CARDIOVASCULAR CONSEQUENCES AFTER SPINAL CORD INJURY AND THE EFFECTS OF ELECTRICAL STIMULATION

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Abstract

A spinal cord injury (SCI) is an extremely complex condition and is associated with numerous adverse health complications. One concern in particular is an increased prevalence and risk of cardiovascular disease (CVD). Cardiovascular disease, which encompasses pathologies of the heart and vascular tree, is one of the leading causes of morbidity and premature mortality in this population. Current clinical practice guidelines for CVD risk estimation in SCI use traditional cardiovascular risk factors and risk algorithms validated in able-bodied populations. However, despite the increased prevalence of CVD in people with SCI, research has demonstrated no difference in traditional cardiovascular risk factors when compared to the able-bodied population. The aim of *Chapter 3* was to explore whether traditional models of CVD risk prediction are accurate in estimating CVD events in people with SCI. Firstly, this study used retrospective data from 200 individuals with a SCI to prospectively estimate their 5-year risk of developing CVD using the FRS. The difference in clinical outcomes of individuals with a below median FRS (≤1.36%) were compared to individuals with an above median FRS (>1.36%) using Kaplan-Meier curves and log-rank test. The end point was defined as either a CVD event or CVD mortality. Across the 5-year period, 39 (19.5%) participants developed a CVD event, 10 of which were fatalities. The log-rank test demonstrated that individuals with >median FRS vs. <median FRS had a 3.2 fold greater risk for developing a CVD event. Secondly, this study used receiver operating characteristic curves (ROC) with corresponding area under the curve (AUC) to explore the prognostic accuracy of the FRS in being able to predict CVD events in people with SCI. The ROC-AUC suggests acceptable accuracy of the FRS in identifying individuals with increased risk for CVD events (ROC-AUC of 0.71, 95% CI 0.62-0.82). Finally, SCI-related factors were explored using univariate cox proportional hazard regression to try to determine individual predictors for CVD. These factors were separately added to the FRS in an attempt to improve the prognostic capability of the FRS when used for people with SCI. Adding ASIA impairment (0.74; 95% CI 0.66-0.82), motor impairment (0.74; 95% CI 0.66-0.83), level of injury (0.72; 95% CI 0.63-0.81) or active engagement in sport prior to injury (0.72; 95% CI 0.63-0.88) to the FRS did not improve the level of discrimination.

The findings in *Chapter 3,* together with other research, suggest that traditional CVD risk factors cannot solely be accountable for the increased CVD morbidity and mortality in SCI. Instead, the effects of chronic physical inactivity and extreme vascular deconditioning resulting from lower limb paralysis have been proposed as an alternative mechanism explaining the greater CVD prevalence in the absence of abnormal CVD risk factors. This provides a rationale for needing appropriate interventions targeting the deconditioned vasculature. Reactivating the muscles

using electrical stimulation has shown to reverse the rapid vascular and muscular deconditioning that occurs in people with SCI. However, the majority of these ES methods are not easily accessible for individuals with SCI or practical for regular homebased use. The primary aim of the study outlined in *Chapter 4* was to examine the acute effects of low intensity ES using a wearable clothing garment on macrovascular (femoral) and microvascular (skin) perfusion and oxygen consumption in people with SCI. This particular method of ES has significant clinical potential because it can be applied for longer periods and in a home based environment. Eight participants with a motor complete SCI received 4x3-minutes of unilateral gluteal and hamstring ES. Deep femoral artery (DFA) blood flow, oxygen consumption, and gluteal and hamstring cutaneous perfusion were measured at baseline and during each block of ES. DFA blood flow increased by 18.1% with the application of ES. Skin perfusion did not change during an individual block of stimulation but did progressively increase over time with repeated stimulation and was 80% higher during the last 3-minute block compared with the first 3-minute block of ES. There was no change in DFA blood flow in the contralateral control leg or in oxygen consumption.

Individuals with SCI are subject to additional secondary complications such as skin ulcers resulting from poor microvascular perfusion and prolonged sitting pressure. This, along with the findings from *Chapter 4,* provide a compelling rationale for studies to examine the long-term effects of low-level ES on the vasculature, pressure ulcer risk factors, and the feasibility of home based ES devices such as a wearable clothing garment. The aim of *Chapter 5* was to explore the changes in femoral artery diameter, basal blood flow, cutaneous microvascular function, limb volume and sitting pressure in people with SCI after 12 weeks of daily low-level ES using a wearable clothing garment. Twelve participants were instructed to wear the ES garment every day or night. The ES protocol lasted for 6 hours and consisted of 8x30 minutes of active ES with 15 minutes rest in-between. Three participants did not complete the study therefore data analysis was performed on the nine remaining individuals. After 12 weeks of ES, resting diameter and mean basal blood flow of the common femoral artery increased by 8.3% ($P<0.001$) and 43% ($P<0.001$), respectively. In response to graded local heating a significant interaction of heating stage and time was observed for gluteal CVC ($P = 0.02$) and $\%CVC_{max}$ ($P <0.001$) but not for hamstring CVC ($P = 0.86$) or %CVCmax. There was a significant increase in average thigh circumference ($P = 0.008$) and calculated thigh volume ($P = 0.01$) after 12 weeks of ES. A significant decrease in peak sitting pressure ($P = 0.003$) and pressure gradient ($P = 0.007$) was observed. Questionnaires were used to examine the feasibility of using the ES garment on a daily basis. Majority of participants wore the device at night whilst sleeping and indicated it did not disrupt their sleep quality.

Applying the garment takes 10 minutes or less, can be worn under suitable clothing and does not disrupt daily living activities. More importantly, all the individuals who completed the intervention indicated they would be happy to continue using the device.

The findings from this thesis suggest that, firstly, traditional cardiovascular risk factors and the FRS are able to distinguish individuals who are at a higher vs. lower risk for CVD, but the absolute prediction values markedly underestimate the occurrence of CVD in people with SCI. Additionally, the increased risk of CVD in people with SCI appears independent of level and severity of injury. Secondly, this thesis indicates that acute low-intensity ES using a wearable clothing garment improves micro- and macrovascular perfusion. Finally, the research undertaken in this thesis provides evidence that home based, daily, low-intensity ES using a wearable clothing garment represents a practical and effective strategy to reduce the risk of pressure ulcers, improve vascular perfusion and help prevent vascular complications in people with SCI.

Acknowledgments and Person Comments

The 3-year long journey I have taken to complete this PhD has been extremely challenging, but yet a very rewarding and enjoyable process (now that it is over). It has given me the confidence and self-belief that I can turn my hand to absolutely anything and be successful. However, I would never have come this far without the guidance, help, and support from a few very special people.

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Declaration

I declare that the work presented in this thesis was performed in accordance with regulations of Liverpool John Moores University and has not been concurrently used for application of any other form of degree or professional qualification at this same University, or any other institute. Except where otherwise stated by reference and acknowledgement, the work presented in this thesis is entirely my own.

Publications directly based on the work described in this thesis

Barton, T.J., Low, D.A., Janssen, T.W.J., Sloots, M., Smit, C.A.J. and Thijssen, D.H.J. (2018) Femoral artery blood flow and microcirculatory perfusion during acute, low-level functional electrical stimulation in spinal cord injury. *Am J Phys Med Rehabil*.

Submitted manuscripts directly based on the work contained in this thesis

Barton, T.J., Low, D.A., Bakker, E.A., Janssen, T.W.J., de Groot, S., Van der Woude, L.H. and Thijssen, D.H.J. (2018) Traditional cardiovascular risk factors strongly underestimate the 5-year occurrence of cardiovascular morbidity and mortality in persons with spinal cord injury. Under Review. *Journal of Physiotherapy*

Book Chapters

Barton T.J., Low D.A., Thijssen D.H.J. (2016) Cardiovascular Responses to Exercise in Spinal Cord Injury. In: Taylor J. (eds) The Physiology of Exercise in Spinal Cord Injury. Physiology in Health and Disease. Springer, Boston, MA

List of Abbreviations

AB	Able-bodied
Angiotensin II	ANG II
ASIA	American Spinal Cord Injury Association
ATP	Adenosine triphosphate
AUC	Area under the curve
AVAs	Arteriovenous anastomoses
BMI	Body mass index
CFA	Common femoral artery
CNS	Central nervous system
CV	Cardiovascular
CVC	Cutaneous vascular conductance
CVD	Cardiovascular disease
DEXA	dual-energy x-ray absorptiometry
DFA	Deep femoral artery
DVT	Deep vein thrombosis
EDHFs	Endothelial-derived hyperpolarising factors
eNOS	endothelial nitric oxide synthase
ES	Electrical stimulation
ESV	End systolic volume
ESV	End systolic volume
ET-1	Endothelin-1
FES	Functional electrical stimulation
FMD	Flow mediated dilation
FRS	Framingham risk score
FSA	Force sensitive array
HDL	High density lipoprotein
Hz	Hertz
IT	Ischial tuberosities
L-NAME	NG-nitro-L-arginine methyl ester
LV	Left ventricle
mA	Milliamperes
MAP	Mean arterial pressure
NEPI	Norepinephrine
NOS	Nitric oxide synthase
NO	Nitric oxide
NPY	Neuropeptide-Y
PSP	Peak systolic pressure
RBCF	Red blood cell flux
ROC	Receiver operating characteristic curves
ROI	Region of interest
SCI	Spinal cord injury
TRPV-1	Transient receptor potential vanilloid type 1
WT	Wall thickness

Chapter 1
Introduction

The spinal cord is part of the central nervous system (CNS) and is a long tubular bundle of nervous tissue that resides within the spinal canal of the vertebral column. It is approximately 45cm and 43cm long in men and women, respectively, and runs continuously extending from the foramen magnum through to the conus medullaris at the level of the first or second lumbar vertebrae. Beyond the conus medullaris and into the lumbar, sacral and coccygeal region is the cauda equina. The cauda equina is a bundle of spinal nerves that distribute out from the spinal cord and innervate the pelvic organs and lower limbs. An extension of the spinal cord, called the filum terminale, is a fibrous strand of tissue and continues down to the coccyx to anchor the cord in place.

The spinal cord consists of 31 segments (8 cervical, 12 thoracic, 5 lumbar, 5 sacral and 1 coccygeal) from which 31 pairs of spinal nerves distribute out. The spinal nerves connect the spinal cord to the peripheral nervous system and are responsible for the transmission of efferent nerve signals from the cerebral cortex to the body via the ventral root and afferent nerve signals from the sensory neurons to the sensory cortex via the dorsal root. In the cervical region (C1-8), the spinal nerves control the transmission of signals to the neck, shoulders, arms, hands and the diaphragm. Signals to the intercostal muscles, the abdomen and the trunk are controlled by the spinal nerves in the thoracic region (T1-12) and the lumbar spinal nerves control signals to parts of the lower limbs and some of the external genital organs. Finally, the sacral spinal nerves (S1-5) control signals to parts of the lower limbs such as the ankles, feet and toes and the external genital organs.

The autonomic nervous system regulates the function of all internal organs. It is modulated by the hypothalamus within the brain, transcends the spinal cord, exits at the various spinal cord segments and innervates the target organ. As the name suggests, the system works autonomously without the need for any conscious effort or voluntary input from a person. The autonomic nervous system receives feedback from environmental cues and responds accordingly by stimulating bodily processes via the sympathetic or parasympathetic nervous system. The parasympathetic

nerves, namely the vagus nerve and cranial nerves, exit the medulla as long preganglionic neurons and synapse with short post-ganglionic neurons within the target tissue. The sympathetic nerve fibres exit the medulla and travel down the spinal cord to the appropriate spinal segment. Relatively short pre-ganglionic fibres travel out of the spinal cord, synapse within sympathetic ganglia and continue as post-ganglionic efferent fibres where they synapse with their effector organ.

Due to the sympathetic division of the autonomic nervous system transcending the spinal cord, injury to the spinal cord results in dramatic changes in the regulation of sympathetically innervated organs and bodily functions. A spinal cord injury is the consequence of damage to any part of the spinal cord within the neural canal and can arise from either a traumatic (e.g. falls, road traffic collisions, sporting injuries & violence) or non-traumatic (infectious disease, tumour, musculoskeletal disease & congenital disease) incident. The result depends on the extent and severity of the injury, but can include motor and/or sensory impairments of the upper and/or lower limbs, as well as loss in the autonomic regulation of certain internal organs and bodily functions.

All sensory and motor information travels between the brain and the body via the length of the spinal cord. Therefore, functional impairment worsens in a lesion that is more rostral. A spinal cord injury (SCI) in the cervical region is associated with an extensive loss in sensory and motor function affecting the arms, trunk and legs and is a condition referred to as tetraplegia. An individual who sustains a (incomplete) C1-C4 SCI will likely require mechanical ventilatory support due to the direct interference of autonomic control as well as paralysis of the diaphragmatic, intercostal and abdominal muscles. A person with a SCI sustained below the cervical region in the thoracic or lumbar segments is referred to as paraplegic and will experience a loss in sensory and/or motor function in the trunk, hips and legs. Additional to the level of the neurological injury, the extent of sensory, motor and autonomic dysfunction is also dictated by whether the lesion is classified as ‘complete’ or ‘incomplete’. The latter term is used when the presence of sacral

sparing remains i.e. preservation of any sensory and/or motor function below the neurological level.

A SCI has a significant impact on health. For example, mortality rates in SCI are elevated by 47% compared to the able-bodied (AB) population, with cardiovascular disease (CVD) being a leading cause in all-cause mortality (Garshick et al, 2005). This may be explained by a number of factors, such as changes in autonomic control and sensorimotor deficits, which lead to altered control of the heart and vasculature. The loss of voluntary muscle contractions in the lower limbs, and therefore an extreme case of physical inactivity, likely contributes to structural changes and impairment in vascular function, which predisposes individuals with SCI to an increased risk of poor cardiovascular health. Additionally, the lack of sympathetic innervation in large vascular beds (such as skeletal muscle and the splanchnic region) coupled with reduced venous return (due to the loss of skeletal muscle pumping activity) contribute to arterial hypotension. Moreover, in individuals with SCI above T6, brief periods of sympathetic hyper-stimulation result in sudden periods of dangerously high blood pressure, a phenomenon known as autonomic dysreflexia. Exposure to these bouts of hypertension can potentially lead to cardiovascular morbidity and a myriad of cardiovascular complications such as myocardial infarction, pulmonary edema and intracerebral haemorrhage. In conclusion, SCI individuals, due to their lesion, are at increased risk for CVD.

Based on the increased CVD risk, it is important that clinicians are able to identify those individuals at an increased risk for CVD, enabling the timely prescription of health care interventions potentially leading to the prevention and improved treatment of CVD. Current screening tools, such as the Framingham Risk Score (FRS), have been validated in the able-bodied population only. This raises the question whether such screening tools are able to provide accurate risk prediction for individuals with a SCI. Therefore, the first aim of this thesis is to determine the accuracy of the FRS in predicting the 5-year occurrence of cardiovascular morbidity and mortality in a SCI population.

In persons with SCI, due to the lower limb paralysis and consequent inactivity of the lower limbs, demonstrate marked atrophy and impairment of the lower limb vascular function and structure. Functional electrical stimulation (FES) is a technique that uses electrical currents applied to peripheral nerves to induce a contraction of the paralyzed muscles. Applying regular FES has the ability to counteract many of these detrimental effects. The application of FES, or alternatives such as FES-cycling, hybrid FES-cycling (leg stimulation with simultaneous arm crank exercise) and FES-rowing, are widely used to enable individuals to regain the benefits of regular aerobic exercise and reverse many detrimental effects of chronic deconditioning of the vasculature. FES-exercise is associated with episodic increases in blood flow through the vessel lumen, supplying the paralyzed muscles and are a key stimulus to initiate vascular remodeling. Specifically exploring the vascular changes that occur with FES, majority of the work uses FES-based exercise training interventions (Gerrits et al, 2001a; Hopman et al, 2002; Thijssen et al, 2005; Van Duijnhoven et al, 2009). These vary from 4 to 8 weeks in duration, with FES-exercise bouts being performed 2-3 times a week using high amplitudes to induce muscle contractions. Despite the positive effects on vascular structure and function, which typically occur within weeks, the procedures associated with FES-based exercise training are limited by their laboratory-based nature, need for trained personnel and expensive machinery, thus limiting their practicality making regular application prove difficult.

Many of the barriers associated with ES (electrical stimulation) methods mentioned above are overcome with the use of wearable clothing garments with embedded surface electrodes. The novelty of this approach could in theory allow for low level ES to be administered throughout the day and/or night on a day-to-day basis. Some preliminary work on this method, targeting pressure sore prevention, has recently been undertaken and has shown promising results. For example, reductions in pressure over the ischial tuberosity and a larger pressure distribution when sitting were reported during low-intensity ES on the gluteal region (Smit et al, 2013a). Additionally, adopting the same ES strategy, tissue oxygenation levels (representative of tissue blood flow) were increased in areas at risk of developing

skin abnormalities (Smit et al, 2013b). Whether the increase in (sub)cutaneous oxygen levels also translates to increase blood flow in superficial (skin) and deeper (conduit arteries) layers has yet to be tested. The second aim of this thesis is to examine this topic and examine the acute changes in lower limb cutaneous and conduit haemodynamics during low level gluteal and hamstring ES using a wearable clothing garment.

Assuming this simple, non-invasive protocol of low-intensity ES indeed continuously elevates blood flow, this method could have important clinical implications for individuals with SCI. These benefits would involve: *1)* Increased conduit and skin microvessel perfusion and vascular health, *2)* improved regulation and stabilization of blood pressure through increased venous return, and *3)* reduced risk of vascular complications, such as deep vein thrombosis and skin ulcers. The third aim of this thesis will therefore look to address the long-term physiological effects of daily ES on cutaneous and conduit vasculature in the lower limbs of individuals with SCI.

Aims and Objectives of the Thesis

The overall aim of this thesis is to investigate the accuracy of the FRS to predict CVD and the effects of lower limb ES on micro- and macro-vascular complications which likely contribute to the increased CVD risk in SCI individuals.

The objectives listed below will be explored in an attempt to satisfy the aims of this thesis:

1. To determine if traditional cardiovascular risk factors, captured in the FRS screening tool, can accurately predict the 5-year occurrence of cardiovascular morbidity and mortality in persons with a SCI, and whether modifying the current FRS model by adding characteristics pertaining to the SCI can improve its predictive accuracy.

2. To determine the acute effects of low intensity gluteal and hamstring ES, using a wearable clothing garment, on femoral artery blood flow and skin microcirculatory perfusion in people with SCI.

3. To determine the feasibility of 12 weeks, daily gluteal and hamstring ES using a wearable clothing garment and the physiological changes in femoral artery diameter and blood flow, skin microcirculation, anthropometric limb volume and sitting pressure in subjects with SCI.

Chapter 2
Literature Review

2.1. The Spinal Cord

The spinal cord together with the brain forms the CNS and serves as the major signalling conduit through which motor commands and sensory information travel to and from the brain. The spinal cord is surrounded by three layers of membranous tissue called meninges (dura mater, arachnoid mater and pia mater) and is protected by the vertebral column. The spinal cord begins at the occipital bone of the skull and extends down to the space between the first and second lumbar vertebrae. From a cross sectional view, the internal regions of the spinal cord consist of a peripheral white matter containing sensory and motor neurons and a central butterfly shaped region made up of nerve cell bodies called the grey matter. This central grey matter region surrounds the central canal, which contains the cerebrospinal fluid (figure 2.1).

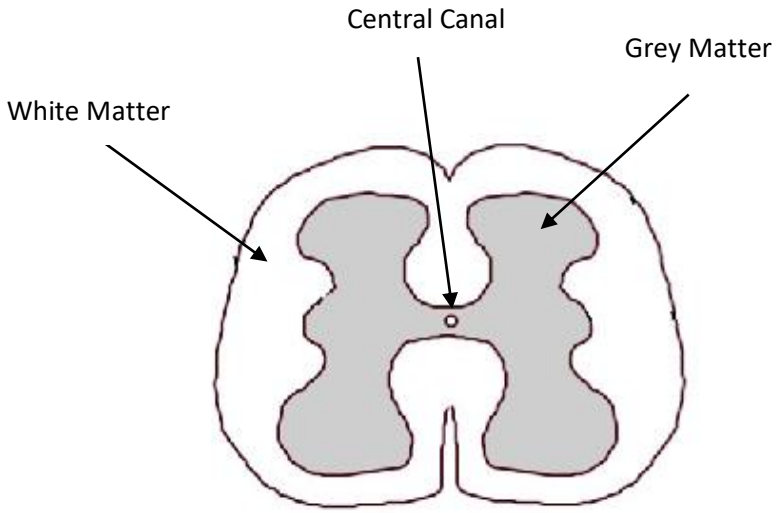


Figure 2.1 Cross sectional view of the spinal cord illustrating the central canal and the white and grey matter regions.

The human spinal cord consists of 31 left-right pairs of spinal nerves that send motor, sensory and autonomic signals between the CNS and the body. Each spinal nerve is formed from a combination of nerve fibres that emerge from the anterior and posterior roots of the spinal cord. The anterior (ventral) root conducts efferent or motor information from the brain to the body and the posterior (dorsal) root conducts afferent or sensory information from parts of the body to the brain. Each spinal nerve roughly corresponds to each segment of the vertebral column and emerges through the intervertebral foramen between adjacent vertebrae. This is with the exception of the first spinal nerve pair, which emerges between the occipital bone and the first vertebrae. The spinal cord is subdivided into five regions; cervical (nerve pairs C1-C8), thoracic (T1-T12), lumbar (L1-L5), sacral (S1-S5) and coccygeal. Figure 2.2 illustrates the longitudinal organisation of the spinal cord and a rough representation of its major functions at each level.

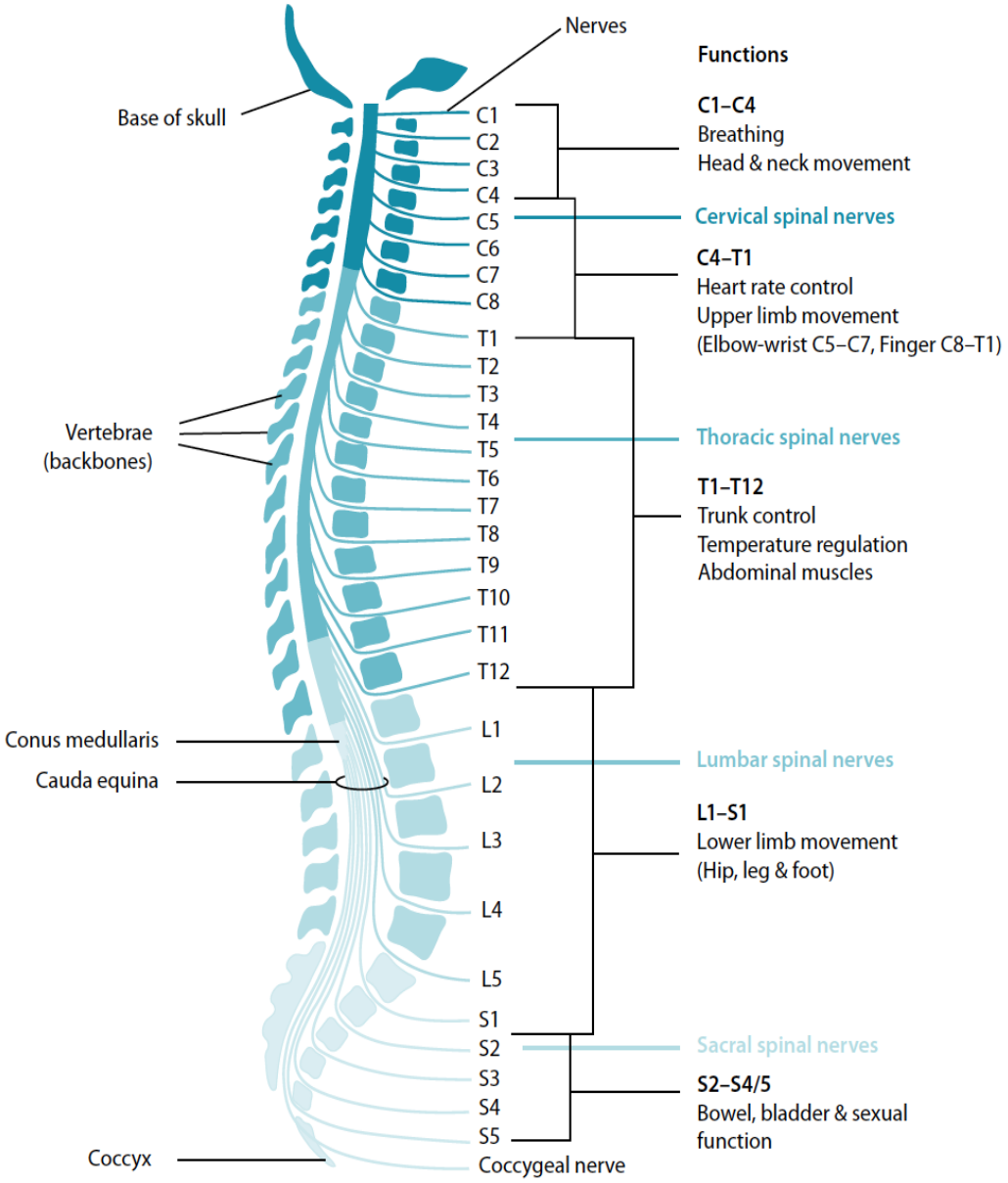


Figure 2.2 Organization of the spinal cord highlighting the cervical, thoracic, lumbar and sacral spinal nerves and their major function

2.2. Spinal Cord Injury

A spinal cord injury is a medically complex and life changing condition. When the spinal cord becomes damaged the transmission of all information from the CNS to the rest of the body becomes disrupted below the level of the lesion. The result is a loss of sensory and motor control of the lower limbs, trunk, upper limbs and

autonomic regulation of normal bodily functions such as breathing, heart rate, blood pressure, temperature control, bowel and bladder control and sexual function. The exact symptoms and areas affected depend on the extent of the injury but in general, the higher the injury the more extensive the range of impairments will be. The American Spinal Cord Injury Association (ASIA) Impairment Scale is a standardised method of assessing and measuring the level and extent of neurological damage sustained (table 1). The international standards examination systematically measures the key sensory points and key muscle functions on both sides of the body. Motor function testing incorporates 10 myotomes corresponding to 5 key muscles in the arms and legs on both the right and left sides. Sensory examination comprises testing of 28 dermatomes on both left and right sides of the body which are recognised as key points within a defined area of skin. Each point is tested for light touch sensation and pain sensation using a pin prick or similar sharp object. Subsequently, based on the sensorimotor scores several measures of neurological damage are generated and a definitive and accurate measure of the level and severity of the spinal cord injury can be established.

Individuals who present with a cervical spinal cord injury were the outcome is a total or partial loss of motor and/or sensory control of all four limbs are referred to as tetraplegic. In the absence of any apparent motor or sensory preservation, a poor prognosis is generally expected. Due to the extensive nature of their depressed functional movement and immobility, individuals with tetraplegia are more vulnerable and predisposed to developing osteoporosis, fractures, pressure sores, deep vein thrombosis, respiratory complications and have a shorter life expectancy. For example, a ventilator-dependant individual with a C1-C4 injury has a 20-year survival of ~23% compared to ~55% for non-ventilator dependant individuals with a C5-C8 injury (Savic et al, 2017b). An individual with a spinal cord injury in the thoracic or sacral regions and who retains useful motor and sensory function of their upper limbs is referred to as paraplegic. The longevity and health of someone with a paraplegia injury is generally considered as being more prosperous due to remaining more functionally independent and physically active.

Table 2.1 American Spinal Injury Association Impairment scale and Neurological Classification of Injury

ASIA	Completeness	Clinical Result
A	Sensory and motor complete	No sensory or motor function remains in the sacral segments S4-5
B	Sensory incomplete	Sensory but no motor function remains below the neurological lesion an includes the sacral segments S4-5 and no motor function is preserved more than three levels below the motor level on either side of the body
C	Motor incomplete	Motor function is preserved below the neurological level and at least half of the key muscle functions below the neurological level of injury have a muscle grade <3**
D	Motor incomplete	Motor function is preserved below the neurological level and at least half of the key muscle functions below the neurological level of injury have a muscle grade ≥3**

**Muscle function grading 0= total paralysis; 1= palpable or visible contraction; 2= active movement, full range of motion (ROM) with gravity eliminated; 3= active movement, full ROM against gravity; 4= active movement, full ROM against gravity and moderate resistance in a muscle specific position; 5= active movement, full ROM against gravity and full resistance in a functional muscle position expected from an otherwise unimpaired person; 5*= active movement, full ROM against gravity and sufficient resistance to be considered normal providing no pain is present.

2.2.1. Epidemiology

It is estimated that every year, between 250,000 and 500,000 people sustain a spinal cord injury worldwide. This number equates to a prevalence of 280 to 1,298 cases per million population (Bickenbach et al, 2013). The age- and sex-specific incidence rates for adult traumatic SCI indicate that a considerably higher number of events occur in males compared to females. Typically, studies show a minimum male-to-female incidence ratio of 2:1 with some studies reporting rates as high 7:1 (Divanoglou and Levi, 2009) and 8:1 (Lee et al, 2014) in Greece and Qatar, respectively. It is likely that the greater male SCI incidence is in part related to gender

specific behavioural characteristics, such as alcohol consumption, driving behaviour and participation in high-risk sports and activities. Young adults (15-29 years) and older individuals (60+ years) are the two most common age groups were traumatic SCI incident rates are at their highest. The growing traumatic SCI incidence rates in the older population is a relatively new observation owing to global ageing, people living longer and a greater number of falls amongst the elderly. For example, in the last 25 years Australia has seen a 3-fold increase in the proportion of traumatic SCIs among people 65 years and older (Middleton et al, 2012). Unlike traumatic SCI, the incidence of non-traumatic SCI increases steadily with age and is more common in older age groups. This is to be somewhat expected, considering non-traumatic SCI are usually influenced by ill health and other associated comorbidities which are ever more apparent with increasing age.

2.2.2. Aetiology

Regarding the aetiology of traumatic spinal cord injury the primary causes are transport related. This involves all road traffic crashes, including drivers, occupants, pedestrians and cyclists injured during the accident. The remaining most common causes are falls and violence, although the prevalence of each varies between regions and countries. For example, falls from elevation or ground level are typically the second most common aetiology. However, in some American states violence (gunshot wounds and stabbings) make up a larger percentage than injury due to falls. This is contrast to Canada where violence related injuries are negligible and sporting and recreational incidences occupy a larger proportion of traumatic spinal cord injury cases. The data available on non-traumatic spinal cord injury aetiology is far more scarce than traumatic spinal cord injury. Although reports suggest neoplastic tumours, degenerative conditions of the spinal cord, vascular and autoimmune disorders are the leading causes (Garshick et al, 2005; Werhagen, Hultling and Molander, 2007; Osterthun et al, 2009).

