, over a period of at least fourteen days. Studies that combined BfRT with interventions other than physical exercise and/or passive/active limb movement (such as acupuncture or electrotherapy) were excluded.

Comparator(s)/control

Control/comparator groups must have received either no intervention, or a low-intensity exercise intervention that did not include BfRT. Where no control or comparator group existed, a suitable within-subject comparator must have been measured (such as comparison between left and right leg muscle size/strength).

Outcome Measures

Primary outcomes:

- Muscular strength, determined via isokinetic dynamometry or one repetition maximum.
- Muscular size, determined via medical imaging or flexible tape measurement of limb girth.
- Muscular endurance, determined via isokinetic dynamometry or total amount of repetitions completed until failure.

Secondary outcomes:

- Objective physical function, assessed via a validated outcome measure such as the Timed Up and Go [TUG] Test.
- Subjective physical function, assessed via a validated outcome measure such as the Lower Extremity Functional Scale [LEFS].

2.2.3 Information Sources

Seven academic databases were used to obtain literature for this review; AMED, CINAHL, Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, ScienceDirect, Scopus and SPORTDiscus. Each database was initially searched for literature on the 2nd December 2015 to detect all articles published with a peer-reviewed journal from the 1st January 1995 to the 2nd December 2015. Due to the rate at which BfRT research was being published following this search, the search strategy was later repeated to cover the period of 3rd December to 5th October 2016 to ensure the review remained contemporaneous.

2.2.4 Search Terms

Five preliminary searches were completed to assess the scope of relevant BfRT literature and to assess the suitability of search terms. A final search strategy was then formulated.

Search terms and Boolean operators were entered into each academic database as follows;

Search Box 1 = (Blood flow OR Vascula*) AND (Restrict* OR Occlu*) [In record title]

AND

Search Box 2 = Exercis* OR Train* OR Rehab* OR Therap* OR Protocol* OR Program* OR

Atroph* OR Disuse* [In record title]

OR

Search Box 3 = Kaatsu¹ [In record title or abstract]

¹*Kaatsu is a patented form of Blood-flow Restriction Training*

2.2.5 Study Selection

Two reviewers (the primary researcher and the Director of Studies) independently performed the search strategy. The records retrieved from each database were collated and inclusion/exclusion criteria were applied sequentially to the retrieved records by title, abstract and full text. Records deemed suitable for data extraction were then compared between reviewers to ensure a consensus.

2.2.6 Data Collection Process

The primary researcher created a bespoke data entry spreadsheet containing fields for all the data items listed in chapter 2.2.7. The primary researcher populated the spreadsheet by extracting data from the selected records. Where information relating to data items was missing or unclear within study texts, the primary researcher attempted to obtain this information via contact with study authors.

2.2.7 Data Items

The following data was extracted from each of the selected studies, where possible.

The Type of Study Design

Population

The total number of participants and the number of male and female participants within the study. The type of injury or surgery, if applicable. The mode of none weight-bearing (crutch ambulation, for example).

Intervention

Length of the BfRT intervention. The number of BfRT sessions completed per week. The type(s) of exercise completed and their intensity, if applicable. The number of repetitions per exercise set and number of sets per exercise. The duration of inter-set rest periods.

BfRT Methodology

The type and width of the restriction cuff used, and the method of its inflation. The main (or mean) cuff pressure applied during the intervention and how this was derived or justified. Whether the cuff was inflated continuously or intermittently during exercise sessions.

Outcomes

All primary and secondary outcome measures listed in chapter 2.2.2, including the direction and magnitude of changes in these variables and the statistical significance level of each outcome.

2.2.8 Risk of Bias within Studies

For randomised controlled trials [RCTs], risk of bias within studies was determined via the use of a tool derived by the Cochrane Collaboration (Higgins et al, 2011a). Reviewers independently assessed each RCT, classing studies as at low, medium or high risk of bias for each of the tool's eight domains. Reviewers then compared their findings to ensure consensus.

2.3 Results

2.3.1 Study Selection

The initial search strategy was completed by both reviewers on the 2nd December 2015. A PRISMA flowchart (Figure 2.3.1) displays the results of the sifting process. Of n=271 articles available after the removal of duplicate records, a total of n=9 were selected for inclusion within the review. At later date, the search strategy was repeated by the primary researcher to cover the period from 3rd December 2015 to the 5th October 2016. This yielded no further eligible studies. However, one of the 9 studies selected from the initial search (Hackney et al, 2012) had been converted from a conference paper to a published article (Hackney et al, 2016). The more recent article was used within the final review.

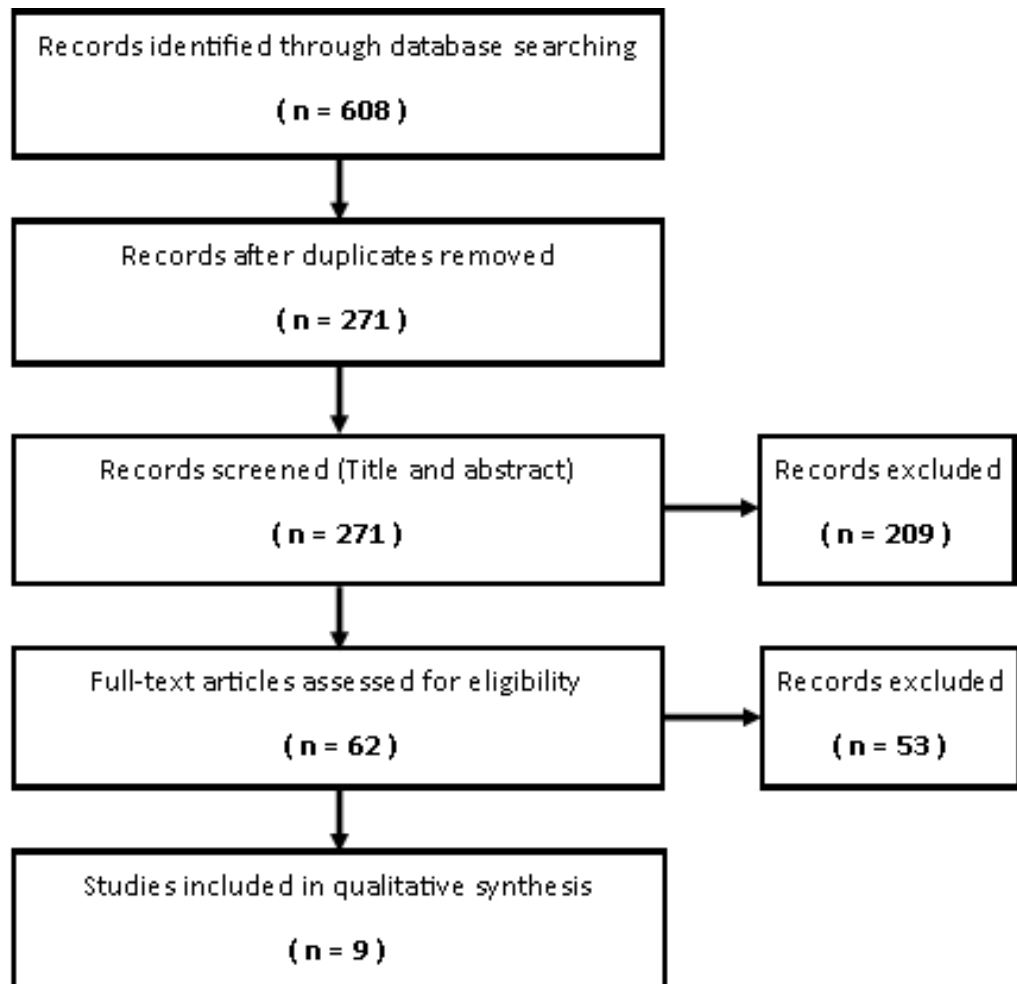


Figure 2.3.1. A PRISMA flowchart documenting the selection of studies included within the systematic literature review for this project.

2.3.2 Study Characteristics

Seven controlled trials (Cook et al 2010; Cook et al 2014; Iversen et al, 2015, Kubota et al 2008; Kubota et al 2011; Ohta et al, 2003; Takarada et al, 2000b), one head-to-head study (Hackney et al, 2016) and one single case study (Lejkowski and Pajaczkowski, 2011) were selected for inclusion. Four studies utilised injured participants, all recovering acutely from a unilateral ACL reconstruction. The five remaining studies utilised uninjured participants who voluntarily underwent a period of unilateral lower-limb suspension [ULLS] and crutch ambulation. Across studies, reported thigh-cuff inflation pressures ranged from 50mmHg (Kubota et al, 2011) to 260mmHg (Takarada et al, 2000b) whilst cuff widths ranged from 6cm (Hackney et al, 2016) to 15.2cm (Lejkowski and

Pajaczkowski, 2011). Intervention periods ranged from fourteen days to 16 weeks (Ohta et al, 2003). A data synthesis table details the methodological characteristics of each particular study (Appendix Va). Within this table, the studies by Cook et al (2010; 2014) have been amalgamated into one record as both relate to the same experiment.

2.3.3 Risk of Bias within Controlled Trials

Risk of bias within the selected controlled trials was often unclear due to inadequate reporting of study methodology. This was particularly apparent in terms of randomisation procedures and the concealment of group allocations among researchers and participants (Appendix Vb). Contact with authors was attempted to clarify methodological points for all studies, but only two responded. Iversen et al (2015) provided sufficient information to score 'low risk' across five of the seven potential sources of bias, measured using the Cochrane tool. Cook et al (2010) and Cook et al (2014) scored 'low risk' across three of the seven potential sources of bias. The remaining texts (Kubota et al 2008; Kubota et al 2011; Ohta et al, 2003; Takarada et al, 2000b) scored 'unclear' risks of bias across most potential sources of bias, with Ohta et al (2003) scoring 'high risk' for selection bias.

2.4 Discussion

2.4.1 Summary of Evidence

2.4.1.1 Changes in Lower-limb Muscle Size and/or Girth

Seven studies determined pre-post changes to lower-limb muscle size via magnetic resonance imaging [MRI] (Cook et al 2010, Cook et al 2014; Hackney et al, 2016; Iversen et al 2015; Ohta et al, 2003; Takarada et al, 2000). The use of tape measurement to monitor changes in limb girth, as a purported indicator of muscle mass, was also evident in three studies (Kubota et al, 2008; Kubota et al, 2011; Lejkowski and Pajaczkowski,

2011).

The earliest selected study (Takarada et al, 2000b) investigated the effect of intermittent bouts of BfR delivered without simultaneous exercise, among eight participants (n=4 female) recovering acutely from ACL reconstruction surgery. BfR sessions were completed twice-daily for 10 consecutive days. Each session consisted of five, 5-minute bouts of BfR, with each bout separated by a three-minute rest period. A 9cm-wide cuff was used, progressively inflated over sessions from 180-260mmHg (mean 238mmHg). A further eight participants (n=4 female) were allocated to a control group, in which the BfR cuff remained deflated throughout sessions. After the ten-day intervention period, knee-extensor had CSA reduced significantly less in the experimental (BfRT) group ($9.4 \pm 1.6\%$) when compared to the control group ($20.7 \pm 2.2\%$; Mann-Whitney U Test, $p < 0.05$). However, knee-flexor CSA reduced by similar amounts between groups ($11.3 \pm 2.6\%$ vs. $9.2 \pm 2.6\%$). A clear explanation for why atrophy attenuation only appeared within the knee extensors was not given, but it was indicated that differences in their function against gravity or muscle fibre composition may have been a factor (Lieber et al, 1992). Presumably, that anti-gravity muscles which cross one articular joint and contain larger proportions of slow-twitch/Type I muscle fibres (such as vastus intermedius and vastus medialis) are more susceptible to immobilisation-induced atrophy than poly-articular muscles that do not have an anti-gravity role, such as the hamstring group. Within Takarada et al (2000b), the accuracy of how researchers determined the same, mid-femur measurement sites within-subject MRI images was unclear. Blinding of outcome assessors was not reported. Either of these factors may cast doubt upon the reliability and internal validity of study findings.

Work by Kubota et al (2008) repeated the BfR protocol of Takarada et al (2000b). Healthy males undertook fifteen days of voluntary unilateral lower-limb suspensions [ULLS], with pre-post thigh and lower-leg circumference being recorded via flexible tape measurement. Post-intervention thigh and leg circumferences, measured by tape measure, were preserved in the experimental group (n=5) and decreased by 2.3-2.8% within the control group (n=6). Further work by Kubota et al (2011) retained the same experimental protocol, but reduced the thigh-cuff pressure used to 50mmHg. On this occasion, thigh and leg circumferences decreased significantly within each group, with no significant between-group difference. Risk of bias was unclear across both studies due to a lack or unclear reporting of any blinding or randomisation procedures.

The work of Kubota et al (2008; 2011) and Takarada et al (2000b) showed that intermittent bouts of BfR performed without exercise could reportedly attenuate lower-limb muscle atrophy during non-weight bearing, providing a supra-systolic cuff pressure was used. A contradiction to this was later provided by Iversen et al (2015), who repeated the intermittent BfRT protocol derived by Takarada et al (2000b) within a larger cohort (n=24) recovering acutely from ACL reconstruction. Authors also added twenty repetitions of isometric quadriceps contractions, end-range knee extensions or straight leg raises during each BfR bout. Over eighteen days, MRI cross-sectional area of the knee extensors (quadriceps) reduced by $13.8 \pm 1.1\%$ in the experimental group (n=12) and $13.1 \pm 1.0\%$ within the control group (n=12); a non-significant between-group difference ($p = 0.626$). The confliction between this work and that of Kubota et al (2008) and Takarada et al (2000b) may be due to differences in methodological rigour across studies.

Randomisation during group allocation and blinding of the outcome (MRI) assessor took place in Iversen et al (2015), which may have minimised the risk of selection and

performance bias. In contrast, no blinding or randomisation was reported in Takarada et al (2000b). Randomisation occurred within Kubota et al (2008), but no blinding was reported. These factors may have introduced bias which confounded the results of Takarada et al (2000b) and Kubota et al (2008). Low group sizes within the BfR and control groups of Kubota et al (2008) (n=5 and n=6) may also limit the extent to which their results can be generalised to other populations. Iversen et al (2015) added in un-resisted exercises during intermittent BfR bouts. It is possible that this volume of exercise was still insufficient when coupled with rests period without BfR to generate enough acute metabolic stress over the course of each BfR session to stimulate a muscular adaptive response. At present, it is justified to accept the null findings of Iversen et al (2015) over Takarada et al (2000b) and Kubota et al (2008) due to evidence of superior methodological rigour, casting doubt upon the efficacy of intermittent BfRT (with or without un-resisted exercise) at preventing acute losses to lower-limb girth and thigh muscle size.

Compared to intermittent BfRT, the use of continuous BfR superimposed over low-intensity resistance exercise [LiBfRT] was more prevalent within the selected studies. Cook et al (2010) recruited sixteen participants, who volunteered to undertake a 30-day period of ULLS. In the experimental group (n=8), continuous BfR was applied during the rest and exercise periods of a seated knee extension exercise performed at 20% MVC. Each of the three sets was performed to volitional failure. The control group (n=8), who received neither exercise or BfR, experienced a 7.5% (range 4-15%) loss in knee-extensor muscle CSA via MRI, and an 8.5% (range 0-16%) loss in plantar flexor CSA over this thirty-day period. In contrast, the LiBfRT group experienced minimal change to their knee-extensor CSA (~1%, range 0-6%), and a 5.4% (range 2-9%) loss in plantar flexor CSA.

Statistically significant sparing of knee extensor muscle CSA in the experimental group was evident compared to the control group ($p = 0.04$), but was insignificant in regards to the plantar flexors ($p > 0.05$).

Whilst Cook et al (2010) reported significant attenuation of knee-extensor CSA, the authors highlighted the lack of a third experimental group completing only low-intensity resistance exercise without blood-flow restriction [LiRT]. This prevented authors from determining the specific contribution that BfR may (or may not) have made towards the attenuation of atrophy during the thirty-day none weight-bearing period. This point is of particular importance. Recent evidence has demonstrated that conventional resistance exercise (without BfR) performed at a low-intensity (20-30% 1RM) is as effective at generating muscle hypertrophy as conventional high-intensity resistance training [HiRT], providing sets are performed to volitional failure (Burd et al, 2011; Morton et al, 2016; Schoenfeld et al, 2015). Therefore, it is not unreasonable to suggest that the attenuation of atrophy seen in Cook et al (2010) was generated exclusively by the completion of low-intensity resistance exercise to volitional failure, rather than the addition of BfR.

A study by Hackney et al (2016) compliments the work of Cook et al (2010), delivering a thrice-weekly programme of leg press and calf raises exercises to thirteen healthy participants during 25 days of unilateral lower-limb suspension. The experimental group ($n=7$) undertook LiBfRT performed at 20-30% 1RM to volitional failure, whilst the second group ($n=6$) completed traditional HiRT (70-80% 1RM) to volitional failure. Post-intervention, the HiRT group increased knee extensor CSA in the none weight-bearing limb by approximately 3.6%, whilst this decreased in the experimental group by approximately 2.9% (Exercise Condition x Time interaction; $p = 0.002$). The lack of a 'no-

intervention' control group prevented a within-study comparison of whether the small decrease in CSA among the LiBfRT was indicative of atrophy-attenuation within the knee extensors. However, the authors noted that the no-intervention control group in Cook et al (2010) experienced twice the loss in knee-extensor CSA (7.5%) than that seen in the BfRT group of Hackney et al (2016). This comparison would suggest atrophy attenuation of knee-extensor CSA in Hackney et al (2016), but the comparison is confounded by the studies having employed different lower-limb exercise programmes. Therefore, the question still remains as to whether BfR was responsible for this attenuation, or just the completion of low-intensity resistance exercise to volitional failure.

Clarification may be sought by looking beyond the selected literature, to that of Barcelos et al (2015). Authors studied forty-seven untrained, males over an 8-week intervention period. All participants were uninjured and were permitted to mobilise freely throughout the experiment. Each participant was randomly assigned into one of five groups; either a no-intervention control group (n=8) or a protocol of seated knee extensions performed at 20% 1RM over one set (n=10) or over three sets (n=10), or 50% 1RM over one set (n=10) or three sets (n=10). Within the experimental groups, BfR was applied to one leg during exercise, whilst the other was exercised separately without BfR. All experimental participants completed their allocated exercise modality to volitional failure. Post-intervention, all training groups had significantly increased knee-extensor CSAs compared to the no-intervention control group ($p < 0.05$). There were no significant differences between training groups in the percentage increase of knee-extensor muscle CSA. Acutely, the levels of blood lactate generated by all exercise protocols were also similar. Importantly, Barcelos et al (2015) encompassed exercise protocols that closely resembled those delivered in Cook et al (2010) and Hackney et al (2016); three sets of exercise at

20% 1RM to failure under continuous BfR. In Barcelos et al (2015), this produced equal muscle hypertrophy and similar acute lactate responses with and without BfR, suggesting that simply performing resistance exercise to volitional failure was responsible for generating favourable muscular adaptations. It is possible that a similar situation also occurred in Cook et al (2010) and Hackney et al (2016), but within the context of atrophy attenuation. In effect, exercising to volitional failure produced atrophy attenuation irrespective of whether BfR was applied.

During longer-term recovery, evidence suggests that rehabilitation programmes involving BfRT maintain the size of thigh musculature, or expedite the reversal of muscular atrophy. Ohta et al (2003) conducted a sixteen-week study investigating the effect of adding BfR into the rehabilitation programme of a patients recovering acutely from ACL reconstruction. Two weeks post-surgery, half of the cohort (n=22) introduced BfR during their low-intensity rehabilitation and resistance exercises. Pre-post intervention MRIs of each thigh were then analysed to determine any change in the CSA of the operated leg, by comparing it to the CSA of the un-operated leg via a ratio. Knee-extensor CSA ratio improved in the experimental group from 0.92 to 1.05, whilst in the control group it remained unchanged at 0.92; a significant between-group difference ($p = 0.04$). Improvements were seen in the knee-flexor and adductor CSAs of both groups, without a significant between-group difference. Whilst these findings support the ability of BfRT to mitigate and even reverse atrophy during long-term rehabilitation, results must be interpreted with caution. Randomisation into groups was performed by whether the last digit of the patient's ID number was odd or even. This is deemed to introduce an element of non-randomness into the process and carries a high risk of selection bias (Higgins et al, 2011b). A lack of reporting in terms of participant and personnel blinding also makes the

risk of performance and detection bias unclear. Raw values for thigh muscle CSA were also omitted from the results, making it impossible for the reader to draw direct comparisons with the MRI findings of other BfRT studies.

A case study Lejkowski and Pajaczkowski (2011) incorporated BfRT into a female athlete's twelve-week rehabilitation programme following a surgical ACL reconstruction. BfR was applied in intermittent bouts (without exercise) from four to seven days post-surgery, as per Takarada et al (2000b), with the remainder of the recovery programme utilising continuous BfR during rehabilitation exercises. Thigh and leg girth was preserved across the rehabilitation programme in both limbs when measured every three weeks via tape measure. This suggests an attenuation of post-surgical muscle atrophy due to the application of BfR to the operated limb. However, the absence of medical imaging to substantiate changes in thigh-muscle CSA and a lack of reporting as to blinding of the outcome assessor, blurs the significance and validity of these girth measurements. Given that this is a single case study lacking a case-control, the potential risk of bias inherent to this methodology also places it at the bottom of the hierarchical pyramid of evidence-based medicine (Murad et al, 2016). Therefore, whilst this study provides valuable information as to how lower-limb BfR can be applied safely and practically within an injured individual, the strength of its evidence in regards to atrophy attenuation is lower than that of larger cohort studies or controlled trials, such as Cook et al (2010) and Iversen et al (2015).

In summary, critical appraisal of the selected evidence suggests that intermittent BfR is not efficacious at attenuating longitudinal losses in lower-limb muscle size girth or lower-limb girth. The use of LiBfRT can attenuate these losses. However, it is plausible to

suggest that the LiRT component of this caused this attenuation via the completion of chosen exercises to volitional failure, rather than BfR.

2.4.1.2 Changes in Lower-limb Muscular Performance

Several studies within the selected literature objectively measured muscular performance at pre-intervention and post-intervention time points (Cook et al 2010; 2014, Ohta et al, 2003; Kubota et al, 2008; 2011). Within the realm of ACL reconstruction, Ohta et al (2003) utilised isokinetic dynamometry [IKD] to calculate knee-extensor strength ratios between the injured and uninjured limbs of participants prior to surgery. Ratios were then re-assessed sixteen-weeks post-surgery and compared with pre-surgery values. All participants experienced a worsening of their knee-extensor strength ratio, whether they completed their traditional rehabilitation programme with or without BfRT. However, pre-post ratio reductions in the BfRT group were significantly smaller than those of the control group in the knee extensors ($p < 0.01$) and knee flexors ($p \leq 0.05$) during both eccentric, concentric and isometric contractions. IKD was likely contraindicated during early and mid-term rehabilitation due to the risk of re-injury, therefore it is difficult to determine a within-study trajectory of strength loss and/or its attenuation during the initial stages of the intervention.

The application of BfR over shorter intervention periods has also found favourable effects relating to the preservation of muscular knee strength. Kubota et al (2008) compared muscular knee torques via IKD, before and after fourteen days of ULLS. The experimental group undertook twice-daily sessions of intermittent BfRT without simultaneous exercise, similar to Takarada et al (2000b). Depending upon muscular contraction type and speed, the control group experienced post-intervention losses in knee-extensor torques between

22.1% and 26.9%, whilst knee-extensor torque was largely preserved in the BfRT group, resulting in losses ranging between 0.6% and 4.7%. This between-group differences reached statistical significance in four of the six tested muscular contraction types/speeds ($p \leq 0.05$). A trend also existed towards the preservation of knee-flexor torques in the BfRT group compared to the control group, reaching statistical significance at two contraction types/speeds ($p < 0.05$).

Later work by Kubota et al (2011), using a lower 50mmHg cuff pressure but an otherwise identical protocol to Kubota et al (2008), reported similar results in terms of knee-flexor torque preservation. However, a between-group statistical difference in knee-extensor torque loss was only seen at one of the six tested contraction types/speeds. Therefore, it could be suggested that a low cuff pressure was less efficacious at preserving knee-extensor torque than the 200mmHg cuff pressure used in Kubota et al (2008).

Interestingly, Kubota et al (2008) included a second experimental group that completed only isometric exercises without BfR. This training modality failed to attenuate knee-extensor torque loss, and provided only minor attenuations in the knee-flexor torque loss. In combination, the results of Kubota et al (2008) and Takarada et al (2000b) studies suggest that intermittent bouts of BfR sessions (without exercise) can maintain muscular knee torques where a participant is unable to fully weight-bear for a period of up to fourteen days, above and beyond that achievable by isometric exercise training alone. However, a supra-systolic cuff pressure should be used. Caution must still be taken in interpreting these findings due to particularly to low group sizes and a lack of reporting in terms of blinding within Kubota et al (2008; 2011). Blinding and randomisation is also unreported within Takarada et al (2000b), introducing the potential for selection bias.

The application of LiBfRT over a thirty-day period of ULLS appears to protect against longitudinal losses in knee muscular performance. Cook et al (2010; 2014) demonstrated a between-group difference in the percentage loss of knee-extension MVC and 1RM that occurred following ULLS. In the BfRT group, losses in knee-extensor MVC were typically prevented, whereas a 15.6% mean reduction occurred in the non-exercising control group without BfR. Knee-extension 1RM was reduced by 1.5% in the BfRT group, but reduced by 21% in the control group, a statistical between-group difference ($p = 0.02$). Hackney et al (2016) repeated the BfRT and ULLS protocol used in Cook et al (2010; 2014), albeit with different lower-limb exercises. The BfRT protocol was unable to preserve lower-limb 1RM values, which reduced by an average of 12% during leg press and 11.9% during plantar flexion tests. However, the magnitude of these 1RM reductions were less than the non-exercising control group of Cook et al (2010; 2014), indicative of some attenuation of strength loss.

As discussed previously in regards to atrophy, the absence of a group completing only low-intensity resistance exercise in either Hackney et al (2016) or Cook et al (2010; 2014) makes it difficult to isolate whether any attenuation in strength loss was attributable specifically to BfR. It is possible that simply exercising to volitional failure exposed participants to sufficient neuromuscular activation to preserve longitudinal strength. Barcelos et al (2015) found that increases in strength could be generated to similar magnitude via the completion of resistance training to volitional failure over eight weeks, irrespective of the load used, or whether BfR was applied.

A notable finding was the overall inability of BfR (performed without exercise) to prevent losses in muscular strength of the plantar flexors during periods of ULLS. Whilst Kubota et

al (2008) found that BfRT provided a sporadic protective effect within these muscles, this effect was largely absent in the later study (Kubota et al, 2011). Cook et al (2010; 2014) also saw significant MVC and 1RM reductions of the plantar flexors across the whole cohort during the ULLS period, regardless of whether they received BfR. This creates a discrepancy; if BfR without exercise attenuates knee extensor/flexor strength losses during ULLS, it should arguably attenuate plantar flexor strength loss.

It could be deliberated that Kubota et al's (2011) findings regarding preservation of knee torque were not the result of applying just BfR (without exercise). For example, Clark et al (2004) reported that during ambulation with crutches, muscle activation within the biceps femoris of a non-weight bearing limb increases to 186% of that generated by normal ambulation whilst soleus activity decreases to 33%. Therefore, it may be possible that regular bouts of raised neuromuscular activation within the hamstring musculature during ULLS (occurring outside of structured BfR sessions) helped to preserve knee flexor torque over the study period in used in Kubota et al (2011). In contrast, the reduced activation of soleus during ULLS and ankle immobilisation may have allowed plantar flexor torque loss to occur unimpeded. Further to this, Cook et al (2010) found that the CSA of the rectus femoris in the suspended limb appeared to be preserved in both their experimental and control group, following a thirty-day period of ULLS. Whilst speculative, it may be that the unweighting of the suspended limb via a thick-soled boot caused all participants to maintain their hip in slight flexion during crutch ambulation. Regular neuromuscular activation and mechanical tension through the rectus femoris, beyond that typically experienced during normal gait patterns, may have triggered muscular adaptations and a novel preservation of the rectus femoris despite limb unloading, independent of BfRT.

In terms of muscular endurance, both Hackney et al (2016) and Cook et al (2010) noted post-intervention changes in the number of repetitions that could be completed following 25-30 days of during ULLS. Within the unweighted limb, knee-extensor endurance improved by 28% in the BfRT group whilst decreasing by 24% in the control group (Cook et al, 2010). Leg press endurance improved by 22.5% and calf raise endurance by 48.2% within Hackney et al (2016). This gives the suggestion that an element of fatigue resistance can be generated longitudinally via exercise to volitional failure. Again, whether the process of restricting blood-flow specifically contributed to this effect is speculative.

In summary, there is some evidence to support the use of intermittent BfR to preserve knee flexor and extensor strength over a non-weight bearing period of fourteen days, where a supra-systolic cuff pressure is utilised with the study's methodology. Combining BfR with LiRT performed to volitional failure can preserve quadriceps strength during non-weight-bearing periods of up to thirty days. As with findings in regards to lower-limb muscle size, however it is plausible to suggest that the LiRT component of this caused this attenuation via the completion of chosen exercises to volitional failure, rather than BfR itself.

2.4.1.3 Subjective Reporting of Physical Function

This was rarely present across the selected literature. Only one case study by Lejkowski and Pajaczkowski (2011) tracked subjective function via the use of the Lower Extremity Functional Scale [LEFS] and the Knee injury and Osteoarthritis Outcome Score [KOOS]. Whilst both scales have shown validity and reliability for use in post-surgical cases (Alcock et al, 2012; Binkley et al, 1999; Roos et al, 1998; Salavati et al 2011), the study itself

consisted of a single athletic participant with no case-control. Therefore, it is not feasible to generalise the findings of this study into other populations. All five KOOS domains showed pre-post improvements in subjective function. However, the minimal detectable change for each domain was not exceeded. The post-intervention LEFS score (65/80) was similar to the score typically reported at twelve weeks after ACL surgery (Cupido et al, 2014), suggesting adequate recovery. However, no LEFS scores were recorded during the intervention programme itself. Therefore, the rate of subjective recovery cannot be compared against the expected 12-week trajectory of LEFS scores seen within traditional ACL rehabilitation protocols (Fowler Kennedy, 2015; Cupido et al, 2014). It is therefore unclear whether subjective recovery was initially expedited by the addition of BfRT into the rehabilitation programme.

2.4.1.4 BfRT Equipment and Methodology

Large between-study variations existed in terms of the equipment used to induce lower-limb BfR, and in the modality by which it was delivered. Thigh cuffs were inflated automatically or manually to between 50mmHg and 260mmHg, with cuff widths ranging from 6cm to 15.2cm. Only three of the 9 selected studies stated the use of a formula to calculate and tailor the cuff pressure applied to their study participants (Cook et al, 2010; Cook et al, 2014; Hackney et al, 2016). Studies endorsed the use of both continuous BfR with exercise and intermittent BfR without exercise, albeit with conflicting evidence for the latter. There was no clear evidence towards a particular cuff type, or cuff width, being more or less effective than others in terms of the attenuation of thigh atrophy or strength loss. However, limited evidence supported the use of cuff pressures ≥ 100 mmHg.

Lejkowski and Pajaczkowski (2011) utilised a relatively inexpensive blood pressure cuff

and manual sphygmomanometer to induce lower-limb BfR. However, studies both within and beyond this review tend to use proprietary cuff equipment combined with automated rapid-cuff inflation systems (Hokanson[®], 2017; Kaatsu Global[©], 2017). It had been noted that the cost and accessibility of these systems may limit their practicality in settings outside of the laboratory (Lowery et al, 2014). An inexpensive, practical alternative involves the use of elastic knee wraps to induce BfR (Loenneke et al, 2010; Wilson et al, 2013). Yet the lack of a simple objective method to determine the degree of thigh compression and BfR induced by an elastic wrap is apparent. This is currently limited to a subjective determinant, requesting participants to pull the wrap to a '7 out of 10' tightness around their limb. Between-participant differences in the perception of tightness may have arguably contributed to disparities in the degree of BfR occurring and the conflicting results seen in regards to strength and hypertrophy responses among longitudinal studies utilising knee wraps (Head et al, 2015; Luebbbers et al, 2014; O'Halloran et al, 2014).

2.4.2 Strengths and Limitations of this Review

2.4.2.1 Strengths

This review was systematic in its design and execution, with prior registration of the search strategy within PROSPERO and reporting in line with PRISMA statement guidelines (Liberati et al, 2009). This transparency allows for objective confirmation that the planned and actual search strategy were identical, whilst enabling the search to be reproduced by others (Biondi-Zoccai et al, 2011). The use of five preliminary searches beforehand allowed the results of different search-term combinations to be explored, helping to refine and inform the final search strategy. In the final search, broad search terms were applied across seven academic databases and two reviewers independently applied

inclusion and exclusion criteria to retrieved search records, before comparing their records to ensure a consensus. This enabled an extensive search to be completed across many peer-reviewed sources, whilst reducing the possibility of pertinent studies being excluded incorrectly. Utilising two researchers to independently assess risk of bias within controlled trials, whilst contacting study authors to clarify points of methodological rigour where possible, also enhanced the objectivity of any judgements regarding bias risk within studies.

2.4.2.2 Limitations

Within the selected studies, it was often difficult to ascertain the risk of bias across the reviewed literature due to lack of appropriate reporting within article texts. Within controlled trials, the method of randomisation was largely unreported, as was the level and extent of participant and researcher blinding. Group sample sizes tended to be small, effect sizes and confidence intervals were not routinely reported and there was heterogeneity in BfRT methodologies and outcome measures utilised across the literature. A meta-analysis of study data was not possible. Therefore, the findings of the review and their potential transposition into clinical practice must be interpreted with caution.

Only four of the retrieved studies investigated the use of BfRT in participants with an actual, acute, lower-limb musculoskeletal injury. Whilst the search strategy also permitted studies involving healthy participants undertaking ULLS for extended periods time, thus replicating the non-weight bearing conditions of a significant acute MSK injury, this only expanded the total to nine studies. It is possible that this search was too restrictive. For example, relaxing the secondary outcome measures to include non-

validated measures of physical function would have allowed inclusion of an acute injury case study by Loenneke et al, 2013b. However, the author believes that it is a paucity of peer-reviewed evidence on this topic which is the causative factor of the low number of retrieved records, rather than an excessively restrictive search strategy.

Evidence regarding the use of BfRT to restore muscle strength, size and function within other rehabilitation contexts is rapidly becoming established. This includes the use of BfRT within patients at risk of knee OA (Segal 2015a, Segal 2015b) and patients with long-term weakness due to traumatic injury (Hylden et al, 2015). It was of the author's initial opinion that a subsequent systematic review exploring the utility and efficacy of BfRT within these populations would be of additional clinical value. This has since been fulfilled by Hughes et al (2017), who provides a systematic review of controlled-trial and quasi-experimental evidence within this context.

2.5 Review Conclusions

This review aimed to answer two research questions:

- I. In adults with an acute lower-limb musculoskeletal injury, does the addition of low-intensity BfRT to an exercise or rehabilitation programme attenuate losses in lower-limb muscular size and/or performance?
- II. Using existing academic evidence, is it possible to identify or propose a valid low-intensity BfRT protocol for use within individuals with an acute lower-limb musculoskeletal injury?

In relation to the first research question, over short-term applications (≤ 15 days), evidence conflicts as to whether intermittent BfRT delivered twice daily can attenuate the

loss of thigh muscle/knee-extensor CSA. The most methodologically robust evidence (Iversen et al, 2015) suggests that it does not. Minimal evidence, with often unclear risks of potential bias, suggests that intermittent BfRT performed without exercise may attenuate losses in isokinetic and isometric knee extensor and flexor strength over this same time period. 200mmHg of thigh-cuff pressure delivered via a 7.7cm-wide would be sufficient to achieve this attenuation. 50mmHg of thigh-cuff pressure may still preserve this effect within the knee flexors, but not the knee extensors.

In mid-term applications (~30 days), LiBfRT performed to volitional failure appears sufficient to attenuate thigh muscle atrophy and knee-extensor strength loss during periods of non-weight-bearing. However, it is very possible that the process of exercising to volitional failure alone, rather than the application of BfR, produces these effects. In long-term applications (twelve to sixteen weeks) there is limited evidence to suggest that thigh girth can be maintained, or the return of thigh muscle CSA expedited, if BfRT is added to a structured lower-limb ACL rehabilitation programme. Pre-post measures of physical function beyond isokinetic dynamometry were rarely employed within the selected literature.

In relation to the second research question, significant methodological variations between the studies included in this review, and conversely, a lack of variety in terms of the injuries investigated, make it impossible to generate a robust BfRT protocol for use specifically during the acute stages of lower-limb injury rehabilitation.

To progress the evidence base regarding the use of BfRT within lower-limb injury rehabilitation, several points should be addressed within future research.

- There should be a consistency across research studies in respect of the apparatus used to induce lower-limb BfR, within the context of injury rehabilitation. The apparatus used should be relatively inexpensive and accessible to healthcare professionals [HCPs], but with an objective method of quantifying the amount of thigh pressure applied to the limb. Combined, this would prevent the need for HCPs who cannot afford or access bespoke pressure-controlled cuff systems from having to compromise or make assumptions as to the effect using another device to induce BfR among injured individuals.
- Whilst variations in the frequency, intensity, type and duration of exercises performed under BfR should be encouraged during injury rehabilitation, greater consistency is required in the amount(s) of thigh-cuff pressure applied to the lower-limb during BfRT, or in how this cuff pressure is derived. Developing a BfR protocol which is universal in terms of the points at which the cuff is inflated and deflated, with a simple way of reasoning or calculating an appropriate cuff pressure, may increase the external validity of research and boost its clinical uptake further. This may also allow HCPs and researchers to better compare the effect of adding BfR to different (or even similar) exercise programmes across the evidence base.
- Research is lacking into the acute physiological and perceptual effect(s) of superimposing BfR over un-resisted (or 'no-load') lower-limb exercises, such as those potentially employed during the initial stages of rehabilitation from orthopaedic surgery or traumatic MSK injury. Determining whether BfR would be efficacious in amplifying the acute metabolic demand of this mode of exercise would help to determine its potential utility as a muscle-preserving treatment adjunct.
- The creation of further 'real-world' case studies, case-series, cohort studies or RCTs that investigate the use of BfRT across a range of common lower-limb musculoskeletal

injuries beyond ACL reconstruction and which impair weight-bearing. Examples include ankle fracture, Achilles tendon repair and knee meniscal repair. Studies which investigate either lower-limb BfR superimposed over un-resisted exercise, or lower-limb LiBfRT, would expand the evidence base available to clinicians and may provide further treatment rationale for the use of BfRT amongst their own clinical populations.

2.6 Project Aims and Objectives;

In response to the findings of this systematic review, the overall aim of this doctoral research was to develop and refine the use of BfRT within the context of lower-limb musculoskeletal injury rehabilitation. Two objectives were formed to achieve this;

1. To develop an externally valid lower-limb BfRT methodology using relatively inexpensive BfR equipment and protocols that may be replicated within clinical settings.
2. To determine the physiological and perceptual effects of combining BfRT with un-resisted ('no-load') lower-limb exercise.

This aim and its objectives would be met by splitting the project into three separate phases, defined below;

2.6.1 Phase I

2.6.1.1 Aim

To investigate whether the physical size characteristics of individuals were associated with, or could help clinicians to predict, the degree of initial lower-limb BfR initially created by different blood-pressure thigh-cuff inflation pressures (chapter four).

2.6.1.2 Hypotheses

H0 – There will be no significant bivariate correlation between the degree of lower-limb BfR occurring and any measured physical characteristic during the application of different thigh-cuff inflation pressures.

H1 – There will be a significant bivariate correlation between the degree of lower-limb BfR occurring and at least one measured physical characteristic during the application of different thigh-cuff inflation pressures.

2.6.2 Phase II

2.6.2.1 Aim

To investigate whether the addition of lower-limb BfR to an un-resisted ‘no-load’ knee exercise produces a significant change to the acute metabolic demand or perceptual response to the exercise session, across a range of thigh-cuff inflation pressures (chapter five).

2.6.2.2 Hypotheses

H0 – The addition of lower-limb BfR to a ‘no-load’ knee extension exercise will produce no significant change in the acute metabolic demand or perceptual response to the exercise.

H1 - The addition of lower-limb BfR to a ‘no-load’ knee extension exercise will produce a significant change in the acute metabolic demand or perceptual response to the exercise.

2.6.3 Phase III

2.6.3.1 Aim

To examine the haemodynamic, perceptual and physical responses to the application of a

'no-load' lower-limb BfRT programme among a case-series of participants recovering from a significant lower-limb musculoskeletal injury (chapter six).

With the aims and objectives of this doctoral research now defined, the chapter three will introduce and detail the research methodologies and outcome measures used across the project's three study phases.

CHAPTER THREE

Research Methodologies and Outcome Measures

3.1 Chapter Introduction

This chapter details the methodologies and outcome measures selected for use during this doctoral research project. Specifically, section 3.2 describes the processes involved in the recruitment and health screening of study volunteers, whilst section 3.3 describes all anthropometric, physiological and subjective outcome measures selected for purposes of data collection. Sections 3.4 and 3.5 detail the equipment used to deliver lower-limb BfR and the lower-limb exercise protocol, respectively. To aid the reader, a summary of the relevant methods, outcome measures and testing protocols used within each phase is also given within their respective thesis chapters. Further to this, a tabulated list of the outcome measures used within each phase is given within Table 3.2 (page 114).

3.2 Research Participants

3.2.1 Participant Recruitment

During Phases I and II, staff and students belonging to universities within the Greater Manchester area were recruited by way of electronic and poster advertisements. Phase I study advertisements were linked to a bespoke website, created by the primary researcher (Philip Smith), that contained both the contact details for the researcher and the participant information sheet for the study. During Phases I and III, professional rugby league players from Warrington Wolves Rugby League Club were recruited by way of a gatekeeper. The gatekeeper had been employed by the club for several years as part of their performance team and was an established Physiotherapist experienced in the

concepts and delivery of rehabilitation-specific BfRT.

3.2.2 Testing Locations

Testing and data collection for Phase I and Phase II were undertaken within a quiet, temperature-controlled room of Manchester Metropolitan University's Muscle Function Laboratory. Testing and data collection for Phase III was completed at Warrington Wolves Rugby League training facility, based within the grounds of Chester University's Padgate Campus. This allowed data collection for Phase III to occur within a busy 'real-world' clinical setting, thus reflective of how BfRT may typically be delivered within an elite-sporting environment.

3.2.3 Participant Consent and Ethical Approval

The primary researcher gained written informed consent from all participants prior to their involvement within a project phase (Appendix Ib). The researcher adhered to the principles outlined within the Declaration of Helsinki (World Medical Association, 2013) throughout this doctoral project. Ethical approval was granted prior to commencement of data collection via the Faculty Academic Ethics Committee of Manchester Metropolitan University (Application Number 1269). If changes to project methodologies were required, revisions to the original ethics application were submitted to the Ethics committee for approval prior to their implementation.

3.2.4 Participant Health Screening

To minimise the risk of adverse or unexpected health-related events during or following BfR and/or lower-limb exercise, participants were required to complete a health screening questionnaire prior to undergoing any project testing protocol (Appendix Ia). At

the onset of this doctoral research project, no universal screening method or health questionnaire existed to aid clinicians or researchers in the safe application of BfR to patients or participants. Therefore, to determine relevant and appropriate screening questions for the project in a thorough manner, the primary researcher (Philip Smith) synthesised all health-related exclusion criteria reported within lower-limb BfRT experimental studies published between 1st January 1995 and 31st December 2014.

The search strategy was performed during w/c 5th January 2015 using the same seven academic databases and search terms described in chapters 2.23 and 2.24 (page 50). A 'health-related exclusion criteria' was defined as a physical or cognitive condition/event, current or historical, that was used to prevent a participant from partaking in a BfR study on the grounds of participant safety or wellbeing. Examples would include a personal history of angina or myocardial infarction. Health criteria used to exclude participants for reasons other than safety or wellbeing were not extracted from retrieved articles. For example, if a study wished to investigate the effect of BfR upon vascular endothelial function within a small cohort, and the inclusion of participants who were long-term tobacco-users may have confounded the results. If ambiguity existed within an article's text as to the reason for the exclusion criteria, it was presumed to be for reasons of participant safety or wellbeing.

BfRT articles published on any date within an academic journal and available in the English language were initially included. Review articles, experiments conducted upon only the upper limbs and experiments not involving human participants were excluded. Following the application of these inclusion and exclusion criteria, the full texts of 125 relevant articles were retrieved. Each text was reviewed by the primary researcher and

health-related exclusion criteria were extracted and tabulated into a bespoke spreadsheet. The types and prevalence and these criteria would then inform the researcher as to the design and content of the bespoke health screening questionnaire. The number of articles in which no health-related exclusion criteria were described was also monitored.

Within the reviewed texts (n=125), the attainment of ethical approval and informed consent from participants was universally reported. Use of the word 'healthy' to describe recruited participants was widespread, however, 45.6% of articles (n=57) reported no explicit health-related exclusion criteria within their texts. Of the remaining studies (n=68), the type and prevalence of reported health-related exclusion criteria are displayed in Table 3.1 (page 82).

No adverse health events were located within the reviewed experimental studies published between 1995 and 2014. Outside of an experimental setting, one published case study has documented an episode of exertional rhabdomyolysis (skeletal muscle injury and necrosis, and leakage of cell contents into the circulation (Bosch et al, 2009) following a LiBfRT session at a physiotherapy centre (Iversen and Røstad, 2010). A healthy male had attempted supervised LiBfRT to regain strength following an 11-month injury period in which unilateral quadriceps atrophy and weakness had occurred. Following his recovery from rhabdomyolysis, he subsequently returned to using LiBfRT without incident and returned to competitive sport after seven weeks. As of 2017, two further case studies have documented the occurrence of isolated adverse events. In Tabata et al (2016), one sedentary male suffered an episode of rhabdomyolysis following BfRT involving bilateral squats and upper-limb resistance training without BfR. This male was not involved within

an experimental BfRT study, but had been under the supervision of a qualified fitness professional at the time of the incident. According to authors, the severity of the rhabdomyolysis may have been exacerbated by a bacterial infection (acute tonsillitis) and several recently prescribed medications. A second male suffered two episodes of unilateral central retinal vein occlusion, followed by blindness in the affected eye (Ozawa et al, 2015). This male was participating in an ethically approved BfRT study at the time of these adverse events. The authors believed that the male's existing diabetes, hypertension and diabetic retinopathy may have raised the relative risk of adverse events occurring from the undertaking BfRT.

One epidemiological study has attempted to quantify the incidence of adverse health events among a Japanese population undertaking BfRT within real-world settings (Nakajima et al, 2006). Instructors based within 105 facilities across Japan were surveyed regarding their use of Kaatsu among their clients and patients. Of the 12,642 persons having received Kaatsu through these facilities, the incidence of serious adverse events was low. Adverse events included venous thrombus (0.055%), pulmonary embolism (0.008%) and rhabdomyolysis (0.008%). The incidences of bruising (13.1%) and numbness (1.30%) were more prevalent, however. The presented results indicated that all adverse events occurred in facilities other than 'training gyms', such as hospitals, clinics and rehabilitation centres. It could be suggested, therefore, that the inherent presence of disease or injury in patients at these locations may have increased their relative risk of experiencing an adverse event during BfRT. Whilst serious adverse events are both isolated and rare in comparison with the number of BfRT sessions completed without incident, evidence does support the need for sound clinical reasoning and health-

screening when considering the application of BfRT to sedentary, comorbid or injured individuals.

Table 3.1. The prevalence of health-related exclusion criteria within lower-limb BfRT experimental studies from 1995-2014.

Health-related Exclusion Criteria	Percentage of Studies Listing the Exclusion Criteria
Current Vascular-specific Disease or Clotting Disorder	53%
Current Cardiac-specific Disease	51%
Current Orthopaedic Disease or Limitation	47%
Current or Recent Smoker (of tobacco)	29%
Current Medication Use (including contraceptives)	25%
Obesity (a Body Mass Index > 30 kg/m ²)	22%
Current or Recent Musculoskeletal Injury	22%
Current Hypertension (> 140/90mmHg)	19%
Historical Deep Vein Thrombosis/Thromboembolism	16%
Current Pulmonary-specific Disease or Disorder	15%
Physical Activity Readiness Questionnaire [PAR-Q] ¹	13%
Ankle-Brachial Index ≤ 0.90	12%
Current Neurological-specific Disease or Disorder	10%
Use of Ergogenic Aids (including anabolic steroids)	9%
Current Pregnancy	4%
Current Hypotension	3%
Current Alcoholism	3%
Current Cancer Diagnosis	3%
Current Lack of Mental Capacity	3%
Currently Following a Weight Loss Programme	3%
Historical Angina or Myocardial Infarction	1%

¹The PAR-Q is itself a health-screening tool, used to ascertain the medical suitability of a person to undertake general exercise.

In respect of the findings noted in Table 3.1 the project’s Health Screening Questionnaire was created (Appendix Ia). Questions were formulated to detect health-related exclusion criteria either specifically, or by way of ‘catch-all’ questions designed to cover multiple criteria. Answering ‘Yes’ to any of the first five questions of the Health Screening Questionnaire would exclude that participant. Participants answering ‘Yes’ to questions 6, 7 or 8 would be considered by the primary researcher on a case-by-case basis and included or excluded as deemed ethically and medically appropriate.

It should be noted that the project used a common Body Mass Index cut-off to indicate the possibility of obesity ($>30 \text{ kg/m}^2$) (World Health Organisation, 2016) and thus exclusion from study participation. However, as the accuracy of using Body Mass Index to diagnose obesity can be limited in both the general population (Romero-Corral et al, 2008) and athletes (Ode et al, 2007), participants displaying a Body Mass Index ($>30 \text{ kg/m}^2$) were not excluded if a reason unrelated to adiposity could be demonstrated. For example, the presence of significant muscle mass in an elite athlete.

3.3 Project Outcome Measures

The primary researcher (Philip Smith) selected, collected and processed all the anthropometric, physiological and subjective outcome measures (and their resultant data) within this doctoral research project. These outcome measures are detailed in the remainder of this chapter section.