2.2.3. Mortality

Advances in medicine, emergency services, and specialist SCI facilities have significantly improved life expectancy after SCI since World War II. Despite this, higher rates of morbidity and premature mortality still remain for SCI individuals who are 2 to 5 times more likely to die early compared to the general population (O'Connor, 2005; Sabre et al, 2013). In the majority of cases, the first year after injury is associated with the highest risk of mortality. Advancing age, lesion type and level of injury, comorbidities (Varma et al, 2010), and complications such as pulmonary embolism (Jones et al, 2005; Selassie, Varma and Saunders, 2011) and polytrauma (Saunders et al, 2009) are key determinants of in-hospital, acute mortality. Additional to the aforementioned, in low in-come countries who have poor availability to resources, people with SCI continue to die from preventable secondary complications such as urogenital disorders and sepsis or other fatal infections resulting from untreated and poorly managed pressure ulcers. These secondary complications are no longer the main causes of mortality in higher income countries, who have seen a shift towards causes of death similar to the general population. The principle causes of death for people with SCI living in the United Kingdom are respiratory and cardiovascular disorders, which account for 29.3% and 26.7% of all documented deaths, respectively (Savic et al, 2017b). However, people with SCI die of these conditions more frequently than the general population. For example, in a community-based Canadian health study, Cragg and colleagues reported that people with SCI have significantly increased odds of heart disease and stroke by more than 2- and 3-fold, respectively, compared to the able bodied population (Cragg et al, 2013). Similarly, risk associated with respiratory disease such as pneumonia and influenza is twice as high among the SCI population (Hagen et al, 2010). These studies suggest special attention should be paid to cardiovascular disease (CVD) prevention strategies and improved management of respiratory dysfunction and infection control.

2.2.4. Cost

The economic impact of a SCI is a substantial burden for the injured individual, their families and for health care providers. The exact cost estimates of a SCI vary considerably and are difficult to compare between countries due to different statistical techniques and inconsistencies in the data available. When taking into consideration the average costs of hospitalisations, physician services and home care, the costs in the first year after SCI in Canada are reported to be $121,600 per person with a complete SCI and $42,100 per person with an incomplete SCI. Thereafter, annual costs for continual treatment of long term complications such as wound infections, pressure ulcers and pain management are $5,400 and $2,800 for people with complete and incomplete injuries, respectively (Dryden et al, 2005). This equates to an estimated lifetime economic burden ranging from $1.47 million per individual with an incomplete paraplegia injury to $3.03 million per individual with a complete tetraplegia injury and a gross annual economic expense of $2.67 billion (Krueger et al, 2013).

2.3. Cardiovascular Disease

Over the last 30 years, research has demonstrated continuing trends towards improvements in acute survival rates after SCI (DeVivo, 2007). However, similar reductions in long-term mortality after a SCI are not as apparent and the risk of dying is still higher in SCI than in the general population (Strauss et al, 2006; Shavelle et al, 2015). Cardiovascular diseases; which encompass all coronary events (e.g. myocardial infarction, coronary death, coronary insufficiency, and angina), cerebrovascular events (e.g. ischemic stroke, haemorrhagic stroke, and transient ischemic attack), heart arrhythmias, valvular disease, aortic aneurysms, peripheral artery disease, thromboembolic disease and venous thrombosis, represent one of the leading causes of death in those with SCI (Garshick et al, 2005; Osterthun et al, 2014; Savic et al, 2017a). For example, in a prospective assessment of mortality in 361 males with chronic SCI, mortality rates (observed/expected deaths) were elevated by 47%. Of all the deaths observed, cardiovascular disease was reported as

the underlying or contributing cause in 40.5% (Garshick et al, 2005). These findings are consistent across numerous epidemiological studies, with recent evidence suggesting cardiovascular mortality rates are twice as high in people with a SCI compared to the general population (Savic et al, 2017a). Despite CVD risk being higher with advancing age, a SCI appears to be independently associated with increased risk of both heart disease (2-fold) and stroke (3-fold) regardless of age or sex (Cragg et al, 2013). To put this into perspective, the relationship between a SCI and risk for CVD is similar in magnitude to the risks associated with smoking, diabetes, hypertension or abdominal obesity and the occurrence of a myocardial infarction in the general population (Yusuf et al, 2004).

Modifiable risk factors for CVD in the general population are well established and include, but are not limited to; abnormal lipid profiles, high blood pressure, sedentary lifestyle, being overweight, and smoking. Whilst these risk factors are still applicable to individuals with SCI, it is questionable whether they are solely accountable for the increased risk and prevalence of CVD after SCI. There is conflicting evidence from several studies demonstrating that persons with SCI have abnormal lipid profiles. For example, some studies report lower high-density lipoprotein (Bauman et al, 1999), higher low-density lipoprotein and higher total cholesterol levels in people with SCI (Zlotolow, Levy and Bauman, 1992; Demirel et al, 2001), whilst others found no difference or lower levels of total cholesterol and low-density lipoprotein compared to the able-bodied population (Bauman and Spungen, 1994; Bauman et al, 1999; Liang et al, 2007; Wang et al, 2007). When estimating the risk for coronary heart disease using traditional risk factors such as lipid profiles, Cardus *et al.* found that the risk in individuals with SCI was comparable to that of non-trained, able-bodied individuals (Cardus, Ribas-Cardus and McTaggart, 1992a). Similarly, the reported increase in CVD in persons with SCI was unexplained by differences in blood pressure and total cholesterol (Krum et al, 1992), or the prevalence of metabolic syndrome (Liang et al, 2007) suggesting that other non-traditional risk factors contribute to the increased mortality from CVD in SCI. Possibly, lower limb deconditioning and sedentary behaviour, which are amplified in persons

with SCI, exert a direct effect on the vasculature thus increasing the risk for CVD in the absence of elevated traditional risk factors (Green et al, 2017).

The disconnection between autonomic circuits and supraspinal control results in a significantly volatile blood pressure in SCI. Individuals with SCI are exposed to episodes of extreme hypotension during postural changes and episodes of extreme hypertension during autonomic dysreflexia. It is speculated that this large blood pressure variability has an atherogenic effect on blood vessels due to repeated contractions, causing vascular injury and thus contributing to a greater risk for arterial disease and mortality outcomes in people with a SCI (Mancia et al, 1988; Stevens et al, 2016). Considering the negative effects of altered autonomic function and physical activity levels on CVD risk, it is reasonable to assume that the prevalence of CVD is greatest in individuals with a more rostral and neurological complete level of injury. Data from Savic and colleagues (Savic et al, 2017a) however, refute this suggestion. They observed CVD mortality rates that were highest in persons with paraplegia and functionally incomplete SCI (ASIA Ds). This is the case in other chronic SCI mortality studies, who also report no difference in mortality based on neurological level and completeness of injury (Hartkopp et al, 1997; Garshick et al, 2005). Although the risk of CVD appears to increase with altered autonomic function, the severity of autonomic dysfunction imposed by a SCI and its influence on CVD risk remains unclear.

2.3.1. Framingham Risk Score

The Framingham Risk Score was originally developed from the longitudinal cohort Framingham Heart Study initiated in 1971 and included 2489 men and 2646 women. Originally, the Framingham study examined the relationship between individual risk factors and the development of CVD. Within a decade of the initiation of the study a clear trend had been established and it was obvious that the hypothesised risk factors did contribute to CVD. Moreover, it became clear that the presence of multiple risk factors increased the risk further. For example in 1961, (Kannel et al,

1961) reported 6-year follow-up data and stated that combinations of 3 risk factors further augment the subsequent development of CVD. It was from this point on that additional risk factors were determined and combined into a multivariate function to give an assessment, or probability of developing a CVD event over a predetermined time period (10 years) (Anderson et al, 1991; D'Agostino et al, 2008). The multivariable risk formulations assign specific weights to the following major CVD risk factors: sex, age, blood pressure, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, smoking behaviour, family history of CVD and diabetes status. The calculated risk estimates are then used to place patients into specific risk categories. These thresholds can be defined as low risk (<10% probability of developing a CVD event within 10 years), medium risk (10-20%) and high risk (>20% risk). Table 2.2 illustrates the standard modifiable risk factors and accepted thresholds for CVD in the general population (World Health Organisation).

Despite the wide variety of CVD risk calculators available, the FRS has demonstrated good discrimination (C statistic, 0.73 [men] and 0.79 [women]) and calibration compared with other CVD risk prediction tools (D'Agostino et al, 2008; Cook et al, 2012). Furthermore, the FRS has been validated in multiple populations, including whites and blacks in the United States (D'Agostino et al, 2001; Ridker et al, 2007) and other populations in Europe and the Mediterranean region (Menotti et al, 2000). The FRS is used in the majority of risk assessment studies and has previously been used in risk assessment for SCI populations (Cardus, Ribas-Cardus and McTaggart, 1992a; Finnie et al, 2008; Wahman et al, 2011). However, because the risk prediction tool is derived from able-bodied populations, it has been suggested that it may not be sensitive enough to detect the increased CVD risk in persons with SCI. Cardus *et al* estimated the probability of developing coronary heart disease within six years in 96 SCI patients and compared this with data obtained from 96 non-trained, age matched, able-bodied men. The results from their study suggest that, based on the FRS, individuals with SCI have a similar risk of developing CVD to non-trained able bodied individuals (Cardus, Ribas-Cardus and McTaggart, 1992a). Data from other studies also indicated a significant underestimation of CVD with 70% (Wahman et al,

2011) and 80% (Finnie et al, 2008) of the SCI participants allocated to a 'low risk' category based on their FRS. Further research is warranted to determine the generalisability of the FRS to specific patient populations such as SCI. The inclusion of additional risk factors specific to the SCI may aid in improving its prognostic strength.

Table 2.2 Modifiable risk factors and accepted threshold for able bodied population

Risk Factor	Healthy	High Risk
Blood Pressure	120/80 mmHg	160/100
TC	5.2 mmol	≤8 mmol
LDL	3.5 mmol	≤6 mmol
HDL	2 mmol	≤1 mmol

TC=Total Cholesterol, LDL=Low density lipoprotein, HDL=High density lipoprotein

2.4. Cardiovascular Adaptations

Mortality rates after a spinal cord injury are elevated by as much as 47 % compared to the AB population, with CVD being a leading cause of all-cause mortality (Garshick et al, 2005). A first logical explanation for this observation relates to changes in cardiovascular risk factors. However, as mentioned above, several studies found that those with SCI and AB individuals demonstrate no differences in the presence of traditional CVD risk factors, such as blood glucose, cholesterol and triglycerides (Bauman and Spungen, 1994; Bauman et al, 1999; Jones, Legge and Goulding, 2004; Liang et al, 2007). Therefore, increased risk for CVD in the population of individuals with SCI is unlikely explained through traditional cardiovascular risk factors. An alternative explanation resides in the concept that a SCI has a direct impact on the cardiovascular system. The (partial) loss of motor function after a SCI results in lower limb deconditioning and extreme physical inactivity below the level of the lesion. As a result, arteries show marked remodelling and changes in vascular function, both

below and above the level of the lesion. These detrimental effects of lower limb physical inactivity on cardiac and vascular structure and function may contribute to the increased risk for CVD in SCI.

2.4.1. Cardiac Structure

The heart is highly adaptable and is subject to morphological changes in response to different loading conditions. Several studies have described the presence of myocardial hypertrophy in response to prolonged periods of (extensive) exercise training, both related to resistance and endurance training (Pluim et al, 2000). Opposite to these changes, prolonged physical inactivity in able-bodied subjects, such as spaceflight (Perhonen et al, 2001) and bed rest (Levine, Zuckerman and Pawelczyk, 1997; Perhonen et al, 2001), decrease myocardial size and mass. In parallel with these changes, individuals with SCI demonstrate smaller cavity sizes and mass compared to AB controls (Kessler et al, 1986; de Groot et al, 2006a; West et al, 2012). More specifically, a significant reduction in left ventricle (LV) mass and chamber dimensions are found in individuals with SCI (de Groot et al, 2006a; West et al, 2012). The explanation for this remodelling likely relates to changes in cardiac workload. Due to paralyses of lower limb muscles only a relatively small upper limbs muscle mass is available to perform exercise, leading to a small cardiac workload. Furthermore, those with SCI show reductions in total blood volume and haemoglobin mass, and increased venous pooling below the injury (Houtman, Oeseburg and Hopman, 2000). These findings, coupled with an inactive skeletal muscle pump, impair venous return and cardiac preload in SCI. These haemodynamic changes (West et al, 2012) likely further contribute to remodelling of the heart in individuals with SCI.

2.4.2. Cardiac Function

In the presence of reduced contractile pressure due to a lower blood volume, myocardial wall stress is significantly diminished in individuals with SCI. As a result,

one may also expect changes in cardiac function. However, previous studies provide little evidence to support that measures of LV function, such as ejection fraction, are altered after a SCI. Doppler echocardiographic measurements of mitral inflow velocities, determined by the early LV relaxation (E-wave) and the subsequent contribution of atrial contraction (A-wave), are popular indices of diastolic function (i.e. E/A wave velocity). No difference in diastolic function is present when comparing SCI to AB controls (de Groot et al, 2006a; West et al, 2012; West et al, 2014). Similarly, systolic function and overall ventricular performance, quantified by ejection fraction (EF), end-systolic volume (ESV) and peak systolic pressure/ end-systolic volume (PSP/ESV) ratio, do not differ between those with SCI and ambulatory controls (Kessler et al, 1986; de Groot et al, 2006a; West et al, 2012). These results of preserved cardiac function may indicate that changes in the structural characteristics of the heart (i.e. wall size and chamber size) after a SCI likely represent a normal physiological response to maintain myocardial function.

2.4.3. Macrovascular Structure

Conduit Arteries: Conduit arteries are large compliant vessels, responsible for supplying blood to the internal organs and limbs. These arteries have important distensible properties giving them the ability to expand and provide a low resistance vessel for blood. Models of extreme physical inactivity, such as bed rest (Bleeker et al, 2005b; van Duijnhoven et al, 2010), leg casting (Sugawara et al, 2004), lower limb suspension (Bleeker et al, 2005a) and SCI (De Groot et al, 2003; de Groot et al, 2004b; de Groot, Bleeker and Hopman, 2006), result in a marked decrease in femoral arterial lumen diameter. Examining the impact of physical inactivity on conduit artery remodelling, changes in diameter turn out to be specific for arteries supplying the inactive areas. For example, unilateral limb suspension causes a decrease in femoral artery in the suspended leg, but not in the weight-bearing limb (Bleeker et al, 2005a). Similarly, bed rest causes a marked decline in artery diameter in the lower limbs, whilst conduit artery diameter of the upper limbs is largely unaffected (van Duijnhoven et al, 2010). Interestingly, changes in conduit artery diameter are

strongly correlated to the size of the muscle mass that it supplies. Indeed, a 37 % reduction in femoral artery diameter was found in those with SCI relative to AB controls (Olive, Dudley and McCully, 2003). However, when femoral artery diameter was expressed per unit of muscle volume, no differences were apparent between groups. Even changes in femoral artery diameter and upper limb volume seem to follow a similar path during the first 6 weeks after a SCI, since no changes were observed when arterial diameter was corrected for limb volume changes.

Conduit arterial wall thickness (WT), measured in both carotid and peripheral arteries, is commonly recognized as a surrogate marker for atherosclerosis severity and has strong predictive value for future cardiovascular events (de Groot et al, 2004a; Lorenz et al, 2007; Simon, Megnien and Chironi, 2010). Remodelling of conduit arterial WT is an apparent response to physical (in)activity and is well established in both AB subjects after prolonged periods of severe physical inactivity (i.e. bed rest) (Rowley et al, 2011; Rowley et al, 2012) and in individuals with SCI (Matos-Souza et al, 2009; Bell et al, 2011; Rowley et al, 2012; Paim et al, 2013). For example, 60 days of bed rest in AB subjects resulted in a significant increase in carotid and superficial femoral artery wall thickness (van Duijnhoven et al, 2010), strongly supporting the presence of a systemic impact of physical inactivity on conduit artery wall thickness. The ability to partly prevent this increase in wall thickness through (resistive) exercise training highlights the importance of physical inactivity to mediate these changes in wall thickness. In parallel, individuals with SCI demonstrate larger carotid intima media thickness relative to controls (Matos-Souza et al, 2009). Taken together, physical inactivity is a potent stimulus for remodelling of conduit arteries, leading to a smaller artery diameter and thicker walls.

Repeated increases in blood flow represent a key stimulus for vascular adaptation. Studies in animals (Langille and O'Donnell, 1986; Tuttle et al, 2001), but also in humans (Hambrecht et al, 2003; Tinken et al, 2010), show that elevations in blood flow and shear stress are required for improvement in vascular function and an outward remodelling (increase) of the artery lumen. The shear stress mechanism

leading to artery remodelling involves a process, starting with exposure of the endothelium to an increase in viscous drag. Any increase in blood flow which results in an increase in drag initiates mechanotransduction at the luminal surface of the endothelium. The detection of shear stress by ion channels, cell membrane receptors, G proteins and glycocalyx initiates the production of nitric oxide (Ando and Yamamoto, 2013) and subsequently, acute vasodilation. When exposed to prolonged periods of increased blood flow and shear, a vessel remodelling occurs whereby the increase in drag is returned to normal by virtue of increased lumen diameter.

Previous work has examined the impact of bilateral handgrip exercise training (8 weeks) on vascular function and structure. Using a blood pressure cuff, they unilaterally attenuated the increase in shear stress during each handgrip exercise bout. Adopting this within-subject design, 8 weeks of bilateral handgrip exercise training resulted in significant, time-dependent changes in vascular function and structure of the brachial artery in the non-cuffed arm. In contrast, exercise training-related adaptations were non-existent in the cuffed arm. The importance for elevations in shear stress in individuals with SCI was indirectly demonstrated by a cross-sectional comparison of conduit artery size between sedentary paraplegics and athletes with paraplegia (Huonker et al, 1998). The athletes demonstrated a significantly larger subclavian artery cross-sectional area, which is most likely a direct result from the intensive wheelchair training and repeated exposure to higher shear stress (Huonker, Halle and Keul, 1996). Despite the large training volume, blood flow only marginally changes in the lower limbs during arm crank exercise (Thijssen et al, 2009b). Accordingly, sedentary wheelchair users and wheelchair athletes both possessed a similarly reduced femoral artery diameter relative to AB individuals, supporting the presence of local adaptations in conduit artery diameter that are most likely linked to changes in shear stress.

In contrast to changes in diameter, little evidence supports a direct role of repeated elevations in shear stress to mediate changes in conduit artery wall thickness. Subjects that underwent 60 days of bed rest were able to largely prevent changes in

wall thickness through exercise training in both the femoral and carotid arteries, whilst exercise only minimally influences carotid artery blood flow and shear rate (van Duijnhoven et al, 2010). Furthermore, a significantly smaller carotid and femoral WT was found in physically active individuals with SCI compared to their sedentary peers (Jae et al, 2008; Paim et al, 2013). These data provide indirect evidence that physical inactivity leads to systemic adaptations in conduit artery wall thickness, whilst these adaptations seem largely independent of repeated elevations in shear stress.

Resistance Arteries: Assessing changes in resistance artery structure traditionally involves measuring hyperaemic blood flow responses to a maximal vasodilator stimulus, since maximal blood flow is restricted by structural aspects of the vessels. These measures also represent independent predictors for CVD (Anderson et al, 2011; Lind et al, 2011). Individuals with a SCI demonstrate a 40–60 % lower peak reactive hyperaemic response in the lower limbs than AB controls (Olive, Dudley and McCully, 2003; de Groot, Bleeker and Hopman, 2006). Some speculate these changes in vascular resistance and blood flow result from a loss of supraspinal sympathetic vascular tone. However, no such changes in vascular resistance are observed after long-term sympathectomy (Lepori et al, 1999). Studies using limb immobilization (Bleeker et al, 2005a)and bed rest (Bleeker et al, 2005b) have also reported a decrease in lower limb reactive hyperaemic blood flow, predominantly present in the physically inactive limbs. This indicates that the extent of reactive hyperaemic impairment is dose dependent. For example, 5 and 52 days of bed rest lead to a 22 and 38 % reduction in superficial femoral artery peak reactive hyperaemia, respectively (Bleeker et al, 2005b; Hamburg et al, 2007), whereas chronic SCI is linked to a 40–60% lower response. Taken together, physical inactivity leads to a marked reduction in resistance artery structure, which is likely related to local processes in the physically inactive areas.

2.4.4. Macrovascular Function

Conduit Arteries: Flow mediated dilation (FMD) is a simple, non-invasive technique used to assess conduit artery endothelium-dependent, nitric oxide (NO) mediated vascular function following brief periods of reactive hyperaemia. A lower FMD response is indicative of endothelial dysfunction and is directly associated with cardiovascular morbidity and mortality (Green et al, 2011). It is well established that exercise training reduces the risk of CVD and improves baseline NO production and FMD (Clarkson et al, 1999; Fuchsjager-Mayrl et al, 2002; Green et al, 2003). Remarkably, a higher FMD of conduit arteries in the lower limbs of individuals with SCI is reported (de Groot et al, 2004b; de Groot et al, 2005; de Groot et al, 2006a), although some data is conflicting (Stoner et al, 2006). In AB subjects, most studies reveal that FMD is preserved or even increases after a period of physical inactivity induced by bed rest (i.e. femoral artery) (Bleeker et al, 2005b), unilateral lower limb suspension (i.e. femoral artery) (Bleeker et al, 2005a) and unilateral upper limb inactivity (i.e. brachial artery) (Birk et al, 2013). Remarkably, the increase or preserved FMD is typically observed in the inactive limbs, whilst leaving the normally active limbs largely unaffected (de Groot et al, 2004b). Therefore, based on the generalized increase in FMD after periods of lower limb or whole body exercise, the localized increase in FMD in the inactive areas after prolonged physical inactivity is somewhat counter-intuitive. At least, these observations suggest that the effects of deconditioning on conduit artery function are not simply the inverse of exercise training.

A possible explanation for a preserved or increased FMD relates to the inverse relation between baseline arterial size and FMD (Schroeder et al, 2000; Pyke and Tschakovsky, 2005; Thijssen et al, 2008a). The significant inward remodelling of the femoral artery in individuals with SCI, consequently, is expected to result in an increase in FMD. As a consequence of the smaller sized vessels, arteries are exposed to an increase in reactive hyperaemic shear stress stimuli during FMD testing. Correcting for differences in baseline diameter, but also potential differences in the post-occlusion reactive hyperaemia, may contribute to the preserved or even increased FMD after prolonged physical inactivity (de Groot et al, 2004b; Thijssen et

al, 2008b). Another potential explanation for the differences in FMD between both groups relates to differences in the wall-to-lumen ratio, especially since arteries with a larger wall-to-lumen ratio possess larger responsiveness to vasodilator stimuli (Thijssen et al, 2011). Based on the larger wall-to-lumen ratios in those with SCI relative to AB controls, such differences may further contribute to the observations between groups. Therefore, lower limb deconditioning in individuals with SCI does not lead to a decrease in FMD, an observation that may, at least partly, be related to marked remodelling of the vessel (i.e. smaller diameter, larger wall-to-lumen ratio).

The functional capacity of an artery can also be examined using the compliance and stiffness, which reflects the change in diameter across the cardiac cycle in response to an increase in blood pressure. Stiffening of conduit arteries reduces its buffering capacity and is associated with an increase in cardiovascular risk (Laurent et al, 2006). Previous studies have reported that individuals with a SCI demonstrate an increase in arterial stiffness in both central (Miyatani et al, 2009; Phillips et al, 2012) and peripheral (Schmidt-Trucksass et al, 2000; Wecht et al, 2004; de Groot et al, 2005) arteries. The decrease in femoral artery blood flow and pulse pressure in individuals with SCI, as a consequence of physical inactivity and deconditioning, may alter the elastin properties in the arterial wall. These changes may contribute to stiffening of the artery and impeding its functional ability. Differences in artery stiffness were not present when comparing physically active individuals with SCI and age matched AB individuals (Jae et al, 2008), whilst localized electrostimulation to activate the paralyzed muscles of individuals with SCI reduces femoral artery stiffness. These observations suggest that stiffening of conduit arteries is a direct consequence of physical inactivity, whilst systemic haemodynamic stimuli are most likely responsible for these changes.

Resistance Arteries: Various models of physical inactivity, such as limb casting (Green et al, 1997), bed rest (Christ et al, 2001; Pawelczyk et al, 2001) and chronic SCI (Hopman et al, 2002; Kooijman et al, 2003; Thijssen et al, 2007) have detrimental effects on resistance artery function, manifested by a characteristic increase in basal

vascular resistance. Considering the importance of vasodilators for vascular health, in particular NO, increases in vascular resistance in the paralyzed limbs of individuals with SCI may be the result of impairment in the NO pathway. A number of previous studies have examined the contribution of NO dilator pathways to vascular resistance. For example, by examining blood flow responses to intra-femoral infusion of incremental doses of a NO-synthase blocker, Bleeker et al reported no difference between individuals with SCI and AB controls in the contribution of NO to lower limb vascular tone. Furthermore, no difference in the responses to a NO-synthase blocker were found after 4-weeks of unilateral lower limb suspension (Bleeker et al, 2005c). In parallel, individuals who underwent casting-induced immobilization of the forearm had comparable forearm vascular responses during intra-arterial infusion of a NO-synthase blocker immediately and 6 weeks after cast removal (Green et al, 1997). Collectively, these studies suggest that the increased vascular resistance due to deconditioning cannot be explained by impairment in vasodilator pathways.

Alternatively, upregulation of vasoconstrictor pathways and endothelium derived constricting factors may better explain the increased vascular resistance observed in deconditioned muscles. In support of this hypothesis, previous studies have reported an increase in circulating vasoconstrictor substances such as endothelin-1 (ET-1) (Robergs et al, 1993; Maeda et al, 2001) and angiotensin II (ANG II) (Bestle, Norsk and Bie, 2001; Maeda et al, 2001) using various models of deconditioning. However, circulating levels of vasoconstrictors do not simply reflect their role in the regulation of vascular tone (Thijssen et al, 2008c). Therefore, to truly understand the role of vasoconstrictors, Thijssen et al used local intra-arterial infusion of an ET-1 receptor blockade in the deconditioned legs of individuals with SCI (Thijssen et al, 2007). They observed a larger vasodilatory response compared with age matched AB controls, indicating that ET-1 contributes to the increased vascular tone in the lower limbs of individuals with SCI. Furthermore, another study examined the role of ANG II in the regulation of vascular tone in the lower and upper limb of paraplegic subjects as well as AB controls (Groothuis et al, 2010). Whilst blockade of ANG II-receptors did not alter blood flow responses in upper or lower limbs in AB controls, a significant

increase in blood flow was observed in the lower limbs of paraplegic subjects. This data provides further support that vasoconstrictors, such as ET-1 and ANG II, contribute to the increased vascular resistance in the lower limb of individuals with SCI.

2.4.5. Deep Vein Thrombosis and Venous Thromboembolism

A predominant secondary complication resulting from changes in macrovascular structure and function is deep vein thrombosis (DVT) and venous thromboembolism (VTE). The high risk arises from the presence of endothelial abnormality, hypercoagulability, and stasis of blood, with the latter being of greatest concern (Rouleau and Guertin, 2007; Teasell et al, 2009). According to various medical reports, the incidence of DVT among patients with SCI is extremely variable ranging from 47 to 100% when no preventive measures are applied (Jones et al, 2005; Teasell et al, 2009). VTEs most commonly begin with a calf DVT. However, the risk of a calf DVT extending to the proximal veins (i.e., at the level of the knee or above) is a primary source of concern. For example, pulmonary embolism is reported in 8 to 14% of patients with an acute SCI and mortality rates of up 5% (Geerts et al, 2004) . Individuals with SCI experience a loss of voluntary 'muscle pump' which, is essential for maintaining pulsatile flow as well as ensuring normal venous return. As blood flow slows through the venous circulation and oxygen tension declines, an increase in haematocrit and procoagulant proteins arise. As such, the hypercoagulable micro environment downregulates certain antithrombotic proteins and promotes the formation of thrombus (Brooks et al, 2009). Therefore, mechanical treatments designed to limit blood stasis in the lower paralysed limbs such as electrically-induced muscle contractions may help reduce the incidence of DVT post SCI. Indeed, electrical stimulation has demonstrated increased blood flow velocity and volume in the popliteal vein of able bodied, intensive care unit patients (Ojima et al, 2017) and increase fibrinolytic activity and venous blood flow in patients with SCI (Katz et al, 1987).

2.4.6. Microvascular structure

The microvasculature is comprised of the smallest vessels (<15 μm in diameter) within the vascular network and consists of terminal arterioles, capillaries and venules. The microvasculature primarily serves as an exchange site to facilitate the movement of nutrients between the blood and localized tissue as well as being a key

regulator of homeostasis for body temperature (Smith and Fernhall, 2011). Microvascular structure in humans is typically examined in muscle tissue. Several studies have identified changes in the muscle microvascular bed in individuals with SCI, characterized by a reduced capillary-to-fibre ratio (Martin et al, 1992; Chilibeck et al, 1999b). Similar findings have also been reported following periods of inactivity in able-bodied subjects (Edgerton et al, 1995; Qin et al, 1997; Degens and Alway, 2006). These changes in capillary-to-fibre ratio were accompanied by a decrease in maximal perfusion of the muscle tissue, which further supports the presence of remodelling in the microvasculature in response to physical inactivity. In addition to the muscle, studies have also examined the skin microcirculation. Given the different role and regulation, the skin microcirculation represents a completely different vascular bed compared to the muscle microcirculation and cannot be simply used as a surrogate for the skeletal muscle microcirculation and *vice versa*. Although skin microcirculation is relevant for thermoregulatory control as well as in the development of secondary complications in those with SCI [e.g. skin ulceration, poor wound healing (Deitrick et al, 2007), few studies examined cutaneous microvascular structure following SCI.