3.3.1 Anthropometric Outcome Measures

3.3.1.1 Height/Body Weight

All height measurements were recorded using a stadiometer to the nearest 0.1cm, with participants' footwear removed. Body weight measurements were recorded using digital scales to the nearest 0.05kg, with footwear and heavy clothing removed.

3.3.1.2 Brachial Blood Pressure

Resting brachial blood pressure was recorded from the left arm of every participant in a relaxed, supine position. An arm blood-pressure cuff [MDF2100451; MDF Instruments®, California, USA], hand-held sphygmomanometer [MDF848XPD, MDF Instruments®, California, USA] and stethoscope [MDF747XP, MDF Instruments®, California, USA], were

used to take two readings, from which mean systolic and diastolic values were calculated. Mean arterial pressure was also derived using a standard calculation (Sherwood, 2012);

$$\text{Mean arterial pressure} = \text{diastolic pressure} + 1/3 \text{ pulse pressure}$$

3.3.1.3 Leg Dimensions

Participant leg dimensions were recorded using a generic flexible tape measure. If leg dimensions were discovered to be of importance in predicting the level of BfR that may occur at given cuff pressures, this measurement method would provide clinicians with the ability to inexpensively replicate leg dimension measurements within future clinical practice. Flexible tape measures were regularly compared with fixed rulers by the primary researcher to ensure their accuracy over repeated use. None were replaced during the research project. All tape measurements of leg dimensions were taken in triplicate to the nearest 0.5cm and a mean value calculated.

Leg length was measured from the anterior superior iliac spine [ASIS] to the medial malleolus in supine (Magee, 2014). This method has displayed high inter-rater and intra-rater reliability and concordance with computed tomography scans that measured leg length (Jamaluddin et al, 2011; Neelly et al, 2013). Thigh length was measured from the anterior superior iliac spine [ASIS] to the superior pole of the patella in supine. Thigh circumference was determined by marking a point on the anterior upper thigh in supine, 40% of the distance between the ASIS and superior pole of the patella in a caudal direction. Participants then stood and placed equal weight on both lower limbs. Thigh circumference was immediately measured at the level of the marker, with the tape measure wrapped perpendicular to the femur.

In regards to thigh circumference, work by Loenneke et al (2012c; 2015a; 2016) typically

selects a distance of 33% between the inguinal crease and the superior pole of the patella from which to source this measurement. It was of the primary researcher's opinion that this method may induce confounding, as the inguinal crease is not a fixed anatomical point. The crease is several centimetres in length and angled obliquely to the shaft of the femur. Accurately marking the midpoint of the crease (to improve measurement consistency) would have also required removal or adjustment of clothing close to the groin of participants. In contrast, the ASIS is visually prominent and easily palpable (Muscolino, 2008) whilst lying adjacent to the inguinal crease. Its rounded shape meant no marking of the skin was required. The ASIS was therefore selected as the preferred proximal anatomical landmark. The percentage distance value along the thigh at which the marker point was placed was adjusted from 33% to 40%. This compensated for the ASIS lying slightly superior of the inguinal crease and prevent inadvertent inclusion of gluteal muscle fibres in the thigh circumference measurement. The thigh circumference measurement therefore encompassed all four quadriceps, sartorius, the hamstrings (except for the short head of biceps femoris), the adductor muscles, gracilis, the femur, and all surrounding neural, vascular and connective tissues.

3.2.1.4 Body Composition

Dual-energy X-ray Absorptiometry [DXA] can rapidly quantify body composition *in vivo* using a three-compartment model, separating scanned body tissues into fat, lean mass and bone (Albanese et al, 2003). Whilst a more accurate four-compartment model exists (Toombs et al, 2012), accessing and performing the additional battery of tests required to achieve this prohibited its use within this project. All Phase I participants underwent one full-body DXA scan [Lunar Prodigy Advance; GE Healthcare] to determine body composition. To minimise sources of error and variability in body composition data, the

primary researcher adhered to the DXA scanning and analysis recommendations of Nana et al (2014). Prior to each full-body scan, the DXA scanner was calibrated using a multi-density phantom block and quality-assurance process. Participants were requested to remove any dense or metal-containing items likely to confound scan results, before lying in a relaxed supine position. Upper limbs were positioned away from the trunk and lower limbs were fixed into an internally-rotated position using ankle straps. Post-scan, the researcher aligned the detected borders of each body region in each scan image to correspond with predetermined skeletal landmarks (Appendix II). Measurements of tissue fat percentage, fat mass and lean mass were then extracted from scan results. These measurements were used in Phase I, via bivariate correlational analysis, to determine any relationships between participants' physical characteristics the degree of lower-limb BfR generated by different thigh-cuff pressures.

3.3.2 Physiological Outcome Measures

Physiological measurements across the doctoral research project were recorded from participants adopting the same seated testing position, and are described across the remainder of this chapter section. These physiological measures were as follows;

- Popliteal Artery Diameter
- Popliteal Artery Cross-sectional Area
- Mean Popliteal Artery Blood-flow Velocity
- Popliteal Artery Blood-flow
- Popliteal Artery Pulsatility Index [PI]
- Popliteal Artery Resistive Index [RI]
- Vastus Lateralis Muscle Microvascular Oxygenation Saturation [SmO₂]
- Vastus Lateralis Muscle Total Haemoglobin Mass [tHb]

- Vastus Lateralis Muscle Deoxygenated Haemoglobin Mass [HHb]
- Heart Rate [HR]

Participants were first fitted with a 21cm-wide thigh blood-pressure cuff [MDF2090471; MDF Instruments®, California, USA] whilst standing, with the cuff situated as high on the upper thigh as possible without contacting the groin (Figure 3.3.1). During Phase I and Phase II, the limb to be fitted with the thigh cuff and undergo BfR was selected randomly beforehand via the use of a Microsoft Excel macro. This was not required during Phase III as both lower limbs received BfR consecutively in an alternating left/right sequence.



Figure 3.3.1. The initial positioning of the thigh cuff in standing.

Participants then sat centrally upon a height-adjustable plinth with their back unsupported. The height of the plinth was adjusted to achieve approximately 90° hip flexion and 80° knee flexion with both feet placed flat on the floor (Figure 3.3.2). Where required, the fit of the deflated cuff was adjusted to prevent any resting compression of thigh tissues and potential confounding of blood-flow measurements. When required

during a testing protocol, thigh cuffs were inflated manually by the researcher via an aneroid, hand-held sphygmomanometer [MDF848XPD, MDF Instruments®; California, USA].



Figure 3.3.2. A sagittal view of the seated testing position used throughout the project.

3.3.2.1 Blood-flow Measurements

Blood-flow measurements were extracted from data captured using an ultrasound imaging machine [MyLab™ 70; Esaote SpA, Italy] equipped with a linear, phased-array ultrasound probe [Biosound LA523; Esaote SpA, Italy] during Phase I and Phase II of the project. With the participant in the seated test position, the probe was placed in a

longitudinal direction against the popliteal fossa of the selected lower limb. Minimal pressure was applied through the probe to prevent compression of the vasculature and potential confounding of arterial diameter measurements. A water-based transmission gel was used to provide a consistent interface between the probe and skin surface [Aquasonic® 100; Parker Laboratories, Inc., USA]. Care was also taken to image the artery through its centre-line, as images recorded off-centre could confound artery diameter measurements.

Data was captured using a mixture of 2-dimensional [2D] and pulsed-wave Doppler ultrasound [PWD] modes. This allowed capture of both static images of the popliteal artery (Figure 3.3.3) and spectral representations of the blood velocities generated within it (Figure 3.3.4). Simultaneous measurement of popliteal venous flow distal to the thigh cuff, or femoral venous outflow proximal to the thigh cuff, may have provided additional insight as to the haemodynamic effects of BfR. However, this was not practicable with the resources and personnel available during the doctoral research project.

2D images of the popliteal artery were typically recorded at depths of 2-4cm. All PWD spectral images were taken from the same arterial segment from which 2D images were captured. If required, colour-flow Doppler was employed beforehand to ensure that the measurement site did not contain collateral arterial branches. These branches can create turbulent flow, confounding subsequent blood-flow velocity calculations (Holland et al, 1998).

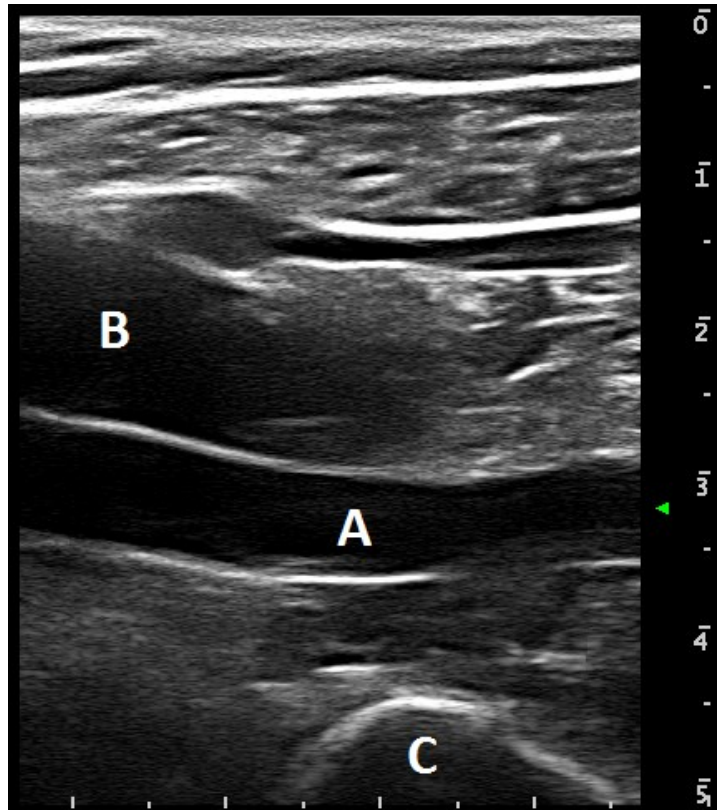


Figure 3.3.3. A sample 2-dimensional ultrasound image of the popliteal region. The popliteal artery [A], the popliteal vein [B] and the posterior, superior aspect of a tibial condyle [C] are visible. Image depth is displayed in centimetres to the right of the image.

Care was taken to ensure that the angle at which the Doppler beam intersected the artery, known as the isonation angle, was as acute as practicable. It is known that an isonation angle of greater than 60° can produce large errors in blood flow velocity calculations (Holland et al, 1998). Therefore, PWD beam steering and angle correction were used to achieve an isonation angle $\leq 60^\circ$. As arterial flow is laminar in normal undiseased arteries, sampling flow only from the centre of the lumen can also cause overestimation of mean arterial blood flow velocity during analysis (Blanco et al, 2015; Buck et al, 2014; Gerhard-Herman et al, 2012). The sample volume gate was therefore adjusted for each participant to encompass their entire lumen diameter (Figure 3.3.5). A wall filter of 65Hz was applied to prevent unwanted low-frequency signals originating

from slow-moving soft tissues from being included within the spectral signal (Pozniak et al, 1992).

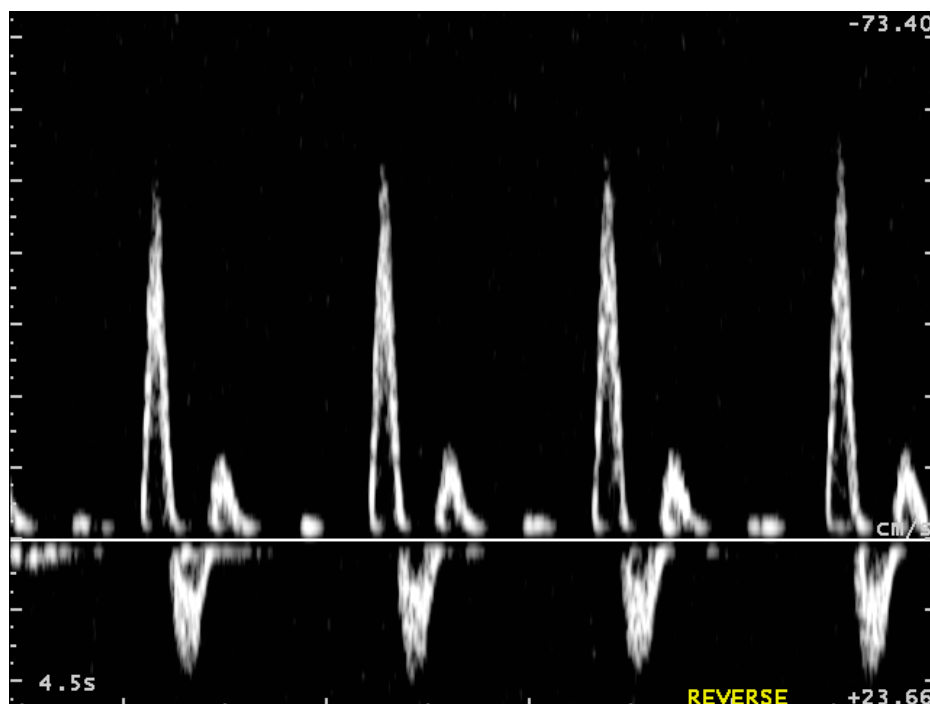


Figure 3.3.4. A typical pulsed-wave Doppler ultrasound image taken from the popliteal artery. Note the normal, tri-phasic waveform of each cardiac cycle. Blood-flow velocity is displayed as a spectral flow over a period of 4.5 seconds. Velocity is represented in centimetres per second on the scale to the right of the image.

Blood-flow data was obtained by collecting blocks of sequential 2D and PWD ultrasound images over periods of thirty seconds. Blood-flow measurements could then be extracted from each image block and compared quantitatively with other image blocks taken at different points of a testing protocol. A visual representation of one image block is shown in Figure 3.3.6.

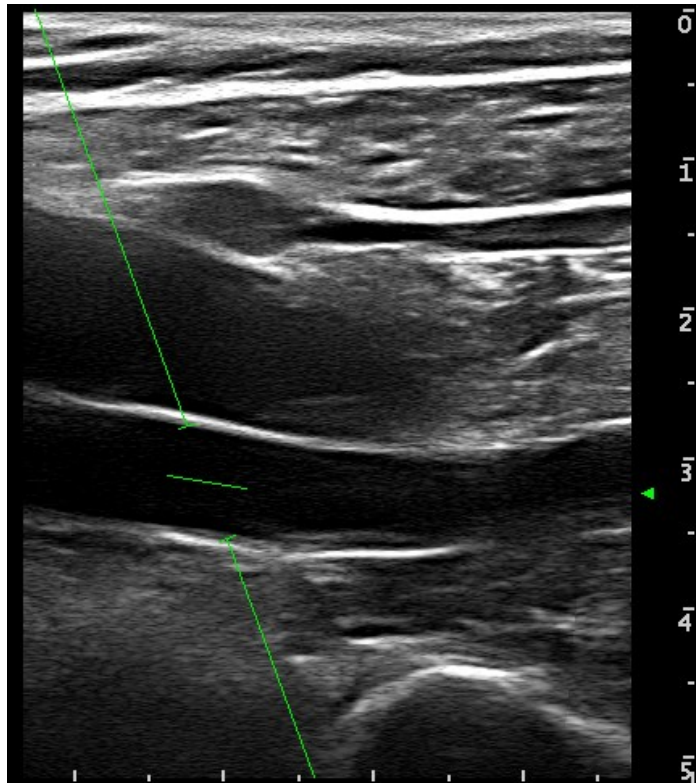


Figure 3.3.5. A 2D ultrasound image showing the alignment and path of the Doppler beam during blood-flow measurements. The sample volume gate has been adjusted to encompass the entire artery lumen. The Isolation angle here is 58°.

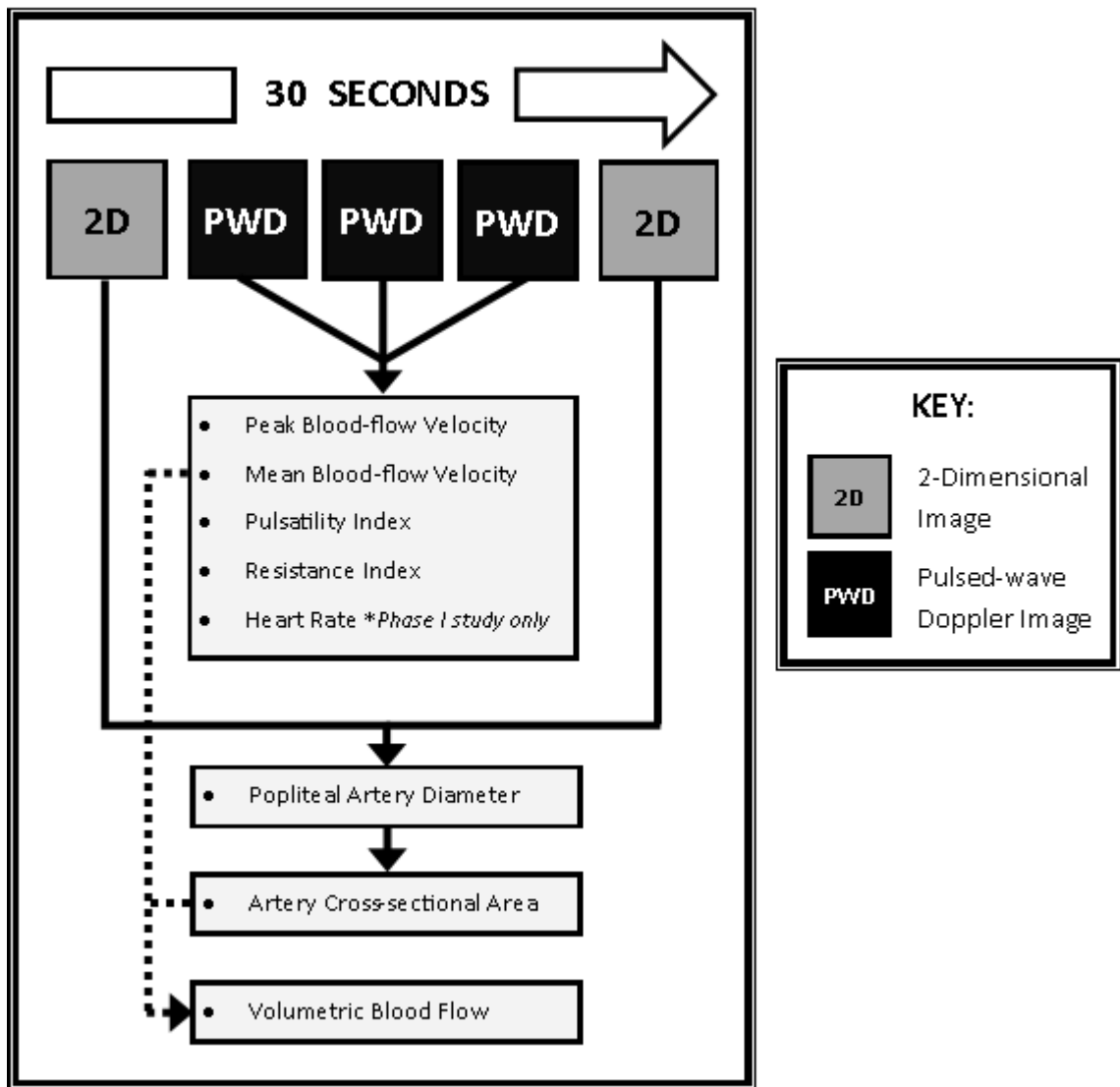


Figure 3.3.6. A flow chart depicting the collection sequence of 2-dimensional and pulsed-wave Doppler ultrasound images within one 'image block'. Black solid arrows depict the image source(s) of each blood-flow measurement. The dashed arrow depicts the measurements from which blood flow was derived.

Using commercial analysis software [MyLab™ Desk, version 8.0; Esaote SpA, Italy], the following blood-flow measurements could be derived from one imaging block.

Popliteal Artery Diameter

Electronic callipers (white crosses, Figure 3.3.7) were placed within the innermost borders of the popliteal artery wall at three locations of a 2D image, spaced equally within the boundaries of the Doppler beam sample gate. All 2D images were captured during cardiac

diastole. This prevented the transient changes in arterial diameter that occur during systole from confounding measurement values. Arterial diameter was expressed in millimetres (mm) and was a mean of the six values recorded over the two 2D images within an image block.

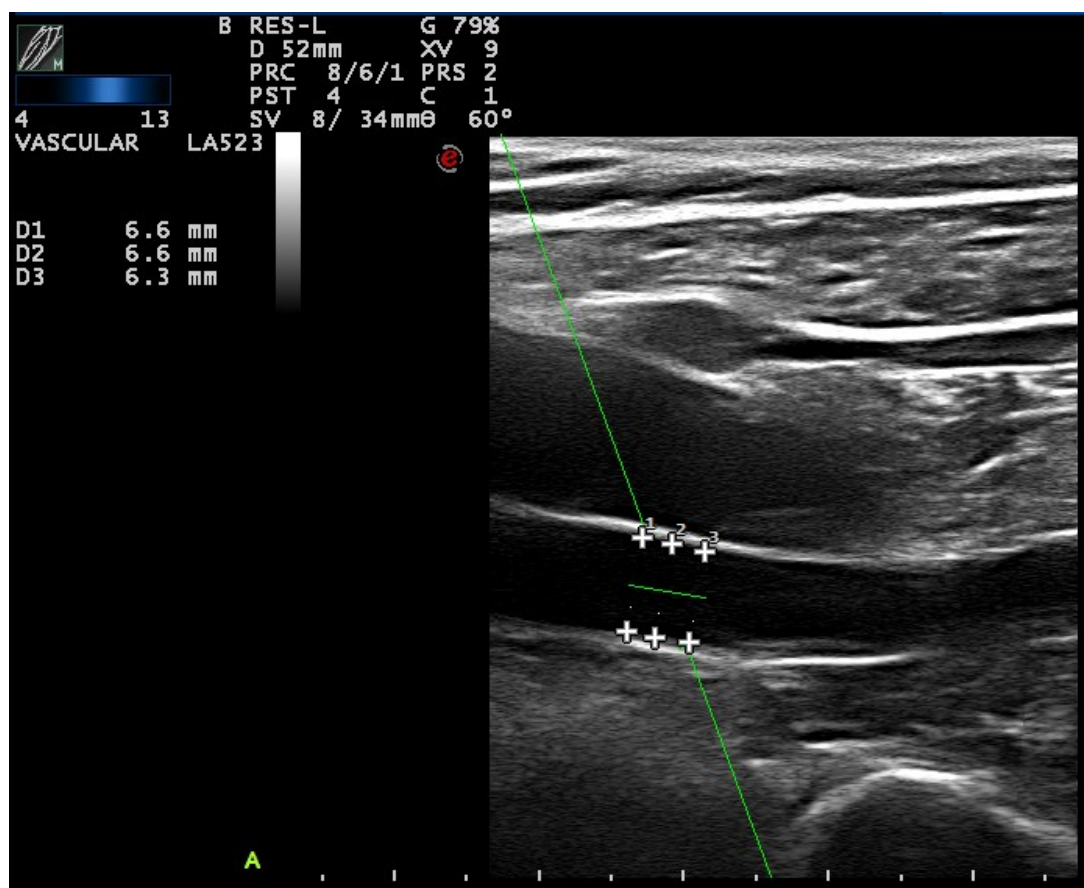


Figure 3.3.7. A 2-dimensional ultrasound image, demonstrating arterial diameter measurement. The average artery diameter here is 6.5mm.

Popliteal Artery Cross-sectional Area

Popliteal artery diameter readings were divided by two to obtain artery radius (r). The formula, πr^2 was then applied to achieve a measure of popliteal artery cross-sectional area, for each imaging block, expressed in millimetres squared (mm²).

Peak Blood-flow Velocity

Peak blood-flow velocity in the popliteal artery was determined by detecting the highest

recorded peak of any cardiac cycle within a PWD image (Figure 3.3.8, page 96). The highest peak value recorded within every PWD image of an image block were then combined and a mean value was calculated. Peak blood-flow velocity was expressed in centimetres per second (cm/s).

Mean Blood-flow Velocity

The time-averaged mean velocity [TAMV] of blood flowing through the popliteal artery was determined by manually tracing the spectral flow within a PWD image. Whilst automatic 'real-time' tracing of waveforms was possible with the ultrasound machine available, the accuracy of the automatic method was highly dependent upon spectral image quality. The slightest noise or artefact within a spectral image would create obvious errors in the automatic tracing path and confound the velocity measurement. Thus, whilst time-consuming, manual tracing allowed the primary researcher to easily discount spectral noise and artefacts from traces and the resultant TAMV measurements.

Spectral tracing began at the first full cardiac cycle of the image and finished at the end of the last full cardiac cycle (Figure 3.3.9, page 97). Deriving TAMV in this way, across several sequential cardiac cycles, is an accepted method within ultrasound imaging and has been used within previous literature (Holland et al, 1998; Nelson and Praetorius, 1988) Care was taken to trace through the centre of each spectral flow to accurately represent TAMV in the sampled artery. Mean blood-flow velocity was expressed in centimetres per second (cm/s) and was an average of the TAMV values recorded across all PWD images within an image block.

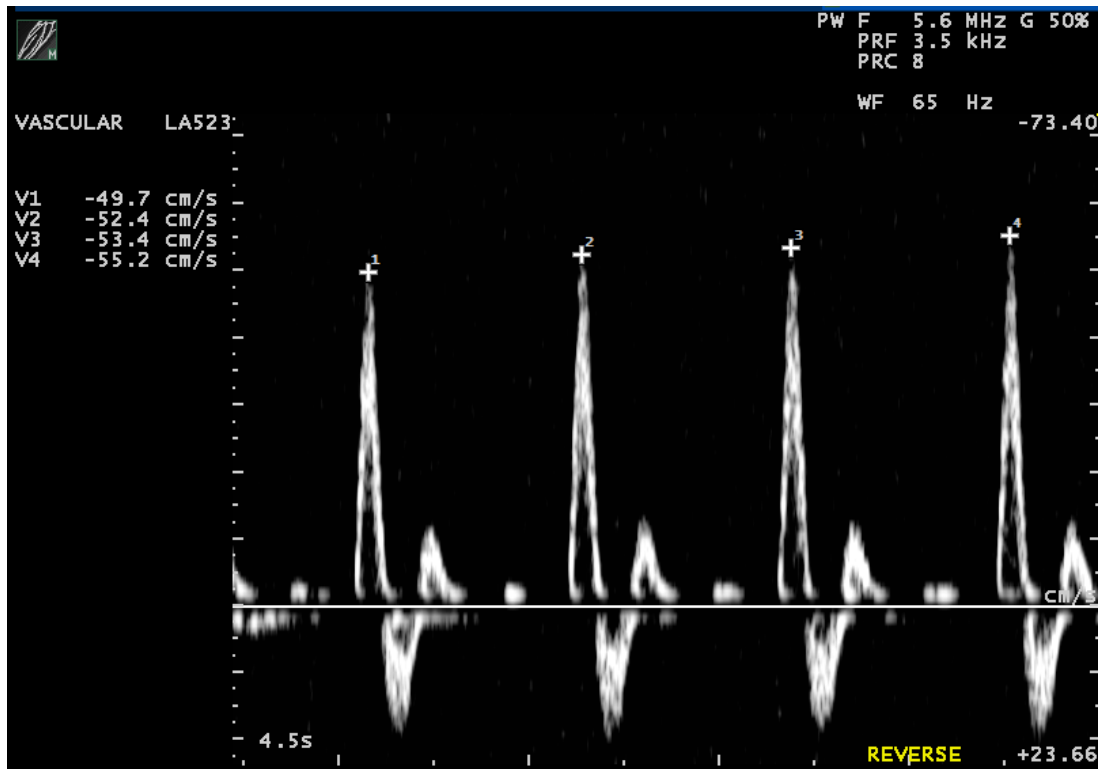


Figure 3.3.8. A Doppler image demonstrating peak blood-flow velocity measurement. The highest value (55.2 cm/s in this instance) would be selected for analysis.

Blood Flow

The amount of blood flowing through an artery over a period of one minute (Blanco, 2015) can be expressed using the following calculation;

$$\text{Arterial blood flow} = \text{Artery cross-sectional area} \times \text{TAMV} \times 60 \text{ (seconds)}$$

The TAMV and artery cross-sectional area recorded from an imaging block were input into this calculation to express an arterial blood-flow reading in millilitres per minute.

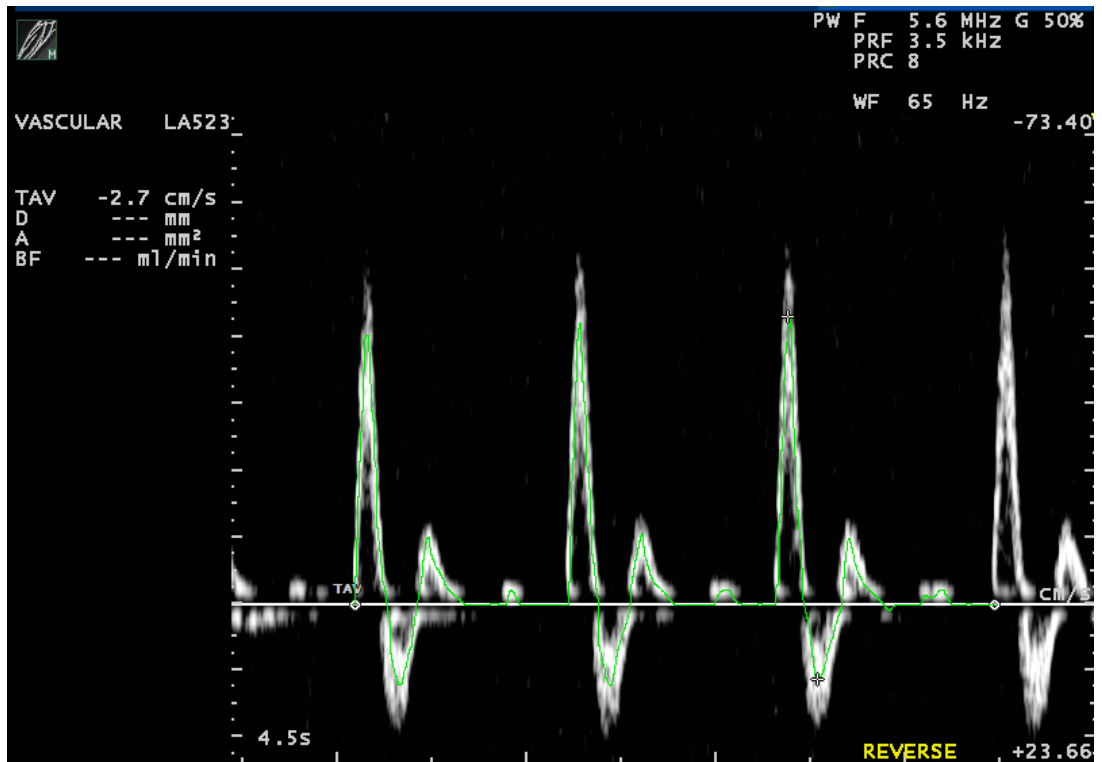


Figure 3.3.9. A Doppler image demonstrating mean blood-flow velocity measurement. The mean velocity here is 2.7 cm/s.

Pulsatility Index

Pulsatility Index [PI] is a ratio measure of peak blood flow velocity, divided by the mean blood-flow velocity over a full cardiac cycle (Gosling and King, 1974). A decrease in PI may indicate the presence of arterial stenosis proximal to the site being sampled by pulsed-wave Doppler (Johnston and Teraschuk, 1976). In the context of this doctoral research, a reduction in popliteal artery PI may indicate arterial stenosis of the femoral artery (underlying the blood-pressure cuff) as higher cuff pressures begin to compress the artery walls. As it is acknowledged that the cuff pressure applied during BfRT should occlude venous return whilst leaving arterial inflow predominantly intact (to encourage local metabolite accumulation and provoke venous pooling/cell swelling; Loenneke et al, 2014; Pope et al, 2013), changes in PI could indicate at what cuff pressure(s) this state was met or exceeded during participant testing. PI was determined by tracing the outline of the

first three cardiac cycles appearing in a Doppler image (Figure 3.3.10). The PI values from all PWD images within a block were then averaged to give a mean PI.

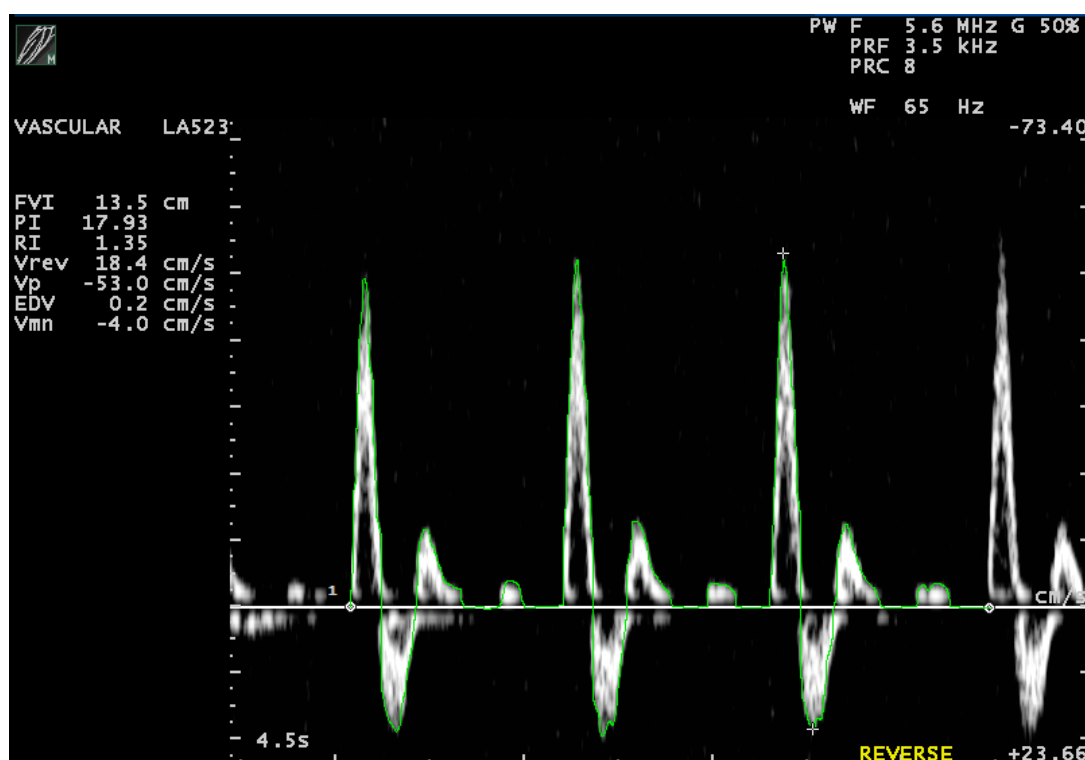


Figure 3.3.10. A Doppler image demonstrating Pulsatility Index and Resistive Index measurements. The Pulsatility Index here is 17.93. The Resistive Index is 1.35.

Resistive Index

Resistive Index [RI] within peripheral arteries is a ratio measure of peak systolic blood-flow velocity, divided by peak reverse diastolic blood-flow velocity during one cardiac cycle (Pourcelot, 1974). Changes in RI indicate changes in vascular resistance and compliance distal to the site being sampled by pulsed-wave Doppler (Bude and Rubin, 1999). In the context of this project, an increase in popliteal RI may occur as thigh-cuff pressure is applied and venous outflow from the limb is occluded. Hence, its inclusion as an outcome measure. RI was determined by tracing the outline of the first three cardiac cycles appearing in a Doppler image (Figure 3.3.10). The RI values from all Doppler images within a block were then averaged to give a mean RI.

Determining the Degree of Lower-limb Blood-flow Restriction

Throughout the studies in which ultrasound imaging was employed (Phases I and II), the percentage of blood-flow remaining in the popliteal artery during the compressive effect of an inflated thigh cuff could be quantified. This was achieved by comparing the blood flow value recorded immediately before inflation of the thigh cuff to a target pressure [x], with the blood flow value recorded immediately upon reaching the target cuff pressure [y] (Figure 3.3.11). Dividing y/x , then multiplying this value by 100, gives the percentage of popliteal arterial blood-flow remaining [%PBfR] during the cuff pressure.

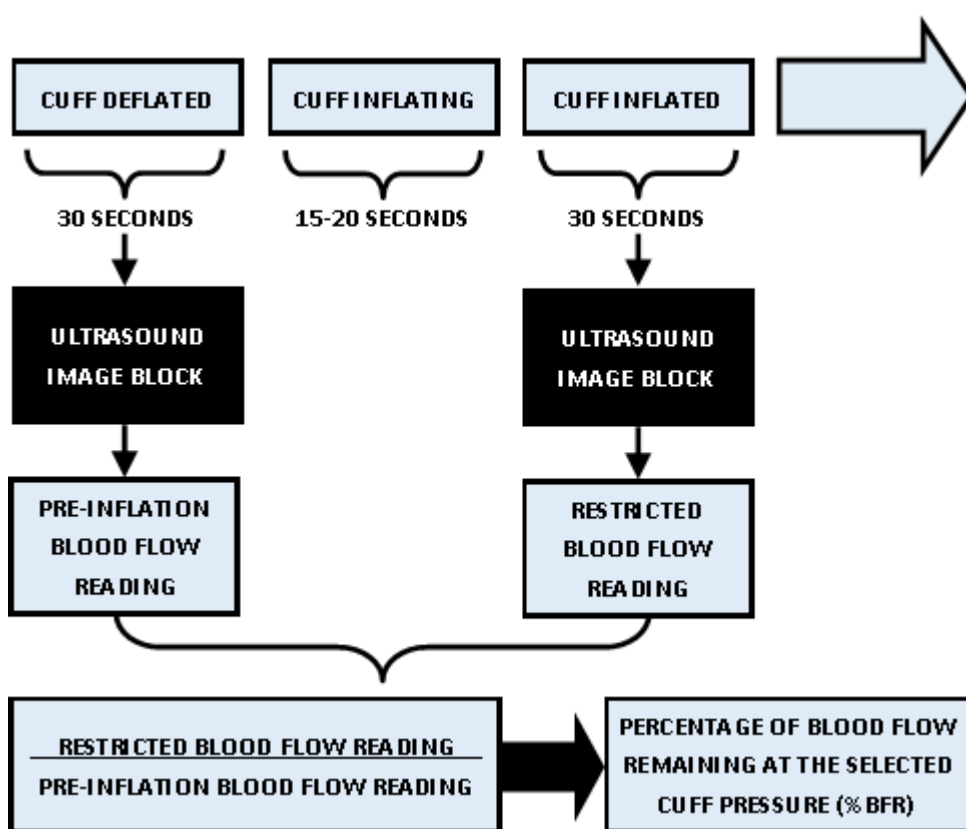


Figure 3.3.11. A flowchart depicting the process by which the degree of blood-flow restriction occurring within the popliteal artery was determined.

3.3.2.2 Microvascular Oxygenation Saturation of Tissue [SmO₂]

Developed from early work by Jobsis (1977), Near Infrared Spectroscopy [NIRS] is now an established non-invasive method of quantifying haemoglobin levels within living tissue (Boas et al, 2014; Jue and Masuda, 2013; Vardi and Nini, 2008). Briefly, a NIRS device

emits light into a portion of tissue across several wavelengths ranging between 700 and 1000nm. It is known that haemoglobin molecules are chromophores; absorbing near infrared light across these light spectra dependent upon whether they are in an oxygenated or de-oxygenated state (Figure 3.3.12).

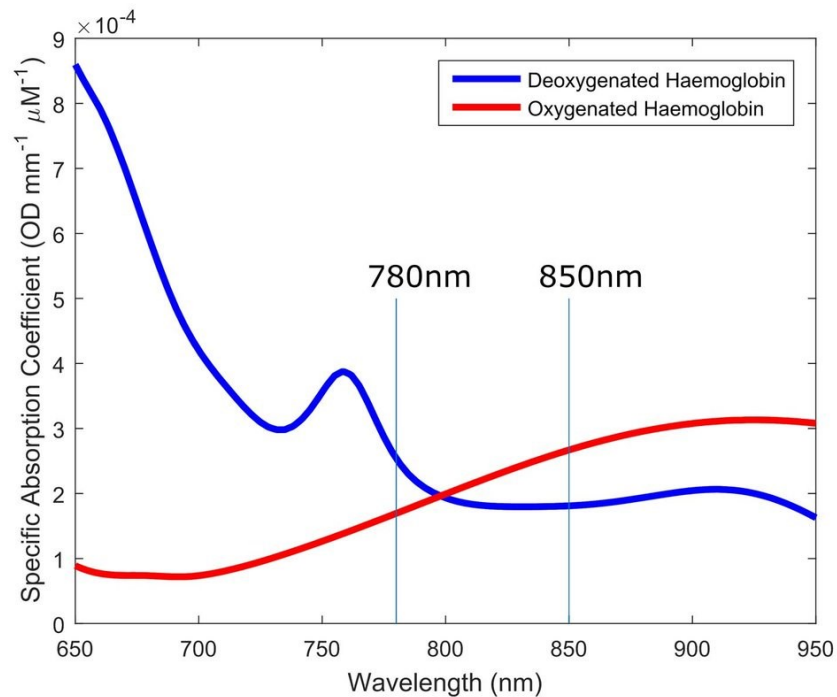


Figure 3.3.12. A graph displaying the absorption spectra of oxygenated [red] and de-oxygenated haemoglobin [blue] across near infrared light wavelengths.
(Image: Gowerlabs Ltd., 2016)

Using Beer-Lambert's Law and mathematical modelling, NIRS devices use the amount of light returning from the sampled tissue at various wavelengths to quantify levels of oxygenated and deoxygenated haemoglobin (Soul and du Plessis, 1999). From this, microvascular oxygen saturation of tissue [SmO_2] can be determined and expressed as a percentage via the following calculation:

$$SmO_2 = \left(\frac{\text{Total Haemoglobin Mass} - \text{De-oxygenated Haemoglobin Mass}}{\text{Total Haemoglobin Mass}} \right) \times 100$$

In Phases II and III, all microvascular oximetry measurements were recorded from the distal vastus lateralis muscle using a commercially available NIRS device [Moxy Monitor, Fortiori Design LLC; Minnesota, USA] (Figure 3.3.13). The device emits near infrared light at 680, 720, 760 and 800nm wavelengths whilst two sensors, spaced 12.5mm and 25mm from the light emitting diode, allow tissue sampling depths of approximately 12mm. A proprietary algorithm is utilised by the device to maximise sensitivity to the muscle layer whilst minimising sensitivity to skin and fat layers (Fortiori Design LLC, 2016). At the commencement of this project, published evidence as to the reliability and validity of this device's use in within experimental studies was absent. Therefore, data recorded from this device during the Phase II and Phase III pilot studies (with the participants at rest) were checked for concordance against normal reference values reported in other NIRS literature. The coefficient of variation in resting NIRS values recorded over periods of three minutes were also reviewed for their magnitude, and variability across testing sessions. This data is presented in results sections of chapters 5.4.3 (page 164) and 6.3.3 (page 203). As the research project progressed, academic studies utilising this device in the quadriceps or plantar flexors have emerged (Bau et al, 2015; Born et al, 2016; Crum et al, 2017; Luck et al, 2017). Each study has demonstrated the ability of the Moxy Monitor to detect changes in SmO₂ during exercise. In particular work by, Crum et al (2017) reported that SmO₂ data collected by the Moxy Monitor appeared to be an acceptable index of metabolic demand in the working vastus lateralis muscle during an incremental cycling exercise (n=10). In Crum (2017), SmO₂ data displayed strong correlations between repeated trials for all study participants (n=10) (Spearman's Rank $r = 0.842-0.993$; ICC $r = 0.773-0.992$, $p < 0.01$) and moderate correlations with other determinants of exercise intensity (VO₂; $r = 0.73$, $p < 0.01$, heart rate; $r = 0.71$, $p < 0.01$).



Figure 3.3.13. The Moxy Monitor device.

Using medical adhesive tape [Transpore™, 3M™; Bracknell, UK], the Moxy Monitor device was positioned to overly the distal vastus lateralis muscle whilst the participant held full active knee extension (Figure 3.3.14). As the muscle belly of the vastus lateralis shortens during concentric knee extension, this positioning method ensured no loss of contact between the device and the distal vastus lateralis during the exercise protocol. To prevent ambient light from reaching the Moxy Monitor and confounding readings, the device and lower thigh were lightly wrapped with black cohesive bandage [PowerFlex®, Andover Healthcare; Massachusetts, USA] (Figure 3.3.15). Care was taken not to compress the vascular or soft tissues of the thigh, to prevent potential confounding of ultrasound blood-flow measurements.

Data captured by the Moxy Monitor was both stored within the device and broadcast wirelessly in real-time. During participant testing, Golden Cheetah (version 3.3) was utilised to display real-time SmO₂ values at sample rates of 1Hz. This allowed the primary researcher to detect any gross problems in regards to signal-acquisition or device positioning and rectify these before the commencement of a testing protocol. Prior to each exercise session, a three-minute baseline period was used to record SmO₂ of the

vastus lateralis muscle whilst the participant quietly maintained the seated testing position (Figure 3.3.15). SmO₂ values were then collected continuously throughout the exercise session. To determine the pattern and magnitude of change between pre-session and mid-session SmO₂, data was extracted directly from the Moxy Monitor post-session by the primary researcher and transferred into a bespoke Microsoft Excel spreadsheet for analysis. Analysis of within-device data was preferred over the real-time data broadcast to Golden Cheetah, as the former was sampled at higher rate of 2Hz and was unaffected by transient losses in wireless signal. This allowed for greater fidelity and reliability among collected data and any resulting analysis.



Figure 3.3.14. Placement of the Moxy Monitor in a position of full active knee extension [above].



Figure 3.3.15. Wrapping of the Moxy Monitor and thigh with black cohesive bandage [right].

3.3.2.3 Heart Rate

In Phase I, heart rate was determined by analysing the spectral flows of PWD images. The RR interval (the time taken between the cardiac systole peaks of two adjacent cardiac cycles in milliseconds) could be measured using electronic callipers and then converted into a heart rate value by the ultrasound machine's in-built software. The first three RR intervals were measured within a PWD image, giving three heart rate values per image

(Figure 3.3.16). Mean heart rate was then calculated from all the recorded heart rate values of each PWD image within an image block.

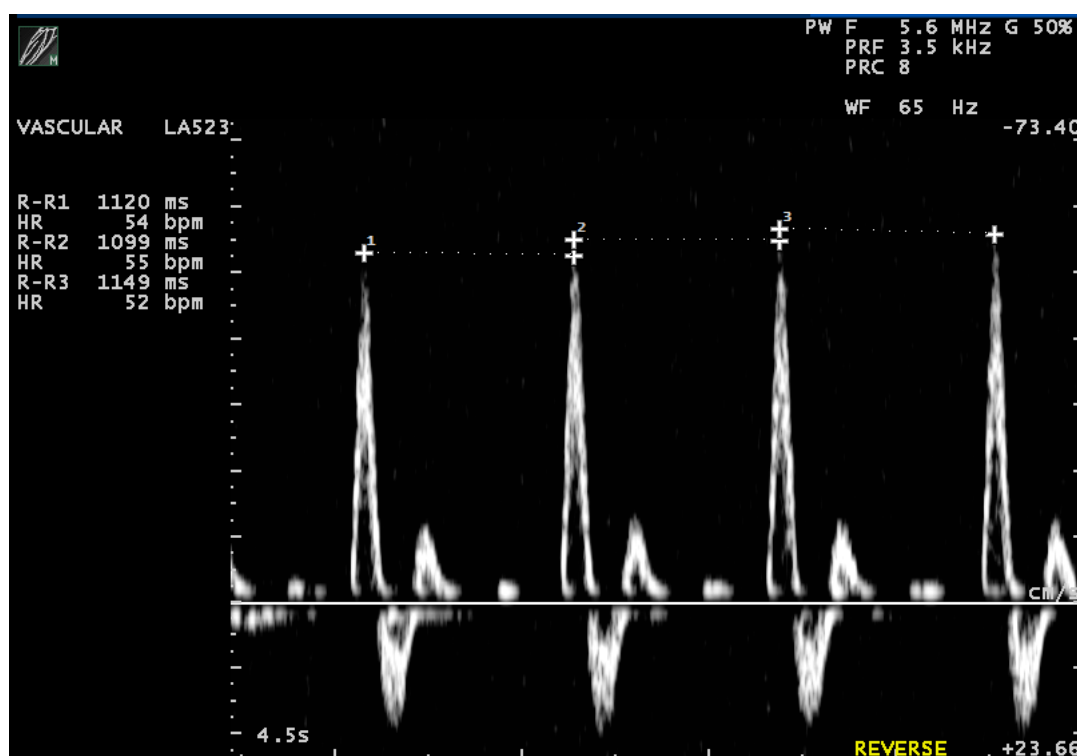


Figure 3.3.16. A Doppler image showing the measurement of RR intervals and corresponding heart rate values.

It was not possible to collect valid PWD images whilst the limb was in motion during lower-limb exercise. Therefore, heart rate before and throughout exercise sessions within Phases II and III was determined using a commercially-available heart rate chest strap [Garmin International; Kansas, USA]. Transmission gel was placed upon the two sensor contacts before securing the strap at the level of a participant's xiphisternum. Heart rate signals were broadcast from the chest strap wirelessly and recorded using freely-available software [Golden Cheetah, version 3.3] at a sample rate of 1Hz. Post-session, the primary researcher transferred heart-rate data to a bespoke Microsoft Excel spreadsheet, aligning the time-stamped HR data to NIRS data obtained from the Moxy Monitor.

3.3.3 Subjective Outcome Measures

3.3.3.1 Pain

Pain is a multi-dimensional experience modulated by a range of environmental, biological and cognitive factors (Melzack, 2001; Moseley and Vlaeyen, 2015). Despite these complexities, a method of quickly quantifying pain intensity was required throughout data collection to safeguard participant wellbeing. Quantitative analysis of pain scores may have also provided rationale as to the appropriate cuff pressures to apply in future populations receiving BfR. The Numerical Rating Scale [NRS] was selected to monitor participant peak pain intensity, in preference to either the Verbal Rating Scale or Visual Analogue Scale. Whilst all three scales have been deemed valid and reliable for use in clinical contexts to quantify pain intensity (Williamson and Hoggart, 2005), the NRS surpasses the Verbal Rating Scale and Visual Analogue Scale in terms of sensitivity to change and responsiveness (Ferreira-Valente et al, 2011; Hjermstad et al, 2011; Williamson and Hoggart, 2005).

The primary researcher supplied each participant with an NRS sheet for Phase I (Appendix Ic) and familiarised them with its completion. Immediately after each cuff inflation, participants were asked to mark a cross on the NRS corresponding to the represent the peak intensity of pain experienced during the cuff inflation, if any. Phase II and III participants were supplied with a similar NRS sheet for each of their sessions and were asked to mark a cross on the corresponding NRS immediately after every exercise set. NRS scores were immediately checked by the primary researcher. A pain score of 60 or more (out of 101), or a request to stop from the participant at any time due to pain, were set as criteria to cease the testing protocol. To reduce confounding during Phase II and Phase III, participants were not given access to NRS scores they recorded during previous

exercise sessions. Scales were set to 10 centimetres in width and pain scores were determined by recording the distance from the zero-point to the cross mark in millimetres using a ruler.

3.3.3.2 Discomfort of the Testing Procedure

In addition to the NRS for pain, Phase I participants were asked to complete a modified NRS, in which the text headings were amended to relate to any discomfort experienced during applied cuff pressures (Appendix Id). A peak discomfort score of 80 or more (out of 101), or a request to stop from the participant at any time due to discomfort generated by the application of thigh-cuff pressure, were set as criteria to cease the testing protocol. The NRS was set to 10cm in width and discomfort scores were determined by recording the distance from the zero-point to the cross mark in millimetres using a ruler.

3.3.3.3 Rate of Perceived Exertion

Perceived exertion integrates peripheral and central nervous sensations received from all bodily systems into one subjective rating, and has been opined as the best single indicator of the degree of physical strain occurring during a physical activity Borg (1982). Recording perceived exertion in this doctoral research project was important for two reasons.

Firstly, as a method of maintaining participant wellbeing during BfRT sessions. Exceeding a pre-determined level of perceived exertion would warrant interruption or cessation of a testing protocol to minimise the risk of adverse events or excessive discomfort. Secondly, as a means of comparing the perceptual effect of adding greater degrees of BfR to an exercise, particularly if exertion scores aligned with the degree of change in acute physiological measures such as muscle tissue oxygenation. This may allow clinicians to use perceived exertion to broadly indicate the degree of acute metabolic demand that occurs

during the prescribed BfRT session.

Scales developed specifically by Borg (1982) were considered as a means to quantify the perceptual effort of BfR sessions during Phases II and III. Borg exertion scales are deemed valid tools to quantify exercise intensity (Chen et al, 2002; Scherr et al, 2013). However, the standard Borg scale was derived via ramped aerobic exercise (cycle ergometry), from which subjective scores when multiplied by ten broadly correspond with participant heart rates between 60 and 200 beats per minute (Borg, 1982). In contrast, evidence suggests that lower-limb LiBfRT at intensities greater than those proposed within this project only raised heart rate by approximately 25-30 beats per minute (Downs et al, 2014). It was considered, therefore, that the sensitivity of the standard Borg scale may be inadequate for this project. The modified Borg CR-10 scale was also reviewed, yet it was disregarded due to the need to establish a license agreement and proprietary fee for its use (Borg Perceptions, 2016).

The Omnibus Resistance Exercise Scale [OMNI-RES] (Robertson et al, 2003) was selected for use during this project. Development of the scale involved exposing participants to a seated knee extension exercise, which bore similarities to the exercise planned for use within this doctoral research. Participants exercised at an intensity corresponding to 65% 1RM during the study; higher than that proposed within this project. However, a subsequent study utilised a lower boundary of 40% 1RM whilst retaining high levels of construct validity compared to the Borg Scale (Legally and Robertson, 2006). A study by Duncan et al (2006) also delivered seated knee extensions at 30% 1RM and found that OMNI-RES scores were positively correlated with levels of quadriceps muscle activity.

No studies appear to have investigated the validity of the OMNI-RES during unweighted lower-limb exercise. However, Hollander et al (2010) used the scale during lower-limb LiBfRT at an intensity of 20% 1RM and detected within-group, between-set statistical differences in OMNI-RES scores despite a small sample size (n=7). A study by Neto et al (2016) also used OMNI-RES to describe exertion levels during LiBfRT. Whilst the validity and sensitivity of the scale was not investigated specifically in this study, the presented results suggested that OMNI-RES scores recorded during LiBfRT were similar and reflective of OMNI-RES scores recorded during traditional resistance exercise. In respect of these findings, it was considered justified to employ the OMNI-RES during Phase II and III of this doctoral research. Following the commencement of data collection for Phases II and III, a study by Yasuda et al (2016) has further reflected the broad similarity in OMNI-RES scores between lower-limb LiRT, LiBfRT and HiRT.

The primary researcher gave Phases II and Phase III participants an OMNI-RES data sheet prior to each exercise session and familiarised them with its completion (Appendix Ie). At the end of each exercise set, participants would quantify 'how hard their thigh muscles worked' using the scale and report a numeric value on the provided sheet. To minimise potential sources of bias, participants were not given access to OMNI-RES scores recorded on prior exercise sessions.

3.3.3.4 Subjective Physical Function

Obtaining a subjective measure of physical function allowed the primary researcher to quantify the return of function in Phase III (injured) participants across the whole BfRT intervention period. This was important, as the presence of injury would contraindicate objective measures of physical function, such as MVC via isokinetic dynamometry or one

hundred-metre sprint time, from being utilised during the intervention period. If possible, subjective function data could then be compared against normative or reference values from existing academic literature.

Developed by Binkley et al (1999), The Lower Extremity Function Scale [LEFS] was selected to track longitudinal changes in subjective physical function within Phase III participants. The LEFS consists of twenty questions in which participants rate their actual, or perceived, difficulty in completing a range of functional tasks. Total scores can range between 0 and 80, with a minimal clinically-important change (and difference) between two questionnaires of 9 points (Binkley et al, 1999). In separate studies, the LEFS has demonstrated responsiveness and high test-retest reliability within patients with ankle fracture (Lin et al, 2009), anterior knee pain (Watson et al, 2005) lower-limb ligament injury and muscle strain (Cacchio et al, 2010). This supports the use of the LEFS in settings in which the types of musculoskeletal injury encountered are wide-ranging, such as Rugby League (Hoskins et al, 2006; King et al, 2010). The LEFS also demonstrates superior sensitivity to change than the general Short Form Health Survey [SF-36] (Binkley et al, 1999) whilst encompassing a broader spread of injuries than more specialised questionnaires such as the Knee Injury and Osteoarthritis Outcome Score (Roos et al, 1998) and the Anterior Knee Pain Scale (Kujala et al, 1993).

Phase III participants were given the LEFS at their first study appointment and were directed by the primary researcher to read the standardised instructions accompanying the scale. The primary researcher then checked participant understanding and allowed them to self-complete the LEFS. Following each testing appointment, completed LEFS were reviewed by the primary researcher and the total score calculated. Participants self-

completed a new LEFS at regular intervals throughout the study, typically once every two calendar weeks. To minimise sources of bias, participants were not given access to their previous LEFS scores prior to completing a new LEFS.

3.3.3.5 Habitual Physical Activity

A measure of habitual physical activity was selected to indicate potential associations between long-term activity levels and the acute physiological effects of BfRT among Phase II participants. Objective long-term measurement of activity levels was not possible within the scope and resources available to this project. Therefore, activity levels were quantified via the completion of a Habitual Activity Questionnaire designed by Baecke et al (1982). Despite their widespread use, physical activity questionnaires can show limited reliability and validity when compared to objective measures such as pedometry or physiological testing (Shephard, 2003). Evidence does exist, however, to specifically support the validity of the Baecke Questionnaire across a range of populations (Florindo et al, 2003; Hertogh et al, 2008; Phillippaerts et al 1999; Sadeghisani et al, 2016). The questionnaire is also quick to administer and gives the ability to compare different activity sub-domains such as ‘work activity level’ with the physiological effects of BfRT. Higher scores relate to higher levels of habitual physical activity within each sub-domain, with the total overall score for the questionnaire ranging from three to fifteen.

Phase II participants were given the questionnaire to complete upon their arrival for their first testing appointment. Participants were asked to answer each question by considering their activity levels over the previous twelve months. Scores were calculated using the scoring system described in Baecke et al (1982) and collated using a bespoke Microsoft Excel spreadsheet. Scores were then be used to determine correlations

between reported physical activity levels and the acute physiological or perceptual responses that occurred during BfR sessions.

3.4 Lower-limb Blood-flow Restriction Equipment

3.4.1 Cuff Types

Two models of thigh blood pressure cuff were used during this project; A 21cm non-elastic Flexiport® cuff manufactured by Welch Allyn® and a 21cm-wide non-elastic cuff manufactured by MDF Instruments® [MDF2090471]. Whilst the Welch Allyn® cuff offered a quick-release air valve and was already in use at Warrington Wolves to deliver BfRT, the inflated cuff would not consistently remain attached during exercise where a player's upper thigh circumference exceeded ~70cm. The cuff manufactured by MDF Instruments® consistently remained attached during exercise, hence this cuff was selected for primary use during all main studies of the project. Before and after each use, cuffs were examined for damage or air-leakage following inflation. No thigh cuff required replacement during the research project.

3.4.2 Cuff Inflation Devices

Thigh blood pressure cuffs were inflated using hand-held aneroid sphygmomanometers manufactured by Welch Allyn® [DuraShock™ DS66] and MDF Instruments® [Bravata™; MDF848XPD]. To ensure accuracy and consistency of pressure readings, sphygmomanometers were matched to their corresponding models of thigh cuff. Both sphygmomanometers were certified to a calibrated accuracy level of ± 3 mmHg. As per manufacturer instructions, each sphygmomanometer was checked before each use to ensure that the measurement needle rested at 0mmHg. One sphygmomanometer (MDF Instruments®) lost calibration during pre-testing sessions and was replaced. Data

collection within pilot and main study phases was unaffected.

3.5 BfR Exercise Protocol

Seated knee extension is a commonly-utilised exercise within BfRT literature, appearing in at least 40% of peer-reviewed, lower-limb studies between 1995 and 2014. Within the context of lower-limb injury, this exercise commonly appears among rehabilitation protocols and programmes designed to maintain or regain muscular strength and function. This includes ACL reconstruction (Oxford University Hospitals NHS Trust, 2012; Palmieri-Smith and Thomas, 2008) knee arthroscopy (East Kent Hospitals University NHS Foundation Trust, 2016; University Hospital Southampton NHS Foundation Trust, 2012) and total knee replacement (Bade and Stevens-Lapsley, 2011; Guy's and St Thomas' NHS Foundation Trust, 2013). Seated knee extension was also an established method of delivering BfRT at Warrington Wolves Rugby Club, as part of players' multi-modal rehabilitation programmes. Thus, the parameters of the exercise used throughout study Phases II and III were drawn directly from those established by Warrington Wolves. One BfRT session typically consisted of three sets of un-resisted, seated knee extensions, with each set lasting for one minute. Inter-set rest periods were also one minute in length. In the absence of an established repetition speed both at the Club and within the academic literature, the researcher set this to a pace of forty repetitions per minute. Participants therefore completed 120 un-resisted knee extensions over the course of one exercise session. The start and end positions for each repetition of the exercise are shown in Figure 3.5.1 and Figure 3.5.2.



Figure 3.5.1 [left] and Figure 3.5.2 [below] showing the respective start and end positions of the seated knee extension exercise.



Participants were requested to move their limb at a constant steady pace throughout each exercise set, with no pause at the start and end of each repetition. To ensure participants completed the prerequisite number of repetitions, metronome software [Metronome, version 4.5.4.0; Zhaobang, China] was used to provide visual and auditory cues at a speed of eighty beats per-minute. Each beep denoted the start or end position of a repetition.

3.6 Chapter Summary

This chapter has provided a detailed description of the outcome measures and general methodologies used across this research project's three study phases. For added clarity, the objective and subjective measures used within each particular phase of the project have been tabulated (Table 3.2). The following three chapters will present and discuss the results of the studies conducted within each phase of this doctoral research. To assist the reader, each chapter will briefly summarise the methods and outcome measures involved within that particular study.

Table 3.2. A tabulated reference of the objective and subjective measures collected within each phase of this doctoral research. A shaded box indicate that the measure was collected.

			STUDY PHASE		
			I	II	III
OBJECTIVE MEASURES	ANTHROPOMETRIC	Gender			
		Age			
		Height (via stadiometer)			
		Weight (via electronic scales)			
		Body Mass Index			
		Leg Length (via Tape Measure)			
		Thigh Length (via Tape Measure)			
		Thigh Circumference (via Tape Measure)			
		Supine Brachial Blood Pressure			
		Mean (Brachial) Arterial Pressure			
	DXA Body Composition Values				
	PHYSIOLOGICAL	Blood Flow Measurements (via Ultrasound)			
		Heart Rate (via Ultrasound)			
		Heart Rate (via Chest Strap)			
Near Infrared Spectroscopy Measurements					
SUBJECTIVE MEASURES	Pain NRS				
	Discomfort NRS				
	OMNI-RES				
	Lower Extremity Functional Scale [LEFS]				

CHAPTER FOUR

PHASE I

Associations Between Participant Physical Characteristics, Thigh-Cuff Pressure and the Degree of Lower-limb Blood Flow Restriction Being Delivered

4.1 Chapter Introduction

This chapter provides details of the first phase of this doctoral research project. A summary of existing academic evidence regarding the selection and/or calculation of cuff pressures for individuals undertaking BfRT is first given, providing rationale and justification for further research on this topic. Following this, the remainder of the chapter describes the implementation and outcomes of a pilot study involving a cohort of nine participants, and a main study involving a larger cohort (n=61). Findings of the main study were subsequently presented at 4th (European Region) World Confederation for Physical Therapy Congress, in November 2016 (Appendix VIa).

4.2 Phase I Aim

To investigate whether the physical size characteristics of individuals were associated with, or could help clinicians to predict, the degree of initial lower-limb BfR initially created by different thigh-cuff inflation pressures

4.3 Background

There is great disparity concerning the appropriate amount of thigh-cuff inflation pressure to be used during lower-limb BfRT, and in how this is derived. Inflation pressures can appear to be set arbitrarily (Fujita et al 2007; Neilsen et al, 2012; Mueller et al 2014).

Where some form of pressure calculation is employed, the absolute cuff pressure values utilised within a study cohort can be left unreported (Kubo et al, 2006; Conceição et al, 2016; Fatela et al, 2016). Discrepancies can also arise during the justification of cuff pressures via the citation of previous BfRT literature. For example, Cook et al (2007) justified the thigh-cuff pressure used within their lower-limb BfRT study with the following text:

“It has been suggested that a pressure 1.3 times systolic blood pressure (partial occlusion) impedes venous blood flow causing blood to pool in the capacitance vessels distal to the cuff while restricting some arterial blood flow.” Cook et al (2007:1710)

Yet the citation used to support this statement, a study by Takarada et al (2000c), applied only upper-limb BfR using sub-systolic cuff pressures. In a second example, Abe et al (2006) delivered a treadmill-walking programme of BfRT utilising 160-230mmHg of thigh-cuff pressure. The authors stated that:

“The restriction pressure of 160–230 mmHg was selected for the occlusive stimulus, as this pressure has been suggested to restrict venous blood flow and cause pooling of blood in capacitance vessels distal to the belt, as well as restricting arterial blood flow...” Abe et al (2006:1461)

Takarada et al (2000c) and Burgomaster et al (2003) were used to justify this statement, despite both studies investigating upper-limb BfRT and utilising cuff pressures below 120mmHg. Whilst Takarada and colleagues have used thigh-cuff pressures in the region of 200mmHg within lower-limb BfRT studies, none directly measured the degree of lower-limb BfR that this cuff pressure actually induced (Takarada et al, 2000a, 2000b, 2002).

Although these disparities exist, the process by which cuff pressure is determined during lower-limb BfRT has steadily evolved over the last sixteen years. Early literature tended to apply lower-limb BfRT through a combination of supra-systolic thigh-cuff inflation

pressures and narrow cuff widths (Ohta et al, 2003, Takarada et al 2000a, 2000b, 2002; 2004). The degree of compression produced was presumed to inhibit both arterial and venous blood flow through the lower limb (Takarada et al, 2000b), creating local tissue hypoxia, a regional accumulation of metabolites and systemic increases in plasma growth hormone levels (Takarada et al, 2000a). Later work by Takano et al (2005) demonstrated that the application of a cuff pressure 130% that of systolic brachial blood pressure (160-180mmHg) via a 3.3cm-wide cuff did not fully occlude blood flow at rest; instead reducing blood flow in the superficial femoral artery to 30% of baseline within five participants. The same work demonstrated that adding this degree of BfR to eleven participants completing a low-intensity knee extension exercise significantly amplified acute plasma growth hormone, insulin growth factor and vascular endothelial growth factor responses. With Abe et al (2006) concluding that a similar degree of cuff pressure induced comparative increases in thigh muscle size and strength when added to a treadmill-walking programme, the use of supra-systolic pressure applied via narrow cuffs was established and proposed as efficacious.

Whilst the benefits of BfRT using high-pressure, narrow-width combinations continued to be ratified within both acute-response studies (Fujita et al, 2007; Drummond et al, 2008; Fry et al, 2010) and longitudinal programmes (Kubota et al, 2008; Karabulut et al, 2010a, Abe et al, 2010a; Cook et al, 2010) the use of wider-width cuffs began to develop within the literature. Cuff widths ≥ 13 cm were initially combined with a supra-systolic pressure to increase the rate of fatigue during a low-intensity knee-extension exercise (Wernbom et al, 2006). However, this combination produced high levels of subjective pain and exertion amongst their sixteen healthy participants (n=13 male, mean age; 27.9 years). Building upon a non-BfRT study by Crenshaw et al (1988), Loenneke et al (2012b)

demonstrated that the thigh-cuff pressure required to completely occlude lower-limb arterial flow was dependent upon cuff width. Among healthy participants (n=116), a 13.5cm-wide thigh cuff eliminated pulsatile flow within the tibial artery of all participants at mean cuff pressure of 144 (\pm 17) mmHg, whilst a narrow (5cm) cuff eliminated the tibial pulse at 235 (\pm 42) mmHg. This difference does not take into account forty-three participants who, during the use of the narrow cuff, still possessed a tibial pulse at the study's ceiling pressure of 300mmHg. Study findings implicated thigh circumference as a moderate predictive indicator of tibial occlusion pressure. A negligible relationship between systolic brachial blood pressure and tibial pulse elimination pressure also led to the conclusion that thigh-cuff pressures derived simply from systolic blood pressure values should be discouraged. Loenneke et al (2015a) completed subsequent work among a cohort of 171 participants producing similar results and conclusions to Loenneke et al, 2012b.

A tendency to utilise thigh-cuff pressures based upon a percentage of total arterial occlusion pressure then developed (Loenneke et al, 2013a; Fahs et al; 2014; Lixandrão et al; 2015). Bi-directional Doppler was used to detect the cuff pressure required to completely interrupt the tibial pulse at rest, then 40% to 80% of this value would often be applied during BfRT itself. This allowed cuff pressure to be adjusted to each individual whilst accommodating for cuff width. When wide thigh cuffs (\geq 13cm) were utilised, the cuff pressures applied to participants became almost exclusively sub-systolic. Yet the acute and chronic effects of adding lower-limb BfR to traditional low-intensity resistance were still evident and deemed to be efficacious (Laurentino et al, 2012; Libardi et al; 2015, Vechin et al, 2015; Tennent et al, 2017).

The work by Loenneke et al (2012b; 2015a) detected a positive relationship between total occlusion pressure and thigh circumference. However, there is no guarantee that if each individual within a cohort were given 80% of their own arterial occlusion pressure, for example, that the whole cohort would subsequently experience a similar degree of actual BfR. This may vary between individuals and could itself be related to physical characteristics such as thigh circumference or vascular characteristics such as arterial stiffness. Hunt et al (2016), was the first to publish evidence investigating relationships between physical characteristics and the percentage of actual BfR occurring at sub-occlusive thigh-cuff pressures. Hunt et al (2016) did indeed find variation in the thigh-cuff pressure required to achieve the same degree of sub-occlusive BfR across individuals. However, physical characteristics inputted into a hierarchical regression model were unable to explain the majority of this variance, with mean arterial pressure alone providing the strongest model ($r = 0.58$, $r^2 0.34$, $p < 0.05$). Hunt et al (2016) acknowledged potential limitations within their study, including a lower sample size than Loenneke et al (2012b; 2015a) and lack of generalisability regarding their findings to individuals who had a body mass index above 29.3 kg/m², or thigh circumference above 66.7cm. Participants were also tested in the supine position, whereas studies often deliver BfRT in a seated or standing position. It is possible that different findings may have presented themselves if either of these testing positions were investigated.

It is important to note that a significant portion of experimental studies deliver lower-limb BfRT using 'Kaatsu' or similar rapid cuff inflation devices. Whilst these are purpose-built and ideal for experimental settings due to their ability to automatically regulate cuff pressure, they are expensive to purchase. For example, Kaatsu devices currently retail between £2,600 and £5,000 within the United Kingdom (Kaatsu Training™, 2016). This

arguably places those wishing to deliver evidence-based BfRT individuals, but who are unable to afford the restriction equipment, in difficulty. Whilst some evidence exists regarding the use of alternative restriction devices such as elastic knee wraps to deliver BfRT sessions, knee wraps remove the ability to objectively set the degree of cuff pressure being applied. This may present an unacceptable risk of adverse events within settings where BfRT is applied to injured, comorbid or elderly populations, particularly if BfRT were to be prescribed to outpatients completing sessions unsupervised. Evidence as to the efficacy of using elastic knee wraps during structured BfRT programmes can also be conflicting as to its efficacy (Luebbers et al 2014; Head et al, 2015).

In summary, the method by which thigh-cuff pressures have been determined and justified during experimental lower-limb BfRT studies has gradually evolved over time. Currently, inter-subject variations in the degree of BfR experienced at sub-occlusive thigh-cuff pressures are poorly explained by the physical characteristics of individuals. Further research is warranted, particularly regarding the use of relatively inexpensive restriction devices, to determine whether the findings of Hunt et al (2016) are replicated or contradicted among a different population, or within a seated or standing test position.

4.4. Phase I Pilot Study

4.4.1 Pilot Study Objectives

To pilot the suitability of a BfR protocol for use in the main Phase I study. Specifically;

1. To confirm whether each thigh-cuff pressure selected for use within the planned Phase I testing protocol produced a sub-occlusive level of BfR in all participants.

2. To confirm that no participant reached the agreed cut-off values for subjective levels of pain (≥ 40 out of 101) and discomfort (≥ 80 out of 101) during thigh-cuff inflations.
3. To trial the suitability of the Doppler measurement site for obtaining arterial blood-flow measurements, and measure the within-subject variability in baseline (pre-inflation) blood flow readings.

4.4.2 Methods

Following informed consent and completion of a health screening questionnaire (Appendix Ia) a convenience sample of nine participants (n=7 male) were recruited from University staff and students. Participants underwent anthropometric measurements as described in chapter 3.3.1. Participants were then fitted with a 21cm-wide blood-pressure cuff [MDF2090471; MDF Instruments®, California, USA] around their upper thigh and seated in the test position described in chapter 3.3. Four thigh-cuff pressures were applied in a randomised order (40, 70, 100 or 130mmHg). Each cuff inflation lasted thirty seconds, with a rest period of three minutes separating each inflation. No exercise or passive movement of participants' limbs occurred throughout the BfR protocol. Before and during thigh-cuff inflations, physiological measures of heart rate, arterial diameter and blood flow were recorded using the ultrasound 'imaging block' method shown in figure 3.3.6. Measures of subjective pain and discomfort were recorded using the numerical rating scales described in chapter 3.3.3. The percentage of arterial blood-flow remaining at each thigh-cuff pressure, compared to the pre-inflation blood-flow, was then determined as shown in figure 3.3.11.

It is important to note that during the pilot study, ultrasound measurements were taken

from the distal portion of the superficial femoral artery. Images were not taken from the popliteal artery. The superficial femoral artery was initially chosen due to its proximity with the distal portion of the thigh cuff. Arterial diameters and blood flow values are also higher in this location than more distal sampling sites (Holland et al, 1998; Wolf et al, 2006). Therefore, the author believed sampling from the superficial femoral artery may have allowed clearer detection of differences in blood-flow between different thigh-cuff pressures than sampling from lower-leg locations.

4.4.3 Results

Two participants were excluded from the analysis due to difficulties in imaging the superficial femoral artery both before and during inflation of the cuff to target pressures.

Details of the seven remaining participants (n=6 male) are shown in Table 4.4.1.

Participants were normally distributed across the demographics of age, height, weight and Body Mass Index (Shapiro-Wilkes $p \geq 0.279$).

Table 4.4.1. Participant demographics of the Phase I pilot study. Mean values are shown, with standard deviations displayed in brackets.

Age	Height [cm]	Weight [kg]	Body Mass Index
31.6 (7.6)	179.7 (7.6)	83.3 (10.7)	25.7 (1.7)

All participants tolerated all thigh-cuff pressures. No adverse or unexpected events occurred throughout testing. Among participants, mean pre-inflation blood flow in the superficial femoral artery over the test protocol was 70.94 (SD 19.89) mL/min and was normally distributed (Shapiro-Wilkes $p = 0.184$). Baseline (pre-inflation) blood flow values varied moderately within-subjects over the course of the test protocol, displaying a coefficient of variance of 28.03%. Pre-inflation blood flow values also decreased gradually

over the course of the test protocol (Figure 4.4.1). However, this numerical decrease was not statistically significant (Repeated measures ANOVA; $F(3,18) = 1.012$, $p = 0.411$).

During thigh cuff inflations, participants' superficial femoral arterial blood flow decreased in a linear way as cuff inflation pressure increased (Figure 4.4.2). This decrease was statistically significant (Repeated measures ANOVA; $F(1,6) = 55.742$, $p < 0.001$). Three participants came within 20% of total arterial occlusion at 130mmHg thigh-cuff pressure, with no participant becoming fully occluded.

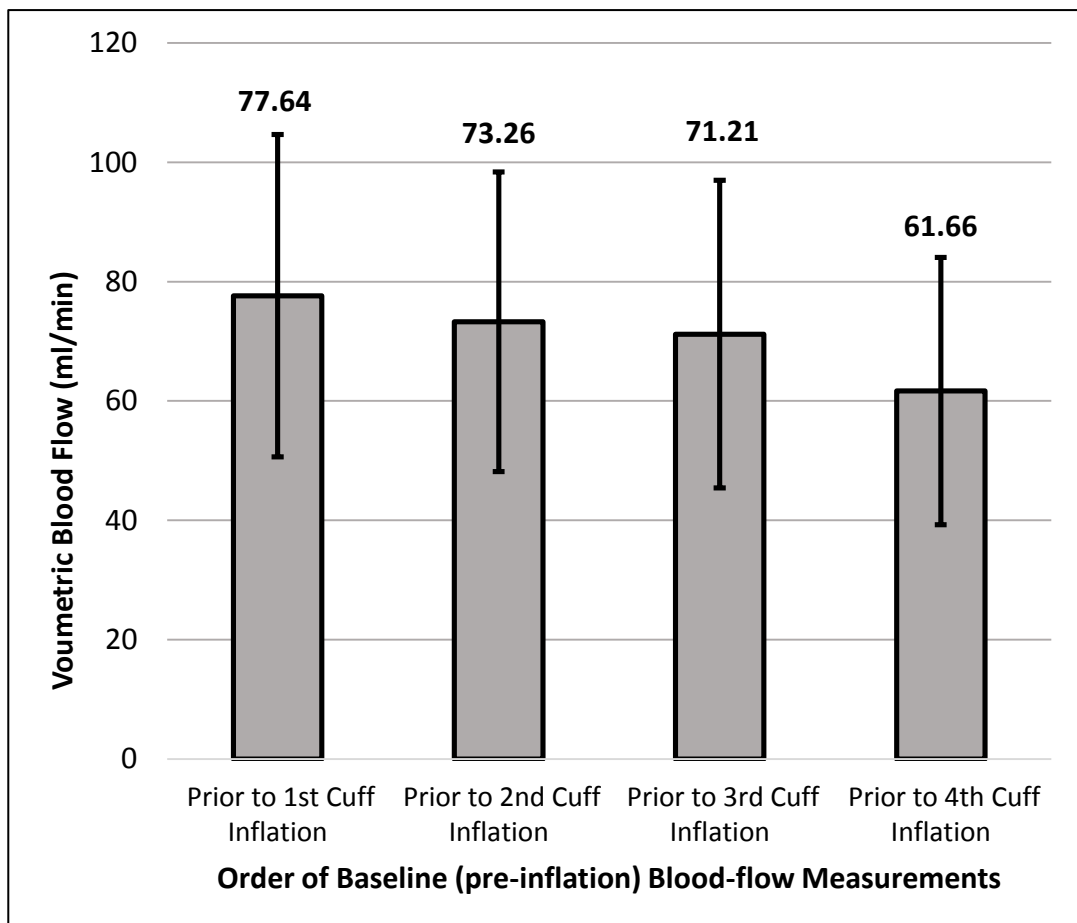


Figure 4.4.1. A bar chart displaying the baseline blood flow readings taken over the course of the Phase I pilot protocol, before each cuff inflation. Mean values are displayed. Whiskers represent standard deviations. No statistically significant difference existed between the cohort mean values recorded for each cuff inflation (Repeated Measures ANOVA; $p = 0.411$).

Measurements of heart rate, arterial cross-sectional area and subjective pain scores were normally distributed at baseline and during all cuff inflations (Shapiro-Wilkes $p \geq 0.184$), remaining similar across all tested cuff pressures (Table 4.4.2). Subjective discomfort scores were only distributed normally at 100mmHg of cuff pressure, and had left-skewed distributions at other thigh-cuff pressures. Subject discomfort scores increased as the cuff pressure applied increased, with reported values ranging between 0 and 40 at 130mmHg cuff.

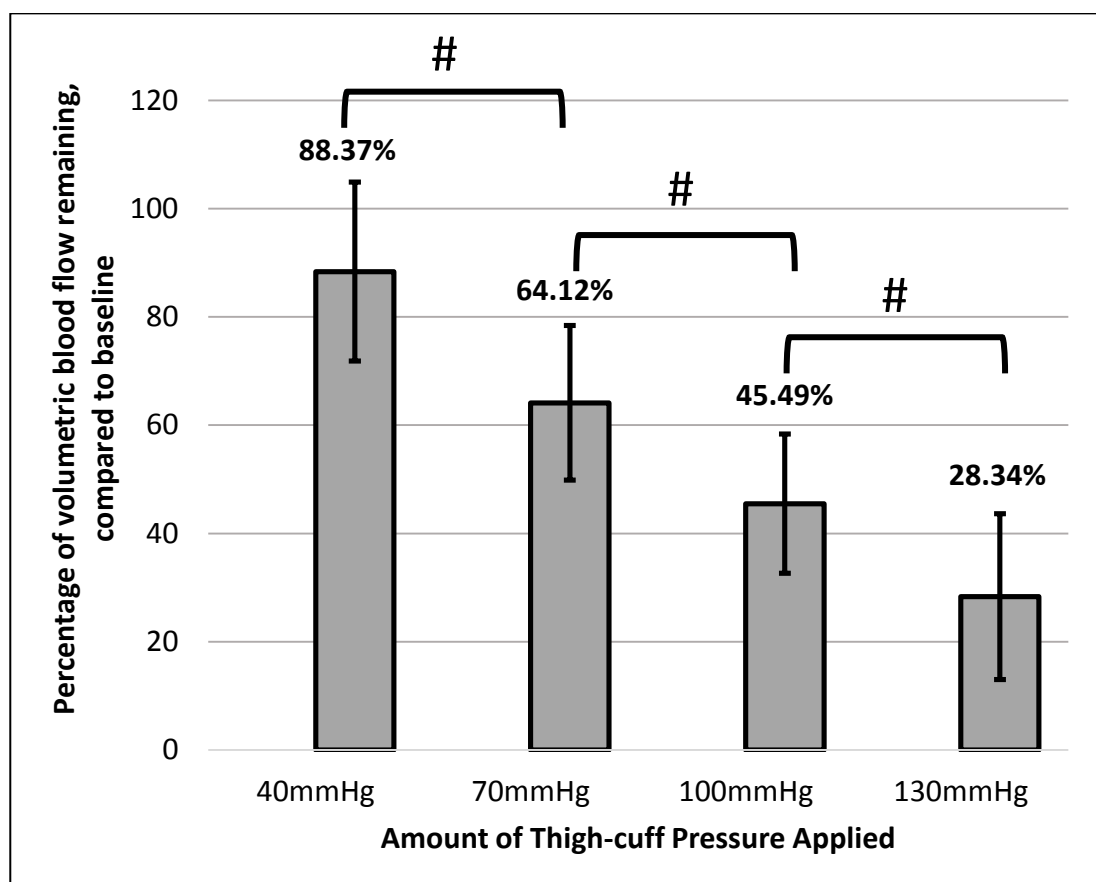


Figure 4.4.2. A bar chart displaying the percentage of superficial femoral artery blood flow remaining at different thigh-cuff pressures during the Phase I pilot study. Mean values are reported. Whiskers represent standard deviations. Statistical differences between adjacent thigh cuff inflation pressures are denoted by the '#' symbol ($p < 0.05$).

Table 4.4.2. Cohort values for heart rate, arterial cross-sectional area and perceptual responses during the Phase I pilot study. Mean values are reported. Standard deviations are shown in brackets.

Thigh-cuff Pressure Applied	Heart Rate [beats per minute]	Arterial Cross-sectional Area [mm ²]	Subjective Pain Score [0-101]	Subjective Discomfort Score [0-101]
0 mmHg*	60.8 (7.6)	35.7 (5.3)	N/A	N/A
40 mmHg	61.4 (6.7)	35.6 (5.7)	0 (0.0)	4.1 (7.5)
70 mmHg	60.7 (7.1)	35.3 (5.6)	0 (0.0)	5.8 (10.0)
100 mmHg	59.8 (7.7)	34.6 (5.2)	0 (0.0)	17.8 (12.6)
130 mmHg	57.5 (7.9)	35.1 (5.4)	1.4 (3.8)	22.4 (15.6)

*The 0mmHg values shown are a mean of the four pre-inflation measurements taken across the pilot protocol.

4.4.4 Discussion

This pilot study looked to assess the suitability of a BfR testing protocol for use in the main Phase II study. The application of thigh-cuff pressures from 40mmHg to 130mmHg did produce increasing degrees of lower-limb BfR within the cohort. Whilst no participant became fully occluded, several were close to arterial occlusion at 130mmHg of cuff pressure. BfRT is not typically performed under full arterial occlusion (Pope et al, 2013). Therefore, occurrence of full occlusion would be of minimal practical use and warranted a reduction in the maximum cuff pressure applied in the main study.

In respect of participants' perceptual responses to thigh-cuff inflations, no participant reached the cut-off values for subjective pain (≥ 40 out of 101) or discomfort (≥ 80 out of 101) at any thigh-cuff pressure. The relative absence of subjective pain across all cuff pressures, the presence of only low levels of subjective discomfort at cuff pressures up to 130mmHg and the absence of adverse or unexpected events were evidence that the testing protocol was tolerable and of minimal risk to participants' wellbeing.

There were difficulties encountered in obtaining Doppler measurements from the

superficial femoral artery during this pilot study. In one participant, the artery could not be located outright in the seated testing position. This participant was petite, which resulted in the thigh cuff largely obscuring the measurement site and exclusion from study results. In another participant, the change in depth and angle of the artery caused by tissue compression during cuff inflations prevented any viable blood-flow measurements from being recorded. When obtaining baseline (pre-inflation) blood flow values, the depth of the artery regularly caused the arterial walls to be poorly defined and the Doppler spectral signal to become faint using the ultrasound equipment available. This may have contributed to the 28% variance in within-subject, baseline blood flow values between cuff inflations. Baseline arterial blood flow values were lower than those reported in existing literature for the superficial femoral artery (Holland et al, 1998; Hussain 1997), however these studies appeared to measure flow in either supine or prone positions, as opposed to a seated position. It is possible that the upright resting position used in the pilot study produced a slowing of venous return from the lower-leg and a relatively lower overall blood-flow through the lower limb.

4.4.5 Conclusions and Protocol Adjustments

In conclusion, all three objectives of the pilot study were met. It was confirmed that each thigh-cuff pressure selected for use within the planned Phase I testing protocol produced a sub-occlusive level of BfR in all participants. It was also confirmed that no participant reached the agreed cut-off values for subjective levels of pain and discomfort during thigh cuff inflations. Doppler measurement at the distal portion of the superficial femoral artery was not possible at all in two of the nine pilot participants, with sporadic difficulties in other participants. In advance of the main study, the following adjustments to the testing protocol were made;

1. The imaging location for blood-flow measurements was switched from the distal superficial femoral artery to the proximal portion of the popliteal artery. Limited testing following the pilot study showed that, in contrast to the superficial femoral artery, the imaging depth and angle of the popliteal artery remained unchanged before and during cuff inflations. The author reasoned that moving the measurement site to the popliteal artery would resolve any confounding of blood-flow measurements caused by changes in artery depth or angle. This could increase the consistency and validity of baseline blood-flow measurements, thus reducing their coefficient of variation. In addition, whereas the superficial femoral measurement site could be obscured by the cuff within a participant with a shorter thigh length, this issue would not arise with a popliteal measurement site.
2. At 130mmHg of thigh-cuff pressure, the smallest pilot participant [weight; 71.0kg, thigh circumference; 55cm] came close to full cessation of blood flow in the superficial femoral artery (%BfR; 16%). If physical characteristics were related to the degree of BfR experienced at a given thigh-cuff pressure, then there may have been potential for physically smaller participants to experience full arterial occlusion at 130mmHg in the main study. A state of full occlusion would not have been unsafe, but may have provided minimal scientific value within the collected results (the actual pressure at which occlusion occurred in the participant would have been unknown, occurring somewhere between 101mmHg and 130mmHg). Therefore, the primary researcher reasoned that reducing the maximum pressure to 120mmHg may decrease the potential of full occlusion occurring, and increase the likelihood of achieving useful sub-occlusive %BfR values across all tested cuff pressures for most, if not all, participants.

3. The increment between each tested thigh-cuff pressure was reduced from 30mmHg to 20mmHg and a fifth cuff pressure was added, producing thigh-cuff pressures of 40, 60, 80, 100 and 120mmHg. It was proposed this would increase the resolution of obtained data allow more robust detection of non-linear patterns in the degree of BfR occurring over the range of cuff pressures tested in each participant. This would also reduce the width of the gap for which blood-flow measurements were missing if blood-flow measurements at one cuff pressure could not be adequately acquired (a gap of 40mmHg would occur as opposed to a gap of 60mmHg).
4. Immediately following each cuff inflation, the participants in the main study would be requested to isotonicly contract their triceps surae, by way of repeated plantar flexion, for at least ten seconds. This was intended to limit the rate at which resting popliteal blood flow decreased over the course of the testing protocol, limiting any degree of confounding within captured data.

4.5 Phase I Main Study

4.5.1 Main Study Objectives

1. To observe and examine the acute haemodynamic responses to lower-limb BfR within a cohort of individuals who differ in height, weight, gender and limb dimensions.
2. To determine whether correlations exist between the physical characteristics of individuals and the degree of lower-limb blood-flow restriction experienced during preselected thigh-cuff pressures.

3. To build a statistical model that predicts the percentage of lower-limb BfR that would occur at a given thigh-cuff pressure, incorporating physical characteristics into the model where appropriate.

4.5.2 Hypotheses

H0 – There will be no significant bivariate correlation between the degree of lower-limb BfR occurring and any measured physical characteristic during the application of different thigh-cuff inflation pressures.

H1 – There will be a significant bivariate correlation between the degree of lower-limb BfR occurring and at least one measured physical characteristic during the application of different thigh-cuff inflation pressures.

4.5.3 Methods

Healthy males and female volunteers aged 18 to 40 were recruited as described in chapter 3.2. Upon arrival at the testing location, a full-body DXA scan was performed and anthropometric measurements of height, weight, supine brachial blood pressure and leg dimensions were taken as described in chapter 3.3.1. In total, eighteen different physical characteristics were derived from these measurements, listed in Appendix III. After the thigh cuff was fitted to one lower-limb, participants adopted the seated test position and the primary researcher applied five different inflation pressures [40/60/80/100/120mmHg]. Each pressure was separated by a three-minute rest period. The leg chosen for testing and the order in which cuff pressures was delivered were randomised via the use of Microsoft Excel macros. The collection of popliteal artery blood-flow measurements and perceptual responses proceeded as described in chapter 3.3.2.

5.5.4 Analysis

Differences between cohort blood flow, HR, PVel, PI, RI and %PBfR during cuff inflation pressures were examined using descriptive statistics and parametric/non-parametric tests. Associations between physical characteristics and %PBfR experienced at different cuff inflation pressures were assessed in three ways;

- 1) Participants were allocated into one of three subgroups; rugby-males, non-rugby males and non-rugby females. A two-way repeated measures ANOVA was used to investigate the effect of a within-subjects factor (the amount of cuff pressure applied) and a between-subjects factor (cohort subgroup) upon %PBfR. These main effects and the two-way interaction effect of inflation pressure x subgroup were reviewed for statistical significance ($p \leq 0.05$).
- 2) For each participant, the %PBfR values recorded for across their five cuff pressures were averaged to produce a participant-mean %PBfR. Where data was normally distributed (Shapiro-Wilkes ≥ 0.05), bivariate correlations between physical characteristics and participant-mean %PBfR were assessed using Pearson's correlation coefficient. If normality was not present, bivariate correlations were assessed via the Spearman's Rank test.
- 3) The %PBfR value recorded for a participant at each cuff inflation pressure was plotted graphically and fitted with a second-order polynomial trend line (Hunt et al, 2016). The resulting polynomial equation was used to calculate the amount of cuff pressure required to theoretically reduce their popliteal arterial blood-flow to 60% of baseline [60% PBfR]. This process was repeated across the cohort. Bivariate correlational analysis was then performed to determine whether 60% PBfR values were associated with participants' physical characteristics.

All statistical analysis was performed using IBM SPSS Statistics for Windows (Version 23.0. Armonk, New York: IBM Corp.) Statistical significance was set at $p \leq 0.05$ and was two-tailed during the analysis of bivariate correlations. An a priori sample-size calculation suggested that a cohort consisting of 72 participants would allow detection of a bivariate correlation with an r value of 0.50, with a 95% confidence interval of 0.304 – 0.655 (Vassarstats, 2017).

The intra-rater reliability of the primary researcher in calculating %PBfR values was also determined. Following the analysis of all participants, seven were selected at random (~10% sample) in a way that blinded the researcher to the identity of the participants, their previous %PBfR values and the order in which cuff pressures were applied during their testing protocol. Ultrasound images for the 10% sample were re-analysed in this blinded state, then compared to %PBfR values recorded during the first analysis. Intra-rater reliability was high, displaying an intra-class correlation coefficient of 0.976 (95% CI 0.954-0.988, $p < 0.001$). It could be proposed that significant confounding of %PBfR due to intra-rater reliability issues, or a lack of blinding during primary analysis of ultrasound images, was unlikely.

4.5.5 Results

4.5.5.1 Participant Anthropometrics

Sixty-one volunteers participated in the study (Table 4.5.1). The three participant subgroups were statistically different across seventeen of the eighteen recorded physical characteristics (one-way ANOVA, $p \leq 0.05$); the exception being total body fat in grams taken from the DXA scan ($p = 0.561$). Post hoc testing, with Games-Howell correction

where required, demonstrated that nine of the eighteen physical characteristics were significantly different across all subgroup combinations ($p \leq 0.05$) (Appendix III).

Table 4.5.1. Phase I cohort and subgroup physical characteristics.
Mean values are displayed. Standard deviations are given within brackets.

SUBGROUP	N=	Age [years]	Height [cm]	Weight [kg]	Body Mass Index
Rugby Males	21	23.9 (3.8)	185.6 (5.6)	96.8 (8.3)	28.1 (1.7)
Non-rugby Males	21	27.7 (5.4)	178.9 (7.5)	82.4 (11.8)	25.7 (3.2)
Non-rugby Females	19	26.6 (5.6)	168.1 (8.6)	63.5 (6.4)	22.5 (1.8)
Total	61	26.0 (5.2)	177.8 (10.1)	81.5 (16.3)	25.5 (3.2)

No serious adverse events occurred during the testing protocol. One participant experienced mild left arm discomfort during their last cuff inflation, which the participant stated was historical musculoskeletal pain not disclosed upon the health screening questionnaire. After subsequent medical review by the participant's GP, the pain was considered to be muscular/postural in origin and unrelated to the testing protocol. A valid Doppler signal could not be acquired during 100mmHg cuff inflations in two participants. In three participants (two at 40mmHg, one at 80mmHg), the resting or restricted blood flow values recorded were anomalous and excluded.

Ultimately, data from ninety-eight percent of cuff inflations (299 out of a possible 305) were included within analysis. The six missing inflations were spread equally across six participants. To prevent these participants' data from being excluded outright during statistical analysis, the six missing %PBfR values were replaced with substitute values. This was achieved by first fitting a second-order polynomial trend line through each

participant’s remaining %PBfR values. The cuff pressure at which the %PBfR value was missing was then input into the polynomial equation to produce a suggested %PBfR value. This value was then entered into the dataset. Therefore, the results for ‘%PBfR’ in chapter 4.5.5 and the results for ‘Repeated Measures ANOVA and ‘Session-mean %PBfR’ in chapter 4.5.5.3 have been presented using a complete data set, with missing values populated.

4.5.5.2 Perceptual Responses to Thigh-Cuff Inflations

The range, means and standard deviations of cohort NRS values provided across each thigh-cuff pressure are shown for discomfort and pain in Table 4.5.2 and Table 4.5.3 respectively. One participant reported pain (NRS = 55 out of 101) and discomfort (NRS = 84) at 100mmHg which exceeded the pre-agreed cut-off values for these measures, therefore the testing protocol was halted and 120mmHg was not attempted by the primary researcher. The participant displayed no visual signs of pain or discomfort during the cuff inflation in question and experienced no other physical symptoms. One participant experienced pain at 120mmHg of cuff pressure (NRS = 49), but wished to continue with the remainder of the testing protocol, giving consent to do so.

Table 4.5.2 Participant-reported values for discomfort during thigh-cuff inflations (NRS Scale 0 to 101)

Cuff Pressure Applied	Cohort Median	Cohort Range	Minimum Value	Maximum Value	Cohort Mean	Std. Dev.
40mmHg	0.0	30	0	30	2.9	7.2
60mmHg	0.0	40	0	40	6.2	9.9
80mmHg	9.5	60	0	60	10.1	13.0
100mmHg	10.0	84	0	84*	16.3	18.7
120mmHg	20.0	74	0	74	21.3	19.5

**This participant reached the pre-agreed cut-off value for discomfort and did not attempt 120mmHg cuff pressure.*

Table 4.5.3 Participant-reported values for pain during thigh-cuff inflations (NRS Scale 0 to 101)

Cuff Pressure Applied	Cohort Median	Cohort Range	Minimum Value	Maximum Value	Cohort Mean	Std. Dev.
40mmHg	0.0	3	0	3	0.1	0.6
60mmHg	0.0	20	0	20	0.5	2.7
80mmHg	0.0	20	0	20	0.8	3.1
100mmHg	0.0	55	0	55*	2.2	9.1
120mmHg	0.0	49	0	49**	1.9	7.7

*One participant reached the pre-agreed cut-off value for pain and did not attempt 120mmHg cuff pressure.

**One participant reached the pre-agreed cut-off value for pain but consented to continue with the remainder of the protocol.

4.5.5.3 Haemodynamic Measurements

Popliteal Arterial Blood Flow

Blood flow values taken prior to each cuff inflation were similar across the testing protocol, Friedman Test, $\chi^2(4) = 6.444$, $p = 0.168$. Table 4.5.4.

Table 4.5.4. Median popliteal artery blood flow values [in mL/min] of the Phase I cohort (n=61) prior to each thigh-cuff inflation pressure. The range for each median value are also displayed.

	Prior to 1st Cuff Inflation	Prior to 2nd Cuff Inflation	Prior to 3rd Cuff Inflation	Prior to 4th Cuff Inflation	Prior to 5th Cuff Inflation
Median	56.4	52.4	53.3	54.7	49.9
Minimum Value	24.2	23.6	22.9	22.4	20.2
Maximum Value	213.3	171.8	167.7	198.4	241.3
Range	189.1	148.1	144.7	175.9	221.0

Cohort blood flow values recorded during each cuff inflation pressure were typically of non-normal (right-skewed) distributions (Shapiro-Wilks, $p \leq 0.05$). Cohort blood flow reduced significantly as cuff inflation pressure was increased, Friedman test, $F(5, 55) = 233.639$, $p < 0.0001$. Table 4.5.5.

Table 4.5.5. Median popliteal artery blood flow values of the Phase I cohort [in mL/min] during each thigh-cuff inflation pressure.
The range for each median value are also displayed.

	0 mmHg	40 mmHg	60 mmHg	80 mmHg	100 mmHg	120 mmHg
Median	53.8	42.3	38.4	29.5	23.7	18.1
Minimum Value	22.9	16.6	15.6	14.0	10.1	7.1
Maximum Value	170.9	163.3	125.8	81.7	66.0	54.7
Range	148.0	146.7	110.2	67.8	56.0	47.6

Popliteal arterial blood flow at rest was positively correlated with participant height (Spearman Rank; 0.41, $p = 0.001$), weight (Spearman Rank; 0.48, $p < 0.001$) and body mass index (Spearman Rank; 0.418, $p = 0.001$).

The Percentage of Popliteal Arterial Blood-flow Remaining [%PBfR]

The percentage of blood-flow that remained at each cuff pressure, compared to baseline, is displayed in Table 4.5.6. %PBfR values were statistically significantly different across inflation pressures (Friedman Test, $\chi^2(4) = 193.875$, $p < 0.001$), decreasing as the inflation pressure increased.

Table 4.5.6. Mean and median %PBfR values of the Phase I cohort during each thigh-cuff inflation pressure.