2.4.7. Microvascular Function

The epithelial layer which lines the internal lumen of blood vessels is called the endothelium and is the primary mechanism for regulating vascular homeostasis (Roustit and Cracowski, 2013). The multiple functions of the vascular endothelium include regulation of vessel integrity, vascular growth, cell adhesion, angiogenesis, vascular permeability as well as maintaining a crucial balance between vasoconstriction and vasodilation. This pivotal role in the regulation of vascular tone is essential for controlling tissue blood flow, maintaining blood fluidity and essentially tissue integrity and quality. The microvascular endothelium encompasses 95% of the whole body's endothelium and is therefore essential for the functioning of many organ systems such as the brain, eyes, kidney, heart, muscle and skin. Since the bodies total vascular endothelium is comprised largely of microvascular

endothelium, it is not surprising that many blood and vascular disorders such as thrombosis, atherosclerosis, pressure ulcers and type 2 diabetes mellitus are characterised by (dis)functional changes to the microvasculature which manifests as impaired endothelial function (Polovina and Potpara, 2014). Microvascular dysfunction often precedes any obvious micro- and macro-vascular complications and any observable symptoms which result from plaque and atherosclerotic problems such as stenosis and ischemic vascular diseases (Levy et al, 2001; Bonetti, Lerman and Lerman, 2003). Therefore, with quantifiable measures, it is possible to detect changes in endothelial (dis)function in the micro-vessels which may later predispose individuals to developing systemic CVD and allow appropriate, early intervention. Several previous studies have used the cutaneous microcirculation for examining microvascular function. It represents an easily accessible, non-invasive vascular bed that is considered a reliable surrogate for overall microvascular function.

A commonly adopted technique to examine skin microvascular function relates to local heat-induced vasodilation, which leads to an initial axon-reflex mediated peak, followed by a partly NO-mediated plateau. The inactive limbs of individuals with a SCI demonstrate a diminished axon-reflex and NO-mediated plateau to local heating compared with able-bodied controls (Nicotra, Asahina and Mathias, 2004; Van Duijnhoven et al, 2009). Interestingly, a similar skin microcirculatory impairment is observed above the lesion in the upper limbs (Van Duijnhoven et al, 2009), suggesting a systemic adaptation and impairment of skin microvascular NO function following SCI. Despite physical (in)activity being fundamental in modulating vascular adaptations in larger vessels, the diminished cutaneous vasodilator responses observed in the upper limbs in individuals with SCI suggests that skin microcirculatory impairment is not a direct result of physical inactivity *per se*. A more conceivable, previously suggested explanation relates to the persistent lack of exposure to sufficient increases in core body temperature and/or changes in skin blood flow usually present during physical activity. Indeed, the impaired vasodilator responses observed during short term physical inactivity in able bodied individuals (Crandall et

al, 2003; Michikami et al, 2004) is prevented by exercise (Shibasaki et al, 2003). To further support this idea, it was found that repeated FES-cycling in individuals with SCI, which induces only marginal increases in core body temperature, could not improve skin microcirculatory function (Van Duijnhoven et al, 2009). Therefore, SCI leads to a generalized and systemic impairment of skin microcirculatory function to local heating, whilst these changes are most likely mediated by mechanisms other than the direct effects of physical activity alone.

2.5. The Skin

The skin is the largest organ in the body and performs many vital functions including serving as a waterproof protective barrier, preventing excess fluid loss from the body and being a crucial component of thermoregulation. The skin is built of three layers, the epidermis, which is a non-vascularised layer and the dermis and hypodermis which are abundantly supplied with a complex vascular network. Most of the cutaneous microvascular network is contained in the papillary dermis 1-2 mm below the epidermal surface and is organised into two (deep and superficial) horizontal intercommunicating plexuses (Johnson, Minson and Kellogg, 2014). The deepest plexus is located close to the dermal-hypodermal junction and consists of vessels which are typically greater in diameter than those in the superficial plexus with 4-5 layers of vascular smooth muscle. Ascending arteries from the lower plexus support the nutritional demands of the hair follicles and sweat glands before they connect and ramify into the superficial subpapillary plexus. Vessels from this plexus then further divide into papillary loops which are made of smaller terminal arterioles ascending vertically towards the surface, arterial and venous capillaries which form the hairpin part of the loop and descending post capillary venules which empty into the horizontal plexus (figure 2.3). The papillary loops have a large surface area and are located in close proximity to the dermal-epidermal junction where a large thermal gradient from the blood to the epidermal tissue exists. An important function of the papillary loops therefore, is to allow blood to flow through and deliver heat to the skins surface where highly efficient heat exchange occurs (Johnson, Minson and Kellogg, 2014). Blood flow through the papillary loops is regulated by

highly innervated arterioles consisting of a dual layer of vascular smooth muscle and a lining of endothelial cells.

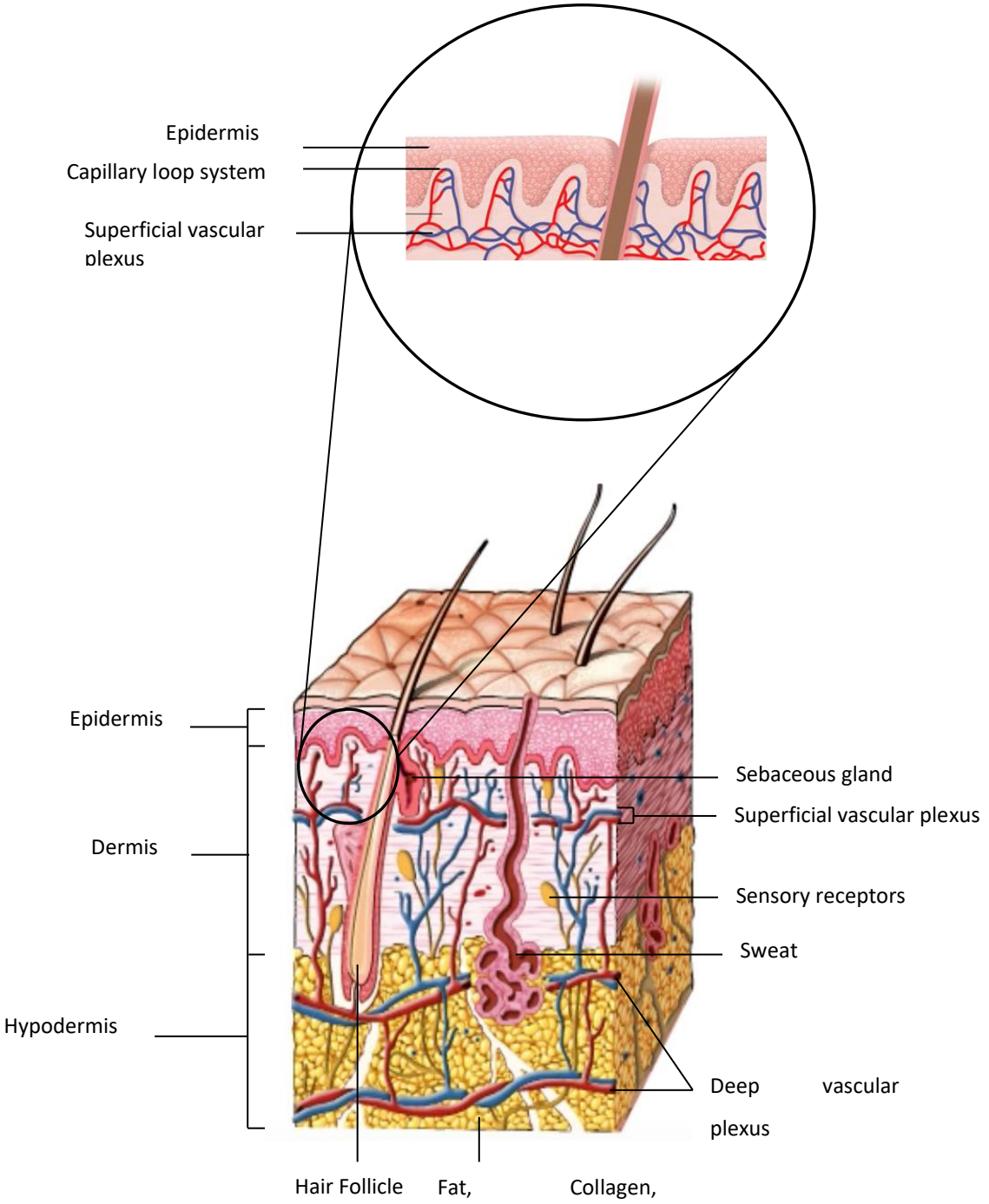


Figure 2.4 Cross-section of the cutaneous membrane and a magnification of the epidermis layer and papillary vascular loop system. The skin is composed of three main layers: the epidermis, made of closely packed epithelial cells. The dermis, made of dense irregular connective tissue and consists of the blood vessels, hair follicles, sweat glands, nerves and smooth muscles. The hypodermis, which lies beneath the dermis and consists mainly of connective and adipose tissue

Although all areas of skin contain the vascular plexus and papillary loop arrangement, differences exist between anatomical regions. For example in glabrous skin which can be identified as 'non-hairy' regions such as palms of the hands, lips, ears and plantar regions of the feet, arteriovenous anastomoses (AVAs) are also found (Johnson, Minson and Kellogg, 2014). AVAs are densely innervated by sympathetic adrenergic fibres and lie deep to the papillary plexus. They bypass the high resistance vessels and provide a direct low resistance connection between arterioles and venules. AVAs play an important role in maintaining thermal homeostasis during extremely cold environmental temperatures. Vasodilation of AVAs brings warm blood to the tissue in an attempt to maintain tissue temperature. Conversely, in conditions of hyperthermia AVAs also dilate as a response mechanism for heat loss from the tissue into the environment (Kellogg, 2006). However, because AVAs are situated deeper in the dermis and have a smaller surface area than papillary loops, they are not as effective in mediating the heat transfer.

Non-glabrous or 'hairy' skin covers areas of the body such as the limbs, head and trunk. Blood flow to the skin in these regions is governed by two sympathetic neural vasomotor branches: noradrenergic vasoconstrictor nerves which induce cutaneous vasoconstriction, and cholinergic vasodilator nerves which elicit cutaneous vasodilation (Charkoudian, 2003; Kellogg, 2006; Johnson, Minson and Kellogg, 2014). Under normothermic conditions, the smooth muscle cells of the cutaneous arterioles receive little neural stimulation and therefore remain at basal tone. During conditions of cold stress, however, a thermoregulatory reflex mediates noradrenergic vasoconstrictor activation in attempt to limit heat loss and conserve body temperature. The increased vasoconstrictor tone subsequently causes arteriolar vasoconstriction to facilitate a decrease in skin blood flow. The vasoconstrictor response involves the release of the neurotransmitters norepinephrine and neuropeptide-Y, which act together to reduce skin blood flow during hypothermia. Alternatively, during conditions of mild heat stress, cooling mechanisms are mediated by small variations in both vasoconstrictor and vasodilator nerve activity to elicit an increase in skin blood flow. Initially, as body temperature

begins to rise, input from tonically active vasoconstrictor neurons is abolished. As temperature continues to rise further, active vasodilator tone to the cutaneous arterioles is increased via cholinergic nerves and co-transmitters, resulting in a decrease in smooth muscle tone, arteriolar vasodilation and increase in skin blood flow (Kellogg, 2006; Johnson, Minson and Kellogg, 2014). The vasodilator pathways described above are responsible for up to 95% of the elevation in skin blood during whole body heat stress. Importantly, when examining skin microcirculation local skin heating is often applied, which elicits different vasodilator pathways and involves local, or 'non-neural', control mechanisms. The mechanisms contributing to whole body heating *versus* local skin heating, therefore, importantly differ and this should be taken into consideration when interpreting data from these experiments.

2.5.1. Local Control of Skin Blood Flow

Skin blood flow will directly change in response to alterations in local skin temperature independent of the aforementioned reflex neural control mechanisms. In response to reductions in local skin temperature local skin blood flow decreases through local activation of the sympathetic active vasoconstrictor system (Rowell, 1977; Kenney et al, 1994; Johnson, Minson and Kellogg, 2014). During local skin warming, the mechanisms involved in cutaneous vasodilation involve a complex interaction of both local neural mechanisms and local chemically mediated vasodilators. The hyperaemic response to local heating of non-glabrous skin is biphasic and is characterised by an initial transient peak in skin blood flow mediated predominantly by sensory neural factors, followed by a prolonged increase and plateau mediated by the local generation of chemical factors (Figure 2.4, Minson, Berry and Joyner (2001). These two mechanisms appear to act independently of each other (Minson, Berry and Joyner, 2001).

The initial vasodilatory response to local heating is mediated by an axon reflex mechanism involving the local activation of afferent sensory nerves. Evidence of this has been demonstrated with the topical application of local anaesthetic which

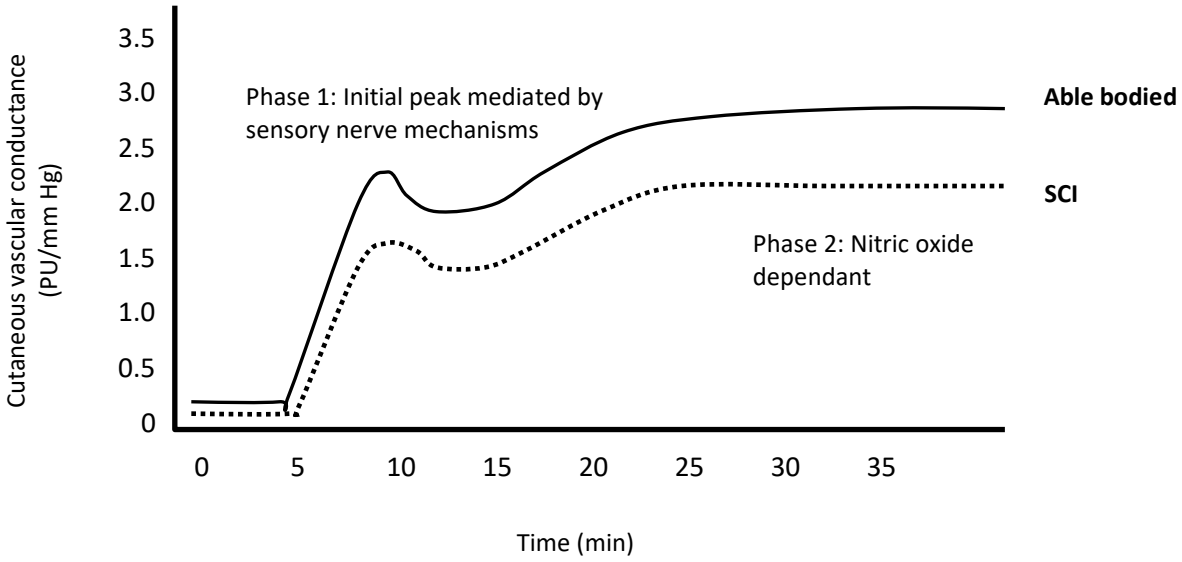
inhibits this initial axon-reflex mediated response (Minson, Berry and Joyner, 2001). Additionally, no effect on skin blood flow was observed in the absence of sympathetically mediated norepinephrine release following neural blockade proximal to the local heating site (Pergola et al, 1993). Although chemical factors are predominately involved in the plateau phase of localised heat induced vasodilation, NO has been suggested to also contribute to sensory neurogenic changes in skin blood flow. For example, studies have reported a delayed (Pergola et al, 1993; Houghton et al, 2006) or absent (Hodges et al, 2008) axon reflex following infused N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor (NOS). Furthermore, transient receptor potential vanilloid type 1 channels (TRPV-1), which are channels located on sensory nerves, are believed to have a substantial role in the initial peak during localised heating and are more effective with the simultaneous contribution of NO than without (Wong and Fieger, 2010). More recently, findings have demonstrated that local sympathetic noradrenergic neurons also play a role in regulating skin blood flow during local heating. Many have demonstrated that with antagonism of vasoconstrictor nerve function the axon reflex is abolished (Houghton et al, 2006; Hodges et al, 2008; Hodges et al, 2009) or reduced (Carter and Hodges, 2011; Tew et al, 2011; Del Pozzi and Hodges, 2015) as well as reducing the overall hyperaemic response (Houghton et al 2006). Taken together, local neurogenic control of skin blood flow involves a complex interaction and communication between the adrenergic system, intact afferent sensory nerves and chemical vasoactive compounds.

The prolonged and sustained increase in blood flow during local heating is largely mediated by locally synthesised NO (figure 2.4). Nitric oxide is generated in endothelial cells from the amino acid L-arginine by endothelial nitric oxide synthase (eNOS). Nitric oxide is a potent vasodilator and is important for modulating vascular dilator tone and maintaining vascular homeostasis and normal endothelial function. In this regard, NO is a major regulator of blood flow within the microcirculation, particularly during local thermal cutaneous vasodilation (Johnson, Minson and Kellogg, 2014). This was demonstrated by Kellogg and colleagues (Kellogg et al, 1999)

who locally infused the NO antagonist L-NAME and observed a 50% reduction in the vasodilation that was present prior to its application. Minson and colleagues later confirmed these findings and also evidenced that during the sustained plateau phase, skin blood flow gradually increased to only 40% of maximal dilation (Minson, Berry and Joyner, 2001). An observation that provides further evidence that NO is predominantly responsible for the secondary rise in skin blood flow during local heating. There is also evidence showing a functional role for EDHF and prostaglandin which are believed to make up the remaining 40% of the vasodilation during the plateau phase following localised heating. It is hypothesised that EDHF may serve as a backup mechanism for cutaneous vasodilation when the NO bioavailability is impaired or reduced as such during eNOS inhibition with L-NAME or in aged endothelial cells (Gaubert et al, 2007).

Although the exact chemically mediated pathways involved in controlling localised temperature dependent changes in skin blood flow remain largely elusive, it is well established and accepted that NO is the primary driving force behind the plateau phase in local heat induced cutaneous vasodilation. The insensate skin below the lesion in individuals with SCI demonstrates small increases in NO dependant skin blood flow. However, although present, this response is greatly attenuated compared to the sensate skin of able-bodied subjects (Figure 2.4). This impaired NO dependant increase in skin blood flow is indicative of endothelial dysfunction and consequently may be predictive of the increased cardiovascular disease risk in individuals with a SCI. Additionally, impaired skin microcirculation, particularly in the sacral region is further exacerbated due to prolonged sitting and increased external pressure loading over areas of significant muscular atrophy and presents a substantial risk factor for developing pressure ulcers. Therefore, skin microcirculation, in particular NO dependant changes in the skin blood in the gluteal region, is an important theme that runs throughout this thesis.

A



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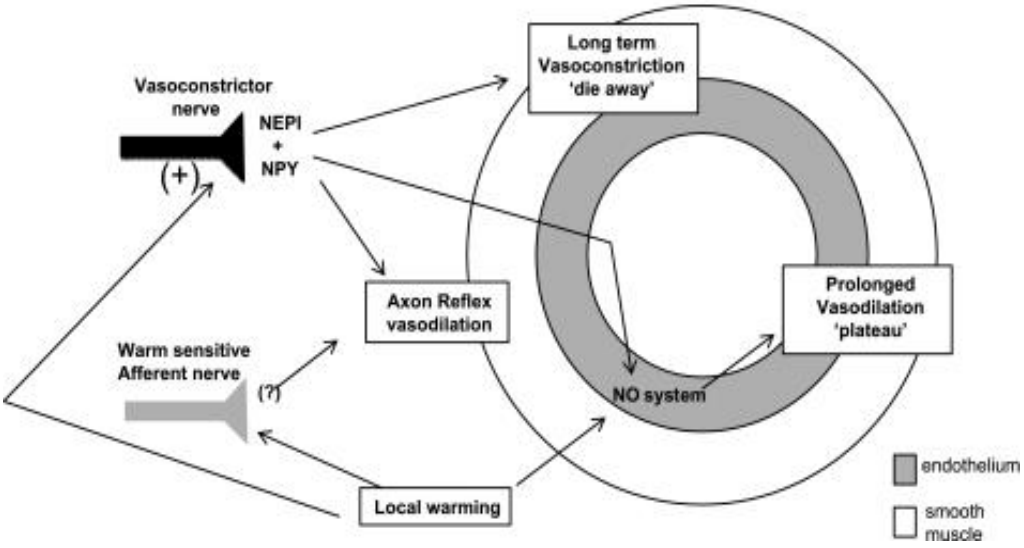


Figure 2.5 A: Representation of the typical cutaneous hyperaemic response to local heating for able-bodied (solid line) and SCI (dashed line) illustrating the initial rapid peak due to the sensory axon reflex and the slower sustained increase in skin blood flow due to nitric oxide. Both phases of the response are impaired in people with SCI; B: The mechanisms involved at each phase of the hyperaemic response to local heating, including the roles of the endothelium and nitric oxide generation, sympathetic transmitters and co-transmitters and warm sensitive afferents. NEPI= Norepinephrine; NPY= Neuropeptide-Y; Cutaneous vascular conductance = cutaneous skin blood flow measured as PU/MAP (red blood cell perfusion units/mean arterial pressure).

2.6. Pressure ulcers

Pressure ulcers, also commonly referred to as decubitus, ischemic ulcers, pressure sores, skin sores or deep tissue injuries, have been defined as a *'localised injury to the skin and/or underlying tissue usually over a bony prominence as a result of pressure or pressure in combination with shear and/or friction'* (Edsberg et al, 2016). Pressure ulcers are a significantly prevalent and troublesome secondary complication amongst individuals with a SCI. In a majority of cases, pressure ulcers may require prolonged periods of bed rest disrupting daily living activities and interfering with physical, psychological and social well-being. Based on severity, pressure ulcers are classified and described using a staging system (figure 2.5). If left untreated or with poor wound care management, the possibility of infection becomes increasingly likely and in extreme cases, can be life-threatening (Kierney et al, 1998; Krause, 1998).

Due to sensory deficit, reduced microcirculation, atrophy of paralysed muscles, absent lower limb voluntary muscle pump function and (lack of) functional independence (Marin, Nixon and Gorecki, 2013), all individuals with a SCI are considered to be at high risk for developing a pressure ulcer at some stage in their life. According to data published in the National Spinal Cord Injury Statistics Centre, the prevalence of a pressure ulcer of grade 2 or higher was 24.6% within the first year post injury. This figure rises to 32.5% over the first 25-year period after injury, with up to 85% of adults with a SCI expected to develop a pressure ulcer at some stage during their lifetime (Garber et al, 2000; Panel, 2009). Additionally, 40%-80% of SCI individuals who develop a pressure ulcer will have at least one reoccurrence (Kierney et al, 1998; Ash, 2002). The overall treatment cost increases with pressure ulcer severity due to the increased healing time, the need for hospital admission and the incidence of additional complications for patients with more server cases. In the UK, the expected cost of treating and healing a pressure ulcer varies from £1214 to £14108 for a stage 1 and stage 4 category wound, respectively (Dealey, Posnett and Walker, 2012). Furthermore, the direct annual medical costs associated with treating pressure ulcers in community dwelling SCI individuals in Canada is between $173

million and $316 million CAD (Chan et al, 2013). Although not all pressure damage can be avoided, it is likely that the incidence rate can be reduced by simply acknowledging and mitigating any substantial risk factors. This would translate into a considerable saving for healthcare systems, releasing more beds, and increasing nurse time allowing for the treatment of more patients with the same overall capacity.

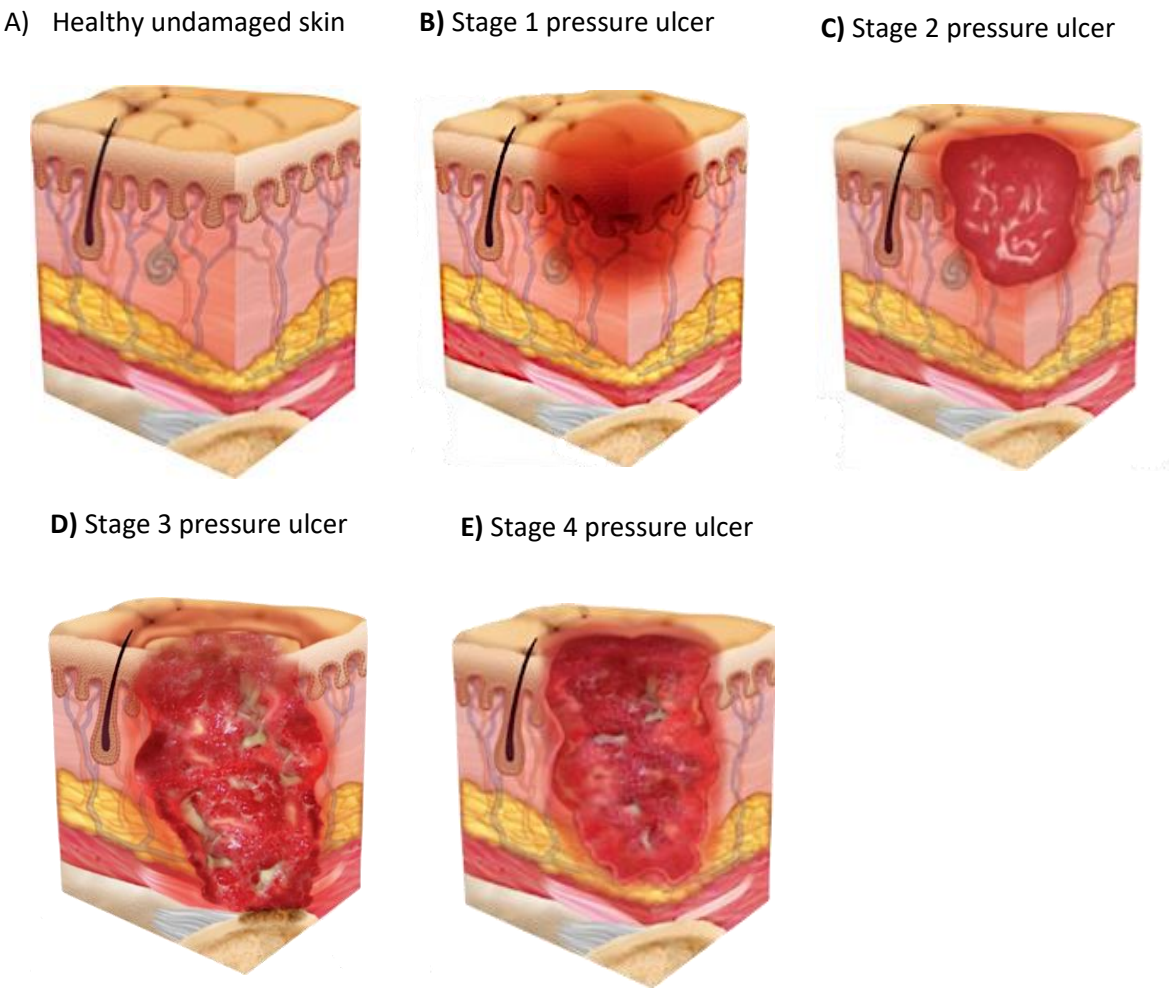


Figure 2.6 A) Lightly pigmented healthy skin providing a reference to illustrate the progressive nature of each pressure ulcer stage. B) Stage 1 pressure ulcer identified as an area of intact skin with non-blanchable redness. C) Stage 2 pressure ulcer identified by partial-thickness skin loss with exposed dermis and a pink or red wound bed. D) Stage 3 pressure ulcer consisting of full thickness skin loss in which adipose tissue is visible but deeper tissue such as muscle, tendon, ligament, cartilage or bone is not. E) Stage 4 pressure ulcer illustrated by full thickness skin and tissue loss with exposed or palpable muscle, tendon, ligament, cartilage or bone in the ulcer. Images taken from (Edsberg et al, 2016)

Pressure ulcers typically originate where prolonged pressure and shear forces are exerted on soft tissue over bony prominences. In SCI, the areas exposed to the greatest risk include the sacrum and ischial tuberosities where reduced mobility and a wheelchair bound lifestyle mean that extended periods of non-movement result in excessive tissue pressures. Although detailed mechanisms involved in pressure ulcer development remain largely unknown (Reddy, Gill and Rochon, 2006; Olesen, de Zee and Rasmussen, 2010), a number of theories exist involving a link between mechanical loading and tissue necrosis. These include poor blood and oxygen supply causing localised ischemia (Herrman et al, 1999; Jan et al, 2012), sustained cell deformation (Bouten et al, 2001), impaired interstitial fluid flow (Reddy and Cochran, 1981) and reperfusion injury (Peirce, Skalak and Rodeheaver, 2000). The localised ischemia theory is widely accepted as the primary cause for developing pressure ulcers and suggests that externally applied pressure to the tissue cause occlusion of blood vessels and subsequently, ischemic damage to the tissue that they supply.

The probability of the development of a pressure ulcer cannot be explained by any one single factor, but rather involves a complex interaction of many different risk factors. This makes it difficult for patients to become properly equipped with the knowledge and tools needed to implement the most effective pressure ulcer prevention strategies. Although current preventative measures such as custom made wheelchairs, pressure relieving mattresses, wheelchair cushions, and performing pressure-reliving movements can disperse pressure forces (Coleman et al, 2013; Smit et al, 2013b), these methods only address a small number of risk factors. Interventions that target multiple risk factors, such as tissue tolerance, tissue blood flow and pressure relief may simplify pressure ulcer prevention strategies for people with SCI. A growing body of evidence and interest surrounding the use of ES for pressure ulcer prevention has emerged over the last decade. Studies examining electrical stimulation induced activation of the paralysed limbs has demonstrated reasonable evidence to suggest its effectiveness in reducing peak sitting pressures, improving tissue tolerance and tissue blood flow (Levine, Zuckerman and Pawelczyk,

1997; Bogie and Triolo, 2003; Crawford et al, 2005; Smit et al, 2013a). A detailed overview of ES and its physiological effects on pressure ulcer risk reduction is provided in the next section of this literature review.

2.7. Electrical Muscle Stimulation

Electrical stimulation over the last 50 years has been refined in such a way that it can be used to induce contractions and re-activate the paralysed muscles in people with SCI. The method of ES involves the application of a series of intermittent stimuli to the superficial skeletal muscle using electrodes placed on the surface of the skin over the muscle motor point. Correct positioning of the stimulation electrodes is critically important as it influences the spreading and density of the current through the motor branches of the peripheral nerve. Non-optimal positioning of the electrodes would require higher current levels to reach and excite the muscle, thus activating pain afferent fibres to a greater degree. Once correct placement has been achieved, an electrical field is generated and a charge is forced to flow through the muscle when voltage is applied between an active electrode and a second non-active or 'reference' electrode. The series of impulses delivered to the muscle imitate the neural triggers that would normally pass through the spinal cord. Sequentially, this provokes an action potential in the peripheral motor nerves and elicits a muscle contraction in the associated muscle fibres (Rattay et al, 2003). Depending on the muscles being stimulated, ES can been used with different modalities and for different purposes. As such, ES is now recognised as an effective method to facilitate exercise, increase independence (Peckham and Knutson, 2005), reduce muscle atrophy (Crameri et al, 2002), improve muscle oxidative capacity and metabolism (Chilibeck et al, 1999b), and counteract some of the structural and (dys)functional changes in the vascular network supplying the paralysed limbs in people with SCI (Thijssen et al, 2006). The magnitude of these benefits are controlled by various factors such as stimulation parameters (pulse width, frequency, duty cycle, amplitude, ramping, program duration), the size/amount of muscle mass activated and the modality or method of application.