Thigh-cuff Inflation Pressure	%PBfR (Mean)	95% Confidence Interval		% PBfR (Median)	Range
		Lower Bound	Upper Bound		
40mmHg	81.6	77.8	85.5	81.7	79.5
60mmHg	68.2	64.2	72.1	67.9	74.1
80mmHg	56.9	53.6	60.2	58.2	63.3
100mmHg	45.6	42.1	49.1	42.0	58.9
120mmHg	33.3	30.6	36.1	31.9	54.3

Heart Rate [HR]

Cohort HR values were normally distributed at each cuff inflation pressure (Shapiro-Wilkes, $p \geq 0.64$). Mauchly's test of sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(14) = 3.343$, $p = 0.159$. A repeated measures ANOVA showed cohort HR was statistically different across inflation pressures, $F(5, 270) = 7.282$, $p < 0.0001$, partial $\eta^2 = 0.119$, displaying a tendency to decrease slightly as inflation pressure increased (Table 4.5.7).

Table 4.5.7. Mean heart rate values (in beats per minute) of the Phase I cohort recorded during each thigh-cuff pressure.

Thigh-cuff Inflation Pressure	Mean	95% Confidence Interval	
		Lower Bound	Upper Bound
0mmHg	67.9	65.2	70.5
40mmHg	66.8	63.7	69.9
60mmHg	66.1	63.2	69.1
80mmHg	65.2	62.4	68.1
100mmHg	65.5	62.6	68.3
120mmHg	65.3	62.4	68.3

Pulsatility Index [PI]

Cohort PI values were typically of non-normal (right-skewed) distribution at each cuff inflation pressure (Shapiro-Wilks, $p \leq 0.05$). A non-parametric Friedman test indicated that cohort PI was statistically different across cuff inflation pressures $\chi^2(5) = 171.800$, $p < 0.001$. Cohort PI tended to increase as inflation pressure increased, up to 100mmHg (Table 4.5.8). Cohort PI decreased at 120mmHg compared to 100mmHg, but post-hoc comparisons indicated that this difference was not statistically significant ($p = 0.359$).

Resistive Index [RI]

Cohort RI values were of a non-normal distribution at two inflation pressures (Shapiro-

Wilks, $p \leq 0.05$). A non-parametric Friedman test indicated that cohort RI was statistically different across inflation pressures $\chi^2(5) = 66.694$, $p < 0.001$. Cohort RI tended to increase as inflation pressure increased up to 80mmHg. Cohort RI decreased slightly at 100mmHg, then remained similar at 120mmHg (Table 4.5.8).

Peak Velocity [PVel]

Cohort PVel values were typically of a non-normal (right-skewed) distribution at each cuff inflation pressure (Shapiro-Wilks, $p \leq 0.05$). A non-parametric Friedman test showed that cohort PVel was statistically different across inflation pressures $\chi^2(5) = 50.801$, $p < 0.001$. Post-hoc comparisons indicated that cohort PVel was lower at 120mmHg compared to 100mmHg to a statistically significant level ($p < 0.001$). PI values are displayed in Table 4.5.8.

Table 4.5.8. Median values for three haemodynamic variables recorded for the Phase I cohort during each thigh-cuff pressure. The range for each median value are displayed in brackets.

	0mmHg	40mmHg	60mmHg	80mmHg	100mmHg	120mmHg
Pulsatility Index	13.27 [24.99]	17.55 [39.19]	22.76 [68.6]	26.73 [108.89]	30.10 [86.82]	28.81 [74.46]
Resistive Index	1.34 [0.58]	1.39 [0.51]	1.41 [0.39]	1.43 [0.50]	1.38 [0.48]	1.40 [0.50]
Peak Velocity	31.78 [38.71]	32.17 [37.77]	33.30 [40.07]	34.17 [41.40]	33.77 [47.27]	26.97 [35.93]

4.5.5.4 Correlations between Physical Characteristics and %PBfR

Repeated Measures ANOVA

The %PBfR for each thigh-cuff inflation pressure within each subgroup were assessed for differences using a two-way repeated measures ANOVA. There were no extreme outliers as assessed by examination of studentised residuals for values greater than ± 3 .

Studentised residuals were normally distributed, as assessed by Q-Q plots. %PBfR values

typically followed a normal distribution across each inflation pressure within each subgroup, (Shapiro-Wilkes, $p > 0.05$). Exceptions were non-rugby males at 100mmHg cuff pressure ($p = 0.014$) and rugby males at 120mmHg ($p = 0.039$). A two-way repeated measures ANOVA, with or without the three participants creating the non-normal distributions, produced negligible differences in generated F, P and partial η^2 values. As there was no suggestion that %PBfR values from these three participants were erroneous, %PBfR results are presented with all participants included.

There was homogeneity of variance in %PBfR values across cuff inflation pressures, as assessed by Levene's test ($p \geq 0.170$). There was homogeneity of covariance, as assessed by Box's M test of equality of covariance matrices ($p > 0.001$). Mauchly's Test indicated that the assumption of sphericity was violated for the two-way interaction of inflation pressure x subgroup, $\chi^2(2) = 40.653$, $p < 0.0001$. Greenhouse-Geisser correction was used to accommodate for this violation.

There was no statistically significant two-way interaction of cuff inflation pressure x subgroup upon %PBfR, $F(6.044, 175.288) = 1.649$, $p = 0.136$, partial $\eta^2 = 0.041$. The main effect of cuff inflation pressure showed a statistically significant difference in %PBfR across inflation pressures, $F(3.022, 175.288) = 215.243$, $p < 0.0001$, partial $\eta^2 = 0.788$) with higher cuff pressure producing a lower %PBfR. The main effect of cohort subgroup showed no statistically significant differences in %PBfR across subgroups, $F(2, 58) = 2.186$, $p = 0.122$, partial $\eta^2 = 0.070$. A boxplot of median %PBfR scores and their distributions across cohort subgroups is displayed in Figure 4.5.1

The Effect of Thigh Cuff Inflation Pressures upon Popliteal Arterial Blood Flow within Different Cohort Subgroups

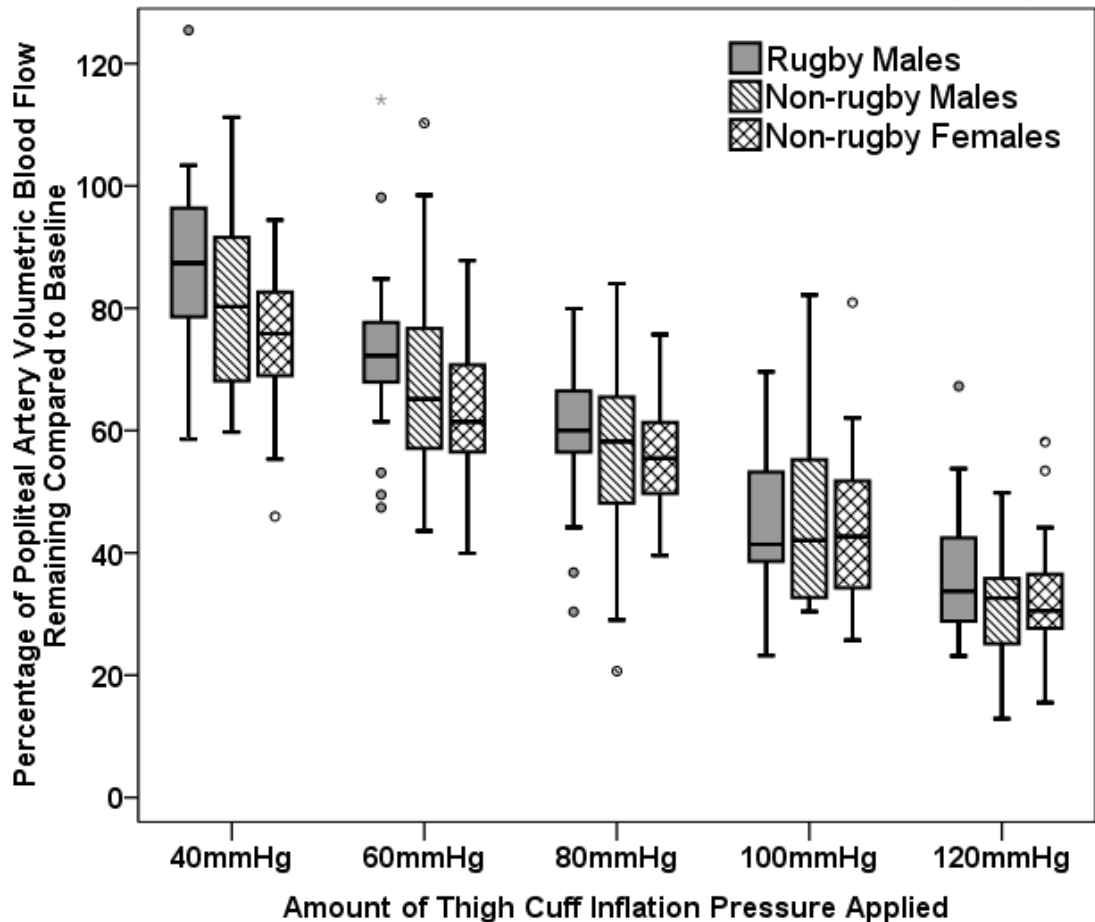


Figure 4.5.1 A box plot displaying median value and distribution of %PBfR within each subgroup at each of the five cuff inflation pressures. No statistically significant between-subgroup difference was found in %PBfR at each thigh cuff inflation pressure ($p > 0.05$).

Participant-mean %PBfR

Participant-mean %PBfR values (the average %PBfR recorded from the five cuff different inflation pressures) were normally distributed for the cohort (Shapiro-Wilks, $p = 0.617$).

For the cohort as a whole, eleven physical characteristics had normally-distributed values (Shapiro-Wilks ≥ 0.05) and seven did not (Shapiro-Wilks ≤ 0.05). Pearson correlation coefficients or Spearman Rank tests were used respectively to determine bivariate correlations between each cohort physical characteristic and participant-mean %PBfR.

Two-tailed significance of bivariate correlations are subsequently displayed in Table 4.5.7.

60% PBfR

One participant was excluded from analysis as a 60% PBfR value could not be extrapolated from their polynomial second-order equation. 60% PBfR values were normally distributed for the remainder of the cohort (Shapiro-Wilks $p = 0.723$). The cohort required a mean cuff pressure of 75.3 (± 18.2) mmHg to induce 60% %PBfR. The cohort median value was similar (76.0 mmHg). Analysis indicated no statistically significant bivariate correlation between any participant physical characteristic and their 60% PBfR value (Pearson's correlation coefficient or Spearman's Rank, $p > 0.05$). Due to these lack of correlations, it was not appropriate to attempt a regression model to explain any inter-subject variance in 60% PBfR values via physical characteristics. However, a second-order polynomial equation could be derived for the cohort, to represent the actual %PBfR that the cohort experienced across all tested cuff inflation pressures;

$$y = -0.0007x^2 - 0.4797x + 100$$

Where y is the percentage of popliteal blood flow remaining compared to baseline, and x is the amount of thigh-cuff inflation pressure applied in mmHg. Figure 4.5.2 displays the trend line relating to this equation.

Table 4.5.7. Bivariate correlations between participant-mean %PBfR and physical characteristics of the Phase I cohort. Bold values indicate statistical significance ($p \leq 0.05$)

Physical Characteristic	Method of Correlational Analysis	r Value	95% Confidence Interval	p Value
Age	Spearman Rank	0.072	-0.183 – 0.318	0.580
Height (cm)	Pearson	0.257	0.006 – 0.477	0.046
Body Weight (kg)	Spearman Rank	0.326	0.081 – 0.533	0.100
Body Mass Index	Pearson	0.226	-0.027 – 0.452	0.080
Thigh Circumference (cm)	Pearson	0.296	0.048 – 0.509	0.021
Leg Length (cm)	Pearson	0.245	-0.007 – 0.467	0.057
Thigh Length (cm)	Pearson	0.255	0.004 – 0.476	0.047
Systolic Blood Pressure (mmHg)	Pearson	0.267	0.017 – 0.486	0.038
Diastolic Blood Pressure (mmHg)	Pearson	0.123	-0.132 – 0.363	0.344
Mean Arterial Pressure (mmHg)	Pearson	0.203	-0.051 – 0.432	0.117
DXA Body Tissue Fat %	Spearman Rank	-0.107	-0.349 – 0.148	0.414
DXA Body Fat Mass (g)	Spearman Rank	0.075	-0.180 – 0.320	0.567
DXA Body Lean Mass (g)	Pearson	0.274	0.024 – 0.491	0.033
DXA Total Body Mass (g)	Spearman Rank	0.335	0.091 – 0.541	0.008
DXA Test Leg Tissue Fat %	Spearman Rank	-0.155	-0.391 – 0.100	0.233
DXA Test Leg Fat Mass (g)	Spearman Rank	-0.054	-0.301 – 0.200	0.680
DXA Test Leg Lean Mass (g)	Pearson	0.326	0.081 – 0.533	0.010
DXA Test Leg Total Mass (g)	Pearson	0.342	0.099 – 0.546	0.007

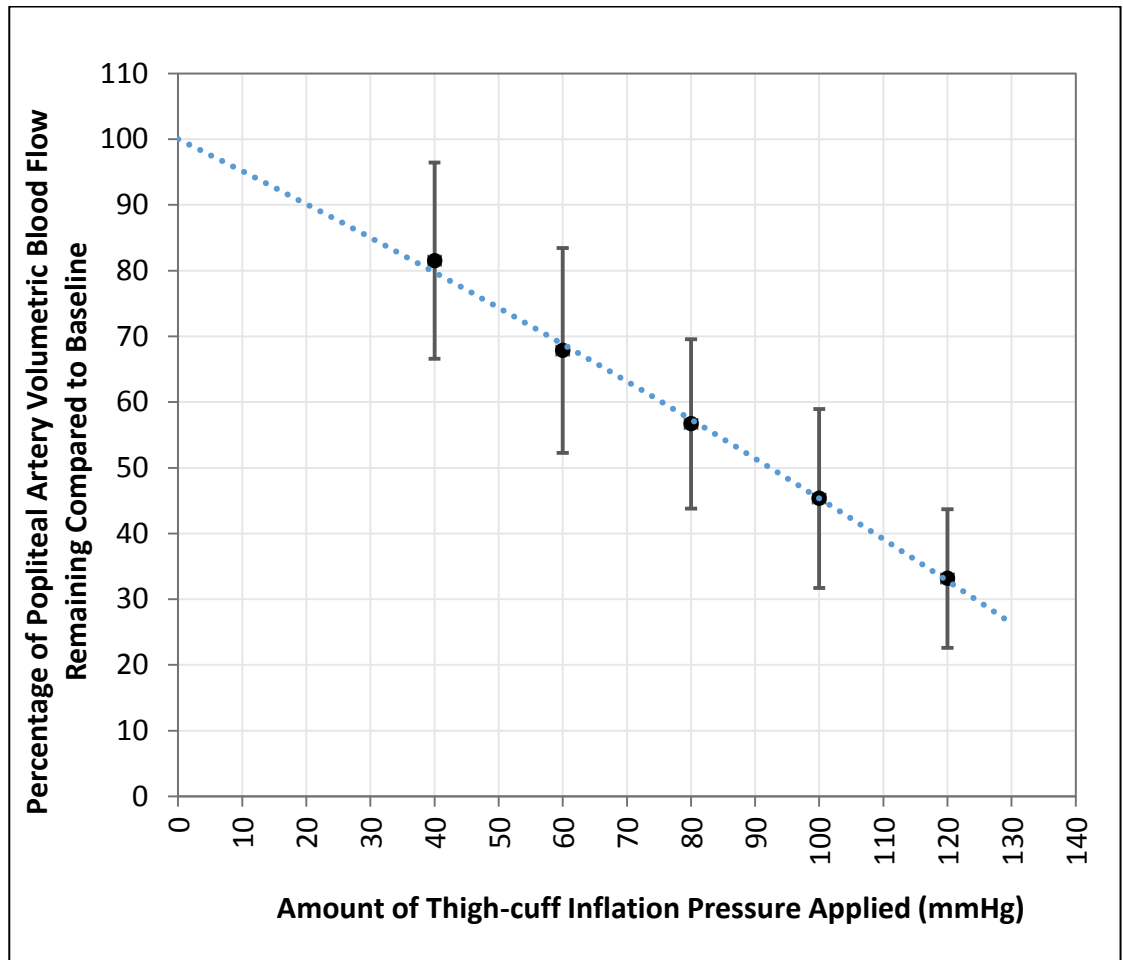


Figure 4.5.2. Phase I cohort %PBfR values fitted to a second-order polynomial trend line. Mean values are used for cohort %PBfR, whiskers represent standard deviations.

4.5.6 Discussion

4.5.6.1 Reliability and Validity of Ultrasound Measurements

The validity of popliteal haemodynamic ultrasound measurements recorded during this study can be endorsed by comparing them to those reported within a reference population (n=40) examined by Holland et al (1998). As seen in Table 4.5.8, mean values recorded from the popliteal arteries of the two cohorts were similar. The order in which cuff pressures were applied was randomised to spread any confounding effect that variance in pre-inflation blood flows had upon results. However, popliteal blood-flow prior to each of the five cuff inflation pressures remained statistically similar across the

testing protocol (Table 4.5.2), in contrast to what occurred during the pilot study, suggesting that any confounding effect due to this factor was minimised.

Table 4.5.8. Inter-study comparisons of reported haemodynamic values within the human popliteal artery at rest. VMn = Mean blood-flow velocity, aCSA = Artery cross-sectional area, BF = Blood flow

	Current Study		Holland et al (1998)	
	Mean	Std. Dev.	Mean	Std. Dev.
Pulsatility Index	13.78	5.46	17.69	13.76
Resistive Index	1.34	0.10	1.41	0.11
VMn (cm/s)	3.38	2.10	4.90	2.20
aCSA (cm²)	0.35	0.09	0.29	0.12
BF (ml/min)	67.62	40.24	72.00	34.00

4.5.6.2 The Acute Haemodynamic Responses to Lower-limb BfR

Whilst blood-flow through the popliteal artery decreased in an almost linear fashion as cuff inflation pressure increased (Figure 4.5.2), the Resistive Index [RI], Pulsatility Index [PI] and peak blood-flow velocity [PVel] values demonstrated different patterns of change (Figure 4.5.3). At 0mmHg cuff pressure, PWD spectral images showed normal (healthy) tri-phasic waveform patterns of peripheral blood flow within their lower limbs (Figure 4.5.3) (Crişan, 2012; Wood et al, 2010). As cuff pressures of up to 100mmHg were added, PVel values did not alter significantly. However, PVel then decreased at 120mmHg cuff pressure compared to 100mmHg.

The stable PVel values up to and including 100mmHg could be explained by considering both the structure of the femoral artery and the intravascular pressure of the blood flowing through it. The presence of elastin fibres within the tunica media of an artery provide structural support (Pugsley and Tabrizchi, 2000) which may allow it to resist deformation at low degrees of extravascular pressure. Whilst intravascular arterial

pressure can vary depending upon blood flow, vascular tone and pulse pressure, normal mean arterial pressure [MAP] in adults is typically between 70mmHg and 100mmHg (Roberts and Hedges, 2009). Within the study cohort, MAP was 88.9 (\pm 7.3) mmHg. It could be suggested, therefore, that up to 100mmHg of extravascular pressure exerted upon the femoral artery by the thigh cuff was not sufficient to universally overcome intravascular MAP in this cohort and deform the shape of the femoral artery. Arterial cross-sectional area [CSA] and pulsatile flow through the femoral artery would have been largely preserved, which in turn maintained peak blood-flow velocity in the downstream popliteal artery. In contrast, 120mmHg was sufficient to overcome MAP in all of the cohort. This may have been sufficient to deform the femoral artery, reduce its CSA and reduce downstream popliteal PVel. It is acknowledged that this explanation depends upon the assumption that upper limb (brachial) blood pressures were similar to lower-limb blood pressures within each study participant, as the latter were not recorded in this study.

In contrast, peripheral veins are susceptible to low levels of extravascular compression by design due to their relatively thin tunica media (Pugsley and Tabrizchi, 2000). Ordinarily, this property combined with the presence of one-way valves within venous vasculature allows adequate venous return of blood from the peripheries of the body (Korthuis, 2011; Meissner, 2005) despite low intravascular pressure and the absence of a pulsatile venous flow. Applying cuff pressures up to 100mmHg could have gradually narrowed and then collapsed the lumen of the femoral vein under the thigh cuff, preventing venous return and causing progressive lower-limb venous congestion within the lower leg. For example, Partsch and Partsch (2005) demonstrated that 50-60mmHg of external calf compression

was sufficient to fully collapse the saphenous vein and posterior tibial vein within participants in a resting seated position.

Whilst there is an apparent lack of evidence that determines the extravascular pressure required to cause complete femoral vein occlusion in sitting, the femoral vein has a larger diameter than veins found more distally (Hertzberg et al 1997; Asbeutah et al, 2014). It could be therefore argued that an extravascular pressure greater than 60mmHg is required for complete occlusion, although narrowing of the lower-limb veins (in a standing position) may begin with as little as 19mmHg of external thigh compression (Mosti et al, 2009).

In the Phase I study, any venous congestion in the lower leg would have increased vascular resistance distal to the popliteal artery measurement site, presenting upon PWD spectral images as an increased magnitude of reverse diastolic flow during cardiac cycles (Figure 4.5.3). It could be this mechanism that raised RI and PI values, and lowered the mean velocity (and thus flow) of blood travelling through the popliteal artery over the time-course of a PWD spectral image.

At 120mmHg of cuff pressure, it is possible that the extravascular pressure exerted upon the segment of femoral artery under the thigh cuff started to exceed intravascular arterial pressure and was sufficient to begin narrowing the femoral artery lumen. This artificially-induced stenosis could have blunted pulsatile flow distal to the thigh cuff and would explain why peak blood-flow velocity significantly reduced at the popliteal measurement site, compared to 100mmHg (Mehra, 2010). In turn, the blunting of pulsatile flow may be responsible for the reduction in magnitude of reverse diastolic flow occurring during

diastole at 120mmHg compared to ≤ 100 mmHg (Figure 4.5.3). Combined, these changes would have modulated RI and PI and explain why RI and PI did not continue to increase beyond the values seen at 100mmHg cuff pressure.

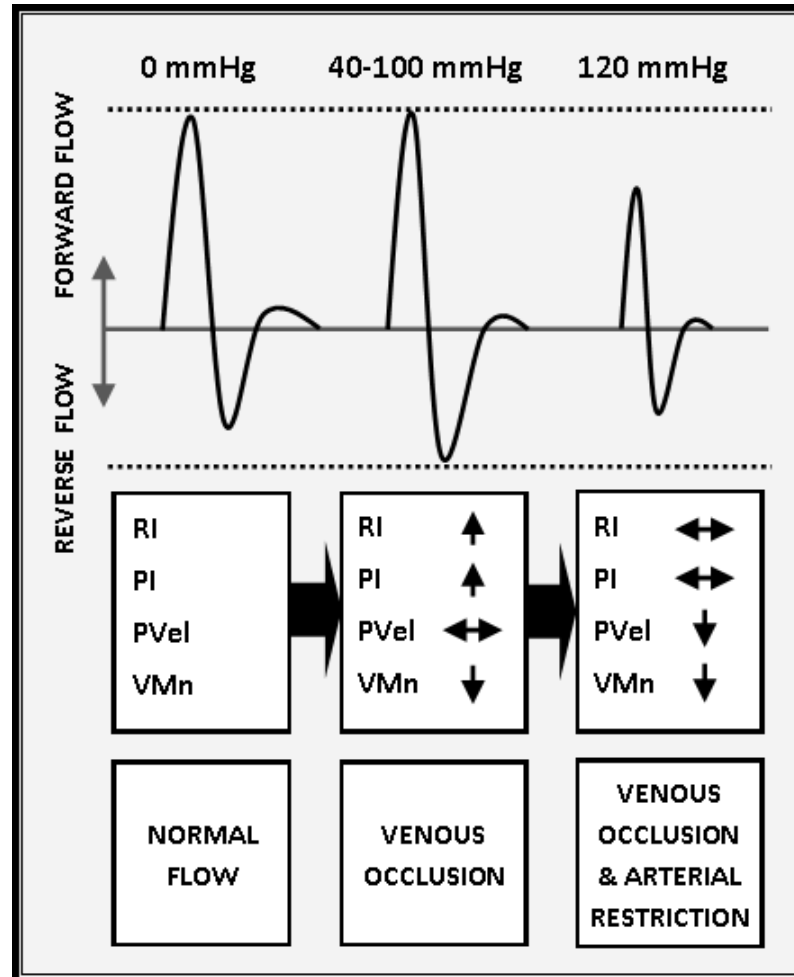


Figure 4.5.3. Acute haemodynamic responses to lower-limb blood-flow restriction, measured at the popliteal artery. Representations of the pulsed-wave Doppler spectral waveforms for one cardiac cycle are displayed below each cuff pressure heading. Note the normal tri-phasic spectral waveform of blood flow at 0mmHg, an increase in the magnitude of reverse flow and slight narrowing of the forward-flow peak at 40-100mmHg, followed by a reduction in both peak blood flow velocity [PVel] and reverse diastolic flow at 120mmHg.

In summary, the author proposes that the reduction in lower-limb mean blood flow velocity at thigh-cuff pressures up to 100mmHg is due predominantly to an increase in peripheral vascular resistance of the lower leg caused by femoral vein

restriction/occlusion. At cuff pressures greater than 100mmHg, up to 120mmHg, further reductions in mean blood-flow velocity are likely caused by partial compression of the femoral artery. Femoral occlusion and thus complete haemostasis of lower-limb blood flow would then occur at a cuff pressure somewhere above 120mmHg. It should be stated that this may not hold true for healthy individuals who are dissimilar to those subpopulations tested within this study. Likewise, individuals who are not healthy due to a disease or comorbidity may also undergo different degrees of lower-limb blood flow restriction at the cuff pressures up to 120mmHg.

The pattern by which %PBfR reduced as thigh-cuff inflation pressure increased was similar to that reported by Hunt et al (2016). A review of their graphical data suggests that 60% %PBfR occurred at approximately 92mmHg among their cohort of 47 participants. Within the Phase I study, 60% PBfR occurred within the cohort at 75.3mmHg. The findings of Crenshaw et al (1988) and Loenneke et al (2012b; 2015a) indicated that the cuff pressure required to fully occlude arterial flow decreases as cuff width increases. As Hunt et al (2016) utilised a narrower-width cuff than the Phase I study (13.5cm vs. 21cm, respectively), an explanation may be that the width-dependent relationship also holds true at sub-occlusive thigh-cuff pressures.

Within-subject heart rate demonstrated a statistically significant change over the course of the five cuff pressures, reducing as cuff inflation pressure was increased. Despite this statistical significance, the actual magnitude of change between 0mmHg and 120mmHg cuff pressure was low; 3.97% or 2.6 beats per minute. Existing BfRT literature is sparse regarding the heart rate response to lower-limb BfR applied without exercise. Karabulut et al (2011b) reported small increases in heart rate as cuff pressure was incrementally

increased. However, the sample size was small ($n=5$) and prevented the use of inferential statistics. Loenneke et al (2012c) applied five, 5-minute bouts of BfR at 70% of participant's arterial occlusion pressure ($n=9$). Results indicated that heart rate increased during cuff inflations, but not to a statistically-significant degree. The fifth cuff inflation increased heart rate by 11 beats per minute compared to baseline ($p = 0.057$). In light of study findings and previous literature, short periods of lower-limb BfR applied at rest, do not appear to adversely raise or lower heart rate within healthy individuals.

4.5.6.3 Correlations between physical characteristics and %PBfR

Participant-mean %PBfR demonstrated statistically significant bivariate correlations across eight cohort physical characteristics ($p \leq 0.05$), particularly those relating to physical mass (Table 4.5.7). Whilst this could be suggestive of physical characteristics having some influence over %PBfR during sub-occlusive thigh-cuff pressures, the generated r values for these correlations were universally low. The Phase I study was insufficiently-powered to provide reasonable 95% confidence intervals for correlations with an r value < 0.50 . Therefore, it would be inappropriate to reject the study's null hypothesis based upon these findings alone.

The amount of thigh-cuff pressure calculated to induce 60% PBfR showed variation between individuals (75.3 mmHg, ± 18 mmHg). Yet this variation was not correlated to, or explained by, the physical characteristics of the cohort. Hunt et al (2016) found greater Pearson r values when relating the cuff pressure required to attain a predetermined %BfR against physical characteristics, such as mean arterial pressure ($r = 0.58$, $p < 0.05$) and Body Mass Index ($r = 0.44$, $p < 0.05$). This may have occurred due to Hunt and colleagues investigating a greater degree of BfR (equivalent to 40% PBfR) and applying each cuff

pressure for longer periods of time than the Phase I study (120 seconds vs. 30 seconds). Hunt et al (2016) also recorded haemodynamic measurements during the last thirty seconds of each 120-second inflation period. Combined, these factors could have increased the rate and magnitude of venous congestion occurring within the lower limb, amplifying associations between %PBfR and physical characteristics in comparison to the current study. Despite these methodological differences, Hunt et al (2016) still found that physical characteristics were unable to explain the majority of between-subject variance in the thigh-cuff pressure required to achieve 40% PBfR.

4.5.7 Study Strengths and Limitations

Strengths

The use of randomisation to vary the order in which thigh-cuff inflation pressures were delivered among the cohort prevented any cumulative confounding effect that repeated bouts of extravascular pressure may have had upon the latter cuff inflations during the testing protocol. For example, if pressures had been applied in ascending order [40/60/80/100/120mmHg], repeated shear stress due to changes in blood flow and extravascular pressure may have caused a late dilatory response within arterioles, potentially confounding the %PBfR values at 100mmHg and 120mmHg. Instead, if any confounding effect did exist, this was spread equally across all tested cuff pressures. The results of intra-rater reliability testing suggest that non-blinded analysis of ultrasound images by the primary researcher should not have significantly confounded blood-flow measurements. The resting ultrasound-derived haemodynamic measurements of the popliteal artery also agreed with those of other work (Holland et al, 1998), which supports the validity of measurements attained within this study. In addition, it was possible to test physical characteristics, such as body mass, across a greater range and a

larger cohort size than the most recent study on the topic (Hunt et al, 2016).

Limitations

This study was under-powered to detect statistically significant bivariate correlations of the degrees seen within this study, without also generating very wide confidence intervals (i.e. thigh circumference; $r = 0.296$, 95% CI 0.048 – 0.509, $p = 0.047$). In particular, the lower limits of these confidence intervals were always close to nil, casting doubt upon whether meaningful correlations truly exist. This limitation could have been addressed through the recruitment of additional participants to narrow the 95% confidence intervals, to provide a more robust indicator of the validity of these correlations. However, this would have taken a much larger cohort ($n=300$) to narrow the confidence intervals by a reasonable degree (i.e. $r = 0.296$, 95% CI 0.19 – 0.395). Future research may address this issue, but the time and resources required to collect and process this amount of data within the doctoral project was beyond that of the primary researcher.

Additional factors may have contributed to a portion of the variance seen in %PBfR across the cuff pressures used in the Phase I study. There are a myriad of methodological pitfalls and spectral artefacts that can confound blood-flow readings taken via Doppler ultrasound (Nelson and Praetorius, 1988; Pozniak et al 1992). In addition, the primary researcher also observed transient variances in resting popliteal blood flow due to talking, coughing, or slight contraction of any lower-leg musculature. Whilst considerable efforts were made by the researcher to pre-empt and prevent each of these factors from confounding Doppler measurements, a small degree of error may have invariably existed within collected data. Unmeasured factors such as inter-subject variations in basal

vascular tone or vasomotor control may have also existed (Korthuis, 2011). These may have affected both the immediate response to the extravascular pressure generated by the thigh cuff and the rate of venous congestion distal to the thigh cuff among individuals to an unknown degree, independent of any measured physical characteristics.

4.5.8 Conclusions

In conclusion, the first two objectives of this Phase I main study were achieved. The acute haemodynamic responses to lower-limb BfR within a cohort of sixty-one individuals who differed in height, weight, gender and limb dimensions were observed and examined. When sub-occlusive thigh-cuff pressures were applied to healthy adults between the ages of 18 and 40 in this study, the physical characteristics of these individuals were not significantly associated with the initial degree of lower-limb arterial BfR that they experienced. In addition, the amount of thigh-cuff pressure required to achieve a specific %PBfR of 60% varied between individuals and was not associated to, or explained by, the eighteen physical characteristics measured within the study. Therefore, it was not possible to fully meet the third objective; to build a statistical model that predicts the %PBfR that would occur at the onset of a given thigh-cuff pressure using physical characteristics. However, it was possible to derive a polynomial equation that provides some indication of the percentage of arterial BfR occurring, based upon thigh-cuff pressure alone. In combination with the results presented by Hunt et al (2016), the utility of physical characteristics alone to accurately induce a predetermined %PBfR appears low. Further research should either seek alternative variables to predict lower-limb %PBfR, or investigate the utility of physical characteristics in determining %PBfR within other populations such as the elderly or comorbid.

Haemodynamic findings suggest that, when cuff pressures of up to 100mmHg are applied to a resting individual via a 21cm-wide blood-pressure cuff, blood-flow through the lower-limb appears to reduce due predominantly to venous restriction or occlusion of the femoral vein(s). At cuff pressures exceeding 100mmHg, arterial restriction of the femoral artery is likely responsible for further reducing lower-limb blood flow. Ultimately, this finding has value to clinicians wishing to utilise a thigh blood-pressure cuff to apply lower-limb BfR to populations similar to those investigated in this study. The proposed intention of BfR is to generate acute lower-limb vascular pooling, local metabolite accumulation and tissue hypoxia by retaining arterial inflow and occluding venous outflow (Loenneke et al, 2014; Pope et al, 2013). In respect of this, Phase I findings suggest that this haemodynamic state occurs in the lower-limb at pressures \leq 100mmHg when using a thigh blood-pressure cuff. Pressures as low as 40mmHg may still provide an adequate state of BfR in some individuals. However, research is required to determine whether BfR induced at these thigh-cuff pressures generates a change in the acute physiological and perceptual responses of low-intensity resistance exercise or un-resisted, or 'no-load' exercise. The next chapter details Phase II of the doctoral research project, which looks to address this point specifically.

CHAPTER FIVE

PHASE TWO

The Acute Physiological and Perceptual Effects of Adding Lower-limb BfRT to a Seated, Unweighted Knee Extension Exercise

5.1 Chapter Introduction

This chapter details of the second phase of this doctoral research project. A summary of existing academic evidence regarding the potential potency of low-load resistance exercise to produce favourable muscular adaptations is first given, with discussion as to why un-resisted ('no-load') exercise alone may struggle to maintain muscular size and performance within a rehabilitation context. Specific focus is given to the acute effects of muscle contraction upon local haemodynamics, providing rationale as to why research into the addition of BfR to 'no-load' exercise is warranted. Following this, the remainder of the chapter describes the implementation and outcomes of a pilot study involving a single participant, and a main study involving a larger, uninjured cohort (n=16). Findings of the main study have subsequently been presented at the World Confederation for Physical Therapy Congress in July 2017 (Appendix VIb).

5.2 Phase II Aim

To investigate whether the addition of lower-limb BfR to an un-resisted, 'no-load' knee exercise produces a significant change to the acute physiological and perceptual responses of the exercise session, across a range of thigh-cuff inflation pressures.

5.3 Background

To maximise strength development and muscle hypertrophy, The American College of Sports Medicine [ACSM] recommends a resistance exercise programme that uses high external loads; typically loads that meet or exceed 70% of an individual's one-repetition maximum [1RM] (ACSM, 2009). Meta-analysis work completed before and after the ACSM's publication broadly supports this recommendation; Rhea et al (2003) proposed that 60-80% 1RM elicits maximal strength gains in untrained individuals, whereas 80% 1RM was more appropriate for trained individuals. Schoenfeld et al (2014) found that loads \geq 65% 1RM were superior than lower loads in promoting strength and hypertrophy response. Schoenfeld et al (2014) did acknowledge, however, that training with loads \leq 60% 1RM still produced a significant strength and hypertrophy response. The described potency of lower-load training was verified experimentally by Schoenfeld et al (2015), who demonstrated that equal muscle hypertrophy occurred in eighteen well-trained males whether they were allocated to a group completing low-load resistance training to failure, or to high-load resistance training. Morton et al (2016) also demonstrated that forty-nine resistance-trained males undergoing a 12-week whole-body resistance training programme significantly increased muscle strength and size. Participants were allocated to either a group using 30-50% 1RM loads or a group using 75-90% 1RM loads. Both groups increased upper and lower limb muscular size and strength to similar extents. Both groups performed each exercise set to volitional failure. The authors suggested that training to volitional failure allowed maximal activation of motor units in both groups, which was the determinant for longitudinal gains in muscular size and performance. Crucially, the amount of mass lifted by participants was not the primary determinant of these gains; external load was essentially unimportant. Meta-analytic work continues to be produced that ratifies the usefulness of lower-load resistance training. For example,

Csapo et al (2016) suggests training at ~45% 1RM is effective at gaining muscle strength within the elderly; although age-related blunting of the hypertrophy response at all exercise intensities seemed apparent.

Following a significant orthopaedic lower-limb injury or surgical intervention, an extended period of impaired weight bearing is often enforced during the acute and sub-acute stages of injury rehabilitation (Logerstedt et al, 2010; Porter and Shadbolt, 2015; Vioreanu et al, 2007). Whilst harnessing the established muscular benefits of resistance training during this time would be ideal, the use of high or low loads are often contraindicated due to pain and the risk of mechanical forces causing re-injury. As a result, the prescription of active rehabilitation exercises can often be limited to un-resisted, or 'no-load' exercise. Although this mode of active movement may transiently improve local blood-flow, synovial fluid exchange and perceived pain (Lederman, 2005), it is very unlikely to result in volitional failure of exercising lower-limb musculature and the generation of significant exercise-induced metabolic stress. For example, Sjøgaard et al (1986) demonstrated that healthy participants performing seated knee extensions at 5% MVC could maintain this continuously for one hour, resulting in only a moderate increase to cohort perceived exertion (1.9 to 4.5) and a mean pre-post reduction in MVC of 12%. Ultimately, the day-to-day metabolic and physiological demands placed upon an injured limb and its muscle tissue are reduced far below normal, producing marked atrophy and loss of strength within the lower-limb musculature (Bodine, 2013; Campbell et al, 2013; Psatha et al, 2012). Thus, clinicians are resigned to a lengthy process of rebuilding muscular strength and size once weight bearing is indicated. This could contribute to an impairment of long-term function. Following an ankle fracture for example, concomitant deficits in muscle torque are still present within the ankle plantar flexors after ten weeks

of rehabilitation following cast removal (Stevens et.al, 2004). Fourteen months after ankle fracture over half of patients report difficulty climbing stairs and 45% of those who enjoyed participating in sport pre-injury had failed to regain a similar level of ability post-injury (Nilsson et al, 2003). Modalities are therefore required to address this. Treatments that prevent or attenuate muscle atrophy and strength loss during impaired weight-bearing periods, by inducing regular exercise-induced metabolic stress without a notable external load needing to be applied, are warranted and desirable.

A factor in determining the ability to perform exercise, and the subsequent degree of exercise-induced metabolic demand and stress that occurs within an exercising muscle, is blood-flow through the muscle itself. At the onset of seated, active knee extensions for example, a rapid increase in peripheral blood flow occurs which is maintained during the exercise period (Paterson et al, 2005; Rådegran and Saltin; 1988). During the rhythmic contractions of the quadriceps, intramuscular pressure is raised, which exerts extravascular pressure upon the microvascular bed of the muscle(s). This vascular compression propels blood through the veins of the lower limb towards the heart (Korthuis, 2011). During the brief rest period between muscular contractions, microvascular patency is restored and muscle tissue is re-perfused with blood. These large, contraction-dependent variations in blood flow have also been demonstrated upstream of the exercising musculature, within the femoral artery (Walløe and Wesche, 1988). With repeated contractions and/or increased metabolic demand from the exercising tissues, local mechanisms such as arterial vasodilation increase the microvascular surface area available for oxygen exchange (Korthuis, 2011; Sarelius and Pohl, 2010). Sympathetic activity also increases cardiac output to increase intra-arterial blood pressure, blood-flow rate and ultimately oxygen delivery rate to exercising

muscle(s) (Korthuis, 2011). This autoregulation of blood flow allows oxygen delivery to exercising muscle tissue to match oxygen demand, allowing the exercise to continue.

The capacity to match oxygen delivery to oxygen demand within exercising muscles via blood-flow is functionally limited, however. For example, Sadamoto et al (1983) demonstrated that intramuscular pressure, and therefore the extravascular pressure exerted within contracting limb musculature, is sufficient to completely cease muscular blood flow at 50-64% MVC. Rådegran and Saltin (1998) also found that femoral arterial blood-flow could be impeded during the contraction phases of a dynamic knee extension exercises performed at ~65% of peak power output.

Understandably, the energy demand of muscle fibres performing high-intensity contractions can be significant. If the oxygen subsequently delivered during the resting (re-perfusion) periods between repeated muscular contractions does not meet the temporal oxygen demands of the exercising muscle fibres, tissue oxygenation within the muscle tissue drops and the fibres rely upon anaerobic respiration to provide energy. This has been demonstrated during rhythmic active knee extensions performed at 70-80% 1RM, which produced rapid drops in tissue oxygen saturation within the vastus lateralis (Miyamoto et al, 2013; Tanimoto and Ishii, 2006) with a subsequent post-exercise increase in blood lactate concentration (Tanimoto and Ishii, 2006). The rate and extent at which this occurs is modulated by the training status of the individual and the parameters of the exercise itself, such as the external load used, the length of exercise sets and the duration of rest period between both exercise sets and muscular contractions.

If sufficient exercise-induced metabolic stress occurs, cellular pathways will mediate

exercise-related adaptations to muscular and vascular tissues. This includes muscle hypertrophy via suppression of the myostatin-Smad pathway and activation of the Akt/mTOR pathway (Egerman and Glass, 2014; Wackerhage, 2014) and angiogenesis through the expression of vascular endothelial growth factor (Bloor et al, 2005; Gavin et al, 2007). Longitudinally, these processes can maintain or improve the exercise capacity and performance of skeletal muscle.

Reviewing the above mechanisms, it is understandable why very-low load, or 'no-load' exercise may generate minimal metabolic stress within exercising muscle tissue.

Blangsted et al (2005) demonstrated that ten minutes of isometric elbow flexion at a 10% MVC raised intramuscular pressure within the exercising muscles. However, tissue oxygen saturation remained constant throughout, suggesting muscular blood flow was sufficient to match oxygen delivery with demand. Sjøgaard et al (1986), whilst demonstrating that healthy participants could perform seated knee extension at 5% MVC for one hour, found that blood flow through the femoral vein of the exercising leg remained patent and unchanged following an initial increase at the start of the exercise. Whilst tissue oxygenation was not directly measured, it could be argued that the ability to complete such a long period of exercise whilst blood throughput within the limb was maintained resulted in adequate oxygen exchange and minimal metabolic stress. Whereas, in studies such as Tanimoto and Ishii (2006) and Miyamoto et al (2013), it is reasonable to suggest that muscular blood-flow was impeded during concentric and eccentric muscular contractions due to the intramuscular pressure that was likely generated from this high-load training. This in turn created the oxygen deficits within the vastus lateralis and exercise-induced metabolic stress, indicated by lactate accumulation.

Through comparison of the work by Blangsted et al (2005) and Sjøgaard et al (1986) against that of Miyamoto et al (2013) and Tanimoto and Ishii (2006), it is reasonable to argue that a high external load is the catalyst towards attaining significant exercise-induced metabolic stress. Yet this assumption can be challenged by examining a recent study by Counts et al (2016). The authors compared within-subject, between-arm changes to brachial muscle thickness and strength after completing eighteen sessions of elbow flexion exercises performed using either a high-load (70% 1RM) or no load. Thirteen participants completed 'no-load' exercise sessions by maximally contracting their biceps through the full range of elbow motion. Pre-post brachial muscle thickness increased in both high-load and 'no-load' arms by a similar magnitude ($p \geq 0.203$). Muscular strength (tested via 1RM) and muscular endurance also increased in both arms but to a greater degree within the high-load arm. It could be argued that the use of maximal voluntary contraction during the 'no-load' exercise may explain why increases in muscle size and strength were seen.

As per Sadamoto et al (1983), contracting at approximately 100% MVC should have induced cessation of muscular blood-flow during muscular contractions via the generation of significant extravascular pressure. Repeated contractions at such a high intensity could have induced significant metabolic demand and an oxygen deficit that was amplified due to the impedance of blood-flow and thus exercise-induced metabolic stress. Longitudinally, cellular signalling pathways responsible for the development of muscular strength and hypertrophy may have been stimulated in similar ways between biceps exercised at 'no-load' and 70-80% 1RM. Within Counts et al (2016), the superiority of the 'high-load' training in terms of muscle strength and endurance may be linked to between-arm differences in exercise parameters. The 'no-load' arms were exercised for

four sets of 20 repetitions, with 30 seconds of rest between sets. The high-load arms were exercised for four sets of 8-12 repetitions, with the external load increased if participants could exceed 12 repetitions. Indeed, over the course of the experiment, the volume of work that the high-load arm completed increased from 786.6 (\pm 308.7) kg in the first half of the programme to 927.5 (\pm 341.0) kg in the latter half. In contrast, participants who completed all twenty repetitions in the 'no-load' arm from the outset were not progressed. It could be argued that high-load arms experienced greater degrees of metabolic stress throughout the experiment, as load was adjusted in a way that participants would have exercised to failure (or close to this). Whereas the 'no-load' arms were unlikely to have been exercised to volitional failure from the outset.

In light of the above, it could be argued that the solution to preventing lower-limb muscle strength and size loss within injured individuals unable to complete loaded resistance training may simply be to ask them to contract maximally whilst completing 'no-load' exercises, to volitional failure. Yet this may still prove difficult to achieve or maintain within individuals inexperienced in exercise, those who are elderly or comorbid, or those whom maximal contraction is prevented due to pain and discomfort from the injury site. A method of artificially raising metabolic demand within muscle tissue, producing significant exercise-induced metabolic stress during the early stage of lower-limb rehabilitation whilst avoiding maximum voluntary contraction, is therefore warranted.

Adding BfR to low-intensity knee extensions has been shown to significantly effect haemodynamics within the lower limb, and the resultant tissue oxygenation of the vastus lateralis. Cayot et al (2016) had seven males perform isometric knee extensions at four exercise intensities; 20, 40, 60 or 80% MVC. These sessions were then repeated with BfR

applied. Total haemoglobin mass within the vastus lateralis was higher at all MVCs when blood flow restriction was superimposed ($p \leq 0.05$). Significantly higher levels of de-oxygenated haemoglobin were also seen during sessions performed 20 and 40% MVC with BfR than without ($p \leq 0.05$) but this was not evident at 60 or 80% MVC. It could be argued that the additional pressure exerted upon thigh tissue by the cuff may have lowered the % MVC required to cease muscular blood flow during muscular contractions from that suggested by Sadamoto et al (1983). This in turn raised or amplified the mismatch in oxygen delivery and oxygen uptake at 20/40%MVC, leading to the increased levels of de-oxygenated haemoglobin. The increasing total haemoglobin mass across sessions may have also indicated venous congestion at the measurement site caused by partial occlusion of the femoral vein. In a separate study, Downs et al (2014) delivered a three-set leg press exercise to healthy participants ($n=13$) across multiple visits. The exercise was performed at 80% 1RM, 20% 1RM, or 20% 1RM with blood flow restriction applied at either 1.3x systolic or 1.3x diastolic brachial blood pressure. BfR significantly blunted femoral and popliteal artery blood flow before and during leg press exercise, compared to blood flows seen at 80% 1RM or 20% 1RM without BfR ($p < 0.05$). Tissue oxygen saturation decreases by approximately 50% during blood-flow restricted sessions, by 35% in the 20% 1RM session without restriction and by 20% in the 80% 1RM session. Tissue oxygen saturation during the rest periods was significantly lower during the blood-flow restricted sessions than sessions completed without restriction ($p < 0.05$). Similar findings in regards to total haemoglobin levels and tissue oxygenation saturation have also been demonstrated within the vastus medialis oblique (Ganesan et al, 2015). In combination, it could be suggested that low-intensity (or low load) BfR does amplify the acute metabolic demand of an exercise session, without having to modulate the parameters of the exercise itself.

At present, however, there appears to be a lack of evidence investigating whether BfR can alter the acute metabolic demand of a 'no-load' lower-limb exercise. Work by Downs et al (2014) and Cayot et al (2016) indicates superimposing BfR over exercise performed at a low intensity or load does amplify local, acute metabolic stress via drops in tissue oxygen saturation. But whether it is possible to replicate this during 'no-load' exercise is unclear. If a favourable effect can be demonstrated, this could bolster the validity of applying 'no-load' BfRT during the acute stages of low-limb injury rehabilitation. Clinicians may ultimately gain a tolerable and low-risk method of attenuating losses to lower-limb muscle strength and size during extended periods of impaired weight bearing.

5.4 Phase II Pilot Study

5.4.1 Pilot-specific Objectives

To pilot the suitability of a BfRT protocol for use in the main Phase II study. Specifically;

1. To observe whether changes in vastus lateralis muscle SmO_2 , haemoglobin and heart rate levels could be detected during an unweighted knee extension exercise, if thigh-cuff pressures of up to 80mmHg were applied to an exercising lower-limb.
2. To determine whether the exercise protocol described in chapter 3.5 was physically achievable under 80mmHg thigh-cuff pressure, without reaching the agreed cut-off values for subjective levels of pain (≥ 40 out of 101).

5.4.2 Methods

Following informed consent and completion of the project's health screening questionnaire, one healthy male (age; 35.8 years, height; 1.83m, weight; 82.1kg) initially participated in the pilot study. The participant was physically active, being a regular fell-runner with and scoring 11.9 (out of a possible 15) on the Baecke Habitual Physical

Activity Questionnaire (Baecke, 1982). Upon arrival, anthropometric measurements of the participant were taken using the methods described in chapter 3.3.1. The participant then completed four exercise sessions, each separated by at least 48 hours.

Exercise Session Methodology

At each exercise session, the pilot participant was taken into the same temperature-regulated room and fitted with a 21cm-wide thigh blood-pressure cuff [MDF2090471; MDF Instruments®, California, USA] in standing, situated as high on the upper thigh as possible without contacting the groin (Figure 3.3.1). The participant was seated upon a height-adjustable plinth to achieve approximately 90° hip flexion and 80° knee flexion with both feet placed flat on the floor (Figure 3.3.2). A near infrared spectroscopy device [Moxy Monitor, Fortiori Design LLC; Minnesota, USA] was fitted to overly the distal vastus lateralis of the test leg to record tissue oxygen saturation percentage [SmO₂], total haemoglobin mass [tHb] and deoxygenated haemoglobin mass [HHb] within the muscle. Heart rate [HR] was recorded via a wireless chest strap [Garmin International; Kansas, USA] fitted at the level of the participant's xiphisternum.

Before the knee exercise commenced, resting HR, SmO₂, tHb and HHb were continuously recorded over a period of three minutes. During the last thirty-seconds of this three-minute period, an ultrasound imaging machine [MyLab™ 70; Esaote SpA, Italy] equipped with a linear, phased-array ultrasound probe [Biosound LA523; Esaote SpA, Italy] was used to determine blood flow within the popliteal artery of the test leg, as described in chapter 3.3.2. The thigh cuff was then left deflated at 0mmHg (control), or inflated to a predetermined pressure by the researcher (40mmHg during appointment 2, 60mmHg during appointment 3, or 80mmHg during appointment 4) via a hand-held aneroid

sphygmomanometer [MDF848XPD, MDF Instruments®; California, USA]. Immediately upon reaching the target pressure, blood flow measurements of the popliteal artery were repeated.

In the seated position, the participant then completed three sets of a unilateral, unresisted knee extension exercise as described in chapter 3.5. If a positive thigh-cuff pressure was applied (40-80mmHg), this was maintained throughout the exercise and rest periods and for one minute following the completion of the third exercise set. Upon cuff deflation, the participant remained in the seated position for three minutes.

Measurements of HR, SmO₂, tHb and HHb were continuously recorded throughout the exercise and rest periods. Immediately after each exercise set, the participant rated the level of perceived exertion experienced within his active thigh muscles using the OMNI-RES (Appendix 1e) and rated any perceived pain using a numerical rating scale for pain (Appendix 1c). For additional clarity, a visual representation of one Phase II testing session is shown in Figure 5.4.1.

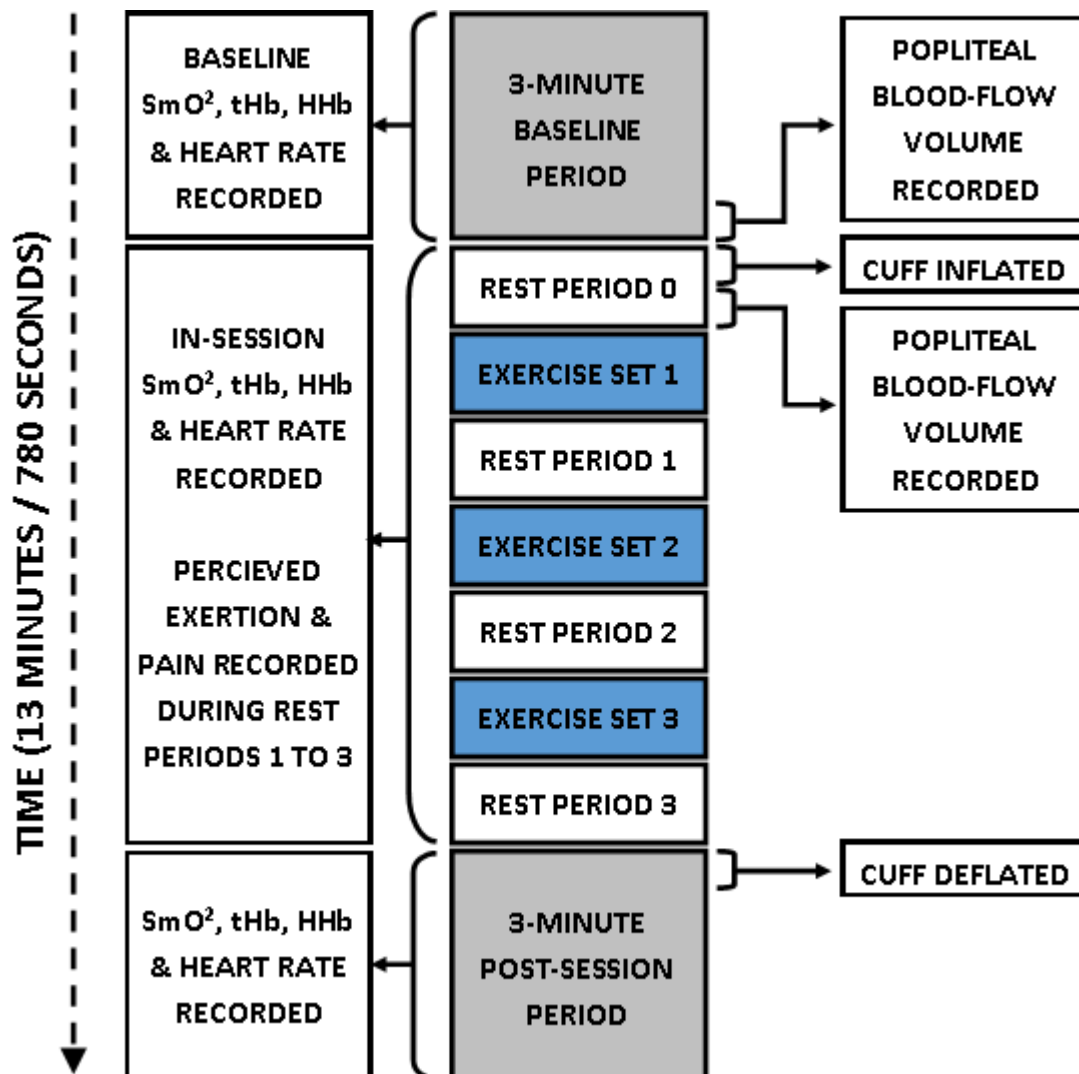


Figure 5.4.1. A flowchart describing the time-course and data collection points of a one testing session within Phase II.

5.4.3 Results

All four exercise sessions were completed without adverse or unexpected events. Mean resting values for vastus lateralis SmO₂, tHb, HHb and HR across the pilot study are shown in Table 5.4.1. The coefficient of variation in resting vastus lateralis SmO₂, tHb, HHb and HR values, taken before each of the four sessions began, are shown in Table 5.4.2. Mean resting blood flow within the popliteal artery of the test leg across the pilot study was 36.91 mL/min (SD 4.94 mL/min).

The application of thigh-cuff pressure reduced blood flow in the popliteal artery

immediately before the first exercise set commenced, generating a %PBfR of 61.8% at 40mmHg, 42.8% at 60mmHg and 49.8% at 80mmHg. During session one, where no thigh-cuff pressure was applied, blood-flow remained stable (%PBfR 101.3%). The mean changes to vastus lateralis SmO₂, tHb, HHb and HR during each exercise session are shown in Table 5.4.3.

Table 5.4.1. Mean values recorded during the three-minute, pre-exercise baseline periods of the Phase II pilot study (n=1).

Outcome Measure	Mean Baseline Value	Standard Deviation
SmO ₂ [%]	49.59	4.79
Total Haemoglobin Mass [g/dL]	13.06	0.07
Deoxygenated Haemoglobin Mass [g/dL]	6.58	0.60
Heart Rate [Beats Per Minute]	74.7	4.09

Table 5.4.2. The coefficient of variation in haemodynamic values taken from each three-minute, pre-exercise baseline period of the Phase II pilot study (n=1).

Outcome Measure	Coefficient of Variation (%)			
	Prior to Session 1 (0mmHg)	Prior to Session 2 (40mmHg)	Prior to Session 3 (60mmHg)	Prior to Session 4 (80mmHg)
SmO ₂ [%]	11.58	9.51	7.00	6.19
Total Haemoglobin Mass [g/dL]	0.82	0.58	0.49	0.59
Deoxygenated Haemoglobin Mass [g/dL]	10.22	6.97	6.76	7.24
Heart Rate [Beats Per Minute]	3.50	8.16	4.88	4.34

Table 5.4.3. The mean relative change in haemodynamic values during the Phase II pilot study exercise sessions, compared to pre-exercise baseline values (n=1). The '+' and '-' symbols denote the direction of change compared to that session's pre-exercise baseline period.

Outcome Measure	Amount of Thigh-cuff Pressure Applied			
	During Session 1 (0mmHg)	During Session 2 (40mmHg)	During Session 3 (60mmHg)	During Session 4 (80mmHg)
SmO ₂ [%]	+ 6.48	+ 3.29	- 6.40	- 11.90
Total Haemoglobin Mass [g/dL]	- 0.06	- 0.02	+ 0.06	+ 0.13
Deoxygenated Haemoglobin Mass [g/dL]	- 0.87	-0.43	+ 0.87	+ 1.62
Heart Rate [Beats Per Minute]	+ 3.87	+ 3.66	+ 3.51	+ 6.78

Second-by-second values for the outcome measures reported in Table 5.4.3 were plotted graphically to visualise any between-session differences in vastus lateralis SmO₂ (Figure 5.4.2) and HR (Figure 5.4.3). Rates of perceived exertion for each exercise set are shown in Figure 5.4.4. The participant reported no pain during any exercise session.

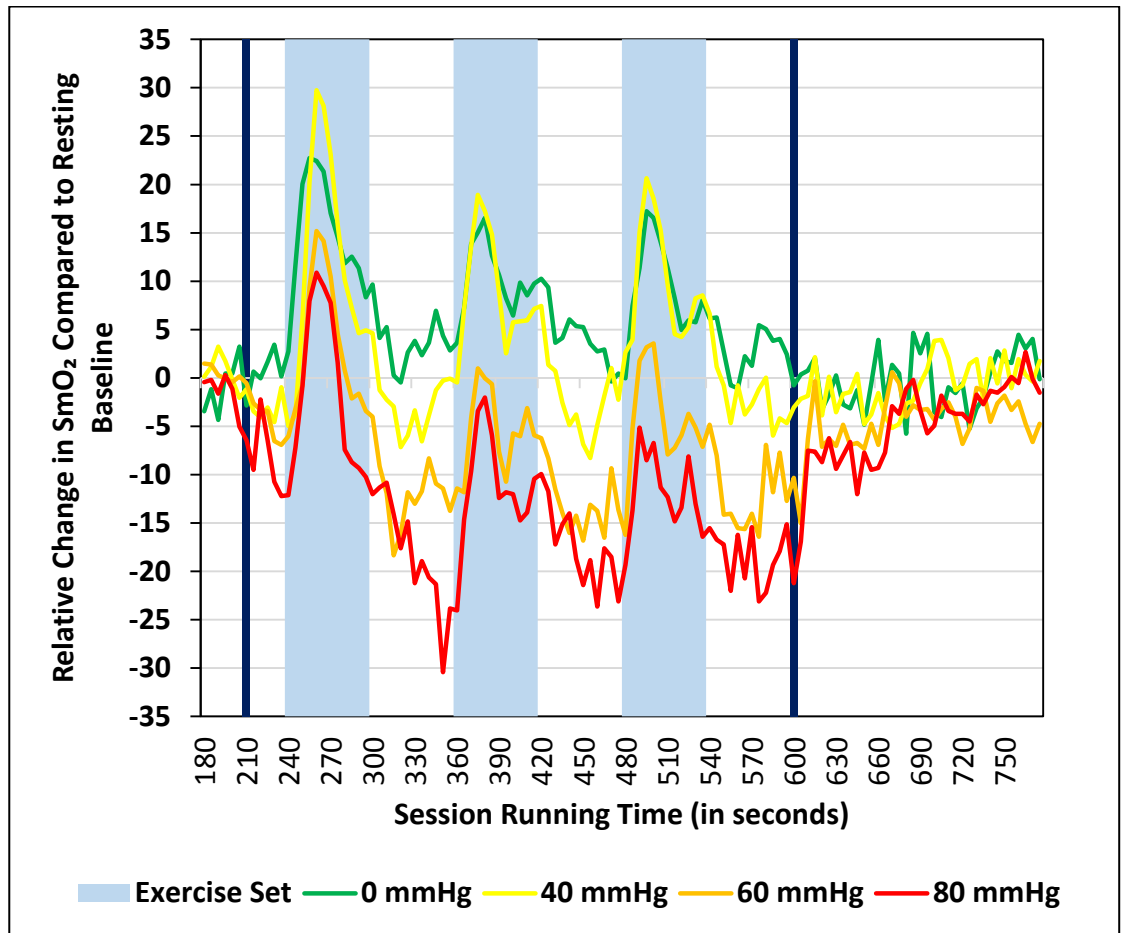


Figure 5.4.2. A graphical representation of in-session changes to vastus lateralis SmO₂ during the Phase II pilot study. The three-minute pre-exercise baseline period (0-180 seconds) is not shown. The cuff was inflated at 180 seconds and deflated at 600 seconds.

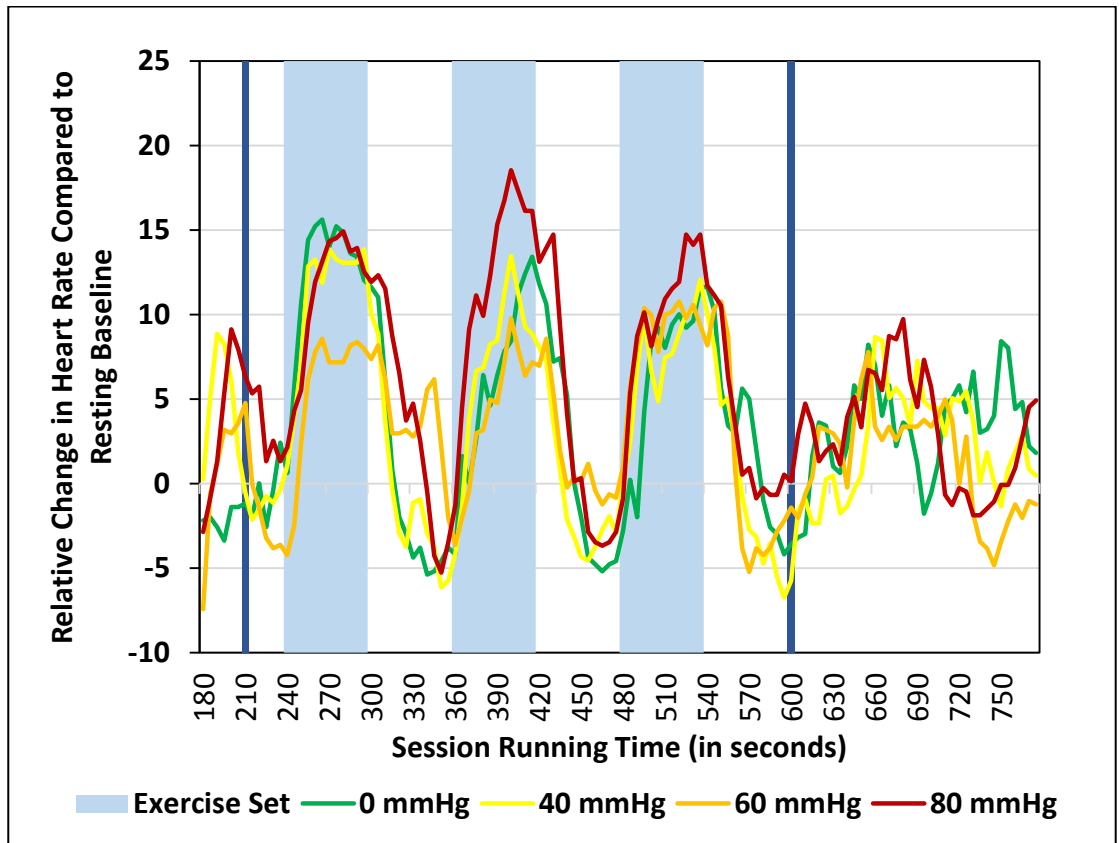


Figure 5.4.3. A graphical representation of in-session changes to heart rate during the Phase II pilot study. The three-minute baseline period (0-180 seconds) is not shown. The cuff was inflated at 180 seconds and deflated at 600 seconds.

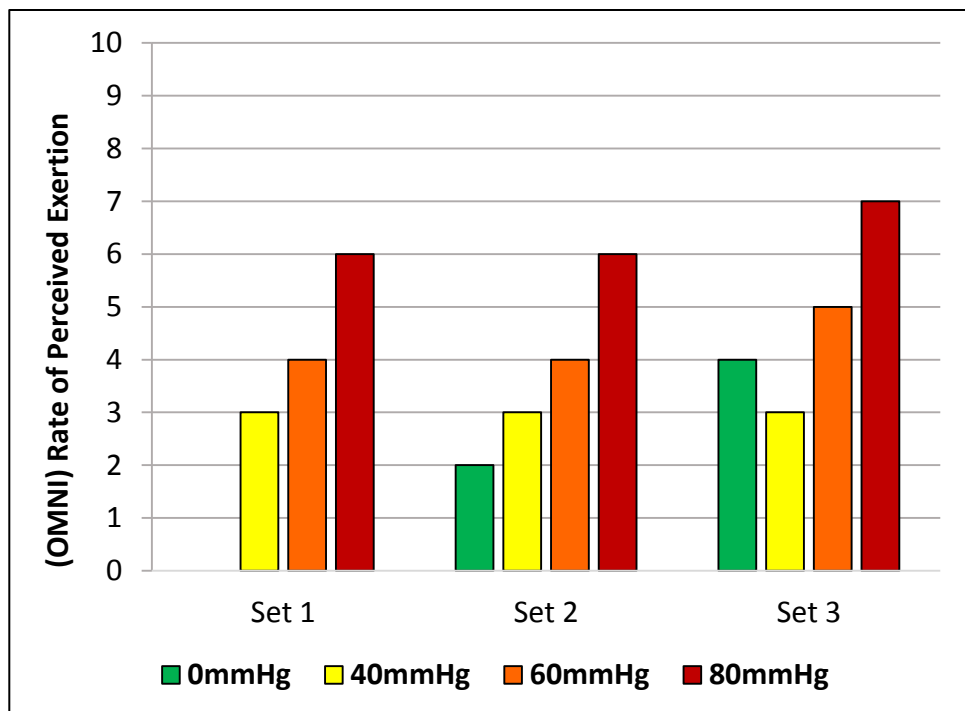


Figure 5.4.4. Set-by-set OMNI-RES scores recorded during the exercise sessions of the Phase II pilot study.

5.4.4 Discussion

The addition of thigh-cuff pressure during an un-resisted knee extension exercise produced numerical and visual differences to vastus lateralis SmO₂, tHb, HHb. At 0mmHg and 40mmHg, SmO₂ increased whilst tHb and HHb decreased. At 60mmHg and 80mmHg, however, this trend was reversed. Heart rate during the unweighted knee exercise increased slightly during all testing sessions, however the mean change in heart rate (Table 5.3.3) and its pattern of change across each testing session (Figure 5.4.3) remained similar.

The values recorded by the wireless chest strap and the NIRS device were all within normal physiological ranges. The coefficient of variation in resting HR derived from the wireless chest strap during resting baseline periods was low within each testing session, whilst being consistent between testing sessions. This was also true of vastus lateralis SmO₂, tHb and HHb derived from the NIRS device (Table 5.4.2). It is very possible that a portion of the within-session variances reported by these devices may have been due to natural physiological fluctuations to maintain homeostasis, rather than any accuracy issue with the devices themselves. Given the presented data, the primary researcher was happy to incorporate the devices into the main study.

The rate of perceived exertion scores indicated that the addition of BfR to the participant's knee extension increased the perceptual effort of the exercise. At 80mmHg thigh-cuff pressure, the reported exertion scores corresponded to between 'somewhat hard' and 'hard' on the OMNI-RES worded headings. The pilot participant reported no pain throughout the four exercise sessions, suggesting that thigh the pressures up to 80mmHg may increase only exercise-related discomfort and not pain due to tissue

compression. Whilst the participant never reached the pain cut-off value (≥ 40 out of 101 on the numerical rating scale), it is possible that individuals with a smaller thigh mass (or less experience in differentiating pain from exercise-related discomfort) could register positive pain scores in the main study. The addition of extra testing sessions to investigate cuff pressures greater beyond 80mmHg may have also discouraged individuals from volunteering, or increased the risk of them withdrawing, due to having to attend 5+ appointments. In light of this, the testing protocol remained at four appointments/testing sessions (0/40/60/80mmHg).

5.4.5 Conclusions

This study looked to pilot the suitability of a BfRT protocol for use in the main Phase II study. Both objectives of the pilot study were met. The protocol demonstrated numerical differences in vastus lateralis muscle SmO_2 and haemoglobin levels between exercise sessions completed with or without the application of thigh-cuff pressures up to 80mmHg. At 60-80mmHg, pilot results appear to indicate that this degree of cuff pressure was adequate to reduce popliteal arterial blood flow by approximately 50%. 'No-load' BfRT sessions were also demonstrated to be safe and tolerable at these cuff pressures. In consideration of the pilot study findings, no changes were made to the testing protocol in advance of the main Phase II study.

5.5 Phase II Main Study

5.5.1 Main Study Objectives

1. To observe and examine the acute haemodynamic and perceptual responses to a 'no-load' knee exercise upon a cohort of individuals, both with and without BfR applied.

2. To determine whether the magnitude of these acute responses are associated with the amount of thigh-cuff pressure applied and/or the physical characteristics of individuals within the cohort.

5.5.2 Hypotheses

H0 – The addition of lower-limb BfR to a ‘no-load’ knee extension exercise will produce no significant change in the acute metabolic demand or perceptual response of the exercise.

H1 - The addition of lower-limb BfR to a ‘no-load’ knee extension exercise will produce a significant change in the acute metabolic demand or perceptual response of the exercise.

5.5.3 Methods and Statistical Analysis

Healthy males and female volunteers were recruited as described in chapter 3.2.

Volunteers completed the bespoke health screening questionnaire and provided informed consent prior to their participation. Upon arrival at the testing location, anthropometric measurements of height, weight, supine brachial blood pressure and leg dimensions were taken (chapter 3.3.1). A Habitual Activity Questionnaire (Baecke et al, 1982) was completed by each participant to acquire a measure of physical activity levels over the previous twelve months (chapter 3.3.3.5). The lower-limb to undergo all four exercise sessions was allocated by way of a Microsoft Excel Macro.

5.5.3.1 Exercise Session Methodology

At each exercise session, participants were taken to the same temperature-regulated room and fitted with a 21cm-wide thigh blood-pressure cuff [MDF2090471; MDF Instruments®, California, USA] in standing, situated as high on the upper thigh as possible without contacting the groin (Figure 3.3.1). The protocol of the exercise session then

proceeded in an identical fashion to the pilot study, already reported in chapter 5.4.2, page 161.

5.5.3.2 The Degree of Lower-Limb Blood-flow Restriction Applied

The percentage of popliteal arterial blood-flow remaining [%PBfR] following the application of a thigh-cuff pressure was calculated for each appointment for each participant (Figure 3.3.11). During the control session, it was expected that blood-flow should remain stable, giving a %PBfR of approximately 100%.

5.5.3.3 The Acute Metabolic Demand of the Knee Exercise

For each appointment, the raw values recorded for HR, SmO₂, tHb and HHb during the three-minute baseline period were collated and mean values calculated for each variable. This process was repeated for HR, SmO₂, tHb and HHb measurements recorded during the exercise session itself. Mean values recorded during the exercise were then subtracted from mean values taken during the baseline period, to provide a direction and magnitude of exercise-related change in each variable. Statistical analysis by way of repeated-measures ANOVA determined whether the addition of thigh-cuff pressures during the un-resisted knee exercise had a significant acute effect upon acute metabolic demand, indicated via HR, SmO₂ or HHb. Statistical differences in tHb between thigh-cuff pressures would indicate whether any acute haemodynamic changes in vastus lateralis muscle blood volume were generated by BfR.

5.5.3.4 Subjective Responses

For each exercise session, the perceived exertion and pain scores recorded during the three exercise sets were collated and a mean score calculated for 'session-RPE' and

'session-pain'. Statistical analysis by way of repeated-measures determined whether thigh-cuff pressure had a significant effect upon 'session-RPE' and 'session-pain'.

Perceived exertion and pain scores for each exercise set were also reviewed to determine whether thigh-cuff pressure had a significant effect upon 'within-set RPE' and 'within-set pain'.

5.5.3.5 Associations with Physical Characteristics

Bivariate correlational analysis was performed to indicate whether associations existed between the anthropometric measurements taken from participants and the %PBfR experienced at each of the four thigh-cuff pressures. Associations between anthropometric measurements and the magnitude of change in HR, SmO₂, tHb and HHb during each testing session were also reviewed. A priori analysis using G*Power (Faul et al, 2017) suggested that sixteen participants provided adequate statistical power ($1-\beta > 0.80$) to detect bivariate correlations with an r value of > 0.60 , using a two-tailed p value of 0.05.

5.5.4 Results

Sixteen participants (n=9 male, n=7 female) completed all four exercise sessions without the occurrence of adverse or unexpected events; Age 32.8 ± 4.3 years, Height 173.2 ± 9.7 cm; Weight 76.4 ± 16.7 kg; BMI 25.2 ± 3.9 kg/m². Participants' Habitual Physical Activity score, via the Baecke Questionnaire, ranged from 5.9 to 11.9 out of 15 (mean 8.9 ± 1.68). Data was obtained for SmO₂, tHb, HHb and RPE for all sixty-four test sessions. Heart rate data was missing for six out of the sixty-four test sessions. During four of these sessions, a consistent HR signal could not be acquired despite repositioning or interchanging of the participant's heart rate strap. Post-session data corruption resulted in the loss of HR data

in the remaining two sessions. Ultimately, full HR data was available for twelve participants. Mean lab temperature across appointments was $22.8 \pm 0.9^\circ$ Celsius. Data are presented as mean \pm standard deviation unless otherwise stated. Statistical significance was set a $p \leq 0.05$.

5.5.4.1 The Degree of Lower-Limb Blood-flow Restriction Applied

Compared to a 100% baseline, 40mmHg of thigh-cuff pressure induced a %PBfR of 73.1 (± 14.3), 60mmHg induced 61.1 (± 14.9) and 80mmHg induced 47.6 (± 8.8). At 0mmHg thigh-cuff pressure, the two blood flow readings were similar, producing a %PBfR value of $108.5 \pm 18.8\%$.

A one-way repeated measures ANOVA was conducted to determine any statistically significant within-subject difference in %PBfR values recorded across the four cuff pressure conditions. There were no outliers in the data, assessed by visual inspection of a boxplot. %PBfR values were normally distributed, as assessed by Shapiro-Wilk's test ($p > 0.05$). Mauchly's test of sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(5) = 4.559$, $p = 0.473$. %PBfR values during the exercise intervention were different across the four cuff pressure conditions, to a statistically significant level; $F(3, 45) = 81.659$, $p < 0.0001$, partial $\eta^2 0.845$. Post hoc analysis with a Bonferroni adjustment revealed that %PBfR values were statistically different between every cuff pressure combination ($p \leq 0.008$), with the exception of 40mmHg vs. 60mmHg ($p = 0.09$).

5.5.4.2 The Acute Metabolic Demand of the Knee Exercise

Resting (baseline) mean values for each haemodynamic variable are shown in Table 5.5.1 for the cohort.

Table 5.5.1. Resting [baseline] haemodynamic mean values for the Phase II cohort [n=16].

	Vastus Lateralis SmO ₂ (%)	Vastus Lateralis tHb (g/dL)	Vastus Lateralis HHb (g/dL)	Heart Rate (bpm)	Popliteal Blood-Flow (ml/min)
Mean	66.78	11.95	4.04	75.08	42.57
Std. Deviation	12.14	0.44	1.49	11.34	20.24

SmO₂

Cohort SmO₂ within the vastus lateralis muscle at rest was similar to that reported within other literature utilising different NIRS devices (Downs et al 2014; Karabulut et al, 2014; Kennedy et al, 2006; Miyamoto et al, 2013). Compared to baseline, vastus lateralis SmO₂ increased by a mean of 6.01 ± 6.40 points. during the control exercise session at 0mmHg cuff pressure. At other cuff pressures, vastus lateralis SmO₂ decreased during the exercise compared to baseline (40mmHg; -1.28 ± 5.03 ; 60mmHg; -7.22 ± 7.39 ; 80mmHg; -12.74 ± 6.81).

A one-way repeated measures ANOVA was conducted to determine whether a statistically significant within-subject difference existed in SmO₂ across the four exercise sessions. There were no outliers in the data, as assessed by inspection of a boxplot. SmO₂ values were normally distributed, as assessed by Shapiro-Wilk's test ($p > 0.05$). Mauchly's test of sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(5) = 3.716$, $p = 0.592$. SmO₂ was different across the four exercise sessions to a statistically significant level; $F(3, 45) = 42.464$, $p < 0.0001$, partial η^2 0.739. Post hoc analysis with a Bonferroni adjustment revealed that SmO₂ values during exercise were statistically different between all cuff pressures combinations ($p \leq 0.033$).

Second-by-second changes to SmO_2 during exercise sessions at each thigh-cuff pressure, compared to resting baselines, are plotted visually in Figure 5.5.2.

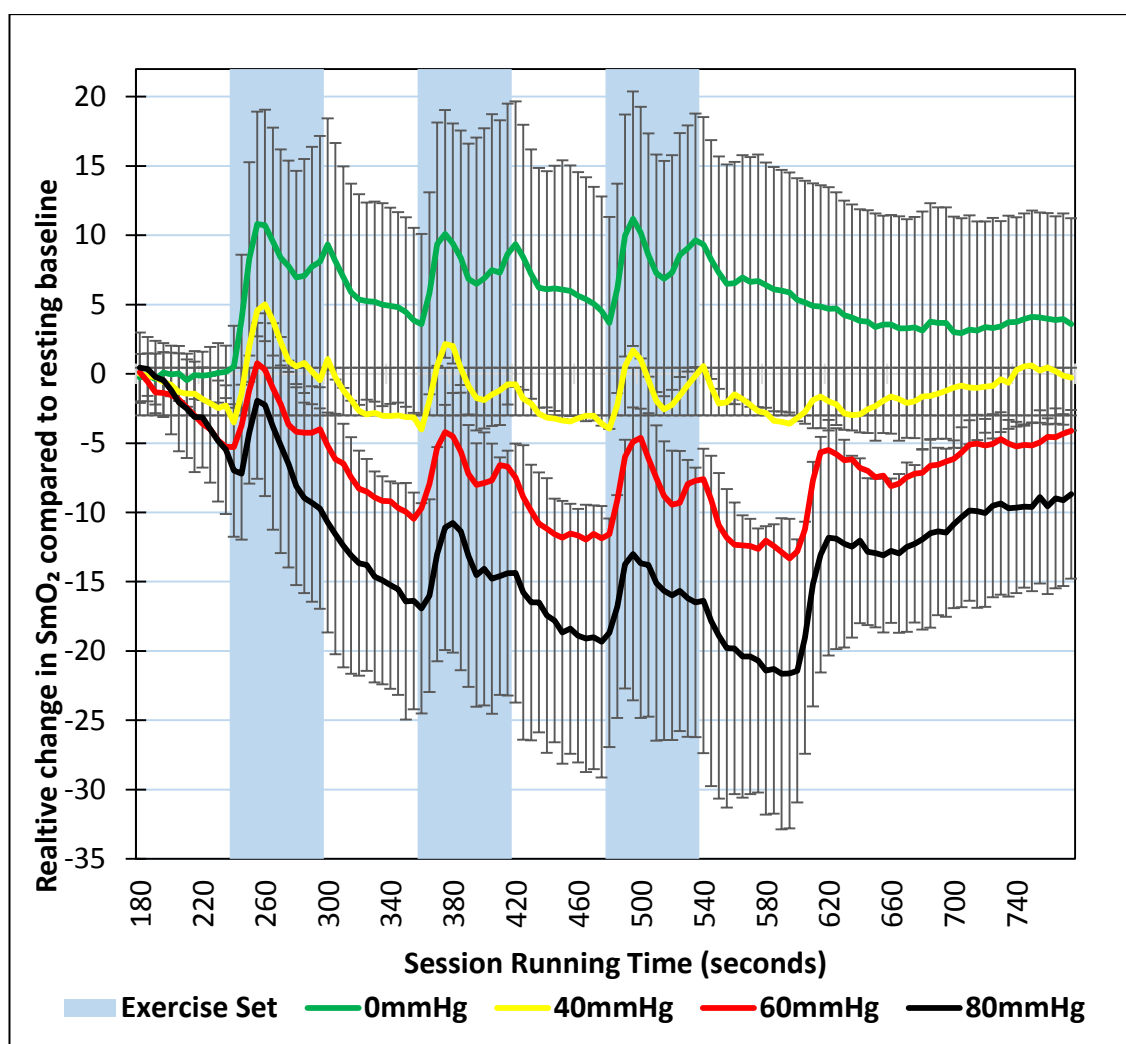


Figure 5.5.2. Changes in cohort vastus lateralis SmO_2 values during the Phase II study exercise protocol performed at different thigh-cuff inflation pressures. Mean values are displayed. Whiskers represent standard deviations. Where positive cuff pressure was applied, thigh cuff began inflation at 180 seconds and was deflated at 600 seconds.

Cohort tHb

Compared to baseline, vastus lateralis tHb decreased during the knee extension exercise performed under 0mmHg and 40mmHg cuff pressure. Vastus lateralis tHb increased during the knee extension exercise performed under 60mmHg and 80mmHg cuff pressure (Table 5.5.2).

Table 5.5.2. Phase II cohort changes in vastus lateralis tHb during un-resisted knee exercise at different thigh-cuff inflation pressures.

	0mmHg	40mmHg	60mmHg	80mmHg
Median	-0.11	-0.01	0.07	0.20
Range	0.44	0.34	0.58	0.56
Mean	-0.11	-0.02	0.10	0.22
Std. Deviation	0.09	0.10	0.15	0.15

There were three outliers in tHb data for 0mmHg cuff pressure, as assessed by inspection of a boxplot. Therefore, the non-parametric Friedman test was run in place of a one-way repeated measures ANOVA to determine any statistical difference in tHb median values during the four exercise sessions. Vastus lateralis tHb during the exercise was statistically significantly across the four cuff pressure conditions, $\chi^2(3) = 30.225$, $p < 0.001$. Pairwise comparisons were performed with a Bonferroni correction for multiple comparisons. Statistical significance was not seen at 0mmHg vs. 40mmHg, 40mmHg vs. 60mmHg or 60mmHg vs. 80mmHg, ($p \geq 0.240$). Statistical significance was seen all other cuff pressure comparisons ($p \leq 0.01$). Second-by-second changes to tHb during exercise sessions at each thigh-cuff pressure are plotted visually in Figure 5.5.3.

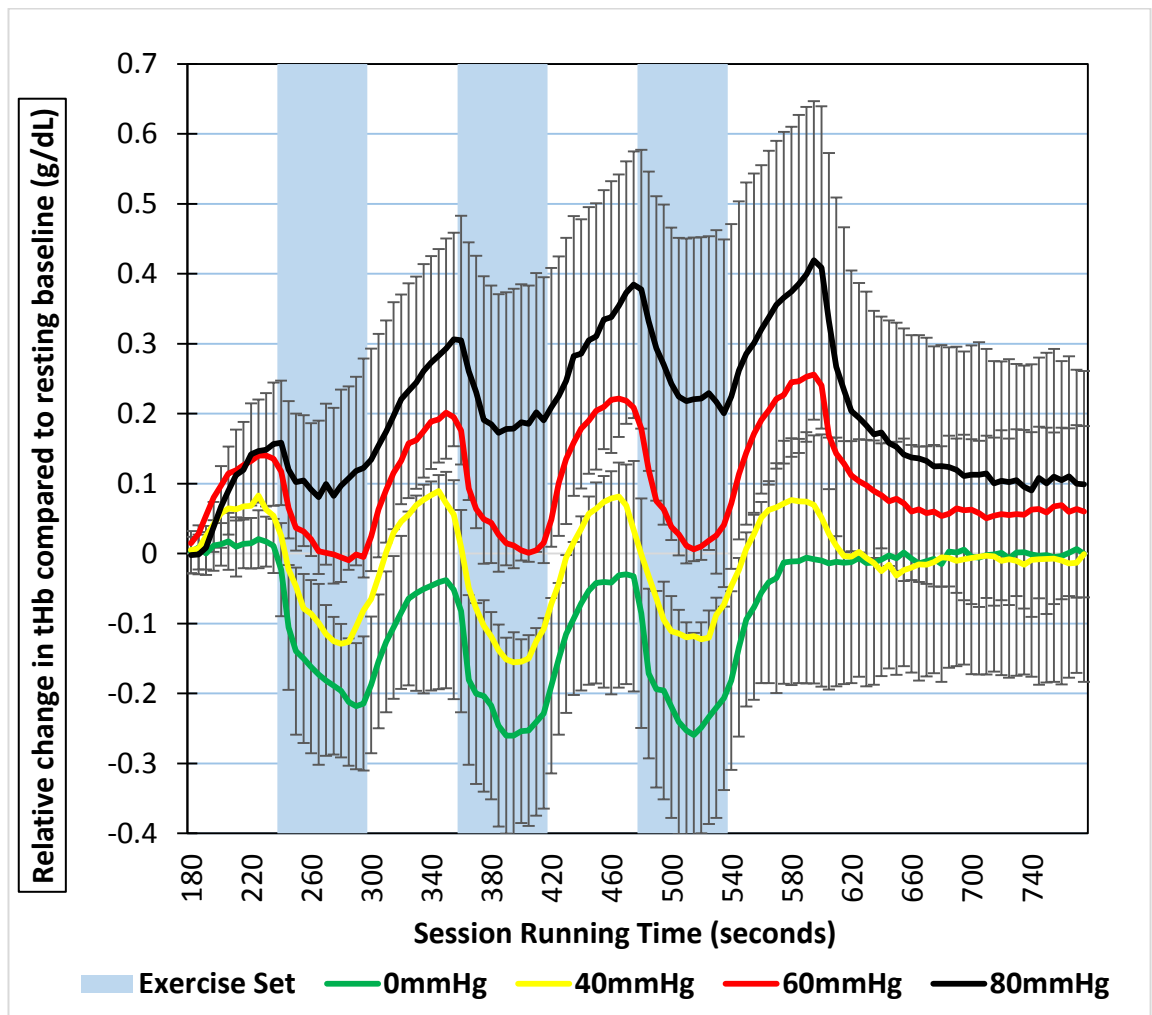


Figure 5.5.3. Changes in cohort vastus lateralis tHb across the Phase II study exercise protocol performed at different thigh-cuff inflation pressures. Mean values are displayed. Whiskers represent standard deviations. Where positive cuff pressure was applied, the thigh cuff began inflation at 180 seconds and was deflated at 600 seconds.

Cohort HHb

Data are mean (\pm standard deviation). Compared to baseline, vastus lateralis HHb decreased during the knee extension exercise performed under 0mmHg cuff pressure; -0.75 g/dL (\pm 0.77). At all other cuff pressures, vastus lateralis HHb increased during exercise compared to baselines [40mmHg; 0.15 (\pm 0.61) g/dL, 60mmHg; 0.91 (\pm 0.90) g/dL, 80mmHg; 1.64 (\pm 0.85) g/dL].

A one-way repeated measures ANOVA was conducted to determine any statistically significant difference in HHb values recorded during the four exercise sessions. There

were no outliers in the data, as assessed by inspection of a boxplot. HHb values were normally distributed, as assessed by Shapiro-Wilk's test ($p > 0.05$). Mauchly's test of sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(5) = 3.524$, $p = 0.621$. HHb values during the exercise intervention were statistically significantly different across the different cuff pressure conditions, $F(3, 45) = 47.400$, $p < 0.0001$, partial $\eta^2 0.760$). Post hoc analysis with a Bonferroni adjustment revealed that HHb values during exercise were statistically different between all four cuff pressures conditions ($p \leq 0.020$). Second-by-second changes to HHb during exercise sessions at each thigh-cuff pressure are plotted visually in Figure 5.5.4.

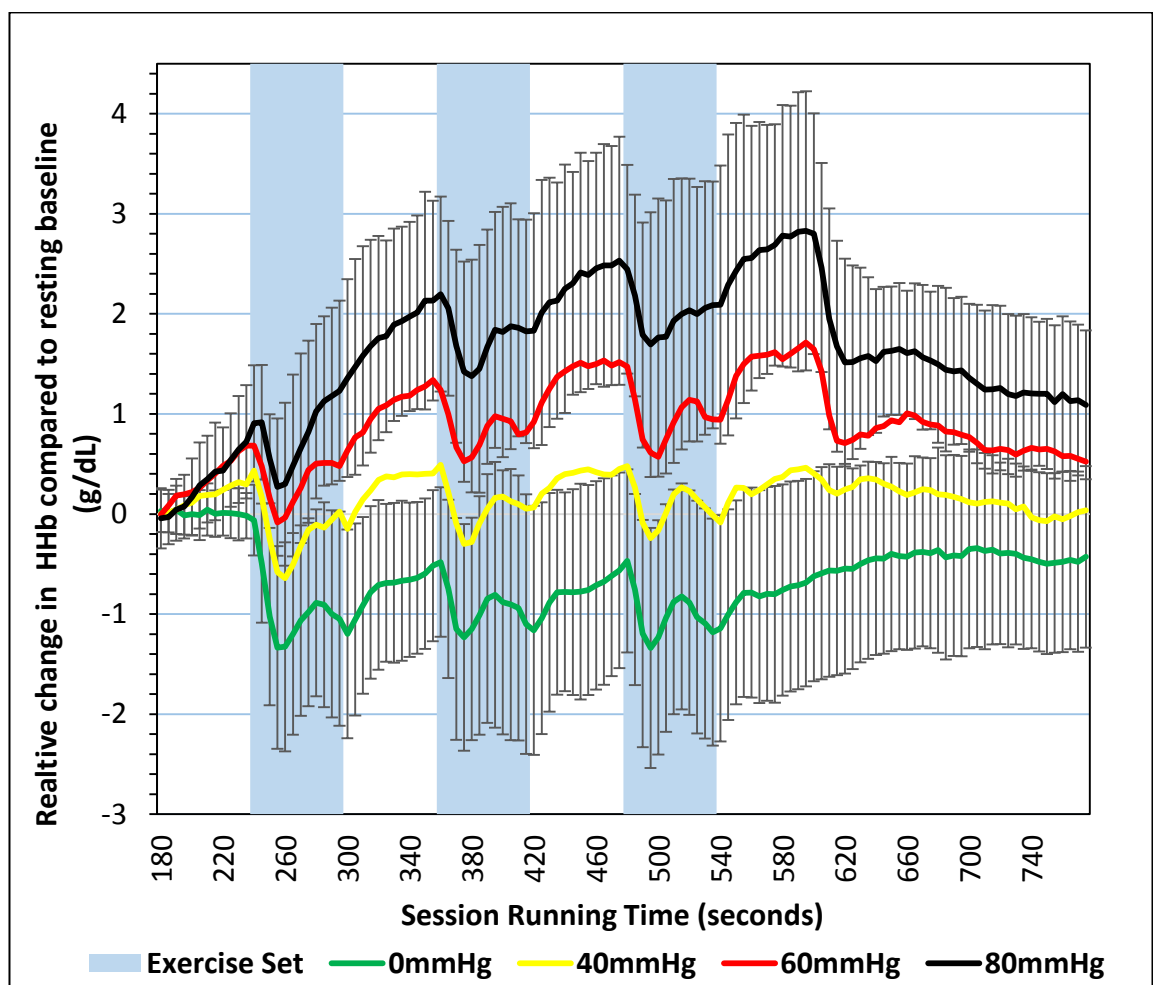


Figure 5.5.4. Changes in cohort vastus lateralis HHb across the Phase II study exercise protocol performed at different thigh-cuff inflation pressures. Mean values are displayed. Whiskers represent standard deviations. Where positive cuff pressure was applied, the thigh cuff began inflation at 180 seconds and was deflated at 600 seconds.

Cohort Heart Rate

Compared to baselines, heart rate increased slightly during all exercise sessions; 0mmHg 3.6 ± 2.8 beats per minute, 40mmHg 3.1 ± 2.5 , 60mmHg 1.8 ± 2.7 ; 80mmHg 3.2 ± 3.7 .

A one-way repeated measures ANOVA was conducted to determine any statistically significant difference in heart rate values recorded during the four exercise sessions. There were no outliers in HR data, as assessed by inspection of a boxplot. Heart values were normally distributed, as assessed by Shapiro-Wilk's test ($p > 0.05$). Mauchly's test of sphericity indicated that the assumption of sphericity had not been violated, $\chi^2(5) = 7.173$, $p = 0.210$. HR change during exercise was not statistically significantly different across the four cuff pressure conditions, $F(3, 33) = 1.013$, $p = 0.399$, partial $\eta^2 0.084$). Second-by-second changes to HR recorded during exercise sessions for each thigh-cuff pressure are plotted visually in Figure 5.5.5.

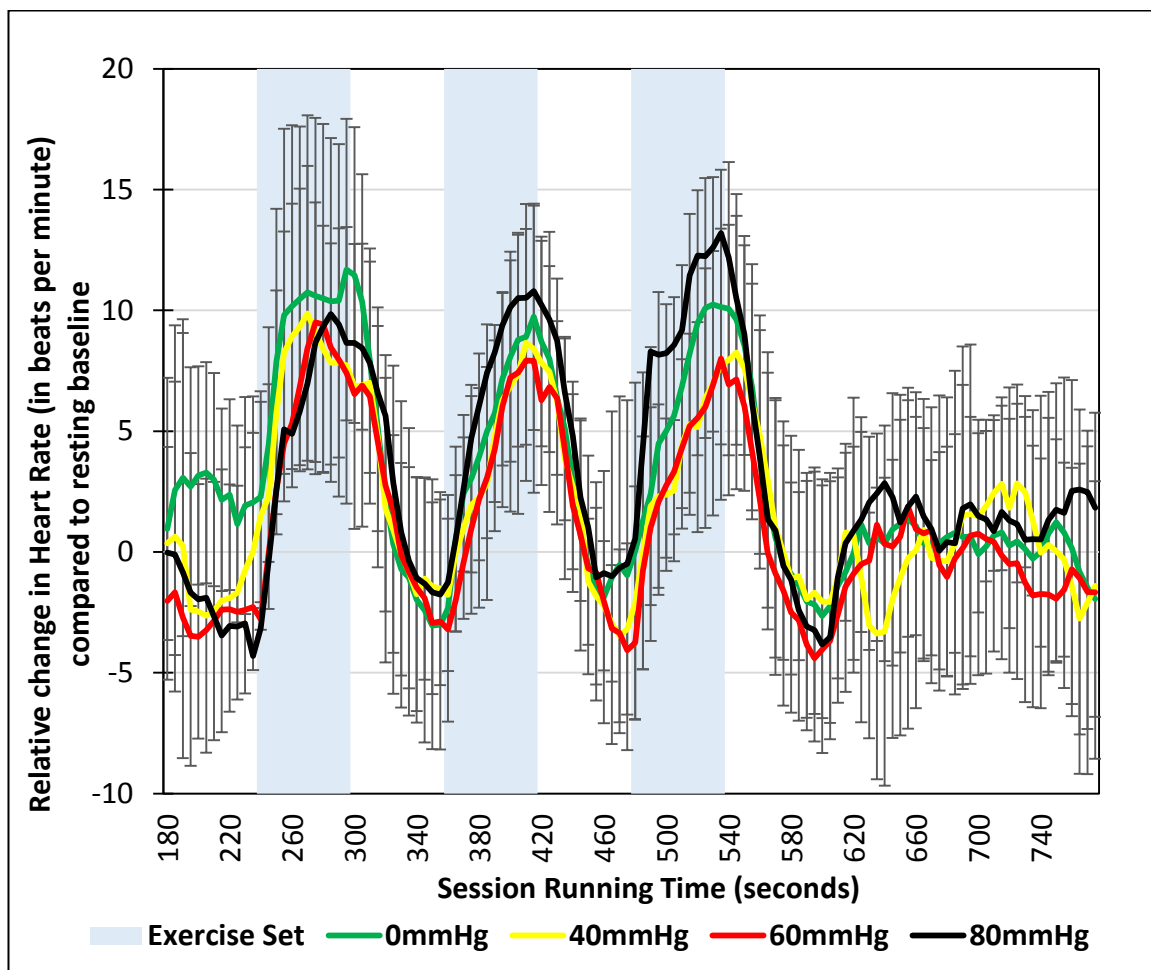


Figure 5.5.5. Changes in cohort heart rate across the Phase II study exercise protocol performed at different thigh-cuff inflation pressures. Mean values are displayed. Whiskers represent standard deviations. Where positive cuff pressure was applied, the thigh cuff began inflation at 180 seconds and was deflated at 600 seconds.

5.5.4.3 Subjective Responses

The rate of perceived exertion recorded for each exercise session [session RPE], reported via the OMNI-RES, tended to increase in the cohort as greater thigh-cuff pressure was added (Table 5.5.3).

Table 5.5.3. Session OMNI-RES* scores reported during un-resisted knee exercise at different thigh-cuff inflation pressures.

	0mmHg	40mmHg	60mmHg	80mmHg
Median	2.0	2.8	3.5	4.8
Range	4.3	4.7	6.7	6.3
Mean	2.1	2.7	3.7	4.8
Std. Deviation	1.3	1.1	1.5	1.5

* The OMNI-RES Scale is scored between 0 and 10.

There were outliers in session RPE data at 60mmHg and 80mmHg thigh-cuff pressures, as assessed by inspection of a boxplot; one participant reported unusually low RPE scores during these sessions. Therefore, the non-parametric Friedman test was run in place of a one-way repeated measures ANOVA to determine any statistical difference in session-RPE median values across the four exercise sessions. Session RPE was statistically different across the four cuff pressure conditions, $\chi^2(3) = 35.837$, $p < 0.001$. Pairwise comparisons were performed with a Bonferroni correction for multiple comparisons. Statistical significance was not seen when comparing session RPE at 0mmHg vs. 40mmHg, 40mmHg vs. 60mmHg or 60mmHg vs. 80mmHg, ($p \geq 0.120$). Statistical significance was seen when comparing session RPE at all other cuff pressure combinations ($p \leq 0.037$).