2.7.1. Electrical Stimulation Parameters

How the paralysed muscles contract, i.e. torque produced, and rate of fatigue is directly influenced by the ES parameters. Stimulation frequency is measured in units of Hertz (Hz) and refers to the number of pulses produced per second. Typically, electrically stimulated muscle activation is provided using higher frequencies (20-50 Hz) to elicit a tetanised muscle contraction that can be used for functional purposes (de Kroon et al, 2005; Kebaetse et al, 2005). Near maximal force is produced at frequencies of approximately 50-60 Hz with negligible force difference when using frequencies up to 100 Hz (Scott et al, 2007; Kesar et al, 2008). Despite higher frequencies producing the greatest force, rapid fatigue of the muscles limits its effectiveness when used over prolonged periods in those with SCI (Kebaetse et al, 2005; Deley, Laroche and Babault, 2014). Alternatively, a lower constant frequency stimulation that produces a smooth contraction at low force levels can be used to avoid rapid fatigue and discomfort. With that in mind however, the lowest stimulation frequency that can effectively induce a strong enough contraction to produce repetitive functional movements is approximately 20 Hz (Kebaetse, Turner and Binder-Macleod, 2002; Kebaetse et al, 2005).

Amplitude, or stimulation intensity, usually reported in milliamperes (mA) is another confounding factor that will contribute to fatigability and strength of a muscle contraction. A depolarisation of motor axons produces contractions by sending signals from the stimulation location to the muscle via a peripheral pathway without any involvement from the CNS. A higher stimulation intensity results in a stronger depolarising drive that travels deeper into the cellular structures under the electrodes and thus depolarises more motor axons producing a more forceful contraction (Mesin et al, 2010; Bergquist et al, 2011). The movement or exercise performed and the extent of muscle fibre degeneration will dictate the stimulation amplitude needed for effective muscle activation.

The majority of work in SCI has used FES, which refers to the process of *'pairing the stimulation simultaneously or intermittently with a functional task'* i.e. cycling, walking, and rowing (Moe and Post, 1962). To elicit contractions that are strong enough to create the involuntary mechanical movement of limbs, studies generally employ high stimulation amplitudes. For example, Crameri and colleagues utilised up to 300 mA during an electrical stimulation training protocol which lasted for a duration of 15-30 minutes three times a week (Crameri et al, 2002). In comparison, other studies using strength training (Belanger et al, 2000) and FES-rowing (Jung et al, 2012) employed stimulation amplitudes of 150 and 140 mA, respectively.

Pulse width duration is the length of time in microseconds (μs) that each electrical impulse lasts for. It has previously been shown that wider pulses generate significantly more torque than narrow pulses and may be less fatiguing (Gregory, Dixon and Bickel, 2007; Bickel, Gregory and Azuero, 2012). Using electrically induced resistance training in people with SCI, a pulse duration of 450 μs was used to effectively increase muscle mass after injury (Dudley et al, 1999; Mahoney et al, 2005). Typically, pulse width durations between 300 and 500 μs using a biphasic waveform (figure 2.6) are used for dynamic FES of the lower limbs in people with SCI. The biphasic waveform involves a secondary pulse of opposite polarity to balance the charge used to stimulate the neurons and is important to avoid tissue damage during high intensity ES training (Peckham and Gorman, 2004). Given the large array of uses for ES and differences in between patient characteristics, it is not possible to design the ideal ES program with a 'one size fits all' approach. Generally speaking, the most common and effective ES programs use a medium to high frequency (30-50 Hz) that is sufficient enough to induce a visible tetanic contraction without excess fatigue, a pulse width between 300 and 450 μs and a stimulation intensity adjusted accordingly to obtain the required torque production. The stimulated muscles, duty cycle, and program length depend on the exercise being performed and the overall purpose/goal of the ES.

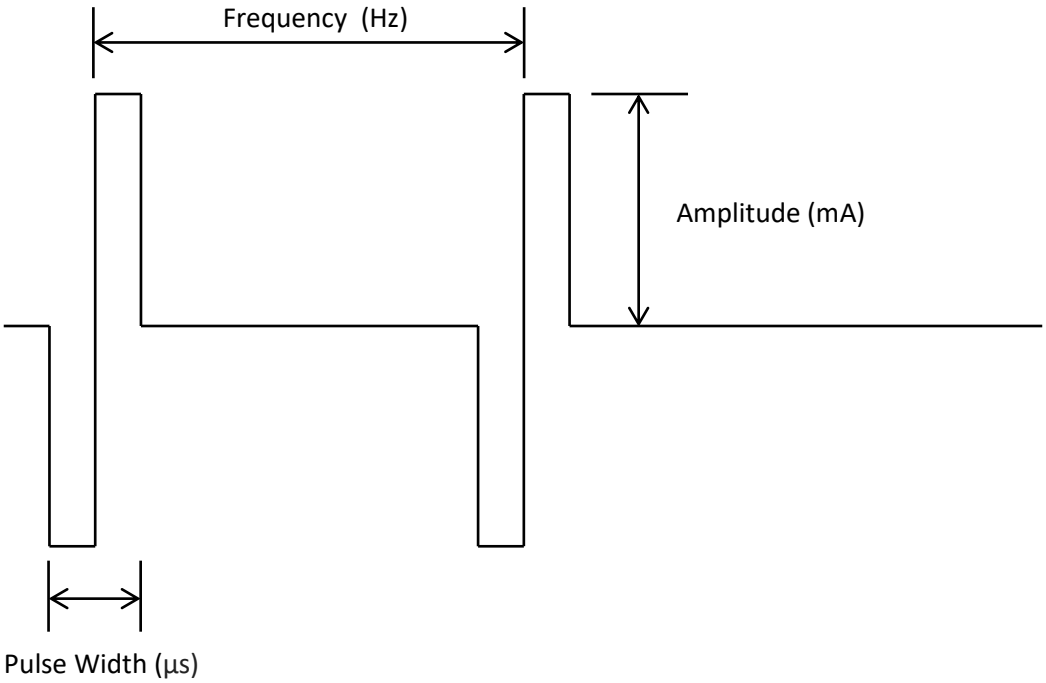


Figure 2.7 Example of a biphasic electrical stimulation waveform and the relevant stimulation parameters

2.7.2. Applications of Electrical Muscle Stimulation

Depending on the targeted actions, electrical stimulation can be used to activate one to several muscles individually or in unison. Its utility depends on the level of SCI and the amount of upper body motor function that remains after injury. For example, individuals with tetraplegia may use ES for restoration of arm and hand function as well as for trunk stability and lower limb movements, whereas individuals with paraplegia will require only lower limb ES. For this reason, there are numerous applications and methods of ES available. Currently, devices have been developed which use FES to facilitate exercise training such as resistance based strength training, cycling, arm cranking combined with FES leg exercise and hybrid rowing that combines voluntary upper body movements with electrically induced lower limb movements. Different to FES, other ES techniques utilise lower limb ES in a static

position (sitting/lying) to improve trunk stability (Momeni et al, 2016), muscle mass and strength (Dudley et al, 1999) and pressure ulcer prevention (Liu et al, 2014).

Studies have indicated the very fatiguing nature of FES due to the high frequency and amplitudes needed to produce the torque necessary for functionally useful movements. A higher frequency of ES has been shown to increase evoked torque by increasing motor unit activation (Gorgey et al, 2006). This causes an increased energy demand that cannot be supplied by the muscle thus preventing the cycling of motor unit activation and muscle contraction. Wheeler and colleagues reported that during FES-rowing, lower extremity strength, endurance and muscular fatigue determined the degree at which a full rowing stroke could continue (Wheeler et al, 2002). Additionally, Jung *et al* reported that the quadriceps muscles in six of ten individuals did not respond to FES due to the high degree of atrophy and loss of strength (Jung et al, 2012). It is therefore recommended that strengthening exercises such as static or dynamic ES induced leg extension and flexion are performed prior to and in parallel to FES exercise training.

FES cycling is possibly the most widely used from of FES exercise training that is currently available. The system applies ES intermittently to the quadriceps, hamstring and gluteus muscles to enable the legs to perform a cycling movement. Many benefits have been observed with FES cycling training such as increase artery diameter and resting mean blood flow (Thijssen et al, 2006), improved bone density (Frotzler et al, 2008), increase in muscle mass (Crameri et al, 2002) and increase aerobic capacity (Gibbons et al, 2016). However, despite its benefits, authors have reported critical drawbacks to FES cycling including low mechanical efficiency and poor power output (Hunt et al, 2012). Hybrid FES exercise, similar to FES cycling, involves electrically assisted lower extremity exercises but combined with voluntary upper extremity movements such as arm cranking or rowing. There is a strong rationale in the literature for using hybrid FES exercises in comparison with exercises only involving the upper or lower limbs. These include activation and strengthening

of larger muscle mass, training at higher oxygen uptake for more effective aerobic conditioning and promoting greater central training effects through higher cardiac volume loading (Hooker et al, 1992; Mutton et al, 1997; Brurok et al, 2011). FES usually involves extremely intricate ES parameters and protocols due to a combination of muscles that need to contract in such a way to create functional limb movement. With this, and the additional need for expensive equipment and technically trained personal assistance, regular use of FES is not an option for many SCI individuals.

The use of ES for static isometric and concentric contractions using only adhesive surface electrodes and a stimulator is an alternative and more simplistic method of ES when compared to FES cycling/rowing/hybrid exercises. Whilst it might not be as effective in improving oxygen uptake or cardiorespiratory fitness, many other benefits such as increased strength, muscle mass (Rodgers et al, 1991; Bogie, Wang and Triolo, 2006) blood flow and tissue oxygenation (Figoni et al, 1991; Mawson et al, 1993a) are observed with static isometric and concentric ES muscle contractions. This particular technique involves placing electrodes over the motor points of muscles and using a stimulator to achieve the desired contraction. More recently, research investigating the use of clothing garments with built in surface electrodes to elicit static isometric contractions has gained considerable interest (Smit et al, 2013a; Barton et al, 2018). Using this innovative technique, application of the electrodes is made relatively simple and avoids the need for skin preparation, thus overcoming some of the disadvantages of traditional surface ES devices.

2.7.3. Physiological effects of electrical stimulation

Muscular: When used regularly and with the appropriate settings, ES is associated with extensive benefits to the health and quality of life of people living with a SCI. One of the most notable changes after SCI is a significant loss in the size/number of muscle fibres and a change in muscle histology of the lower paralysed limbs. This muscle atrophy occurs rapidly after injury with significant changes in cross-sectional

area and muscle volume within 6 weeks of injury (Gorgey and Dudley, 2007). Several studies have found that ES can reverse or limit the rate of muscle atrophy and shift their morphological characteristics more towards that of the able-bodied population (Baldi et al, 1998; Chilibeck et al, 1999a; Dudley et al, 1999; Kjaer et al, 2001). For example, using an implanted gluteal stimulation device, Bogie *et al.* demonstrated a 50% increase in muscle thickness that was maintained at long-term follow up with regular use of gluteal stimulation (Bogie et al, 2000; Bogie, Wang and Triolo, 2006). Further, Dudley and colleagues reported that electrically induced knee extension exercises, twice a week for 8 weeks effectively reversed muscular atrophy of the quadriceps muscles (Dudley et al, 1999). These changes in muscle size are concomitant with a 23% and 39% increase in fibre area and capillary density, respectively (Chilibeck et al, 1999b). Other studies, which explored the effects of ES on muscle histology and fatigue, reported that with daily treatments of ES; resistance to fatigue, speed of contraction, together with oxidative capacity rapidly revert towards normal values (Martin et al, 1992; Rochester et al, 1995). Considering that muscle mass importantly contributes to pressure ulcer prevention and resting metabolism, limiting muscle atrophy with the use of ES should be reinforced as a priority for people with SCI.

Conduit vessels: Electrical stimulation of the paralysed muscles has demonstrated preferential changes in micro- (Mawson et al, 1993a; Levine, Zuckerman and Pawelczyk, 1997; Barton et al, 2018) and macro-vascular (Gerrits et al, 2001b; Thijssen et al, 2005) structure and function. In able-bodied subjects, exercise training is associated with an outward remodelling and enlargement of the conduit arteries supplying the active skeletal muscle (Dinenno et al, 2001b; Thijssen et al, 2006). Data from experimental studies (Langille and O'Donnell, 1986; Tuttle et al, 2001; Tinken et al, 2010) indicate that vascular enlargement is likely related to repeated increases in shear stress, with the magnitude of luminal expansion being proportionate to the level of blood flow and wall shear. Using ES as a way to simulate a voluntary muscle contraction similar to that in the able-bodied, blood flow in the common femoral artery of SCI individuals demonstrated a 46% increase after just 4 weeks (Thijssen et

al, 2005). This finding was accompanied by a 6% increase in resting artery diameter and is comparable with many studies assessing the structural peripheral vascular adaptations to ES (Taylor et al, 1993; Gerrits et al, 2001b; Hopman et al, 2002).

Resistance vessels: Electrical stimulation also appears to modulate vascular tone and blood flow in the resistance vessel of SCI individuals. For example, 2–8 weeks of electrically stimulated exercise training in the chronically deconditioned lower limbs is able to reverse the increase in vascular resistance towards normal values (Hopman et al, 2002; Thijssen et al, 2007). The mechanisms involved in the apparent increase vascular resistance in SCI is most likely explained by modulation of vasoconstrictor pathways. Exercise training has been shown to have a significant role in suppressing humoral vasoconstrictors such as ET-1 and ANG II in patients with CVD (Adams et al, 2005; Maeda et al, 2009). In further support, FES-based exercise training in individuals with SCI reverses the contribution of the ET-1 pathway to regulate baseline vascular tone. These data provide evidence to support the role of ES in reversing the adaptive responses to SCI in resistance vessel structure and function.

Microvessels: Capillary growth is closely linked with exercise training and has been previously shown in the paralyzed limbs of SCI individuals after ES (Chilibeck et al, 1999b; Crameri et al, 2004). Although the majority of research regarding ES and microvessels pertains to the muscle microvasculature, a number of studies have reported an increase in cutaneous microvascular function in older (Black, Green and Cable, 2008) and diabetic (Cohen et al, 2008) able-bodied individuals with exercise training. In persons with chronic SCI, there is an apparent systemic adaptation of the cutaneous microcirculation rather than in the paralysed region only. Van Duijnhoven demonstrated impaired cutaneous vascular function during local heating in the paralysed lower limbs and active forearms of individuals with SCI compared to able-bodied controls (Van Duijnhoven et al, 2009). As paraplegic individuals have normal upper arm mobility, physical inactivity cannot explain the impaired cutaneous function. This was confirmed with 8 weeks of FES cycling, in which the increased

physical activity in the legs did not alter skin blood flow responses to local heating despite an increase in femoral artery diameter. This suggests that structural and functional changes in micro- and macro-vessels in response to exercise training are mediated by different mechanisms, and physical inactivity alone may not explain the impaired cutaneous microcirculatory responses to local heating.

Several studies have measured transcutaneous oxygen levels as an indirect representation of skin blood flow during ES and have reported promising results (Bogie and Triolo, 2003; Bogie, Wang and Triolo, 2006; Smit et al, 2013b). For example baseline mean unloaded tissue oxygen levels were shown to increase by 35% post ES (Bogie and Triolo, 2003). A healthy microcirculation is crucial for tissue viability in terms of supply of oxygen and nutrients as well as removal of waste products. Therefore, with the use of ES, an increase in microvascular perfusion could prevent ischemia and local tissue starvation, sequentially reducing the risk of pressure ulcers. Relating to pressure ulcer prevention, ES can play a critical role in reducing the prolonged mechanical loading at the skin and muscle. Many studies have examined the dynamic short-term effects of ES on interface pressure in SCI and consistently report a pressure redistribution and significant reduction at the ischial tuberosity (Levine et al, 1990; Ferguson et al, 1992; Liu et al, 2006; van Londen et al, 2008). The application of electrical stimulation whilst seated changes the force, tone and shape of the gluteal muscle and exposes the surrounding tissues to pressure relief. The temporary decrease in interface pressure possibly prevents the total occlusion of blood vessels and restores blood flow. Additionally, with prolonged exposure to ES, the gains in gluteal muscle mass increases the seated contact area with the support surface. These structural changes may therefore constitute an effective prevention of pressure ulcers due to the increased muscle cushioning effect and overall pressure distribution.

2.8. Summary

In summary, persons with SCI undergo extreme vascular remodelling, which ultimately leads to an array of cardiovascular complications and increased risk for premature cardiovascular death. Currently, there is insufficient evidence regarding the effectiveness of using traditional cardiovascular risk factors and risk prediction models for calculating CVD risk in persons with SCI. Additionally, electrical stimulation, when used frequently, demonstrates encouraging results in reversing the structural changes to the vascular lumen imposed by physical inactivity and modifying risk factors associated with numerous micro- and macro-vascular complications. The majority of research uses methods that are not readily available or practical for every day, home based usage. Little is known about the practicality of long term, low intensity electrical stimulation using a portable electrical stimulation-clothing device and the effects it has on micro- and macro-vascular structure and function in persons with SCI.

Chapter 3

Traditional cardiovascular risk factors strongly underestimate the 5-year occurrence of cardiovascular morbidity and mortality in persons with spinal cord injury

3.1. Introduction

Cardio- and cerebro-vascular diseases have become a major concern for individuals with a SCI. CVD constitutes 26.7% of all-cause mortality (Savic et al, 2017a) and are responsible for the greatest proportion of morbidity and mortality in the SCI population (Garshick et al, 2005; Osterthun et al, 2014). Assessing a person's risk for developing CVD is typically performed using traditional cardiovascular risk factors and, subsequently, risk is predicted using widely available algorithms such as the FRS. Since these algorithms are based on able-bodied populations, mainly including middle-aged and older Caucasian men from Western countries, one may question its generalizability to other populations (Grundy et al, 1999; Yeo and Yeo, 2001; Matheny et al, 2011), including SCI.

Interpretation of traditional CVD risk factors is complicated in SCI population. For example, elevated arterial blood pressure is recognized as an independent risk factor for CVD in the general population. However, individuals with SCI, in particular those with high thoracic and cervical lesions, exhibit low resting arterial blood pressure that results from autonomic disturbances (West, Mills and Krassioukov, 2012; Phillips and Krassioukov, 2015). Furthermore, despite the increased risk for CVD in individuals with SCI, classic cardiovascular risk factors, such as low-density lipoprotein, plasma triglycerides and fasting glucose, are not different between SCI and able-bodied populations (Cardus, Ribas-Cardus and McTaggart, 1992b; Krum et al, 1992; Bauman and Spungen, 1994; Bauman et al, 1999; Jones, Legge and Goulding, 2004; Liang et al, 2007; Finnie et al, 2008). This raises the question whether traditional cardiovascular risk factors and risk prediction models which use these risk factors can accurately predict future CVD in individuals with SCI.

The aim of this chapter was to examine the predictive value of traditional risk factors for future CVD using the Framingham risk score in individuals with SCI. For this purpose, an observational cohort study was used to 1) determine whether the FRS accurately predicts cardiovascular morbidity and mortality across 5-years after

discharge from in-patient rehabilitation in people with SCI, and 2) if adding SCI-related characteristics (i.e. lesion level) to the FRS can improve the prognostic value of the FRS. Based on the argument raised earlier which suggests that SCI may affect interpretation of traditional CVD risk factors, it was expected that the FRS underestimates future CVD, whilst adding SCI characteristics improves the prognostic value of the FRS in individuals with SCI.

3.2. Methods

3.2.1. Participants

The data used in this study were collected as part of the Dutch prospective multicentre cohort study 'Restoration of (wheelchair) mobility in SCI rehabilitation' (de Groot et al, 2006b) and obtained prospectively. The medical ethics committee of the Stichting Revalidatie Limburg/Institute for Rehabilitation Research in Hoensbroek approved the research protocol in 1999, and the medical ethics committee of the University Hospital of Utrecht approved for the follow-up research protocol in 2006. Participants (n=225) were recruited from 8 specialist SCI rehabilitation centres in the Netherlands. Written informed consent was obtained from all participants prior to the start of this study. Inclusion criteria required participants to have a traumatic or non-traumatic SCI classified as A, B, C or D on the American Spinal Cord Injury Association impairment scale (ASIA) (Kirshblum et al, 2011), expected to remain wheelchair dependent, no evidence of pre-existing cardiovascular diseases and aged between 18-65.

3.2.2. Experimental design

The observation period began at the start of active rehabilitation when the participants could remain seated for a minimum of 3 hours (m=3 months after injury). Participants were asked to eat only a light meal, to abstain from consuming tobacco, caffeine and alcohol at least 2 hours prior to testing and to void their bladders. All participants continued to take their regular medication. Blood samples were collected and analysed for serum concentrations of total and high-density lipoprotein

cholesterol. Resting arterial blood pressure was measured by a physician using a manual sphygmomanometer whilst participants remained seated in their wheelchair (Ravensbergen et al, 2014). Participants were considered to have diabetes when the primary care physician reported this or when medical records indicated the participant was taking diabetes medication. Lesion characteristics (level and completeness) were assessed by a specialist physician and according to the International Standards for Neurological Classification of Spinal Cord Injury (Maynard et al, 1997). Survival status and cardiovascular morbidity and mortality were obtained from medical records up to 5 years after discharge from inpatient rehabilitation. The follow up period of some individuals included in the analysis goes beyond the 5 years. All these individuals did, however, develop CVD within the 5 years, and in some instances additional cardiovascular complications after the 5 years. Cardiovascular complications and causes of death were identified according to the International Classification of Diseases and Related Disorders, 10th revision, volume 2 (codes I00-I99).

3.2.3. Framingham Risk Score

The FRS-calculator is a method that uses equations derived from large prospective cohort studies such as the Framingham heart study and Framingham offspring study (Anderson et al, 1991) to estimate the risk of developing CVD events in the proceeding 5-10 years (D'Agostino et al, 2008). CVD endpoints using the FRS prediction model can be defined as all coronary events (e.g. myocardial infarction, coronary death, coronary insufficiency, and angina), cerebrovascular disease (e.g. ischemic stroke, hemorrhagic stroke, and transient ischemic attack), rheumatic disease, heart arrhythmia, valvular disease, aortic aneurysms, peripheral artery disease, thromboembolic disease and venous thrombosis (D'Agostino et al, 2008). Compared with other risk algorithms, the FRS-calculator is able to discriminate between those who will and will not develop a CV event (Liao, McGee and Cooper, 1999; Liao et al, 1999; Menotti, Puddu and Lanti, 2000; D'Agostino et al, 2001; Grundy et al, 2001) and has been validated in multiple populations (Menotti et al, 2000). For every individual, at the start of active rehabilitation, a 5-year risk for

developing CVD was predicted using the Framingham risk calculator from the Centre for Cardiovascular Sciences at the University of Edinburgh (Payne). This particular tool is a spreadsheet-based calculator that uses age, sex, systolic blood pressure, total cholesterol, HDL cholesterol, smoking status and diabetes status to estimate the percentage based risk of developing CVD over a selected number of years.

3.2.4. Statistical analysis

Participant characteristics were summarized by means and standard deviations for normally distributed continuous variables and percentages for categorical variables. The Kolmogorov-Smirnov test of normality was conducted for the Framingham risk scores. Non-normally distributed data were presented as medians with interquartile ranges. Kaplan–Meier curves and the log-rank test were used to assess the difference in clinical outcome between participants with a FRS >1.36 (median score for the cohort) and FRS $\leq$1.36. For the context of this paper, the group of participants with a FRS $\leq$1.36 will be referred to as the 'low FRS' group and those with a FRS >1.36 will be referred to as the 'high FRS' group. The end-point was a CV event or CV mortality. Patients who did not reach the end-point were censored at the end of the observation period. Hazard ratios with 95% confidence intervals (CIs) were calculated using Cox proportional hazard regression.

The FRS ability to predicted events in patients with SCI was assessed using receiver operating characteristics curves (ROC) with corresponding area under the curves (AUC) and 95% CIs. SCI-related factors associated with CVD-events were explored using univariate Cox proportional hazard regression. Severity of injury as indicated by ASIA impairment, motor completeness and level of injury were included as factors in the regression analysis due to their direct association with impaired CV function (West, Mills and Krassioukov, 2012; Phillips and Krassioukov, 2015). Considering the beneficial effects of physical activity on CV health in the able bodied, this study also included sports participation prior to injury as a factor and its influence on predicting CVD after injury was explored. These factors were separately added to the FRS and

X*β values were calculated using multivariate Cox proportional hazard regression. Using the X*β values, receiver operating characteristics curves with corresponding AUC and 95% CI were determined. All statistical analyses were performed in SPSS 20.0. A *P*-value <0.05 was considered statistically significant.

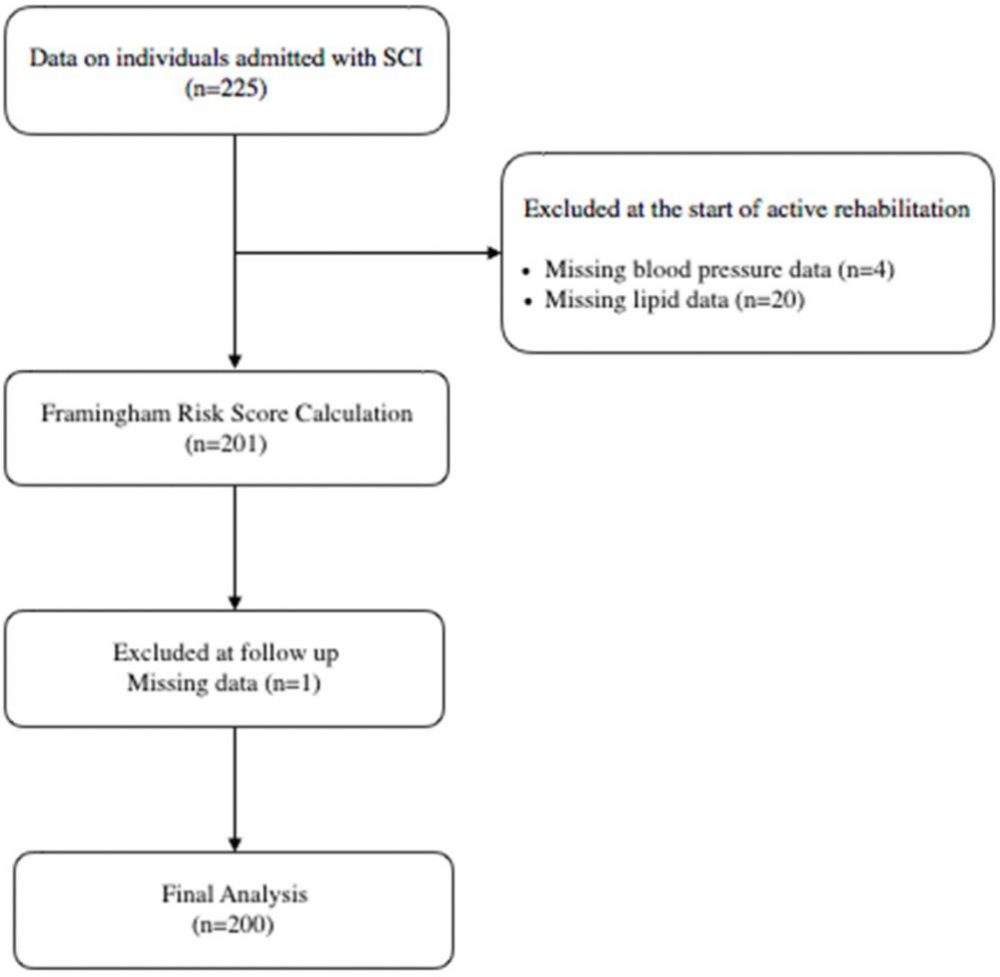


Figure 3.1 CONSORT flow diagram of subject inclusion and retention from the initial measurement period up to follow up

3.3. Results

3.3.1. Test of Normality

Figure 3.2 illustrates the distribution of the FRS for the whole SCI population and indicates the data are skewed to low (<1.36%) risk values. The Kolmogorov-Smirnov test of normality confirms the FRS data are not normally distributed (*P*<0.001).

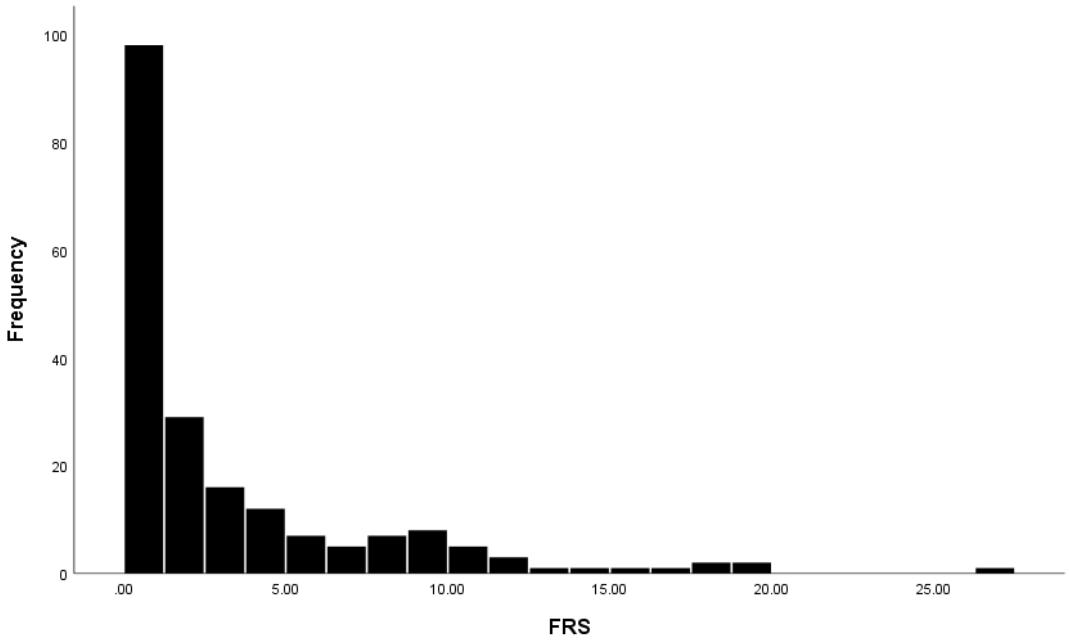


Figure 3.2. Histogram illustrating the distribution of the FRS values for the whole SCI population

3.3.2. Survival analysis

Table 3.1 summarizes baseline characteristics and Table 3.2 indicates the cardiovascular events for the 200 individuals included in the analysis. In the 5 years following discharge from in-patient rehabilitation, a total of 39 participants (19.5%) developed a CVD event, 10 of which were fatal events. Deep venous thrombosis (DVT) was the most commonly observed CVD event with 5% of the study participants having an incidence of DVT. Figure 3.2 shows the survival analysis for the groups with a low FRS (≤1.36 (median)) and a high FRS (>1.36). One individual was excluded from

the survival analysis due to missing follow-up data. The study found a significant difference in CVD events between both groups (hazard ratio for high FRS *vs* low FRS was 3.2, 95% confidence interval [CI] 1.6-6.5; *P*=0.001). A total of 10 and 29 CVD events were recorded in the low and high FRS groups, respectively.