The rate of perceived exertion recorded for each exercise set (within-set RPE) numerically increased as thigh-cuff pressure was increased. There were outliers among the within-set RPE data across all three exercise sets. Therefore, a Kruskal-Wallis H test was performed for each exercise set, to determine whether within-set RPE differed significantly for each thigh-cuff pressure. Distributions of within-set RPE were statistically different between cuff pressures (Set 1, $\chi^2(3) = 25.346$, $p < 0.001$; Set 2, $\chi^2(3) = 22.903$, $p < 0.001$; Set 3, $\chi^2(3) = 18.703$, $p < 0.001$). Pairwise comparisons were performed with a Bonferroni correction. Statistical differences of within-set RPE were evident when comparing 0mmHg vs. 60mmHg during exercise set 1 ($p < 0.007$). Statistical differences were also seen when comparing 0mmHg vs. 80mmHg ($p \leq 0.005$) and 40mmHg vs. 80mmHg ($p \leq 0.005$) across all three exercise sets. All other comparisons were not statistically significant ($p \geq 0.122$).

5.5.4.3 Association with Physical Characteristics

Data for each measured physical characteristic were assessed for outliers and normal

distribution by visual inspection of boxplots and Shapiro-Wilk tests respectively. No significant outliers were present. Only diastolic blood pressure displayed a non-normal distribution (Shapiro-Wilks $p = 0.017$). Data for %PBfR, session SmO₂, session tHb and session HHb for each cuff pressure (0-80mmHg) were also reviewed. Only tHb during the control session showed outliers and a non-normal distribution (Shapiro-Wilks $p = 0.44$). Finally, values for the average change recorded for %PBfR, SmO₂, tHb, and HHb for each participant over the 3 sessions in which positive cuff pressure was applied were reviewed. These are denoted %PBfR₃, SmO₂₃, tHb₃, and HHb₃. No outliers were present. Normal distributions of data were evident throughout (Shapiro-Wilks $p \geq 0.191$). Bivariate correlations between physical characteristics and all other stated variables were then assessed by way of Pearson's correlation or Spearman's Rank as appropriate.

Physical Characteristics and their Relationship to %PBfR

At each individual cuff pressure (0-80mmHg), bivariate correlations between physical characteristics and %PBfR were infrequent. Whilst, Body Mass Index showed statistical significance at 60mmHg cuff pressure (Pearson $r = 0.593$, $p = 0.015$) and near-significance at 80mmHg (Pearson $r = 0.446$, $p = 0.083$), the study was underpowered to robustly detect correlations with an r value < 0.60 . This was also the case with thigh length at 40mmHg (Pearson $r = 0.530$, $p = 0.035$). %PBfR was not significantly correlated with physical characteristics at any other cuff pressures.

There was a statistically significant bivariate correlation between %PBfR₃ and Body Mass Index (Pearson $r = 0.606$, $p = 0.013$). A review of the scatterplot for this correlation showed that one data point may have exerted a high influence upon the Pearson r value

(Figure 5.5.6). Re-analysis without this data point produced a non-significant correlation (Pearson $r = 0.461$, $p = 0.084$).

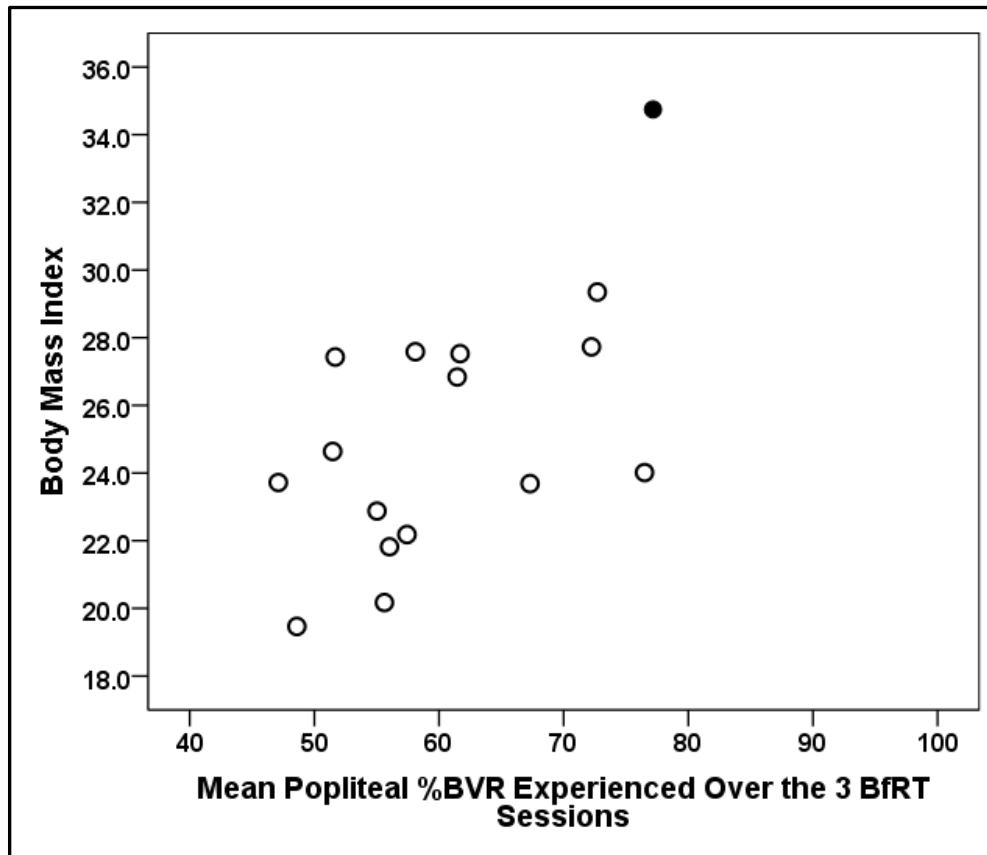


Figure 5.5.6. A scatterplot showing the bivariate association between Body Mass Index and mean %PBfR experienced over the three BfRT sessions. The outlying data point is shown in solid black.

Physical Characteristics and their Relationship to SmO₂ / tHb / HHb

During the control exercise session at 0mmHg, no statistically-significant bivariate correlations existed between any measured physical characteristic and SmO₂, tHb or HHb. During the sessions performed at each cuff pressure, bivariate inverse correlations were seen at 40mmHg (Pearson $r = -0.778$, $p < 0.001$), 60mmHg (Pearson $r = -0.604$, $p = 0.013$) and 80mmHg (Pearson $r = -0.618$, $p < 0.011$) for HHb vs. Body Mass Index. Correlations of similar strength were seen for body weight vs. HHb at 40mmHg and 60mmHg, and for thigh circumference vs. HHb at 40mmHg (Appendix IVc). These correlational findings were largely replicated for SmO₂ values versus physical characteristics (Appendix IVa). This is

understandable as SmO_2 is highly influenced by HHb, the former being partly a function of the latter. Other statistically significant correlations were found (two-tailed p value ≤ 0.05), however this study was underpowered to detect these robustly. There were significant inverse correlations between HHb3 and body weight (Pearson $r = -0.731$, $p = 0.001$), HHb3 and Body Mass Index (Pearson $r = -0.794$ $p < 0.001$) and HHb3 and thigh circumference (Pearson $r = -0.695$, $p = 0.003$). Very similar correlations were seen between SmO_2 and the same physical characteristics (Appendix IVd).

5.5.5 Discussion

5.5.5.1 The Degree of Lower-Limb Blood-flow Restriction Applied

At rest, blood flow within the popliteal artery was slightly lower compared to the Phase I cohort (Mean; 42.57 ± 20.24 ml/min vs. 67.62 ± 40.24 ml/min. Median; 36.60 ml/min vs. 53.10 ml/min). This is understandable, as from Phase I results it is known that popliteal blood flow at rest was positively correlated with physical characteristics of size.

Comparison of the two cohorts' physical characteristics shows that Phase II participants tended to be smaller in mass (Table 5.5.4), and this could explain the lower popliteal blood-flow. There was no significant correlation between resting popliteal blood flow and physical characteristics in the Phase II cohort itself. However, cohort size was comparatively small ($n=16$) compared to the Phase I cohort ($n=61$). It may be that an insufficient number of Phase II participants were tested to elucidate these correlations.

%PBfR values decreased within the cohort as the thigh-cuff pressure employed during exercise sessions was increased. However, %PBfR values were slightly lower than those seen during the Phase I study (40mmHg; 73.1% vs. 81.6%, 60mmHg; 61.1% vs. 68.2%, 80mmHg; 47.6% vs. 56.9%). In real terms, the between-cohort differences in %PBfR at

each cuff pressure equate to only a few millimetres of popliteal blood flow per minute.

Whether these differences would be nullified if additional Phase II participants were tested is unknown.

Table 5.5.4. A comparison of physical characteristics between the Phase I and Phase II study cohorts.

	Height [cm]		Weight [kg]		Body Mass Index	
	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II
Median	177.8	175.3	84.2	78.1	26.2	24.3
Min Value	148.8	158.4	52.7	53.4	19.5	19.5
Max Value	198.3	186.7	113.3	103.1	32.1	34.7
Range	50.0	28.3	60.7	49.7	12.6	15.3
Mean	177.8	173.2	81.5	76.4	25.5	25.2

5.5.5.2 The Acute Metabolic Demand of the Knee Exercise

To understand how the addition of lower-limb BfR to the un-resisted knee exercise affected SmO₂ (Figure 5.5.2), tHb (Figure 5.5.3) and HHb (Figure 5.5.4) of the vastus lateralis muscle, the change in these variables during the control session (0mmHg cuff pressure) will first be discussed.

Following the baseline resting period (0-180 seconds), tHb, HHb remained stable between 180 and 239 seconds. SmO₂ also remained unchanged, itself being a function of tHb and HHb. Stability of these three variables was expected, as the cuff remained deflated and no active movement was performed during this period. Therefore, homeostasis would have been maintained within the lower-limb. At the onset of the first exercise set (240-260 seconds) a decrease in tHb and HHb occurred, more so in HHb, leading to an initial increase in SmO₂ compared to baseline. The action of concentric quadriceps contractions would have compressed the venous vasculature of the whole thigh, creating a muscle-

pump effect that propelled blood towards the femoral vein and ultimately the heart (Sarelius and Pohl, 2010). Within the vastus lateralis itself, increased intramuscular pressure would have mechanically hindered arterial inflow by compression of microvasculature (Rådegran and Saltin, 1998). As the foot was lowered to the floor eccentrically, the vastus lateralis would have been re-perfused with oxygenated blood as intramuscular pressure reduced. A fast vasodilator response would have further increased local tissue perfusion (Korthius, 2011). The combined effects of the muscular pump and the fast vasodilation response would have increased blood flow rates through the vastus lateralis, increasing oxygen delivery rate above that of oxygen extraction and resulting in the early increase of vastus lateralis SmO_2 . As the quadriceps would have been under an amount mechanical tension in both the concentric and eccentric phase of the exercise, a degree of extravascular compression would have been present throughout the majority of each exercise repetition. It could be suggested that this enhanced venous return and would explain why the rate of blood flow through the vastus lateralis may have increased, but the actual mass of blood contained within the muscle decreased.

As the exercise continued (261-299 seconds), the decrease in tHb slowed whilst HHb stabilised, preventing a further increase in vastus lateralis SmO_2 . During this period, a degree of metabolite accumulation would have likely lowered the partial of pressure of oxygen within the exercising muscle tissue (Korthius et al, 2011). Oxygen demand of the active muscle tissue would have also risen to generate sufficient ATP to continue muscular contractions (Chiras, 2013). Oxygen diffusion across, and extraction from arterioles would have therefore increased. It could be suggested that blood flow and thus oxygen delivery to the vastus lateralis was still sufficient to meet oxygen demand, which is why stabilisation of SmO_2 occurred during this period rather than further SmO_2

reduction.

Upon completion of the first exercise set and the subsequent rest period (300-359 seconds), tHb, HHb and SmO₂ began returning to baseline levels. Oxygen demand within the vastus lateralis muscle would have decreased with a concomitant reduction in the concentration of metabolites, leading to reduced oxygen extraction rates, displayed as falling HHb and rising SmO₂ levels. Absence of the muscular pump would have likely reduced venous return through the lower-limb, leading to a temporal slowing of blood-flow clearance from the muscle tissue and a return of tHb to near-baseline levels.

Throughout the remaining exercise and rest periods, the haemodynamic patterns were repeated. It is important to note the lack of HHb build-up throughout this control session. In fact, HHb during the actual exercise session (240-600 seconds) appeared to decrease compared to baseline (-0.75 ± 0.77 g/dL) contributing to an overall increase in SmO₂ ($6.01 \pm 6.40\%$). This may indicate that the conventional un-resisted exercise session caused minimal disruption to the homeostasis of the vastus lateralis muscle tissue. Oxygen demand matched oxygen delivery through the auto-regulation of lower-limb blood flow and tissue perfusion, leading to a negligible metabolic disturbance. Without these disturbances, such as the tissue hypoxia and lactate accumulation seen during high-intensity knee extensions (Tanimoto and Ishii, 2006), exercise-induced metabolic stress to quadriceps muscle tissue may have been minimal.

In contrast to the 'no-load' exercise performed without BfR, the addition of thigh-cuff pressure altered haemodynamics within the lower-limb during exercise sessions. At the onset of cuff inflation (180-209 seconds) and the maintenance of the target cuff pressure

before the exercise began (210-239 seconds), vastus lateralis tHb increased. As described in chapter four, narrowing of large lower-limb veins at rest can occur with a little as 19mmHg extravascular pressure (Mosti et al, 2009). The extravascular compression created proximally by the thigh cuff would have compressed venous vasculature within the thigh, leading to venous congestion and a build-up of blood within the distal vastus lateralis. HHb also increased slightly during this time. It could be suggested that the oxygen extraction rate of the vastus lateralis remained stable, but the rate of blood-flow through the muscle slowed. More oxygen had to be extracted per unit of blood, leading to a slow drop in SmO₂ within the sampled muscle tissue. These effects appeared to be amplified as the thigh-cuff pressure was increased (Figure 5.5.2) (Repeated measures ANOVA; main effect of cuff pressure between 210-239 seconds upon tHb, $p < 0.0001$), but the difference in the rate and extent of tHb increase between 60mmHg and 80mmHg cuff pressure was not significant (post-hoc Bonferroni with adjustment for multiple comparisons, $p = 1.00$).

Other evidence has demonstrated that 50-60mmHg of external calf compression was sufficient to fully collapse the saphenous vein and posterior tibial vein within participants in a resting seated position (Partsch and Partsch, 2005). It may be that 60mmHg of thigh-cuff pressure was sufficient to achieve venous closure in the femoral vein during the current study, and would explain why 80mmHg did not further amplify the increase of tHb in the period prior to the first exercise set. However, further research would need to quantify venous closure via MRI scans or Doppler ultrasound of the femoral artery proximal to the thigh cuff.

At the onset of the first exercise set (240-260 seconds) both tHb and HHb decreased in a similar way to that seen during exercise without BfR. This occurred at all cuff pressures,

despite venous occlusion caused proximally by the thigh cuff. This suggests that the muscular pump effect overcame the extravascular pressure exerted proximally and still generate venous outflow during active exercise. During the remainder of the exercise set (261-299 seconds) tHb continued to decrease and then stabilise, whilst HHb began to increase. Again, in a way similar to that seen during the control session.

During the first rest period (300-359 seconds), tHb and HHb both increased, more so with HHb, leading to a continuing drop in SmO_2 despite the cessation of active muscle contractions. Absence of the muscular pump likely led to a state of venous restriction or occlusion proximally, impeding venous outflow from lower-limb and musculature distal to the cuff, including the vastus lateralis. Oxygen extraction per unit of blood passing through the sampled muscle tissue then had to increase, leading to a progressive increase in HHb and a drop in SmO_2 . These haemodynamic patterns were then replicated during the remaining exercise and rest periods (360-600 seconds).

In summary, addition of BfR at thigh pressures of 60-80mmHg caused a build-up of HHb and reduction of SmO_2 within the exercising vastus lateralis; an effect that was absent from the control session and negligible at 40mmHg cuff pressure. Indeed, reductions in SmO_2 and increases in HHb are associated with the need for anaerobic respiration and lactate accumulation within exercising lower-limb musculature (Miura et al, 2000; Tanimoto and Ishii 2006). It could be proposed that increased HHb and reduced SmO_2 levels during BfR sessions at 60mmHg and 80mmHg indicated an increased metabolic demand within the vastus lateralis, compared to the control session performed at 0mmHg cuff pressure. This was driven by the restriction of blood flow, and thus oxygen delivery, to contracting muscle fibres. Greater oxygen extraction from the blood that was

still available may have occurred to maintain the contraction of these active fibres, or to allow additional muscle fibres to take up this duty. Speculatively, this may have led to greater degrees of exercise-induced metabolic stress within the vastus lateralis overall, but further research would need to quantify this via measurements of blood lactate post-exercise, for example. BfR at 80mmHg also raised tHB progressively above baseline across the exercise session, indicating the accumulation of blood within the muscle, as seen within other studies (Cayot et al, 2016; Ganesan et al, 2015).

Heart Rate Response

Heart rate tended to increase compared to baseline during the performance of each exercise set, with a subsequent return to baseline during each minute-long rest period. The magnitude and patterns of change in HR were visually similar for the cohort at each thigh-cuff pressure (Figure 5.5.6), with HR rarely increasing by more than 20 beats per minute during exercise. Combined with a lack of statistical within-subject differences in heart rate between the four exercise sessions, it could be suggested that adding BfR to the chosen 'no-load' knee exercise did not increase the systemic cardiovascular demand of the exercise session.

5.5.5.3 Subjective Responses

In this study, the low RPE scores reported during the control exercise session may reflect the minimal metabolic demand that this type of exercise appeared to generate. By adding BfR, within-subject RPE was increased as greater cuff pressures were employed, without the reporting of any pain. It could be reasonable to presume that the addition of BfR resulted in a greater degree of acute metabolic demand via mild hypoxia and some accumulation of hydrogen protons (de Frietas et al, 2017). In turn, free nerve endings

within muscle tissue were stimulated by a change in pH to a degree that raised perceived exertion (Mense, 2008).

5.5.5.4 Associations with Physical Characteristics

A trend was seen towards body mass, and possibly thigh mass (indicated via thigh circumference) being associated with the amount of HHb that accrues within the vastus lateralis during un-resisted knee extensions performed with BfR. Several explanations for this could be suggested.

As cuff width was fixed at 21cm, smaller individuals would have experienced thigh-cuff compression across more of their thigh length than larger individuals, and had less soft tissue mass over which to dissipate this compression. Whilst thigh mass was not measured directly in Phase II to substantiate this, Phase I study data demonstrates that thigh circumference taken via tape measure is strongly, positively associated with leg mass (Pearson $r = 0.890$, $p < 0.0001$). Due to these potential differences in thigh mass, the superficial vasculature within and around the distal vastus lateralis may have been compressed to a greater degree within smaller individuals, with a greater degree of restriction of the proximally-situated femoral vein. This could have produced a comparatively greater slowing of blood through the vastus lateralis at rest in smaller individuals. More oxygen would have then been extracted per unit of blood passing through, or pooling within, the vastus lateralis. This may have amplified the magnitude by which HHb increased and SmO₂ dropped over the course of the entire exercise session.

Larger individuals also had greater lean tissue mass within their lower-limb than smaller individuals. This can be demonstrated from Phase I study data, where lean leg mass

recorded via DXA was positively associated with thigh circumference taken by tape measure (Pearson $r = 0.716$, $p < 0.0001$) and Body Mass Index (Pearson $r = 0.680$, $p < 0.0001$). As lean mass includes muscle tissue, larger individuals may have had a greater number of thigh muscle from which to draw from during the 'no-load' exercise. Thus, at such a low-intensity of exercise, larger Phase II individuals may have had to recruit a lower proportion of their available thigh muscle fibres to generate un-resisted knee extensions than smaller individuals, leading to comparatively lower oxygen uptake over the course of an exercise set. In addition, if less compressive force was generated by the cuff per cubic unit of thigh muscle in larger individuals, then the intravascular pressure generated by the contraction of thigh musculature distal to the cuff may have been better able to overcome the extravascular pressure exerted proximally by the cuff. This would have allowed a greater percentage of blood to exit the vastus lateralis (and the thigh as a whole) during exercise periods. Ultimately, better maintenance of blood throughput and reduced muscle fibre recruitment could have blunted the rise in HHb and drop in SmO₂ within larger individuals over the course of the entire exercise session.

Initially, there was a significant bivariate correlation between the average %PBfR experienced by participants across the experiment's three BfR session and Body Mass Index within the Phase II cohort (Pearson $r = 0.606$, $p = 0.013$). This was in direct contrast to the findings of the Phase I study, which demonstrated no significant correlation between average %PBfR and Body Mass Index (Pearson $r = 0.226$, $p < 0.08$). It is presumed that the strength of the correlation seen in the Phase II cohort was misleading as the data contained one highly-influential data point. The removal of which produced a non-significant correlation (Pearson $r = 0.461$, $p = 0.084$). This is further substantiated by the absence of other physical characteristics displaying robust correlations with average

%PBfR, and the lack of Body Mass Index showing significant correlations with %PBfR recorded at each individual thigh-cuff pressure. It is possible that physical characteristics of mass have some minor association with the %PBfR experienced at rest, before exercise. Determining this, however, would take a substantial number of additional participants to elucidate.

5.5.6 Study Strengths, Limitations and Future Research Directions

The changes to tHb, HHb and SmO₂ seen in the distal vastus lateralis during BfR may not be indicative of what was occurring within quadriceps muscle tissue physically under compression by the thigh cuff. It was impossible to quantify this with the equipment used. It is known that tissue oxygenation of the vastus lateralis can vary depending upon the sampling location (Kennedy et al, 2006; Miyamoto et al, 2013). Work by Sjøgaard et al (1986) also found that variations in knee-extensor intramuscular pressure during 5% MVC knee-extensions was related to alternating recruitment of various parts of the knee-extensors as the exercise progressed. This may induce heterogeneity in blood flow and oxygenation within and among each contracting quadricep, further complicating matters in respect of repeatable measurements. Further studies could modify the thigh cuff to incorporate a transparent window may allow NIRS to be used to detect changes to tHb, HHb and SmO₂ under the cuff itself. This could be combined with the use of additional NIRS monitors (placed on the vastus medialis, gastrocnemius or hamstrings for example) to address and further investigate variations in lower-limb tissue oxygenation during exercise, both with and without BfR.

The order in which cuff pressures were delivered were not randomised. To the author's knowledge, the blood-pressure cuff utilised within the Phase II study was wider than

restriction devices implemented within previous BfRT literature. A progressive, sequential increase in cuff pressure over several sessions was also common practice in Warrington Wolves Rugby League Club and implemented practically within longitudinal BfRT studies (Corvino et al, 2014; Iversen et al, 2015; Sakamaki et al, 2011; Takarada et al, 2000b). It therefore made ethical and clinically-relevant sense to introduce cuff pressures in increasing order, rather than randomly. It is acknowledged that this method may have potentially confounded RPE scores, due to participants becoming accommodated to blood-flow restricted exercise and thus diminishing the RPE scores reported at the higher cuff pressures.

Further objective measures may have provided a more complete picture of the actual metabolic stress occurring during and after 'no-load' exercise sessions, both with and without BfR. Whilst drops in muscle tissue oxygenation of the vastus lateralis are related to an increase in lactate (Tanimoto and Ishii, 2006), the presence of lactate or other metabolites was not directly measured within the Phase II study. If the study were to be repeated, the measurement of post-exercise lactate levels via a blood sample may strengthen the proposed link between drops in tissue oxygenation during blood-flow restricted exercise and exercise-induced metabolic stress. LiBfRT has previously been shown to alter post-exercise gene expression, increasing muscle protein synthesis and causing inhibition of catabolic cellular signalling pathways (Fry et al, 2010; Fujita et al, 2007; Laurentino et al, 2012). Therefore, the use of muscle biopsies to quantify whether this occurs following 'no-load' BfRT would be both favourable and warranted in future.

Finally, modifying the training protocol in future research to increase cuff pressure beyond 80mmHg and/or increasing the duration of the exercise sets to achieve volitional

failure, may produce greater drops in tissue oxygenation and a greater magnitude of exercise-induced metabolic stress to occur during 'no-load' exercise. If this can be achieved within injured individuals particularly, the likelihood of the exercise generating a longitudinal attenuate of muscle strength and size loss during impaired weight-bearing periods may well be improved.

5.5.7 Conclusions

In summary, this study looked to observe and examine the acute haemodynamic and perceptual responses to a 'no-load' knee exercise upon a cohort of healthy individuals, both with and without BfR applied. The conventional 'no-load' knee exercise session appeared insufficient to produce drops in tissue oxygenation of the distal vastus lateralis. Arguably, this translates to the ineffectiveness of such an exercise to produce an acute, local metabolic stress within the exercising muscle of the tested population. However, adding BfR at thigh-cuff pressures of 60-80mmHg altered lower-limb haemodynamics and increased perceived exertion, whilst producing statistically-significant reductions in tissue oxygenation of the vastus lateralis across exercise and rest periods. Therefore, the null hypothesis of this study is rejected, and the alternative hypothesis is accepted.

it is proposed that adding BfR to a 'no-load' lower-limb exercise increases the acute metabolic (and perceived) demands of the exercise, without the need to alter other parameters such as the addition of an external load. This proposal may be of significant importance to HCPs if this type of exercise can also produce a degree of metabolic stress that stimulates muscle protein synthesis or attenuates muscle protein degradation. 'No-load' BfRT could be used as a treatment adjunct during lower-limb injury rehabilitation to attenuate muscle atrophy and strength loss during periods where resistance exercise is

contraindicated or impossible. During blood-flow restricted exercise, there may be an association between individuals' physical characteristics of mass and the magnitude of change they experience in the chosen indicators of acute metabolic stress. This may indicate a need to modulate cuff pressure, delivering higher pressures to larger individuals, to achieve more consistent results across populations. Further research is recommended to directly measure the acute haemodynamic effect upon musculature directly under the thigh cuff, and the quantification of metabolic stress through methods additional to NIRS.

The next chapter will discuss the implementation and outcomes of using 'no-load' BfRT within the rehabilitation programmes of a case series of athletes with lower-limb injuries.

CHAPTER SIX

PHASE THREE

The Application of Lower-limb BfRT among Injured Elite Rugby-League Players: A Real-world Case Series

6.1 Chapter Introduction

This chapter details the third phase of this doctoral research project, describing the implementation and outcomes of a pilot study involving one elite rugby-league player, followed by a subsequent case series of three players. Results have been accepted for presentation at the national Chartered Society of Physiotherapy conference (Physiotherapy UK) in November 2017.

6.2 Phase III Aim

To examine the haemodynamic, perceptual and physical responses to the application of lower-limb BfRT programmes among a case-series of participants recovering from a significant lower-limb musculoskeletal injury

6.3. Phase III Pilot Study

6.3.1 Pilot-specific Objectives

To pilot the suitability of a BfRT protocol for use in the main Phase III study. Specifically;

1. To observe whether changes in vastus lateralis muscle SmO_2 , haemoglobin and heart rate levels could be detected during an unweighted knee extension exercise, if thigh-cuff pressures of up to 120mmHg were applied to an exercising lower-limb.

2. To determine whether the exercise protocol described in chapter 3.5 is physically achievable under 120mmHg thigh-cuff pressure, without reaching the agreed cut-off values for subjective levels of pain (≥ 40 out of 101).

6.3.2 Methods

The existing evidence-based BfRT protocol used among injured Warrington Wolves Rugby League Club players involved stepped increases in the degree and duration of BfR applied over several rehabilitation sessions (Table 6.3.1). A maximum thigh-cuff pressure of 130mmHg was employed as existing evidence suggested that this was pressure was sufficient to interrupt the pulse within the tibial artery at rest (Laurentino et al, 2008). Thus, pressures higher than this may have had no additional occlusive effect upon players. Progression through the protocol was dependent upon injury status and the completion of the previous BfRT session without pain or indicators of potentially-adverse events (such as prolonged numbness or altered lower-limb sensation). Five minutes of BfR at the target cuff pressure, without exercise, also preceded each of the sessions described in Table 6.3.1. If this five-minute pre-session BfR period made the subsequent BfRT session too difficult to tolerate, it was moved to occur after the BfRT session instead. The parameters of the seated knee exercise used at the club were retained by the researcher and are described in chapter 3.5. The stepped nature of the BfRT protocol was also retained. Maximum cuff pressure was reduced from 130mmHg to 120mmHg to reduce the potential occurrence of full arterial occlusion, as full arterial occlusion is now considered to be unnecessary during BfRT (Laurentino et al, 2012; Pope et al, 2013). To further simplify the protocol, the five-minute period of resting BfR applied before or after the exercise session was abandoned. These revisions produced the BfRT pilot protocol described in Table 6.3.2.

Table 6.3.1. The existing stepped blood-flow restriction training protocol in place at Warrington Wolves Rugby League Club at the commencement of the project.

Session Number	Target Cuff Pressure	Exercise Sets/Duration	Inter-set Rest Period	Cuff deflated between sets?
1	100mmHg	3 x 1 minute	1 minute	Yes
2	100mmHg	3 x 1 minute	1 minute	Yes, for 30 seconds of the rest period
3	100mmHg	3 x 1 minute	1 minute	Yes, for 30 seconds of the rest period
4	130mmHg	3 x 1 minute	1 minute	Yes, for 30 seconds of the rest period
5+	130mmHg	3 x 1 minute	1 minute	No

Table 6.3.2. The revised BfRT protocol used within the Phase III pilot study.

Session Number	Cuff Pressure	Sets/Duration	Inter-set Rest Period	Cuff deflated between sets?
1	0mmHg	3 x 1 minute	1 minute	N/A
2	100mmHg	3 x 1 minute	1 minute	Yes
3	100mmHg	3 x 1 minute	1 minute	No
4	120mmHg	3 x 1 minute	1 minute	Yes
5+	120mmHg	3 x 1 minute	1 minute	No

Following informed consent and completion of the project's health screening questionnaire, one professional male rugby player participated in the Phase III pilot study (age; 28.0 years, height; 1.87m, weight; 101.3kg). Whilst no acute lower-limb injuries were present, a left posterior cruciate ligament reconstruction and femoral condylar micro-fracture had been performed twenty-two weeks earlier, from which the player had recovered well. The player was due to undergo two separate shoulder arthroscopies in the weeks following recruitment into the pilot study, followed by appropriate upper limb rehabilitation programmes.

The player was selected despite the lack of an acute lower-limb injury due to being contraindicated from normal team training programmes for several weeks per upper-limb surgery. This allowed initial piloting of the lower-limb BfRT protocol within the context of a daily rehabilitation routine, without risking reoccurrence or exacerbation of an acute lower-limb injury.

Before each exercise session the participant was fitted with a 21cm wide thigh-cuff [MDF2090471; MDF Instruments®, California, USA] around their upper thigh and seated in the test position described in chapter 3.3.2. A near-infrared spectroscopy device [Moxxy Monitor, Fortiori Design LLC; Minnesota, USA] was affixed to the right distal vastus lateralis to record microvascular tissue oxygenation [SmO_2], total haemoglobin mass [tHb] and deoxygenated haemoglobin mass [HHb]. A wireless heart rate monitor was affixed around the chest of the participant to record heart rate [HR]. The participant maintained the resting seated position for three minutes to obtain baseline values for vastus lateralis SmO_2 , tHb, HHb and heart rate. The participant then completed three sets of the seated, unweighted, unilateral knee extension exercise as described in chapter 3.5. SmO_2 , tHb, HHb and HR values were recorded continuously during the exercise and for four minutes after completion of the last exercise set. Immediately after each exercise set, any subjective pain experienced during the previous set was recorded via a numerical rating scale (Appendix Ic). Rate of perceived exertion for the previous set of exercise was recorded using the OMNI-RES scale (Appendix Ie).

During the first exercise session the thigh cuff remained deflated. During all subsequent sessions, the thigh cuff was inflated to reach a preselected pressure (100 or 120mmHg) thirty seconds before the beginning of the first exercise set. The cuff was either deflated

during each inter-set rest period, or remained inflated throughout the whole exercise session, depending upon the session number (Table 6.3.2).

6.3.3 Results

All five exercise sessions were completed without adverse or unexpected events. Mean resting values for vastus lateralis SmO₂, tHb, HHb and HR across the pilot study are shown in Table 6.3.3. The coefficient of variation in resting vastus lateralis SmO₂, tHb, HHb and HR values for each of the four sessions are shown in Table 6.3.4. The mean change to vastus lateralis SmO₂, tHb, HHb and HR during each exercise session are shown in Table 6.3.5. Second-by-second values for the outcome measures reported in Table 6.3.5 were plotted graphically to visualise between-session differences in regards to vastus lateralis SmO₂ (Figure 6.3.1), heart rate (Figure 6.3.2). Rates of perceived exertion for each exercise set are shown in Figure 6.3.3. The participant reported no pain during any exercise session.

Table 6.3.3. Mean values recorded during the three-minute, pre-exercise baseline periods of the Phase III pilot study.

Outcome Measure	Mean Baseline Value	Standard Deviation
SmO ₂ [%]	43.50	6.67
Total Haemoglobin Mass [g/dL]	12.61	0.12
Deoxygenated Haemoglobin Mass [g/dL]	5.96	0.90
Heart Rate [Beats Per Minute]	66.45	3.38

Table 6.3.4. The coefficient of variation in values taken over each three-minute, pre-exercise baseline period during the Phase III pilot study.

Outcome Measure	Coefficient of Variation %				
	0mmHg	100mmHg IM	120mmHg IM	100mmHg C	120mmHg C
SmO ₂ [%]	7.12	4.97	6.12	5.27	4.37
Total Haemoglobin Mass [g/dL]	0.20	0.23	0.19	0.18	0.32
Deoxygenated Haemoglobin Mass [g/dL]	3.99	4.04	5.34	3.10	4.67
Heart Rate [Beats Per Minute]	6.25	2.99	3.79	5.39	4.41

C = Continuous BfR, IM = Intermittent BfR.

Table 6.3.5. The mean change in outcome measure values over the course of each exercise session, compared to pre-exercise baseline values, during the Phase III pilot study.

Outcome Measure	Amount of Thigh-cuff Pressure Applied and Mode of Application				
	0mmHg	100mmHg IM	120mmHg IM	100mmHg C	120mmHg C
SmO ₂ [%]	+ 5.99	- 6.16	- 8.13	- 15.63	- 19.80
Total Haemoglobin Mass [g/dL]	- 0.05	+ 0.15	+ 0.25	+ 0.30	+ 0.42
Deoxygenated Haemoglobin Mass [g/dL]	- 0.79	+ 0.87	+ 1.17	+ 2.22	+ 2.78
Heart Rate [Beats Per Minute]	+ 0.88	+ 0.31	+ 1.92	+ 4.61	+ 6.19

C = Continuous BfR, IM = Intermittent BfR. The '+' and '-' symbols denote the direction of change compared to that session's pre-exercise baseline period.

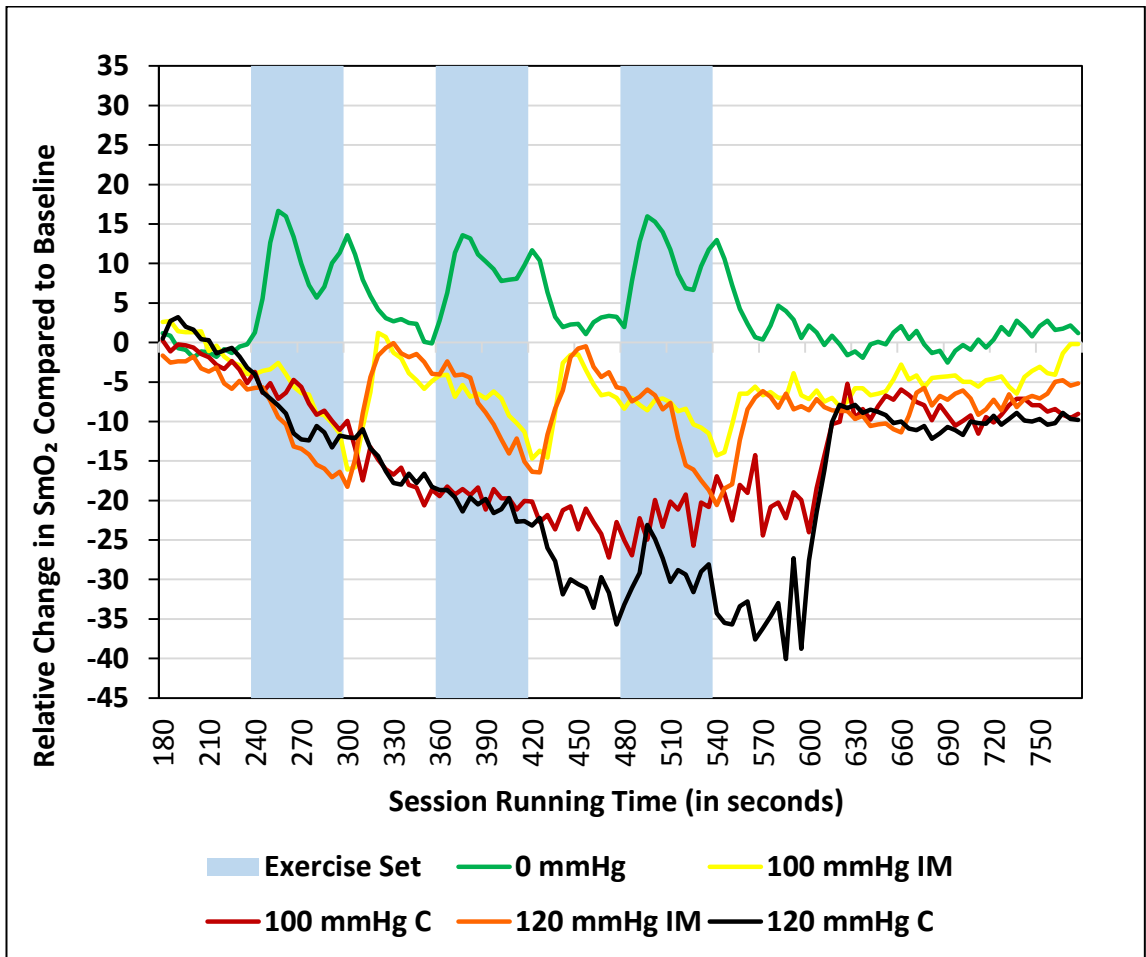


Figure 6.3.1. A graphical representation of in-session changes to vastus lateralis SmO₂ during the Phase III pilot study. The three-minute baseline period (0-180 seconds) is not shown. C = Continuous BfR, IM = Intermittent BfR.

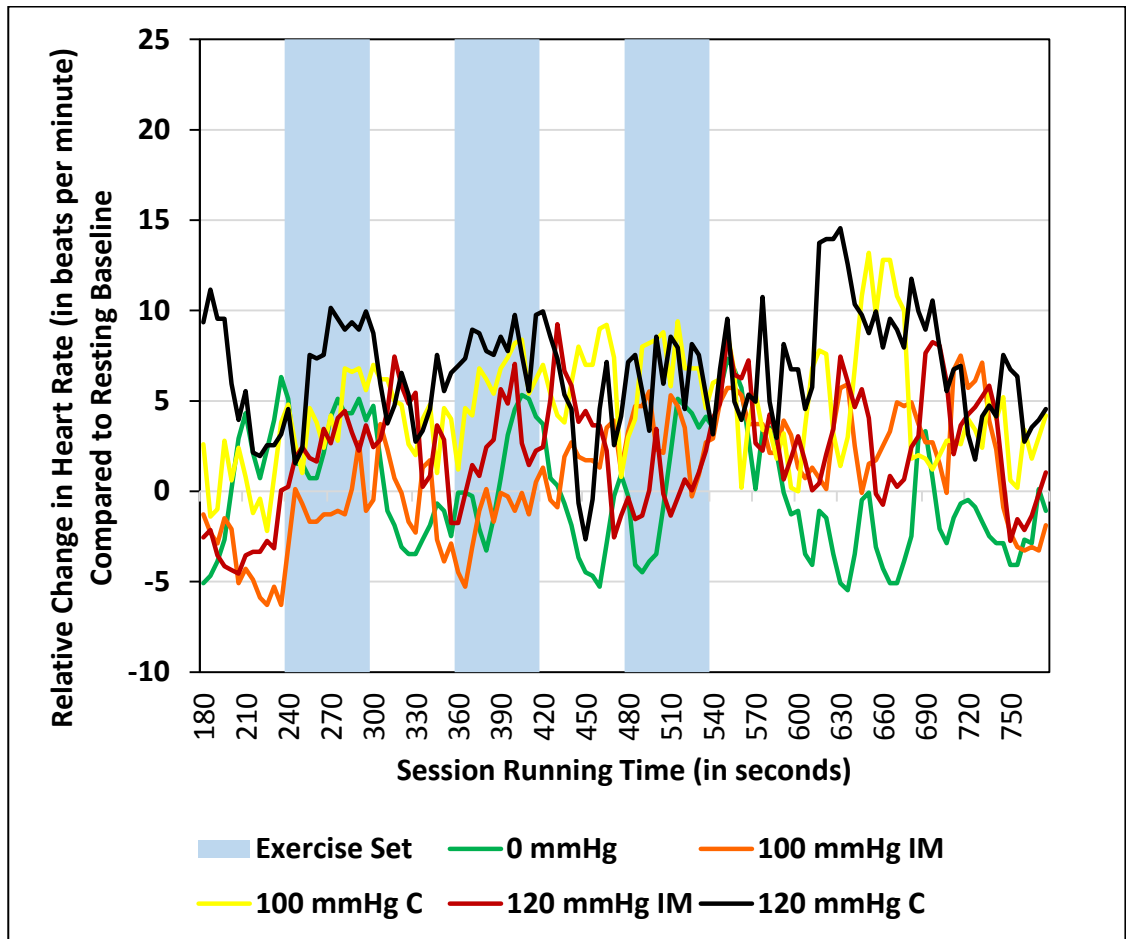


Figure 6.3.2. A graphical representation of in-session changes to heart rate during the Phase III pilot study. The three-minute baseline period (0-180 seconds) is not shown. C = Continuous BfR, IM = Intermittent BfR.

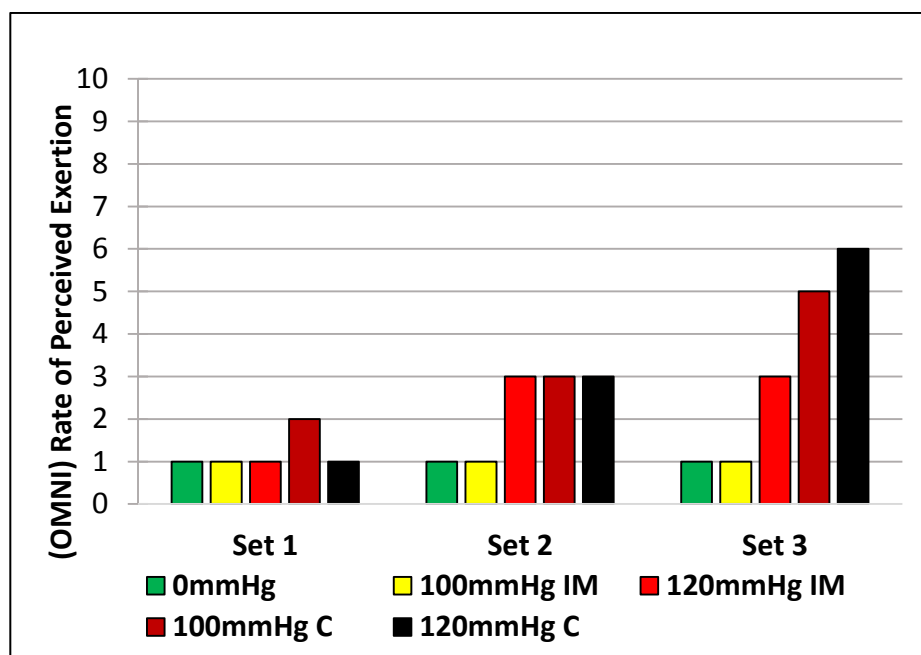


Figure 6.3.3. Set-by-set OMNI-RES scores recorded during exercise sessions of the Phase III pilot study. C = Continuous BfR, IM = Intermittent BfR.

6.3.4 Discussion

The mean values recorded during pre-exercise baseline periods of the Phase III pilot were similar to those seen in the Phase II pilot in respect of vastus lateralis SmO₂, tHb, HHb and HR. The overall effect of applying continuous thigh-cuff pressure during unweighted knee extensions upon NIRS data, namely the direction of in-session change in SmO₂, tHb and HHb, is similar between the Phase II and III pilot participants. Thus, it could be proposed that the mechanisms generating these changes were also similar. However, the magnitudes of change in SmO₂, tHb and HHb during exercise were higher in the Phase III participant at 100mmHg and 120mmHg compared to the Phase II participant at 60mmHg and 80mmHg.

The rate of perceived exertion scores indicated that the addition of BfR to the participant's knee extension increased the perceptual effort of the exercise. Despite the use of higher cuff pressures, the Phase III pilot participant tended to record lower perceived exertion scores than the Phase II pilot participant. This could arguably be due to differences in pilot participants' occupations and typical training modalities providing different references of perceived exertion from which to base their scores.

6.3.5 Conclusions and Protocol Adjustments

This study looked to pilot the suitability of a BfRT protocol for use within the main Phase III study. The protocol demonstrated the ability to alter popliteal blood flow and vastus lateralis muscle SmO₂, tHb and HHb values across thigh-cuff pressures up to 120mmHg. Applying a stepped protocol consisting of intermittent or continuous lower-limb BfRT, in a very similar way to that previously applied by Warrington Wolves, did appear to induce progressively greater exercise-induced metabolic stress in this pilot participant, without

changing the parameters of the exercise itself. The BfRT sessions were demonstrated to be safe and tolerable.

In consideration of the pilot study findings, only one adjustment was made in advance of the main study. Of the protocol shown in Table 6.3.5, session 3 (100mmHg applied continuously) appeared to produce greater changes in SmO₂, tHb and HHb than session 4 (120mmHg applied intermittently). Therefore, these sessions were swapped to ensure that other participants experienced a more consistent increase in session-difficulty as they progressed through the protocol.

6.4 Phase III Main Study

6.4.1 Main Study Objectives

1. To examine the acute haemodynamic and perceptual responses to four different grades of lower-limb BfR, applied during an unweighted 'no load' knee exercise.
2. To document longitudinal changes to thigh girth and subjective function during the application of rehabilitation programmes incorporating lower-limb BfRT.

6.4.2 Methods

The CARE reporting guidelines for case reports were consulted, to aide completeness and transparency of reporting across this case series (Riley et al, 2017). Following informed consent, three male elite rugby-league players agreed to incorporate lower-limb BfRT into their injury rehabilitation programmes. Players were screened using the bespoke Health and Eligibility Questionnaire (Appendix Ia). Consultation with the Club's performance team was also undertaken to ensure the use of BfRT was not contraindicated. Each player

had experienced a unilateral, lower-limb musculoskeletal injury within the last thirty days which continued to prevent normal training their ability to fully weight bear.

Every exercise session consisted of three sets of unweighted, seated, unilateral knee extensions whilst sat upon a height-adjustable plinth (chapter 3.5). The order in which each leg was trained (injured or uninjured) was alternated at each session. The extent of BfR applied to the lower-limb was increased incrementally over several exercise sessions. This was achieved by modulating the amount of cuff pressure applied and whether the cuff was deflated during inter-set rest periods (Table 6.4.1).

Table 6.4.1. The staged BfRT protocol used within the Phase III study. CON = Control, C = Continuous BfR, IM = Intermittent BfR.

Session Number	Session Code	Thigh-cuff Pressure [mmHg]	Number of Exercise Sets	Inter-set Rest Period	Thigh Cuff deflated between sets?
1	CON	0	3 x 1 minute	1 minute	N/A
2	100 IM	100	3 x 1 minute	1 minute	Yes
3	100 C	100	3 x 1 minute	1 minute	No
4	120 IM	120	3 x 1 minute	1 minute	Yes
5	120 C	120	3 x 1 minute	1 minute	No

Upon completion of the fifth session without incident, players repeated this session four to five times per week until it was deemed that BfRT was no longer required. For example, upon the commencement of normal weight bearing and/or traditional low-intensity resistance training.

6.4.2.1 Lower-limb Measurements

Thigh girth of both lower limbs were recorded at fortnightly intervals by the primary researcher. From a relaxed, supine position, a flexible tape measure was used to determine thigh girth at a point 40% distal from the ASIS to the superior pole of the patella, and from the widest portion of the vastus medialis oblique muscle. Each measurement was taken in triplicate to the nearest 0.5cm and a mean measurement then determined. Fortnightly mean measurements were used to monitor changes thigh girth between and within legs as an indicator of potential thigh-muscle atrophy (Lejkowski and Pajaczkowski, 2011).

6.4.2.2 Haemodynamic Variables

A near-infrared spectroscopy [NIRS] device [Moxy Monitor, Fortiori Design LLC; Minnesota, USA] was applied to the distal vastus lateralis of the leg to being trained prior to commencement of an exercise session (Figures 3.3.14 and 3.3.15, page 103). The NIRS device recorded tissue oxygen saturation percentage [SmO_2], total haemoglobin mass [tHb] and deoxygenated haemoglobin mass [HHb] within the vastus lateralis muscle. Heart rate [HR] was recorded via a wireless chest strap [Garmin International; Kansas, USA] fitted at the level of the participant's xiphisternum. Before the knee exercise commenced, resting HR, SmO_2 , tHb and HHb were continuously recorded over a three-minute baseline period. Measurements of HR, SmO_2 , tHb and HHb were then continuously recorded throughout the exercise and rest periods. Mean values recorded during the exercise were then subtracted from mean values taken during the baseline period, to provide a direction and magnitude of exercise-related change in each haemodynamic variable.

6.4.2.3 Subjective Outcome Measures

Players were familiarised with a numerical rating scale to record pain [NRS Pain] (Appendix 1c) and the OMNI-RES scale (Roberston et al, 2003) to record rate of perceived exertion [RPE] (Appendix 1e) prior to each exercise session. NRS Pain and RPE scores were recorded by the player immediately after the completion of each exercise set. Scores were collated to provide mean and NRS Pain and RPE scores for each session listed in Table 6.4.1. To provide a subjective measure of lower-limb function, each player also completed a Lower Extremity Functional Scale [LEFS] (Binkley et al, 1999) at fortnightly intervals.

6.4.3 Results

6.4.3.1 Case One

A player [age; 26.7 years, height; 185cm, weight 106.3kg] experienced a tibia and fibula fracture of the left leg during competitive match play. Surgical fixation was performed within twenty-four hours of the injury, followed by immobilisation of the limb via a rigid cast. The cast was removed one week post injury and replaced with a removable boot. Clot-prevention medication was administered daily for thirty days following surgery. A range of therapeutic interventions formed the player's initial lower-limb rehabilitation programme, including soft tissue mobilisation, neuromuscular electrical stimulation, therapeutic ultrasound and passive joint mobilisation. BfRT was introduced at four weeks post-injury, where baseline limb measurements and a LEFS score were obtained by the researcher. Upon initiation of BfRT the player remained non-weight bearing upon the injured limb and ambulated using elbow crutches.

Lower-limb BfRT was delivered four to five times per week for 12 continuous weeks, with

an intermission in the third week due to player vacation. The initial seven BfRT sessions were supervised by the primary researcher. Remaining BfRT sessions were supervised by the researcher or a member of the Club's performance team.

The player was permitted to fully-weight bear without a boot at eight weeks post-injury. Separate to BfRT, cardiovascular conditioning, resistance exercise and agility work were gradually introduced as recovery allowed. No adverse or unexpected events occurred during the BfRT protocol. Longitudinal lower-limb measurements and LEFS scores are displayed in Table 6.4.2. The player returned to first-team competitive fixtures approximately fifty-four weeks after the initial injury.

Table 6.4.2. Longitudinal thigh girth measurements and LEFS scores; Phase III, case one.

BfRT protocol length	Baseline (0 weeks)	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks		
Time since injury	4 weeks	6 wks	8 wks	10 wks	12 wks	14 wks	16 wks	20 wks	25 wks
Thigh Girth (in centimetres)									
Injured Limb	65.5	66	67.5	68.5	68.5	69.5	69.0	68.0	68.5
Uninjured Limb	67.5	67.5	68	68.5	69.0	69.5	69.5	68.5	69.5
Vastus Medialis Oblique Girth (in centimetres)									
Injured Limb	44.5	46.0	47.5	48.5	48	47.5	48	49.0	48
Uninjured Limb	48.0	48.0	48.0	49.0	49.0	49.5	49.5	49.5	49.5
Lower Extremity Functional Scale									
LEFS Score	6	29	49	53	61	69	64	68	73

6.4.3.2 Case Two

A player [age; 26.4 years, height; 178cm, weight; 92.4kg] experienced an Achilles tendon rupture of the left lower leg during competitive play, followed by a surgical repair of the

tendon. A removal boot with a heel wedge was applied and ambulation was permitted using elbow crutches. Clot-prevention medication was administered daily for thirty days following surgery. A range of therapeutic interventions formed the player's initial lower-limb rehabilitation programme, including soft tissue mobilisation, neuromuscular electrical stimulation and active range-of-movement exercises.

Lower-limb BfRT was performed four to five times per week for 10 weeks, commencing at five weeks post-injury. BfRT was not applied during weeks eight and nine due to player vacation. The initial six BfRT sessions were supervised by the primary researcher.

Remaining BfRT sessions were supervised by the researcher or a member of the Club's performance team.

Full weight bearing without the boot was achieved at ten weeks post-injury. Separate to BfRT, cardiovascular conditioning, isometric and isotonic resistance exercise and agility work were gradually introduced as recovery allowed. No adverse events occurred during the BfRT protocol. During session five, the player reported mild tingling in his left (injured) foot at the end of the final set of the exercise. This resolved immediately upon deflation of the thigh cuff. Longitudinal thigh girth measurements and LEFS scores are displayed in Table 6.4.3. The player returned to first-team competitive fixtures approximately fifty-two weeks after the initial injury, at the beginning of the following season.

Table 6.4.3. Longitudinal thigh girth measurements and LEFS scores; Phase III, case two.

BfRT protocol length	Baseline (0 weeks)	2 wks	4 wks	6 wks	8 wks	10 wks		
Time since injury	5 weeks	7 wks	9 wks	11 wks	13 wks	15 wks	19 wks	23 wks
Thigh Girth (in centimetres)								
Injured Limb	61.5	63.0	64.0	64.0	-	63.5	64.0	64.5
Uninjured Limb	63.5	64.5	64.5	65.5	-	64.5	65.0	65.5
Vastus Medialis Oblique Girth (in centimetres)								
Injured Limb	41.0	40.5	40.5	40.5	-	40.5	41.0	42.0
Uninjured Limb	41.5	41.0	40.5	41.0	-	40.5	41.0	42.0
Lower Extremity Functional Scale								
LEFS Score	28	35	38	41	-	52	51	54

6.4.3.3 Case Three

A player [age; 24.6 years, height; 188cm, weight; 102.1kg] experienced pain in the left knee during the latter stages of rehabilitation from a gross ankle dislocation and high fibula fracture. Following medical review and magnetic resonance imaging [MRI], the player was diagnosed with an osseous stress injury below the left medial tibial plateau (Grade II Fredericson) with patchy bone oedema within the medial femoral condyle. The player was barred from heavy resistance training and impactful activities for four weeks to prevent progression of the osseous stress injury and to allow the bone oedema to resolve. Full weight bearing without crutches was permitted when ambulating.

BfRT commenced immediately and was performed 4 to 5 times per week for four weeks. No adverse events occurred during the BfRT protocol. A range of additional therapeutic interventions formed the player’s lower-limb rehabilitation programme, including

neuromuscular electrical stimulation, proprioception exercises and low-impact cardiovascular conditioning (swimming and ski-ergometer). A follow-up MRI scan was performed shortly after cessation of the BfRT protocol. The scan displayed a significant reduction in the signal intensity and extent of bony oedema within the medial half of the proximal tibia, with near complete resolution of the stress injury within the medial femoral condyle. The player began returning to traditional lower-limb resistance training and cardiovascular conditioning with speed and contact drills. Eight weeks post-injury, a third MRI scan demonstrated complete resolution of the femoral condyle stress injury and continued healing of the proximal tibial stress injury, with no new injury demonstrated. The player has subsequently returned to competitive first-team Rugby League. Longitudinal thigh girth measurements and LEFS scores are displayed in Table 6.4.4. The player returned to first-team competitive fixtures approximately forty-four weeks after the initial injury, at the beginning of the following season.

Table 6.4.4. Longitudinal thigh girth measurements and LEFS scores; Phase III, case three.

BfRT protocol length	<i>Baseline (0 weeks)</i>	<i>2 wks</i>	<i>4 wks</i>	
Time since injury diagnosis	<i>0 weeks</i>	<i>2 wks</i>	<i>4 wks</i>	<i>6 wks</i>
Thigh Girth (in centimetres)				
Injured Limb	65.5	64.5	64.5	66
Uninjured Limb	65	65	64.5	66
Vastus Medialis Oblique Girth (in centimetres)				
Injured Limb	46.5	46	46.5	47
Uninjured Limb	46	46.5	46.5	47
Lower Extremity Functional Scale				
LEFS Score	55	58	69	68

6.4.3.4 Haemodynamic Variables

The mean resting values for the exercising vastus lateralis SmO₂, tHb, HHb and HR

recorded across thirty BfRT sessions (five sessions per leg, per player) are shown in Table 6.4.5. Standard deviations and within-subject, between-session coefficients of variation for each of these variables are also displayed. Resting haemodynamic values of the vastus lateralis were similar to those seen in the Phase II study and literature utilising other NIRS devices. (Downs et al 2014; Karabulut et al, 2014; Kennedy et al, 2006; Miyamoto et al, 2013). The mean change in haemodynamic variables during the exercise sessions, compared to resting values, are shown in Table 6.4.6. A trend can be seen towards greater degrees of BfR generating greater increases of in-session tHb, HHb and HR, with an inverse decrease in SmO₂.

Table 6.4.5. Resting mean values for haemodynamic variables recorded during the Phase III study.

	Mean	Standard Deviation	Within-subject Coefficients of Variation
SmO ₂ (%)	54.32	7.04	7.14% - 13.87%
tHb (g/dL)	12.72	0.37	0.53% - 1.80%
HHb (g/dL)	5.82	0.98	8.09% - 20.59%
HR (beats per minutes)	75.07	8.93	7.43% - 15.79%

Table 6.4.6. The mean in-session change to haemodynamic variables recorded during Phase III exercise sessions, compared to resting values. Standard deviations are in brackets.

	SmO ₂ (%)	tHb (g/dL)	HHb (g/dL)	HR (bpm)
0mmHg (CON)	+4.68 (4.29)	-0.04 (0.10)	-0.61 (0.55)	+0.6 (4.4)
100mmHg IM	-6.62 (5.02)	+0.10 (0.12)	+0.90 (0.65)	+3.4 (2.2)
120mmHg IM	-10.45 (3.56)	+0.05 (0.15)	+1.35 (0.45)	+3.7 (2.7)
100mmHg C	-12.87 (4.93)	+0.17 (0.20)	+1.73 (0.69)	+4.2 (3.6)
120mmHg C	-22.31 (7.64)	+0.23 (0.15)	+2.95 (0.94)	+6.2 (3.9)

NIRS data collated from all thirty BfRT sessions could be amalgamated, to display in-session changes to SmO₂ (Figure 6.4.1), tHb (Figure 6.4.2), HHb (Figure 6.4.3) and HR

(Figure 6.4.4). Standard deviations for each five-second measurement block are represented by whiskers. In all figures, cuff inflation began at 180 seconds and reached target pressure at approximately 210 seconds. During intermittent BfRT sessions, cuff pressure was released immediately after each exercise set. Re-inflation began thirty seconds before the next exercise set. During continuous BfRT sessions, cuff pressure was maintained until 600 seconds (one minute after completion of the final exercise set).

6.4.3.5 Perceptual Responses to Exercise

No pain was reported by any player during any BfRT session. The mean RPE values reported for each BfRT modality were similar for both the injured and uninjured lower limbs (Figure 6.4.5).

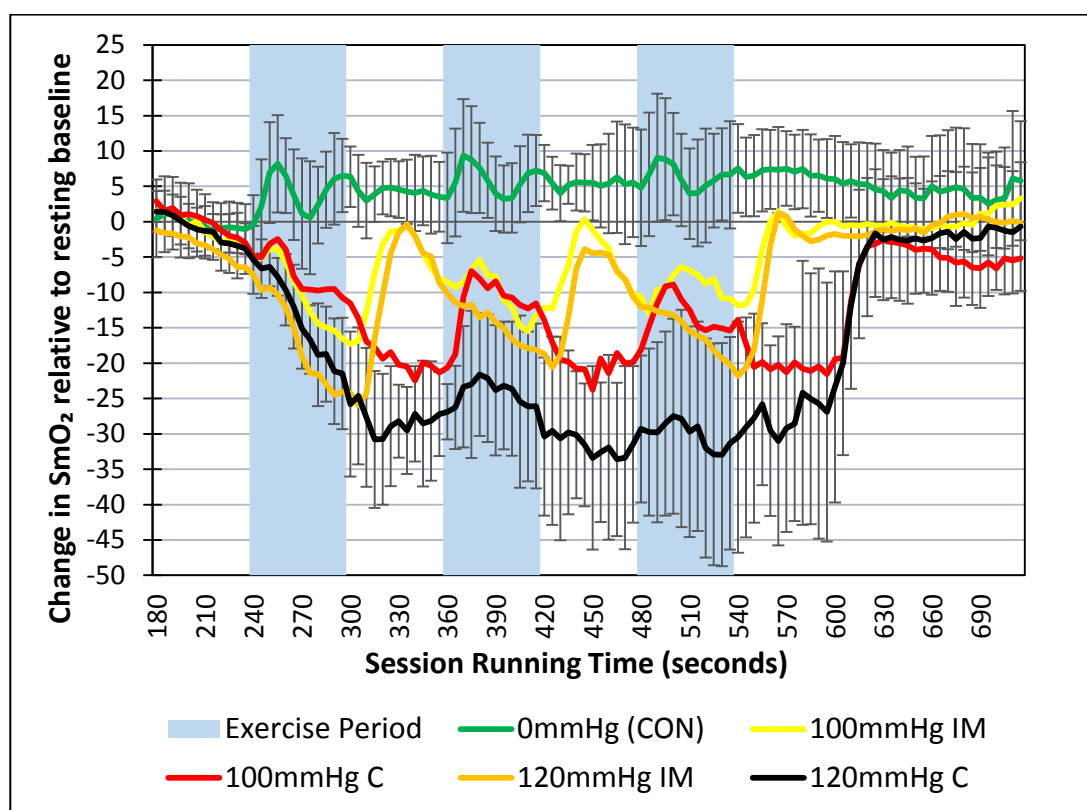


Figure 6.4.1. Changes in vastus lateralis tissue oxygen saturation of Phase III case studies [n=3], relative to baseline, during unweighted knee extension exercise sessions performed under different modalities of blood flow restriction. CON = Control, C = Continuous BfR, IM = Intermittent BfR.

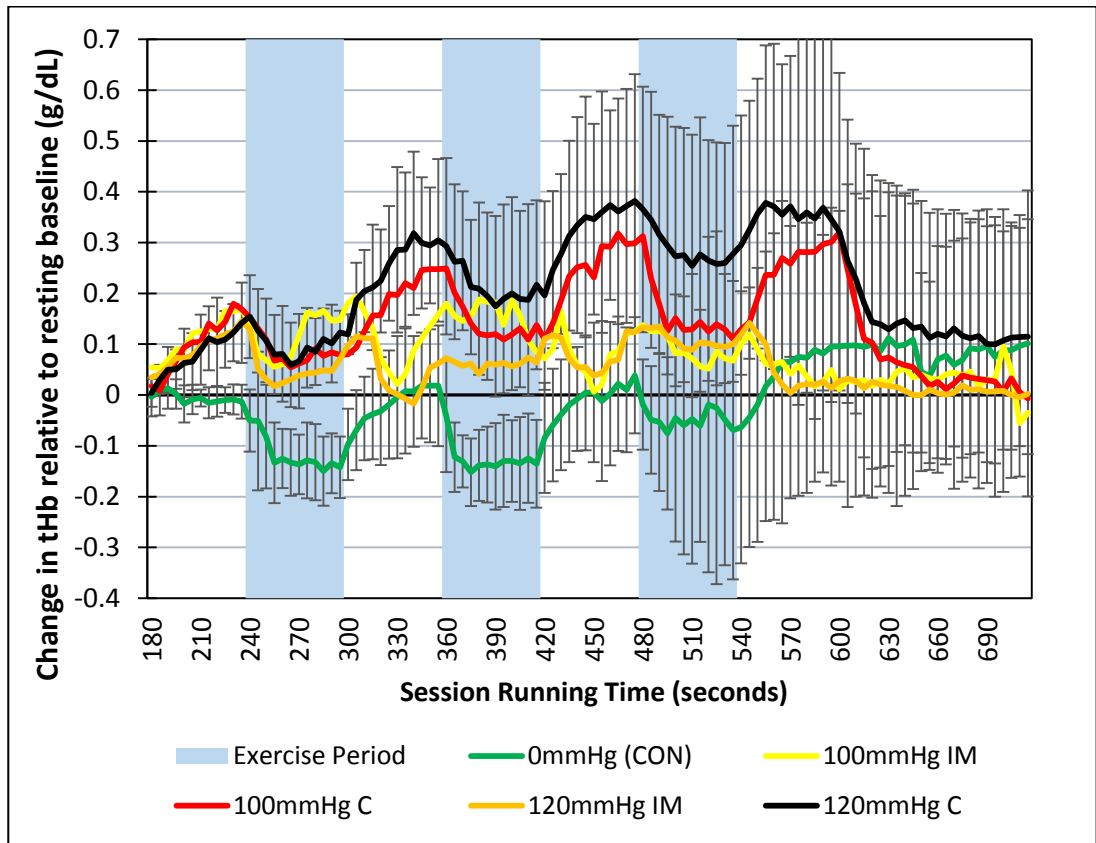


Figure 6.4.2. Changes in vastus lateralis total haemoglobin levels of Phase III case studies [n=3], relative to baseline, during unweighted knee extension exercise sessions performed under different modalities of blood flow restriction. CON = Control, C = Continuous BfR, IM = Intermittent BfR.

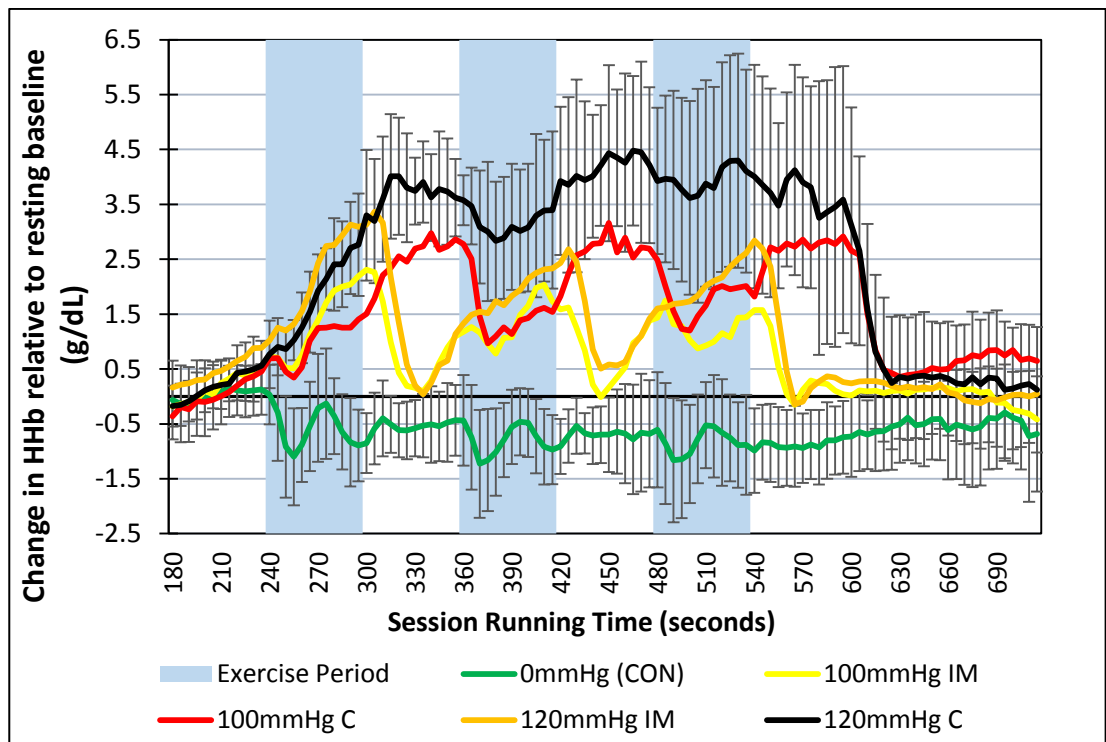


Figure 6.4.3. Changes in vastus lateralis deoxygenated haemoglobin levels of Phase III case studies [n=3], relative to baseline, during unweighted knee extension exercise

sessions performed under different modalities of blood flow restriction. CON = Control, C = Continuous BfR, IM = Intermittent BfR.

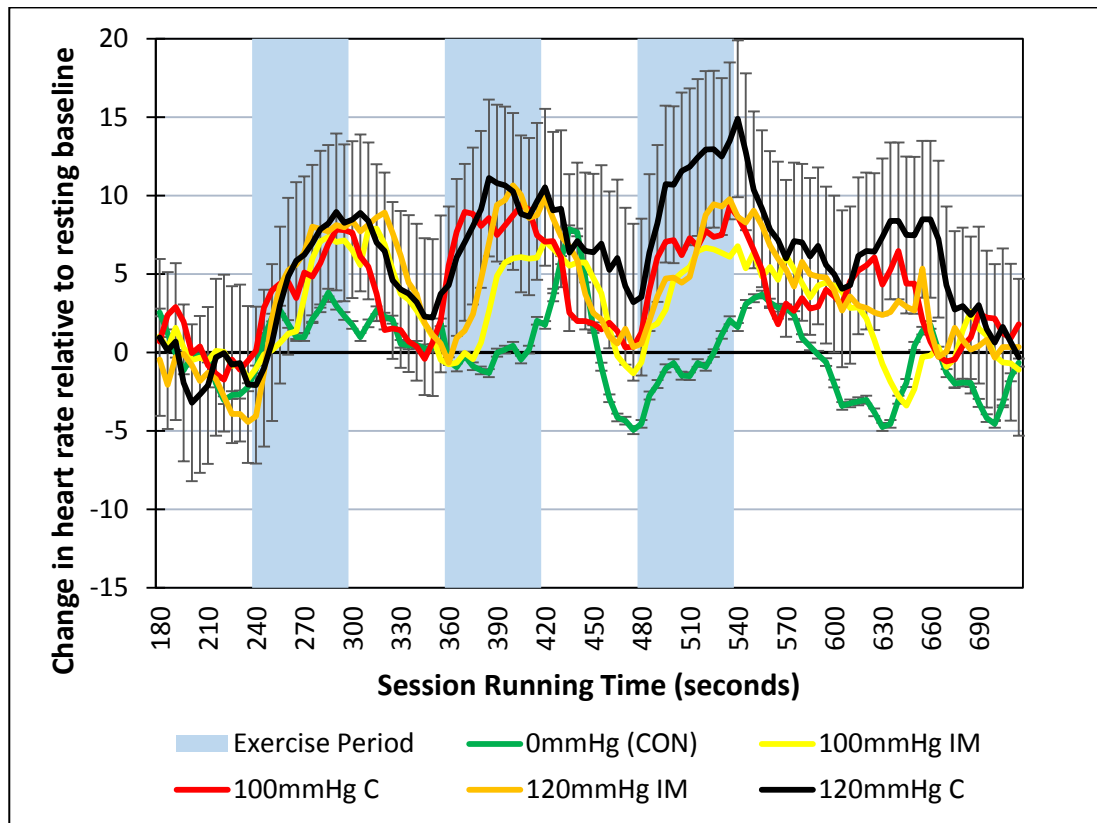


Figure 6.4.4. Changes in the heart rate of Phase III case studies [n=3], relative to baseline, during unweighted knee extension exercise sessions performed under different modalities of blood flow restriction. CON = Control, C = Continuous BfR, IM = Intermittent BfR.

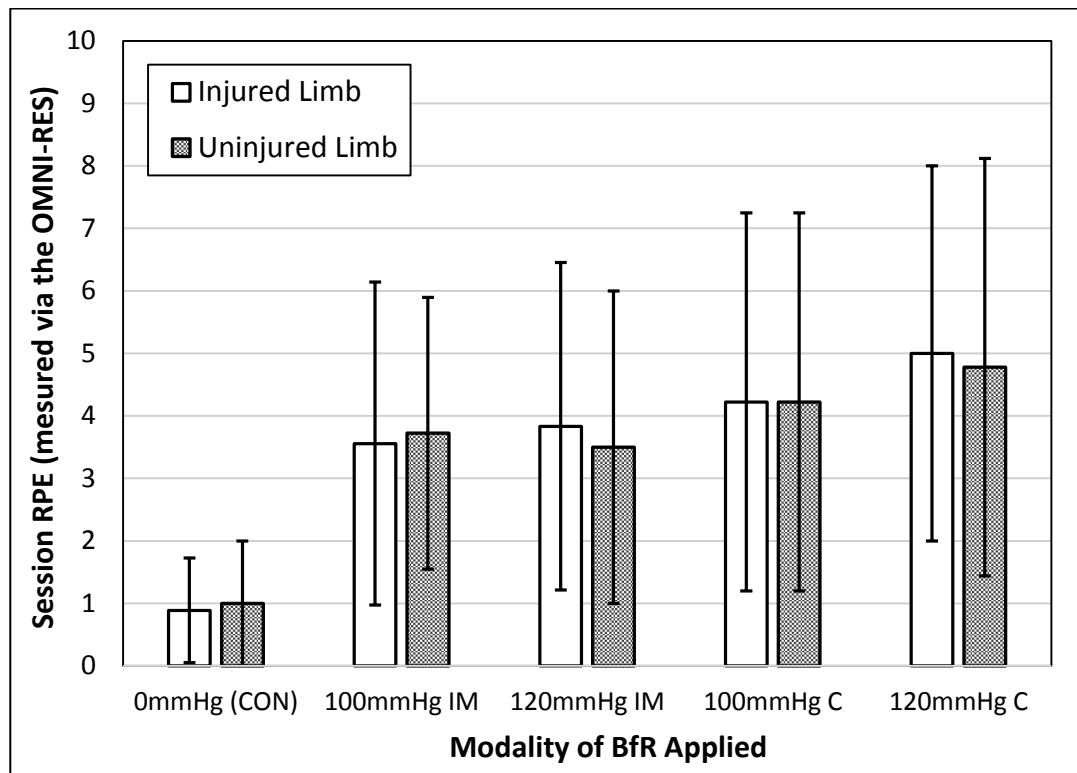


Figure 6.4.5. Mean rate of perceived Exertion of the Phase III case studies (n=3), during unweighted knee extension exercise sessions performed under different modalities of blood flow restriction. CON = Control, C = Continuous BfR, IM = Intermittent BfR.

6.4.4 Discussion

6.4.4.1 Lower-limb Measurements

All cases demonstrated at least longitudinal maintenance of thigh measurements relating to girth in both limbs throughout delivery of the BfRT protocol, which persisted upon the withdrawal of BfRT and a return to more traditional training regimes. This may indicate an ability of BfRT to attenuate atrophy as suggested in earlier studies (Kubota et al, 2008; Lejkowski and Pajaczkowski, 2011) or contribute to muscular hypertrophy. However, alternative explanations must also be considered.

Firstly, tape measurement cannot to differentiate between factors other than muscle mass that may have contributed to girth changes, such as fluid content of the limb or longitudinal changes in fat mass. Secondly, rehabilitation interventions in addition to BfRT

were delivered to the injured players that potentially contributed to atrophy prevention or hypertrophy. Neuromuscular electrical stimulation, for example, appears particularly effective at reducing quadriceps atrophy in circumstances where normal limb use has been prevented (Dirks et al, 2014; Dirks et al, 2015; Stevens-Lapsley et al, 2012). Whilst the maintenance or increase of thigh muscle mass may certainly have contributed to the longitudinal changes in girth measurements, this cannot be proven from the results of this study.

6.4.4.2 Haemodynamic Variables

During the control exercise session, a decrease in tHb occurred accompanied by a decrease in HHb of greater magnitude. Thus, a relative increase in vastus lateralis SmO₂ occurred. This is a very similar response to that seen during the Phase II study and could be explained via the same haemodynamic and mechanical factors discussed in chapter 5.5.5.2 (page 187-189). Briefly, repeated thigh muscle contractions provided a haemodynamic pump effect to generate increased venous outflow from the muscle and lower-limb itself during exercise sets, lowering vastus lateralis tHb. A simultaneous increase in the rate of blood flowing through the vastus lateralis likely occurred due in part to rapid vasodilation and drops in intravascular pressure between muscle contractions. Oxygen delivery outweighed oxygen uptake and vastus lateralis SmO₂ increased.

Likewise, exercise with continuous BfR applied produced very similar patterns of change in vastus lateralis SmO₂, tHb and HHb to those seen during the Phase II study. BfR applied continuously appeared sufficient to reduce blood throughput of the vastus lateralis during the exercise sets, by likely impairing any muscular pump effect and limiting venous

outflow proximally. During the inter-set rest periods, the absence of any muscular pump effect combined with the extravascular pressure provided by the cuff was likely sufficient to prevent venous outflow proximally, but did not prevent arterial inflow into the lower limb itself. This led to a local increase in vastus lateralis tHb. An overall slowing of the rate of blood flowing through the vastus lateralis resulted in increased oxygen extraction from that was blood available to tissues, leading to an increase in vastus lateralis HHb and a decrease in vastus lateralis SmO₂.

During the exercise sessions performed with intermittent BfRT, vastus lateralis SmO₂, tHb and HHb returned rapidly to baseline during the inter-set rest periods where cuff pressure was released. This would be indicative of lower-limb venous outflow being restored upon cuff deflation, allowing blood throughput of the vastus lateralis to be normalised and tissue homeostasis achieved via adequate oxygen delivery.

The undulation of heart rate in correspondence with exercise and rest periods was expected. During exercise sets, heart rate would have increased in response to raised venous return from the previously static lower limb. An increase in heart rate also works to raise cardiac output as a method of raising oxygen delivery rate to exercising muscles (Korthius, 2011). As the body returns to homeostasis during rest periods, heart rate then returns to baseline. Interestingly, the addition of BfRT did not greatly affect heart rate response, with magnitudes of change very similar to the lower cuff pressures utilised within the Phase II study. Phase III findings to therefore appear to reinforce the principle that BfR added to an unweighted knee exercise produced insignificant cardiovascular demand beyond that created by the knee exercise alone.

6.4.4.3 Subjective Outcome Measures

Pain and RPE

A promising finding was the absence of reported pain during Phase III BfRT sessions. Adding BfR to the unweighted knee exercise also raised RPE above that reported during the control sessions, suggesting an ability for the modality to increase the perceptual difficulty of the exercise without the need to increase exercise volume or add an external resistance.

LEFS

In relation to case one, work by Lin et al (2009) collated the results of three studies utilising the LEFS to quantify functional recovery from unilateral ankle fracture (n=306). The player, who experienced a fracture of the lower tibia and fibula, reported a similar trajectory of LEFS scores as those reported in Lin et al (2009) within one week of cast/boot removal (LEFS = 29 vs. 30.7, respectively), 4 to 6 weeks after cast/boot removal (LEFS = 53 vs. 53.8, respectively) and LEFS = 73 at 20 weeks after boot removal vs. 69.4 at 24 to 26 weeks after cast removal, respectively).

With case two, limited academic evidence exists as to LEFS trajectory scores following surgical repair of an Achilles tendon rupture. A study by Peng et al (2017) involving fifteen participants reported a mean LEFS score of 74.0 (95% CI, 67.9-74.9) at 4.2 (SD 1.1) months after surgery. This is notably higher than the score reported by the player a similar time point (51 at 19 weeks post-surgery). However, the player scored himself consistently low on questions with a sporting context, such as difficulty completing 'your usual hobbies, recreational or sporting activities' or 'running on even ground'. Given that Peng et al (2017) studied older participants (45.1 years) of a nondescript sporting ability, the

discrepancy in LEFS scores between this study and the player may have been due to a difference in pre-injury sporting ability and thus the perceived distance from regaining this level of ability at 18 to 19 weeks post-surgery.

No academic evidence appears to exist from which case three's LEFS can be directly compared. Reviewing the player's baseline LEFS score however, the minimal clinically important change for the LEFS (9 points; Binkley et al, 1999) was exceeded after two weeks of the BfRT protocol, and again at four weeks. Supported by MRI findings showing the continued healing of the osseous stress injury, it could be surmised that the wider rehabilitation programme provided was appropriate and that BfRT element was non-detrimental to bone healing. This appears to concur with an observational case review by Loenneke et al (2013b), who documented the non-supervised use of BfRT in a male bodybuilder with an osteochondral fracture. A repeat MRI and examination after approximately two weeks of BfRT suggested continued bone healing and a resolution of knee joint effusion and tenderness. Experimentally, both Karabulut et al (2011a) and Kim et al (2012) have investigated the longitudinal effect of BfRT upon bone turnover. Each study monitored serum levels of C-terminal cross-linking telopeptide of type 1 collagen [CTX] and bone alkaline phosphatase [BALP], indicators of bone resorption and osteoblast activity respectively. Three weeks of low-volume LiBfRT (20 repetitions per session at 20% 1RM) did not alter pre-post CTX and BALP levels of 30 males studied within Kim et al (2012). However, six weeks of higher volume LiBfRT (75 repetitions per session at 20% 1RM) in 37 older males saw significant pre-post increases in BALP ($p = 0.03$) compared to a non-exercising control group (Karabulut et al, 2011a). CTX also decreased in the LiBfRT group whilst increasing in the control group, however the difference was not statistically significant. Whilst large-scale studies investigating the use of BfRT within participants with

bony injuries are currently lacking, the existing evidence does appear to support its use under these conditions.

6.4.5 Study Strengths and Limitations

This study was able to draw upon an existing 'real-world' BfRT protocol during lower-limb injury rehabilitation, simplify and refine it, then deliver the protocol safely within the same clinical environment to injured participants. The lack of adverse events, the tolerability of the protocol by study participants, and the use of relatively inexpensive restriction equipment, all pertain to the suitability of this protocol for use within the elite sporting environment of rugby-league.

This study was a case series. By design, a case series does not address risks of bias within its methodology as competently as other research designs, such as a blinded, randomised controlled trial. A sample size of three elite, male rugby-league players from the United Kingdom, combined with a lack of case-controls, currently prevents findings from being generalised to other sporting, non-sporting populations. The use of medical imaging or biopsy to quantify longitudinal changes in muscle CSA, volume and fibre-type would have enabled more robust confirmation of muscle atrophy attenuation during the BfRT intervention. Research that addresses these limitations is warranted, to increase the clinical relevance of the chosen BfRT methodologies and strengthen the validity of its proposed longitudinal effect upon muscle disuse atrophy.

6.4.6 Conclusions

In a case series of injured male rugby-league players, NIRS and RPE data indicates that adding BfR to an unweighted 'no load' knee exercise increases the acute, local metabolic

demand and perceptual exertion of this exercise. Over a period of up to twelve weeks, a staged lower-limb BfRT protocol applied 4 to 5 times per week can be delivered in a safe and tolerable way within a clinical (non-laboratory) environment via the use of a thigh blood-pressure cuff and manual sphygmomanometer. Results suggest that the holistic rehabilitation programme delivered to each player, of which 'no-load' BfRT was a part, prevented longitudinal thigh muscle atrophy and provided a return towards normal function and a return to competitive play. From this study alone, however, the extent to which 'no-load' BfRT contributed to this remains unclear. Further research by way of larger case-control study or a randomised-controlled trial to elucidate this information is warranted.

The next chapter will summarise the aims, objectives and key findings of each of the three phases of this doctoral research project. Discussion will be made as to the clinical and research implications of these findings, with suggestions as to future pathways for BfRT research.

CHAPTER SEFVEN

Project Conclusions and Implications

7.1 Chapter Introduction

This chapter begins by re-stating the overall aim and objectives of this doctoral research project. The key findings of each phase of this doctoral project are then described.

Discussion is made as to how the objectives of this project have been met, combined with consideration of the potential clinical and research implications of study findings. Finally, suggestions upon which directions to proceed with future research are presented.

7.2 Project Aim and Objectives

The overall aim of this doctoral research was to develop and refine the use of BfRT within the context of lower-limb musculoskeletal injury rehabilitation. Two objectives were developed to achieve this, supported by the findings of the reviews described in chapters one and two;

1. To develop an externally-valid lower-limb BfRT methodology using relatively inexpensive BfR equipment and protocols that may be replicated within clinical settings.
2. To determine the physiological and perceptual effects of combining BfRT with un-resisted ('no-load') lower-limb exercise.

Across the three phases of this doctoral research project, the following key findings were obtained;

7.3 Key Findings

7.3.1 Phase I

- Within sixty-one healthy adults, resting in a seated position, lower-limb BfR could be applied in a safe and tolerable way using a thigh blood-pressure cuff to reduce the flow into the limb, distal to the cuff. The greater the cuff pressure applied (up to 120mmHg), the greater the degree of initial BfR that occurred.
- Using ultrasound imaging techniques, results suggested that a thigh blood-pressure cuff inflated to up to 80mmHg generated BfR predominantly via venous restriction or venous occlusion at thigh level. At 100mmHg to 120mmHg, an element of arterial restriction may have begun to contribute to further degrees of BfR. 120mmHg of thigh-cuff pressure did not appear to cause total occlusion (haemostasis) in any participant.
- A degree of between-subject variance, unexplained by physical characteristics, was present in the %PBfR experienced at each tested cuff pressure. Physical characteristics could not be used to explain the variance in cuff pressure required to induce 60% PBfR (a reduction in popliteal arterial blood-flow of 40%.)
- An equation was produced to provide a broad indicator for the degree of BfR that occurred in this cohort at any given cuff pressure between 40mmHg and 120mmHg.

7.3.2 Phase II

- In sixteen healthy adults, applying continuous BfR during one-hundred-and-twenty repetitions of a seated, un-resisted ('no-load') knee extension exercise appeared to increase the acute metabolic demand and perceptual effort of the exercise session. This was demonstrated by;
 - Significantly greater reductions in vastus lateralis tissue oxygenation during the exercise session by the addition of BfR, which increased in magnitude as the cuff pressure was increased from 40mmHg to 80mmHg.
 - Statistically significant increases in rates of perceived exertion reported during the exercise by the addition of BfR, with greater thigh-cuff pressures producing higher levels of perceived exertion.
- In the tested population, the use of BfR during 'no-load' exercise appeared safe and tolerable;
 - No pain during BfR was reported and all participants completed all exercise sessions.
 - BfR at any tested cuff pressure did not significantly alter heart rate response compared to the exercise performed without BfR.
- A negative correlation existed between physical characteristics of size and the mean magnitude of change in the exercising vastus lateralis across BfR sessions. Participants with a lower body weight, Body Mass Index or thigh circumference tended to experience greater magnitudes of change in vastus lateralis SmO₂ and HHb during exercise performed under BfR.

7.3.3 Phase III

- Among three male elite rugby-league players with a unilateral low-limb injury, lower-limb BfR could be safely combined with the un-resisted knee extension exercise used in Phase II, delivered four to five times per week, for periods of up to twelve weeks;
 - No pain during BfR was reported and all players completed all exercise sessions.
 - No adverse events occurred within any player during any BfRT session.
- Thigh-cuff pressures of up to 120mmHg produced similar patterns of change in vastus lateralis SmO₂, HHb and tHb, heart rate and perceived exertion to those seen in Phase II at lower thigh-cuff pressures.
- Regular tape measurements indicated that no player lost thigh mass during the course of their BfRT intervention period, suggesting that thigh muscle atrophy was attenuated in these players as a result of their overall injury rehabilitation programme.

7.4 Key Messages of This Doctoral Research Project

- A thigh blood-pressure cuff and manual sphygmomanometer can be used to deliver lower-limb BfR in a safe and tolerable way, before and during an un-resisted lower-limb exercise. This can be achieved among individuals with and without a lower-limb musculoskeletal injury, who are otherwise healthy.
- Adding BfR to an un-resisted lower-limb knee extension exercise appears to increase the acute metabolic demand of the exercise within the vastus lateralis

muscle, without the need to add any external load or change other parameters of the exercise.

- There is no current evidence to suggest that physical characteristics are highly-correlated with, or can predict, the between-subject variance in the initial degree of lower-limb BfR that occurs following the application of a sub-occlusive thigh-cuff pressure.
- However, physical characteristics do appear to be correlated with the magnitude of change in acute metabolic demand that occurs in response to un-resisted exercise performed with BfR at sub-occlusive thigh-cuff pressures. Further research is required to substantiate this, with a view to providing individualised thigh-cuff pressures and a consistent degree of acute exercise-induced metabolic stress across individuals.
- The findings of this doctoral research project are specific to the type and width of cuff used within it. Caution must therefore be taken when interpreting results, as the effects of BfR delivered via alternative cuff apparatus may differ to those seen within this project.

7.5 The Achievement of Project Objectives

How the key findings of this doctoral research project have met each of the project's two objectives can be discussed in further detail, with further discussion as to the clinical implications of these findings.

7.5.1 Objective One

'To develop an externally-valid lower-limb BfRT methodology using relatively inexpensive BfR equipment and protocols that may be replicated within clinical settings.'

Thigh blood-pressure cuffs, combined with manual aneroid sphygmomanometers, were used throughout all parts of this research project. The current retail price of the equipment used within the main studies of this doctoral research project is \$104.27 (US Dollars) (MDF Instruments, 2017a). Although the model of aneroid sphygmomanometer used has become unavailable in the UK at present [MDF848XPD, MDF Instruments®; California, USA], a very similar model is available [MDF848AR, MDF Instruments®; California, USA] and can be paired with the same thigh cuff for £77.14 (MDF Instruments, 2017b). This equipment costs considerably less than the current Kaatsu Master (\$4,795.00 USD, £5,000.00 GBP) and Kaatsu Nano (\$2,850.00 USD, £2,600.00 GBP) devices (Kaatsu Global©, 2017) and the Hokanson® system often used in BfR academic research (£3,210 GBP; PMS Instruments, 2017). Whilst the latter systems have additional functionality, such as rapid cuff inflation and automatic pressure regulation, the results of this doctoral research project demonstrate that generic blood-pressure equipment can also be used to safely maintain states of BfR before and during a lower-limb exercise. The degree of BfR can also be modulated objectively through the use of the manual sphygmomanometer's pressure dial, giving a potential superiority in accuracy over other inexpensive options such as manual tourniquets bands (\$17.97 USD; BfR Bands®, 2017). The author proposes that the data generated from this project will enable HCPs to access and deliver evidence-based BfRT in a way which is less expensive than most BfR systems, but which offer greater objective control than cheaper methods.

The primary researcher is aware that new BfR-specific devices are emerging. At least one device now utilises a manual sphygmomanometer to inflate a cuff and maintain its pressure, much like the generic blood-pressure equipment used within this doctoral research project. The Occlusion Cuff® retails at between £79.99 and £138.00 (Occlusion

Cuff, 2017; Perform Better Ltd., 2017). This cost is similar to the equipment used within this doctoral research, which may itself help to boost the clinical accessibility and uptake of BfRT. However, the Occlusion Cuff® is narrow in width (8cm) and has a suggested lower-limb operating pressure of 150-250mmHg. A similar width-pressure combination can create bruising in 13.1% of users and numbness in 1.3% of users (Nakajima et al, 2006). There was an absence of bruising or adverse events when utilising generic blood-pressure equipment, conceivably due to lower operating cuff pressures (40-120mmHg) and a comparatively wide cuff (21cm). Therefore, beyond cost, a superiority of this latter equipment over the new BfR-specific system may still exist clinically. Distributing comparatively less pressure over a comparatively greater volume of tissue may generate a similar state of BfR within clinical populations whilst minimising the risk of compression-related tissue damage.

This project has generated and documented a method of applying BfR and delivering 'no-load' lower-limb BfRT sessions to individuals. Firstly, this consists of a new BfR-specific, evidence-based, health screening questionnaire. Whilst of significant use within the project itself, this questionnaire may also aide HCPs to identify and screen for common BfR contraindications within their own clinical settings. It also provides a base from which HCPs can develop or add their own condition-specific or setting-specific contraindications. Previous work has discussed the safety aspects of BfRT in detail (Loenneke et al, 2011c) and work published after the generation of this project's health screening questionnaire has created a risk assessment tool (Kacin et al, 2015). However, neither has collated and expressed the incidence of contraindications across the literature to interested readers. Given the scarcity of adverse events within BfRT studies, the author proposes that the project's tabulated list of the most common contraindications, and the screening

questionnaire that incorporates these, is of significant value to clinicians.

Secondly, Phase I results now provide reference data for the degree of initial lower-limb BfR that occurs across a range of individuals when utilising a blood-pressure thigh cuff at sub-occlusive thigh pressures up to 120mmHg. In addition, this reference data was collected from participants in a seated position - a position from which a range of other exercises could be delivered beyond the seated knee extension exercise used within this project. Prior to this information, HCPs would have had to draw upon data from studies that used narrower restriction cuffs attached to rapid cuff-inflation devices, with participants tested in a supine position (Hunt et al, 2016; Loenneke et al 2012b, 2015a), then make assumptions as to whether these findings would translate into their own clinical practice.

The exercise and cuff-inflation protocols were derived from those already used among injured elite athletes within a clinical setting (Warrington Wolves Rugby League Club). These protocols were subsequently refined by the primary researcher and then redelivered in a safe and tolerable way within the same clinical setting, to a case series of participants from the same injured population. These same protocols were also replicated without adverse or unexpected events within healthy, non-athletic populations during Phase II, albeit with a lower thigh-cuff operating pressures (40-80mmHg). Enough information exists within this thesis to replicate these BfRT protocols directly within other clinical practice settings. This information could even be converted into a simple manual for HCPs if required. Beyond the purchase of the generic blood-pressure equipment, the minimum requirement of a plinth (or at least a stable surface on which achieve the seated position) is common-place within clinical settings. Additional relevant items such as a

stopwatch, a metronome and a goniometer for checking the individual's sitting position, are all available as free software for use with electronic devices such as smartphones if required.

The project used a validated functional subjective outcome measure (LEFS) to monitor the perceived longitudinal recovery of function within Phase III participants. This case series now compliments an existing case study by Lejkowski and Pajaczkowski (2011), who used a thigh blood-pressure cuff to deliver BfR during rehabilitation from ACL reconstruction and utilised the LEFS to measure subjective function. This provides HCPs with an insight as to the trajectory of functional recovery following significant lower-limb MSK injuries that incorporate 'no-load' BfRT into rehabilitation. Once the findings of Phase III are published, HCPs will have a greater evidence base of case studies from which to draw upon and inform their own clinical use of BfRT, both in the number of case studies available and in the types of lower-limb injuries documented.

This doctoral research project also utilised subjective measures of pain and exercise-related exertion that are already available to HCPs without cost. Results from all phases of the research project now provides a reference source from which HCPs can compare pain and perceived exertion scores reported by their service users during 'no-load' lower-limb BfR applied via blood-pressure equipment. In turn, this data provides a further route by which HCPs can clinically justify and refine the cuff pressure(s) delivered to clinical populations.

The time taken to complete a unilateral 'no-load' lower-limb BfRT session can also be important within clinical settings, particularly in time-pressured environments such as an

elite sports club or a busy musculoskeletal outpatient department. Discounting the initial health-screening process, one unilateral 'no-load' BfRT session delivered as per described in this thesis (but devoid of measurement devices such as NIRS) would take approximately eight minutes. This is a very feasible timeframe for use within Warrington Wolves Rugby League Club and it is sensible to propose that other settings and HCPs would concur. Given the comparatively low cost of the blood-pressure equipment, it is also feasible to suggest that some settings would be able to purchase multiple kits. This may allow the use of 'no-load' BfRT sessions within small, supervised MSK outpatient classes, furthering the clinical applicability of BfRT as a potential treatment adjunct.

Beyond 'no-load' BfRT specifically, the creation of the systematic review detailed in chapter two provides HCPs with a succinct summary and critical appraisal of the existing peer-reviewed evidence surrounding the use of BfRT to attenuate lower-limb muscle disuse atrophy following an MSK injury. This is the first systematic review on this specific topic, and once published, will accompany a rehabilitation-focused systematic review and meta-analysis created by Hughes et al (2017). The primary researcher suggests the benefit of this to be two-fold. Firstly, there is less demand upon HCPs to search for and synthesise data themselves from previous individual BfRT studies in order to learn and apply this modality in an evidence-based fashion within rehabilitation. Secondly, the evidence synthesised in both this doctoral project and Hughes et al (2017) highlights the large variations in BfRT methodologies implemented across existing literature, particularly in terms of the cuff widths and cuff pressures utilised. It is hoped that recognition of this will drive HCPs and researchers towards adopting a common overall methodology and BfR device, whilst still allowing for modulation of the cuff pressure(s) utilised to suit each service user and their physical or physiological condition. In turn, this may bolster the

clinical uptake of BfRT and improve concordance between the BfRT methodologies used in research environments and those used in real-world practice.

7.5.2 Objective Two

'To determine the physiological and perceptual effects of combining BfRT with un-resisted ('no-load') lower-limb exercise.'

At this time, only one peer-reviewed study investigating associations between individuals' physical characteristics and the initial degree of lower-limb BfR generated across different sub-occlusive thigh-cuff pressures has been published Hunt et al (2016). Additional studies surrounding this topic do exist, but only the cuff pressure required to fully occlude limb blood flow was investigated (Loenneke et al, 2012b; 2015a). No published evidence exists as to the association between physical characteristics and the initial degree of BfR generated by cuff pressure in a seated position (only supine) or via the use of a blood-pressure cuff as the restriction device, or the incorporation a sub-cohort of elite athletes into their population sample. Work completed in Phase I has addressed this, and is the first to generate an equation that broadly indicates the degree of sub-occlusive lower-limb BfR that initially occurs when utilising a blood-pressure thigh cuff. In tandem with Hunt et al (2016), Phase I findings demonstrate that physical characteristics, be that thigh circumference, systolic blood pressure, or mean arterial pressure, are unable to explain the majority of between-subject variance in the degree of BfR that created by sub-occlusive thigh-cuff pressures.

Phase II of this doctoral research project produced evidence to document the acute physiological and perceptual responses to un-resisted 'no-load' lower-limb exercise

combined with BfR. No peer-reviewed evidence has been published on this topic to date, either with the use of a blood-pressure cuff to induce BfR or via other restriction equipment. More so, Phase II findings suggest that physical characteristics of size or mass may be implicated in degree of acute change in metabolic demand of the 'no-load' exercise. Considering the results of Phase I and Phase II together, the following could be hypothesised. The amount of lower-limb BfR initially induced by a predetermined thigh-cuff pressure is not related to physical characteristics of size, but the effect of that cuff pressure upon lower-limb vasculature and the degree of acute metabolic demand generated during subsequent 'no-load' exercise *is* associated to physical characteristics of size.

The above proposal would need be ratified by further experimental studies. If confirmed, however, the process of trying to set or 'tailor' a cuff pressure for a whole exercise session by only referring to what happens haemodynamically when external pressure is applied to a resting limb the prior to exercise may be an invalid one. For example, determining the cuff pressure required to totally occlude blood flow at rest and then using percentage of this value during exercise (Loenneke et al, 2015b; 2016), or trying to predict the actual %PBfR occurring at rest before the onset of exercise via physical characteristics, as per the Phase I study and Hunt et al (2016). Particularly in circumstances where exercises are not completed to volitional failure, such methods may still produce variations in the resultant degree of acute metabolic stress of the exercise session across physically-different individuals, because the degree of lower-limb BfR actually experienced during exercise sets (and their muscle contractions) may be different.

To address this issue in 'no-load' BfRT, focus would need to shift towards finding the lowest thigh-cuff pressure that occludes venous outflow in an individual both at rest *and during very low-intensity muscular contractions*, whilst still allowing arterial inflow into the lower-limb and its musculature. Physical characteristics such as indicators of thigh and/or muscle mass might be used to predict this 'sweet-spot' cuff pressure, with larger individuals still receiving a higher cuff pressure to maintain the required haemodynamic state of BfR throughout the exercise session. With the cuff used during this doctoral research, this 'sweet-spot' may well be between 60mmHg and 80mmHg for most individuals, but could be higher for very large or muscular individuals.