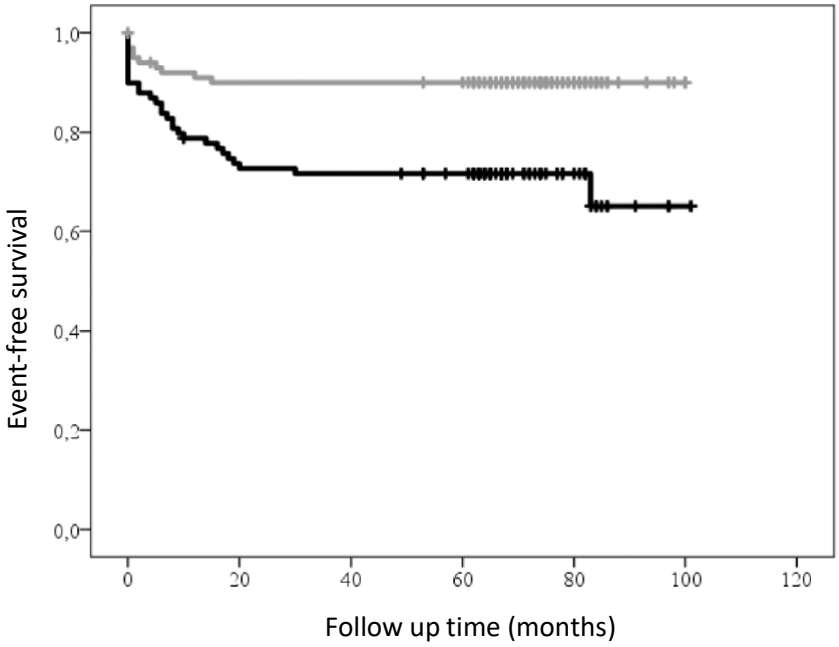


Figure 3.3 Survival analysis for individuals with a spinal cord injury (n=200) across a 5 year follow-up. Subjects were divided into individuals with a Framingham Risk Score (FRS) ≤1.36 (i.e. median; grey line, 10 CVD events) and those with a FRS >1.36 i.e. median; black line, 29 CVD events).

Table 3.1 Baseline characteristics of participants at start of the observation period

Characteristic	
Age (years)	40 (14)
Gender (male)	149 (74%)
Smoking status (yes)	46 (23%)
Systolic blood pressure (mmHg)	118 (16)
Cholesterol	
HDL (mmol/L)	1.02
Total (mmol/L)	4.70
Diabetes	3 (2%)
BMI (kg/m^2)	22.8 (3.8)
ASIA impairment scale (n=197)	
A	91 (46%)
B	47 (24%)
C	42 (21%)
D	17 (9%)
Motor impairment (complete)	139 (69%)
Lesion level (tetraplegia)	80 (40%)
Performed sports before injury (yes)	127 (63%)
FRS (5-year probability (%))	1.36 (0.14-4.49)

HDL=high density lipoprotein, BMI=body mass index, FRS=Framingham risk score, ASIA=American spinal cord injury association

Data is presented in mean (SD), median (Q25-Q75) or indicated as n (%).

Table 3.2 Cardiovascular disease events developed by participants during the observation period (n=39)

Cardiovascular event	Number
Deceased	
Pulmonary embolism	3
Other cardiovascular death	7
Cardiovascular morbidity	
Chronic venous insufficiency	2
Deep venous thrombosis	10
Transient ischemic attack	1
Atrial flutter/fibrillation	4
Peripheral vascular disease	2
Aortic diseases	3
(Aneurysms, valve diseases & dissection)	
Myocardial infarction	5
Angina	2
Total	39

3.3.3. FRS prediction model using SCI characteristics

Table 3.3 illustrates the calculated hazard ratios with 95% CI, regression coefficients and statistical significance for various SCI characteristic individual predictors for CVD events. Each factor is assessed through separate univariate Cox regressions. Older age at time of SCI (1.05, 95% CI 1.02-1.07; *P<0.001*), a higher 5-year FRS (1.10, 95% CI 1.05-1.16; *P<0.001*) and no participation in sport activities before the SCI injury (1.25, 95% CI 0.65-2.41; *P=0.013*) were identified as significant independent predictors for CVD events across the mean 5-year follow-up period. When the predictive value of the FRS alone was assessed by receiver operating characteristic curves (Figure 3.3), the area-under-the-curve was 0.71 (95% CI 0.62-0.82). For the

new models, which included the FRS combined with SCI characteristics, no significant improvement in ROC-curves was found. More specifically, the predictive power of the FRS was not improved when adding ASIA impairment (0.74; 95% CI 0.66-0.82), motor impairment (0.74; 95% CI 0.66-0.83), level of injury (0.72; 95% CI 0.63-0.81) or active engagement in sport prior to injury (0.72; 95% CI 0.63-0.88).

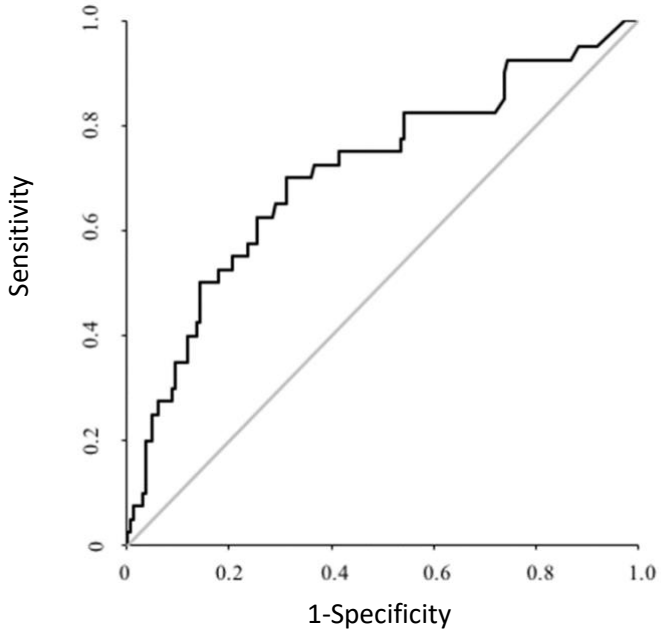


Figure 3.4 Receiver operating characteristics (ROC) curve for the Framingham Risk Score for the prediction of 5-year occurrence of a CVD event individuals with a spinal cord injury (n=200) across a 5 year follow-up.

Table 3.3 Cox regression analysis of individual predictors for cardiovascular disease events

Predictors	**Hazard ratio (95% CI)**	**Beta coefficients**	**P-value**
Age (male)	1.05 (1.02-1.07)	0.044	<0.001
Diabetes (yes)	3.53 (0.85-14.7)	1.26	0.08
Smoking (yes)	0.727 (0.32-1.65)	-0.319	0.44
Total cholesterol (mmol/L)	1.28 (0.97-1.69)	0.248	0.08
HDL cholesterol (mmol/L)	0.96 (0.32-2.91)	-0.040	0.94
BMI (kg/m^2)	1.04 (0.96-1.13)	0.042	0.30
5-year FRS (%)	1.10 (1.05-1.16)	0.099	<0.001
ASIA impairment scale B	1.84 (0.89-3.81)	0.610	0.10
C	0.86 (0.33-3.79)	-0.151	0.75
D	1.10 (0.32-3.79)	0.093	0.88
Motor impairment (complete)	1.21 (0.59-2.48)	0.187	0.61
Level of injury (paraplegia)	1.25 (0.65-2.41)	0.226	0.50
Played sports before injury (no)	0.45 (0.24-0.85)	-0.798	0.01

HDL=high density lipoprotein, BMI=body mass index, FRS=Framingham risk score, ASIA=American spinal cord injury association

ASIA A, Motor incomplete, Tetraplegia = reference categories

Table 3.4 Mean ± SD values for each risk factor included in the FRS for the whole group, below median FRS score, above median FRS, paraplegics and tetraplegics.

Risk factor	Whole Group	<1.36	≥1.36	p value	Paraplegics	Tetraplegics	p value
n=	200	100	100		120 (60%)	80 (40%)	
Systolic BP (mmHg)	118 ± 16	111 ± 12	125 ± 17	<0.001	121 ± 16	113 ± 15	<0.001
TC (mmoL)	4.7	4.3 ± 1.0	5.2 ± 1.0	<0.001	4.8 ± 1.7	4.6 ± 1.1	0.09
HDL (mmoL)	1.0	1.0 ± 0.2	1.1 ± 0.4	0.19	1.0 ± 0.3	1.0 ± 0.3	0.12
LDL (mmoL)	2.9	2.7 ± 0.8	3.3 ± 1.0	<0.001	3.0 ± 1.0	2.9 ± 0.8	0.32
Age at injury	40 ± 14	29 ± 8	52 ± 8	<0.001	41 ± 14	39 ± 13	0.43
Sex (male)	149 (74%)	75 (75%)	71 (71%)		88 (73%)	58 (73%)	
Smoke (yes)	46 (23%)	19 (19%)	25 (25%)		29 (24%)	15 (19%)	
5 year FRS %	3.2%	0.3 ± 0.4	6.2 ± 5.0	<0.001	3.7 ± 5.1	2.5 ± 3.5	0.05

TC=Total Cholesterol, HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein, BP=Blood Pressure

3.4. Discussion

The purpose of this chapter was to investigate whether traditional cardiovascular risk factors, through the calculation of the commonly used Framingham risk score, can predict the occurrence of CVD events over a 5-year follow-up in individuals with SCI. This is the first study to test the accuracy of the FRS to predict future CVD events in individuals with SCI. Firstly, this study found that the FRS markedly underestimates the occurrence of CVD mortality and morbidity in individuals with SCI. Secondly, despite this marked underestimation of the true CVD event rate, the FRS was able to successfully identify individuals with SCI at increased risk for future CVD. These novel observations have important clinical impact, since the findings in the current study suggest that aggressive (pharmaceutical) interventions may be required in individuals with SCI to lower risk for future CVD events, even when traditional CVD risk factors suggest a low-to-moderate risk.

A FRS of <10% in able-bodied individuals is classified to be “low” risk of 10 year CVD (D'Agostino et al, 2008). Although difficult to perform a direct comparison due to the current study using a 5 year CVD risk calculation, it would not seem unreasonable to say a FRS of <5% is a low CVD risk classification. Therefore, based on the FRS risk prediction, one would expect to see very few events in the relatively young population (40±14 years) of SCI individuals across the 5-year period. The distribution data (Figure 3.2), which is skewed towards the lower FRS values, further supports this suggestion. Surprisingly however, 39 CVD events were observed. A total of 9 events occurred in the low risk group and overall there were 10 fatalities. This represent an unexpectedly high rate of CVD events, particularly for such low FRS values. Although previous work suggested that the FRS may underestimate the actual CVD risk in the SCI population (Finnie et al, 2008; Wahman et al, 2011; Sullivan et al, 2017), this study represents the first prospective study to support this hypothesis. Despite the marked underestimation, the FRS was successful in distinguishing individuals who were at an increased risk for a CVD event. When comparing the ‘high’ vs ‘low’ risk group, the survival analysis indicated that the group of SCI individuals with a >median FRS had a 3.2-fold greater risk for developing a CVD event than those with a <median FRS.

When comparing the individual risk factors between the high and low risk group, blood pressure, total cholesterol, LDL cholesterol and age were all greater in the higher risk group (Table 3.4). However, despite this difference, the risk factor values in both high and low risk groups were within the healthy normative parameters recommended for able-bodied individuals. Interestingly, data from the ROC-curve indicates that the ability of the FRS to predict CVD events in SCI (i.e. 0.71) is comparable to that typically observed in able-bodied populations (0.68-0.75) (Erbel et al, 2010; Cook et al, 2012; DeFilippis et al, 2015). Taken together, this indicates that the FRS successfully identifies subjects with SCI who have an increased risk for CVD, but markedly underestimates the true risk.

One potential implication of the observations in the current study is that different cut-off values for factors such as blood pressure and cholesterol should be adopted to calculate the correct CVD risk in SCI (Krum et al, 1992). Indeed, in the current study sample, cholesterol levels were within healthy ranges specified for able-bodied individuals and therefore a low FRS was calculated, despite being at an apparently higher risk for CVD. This finding supports previous work indicating the presence of low-to-normal levels of triglycerides, total and LDL cholesterol for individuals with SCI (Cardus, Ribas-Cardus and McTaggart, 1992b; Bauman et al, 1999; Finnie et al, 2008; Laclaustra et al, 2015). In addition, systolic blood pressure in the subset of individuals who developed a CVD event was also within the normal range. Future studies adopting a prospective design should explore whether adjustment of cut-off values is required for the traditional CVD risk factors.

In addition to adjusting the cut-off values of traditional risk factors, one should also consider alternative risk factors in this population. First, although blood pressure is recognized as a strong predictor for CVD in the able-bodied population, frequent exposure to blood pressure variability may pose an additional risk. Individuals with SCI often experience episodes of autonomic dysreflexia, which represents an important CVD risk factor, independent of basal mean arterial blood pressure (Grove

et al, 1997; Rothwell et al, 2010). Second, current models of CVD risk prediction do not include a measure of physical activity. This is of special importance since recent work has revealed that physical inactivity has overtaken smoking as the leading cause of non-communicable diseases (Lee et al, 2012), whilst individuals with SCI are exposed to marked physical inactivity (Thijssen et al, 2010). Their life-long exposure to an extreme form of sedentary behaviour may accelerate the atherosclerotic processes. A final alternative explanation relates to the detrimental impact of a SCI on vascular health (Thijssen et al, 2010; Green et al, 2017). This is of special importance, since independent from known risk factors, impaired vascular function and structure may increase CVD risk (Taylor et al, 2006; Mora et al, 2007; Chomistek et al, 2011; Hamer et al, 2012).

An important observation to make relating to vascular structure and function is the high prevalence of deep vein thrombosis (DVT) amongst the group of individuals who experienced a cardiovascular event during follow up (Table 3.2). Although there was a high occurrence within this small group of individuals, it is not surprising considering that between 47 and 100% of individuals with a chronic SCI will sustain a DVT during their lifetime (Jones et al, 2005; Teasell et al, 2009). Stasis and hypercoagulability are the two major factors contributing to the development of thrombosis in this patient population. This is supported by studies that demonstrate abnormal coagulation factors and impaired venous return, which predispose to the development of thrombogenesis (Merli et al, 1993). The high rate of this complication suggests the need for effective preventative treatment strategies. Currently, the use single agent pharmacologic anti coagulation therapy in form of heparin is widely prescribed, although with it comes the risk of worsened bleeding. Therefore, a combination of therapies involving pharmacological agents and mechanical methods for improving blood stasis may represent an effective strategy for reducing the incidence of DVTs. However, further large scale studies are required to better identify the best modalities and treatments for the prevention of these complications. This provides a rationale for use of electrical stimulation in the later chapters of this thesis, and how it effects blood flow in the lower limbs of people with

SCI.

This study tested individual predictors for CVD events using separate univariate cox regressions and to establish whether adding SCI characteristics to the Framingham model can improve the accuracy and prognostic value of the FRS. Unlike ASIA impairment and level of the injury, older age at time of injury, no sports participation prior to injury and a higher FRS were all significant predictors for CVD events. When comparing the models' accuracy and ability to identify individuals who will develop a CVD event, adding these individual predictors did not improve the FRS model. This is somewhat surprising considering that CVD risk increases relative to serum HDL levels (National Cholesterol Education Program Expert Panel on Detection and Treatment of High Blood Cholesterol in, 2002) and direct associations have been reported between lipid concentrations (e.g. low HDL) and neurological deficit or severity of the spinal injury (Laclaustra et al, 2015). Possibly, the link between lipids and lesion level may be caused by the strong physical inactivity experienced by individuals with a higher level SCI rather than the lesion characteristics per se. Although SCI lesion characteristics did not improve the accuracy of the FRS, older age at time of injury was a significant independent predictor. These results corroborate with others who report that older age at time of injury accelerates the aging process and is an independent predictor of mortality in the first 5-years after injury (Casper et al, 2017). Additionally, advancing age is associated with a higher prevalence of risk factors such as metabolic syndrome (de Groot et al, 2016), and possibly further accelerates the development of CVD in older individuals after SCI. Taken together, the findings within this chapter do not support adding SCI-specific factors to the FRS to improve the prediction of future CVD events in SCI individuals.

3.4.1. Clinical relevance

Accurate CVD estimation is essential to balancing the risks and benefits of prescribing

preventive therapies and interventions. The findings in the current study may have important clinical consequences as they suggest that individuals with SCI, even in the presence of risk factors that are within the low range of able-bodied individuals, may benefit from (pharmaceutical) interventions to prevent CVD. Some evidence also shows that in the able-bodied population, using interventions that lower the risk of CVD in those with risk factors within the "normal" range can have beneficial effects on overall CVD risk development (Cholesterol Treatment Trialists et al, 2012; Thanassoulis et al, 2016). Although future work is required to better understand this area, adjustment of current risk-prediction models and exploring their clinical implication for individuals with SCI seems warranted. With this in mind, paraplegics and tetraplegics have different FRS values due to the dramatic difference in systolic blood pressure (Table 3.4). Therefore, when considering changes to risk prediction models, differences in blood pressure between individuals with different lesion levels should be accounted for. One should also consider adding novel risk factors (e.g. physical inactivity) and/or alternative screening methods. For the latter, carotid intima-media thickness (CIMT) is a known surrogate marker for CVD in the general population (Folkow, 1978; O'Leary et al, 1999; Bots et al, 2005). In SCI individuals, no correlation was found between lipid profile and CIMT, despite signs of subclinical atherosclerosis (Szlachcic et al, 2014). Possibly, vascular imaging techniques may be an appropriate CVD screening tool that, independent of current risk factors, provide independent predictive capacity.

3.4.2. Conclusion

In conclusion, the findings contained within this chapter suggest that, although a higher FRS corresponds with an increased rate of CVD, the FRS/traditional cardiovascular risk factors significantly underestimate the 5-year risk of CVD morbidity and mortality in individuals with SCI. Furthermore, the increased risk and greater prevalence of a CVD event was independent of SCI lesion characteristics. Therefore, these data suggest that CVD risk estimation using the FRS and/or traditional cardiovascular risk factors should be interpreted with caution in this

vulnerable population of SCI individuals. Given the high risk of CVD in this population, prospective follow-up studies are required to better understand CVD risk estimation in individuals with SCI, but also how this could adjust current medical care in individuals with SCI to prevent future CVD. Moreover, these findings further highlight the importance of establishing interventions to ameliorate the increased risk of CVD in this population.

Chapter 4

Femoral artery blood flow and microcirculatory perfusion during acute, low-level electrical stimulation in spinal cord injury

4.1. Introduction

A spinal cord injury leads to significant changes in sub-lesional vascular structure and function. Most characteristic changes involve a decrease in conduit artery diameter (de Groot, Bleeker and Hopman, 2006), increased vascular resistance (Hopman et al, 2002), increased arterial stiffness (de Groot et al, 2005) reduced capillarisation (Chilibeck et al, 1999b) and impaired cutaneous microcirculation (Nicotra, Asahina and Mathias, 2004; Van Duijnhoven et al, 2009) in the paralyzed, inactive limbs. Collectively, such vascular changes are associated with endothelial dysfunction and the development of cardiovascular disease, which is a primary cause of death in persons with a SCI (Osterthun et al, 2014) and it was demonstrated in the previous chapter that the risk of CVD morbidity and mortality in individuals with SCI is significantly underestimated using FRS/traditional cardiovascular risk factors. Besides the increased risk of cardiovascular disease, below lesion microvascular endothelial dysfunction manifested as impaired cutaneous blood flow also has significant implications for persons with SCI. The incidence and progression of skin breakdown lesions and pressure ulcers in persons with SCI have been attributed to factors that are associated with a reduction in cutaneous microcirculation (Byrne and Salzberg, 1996). Interventions that help reverse macro- and microvascular endothelial dysfunction below, and even above, the lesion are therefore of great clinical significance for persons with SCI.

Studies show that elevations in blood flow and shear stress are required for improvement in vascular function and an increase in artery diameter (Tinken et al, 2010)' (Green et al, 2017). Using electrically stimulated leg exercise in individuals with SCI, Thijssen *et al.* showed evidence of arterial remodelling in areas subject to electrically stimulated muscular contractions, while vascular adaptations were not apparent in the passive, non-stimulated areas of the leg (Thijssen et al, 2005). In addition to conduit remodelling, studies have shown that muscle ES results in increased muscle mass(Dudley et al, 1999), higher muscular oxidative capacity (Erickson et al, 2016), enhanced capillary supply (Chilibeck et al, 1999b) and

improved blood flow (Hopman et al, 2002). This highlights the potency of ES to mediate beneficial adaptations.

Commonly used methods of ES require specialist facilities and trained staff, making regular application difficult, expensive and impractical. A potential alternative is the use of wearable clothing garments with embedded surface electrodes that automatically stimulate muscles when the garment is applied. This also allows for the adoption of low-intensity ES that can be applied for prolonged periods (i.e. during awake hours). Using this approach, an acute bout of ES to the gluteal and hamstring muscles has shown to reduce pressure over the ischial tuberosity (Smit et al, 2013a) and increase transcutaneous oxygen levels (Bogie, Wang and Triolo, 2006). To date, no study has directly examined the acute impact of ES using a wearable clothing garment on both micro- and macro-vascular perfusion in people with SCI.

The purpose of this chapter, therefore, was to examine the acute effects of low-intensity ES (involving the gluteal and hamstring muscles) on deep femoral artery blood flow (i.e. supplying the active muscles) and skin microcirculatory perfusion (i.e. covering the active areas). It was hypothesized that an increase in conduit artery and skin blood flow would occur with muscle stimulation, whilst also having a cumulative effect leading to a gradual increase in baseline perfusion with repeated application of ES.

4.2. Methods

4.2.1. Participants

Eight male individuals with ASIA A or B classified SCI participated in this study. All participants were outpatients and frequently visited Reade rehabilitation centre for check-ups with their physician and to participate in sporting activities. All injuries were traumatic in origin and existed for at least 1 year prior to undergoing the study. None of the participants had any known cardiovascular diseases or took any

medication known to interfere with the cardiovascular system. Exclusion criteria included individuals with flaccid paralysis (i.e. inability to activate the muscles through nerve stimulation), a previous history of autonomic dysreflexia during ES (i.e. for safety purposes) and intolerance or contraindication for the use of ES. The local institutional medical ethical board of Reade Rehabilitation centre approved the study and all participants provided written informed consent after receiving and understanding full details of the research study. This study is reported in accordance with the STROBE guidelines and conforms to all items on the checklist accordingly (see supplementary checklist).

Table 4.1 Characteristics of SCI individuals

Subject	Age (yr.)	Level of injury	ASIA score	Time Since Injury (yr.)	Stimulation level
1	40	T9	A	10	75
2	30	C6	A	16	70
3	57	T1	B	15	60
4	54	C6	A	28	85
5	34	T2	A	10	75
6	29	T8	A	9	85
7	60	T8	A	8	70
8	43	C6	A	16	80
Mean	**43**	**-**	**-**	**14**	**75mA**

4.2.2. Electrical stimulation

Electrical stimulation was applied using a specially developed garment with embedded surface electrodes (Axiobionics, Ann Arbor, MI, USA), connected to a portable battery-operated stimulator (Neuropro, Berkelbikes, Nijmegen, The Netherlands). All wires and leads were embedded within the seam of the garment to prevent them becoming entangled with the patient. The ES garment was made from elastic lycra and secured to the body using foldable Velcro straps (figure 4.1). One surface electrode was positioned at the upper (proximal) part of the gluteal muscle and a second about halfway down the hamstring area, preventing the participants from lying directly on the electrodes with their buttocks. Ultrasound gel was placed

in small Velcro pouches to be used as a conductor between the electrodes and the participants' skin. ES was applied to the right leg only at a standard constant voltage of 150V using 50Hz biphasic impulse frequency to induce a visible tetanic contraction. The amplitude needed to induce a strong muscle contraction depends on muscle denervation and the amount of muscle nerve fibres that can be recruited and activated. Due to the variability between individuals, the current amplitude was subjectively determined by the researcher and individualized for each participant with increments of 5 to 10mA to a level that did not cause discomfort or excessive movement to an extend which causes the individual to become unstable when sitting in their wheelchair. To minimize muscle fatigue and ensure continuous muscle contractions, a 1:4 duty cycle, consisting of 1-second stimulation followed by 4 seconds without stimulation for a period of 3 minutes was used (Smit et al, 2013a).

4.2.3. Protocol and testing procedure

Participants attended the laboratory at Reade rehabilitation centre once to undergo testing. Due to sympathetic nervous system activation and the effects on haemodynamics and blood pressure, all participants were asked to refrain from alcohol and caffeine consumption 24 hours prior to testing. On arrival, the protocol and testing procedures were explained in full to each participant. Participants were transferred from their wheelchair to a bed and positioned comfortably in the supine position. Subsequently, the shorts were applied to ensure correct placement of the electrodes. After a 10-minute rest period and before the start of stimulation, baseline measurements were made for oxygen consumption (VO_2), skin blood flow, and deep femoral artery (DFA) blood flow in the control and intervention leg. After baseline measurements, the protocol included four blocks of stimulation lasting 3 minutes interspersed with 17 minutes of no stimulation (figure 4.2). Four blocks of stimulation were used to determine the response and potential benefits of repeated exposure to ES (i.e. a pattern that would be applied in practice). Recordings for all measures were collected 1 minute before and 3 minutes throughout stimulation. Measurements of DFA diameter and blood flow velocity during stimulation were performed in the intervention leg only. Since it was unlikely that ES would alter blood

flow in the contra-lateral, non-stimulated leg (i.e. a systemic effect), blood flow in the non-stimulated leg was not measured.

Figure 4.1 Example of the electrical stimulation shorts and how they are worn

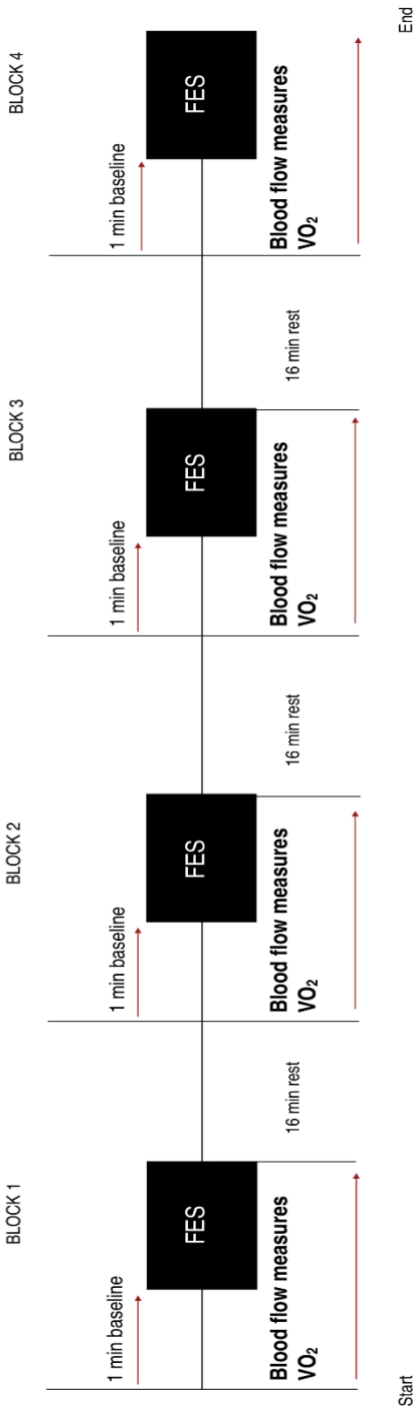


Figure 4.2 Schematic of the stimulation protocol

4.2.4. Experimental Measures

Femoral artery blood flow. Velocity and diameter in the right DFA was measured using a 2-dimensional echo Doppler ultrasound. Using a 10-MHz-multi-frequency linear array probe attached to a high resolution ultrasound machine (T3000, Terason, Aloka, UK), optimal longitudinal B-mode images capturing the lumen/arterial wall interface, along with Doppler velocity measures of the DFA, approximately 2cm from the bifurcation were obtained (Figure 4.3). Following image acquisition, 1 min baseline imaging was performed in the control and intervention leg. The same examiner performed all measurements and images were recorded for later offline analysis.

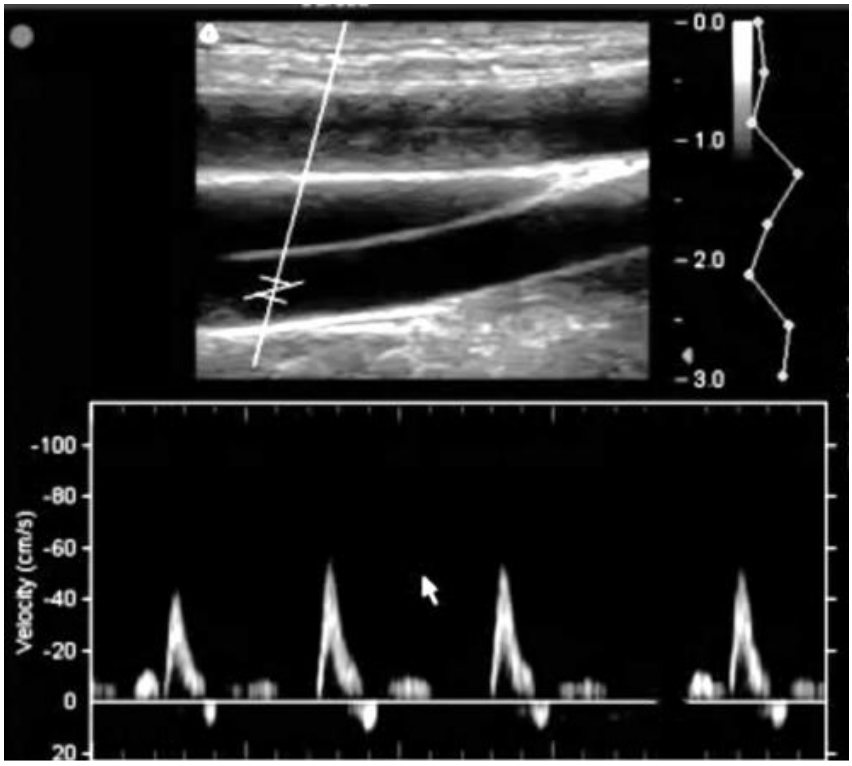


Figure 4.3 Ultrasound image of the deep femoral artery and blood velocity trace

Skin microcirculatory perfusion. Laser Doppler flowmetry (Periflux system 5000, Perimed AB, Järfälla, Stockholm, Sweden) was used to obtain an index of microcirculatory perfusion. This is a non-invasive technique that enables evaluation of skin microvascular blood flow over a period of time and is sensitive at detecting changes in response to a stimulus. The technique uses a beam of laser light that undergoes a change in wave lengths when it detects moving red blood cells. The specific changes in wavelength are characterized by red blood cell concentration and velocity to give a measurement of skin blood flow expressed as arbitrary perfusion units (PU). After the ES shorts had been applied and the participant was comfortably lying in a supine position, the laser Doppler flowmetry probes were placed at the measurement site. Blood flow was continuously measured at the skin covering the gluteal muscle on the stimulated leg. A small incision was made in the shorts to allow placement of the laser Doppler probe in close proximity to the stimulated muscle and to ensure fixation throughout the protocol.