Within LiBfRT, the situation may become more complex. Cuff pressures may have to be further modulated, being increased for higher exercise intensities (such as 30-50% MVC or 1RM) to prevent the force of muscle contractions from overcoming the venous occlusion generated proximally by the cuff and attenuating the efficacy of the BfRT session. Even further adjustment would be needed if the cuff width that used was not universal across research or clinical settings. Whilst purely hypothetical, examples of how these factors may be used determine 'sweet-spot' cuff pressures are demonstrated in Figure 7.5.1 and Figure 7.5.2.

The absence of adverse events, 100% participant compliance with BfR sessions and lack of perceived pain during Phase II and Phase III BfRT sessions alludes to the acute safety and tolerability of this training modality within the populations tested across this research project. This is combined with the ability of BfR to increase the degree of perceived exertion of 'no-load' exercise. Whilst not specifically investigated in this project, it could be proposed that increasing the perceptual difficulty of sessions that involve 'no-load'

rehabilitation exercises, via the addition of BfR, may have a psychological benefit to individuals recovering from injury. Particularly among those who were occupationally active before their injury. Athletes, for example, can experience a range of psychosocial stressors during their injury recovery period Podlog et al (2011). Adding BfR to allow athletes to experience sensations of exercise-related discomfort during 'no-load' exercises which are closer to the sensations that they recall from pre-injury resistance training, for example, may positively impact upon stressors such as re-injury anxiety and the fear of a loss of physical fitness. Further research would be required to substantiate this proposal, however.

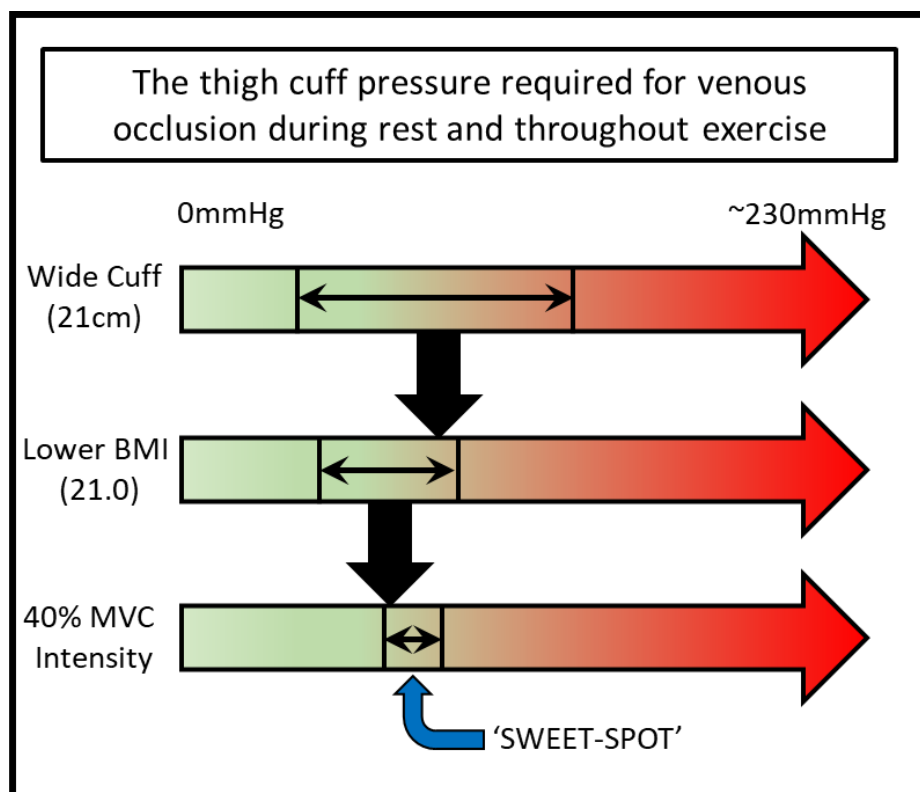


Figure 7.5.1. A visual representation of how a thigh-cuff 'sweet-spot' pressure could be determined for use during LiBfRT, performed with a wide cuff at 40% MVC.

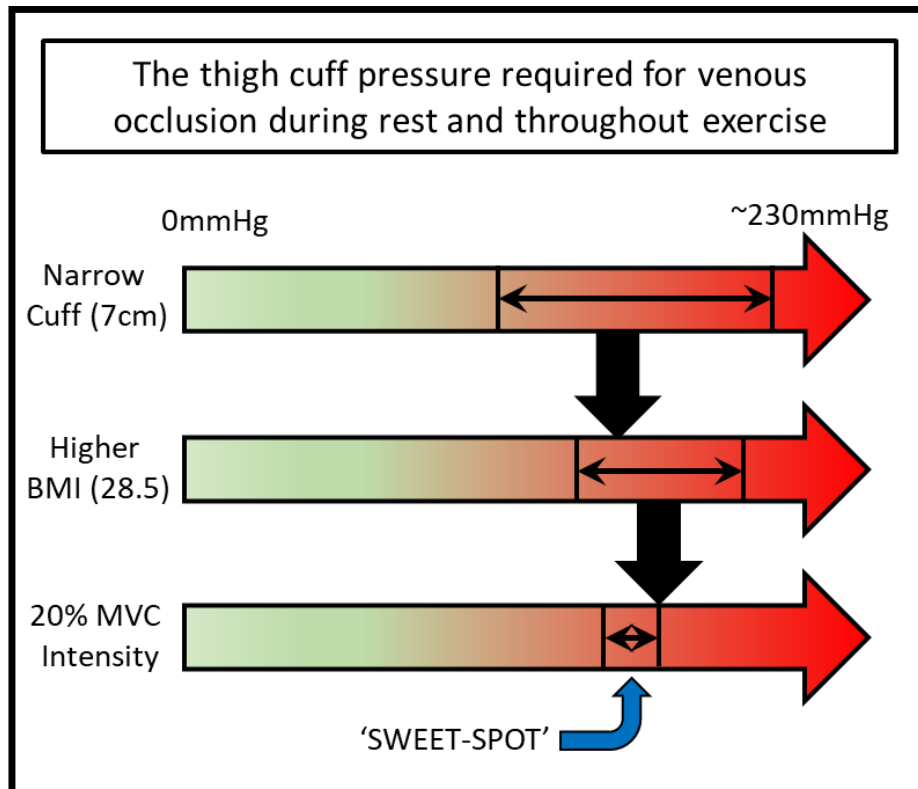


Figure 7.5.2. A visual representation of how a thigh-cuff 'sweet-spot' pressure could be determined for use during LiBfRT, performed with a narrow cuff at 20% MVC.

7.6 Recommendations for Future Research

A number of opportunities to build upon the research produced within the research project are still evident from the discussion and conclusions surrounding each study phase. These are highlighted below.

7.6.1 Phase I

An increase in the diversity of populations investigated and in the total number of participants recruited may further increase the clinical applicability of study findings.

Previous evidence has delivered BfRT safely to clinical populations with hypertension, diabetes and heart failure (Gualano et al, 2010; Pinto et al, 2015; Takahishi et al, 2010; Tanaka et al, 2015). As these chronic conditions can adversely affect vascular and cardiac structure and function, considerable care would need to be taken to adjust the screening

questionnaire and test these populations. Yet, potential differences in vascular compliance and intravascular pressure within these individuals may produce more profound between-subject differences in the degree of initial BfR that a range of thigh-cuff pressures generates within the lower limb, compared to healthy individuals. This may allow either the generation of alternative equations to indicate the BfR generated within these clinical populations, or allow a robust predictive model to be built which encompasses both healthy individuals and those with chronic disease. Alternatively, simply expanding the sample size of healthy participants in Phase I would generate greater statistical power to detect lower bivariate correlations and would narrow confidence intervals. This may elucidate more robust associations between the initial BfR experienced and physical characteristics across thigh-cuff pressures. Dependent upon results, this may again allow rejection of the Phase I null hypothesis and the creation of a pertinent predictive model.

7.6.2 Phase II

Current cohort associations exist between physical characteristics and the degree of change in SmO_2 and HHb of the vastus lateralis across the BfRT sessions delivered. Yet a larger cohort may allow the use of hierarchical linear modelling, with a view to predicting the degrees of change in SmO_2 or HHb that will occur at a given cuff pressure across physically different individuals. If this is possible, the selection or 'tailoring' of cuff pressures can start to be based upon what actually occurs haemodynamically and physiologically during the BfRT session, rather than from what occurs haemodynamically before it.

Scope also exists to improve the study methodologically. Additional physiological

measures such as whole blood lactate could be used to further indicate the degree of metabolic stress occurring during 'no-load' BfRT exercise. Together with post-session measures such as changes in muscle protein synthesis or gene expression, the dose-response of 'no-load' BfRT sessions could be better quantified. This may lead to more robust cuff-pressure recommendations to HCPs using this training modality in clinical practice. Investigating different exercises, such as sit-to-stands or seated hamstring curls, would provide further clinical value.

7.6.3 Phase III

Expanding Phase III to include a greater number of injured case studies may allow statistical inferences to be drawn from collected NIRS data during 'no-load' BfRT sessions, as among the uninjured cohort investigated within Phase II. In addition, bivariate correlations between Phase III cohort physical characteristics and the degree of change in variables such as SmO_2 or HHb could then be investigated. Following this, it may be possible to merge Phase II and Phase III data via hierarchical linear modelling, to start predicting shifts in vastus lateralis SmO_2 and HHb across the two sub-populations and across cuff pressures ranging from 0mmHg through to 120mmHg. As described in the previous section for Phase II, adding physiological measures such as whole blood lactate could be used to better quantify the dose-response of 'no-load' BfRT sessions across cuff pressures. Linking these to longitudinal measures of muscle fibre diameter via biopsy, or overall muscle volume via MRI or other medical imaging, may also substantiate the effect of BfR included within an injury rehabilitation programme has upon muscle atrophy attenuation.

7.6.4 Beyond Project Phases

Finally, research designed to specifically examine the ‘sweet-spot’ cuff pressure proposed in chapter 7.5.2 (page 237-241) is warranted. Across a range of individuals, the study would aim to find a level of cuff pressure that would occlude venous outflow both at rest and during a given low-intensity of lower-limb exercise (such as 5% MVC/1RM), by just barely overcoming the extravascular pressure generated by the muscular pump effect of that particular exercise intensity. With sufficient resources, it may be possible to confirm that this state of BfR occurs throughout exercise sets via ultrasound imaging of the femoral vein and artery immediately proximal to the thigh cuff. Any between-subject variation in this ‘sweet-spot’ pressure may then be checked for associations with physical characteristics such as thigh mass (measured via tape, DXA or MRI) to detect any positive correlations. A predictive model may then allow identification of the ‘sweet-spot’ cuff pressure for differently-sized individuals during ‘no-load’ BfRT. The experiment could be repeated for different intensities of exercise, up to 60% MVC/1RM; the intensity at which BfR may generate no additional acute benefit (Cayot et al, 2015). Finding these ‘sweet-spots’ would allow the state of BfR to be achieved (venous occlusion with maintenance of arterial inflow into the limb) but at the lowest possible cuff pressure for each exercise intensity. From an injury rehabilitation perspective, this could be invaluable in the attenuation or recovery from muscle disuse, where safety and a low re-injury risk is paramount.

If a predictive model is produced, an RCT could then be constructed consisting of an injured population. Whilst the control group completed a ‘traditional’ exercise rehabilitation programme for their named injury or surgery, a second group would complete the same programme with BfR superimposed at ‘sweet-spot’ pressures relevant

to the intensity of the selected exercise. Acute changes to SmO_2 , HHb, tHb and HR could be collected throughout, followed by measures of lactate and muscle protein synthesis. Longitudinal changes in thigh mass, 1RM, isokinetic strength, muscular endurance and physical function could then be compared for within and between-group differences, where appropriate. Whilst such a study may require significant resources to complete, results may greatly inform the clinical application of BfRT within the context of lower-limb rehabilitation.

From the findings of this doctoral research project, it is apparent to the author that many unanswered questions and variations still exist in relation to the delivery of BfRT and its proposed longitudinal effects. Through the author's awareness of current experimental work being completed by other researchers, there is little doubt that BfRT research will continue to emerge at a rapid pace, both within the context of lower-limb injury rehabilitation and beyond it. It is hoped that the findings and discussions presented in this thesis will influence future BfRT research and allow the potential benefits of this training modality to be fully realised.

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APPENDICES

HEALTH & ELIGIBILITY QUESTIONNAIRE

Study title: 'The Effect of Different Thigh-cuff Pressures upon Blood-flow Volume and Thigh Muscle Oxygenation during Unweighted Knee Exercise Sessions.'

TO TAKE PART IN THIS STUDY, YOU NEED TO BE BETWEEN THE AGES OF
18 AND 40

- Please tick ONE box for each question below -

1) Are you pregnant, or have you given birth within the last month?

Not Applicable YES

NO

2) Do you regularly smoke tobacco products?

(More than once a week, most weeks)

YES

NO

3) Have you currently been diagnosed with high blood pressure?

YES

NO

4) Are you currently diagnosed as obese or underweight?

YES

NO

5) Have you ever experienced any of the following;

A Blood Clot (Deep Vein Thrombosis or an embolism)

YES

NO

A Stroke or a Transient Ischaemic Attack (TIA)

YES

NO

Angina or a Heart Attack

YES

NO

Unexplained Chest Pains

YES

NO

6) Do you have any other current health condition, disease, injury or illness?

YES NO

If YES, please write details of these here:

7) Do you have any allergies, or take medication that can affect your blood pressure or circulation? YES NO

If YES, please write details of these here:

8) Do you feel there is any other reason that could affect your ability to take part in this research study? YES NO

If YES, please write details here:

ENTER YOUR GP/DOCTOR DETAILS BELOW. IF YOU WISH TO OPT OUT OF SUPPLYING THESE DETAILS, PLEASE SIGN YOUR INITIALS HERE >>

Name of your GP/Doctor:

Surgery Name:

Surgery Telephone Number or Address:

THIS BOX WILL BE COMPLETED AT YOUR FIRST APPOINTMENT

The information I have entered onto this questionnaire is correct to the best of my knowledge and belief. Where I have had any questions or concerns, I have first spoken to the researcher for advice before signing below.

Name.....

Signature.....
(DD/MM/YY)

Date / /

Next of Kin:

Next of Kin – Phone:

Appendix Ib. A Written Consent Form (Phase I Study Example)



**Manchester
Metropolitan
University**

Participant ID:

CONSENT FORM

Title of Study: Lower-Limb Blood Flow Restriction: The Relationship between Thigh-cuff Pressures and Physical Characteristics when Determining Target Blood Flow Rates

Name of Primary Researcher: MR PHILIP SMITH

- PLEASE INITIAL ALL BOXES BELOW -

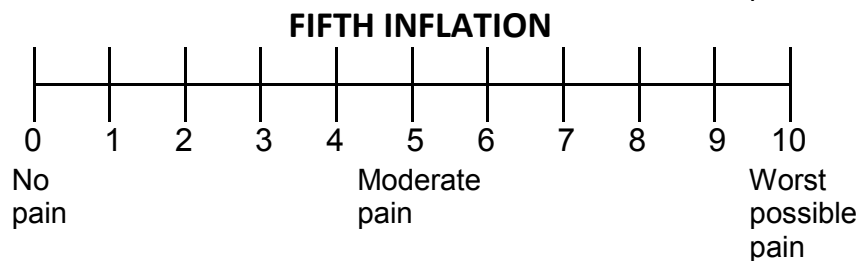
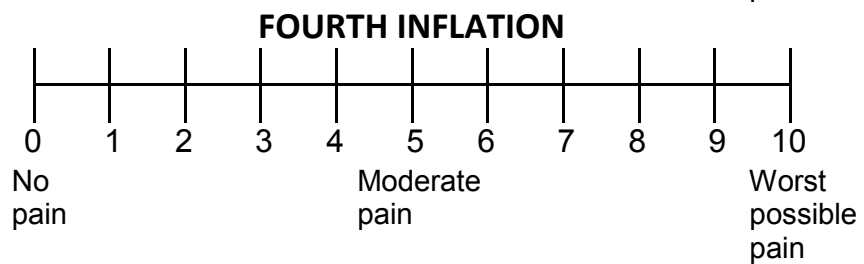
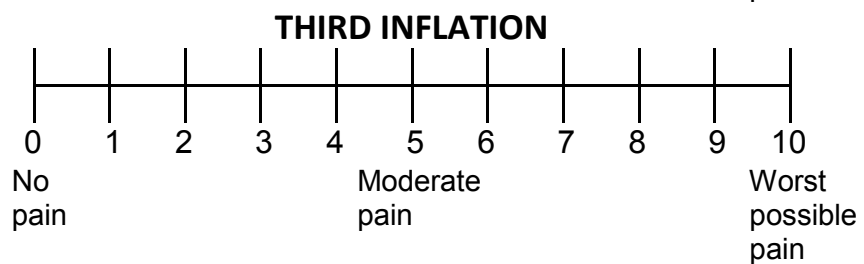
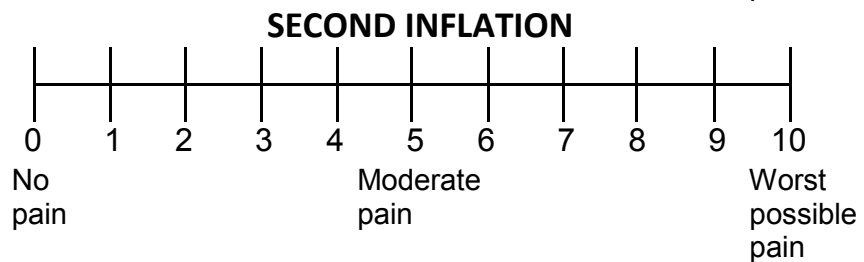
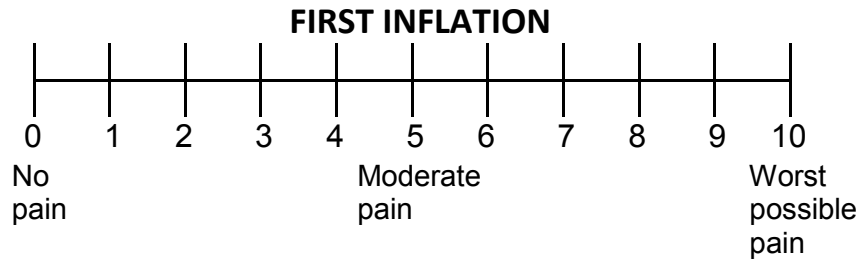
1. I have read and understood the Participant Information Sheet (version 2.3) for this study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
2. I understand that my participation is voluntary. I am free to withdraw at any time without giving a reason, without my medical care or legal rights being affected.
3. I understand that my personal data (from which I can be identified) may be looked at by the Primary Researcher, University supervisors and regulatory bodies where it is relevant. I give permission for this.
4. I agree to take part in the above study.

<input type="checkbox"/>
<input type="checkbox"/>
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<input type="checkbox"/>

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature

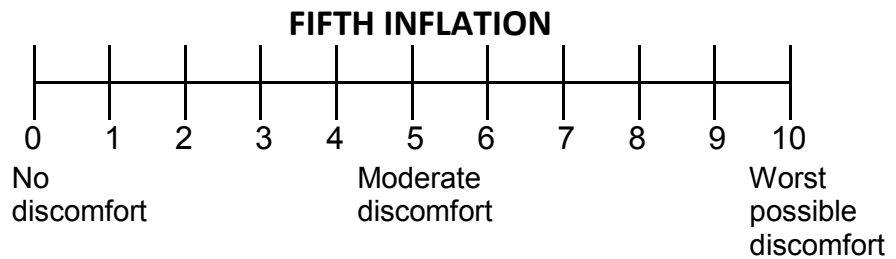
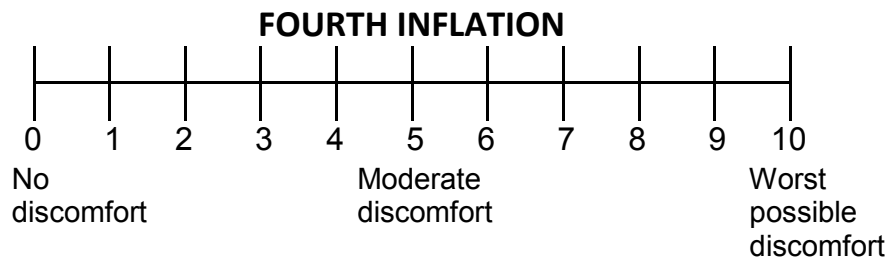
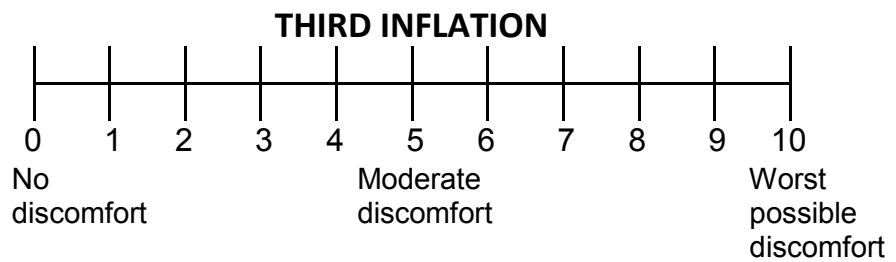
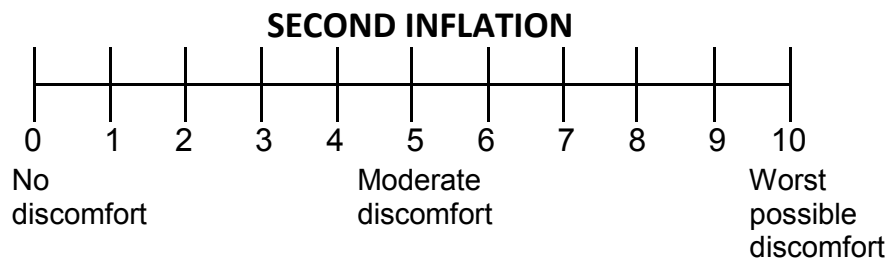
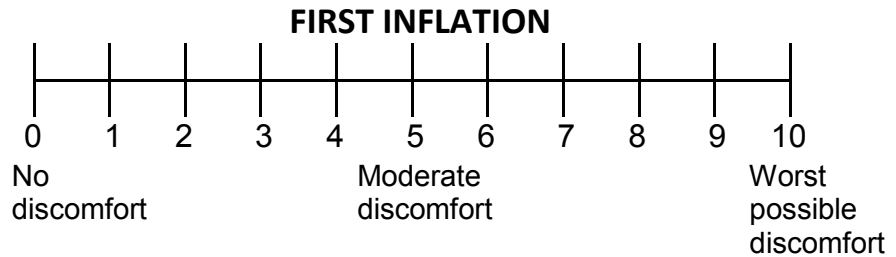
Pain Intensity Scale

At the end of each cuff inflation, **place a cross on the scale** to rate the level of any pain that you experienced

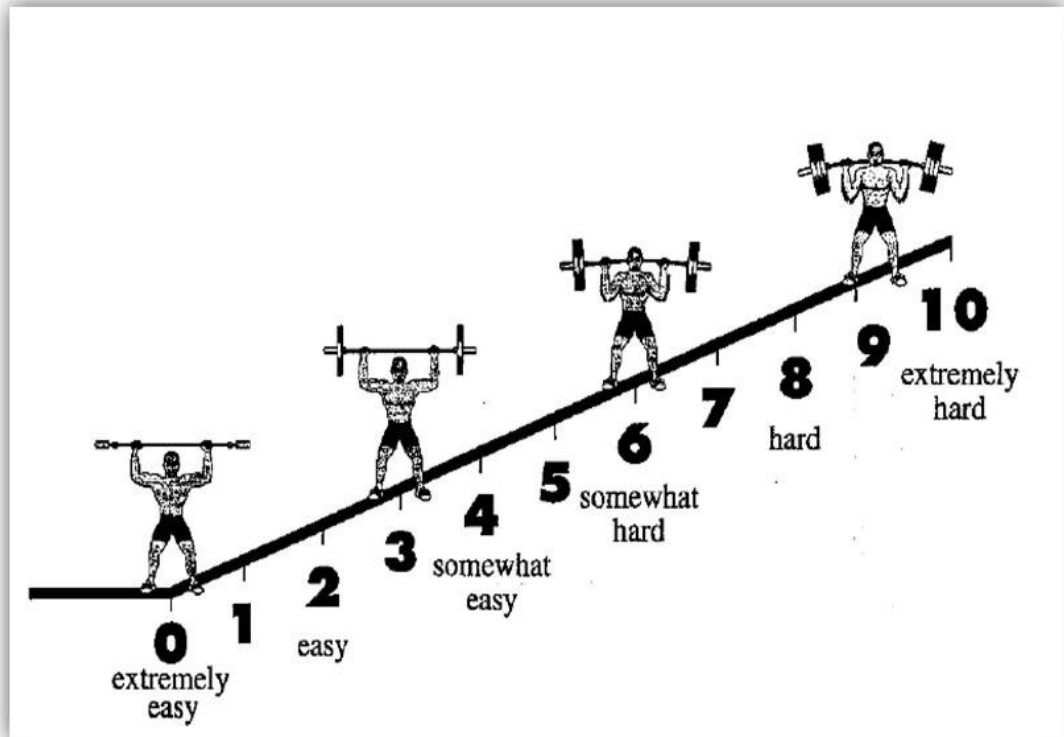


Discomfort Intensity Scale

At the end of each cuff inflation, **place a cross on the scale** to rate the level of any discomfort that you experienced.



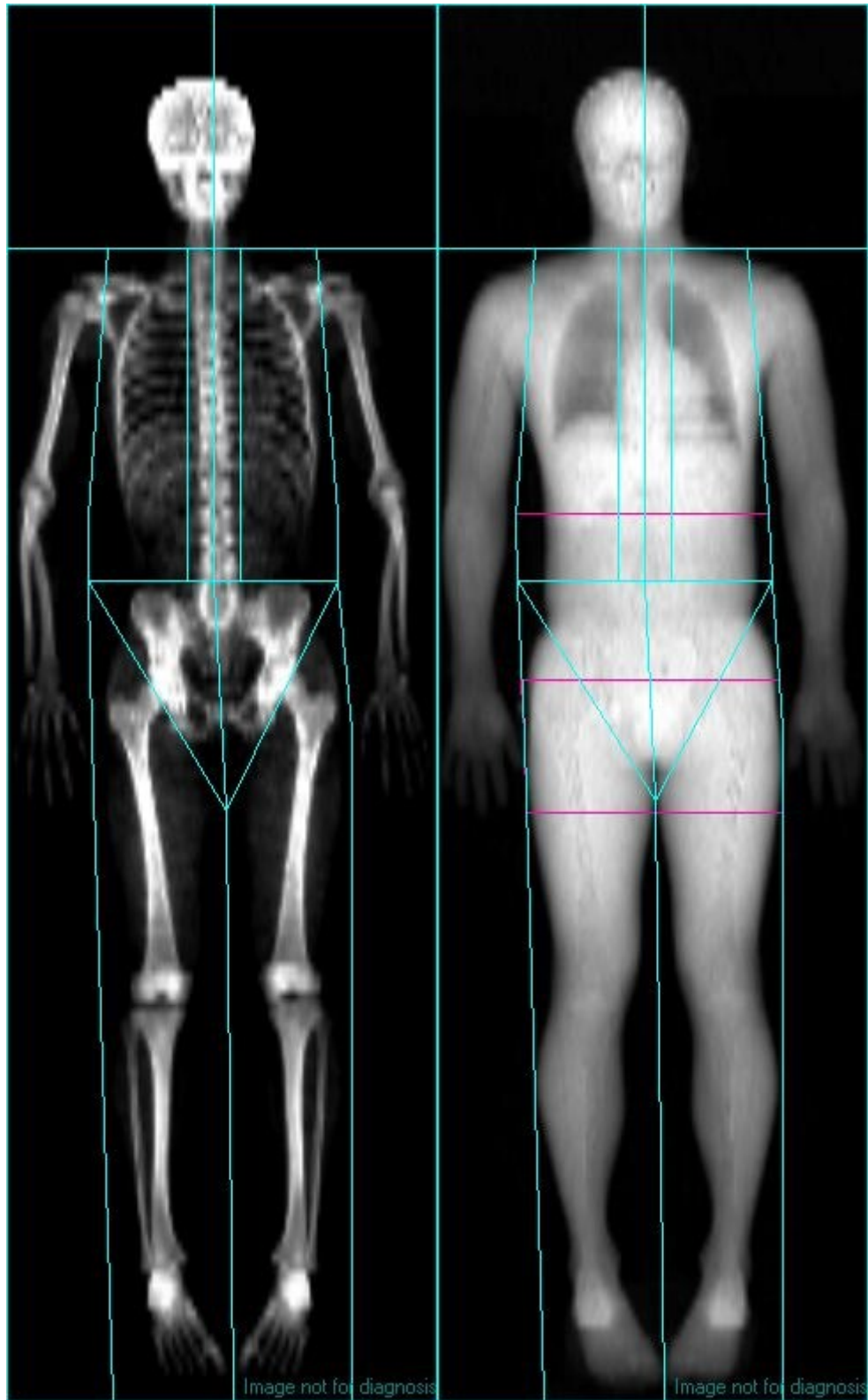
Perceived Exertion



At the end of each set of exercise, look at the chart above. Write down below how hard you feel your muscles worked during the last set, out of 10.

FIRST SET	/10
SECOND SET	/10
THIRD SET	/10

Appendix II. A Dual-energy X-ray Absorptiometry [DXA] Scan Example



An example full-body DXA scan captured during the Phase I study, displaying the placement of all body-region boundaries.

Appendix III. Phase I Cohort Anthropometrics

Physical Characteristic	Cohort Subgroup							
	Rugby Male (n = 21)		Male (n = 21)		Female (n = 19)		Total (N = 61)	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
Age (years)	23.9	3.8	27.7	5.4	26.6	5.6	26.0	5.2
Height (cm)	185.6	5.6	178.9	7.5	168.1	8.6	177.8	10.1
Weight (kg)	96.8	8.3	82.4	11.8	63.5	6.4	81.5	16.3
Body Mass Index	28.1	1.7	25.7	3.2	22.5	1.8	25.5	3.2
Thigh Circumference (cm)	65.1	2.8	60.9	4.7	57.4	3.2	61.2	4.8
Leg Length (cm)	97.3	4.0	93.1	5.1	88.8	5.6	93.2	5.9
Thigh Length (cm)	50.8	2.1	48.6	2.5	47.0	3.4	48.9	3.1
Systolic Blood Pressure (mmHg)	125.0	7.1	120.8	10.3	108.9	5.6	118.5	10.3
Diastolic Blood Pressure (mmHg)	76.0	5.8	74.1	6.6	68.6	6.2	73.0	6.8
Mean Arterial Pressure (mmHg)	92.3	5.6	89.7	6.8	82.0	5.4	88.2	7.3
DXA Body Tissue Fat (%)	17.9	4.5	23.5	9.5	28.0	7.6	23.0	8.5
DXA Total Body Fat Mass (g)	16,692	5,243	19,274	9,468	17,354	4,855	17,787	6,883
DXA Total Body Lean Mass (g)	75,799	6,110	59,699	6,889	43,250	5,717	60,118	14,639
DXA Total Body Mass (g)	97,239	8,434	82,429	11,591	63,206	6,400	81,540	16,546
DXA Test Leg Tissue Fat (%)	17.2	3.8	22.7	9.6	33.5	8.2	24.2	10.1
DXA Test Leg Fat Mass (g)	2,849	765	3,408	1,833	3,968	1,030	3,390	1,357
DXA Test Leg Lean Mass (g)	13,635	1,251	11,045	1,478	7,819	1,269	10,932	2,713
DXA Test Leg Total Mass (g)	17,362	1,575	15,126	2,375	12,277	1,584	15,009	2,787

Appendix IV (a). Bivariate Correlations between In-session Changes to Vastus Lateralis SmO₂ and Physical Characteristics of the Phase II Cohort

Variable & Cuff Pressure		Height (m)	Weight (kg)	BMI	Leg Length	Thigh Length	Thigh Circ.	Systolic BP	Diastolic BP	Mean Arterial Pressure
SmO ₂ 0mmHg	Pearson <i>r</i>	0.426	0.483	0.396	0.462	0.377	0.481	-0.16	-0.166	-0.197
	Sig. (2-tailed)	0.1	0.058	0.129	0.071	0.15	0.059	0.554	0.54	0.465
SmO ₂ 40mmHg	Pearson <i>r</i>	0.344	.696**	.759**	0.399	.506*	.694**	0.447	0.187	0.36
	Sig. (2-tailed)	0.192	0.003	0.001	0.125	0.045	0.003	0.082	0.487	0.171
SmO ₂ 60mmHg	Pearson <i>r</i>	0.443	.634**	.608*	0.269	0.183	.522*	0.185	0.319	0.316
	Sig. (2-tailed)	0.086	0.008	0.012	0.314	0.498	0.038	0.493	0.228	0.233
SmO ₂ 80mmHg	Pearson <i>r</i>	0.084	0.46	.596*	-0.13	-0.205	0.494	0.423	0.443	.524*
	Sig. (2-tailed)	0.758	0.073	0.015	0.632	0.446	0.052	0.103	0.086	0.037

Black and grey boxes indicate sufficiently and insufficiently powered, statistically significant bivariate correlations ($p \leq 0.05$) respectively.

Appendix IV (b). Bivariate Correlations between In-session Changes to Vastus Lateralis tHb and Physical Characteristics of the Phase II Cohort

Variable & Cuff Pressure		Height (m)	Weight (kg)	BMI	Leg Length	Thigh Length	Thigh Circ.	Systolic BP	Diastolic BP	Mean Arterial Pressure
tHb 0mmHg	Pearson <i>r</i>	0.068	0.073	0.032	0.061	0.155	-0.204	0.368	-0.039	0.162
	Sig. (2-tailed)	0.802	0.789	0.906	0.821	0.567	0.449	0.161	0.887	0.549
tHb 40mmHg	Pearson <i>r</i>	-0.405	-0.269	-0.132	-0.346	-0.127	-0.308	-0.002	-0.013	-0.01
	Sig. (2-tailed)	0.12	0.314	0.626	0.189	0.639	0.245	0.993	0.963	0.971
tHb 60mmHg	Pearson <i>r</i>	-0.425	-0.243	-0.078	-0.508*	-0.409	-0.174	-0.292	0.255	0.027
	Sig. (2-tailed)	0.101	0.364	0.775	0.045	0.116	0.519	0.273	0.34	0.92
tHb 80mmHg	Pearson <i>r</i>	-.538*	-.550*	-0.434	-.519*	-0.431	-0.443	-0.485	0.183	-0.122
	Sig. (2-tailed)	0.032	0.027	0.093	0.039	0.096	0.086	0.057	0.497	0.652

Grey boxes indicate insufficiently powered, statistically significant bivariate correlations ($p \leq 0.05$) respectively.

Appendix IV (c). Bivariate Correlations between In-session Changes to Vastus Lateralis HHb and Physical Characteristics of the Phase II Cohort

Variable & Cuff Pressure		Height (m)	Weight (kg)	BMI	Leg Length	Thigh Length	Thigh Circ.	Systolic BP	Diastolic BP	Mean Arterial Pressure
HHb 0mmHg	Pearson <i>r</i>	-0.436	-0.493	-0.404	-0.466	-0.377	-0.49	0.149	0.155	0.184
	Sig. (2-tailed)	0.091	0.052	0.121	0.069	0.15	0.054	0.582	0.567	0.496
HHb 40mmHg	Pearson <i>r</i>	-0.379	-.726**	-.778**	-0.43	-.525*	-.720**	-0.457	-0.197	-0.371
	Sig. (2-tailed)	0.147	0.001	< 0.001	0.096	0.037	0.002	0.075	0.465	0.157
HHb 60mmHg	Pearson <i>r</i>	-0.439	-.628**	-.604*	-0.272	-0.19	-.518*	-0.206	-0.305	-0.317
	Sig. (2-tailed)	0.089	0.009	0.013	0.308	0.482	0.04	0.445	0.251	0.232
HHb 80mmHg	Pearson <i>r</i>	-0.106	-0.485	-.618*	0.103	0.183	-.524*	-0.432	-0.401	-.500*
	Sig. (2-tailed)	0.695	0.057	0.011	0.703	0.499	0.037	0.095	0.124	0.049

Black and grey boxes indicate sufficiently and insufficiently powered, statistically significant bivariate correlations ($p \leq 0.05$) respectively.

Appendix IV (d). Bivariate Correlations between the Mean Three-session Change to Vastus Lateralis Haemodynamic Variables and Physical Characteristics of the Phase II Cohort

Variable		Height (m)	Weight (kg)	BMI	Leg Length	Thigh Length	Thigh Circ.	Systolic BP	Diastolic BP	Mean Arterial Pressure
BfR3	Pearson <i>r</i>	.241	.533*	.606*	.258	.395	.466	.064	.262	.214
	Sig. (2-tailed)	.368	.034	.013	.335	.130	.069	.815	.327	.426
SmO ² 3	Pearson <i>r</i>	.356	.723**	.791**	.199	.160	.685**	.415	.404	.493
	Sig. (2-tailed)	.176	.002	< 0.001	.460	.554	.003	.110	.121	.052
tHb3	Pearson <i>r</i>	-.544*	-.429	-.264	-.555*	-.409	-.362	-.345	.191	-.045
	Sig. (2-tailed)	.029	.097	.323	.026	.116	.168	.191	.479	.869
HHb3	Pearson <i>r</i>	-.369	-.731**	-.794**	-.215	-.171	-.695**	-.428	-.379	-.482
	Sig. (2-tailed)	.160	.001	< 0.001	.424	.526	.003	.099	.148	.059

Appendix V (a). Data Synthesis Table

	Controlled Trials						Other Research Designs	
	Cook et al (2010; 2014)	Iversen et al (2015)	Kubota et al (2008)	Kubota et al (2011)	Ohta et al (2003)	Takarada et al (2000b)	Hackney et al (2016)	Lejkowski and Pajaczkowski (2011)
Cohort size	16	24	15	11	44	16	13	1
Injury Type	No Injury	Acute ACL Reconstruction	No Injury	No Injury	Acute ACL Reconstruction	Acute ACL Reconstruction	No Injury	Acute ACL Reconstruction
Weight bearing Status	Voluntary NWB	Enforced NWB	Voluntary NWB	Voluntary NWB	NWB to FWB	Enforced NWB	Voluntary NWB	Enforced PWB
Outcome Measures	Thigh & calf muscle CSA via MRI; Muscle strength via 1RM, MVC and IKD; Dynamic muscular endurance (# reps until failure)	Thigh muscle CSA via MRI	Muscular force via IKD; Serum growth hormone via blood sampling; Thigh & Leg circumference via tape measurement	Muscular force via IKD; Thigh & Leg circumference via tape measurement	Muscular force (knee) via IKD; Thigh muscle CSA via MRI; Muscle fibre diameter (vastus lateralis) via biopsy	Thigh muscle CSA via MRI;	Thigh (knee extensors) & Calf (planter flexors) muscle CSA via MRI; Muscular strength via 1RM; Dynamic muscular endurance (# reps until failure)	Thigh circumference via tape measurement; Subjective function via KOOS and LEFS

Intervention Duration	30 days	14 days	14 days	14 days	16 weeks	10 days (14 days between pre-post intervention MRI scans)	25 days	12 weeks
Exercise Type(s)	Seated knee extension	Isometric quadriceps, end-of-range knee extensions, straight leg raises (all performed in long-sitting position)	None	None	(Progressive) Straight leg raise, hip abduction, hip adduction, half-squats, knee-bend walking, step-ups	None during occlusion, otherwise followed a 'normal' rehab protocol - exercises not reported	Supine leg press, supine calf raise	Knee extension, heel slides, squats, lunges, step-ups
# of BfR sessions per week	3	14	14	14	6	14	3	4 to 5
# of Sets	3 sets to volitional failure	5 sets of 5 mins of BfR	5 sets of 5 mins of passive BfR	5 sets of 5 mins of passive BfR	4 sets	5 sets of 5 mins of passive BfR	3 sets to volitional failure	2 sets
Number of Repetitions	N/A	20 reps of exercise per 5-minute set	N/A	N/A	30-15-15-15	N/A	N/A	20 repetitions
Rest Period Duration	90 seconds	180 seconds	180 seconds	180 seconds	Not reported	180 seconds	90 seconds	30 seconds
Exercise Intensity	20% MVC	Unknown	N/A	N/A	Various	N/A	20-30% MVC	Various
Cuff Type/Manufacturer	Hokanson	Delphi	Mihuzo	Mihuzo	An 'air tourniquet'	A 'pneumatic occlusion cuff'	Hokanson	Welch Allyn

Cuff Inflation Method	E20 Rapid Cuff Inflator	Manual sphygmomanometer	Not reported	Not reported	Manual Sphygmomanometer	Not reported	E20 Rapid Cuff Inflator	Manual Sphygmomanometer
Cuff Width	6cm	14cm	7cm	7cm	Not reported	9cm	6cm	15.2cm
Main or Mean Cuff Pressure Applied (mmHg)	150 (SD 10)	130-180	200	50	180	238 (Range180-260)	140 (SD 10)	100
Cuff Pressure Calculation Used	1.3x SBP*	None	Not reported	Not reported	Not reported	None (cited earlier work to justify pressures used)	1.3x SBP*	Not reported
BfR Modality	Continuous	Intermittent	Intermittent	Intermittent	Not reported	Intermittent	Continuous	Intermittent, then continuous after 3 days

*SBP = Systolic Blood Pressure

Appendix V (b). Risk of Bias Table

Selected Studies (First Author and Year of Publication)	Potential Sources of Bias						
	Selection Bias		Performance Bias	Detection Bias	Attrition Bias	Reporting Bias	Other Bias
	Inadequate Randomisation	Inadequate Concealment					
Cook et al (2010)	Yellow	Yellow	Yellow	Green	Green	Yellow	Green
Cook et al (2014)	Yellow	Yellow	Yellow	Green	Green	Yellow	Green
Iversen et al (2015)	Green	Yellow	Green	Green	Green	Green	Yellow
Kubota et al (2008)	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Kubota et al (2011)	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
Ohta et al (2003)	Red	Red	Yellow	Yellow	Green	Yellow	Green
Takarada et al (2000b)	Yellow	Yellow	Yellow	Yellow	Green	Yellow	Yellow

KEY: Risk of Bias Judgement	Green	Low Risk
	Red	High Risk
	Yellow	Unclear Risk

Appendix VI (a) A Conference Paper relating to The Phase I Main Study.

Presented at the 4th (European Region) World Confederation for Physical Therapy Congress [ER-WCPT]. 11th and 12th November 2016. Liverpool, United Kingdom.

Citation Information

Smith, P., Azzawi, M., Stirling, B., Stockley, R., Goodwin, P. (2016) 'Lower-limb Blood-flow Restriction Training: using physical size and thigh cuff pressure to predict the amount of restriction being delivered'. *Physiotherapy* 102(S1), pp. e135-136 DOI: <http://dx.doi.org/10.1016/j.physio.2016.10.154>

Conference Abstract

Relevance

The conference theme of 'Research, education and Practice' is relevant to this work. Blood-flow Restriction Training [BfRT] involves the temporary, artificial reduction of blood flow through a limb, often during low-intensity exercise. Evidence suggests that BfRT may attenuate losses in thigh muscle size and strength if a non-weight bearing period occurs following lower-limb injury. However, the restriction equipment often employed in BfRT research can be inaccessible to many clinicians. Variations may also exist between individuals in the amount of blood-flow restriction [BfR] experienced at a given thigh cuff pressure. To maximise the impact of BfRT research upon clinical practice, work is needed to remedy these issues via the use of clinically-accessible restriction equipment and evidence-based, personalised cuff pressures.

Purpose

Using a thigh blood-pressure cuff, this study investigated whether the physical size of an individual was associated with, or could help clinicians to predict, the amount of BfR experienced at different cuff pressures.

Methods/Analysis

Ethical approval was gained from Manchester Metropolitan University. Following informed consent, 61 healthy adults aged <40 years were recruited into three subgroups (21 males, 19 females, 21 professional male rugby players). Physical measurements were recorded for each participant via Dual X-ray Absorptiometry and a flexible tape measure. In a resting seated position, five cuff pressures (40/60/80/100/120mmHg) were applied in a randomised order using a 21cm-wide blood pressure cuff fitted around one thigh. Each cuff inflation lasted thirty seconds, with three minutes separating each inflation. Changes in popliteal arterial blood-flow volume between baseline and each cuff pressure were recorded using Doppler ultrasound, then converted into percentages for each individual. Between-subgroup comparisons were calculated using parametric and non-parametric tests. Relationships between the applied cuff pressure, physical measurements and the percentage of popliteal blood-flow remaining [%PBfR] were analysed via Pearson correlation coefficients and multiple stepwise regression.

Results

A strong linear relationship existed between the cuff pressure applied and %PBfR for the cohort (Pearson $r = -0.77$, $p < 0.001$). Subgroups were statistically different across nineteen physical size measurements ($p < 0.001$). However, a between-subgroup difference in

%PBfR existed only at 40mmHg cuff pressure ($p < 0.05$). For the whole cohort, weak correlations were found across all cuff pressures between %PBfR and seven physical size measurements (Pearson r 0.12-0.15, $p \leq 0.05$). A model was formed to predict %PBfR based predominantly upon the amount of cuff pressure applied, combined with tape measurements of thigh length and circumference (r 0.79, r^2 0.63, $p < 0.001$).

Discussion and conclusions

Results suggest small associations between physical size and the %PBfR experienced at sub-occlusive thigh cuff pressures. Whilst some between-subject variations in the amount of BfR experienced still remain unexplained, the model produced will allow clinicians to begin calculating and justifying their cuff pressure selections. Further work will combine low-intensity exercise with different amounts of BfR to ascertain optimum cuff pressure(s) to use during rehabilitation sessions.

Impact and implications

This is the first study to aide clinicians in using a thigh blood-pressure cuff to induce known amounts of BfR. It will help optimise the delivery and clinical-accessibility of BfRT following lower-limb injury.

Key words

Blood-flow restriction; Lower-limb injury; Low-intensity exercise

Funding acknowledgements

This study was funded via a PhD Studentship from Manchester Metropolitan University.

Appendix VI (b) A Conference Paper relating to The Phase II Main Study.

Presented at the World Confederation for Physical Therapy Congress [WCPT]. 4th July 2017. Cape Town, South Africa.

Citation Information

Smith, P., Azzawi, M., Stirling, B., Stockley, R., Goodwin, P. (2017) 'Increasing the metabolic stress of an unweighted knee exercise via Blood-flow Restriction Training: A potential treatment adjunct for physiotherapists'. Display number RR-PO-06-06-TUE. (Awaiting DOI).

Conference Abstract

Background

Blood-flow Restriction Training [BfRT] involves the temporary, artificial reduction of blood flow through a limb, often during low-intensity resistance exercise. Following lower-limb injury or surgery, evidence suggests that BfRT can be used to minimise losses in thigh muscle size and strength or accelerate their return. However, the restriction equipment used in BfRT research is often inaccessible to frontline clinicians. There is also little evidence as to the acute metabolic effect of adding blood-flow restriction to un-resisted, or 'no load', rehabilitation exercises.

Purpose

Using an inexpensive restriction device, this study investigated whether adding lower-limb blood-flow restriction to a rehabilitation-appropriate 'no load' knee exercise produced a significant change in the acute metabolic stress of the exercise session.

Methods

The height, weight and leg measurements of n=16 healthy participants (n=9 male) were recorded. Participants attended four exercise sessions separated by at least 48 hours. Each session consisted of three, one-minute sets of a single-leg, unweighted knee-extension exercise. Throughout all sessions a 21cm-wide thigh blood-pressure cuff was wrapped around the thigh of the exercising limb. During the first exercise session the cuff was not inflated [control]. Over the remaining three sessions, the cuff was inflated to one preselected pressure [40/60/80mmHg] in order to restrict blood flow through the exercising limb. At the start of these sessions, the percentage of popliteal arterial blood-flow volume remaining after cuff inflation [BfR] was determined using Doppler ultrasound. To indicate metabolic stress, near infra-red spectroscopy was used to record deoxygenated haemoglobin mass [HHb] of the vastus lateralis muscle before and during every exercise session. Cohort differences in BfR and HHb change for each exercise session were then compared.

Results

All participants completed all exercise sessions. BfR decreased as cuff pressure was increased, with 80mmHg inducing a mean BfR of 47.6% (95% CI 42.9% - 52.3%). HHb of the vastus lateralis muscle did not increase during the control session. HHb of the vastus lateralis muscle increased significantly when cuff pressures were applied during the three remaining sessions. (Repeated Measures ANOVA, $p < 0.001$, partial $\eta^2 0.65$). Overall, a

higher Body Mass Index was associated with smaller changes in HHb of the vastus lateralis muscle during sessions in which cuff pressures were applied (Pearson R -0.794, R² 0.630, p < 0.001).

Conclusions

Results indicate that adding lower-limb blood-flow restriction significantly increased the local, acute metabolic stress of a rehabilitation-appropriate knee exercise without the need to increase exercise load or repetitions. At cuff pressures up to 80mmHg, the degree of metabolic stress experienced during 'no load' BfRT differed between individuals and was associated with their Body Mass Index.

Implications

Using an inexpensive blood-pressure cuff as the restriction device, findings support the potential use of lower-limb BfRT as a treatment adjunct following lower-limb injury. To deliver a consistent level of metabolic stress among different individuals, clinicians may need to tailor the amount of thigh cuff pressure that they apply based upon an individual's physical size. Further research is required to determine the potential magnitude of acute metabolic stress required to attenuate the effects of muscle disuse within injured populations.

Keywords

Blood-flow Restriction; BfR; Injury Rehabilitation

Funding Acknowledgements

Manchester Metropolitan University provided the primary researcher with a funded scholarship in order to complete this research.

Ethics Approval

Ethical approval was granted by the Faculty Ethics Committee of Manchester Metropolitan University (Application number 1269).

Brief biography of presenting author

The presenting author is currently a PhD candidate of Manchester Metropolitan University, UK. Having graduated as a Physiotherapist from the same University in 2014, the author is now using his doctoral studies to investigate the development and use of lower-limb blood-flow restriction training within the context of injury rehabilitation.

Information concerning any prior publication of presentation of the work

This work, in part or in whole, has not been previously published or presented at a national or international level.