Oxygen consumption. The Oxygen and Carbon dioxide sensors were calibrated with both ambient air and an ά- gravimetric gas (Oxycon Jaeger, Netherlands) which contained 16 % O_2 and 4 % CO_2 with the balance nitrogen. Calibration of the volume transducer was performed with a three-litre syringe (Model 5330, Hans Rudolf, MO, USA), being pumped through the transducer at varying flow rates to match system designated requirements.

4.2.5. Data Analysis

DFA diameter and blood flow. Post-test analysis of the DFA was performed using custom-designed edge-detection and wall tracking software which is largely independent of researcher bias. Thorough details of the analysis technique have been described elsewhere (Thijssen et al, 2009a). Briefly, data collected on the ultrasound machine were stored as a digital avi file. Subsequent software analysis of the data was performed at 30 Hz using an icon-based graphical programming

language and toolkit. The initial phase of analysis required selecting an optimal region of interest (ROI) on the B-mode image, which allowed for automated calibration of artery diameter. Within the ROI, a pixel density algorithm automatically identified the angle corrected near and far wall e-lines. Finally a ROI was drawn around the Doppler waveform and automatically detected the peak of the envelope for this waveform. The mean diameter measure was calculated from within the B-mode ROI and synchronized with the velocity measure which was calculated from the Doppler ROI at 30 Hz. The product of this (artery cross-sectional area and Doppler velocity) gives a measure of average blood flow (mL/s). Previous work have shown that analysis using this semi-automated method produces reproducible diameter calculations that are significantly better than manual methods and producing an intra-observer coefficient of variation of 6.7% (Woodman et al, 2001).

Skin microcirculatory perfusion. Dedicated software (Perisoft for Windows) was used to collect, store and analyze the skin blood flow data. Unwanted artefact in the data due to participant/wire movement was identified and removed from the data prior to analysis. Resting values were calculated by averaging the last 3 minutes of rest before the start of the next stimulation block, whilst perfusion during stimulation was presented as averages every 30-s.

Oxygen consumption. Expired gas fractions were measured to assess the volume of oxygen uptake and CO_2 excretion. The resultant VO_2 (ml/min) values were calculated from the last minute of rest prior to stimulation and during the entire 3 minutes of stimulation.

4.2.6. Statistical Analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences. All data were expressed as means ± SD and statistical significance was set at $P<0.05$. Linear mixed models were used to examine the impact of ES on femoral

artery and skin microcirculatory blood flow (main effect of “stimulation”: baseline *vs* stimulation), but also whether the stimulation-induced changes differed across the 4 blocks of stimulation (main effect for “blocks”). The repeated covariance type was compound symmetry and stimulation, blocks and stimulation*blocks were specified as fixed effects and as estimated marginal means. The test of fixed effects stimulation*blocks interaction was interpreted. Significant main effects of stimulation, blocks and stimulation*blocks interaction were followed up with a simple main effects analysis and the least significant difference (LSD) approach to multiple comparisons.

4.3. Results

Conduit artery. There was a significant main effect of stimulation on DFA blood flow (*P*=0.02). On average, arterial blood flow increased by 18.1% from 0.28 L·min^{-1} at first baseline (pre-intervention) to 0.33 L·min^{-1} during 3 minutes of ES (Figure 4.4). There was also a significant main effect for "blocks" (*P*=0.004), indicating that perfusion at each subsequent baseline and perfusion during stimulation was different across repeated blocks. More specifically, blood flow in block 2 (*P*=0.02), 3 (*P*=0.01) and 4 (*P*<0.001) were all significantly higher than during block 1. There was no stimulation*block interaction (*P*=0.74). To assess changes in arterial blood flow in the control leg, a paired samples t-test was used. Femoral blood flow in the control leg did not change from pre (0.23 ± 0.09 L·min^{-1}) to post-stimulation (0.22 ±0.09 L·min^{-1}; t_3 = 0.97, *P* = 0.41).

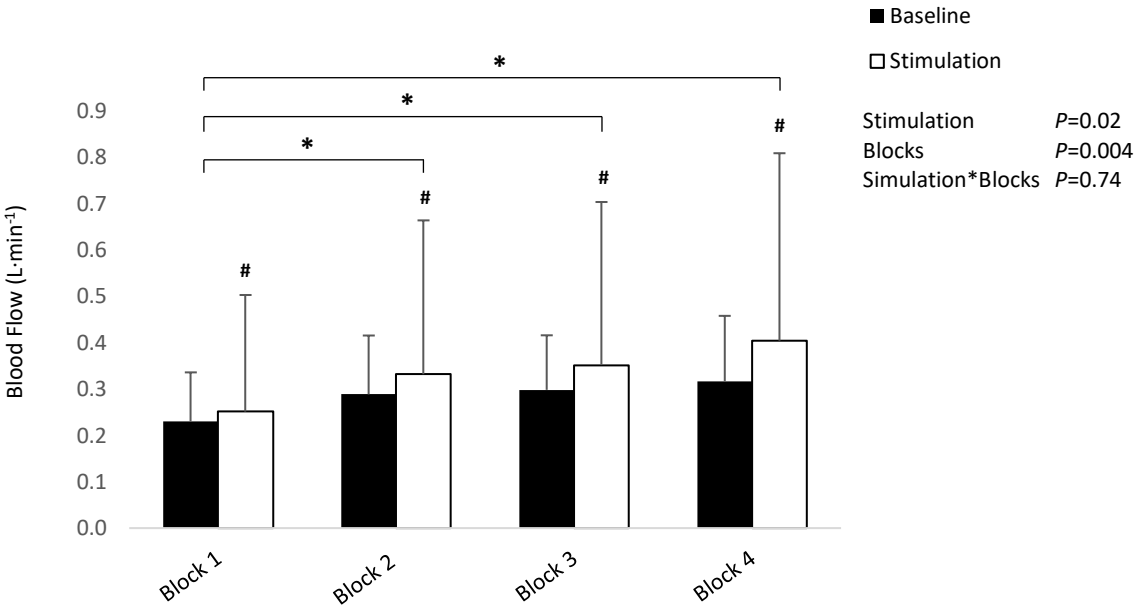


Figure 4.4 Deep femoral artery blood flow at baseline and during stimulation using low-intensity ES in the stimulated leg. Data are presented for each block of stimulation. Error bars represent standard deviations. *P<0.05 vs. Block 1 #P<0.05 vs. Baseline

Skin blood flow. There was no significant main effect for stimulation (Figure 4.5) (*P*=0.66), indicating that there was no immediate change in perfusion with stimulation when compared to baseline. However, perfusion did increase over time with repeated stimulation resulting in a significant main effect for "blocks" (*P*<0.001). Skin blood flow, expressed as perfusion units (PU) significantly increased from block 1 (12±6 PU) to block 2 (17±9 PU; *P*=0.01) and block 3 (22±13 PU; *P*<0.001) and was ~80% higher during block 4 compared to block 1 (22±13 PU; *P*<0.001). Blocks 3 and 4 were also greater than block 2 (*P*=0.004), but plateaued between blocks 3 and 4. There was no stimulation*blocks interaction (*P*=0.99).

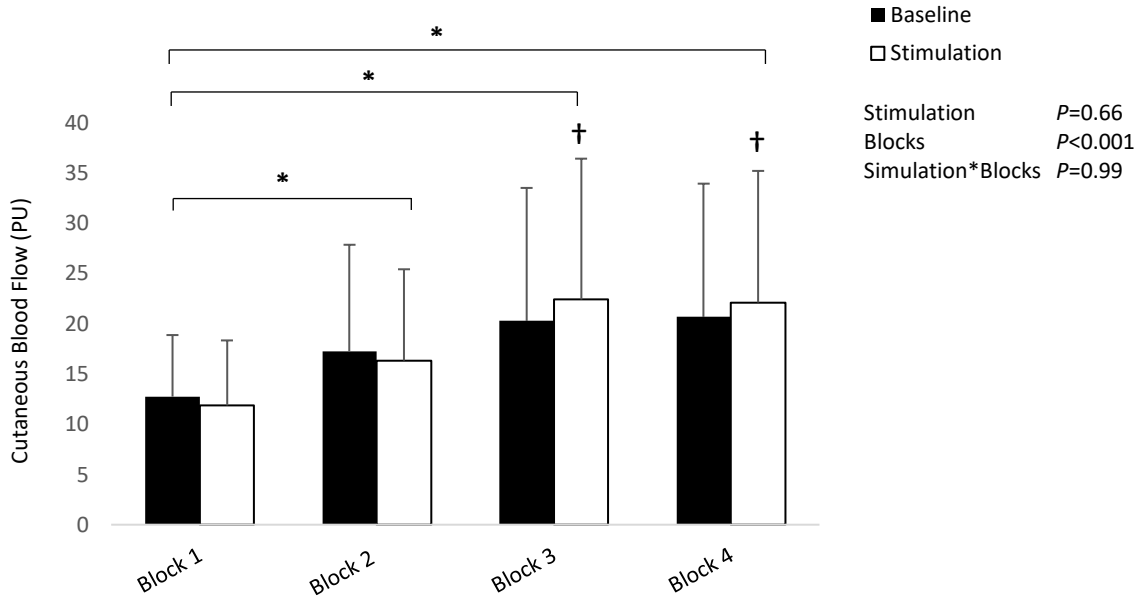


Figure 4.5 Skin blood flow at baseline and during stimulation using low-intensity ES in the stimulated leg. Data are presented for each block of stimulation. Error bars represent standard deviations. *P<0.05 vs. Block 1 †P<0.05 vs. Block 2

Oxygen consumption. Oxygen consumption did not change throughout the stimulation protocol. There was no significant main effect for stimulation (*P*=0.98), the number of stimulation blocks (*P*=0.94) or stimulation* block interaction (*P*=0.87).

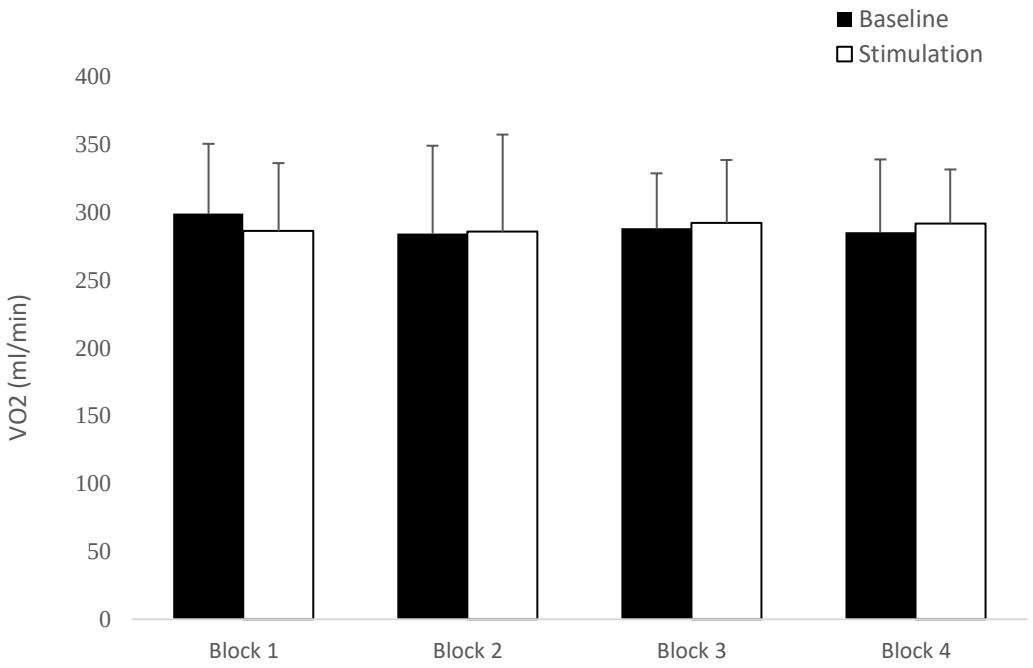


Figure 4.6 Oxygen consumption (VO_2) during baseline and stimulation using low-intensity ES in the stimulated leg. Data are presented for each block of stimulation. Error bars represent standard deviations.

4.4. Discussion

The main finding of this chapter was that a short period (3 minutes) of unilateral ES increased femoral blood flow in the stimulated leg, most likely a direct result of the increased oxygen demand of the activated gluteal muscles. Skin microcirculatory perfusion also increased from pre-intervention baseline, although the response was more gradual and was not evident during the 3-minute stimulation blocks. Additionally, resting femoral artery blood flow and skin perfusion both progressively increased with repeated bouts of stimulation. Collectively, these results indicate that low-intensity ES was effective at inducing haemodynamic changes in the superficial and deep layers of the gluteal region. Since frequent increases in blood flow represent a key stimulus for improvement in micro- and macrovascular function and structure (Green et al, 2017), these observations warrant further research to examine the potential effects of repeated exposure to low-intensity ES on the vasculature in individuals with SCI.

Blood flow in Stimulated Leg: This study is the first to examine conduit artery blood flow and skin microcirculatory perfusion in spinal cord injured following acute application of ES using a wearable clothing garment. As anticipated, the results show an immediate increase in deep femoral blood flow, even when performed using a low-intensity ES protocol. These findings are consistent with previous data from studies in able bodied (Janssen and Hopman, 2003) and individuals with SCI (Scremin et al, 1998). These previous studies observed a 95% increase in blood flow in the femoral artery during ES. Although in the current study, a modest increase of 20% was observed, this difference between studies is most likely attributable to distinct stimulation parameters. Whilst in the current study, only two muscle groups were stimulated using a stimulation level that allowed for muscle contractions without overt limb movement (m=75mA), previous work used whole leg muscle stimulation inducing significant muscle movement and therefore marked oxygen demand of the activated muscles. The co-contractions used in the aforementioned studies are also likely to further increase oxygen demand and contribute to greater arterial inflow and blood distribution throughout the entire limb. Nonetheless, it must be

emphasized that the large muscle stimulation with marked movement can only be applied for ~20 minutes. Muscle fatigue and energy source depletion prevents longer duration stimulation, whereas low-intensity ES can be applied throughout the day and night and on a day-to-day basis. Although the protocol used only increased blood flow by ~20%, the ability for prolonged exposure to low-intensity ES in individuals with SCI make the ES-protocol applied in the present study a physiologically significant and potentially clinically relevant stimulus.

An important question relates to the mechanisms responsible for the increase in perfusion. Since the current study found no changes in DFA blood flow in the non-stimulated leg, the possibility of systemic stimuli affecting perfusion (e.g. blood pressure) can be excluded. During muscular contractions, a number of mechanisms are known to regulate arterial blood flow supplying the active muscles. Firstly, an increase in cell metabolism initiates the localized release of vasodilator metabolites such as NO, prostacyclin, (adenosine triphosphate) ATP, adenosine and potassium from contracting skeletal muscle and the vascular endothelium (Clifford and Hellsten, 2004; Hellsten et al, 2012). The release of such compounds initiates vascular smooth muscle relaxation, vasodilation of the artery and a subsequent increase in blood flow to the stimulated region. During exercise, skeletal muscle blood flow increases in proportion with metabolic activity to meet the oxygen demands of the contracting muscle (Andersen and Saltin, 1985). Considering the direct relationship between skeletal muscle blood flow and metabolic load, it seems sensible to assume that the small, albeit significant, increase in arterial blood flow is due to the low-intensity stimulation protocol used.

Another physiological impact of low-intensity ES must be considered. The dynamic and mechanical effect of muscle contractions and relaxations, or the ‘muscle pump’ mechanism, importantly influences blood flow in the vasculature. During muscle contraction, a decrease in venous pressure occurs as venous blood empties from peripheral areas (i.e. the legs) and is propelled to the central circulation (Folkow, Gaskell and Waaler, 1970; Tschakovsky, Shoemaker and Hughson, 1996). The

emptying of venous segments leads to an increase in arteriovenous pressure gradient facilitating an increase in arterial inflow as the muscle relaxes (Pollack and Wood, 1949; Tschakovsky, Shoemaker and Hughson, 1996). Although this study did not differentiate changes in blood flow during the contraction and relaxation phases of muscle stimulation, the increase in arterial blood flow may, at least partially, be explained through increased muscle pump activity and increases in the arteriovenous pressure gradient.

Microcirculatory Perfusion: Changes in skin microcirculation occur as a reflex thermoregulatory control mechanism during whole body and/or localized changes in temperature (Johnson and Kellogg, 2010). In the current study, little change in skin perfusion during an individual block of stimulation was observed. However, the combined effect of consecutive and repeated exposure to stimulation did result in a successive rise in skin perfusion over the duration of the protocol. Considering there was no change in whole body VO_2, it is unlikely that an increase in core body temperature could explain the progressive rise in skin perfusion. A more likely explanation relates to localized heat production and a subsequent gradual warming of the skin covering the activated muscles. This would result in a sustained rise in skin blood flow during localized heating which, is mediated through the release of NO from the vascular endothelium (Minson, Berry and Joyner, 2001). Regardless of any change in skin temperature, previous work confirms a NO mediated increase in skin perfusion in response to ES (Petrofsky et al, 2007). Petrofsky and colleagues observed an increase in skin blood flow during ES that was prevented with the infusion of L-NAME, a NO inhibitor. Although the current study nor Petrofsky *et al.* controlled for potential changes in skin temperature, its contribution to the gradual rise in skin perfusion should not be excluded. Future research should consider the exact mechanisms involved in the increase in skin perfusion during ES

Oxygen Consumption: There was no change in VO_2 during the stimulation protocol, which is in contrast to other studies using ES whilst sitting or lying (Janssen and

Hopman, 2003; Hsu, Wei and Chang, 2011). In the current study, only two muscle groups were stimulated using low level ES for 3 minutes. Given the increase in blood flow, it seems logical that energy expenditure in these muscles increased. However, energy expenditure has previously been shown to increase in a dose response relationship with stimulation intensity and the number of muscles stimulated. The small dose of stimulation adopted in the current protocol may be insufficient to detect a significant increase in whole body VO_2. Indeed, previous work that reported higher oxygen consumption upon ES adopted higher stimulation (100 mA and 93 mA), but also stimulated a larger muscle mass (Janssen and Hopman, 2003; Hsu, Wei and Chang, 2011). These previous studies confirm that ES has the potential to increase VO_2 and energy expenditure, which is indirectly supported by the current observation of increased perfusion, and therefore oxygen delivery to the large muscle mass in the legs and gluteal region. One should also consider that changes in oxygen consumption in the current study (involving unilateral ES) may increase exponentially more when ES is applied in a clinical situation using bilateral stimulation.

4.4.1. Study Limitations

The small sample size used in the current study may overestimate the true effect of stimulation on vascular perfusion. That said, the data presented in this study clearly show a distinctive increase in perfusion with ES suggesting that the results are representative of the wider spinal cord injured population. Secondly, due to equipment failure, skin blood flow measures in the contralateral, unstimulated leg were unobtainable. However, the low intensity stimulation protocol used was unlikely to induce any systemic effects on cutaneous perfusion. This is supported by the absent changes in the deep femoral artery in the contra-lateral, non-stimulated leg. Finally, ES induced autonomic dysreflexia is a potential side effect that may limit its usage in some individuals. Although this was an exclusion criterion in the current study, it has previously been reported to occur at higher current amplitudes (160mA) during ES-assisted hydraulic resistance training exercise.(Ashley et al, 1993) Blood

pressure monitoring is therefore recommended for novice users.

4.4.2. Conclusion

In conclusion, this chapter clearly shows an increase in superficial and deep vascular perfusion during low level ES. A ~20% increase in blood flow occurred through the deep femoral artery supplying the gluteal muscles, most likely through local increased oxygen demand and muscle pump activation. The results also show a gradual and consistent increase in skin perfusion over the duration of the protocol. This may represent a potent stimulus when this type of low-intensity ES is applied for several hours. Future work is required to ascertain whether such physiological changes translate to clinically relevant effects, as well as the feasibility of low-level ES for home-based, day-to-day use.

Chapter 5

Effects of 12 weeks low-level gluteal and hamstring electrical stimulation on conduit artery structure, microvascular function and risk factors for pressure ulcers in spinal cord injury: A pilot study

5.1. Introduction

A motor complete spinal cord injury (SCI) results in the immediate loss of voluntary muscle contractions and paralysis below the spinal cord lesion. Individuals with a SCI are subject to a lifetime of sedentary behaviour and a myriad of complications, which have profound consequences for psychological and physiological health (Liang et al, 2007; Migliorini, Tonge and Taleporos, 2008; Savic et al, 2018). The (re)occurrence of pressure ulcers is one of the most frequently observed and extremely concerning complications amongst spinal cord injured individuals. In the United Kingdom, the average cost to treat one stage 4 pressure ulcer is estimated at £14,108 (Dealey, Posnett and Walker, 2012). Considering that 85% of the spinal cord injured population will develop at least one pressure wound at some stage in their life (Center, 2005; Haisma et al, 2007), health and social care systems are exposed to a significant cost burden. This emphasises the need for immediate and effective risk reducing interventions for the development of pressure ulcers for individuals with SCI.

Physical deconditioning resulting from lower limb paralysis causes changes in micro- and macro-vascular structure and function which, when combined with prolonged sitting pressure and loss of muscle mass covering bony structures, contributes to subcutaneous tissue ischemia and the development of pressure ulcers (Mawson et al, 1993b; Cragg et al, 2013; Osterthun et al, 2014; Savic et al, 2017a). Reactivating the paralyzed muscles by inducing electrically stimulated muscle contractions (partly) reverses many of these complications in spinal cord injured individuals. For example, FES is found to increase artery dimeter (Thijssen et al, 2005), improve arterial compliance (de Groot et al, 2005), restore contractility in denervated muscle fibres and increase muscle mass (Dudley et al, 1999; Kern et al, 2010), improve muscle oxidative capacity (Erickson et al, 2016), enhance capillary supply (Chilibeck et al, 1999b), increase skin epidermis thickness (Albertin et al, 2018a; Albertin et al, 2018b)

and improve cutaneous microcirculation (Barton et al, 2018). Collectively, FES is an effective and clinically significant tool for improving the health of individuals with SCI.

Despite the many reported benefits, the need for specialist equipment, facilities and trained staff for FES interventions make regular application expensive and impractical for many individuals with SCI. In an attempt to overcome such limitations, wearable clothing garments with embedded surface electrodes to provide long-term, low-intensity muscle activation have been introduced by researchers in Canada (Ahmetovic et al, 2015; Topfer LA, 2015). This type of ES activates muscle tissue, but without the concomitant joint movement as is the case with FES, and has the advantage to be applied for prolonged periods. Using an acute bout of ES, this method has demonstrated many important benefits relevant to pressure wound prevention, such as reducing sitting pressure over the ischial tuberosity and an increase in transcutaneous oxygen levels (Bogie, Wang and Triolo, 2006; Smit et al, 2013a). Additionally, the work described in *Chapter 4* showed that applying acute bouts of low intensity ES using a wearable clothing garment leads to incremental increases in blood flow in the conduit and skin microcirculation, an effect that is sustained in the minutes after ES. Currently, however, no study has examined the effects of long-term, daily home based low intensity ES in individuals with SCI.

The purpose of this study was to examine the effects of 12 weeks, daily gluteal and hamstring ES using a wearable clothing garment on skin (i.e. covering gluteal and hamstring muscles) and conduit artery (i.e. femoral) vascular function, limb volume and sitting pressure in people with SCI. Based on previous experimental work in *Chapter 4*, it is expected that the cumulative effects of 12 weeks, daily ES would result in an increase in femoral artery diameter, improved skin vasculature function, increased limb volume and reduced sitting pressure in areas at risk of pressure wounds. Secondly, adherence and practicality of using a wearable clothing garment for the day-to-day, home-based application of ES for individuals with SCI will be assessed. It is hypothesised that the shorts will be a feasible method for day-to-day application of ES without interfering with daily activities.

5.2. Methods

5.2.1. Participants

Twelve middle aged male (n=10) and female (n=2) individuals (48±14 y) with ASIA A-C classified SCI were recruited to participate in this study. One injury was the result of a non-traumatic thoracic aortic aneurysm and the rest from accidents that caused direct trauma to the spinal cord. Time since injury was at least 1 year (7.1±7.8 y) prior to the study and none of the participants had any known cardiovascular diseases. Participants were not taking any vasoactive medications or supplements and participants were excluded based on having flaccid paralysis (inability to activate the muscles with ES) and a history of autonomic dysreflexia or any other intolerances or contraindications to ES. All procedures conformed to the Declaration of Helsinki, and after receiving and understanding full details of the research study, all participants provided written informed consent. The study was approved by local institutional medical ethical board of Reade rehabilitation centre.

Table 5.1 Participant characteristics

Participant	Sex M/F	Age	Lesion Level	ASIA Score	Time since injury (yrs.)	12 wk mean Stimulation (mA)
1	M	64	L1	C	4	80
2	M	49	T7	A	3	95
3	M	63	T4	A	3	85
4	F	58	C6	A	4	100
5	M	28	T12	A	4	80
6	M	47	T8	B	1	90
7	M	29	T3	A	2	90
8	M	34	C5	A	19	100
9	M	62	C4	A	1	105

ASIA = American Spinal Injury Association; Lesion level C= cervical, T= thoracic, L= lumbar

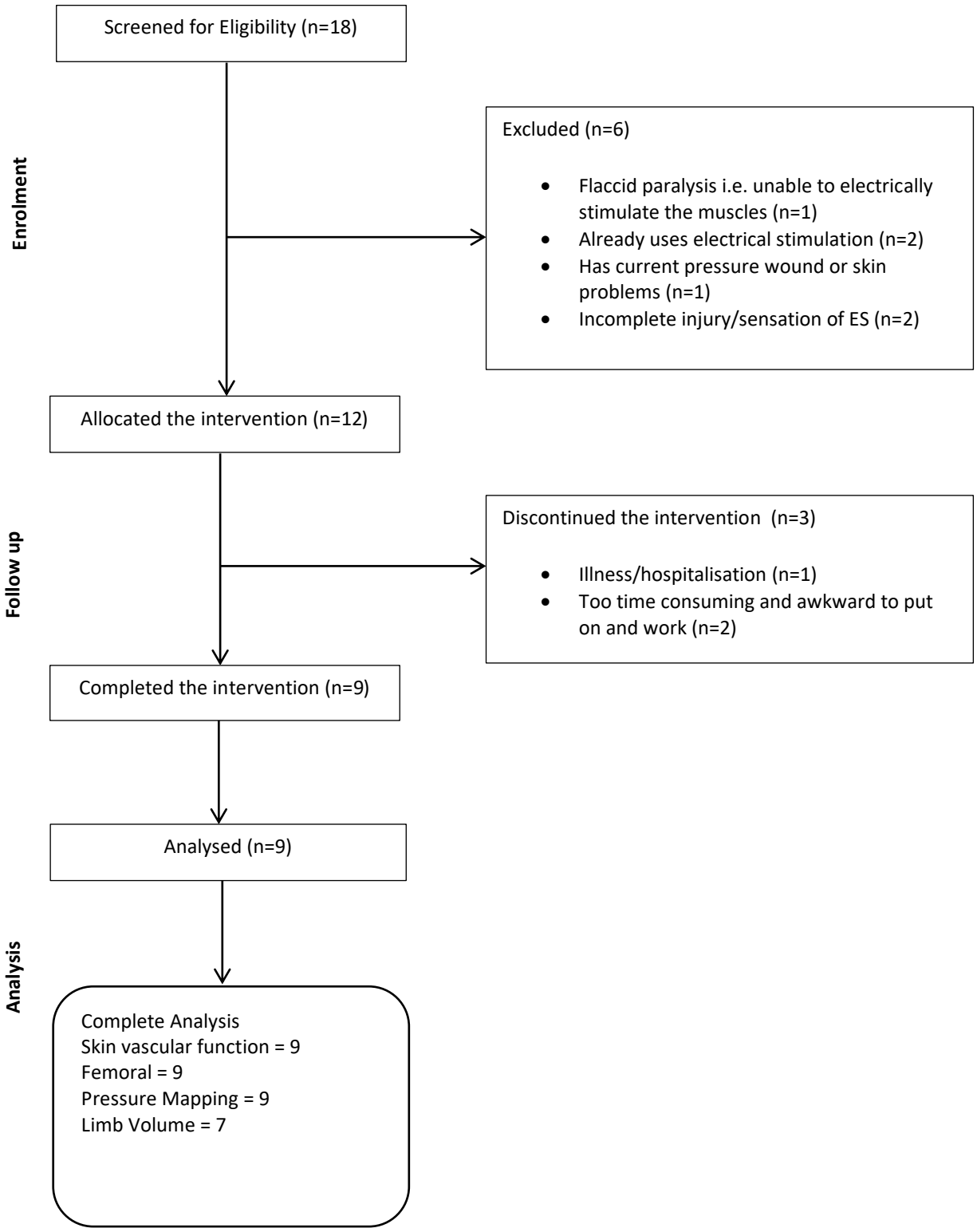
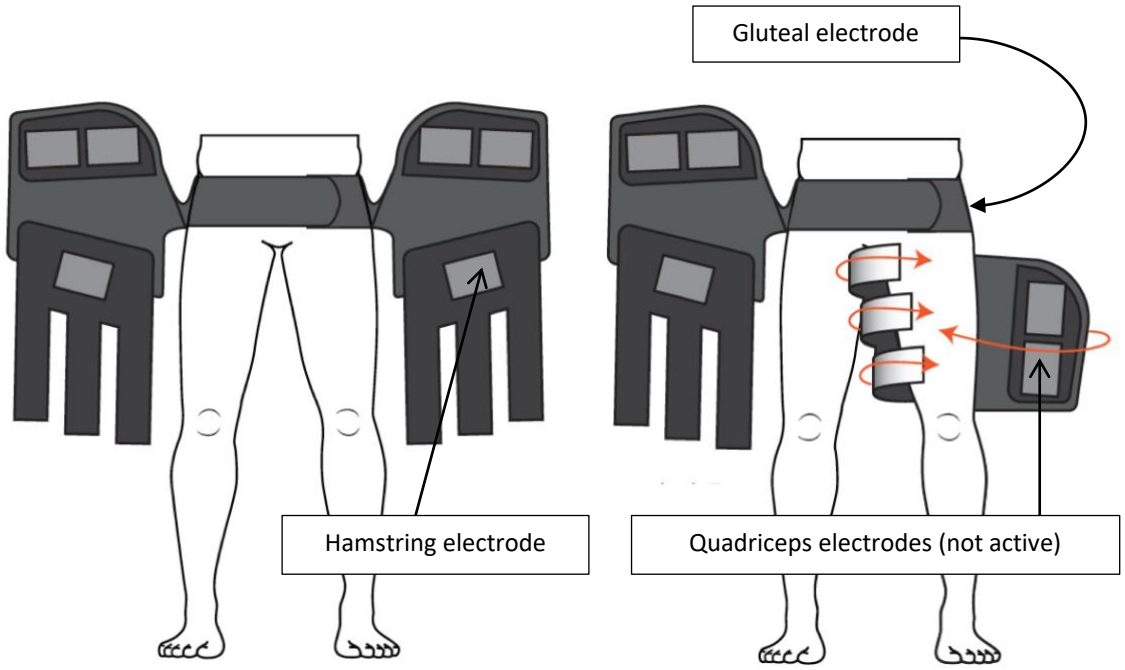


Figure 5.1 CONSORT diagram showing the flow of participants through each stage of the intervention trial

5.2.2. Electrical Stimulation Intervention

Electrical stimulation was applied with an adapted and improved version of a previously used wearable clothing garment (Barton et al 2018) connected to a portable battery operated stimulator (Neuropro, Berkelbikes, Nijmegen, the Netherlands). The clothing garment was a pair of specially developed elasticated velcro lycra shorts with embedded surface electrodes (Axiobionics, Ann Arbor, MI). The foldable velcro design (Figure 5.2) allows the user to independently don the shorts and ensures efficient electrode placement when otherwise might be difficult (i.e. to the backside of the body). All leads and wires were readily connected to each electrode and stowed inside the seam of the shorts to prevent them becoming entangled and to avoid accidental disconnection of individual electrodes. The shorts were applied so that one surface electrode was positioned at the upper part of the gluteal muscle over the motor point located ~2.5 cm above the ischial tuberosity on both buttocks and the other surface electrode placed approximately mid hamstring. Electrical stimulation was applied to both limbs at a standard constant voltage of 150V using 50-Hz biphasic impulse frequency to elicit a strong visible tetanic contraction. The stimulation amplitude was individualised for each participant (preventing movement of the limbs) and progressively increased every 3 weeks throughout the 12-week intervention. This minimised muscular fatigue in the early stages and to compensate for any training adaptations in the latter stages of the intervention. The complete stimulation protocol ran for 6 hours and comprised of blocks of 30 minutes stimulation followed by 15 minutes rest. A duty cycle consisting of 4 seconds rest for every 1-second contraction was used as per previous studies (Smit et al, 2012; Smit et al, 2013a). This particular protocol allows sufficient recovery time between contractions and minimises muscle fatigue. A 2-second ramp up and ramp down before and after maximal amplitude was also used for each muscle contraction to ensure a smooth, comfortable contraction for the user. For the 12-week duration, each participant was instructed to use the shorts as much as possible, during the day and/or night but for a minimum of 6 hours every day and to record their daily usage using a diary provided by the research team.

A



B

Figure 5.2 A) Electrical Stimulation shorts depicting how they are applied and where each of the electrodes are located including one hamstring electrode, two quadriceps electrodes (which were not active in this study) and one gluteal electrode (located inside the fastened waist belt) B) Front side image of the ES shorts with (left) and without (right) the attached NeuroPro electrical stimulator.

5.2.3. Experimental Measures

All participants visited Reade Rehabilitation Centre on 3 occasions. The initial visit was prior to starting the study and used to administer electrical stimulation to ensure all inclusion criteria were met and to explain the study in detail. The following two visits were before and after the 12-week intervention and consisted of each participant undergoing all experimental measures. Prior to arrival, all participants refrained from consuming alcohol and caffeine for at least 24 hours to minimise any external influences on the sympathetic nervous system and changes in haemodynamics. All experimental measures were performed in the same order and at same time of day pre and post intervention to reduce any circadian influences on vascular function.

Femoral Artery Diameter and Blood Flow: The participant was transferred from their wheelchair and comfortably placed onto a bed in the supine position. After a 20-min resting period, a 10-MHz multi-frequency linear array probe, attached to a high-resolution ultrasound machine (T3000, Terason, Burlington, MA) was used to image the common femoral artery (CFA) 2 cm proximal to femoral bifurcation. Once the sonographer (TB) had obtained an optimal longitudinal B-mode image, the probe was held stable and the ultrasound parameters were set to optimize the image of the lumen-arterial wall interface. Continuous Doppler velocity assessments were recorded for 1 minute using the lowest possible insonation angle (always <60°) for later offline analysis.

Skin Vascular Function: Laser Doppler flowmetry (LDF) was used to examine skin vascular function in response to a localised incremental heating protocol. This is a non-invasive technique that is sensitive at detecting changes in cutaneous perfusion. LDF uses the reflection of a laser beam which detects changes in the movement of red blood cells and undergoes a change in wavelength specific to the concentration and velocity of the red blood cells. Before beginning the local heating protocol and with the participant comfortably lying in a prone or partial prone/side position, two heating disks (Perimed 355, Perimed AB, Järfälla, Stockholm, Sweden) were

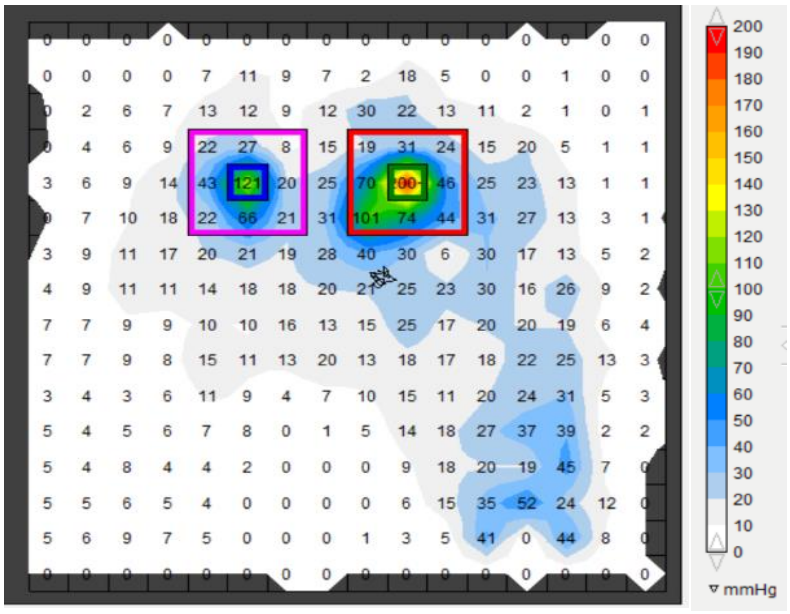
instrumented on the gluteal and hamstring and carefully placed in the same anatomical position as the electrodes in the clothing garment. A 7-laser array probe (PF 413, Perimed AB Järfälla, Stockholm, Sweden) was placed into each heater and securely attached to the skin using adhesive stickers. The heating discs were connected to a heating unit (PeriTemp 4005 heater, Perimed AB, Järfälla, Stockholm, Sweden) and manually set to 33°C for a 20-minute baseline acclimation period. Baseline cutaneous blood flow was then measured as red blood cell flux (RBCF) using a laser Doppler flowmeter (Periflux system 5000, Perimed AB, Järfälla, Stockholm, Sweden) for 10-minutes at 33°C. Subsequently, local skin temperature was increased by 1°C per 5minutes up to 42°C (Black, Green and Cable, 2008). Thereafter, the heating probes were held at 42°C for 30-minutes and finally at 44°C for a remaining 20-minutes to examine maximal cutaneous blood flow (Charkoudian, 2003; Cracowski et al, 2006). Heart rate and mean arterial pressure (MAP) were recorded using an automated sphygmomanometer at 5-minute intervals throughout the protocol. This particular slow heating protocol induces a response that minimises axon reflexes to changes in local temperature and assesses microvascular function using vasodilator pathways that are largely NO mediated (Minson, Berry and Joyner, 2001; Houghton et al, 2006).

Limb Volume: Thigh muscle volume was estimated based on a calculation using anthropometric measures of leg circumferences, leg length and skin folds. This approach has previously been validated against the gold standard H-MRI as a reliable and accurate method of measuring skeletal muscle volume in people with SCI (Layec et al, 2014). All measurements were taken by the same experimenter (TB) with the participant lying on a bed in the supine position with some repositioning when required. Thigh circumferences were measured to the nearest 1 mm at three sites: at the gluteal furrow (proximal), one third of the sub-ischial height up from the tibia-femoral joint space (middle) and the minimum circumference above the knee (distal). The length of the leg was measured to the nearest 1 mm from the greater trochanter to the lateral femoral epicondyle and skinfold thickness was measured using skin callipers at three sites (medial, anterior & lateral) at the midpoint of the thigh. All

assessment sites were marked on the skin with a demographic pencil and digital photographs taken as a reference to ensure accurate re-assessment post intervention.

Sitting Pressure: Interface sitting pressure was measured using a force sensitive array (FSA, Vista Medical, Vancouver, Canada) pressure-mapping device. The device was a 42 x 42 cm, 2mm thick, soft flexible mat consisting of 256 pressure sensors (1.82 cm^2 per sensor) each measuring 0-200 mmHg. Each participant was transferred from their wheelchair to a standardized table chair and sat in a normal position, with feet flat on the floor, a 90° bend at the knee arms rested on the lap and lower back against the backrest and remained seated on the FSA for 2 minutes whist the values were recorded. The ischial tuberosities were defined from the FSA profile as the 3x3 sensors with the highest pressure values (Figure 5.3). The nine sensors were averaged to give a mean IT pressure value for the left and right IT area. Pressure gradient, which is indicative of shear forces within the tissue and is associated with the risk of developing pressure ulcers (Liu et al, 2006; van Londen et al, 2008) was calculated by subtracting the average of the 16 surrounding sensor values from the mean IT pressure.

A



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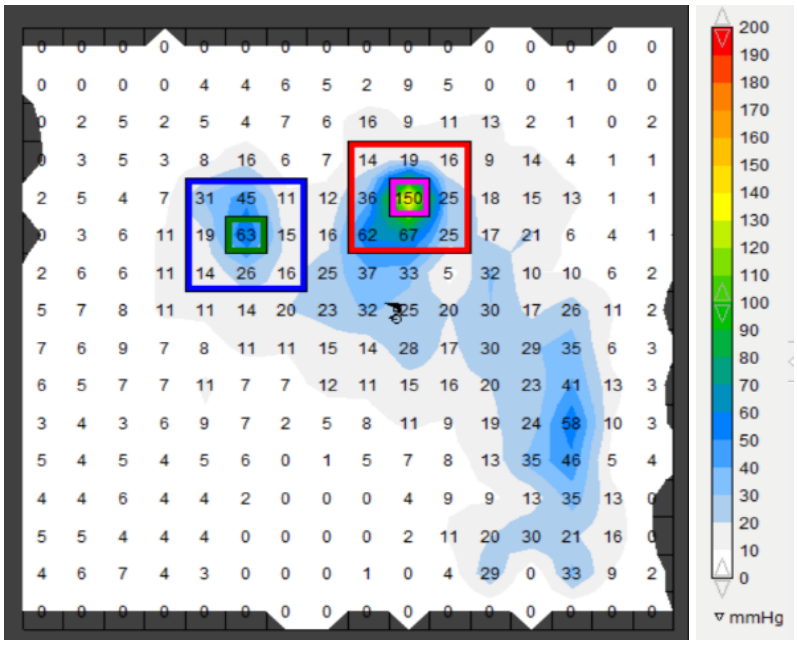


Figure 5.3 Force sensitive array frame of sitting pressure distribution before (A) and after (B) the three-month gluteal and hamstring ES intervention for one individual participant. The highlighted squares are used to determine the peak value (A. Blue and green and B. green and pink) and mean IT pressure (A. purple and red B. blue and red 3x3 squares) of ischial tuberosities. Pressure gradient was calculated as the average of the 16 sensors surrounding the 3x3 square subtracted from the mean IT pressure

5.2.4. Data Analysis

Femoral Artery Diameter and Blood Flow: Femoral artery diameter and velocity recordings for pre and post intervention were analysed using custom-designed edge detection and wall tracking software which is largely independent of researcher bias (Woodman et al, 2001). Image analysis initially involved the identification of regions of interest (ROI) which allowed the automated calibration for diameters on the B-mode image and velocities and on the Doppler strip. The mean diameter derived from the B-mode ROI was synchronized with the velocity measure derived from the Doppler ROI at 30 Hz which allowed for the calculation of mean blood flow averaged over a 1-minute period (the product of cross-sectional area and Doppler velocity). This semi-automated method tracks blood flow over time, as opposed to using cardiac cycles in order to obtain a mean value. It has proven to reproduce diameter and blood flow calculations that are significantly more accurate than manual methods by reducing observer error (Woodman et al, 2001).

Skin Vascular Function: Red blood cell flux was collected and stored using dedicated software (PeriSoft for Windows, Perimed AB, Järfälla, Stockholm, Sweden) and was subsequently averaged every 30 seconds and exported to Excel. Cutaneous RBCF was expressed as cutaneous vascular conductance using Mean Arterial Blood Pressure (CVC=RBCF/MAP) to account for potential changes in skin blood flow resulting from variations in blood pressure. Unwanted artefact in the data resulting from subject movement was identified and carefully removed before analysis. Baseline CVC was averaged during the 10-minute stable baseline recording. Subsequently, CVC was calculated over a stable 60s period for the final minute of each local temperature increment or each local temperature increment and over the last 5 minutes of each plateau phase at 42°C and 44°C. All data at each incremental phase were normalised to the maximal CVC at 44°C ($\%CVC_{max} = [CVC/CVC_{max}]\ 100$).

Limb Volume: The following formula was used to calculate limb volume:(Jones and Pearson, 1969; Layec et al, 2014) $V= (L/12\pi) \cdot (C1^2+C2^2+C3^2) - [(S-0.4)/2] \cdot L \cdot$

[(C1+C2+C3)/3] where L signifies the length; C1, C2 and C3 refer to proximal, middle and distal circumferences, respectively; and S is skinfold thickness of the thigh. When compared to H-MRI, anthropometric calculation has demonstrated to systematically overestimate muscle volume. Therefore, an error correction value of $0.866 \cdot Vol_{anthropo}$ -1750 was applied (Layec et al, 2014)

5.2.5. Statistical Analysis

Statistical analysis were performed using SPSS 25.0 computer based software (SPSS Inc, Chicago, IL, USA). Data were expressed as mean ± SD and statistical significance was set at $P < 0.05$. The effects of 12 weeks ES on gluteal and hamstring skin vascular function was examined using two-way ANOVA with repeated measures (main effects for time (of the heating protocol) and intervention (pre and post)). Paired *t*-tests were used to assess differences in sitting pressure, limb volume, femoral artery diameter and femoral artery resting blood flow before and after the 12-week ES intervention. All data were tested for normal distribution and Wilcoxon signed rank test was used where appropriate. Pearsons correlation was performed to determine the association between changes in thigh circumference and basal femoral artery blood flow after 12 weeks ES. Effect sizes were calculated using Cohen's d formula.

5.3. Results

Average Daily Time of ES During the 12 Weeks: Participants indicated via self-report diary that the shorts were worn for an average of 7 hours per 24-hour daily cycle. For each 7 hours the electrical stimulator was active, a total of nine 30-minute cycles of ES were administered to the gluteal and hamstring muscles.

Femoral Artery Diameter and Blood Flow: Resting diameter of the CFA increased significantly by 8.3% from pre (0.73 ± 0.20 cm) to post 12 weeks ES training (0.79 ± 0.22 cm; t_8 = -6.06 , P <0.001; d = 0.32). Mean baseline CFA blood flow significantly increased from pre (0.28 ± 0.12 L · min^{-1}) to post 12 weeks ES (0.40 ± 0.15 L · min^{-1}; t_8 = -8.78, P <0.001; d = 0.91).

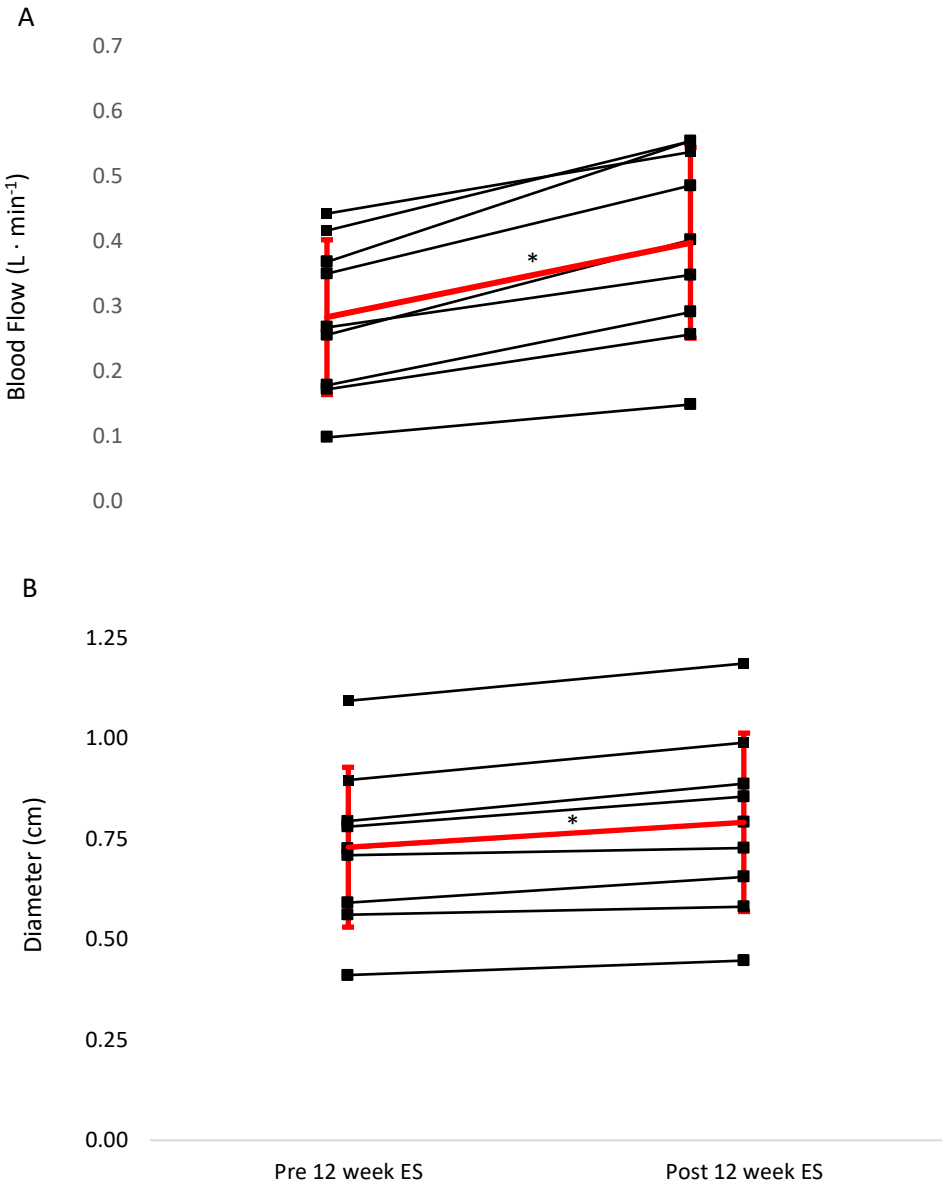


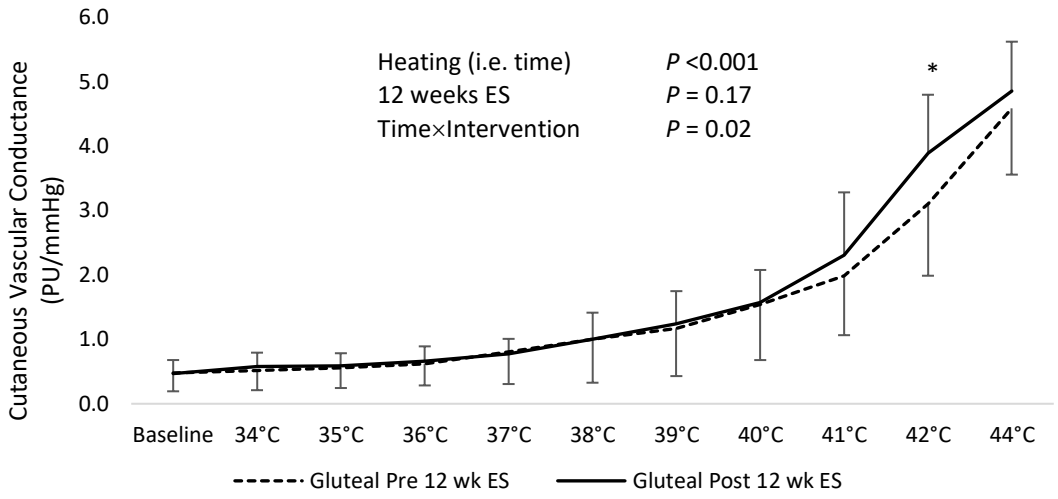
Figure 5.4 A) Individual and group mean common femoral artery resting blood flow and B) resting diameter pre and post 12-week electrical stimulation in six participants. The red line depicts overall mean and error bars represent standard deviation. *Main effect of 12 weeks of electrical stimulation (P = <0.05)

Skin Vascular Function: There was no difference in MAP from pre to post 12 weeks ES (P = 0.21). Local heating induced a gradual, slow heating response (main effect for time for both gluteal and hamstring skin blood flow, P <0.001) with no detectable axon reflex induced peaks or nadirs (Figure 5.5).

There was no difference in the gluteal skin blood flow response to local heating when expressed as either absolute flux (P = 0.23), absolute CVC (P = 0.17; Figure 5.5A) or percentage of maximum CVC (%CVC_{max}, P = 0.19) after 12 weeks of ES. There was no time×intervention-interaction for absolute flux (P = 0.12). However, there was a significant time×intervention interaction for absolute CVC (P = 0.02) and %CVC_{max} (P <0.001) indicating that the change in skin blood flow during local heating from baseline to maximal levels was higher after the 12 weeks of ES. Furthermore, when the individual stages at higher local temperatures were examined, there was a significant increase in absolute CVC (0.01) at 42°C after 12 weeks of ES, but this was not evident at 39°C (P = 0.60), 40°C (P = 0.87), 41°C (P = 0.08) and 44°C (P = 0.30; Table 5.2).

There was no significant difference in hamstring skin absolute flux (P = 0.85), absolute CVC (P = 0.47; Figure 5.5B) or %CVC_{max} (P = 0.41) after 12 weeks of ES. There was also no significant time×intervention-interaction for absolute flux (P = 0.99), absolute CVC (P =0.86), or %CVC_{max} (P = 0.41). No significant effect of ES was observed at local temperatures of 39-44°C (Table 5.2).

A



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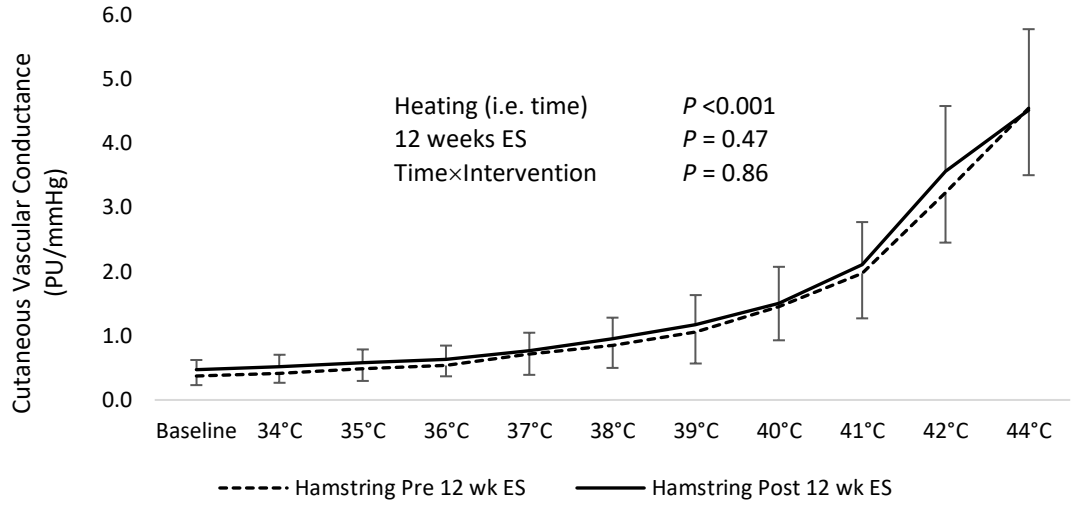

Figure 5.5 Cutaneous vascular conductance (CVC) responses across time points from baseline at 33°C to maximal plateau at 44°C of **A.** the gluteal and **B.** the hamstring before and after 12 weeks of electrical stimulation. Data are presented as means and error bars standard deviation. *Significant interaction

Table 5.2 Gluteal and hamstring CVC at baseline and during local heating at 39, 40, 41, 42 and 44 °C pre and post (and delta change data) 12 weeks ES. Data are means ± SD. *Main effect of 12 weeks ES

	Gluteal				Hamstring			
	Pre	Post	*Δ*	*P* value	Pre	Post	*Δ*	*P* value
Baseline	0.48 ± 0.28	0.47 ± 0.21	0.01	0.87	0.37 ± 0.14	0.47 ± 0.15	0.10	0.09
39°C	1.17 ± 0.74	1.24 ± 0.51	0.07	0.60	1.06 ± 0.49	1.17 ± 0.47	0.11	0.56
40°C	1.55 ± 0.87	1.58 ± 0.50	0.03	0.86	1.45 ± 0.52	1.50 ± 0.57	0.05	0.80
41°C	1.98 ± 0.92	2.31 ± 0.97	0.35	0.08	1.97 ± 0.70	2.10 ± 0.67	0.13	0.59
42°C	3.10 ± 1.11	3.89 ± 0.91	0.79	0.01*	3.23 ± 0.78	3.56 ± 1.02	0.33	0.23
44°C	4.57 ± 1.03	4.92 ± 0.77	0.35	0.30	4.54 ± 1.04	4.51 ± 1.26	0.03	0.92

Sitting Pressure: Peak pressure, which refers to the value of the sensor with the highest pressure within the IT area (Figure 5.3), significantly decreased by 32 ± 23 mmHg (t_8 = 4.14; P = 0.003; d = 0.98; Figure 5.6) with 12 weeks of ES. Interface pressure reduced by 19% from 37 ± 9 mmHg to 30 ± 6 mmHg, although this did not reach statistical significance (t_8 = 2.23; P = 0.06; d = 0.98; Figure 5.7). Pressure gradient significantly decreased from 23 ± 7 mmHg to 16 ± 6 mmHg with 12 weeks of ES (t_8 = 3.61; P = 0.007; d = 1.0; Figure 5.8).

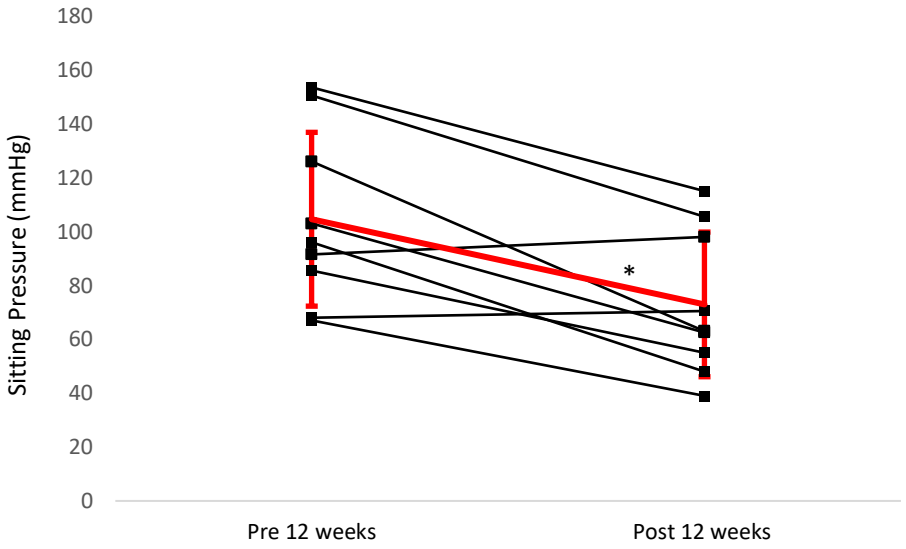

Figure 5.6 Individual and group mean peak pressure of a single sensor in the IT area before and after 12 weeks of electrical stimulation. Red bar indicates group mean and error bars represent SD. *Main effect of 12 weeks of electrical stimulation (P = <0.05)

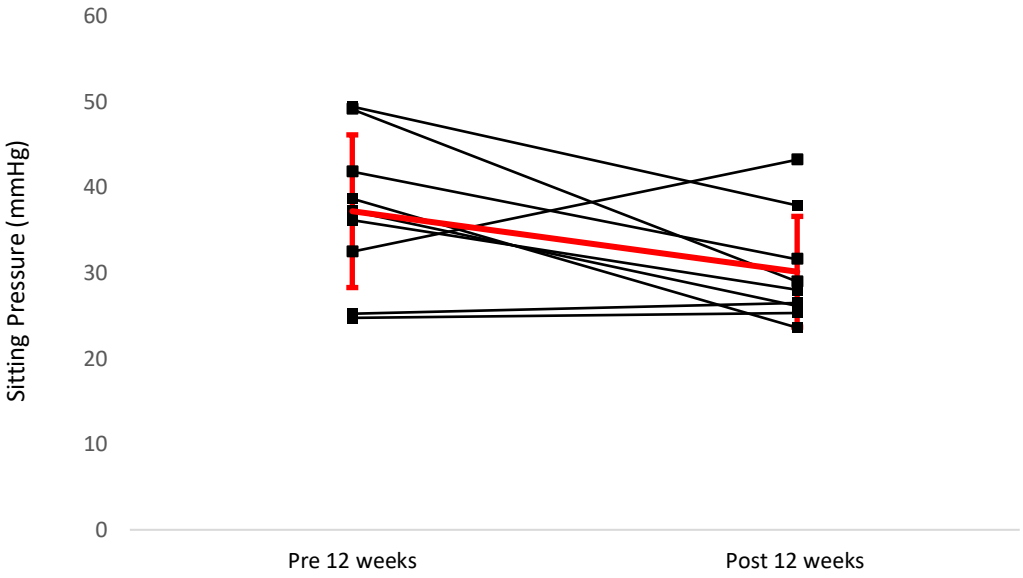

Figure 5.7 Individual and group mean average IT pressure before and after 12 weeks of electrical stimulation. Red bar indicates group mean and error bars represent SD.

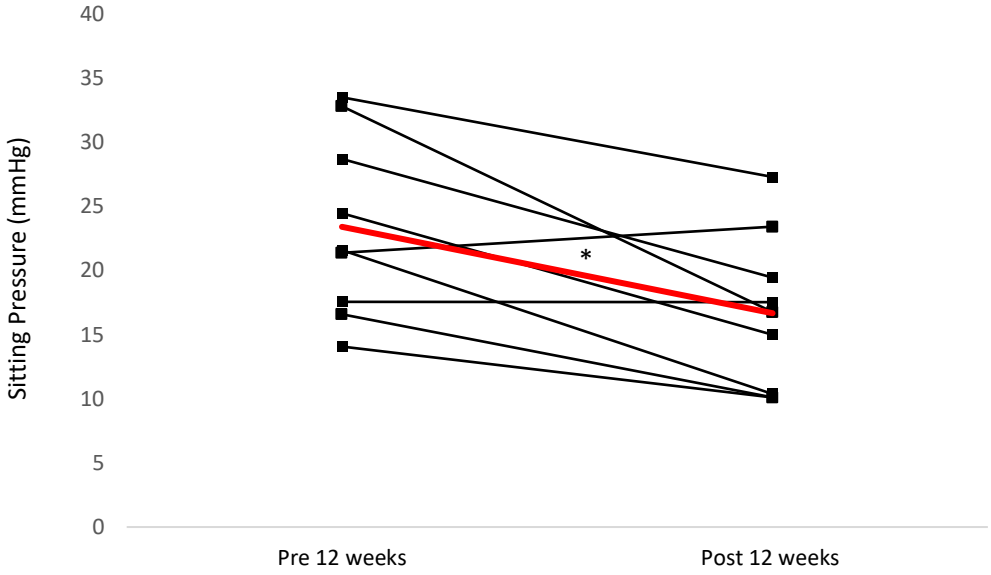

Figure 5.8 Individual and group mean pressure gradient of each individual before and after 12 weeks of electrical stimulation. Red bar indicates group mean and error bars represent SD. *Main effect of 12 weeks of electrical stimulation (P = <0.05).

Limb Volume: Exact measurements for each individual are displayed in Table 5.3. Skin fold measures were unobtainable in one individual, so analyses were performed on thigh circumference as well as overall limb volume. When taking into consideration upper, mid and lower girth measurements, average thigh circumference significantly increased from pre to post 12 weeks of ES (Z = -2.67; P = 0.008; d = 0.9). There was a significant overall increase in thigh volume (t_6 = -3.67; P = 0.01; d = 0.14). The change in upper and mid-thigh circumference was significantly correlated with the change in basal femoral blood flow (r = 0.85; P =0.03; Figure 5.9)

Table 5.3 Upper, mid and combined mean thigh girths, average skin folds for the lateral, medial and anterior part of the thigh and total thigh volume pre and post 12 week electrical stimulation.

	Thigh circumference (mm)						Skin Folds (mm)		Thigh Volume (L)	
	Upper		**Mid**		**Mean**					
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	451	455	329	332	390	394	15.4	15.4	3.3	3.4
2	559	562	471	472	515	517	13	11.6	6.0	6.2
3	533	536	444	446	489	491	×	×	×	×
4	484	490	434	438	459	464	15.6	15.4	4.4	4.5
5	494	496	458	461	476	479	15.7	15.6	5.2	5.3
6	490	491	451	452	471	472	13.8	13.6	5.2	5.2
7	537	546	529	536	533	541	14.9	14.4	7.0	7.3
8	500	508	490	497	495	503	18.3	17.6	5.0	5.3
9	494	498	454	454	474	476	×	×	×	×
	504	**508**	**427**	**430**	**478**	**482***	**14.9**	**14.5**	**4.7**	**5.3***

× = measurements unobtainable * = Main effect for 12 weeks ES
Last row in **bold** represents group mean

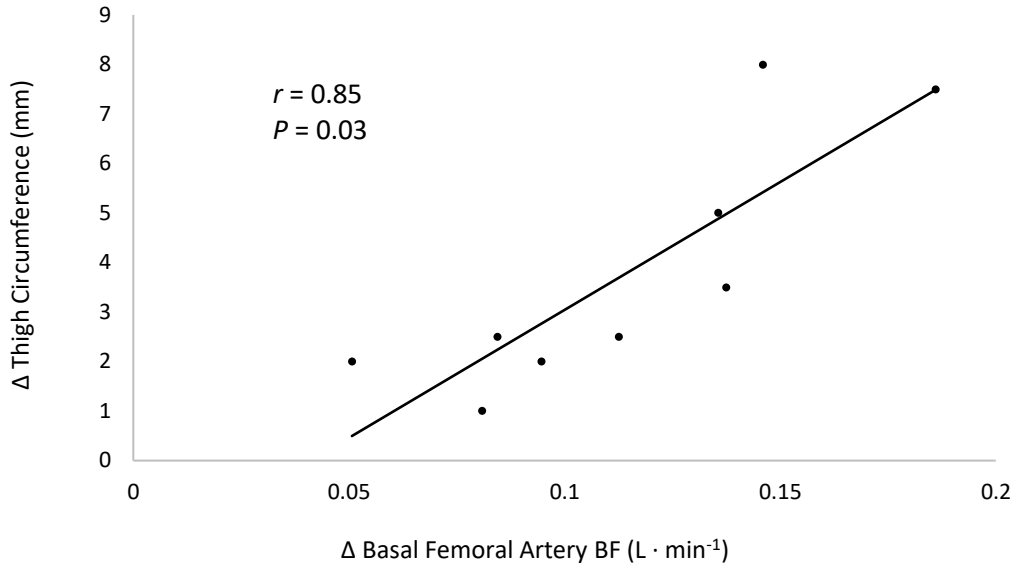

Figure 5.9 Correlation for change in thigh circumference and basal femoral artery blood flow after 12 weeks ES.

Adherence and Practicality: All participants were asked to complete a questionnaire at the end of the intervention (Table 5.4). Three of the twelve participants who started the intervention did not remain in the study giving a 75% retention rate. One individual was unable to continue due to unforeseen health complications and hospitalisation. The other two individuals were in full time employment and found the device too time consuming or difficult to apply on a daily basis. Of the remaining participants, eight indicated that they adhered to the minimum requirement of 6 hours per day 7 days a week with one subject choosing to use it in excess of 10 hours per day. One participant indicated they wore the shorts 6 hours per day for an average of 5 days per weeks. Eight of the nine participants chose to wear the shorts during the night when sleeping and one individual used them both day and night. Most positively, the majority of individuals indicated that they felt using the ES shorts was improving their health and risk for pressure ulcers and indicated that if given the choice, they would continue to use the garment.

Table 5.4 Questionnaire completed by all nine participants on the feasibility of wearing and using the electrical stimulation shorts

Question	Strongly Agree	Agree	Neutral	Disagree	Strongly Disagree	N/A
1. I am able to wear the ES garment under my normal clothing	1	-	-		2	9
2. The ES garment doesn't affect my day to day activities	1	1	-	2	-	8
3. The ES does not disrupt my quality of sleep	9	-	-	-	-	3
4. I am able to apply the garment myself without assistance	7	2	-	-	3	-
5. The ES makes going to the toilet difficult	-	1	1	4	5	1
6. Applying the ES garment takes me 10 minutes or less	3	6	-	2	1	-
7. I didn't experience any skin problems during the 12 weeks	9	-	-	-	-	3
8. I feel like the ES is helping improve my health/preventing pressure ulcers	4	5	2	-	-	1
9. If I had the choice I would continue wearing the ES garment	5	5	-	-	2	-

The two participants who dropped out of the study either disagreed or strongly disagreed with being able to wear the shorts under normal clothing. One individual who wore the shorts during the day found that they did not impede daily activities and those that wore the device during the night said the ES did not disrupt their sleep whatsoever.

5.4. Discussion

The primary aim of this study was to examine the effect of 12 weeks low-level electrical stimulation using a wearing clothing garment on cutaneous vascular function, femoral artery diameter, limb volume, and sitting pressure in areas at risk of pressure wounds. The main findings of this study were that 12 weeks of ES increased femoral artery diameter and basal blood flow, limb volume, and reduced peak ischial tuberosity sitting pressure and pressure gradient. Conversely, despite improved skin responses to local heating at the gluteal area, ES did not appear to cause significant alterations in hamstring cutaneous circulation during localised heating. Secondly, this study utilised participant feedback to explore whether using a wearable clothing garment for day-to-day, home-based electrical stimulation is practical and achievable. Participant feedback indicated that those who completed the study adhered to the protocol and used the shorts most days and/or nights and would be happy to continue using the garment if given the opportunity. Collectively, these findings indicate that regular, low-level electrical stimulation using a wearable garment may help safe guard against pressure ulcers and confer similar cardiovascular health benefits as other, more complicated methods of ES. Future studies are warranted on whether such a garment can ultimately reduce risks for clinically relevant health problems in this vulnerable population.

This study is the first to explore the effects of prolonged daily, low-level electrical stimulation utilising a clothing garment on micro- and macrovascular structure and function in persons with a SCI. In the present study, there was an 8.3% and 43% increase in femoral artery diameter and basal blood flow, respectively. These observations are in line with previous ES training studies that report a 30-50% increase in blood flow and a 6-8% increase in diameter after 4 to 12 weeks of FES cycling or FES assisted Odstock standing in spinal cord injured individuals (Taylor et al, 1993; Gerrits et al, 2001b; Hopman et al, 2002; Thijssen et al, 2005; Thijssen et al, 2006). An important difference, however, is that these previous studies adopted significantly higher levels of ES within the context of the more demanding and time-consuming FES-type exercise. This suggests that daily ES using this practical, home-

based method is just as effective as other well established, but less practical methods of ES.

The process of conduit artery structural modification is dependent on chronic changes in blood flow and is endothelium dependent (Langille and O'Donnell, 1986). In *Chapter 4*, an acute increase in femoral artery blood flow during short, consecutive bouts of ES was observed (Barton et al, 2018). The mechanical effect of blood flow through the arteries induces an increase in luminal shear stress and cyclic wall strain, with a subsequent release of endothelial derived vasodilators such as NO (Tuttle et al, 2001; Tinken et al, 2010). The release of NO from the vascular endothelium triggers an acute vasodilation as a homeostatic response to the initial increase in shear. When exposed to prolonged periods of change in flow and shear, a compensatory vessel remodelling occurs, whereby shear forces are normalised by virtue of the increase in luminal diameter. Previous training studies in humans have suggested that conduit arteries adapt their baseline diameter to peak shear stress (Dinenno et al, 2001b). Taken together, the increase in blood flow and episodic periods of high shear stress during ES in the deconditioned vessels likely contributed to the upregulation of vasodilators (e.g. NO) and the subsequent expansive remodelling of the femoral artery.

Additional to the apparent changes in artery diameter, a 43% increase in resting femoral artery blood flow was observed. When considering changes in basal femoral artery blood flow due to physical inactivity, current literature report inconsistent results (Olive, Dudley and McCully, 2003; Bleeker et al, 2005a; Bleeker et al, 2005b). Despite this, the figures observed in the current study correspond with results from previous able-bodied and Spinal Cord Injured exercise intervention studies assessing basal leg blood flow, which found a 30-50% increase when assessed using plethysmography or echo Doppler (Gerrits et al, 2001b; Hopman et al, 2002; Thijssen et al, 2005; Limberg et al, 2014). Evidence has shown that basal limb blood flow is closely correlated with leg fat-free mass and positively related to lumen diameter (Dinenno et al, 2001a; Dinenno et al, 2001b) which would likely explain the moderate

standard deviation of the basal blood flow data in this SCI population. More specifically, the increases in femoral artery diameter observed with exercise training in able-bodied individuals have been positively related to changes in blood flow, which tended to be higher after exercise training interventions (Dinenno et al, 2001a; Dinenno et al, 2001b). In the current study, one can only speculate the mechanisms involved. However, it is reasonable to assume that the increase in basal limb blood flow may partly be related to the corresponding increase in limb volume (Figure 5.9), and therefore muscle mass and limb oxygen demand during rest. Additionally, blood flow regulation is recognised to occur downstream, primarily at the level of the arterioles, and may contribute to alterations in upstream conduit artery flow patterns. Therefore, other factors altering vascular tone in downstream resistance vessels may contribute to the differences in femoral blood flow and should not be discounted.

Unlike the increase in femoral artery blood flow, and contrary to the original hypothesis, cutaneous microvascular function covering the hamstring muscles did not significantly change, whereas gluteal CVC was higher during the higher temperatures (41-42 °C) of local heating after 12 weeks of ES. Gradual local heating of an area of skin leads to the production of NO and smooth muscle relaxation via hyperpolarisation from EDHFs (Johnson, Minson and Kellogg, 2014). When heating the skin to 42°C, the vasodilation that occurs is largely endothelial and NO mediated (Kellogg et al, 1999; Minson, Berry and Joyner, 2001; Minson et al, 2002; Black, Green and Cable, 2008). Therefore, the heating stage x intervention interaction effect and the small increase in gluteal cutaneous microcirculation at 42°C provides an indication that cutaneous endothelial function, potentially via upregulation of NO, is improved with the application of ES. However, the skin microcirculation is highly complex and governed by a number of local and systemic mechanisms. One potential explanation relates to the presence of structural changes in the skin blood flow regulation. However, blood flow at 44°C, which causes maximal cutaneous vasodilation and may be regarded as a way of estimating structural differences (Johnson and Kellogg, 2010), did not change after the ES intervention. Thus, it

appears that while the NO-dependant phases (42°C) of the response to local heating may be upregulated after ES, this was not accompanied with changes in cutaneous vessel architecture. In contrast to the gluteal cutaneous vascular function, there was a no change in hamstring skin blood flow response to local heating after the 12 weeks of ES. This difference in site responsiveness is unclear but may relate to regional differences in the acute (skin) blood flow responses to ES.

Whilst physical inactivity is a key stimulus for vascular adaptations in conduit and resistance vessels in the paralysed legs of spinal cord injured individuals, previous studies have demonstrated that FES cycling exercise does not alter skin blood flow responses to local heating despite an increase in femoral artery diameter (Van Duijnhoven et al, 2009). The stimulus required for cutaneous microvascular function adaptations may relate to sufficient increases in skin and/or core temperature coupled with the haemodynamic impact of repeated exposure to increases in skin blood flow. For example, 8 weeks of repeated lower limb heating, which was accompanied by increases in core and forearm skin temperatures, improved skin microvascular vasodilation during localised heating (Carter et al, 2014). Interestingly, when skin temperatures were clamped and prevented from rising, skin blood flow responsiveness did not improve (Carter et al, 2014). Similarly, another study demonstrated that unilateral manipulation of skin blood flow and shear stress with pneumatic cuff inflation significantly attenuated the enhanced skin blood flow response to incremental local heating (Green et al, 2010). Although in the present study ES enhanced blood flow in conduit vessels, it is likely that the increase in perfusion during acute ES (*Chapter* 4), and any changes in skin and/or core temperature during prolonged ES were not sufficient to evoke changes in cutaneous microvascular function during localised heating.

Twelve weeks of ES significantly reduced the peak pressure value (-32 mmHg) over the ischial tuberosity and pressure gradient (-7 mmHg), most likely through an increase in muscle bulk at the hamstrings and glutes. This study is one of the few to measure long-term effects of gluteal and hamstring ES on sitting pressure in SCI and

the first to do so using simple, non-invasive ES techniques. Studies measuring the acute pressure changes during ES have reported a reduction in peak values of 25mmHg (van Londen et al, 2008) to 42 mmHg (Liu et al, 2006) and pressure gradient of 11 mmHg(Liu et al, 2006) to 14mmHg (van Londen et al, 2008), which occur *during* ES. Whilst this highlights the potency of ES to improve sitting pressure, these studies are importantly limited in that little is known whether these effects remain present during periods of non-stimulation. Based on the results in the current study, it is apparent that the acute pressure reduction during ES is maintained after continued long-term usage in a resting condition *without* active muscle stimulation. This suggests presence of structural or chronic adaptations that improve sitting pressure, rather than short-term, muscle activity induced changes in pressure. The interface pressure gradient reflects the pressure distribution of the area directly surrounding and containing the ischial tuberosity. When a greater part of the interface pressure is supported by a relatively small surface area of the buttock the pressure gradient increases and is associated with large shear forces and predictive of skin trauma (Mueller, Zou and Lott, 2005; Zou, Mueller and Lott, 2007; Lott, Zou and Mueller, 2008). Moreover, the reduction in peak sitting pressure is clinically important considering the location of pressure ulcers has shown to coincide with the location of peak sitting pressure (Brienza et al, 2001). It is likely that hypertrophy of the hamstring muscles over the 12 weeks ES intervention increased the seated contact area and improved pressure distribution to areas away from the ischial tuberosity. Interestingly, two participants appeared to be non-responders and displayed opposing results to the rest of the group. These individuals are identified as being male, aged 63 and 47, paraplegic T4 and T8 ASIA A and B, respectively. Although the reason for these differing responses are not clearly understood, it can be speculated that these individuals may be less susceptible to increases in muscle mass, possibly due to their slightly older age compared to the rest of the group.

Changes in muscle bulk were estimated using anthropometrically based limb volume calculations as per previously validated SCI studies (Layec et al, 2014). It should be noted, however, that changes in limb volume do not directly reflect changes in

muscle mass, and that limb volume may be considered as an indication of muscle bulk. Nonetheless, an increase in limb volume and thigh circumference were apparent after 12 weeks of ES. These results are congruent with pervious ES studies, which have shown an increase in estimated muscle hypertrophy demonstrated by girth measurements (Ragnarsson et al, 1988; Sipski, Alexander and Harris, 1993) and more sophisticated CT scans (Pacy et al, 1988; Bogie, Wang and Triolo, 2006). The small, but albeit significant changes in limb volume and thigh circumference found in the current study most likely relate to the location of ES. The anthropometric measurements were made at the upper, mid, and lower thigh. Considering that only the gluteal and upper hamstring muscles were stimulated, the changes in thigh circumference are remarkable and support the effect of ES in the current study. Despite no direct measurement of gluteal muscle mass in the current study, Baldi *et al.* used dual-energy x-ray absorptiometry (DEXA) to explore the effects of FES cycling exercise on muscle mass in the gluteal region (Baldi et al, 1998). An average loss of 27% in gluteal lean mass occurred during the first 6 months after SCI that was partly reversed with the application FES cycling exercise. Since also changes in sitting pressure were observed in the current study, one can speculate that changes in gluteal muscle size and shape have likely occurred.

Current methods of ES that are available to individuals with SCI involve either invasive procedures (implanted percutaneous electrodes), or complex equipment that are expensive and require special facilities. The current study aimed to use a method of ES that is relevant for clinical application instead of being limited to a laboratory based setting. The ES garment used in the current study is non-invasive and costs are modest (also because supervision is not required). Since electrodes are readily integrated within the shorts and do not need to be repeatedly applied, this approach of ES is less time consuming than traditional FES. The major benefit of this method, however, is its portability. The ES device can travel with the individual and be worn under normal clothing, during every day activities and whilst sleeping. This likely contributed to the 100% compliance of the intervention amongst the participants who completed the study. It was hypothesised that the shorts would be feasible for

daily ES without interfering with generic living activities. This was not the case for two of the participants who did not complete the intervention. Due to busy schedules and full time employment, two participants found the shorts too time consuming and difficult to comfortably wear under normal clothing. For the purpose of the study, participants were specifically requested to use ES at least 6 h/day. Whether shorter periods of stimulation, which may be more feasible for some spinal cord injured individuals, lead to adaptation seems logical, but remains to be tested. The versatility of this ES method means that the shorts can be worn during day and/or the evening whilst asleep. This still gives individuals an opportunity to benefit from ES when it might otherwise not be practical (i.e. at work). Indeed all nine participants who completed the study decided to wear the shorts during the night whilst sleeping and indicated it did not affect their sleep quality. Minor practical issues concerning the practicality of the electrical stimulator box and wire attachments were acknowledged by some of the participants. With current technological advances, such problems might soon be overcome with 'wireless' connectivity between the electrodes and the stimulator. Nonetheless, seven of the nine participants indicated they felt the ES was improving their health, lowered risk of pressure ulcers, and indicated they would be happy to continue wearing the device. In conclusion, the ES garment used in the current study appears to be a feasible alternative to other ES devices, which unlike many other methods enables continued daily muscle stimulation.

5.4.1. Limitations

Since three participants not complete the study, data analysis only included nine individuals. The relatively small sample size makes it difficult to infer if the results of this study are clinically relevant and can be extrapolated to the entire population of spinal cord injured individuals. Nonetheless, the Cohen's d calculated effect sizes of the primary parameters indicate a medium-to-large effect size, suggesting a significance regardless of sample size. Furthermore, this study was designed as a pilot, which has provided positive results and a clear rationale for further investigation using more rigorous randomised control trials. Another limitation is

that participant compliance and adherence to the protocol was assessed via self-reported usage diaries. Whilst this may be associated with some limitations, the results of the self-reported use and usability are consistent and in agreement with each other. Finally, skeletal muscle mass was estimated using anthropometric measurements and calculations. Whilst this method is highly cost effective and previously been validated against MRI data in persons with SCI, it does not factor in the complete gluteal muscle and may therefore underestimate the true changes in skeletal muscle volume.

5.4.2. Conclusion

In summary, this study demonstrates that 12 weeks of gluteal and hamstring electrical stimulation transduces structural and functional changes in the femoral artery supplying the active skeletal muscle, most likely through repeated increases in hyperaemic shear stress during electrical stimulation. Secondly, the results show a decrease in peak sitting pressure, pressure gradient and limb volume, which all may result from increases in hamstring and possibly gluteal muscle mass. Thirdly, cutaneous microvascular function at the gluteals may be somewhat improved. Finally, participant feedback indicated that the ES clothing device is feasible for prolonged daily electrical stimulation. Future work should expand on the present results using randomised control trials and focus on the potential impact of low-intensity ES in the prevention (and/or treatment) of co-morbidities relates to peripheral deconditioning of muscle and vasculature.

Chapter 6

Synthesis of Findings

6.1. Aims and Objectives

The aims of the research presented within this thesis are twofold. First, the research was designed to explore the accuracy of using traditional risk factors and the Framingham Risk Score to predict CVD in persons with SCI. Secondly, the work aimed to examine the acute and chronic effects of gluteal and hamstring electrical stimulation on micro- and macrovascular structure and function and risk factors for pressure ulcers. The studies described within this thesis are the first of their kind to use a clothing device to administer chronic low-level electrical stimulation to the paralysed muscles of persons with SCI. Below, the major findings of the different chapters of the thesis are first described, which are then followed by a more detailed discussion of the wider implications and future directions.

6.2. Major Findings

6.2.1. Traditional CV risk factors and the FRS in estimating CVD

The study described in *Chapter 3* used retrospective data to prospectively estimate the 5-year risk of developing CVD using the FRS. This study found that the FRS was able to distinguish between individuals who were at a higher *vs.* lower risk for future CVD. However, the absolute prediction values markedly underestimated the true occurrence of CVD mortality and morbidity. The analysis suggests that individuals with SCI are at increased risk for CVD despite having CVD risk factors that are within a comparably low or normal range as the able-bodied population. Furthermore, this study used separate univariate cox regressions to test characteristics pertaining to the SCI to improve the prognostic capacity of the FRS. The separate univariate cox regressions illustrated that ASIA impairment and level of injury were not significant predictors of CVD whereas older age at time of injury and no sports participation prior to injury were. Nonetheless, the predictive power of the FRS did not improve when ASIA impairment, motor impairment, level of injury and/or active engagement in sport prior to injury were added to the model. Collectively, these findings suggest that CVD risk estimation using only traditional cardiovascular risk factors should be interpreted with caution, and support the need to adjust current risk prediction models to make them more accurate for clinical assessment of CVD in persons with

SCI. Moreover, presence of SCI *per se* seems an important factor that must be considered in the prediction of CVD, supported by the markedly higher presence of CVD events as one may expect based on current models of risk prediction.

6.2.2. Conduit artery perfusion during acute low-level electrical stimulation

Chapter 4 of this thesis illustrated that 3 minutes of gluteal and hamstring ES significantly increased blood flow through the deep femoral artery. Furthermore, both resting blood flow and perfusion during stimulation continued to increase with each consecutive 3-minute block of ES. The increase in blood flow was localised to the limb that was subject to ES and not in the contralateral control, inactive limb. Current findings suggest that changes in arterial blood flow during muscle contractions are mediated by mechanisms involving an increase in cell metabolism and the release of vascular vasodilators. Additionally, the mechanical effect of muscle contraction and relaxation causes a change in arteriovenous pressure gradient and facilitates an increase in arterial inflow. Given that regular increases in blood flow are a key stimulus for improvement in macrovascular structure and function, these findings suggest that regular ES may represent an effective strategy to improve vascular health and reduce the risk of atherosclerotic diseases in persons with SCI.

6.2.3. Cutaneous microvascular perfusion during acute low-level electrical stimulation

Chapter 4 also demonstrated an increase in cutaneous microvascular perfusion in areas covering the activated muscles (gluteal and hamstring). Unlike conduit artery perfusion, this increase was not an immediate response and was not evident during each 3-minute block of stimulation when compared with resting pre- stimulation values. Instead, the increase in cutaneous perfusion was a progressive response, which increased with each successive 3-minute block of stimulation when compared with its preceding block. The response was likely mediated by changes in localised

skin temperature in the area directly exposed to ES. The acute changes in cutaneous perfusion observed in *Chapter 4* may aid in the restoration of tissue blood flow during prolonged sitting, thus having a clinically relevant impact on pressure ulcer prevention and/or repair for individuals with SCI.

6.2.4. Chronic low-level electrical stimulation on conduit artery structure and basal blood flow

Chapter 5 demonstrated that 12 weeks of daily gluteal and hamstring ES increased the common femoral artery lumen diameter. Structural arterial adaptations are dependent on chronic changes in blood flow and mediated by the endothelium through NO-dependant pathways. Given that approximately 40% of the cardioprotective effects of exercise are unexplained by modification of traditional cardiovascular risk factors (Mora et al, 2007), the ES induced increases in blood flow and structural adaptations may represent an important cardioprotective mechanism.

An increase in basal femoral artery blood flow was also demonstrated in *Chapter 5* of this thesis. It was postulated that the changes occurred because of increased leg fat free mass, changes in downstream resistance and/or changes in haemostatic variables such as central driving blood pressure. Under conditions of low flow, circulating leukocytes can adhere to the arterial walls. Therefore, an increase in basal leg blood flow may help attenuate the progression of development of atherosclerotic lesions and peripheral arterial disease. These data support the utilisation of ES as a potentially effective cardioprotective strategy for persons with SCI.

6.2.5. Chronic low-level electrical stimulation on cutaneous microvascular function

In *Chapter 5*, gluteal and hamstring cutaneous NO-mediated microvascular function in response to localised heating did not drastically change after 12 weeks of ES. However, gluteal CVC at 42°C revealed a small increase and there was a significant time x temperature interaction, indicating that the change in gluteal skin blood flow

during local heating from baseline to maximal levels was higher after the 12 weeks of ES. Considering the complexity of a SCI and high-risk nature for CVD and skin problems, even the smallest of changes may be relevant. The lack of changes in CVC-responses to heating at the hamstring after the 12 weeks of ES implies that adaptations in the skin microcirculation, despite similar changes in cutaneous microvessels during acute ES in the gluteal and hamstring region (*Chapter 4*), are complex and may depend on other stimuli that interfere with (regional) adaptations.

6.2.6. Chronic low-level electrical stimulation on sitting pressure

Peak sitting pressure and pressure gradient described in *Chapter* 5 were reduced after 12 weeks of ES. These results imply that daily low level ES alters the force acting through the seating support area in people with SCI most likely because of hypertrophy of the stimulated muscles.

6.2.7. Chronic low-level electrical stimulation on limb volume

Chapter 5 also illustrated an increase in upper leg volume and thigh circumference after 12 weeks of ES. Although there was no direct measure of gluteal muscle mass, the lower sitting pressure values most likely relate to changes in muscle size and/or shape. Gluteal muscle mass is important for pressure ulcer prevention as it helps to increase cushioning between the ischial tuberosity and the sitting support surface area. Consequently, the underlying vessels are able to withstand prolonged loading, thus minimising tissue deformation, cell occlusion and maintaining tissue blood flow. Daily low-level gluteal and hamstring ES may therefore represent an effective stimulus for pressure ulcer prevention in people with SCI.

6.2.8. Feasibility of daily low-level gluteal and hamstring electrical stimulation

The study in *Chapter 5* is the first to use a wearable clothing device for prolonged (12 weeks) daily ES outside of a laboratory based setting. The study demonstrates that

prolonged daily gluteal and hamstring ES is achievable and practical when applied using a wearable clothing garment. Nine of the original twelve participants completed the intervention, adhered to the protocol, and stated they would be happy to continue using the device. Although three individuals did not complete the study, one of these was unavoidable due to ill health and hospitalisation (not related to the use of the device). The other two individuals found that the daily use of the garment (i.e. 6-h) in combination with a full-time job imposed a too large time commitment. An important observation was that all participants who maintained functional mobility of their upper limbs were able to apply the garment without assistance. This latter topic is a key limitation of many other ES devices and often restricts the accessibility and application of ES for many individuals with SCI.

6.3. General Discussion

A SCI by virtue confines individuals to a lifetime of sedentary behaviour. Considering the strong link between physical inactivity and CVD, it is not surprising that the risk and prevalence of premature CVD morbidity and mortality are increased in persons with SCI (Garshick et al, 2005; Cragg et al, 2013; Osterthun et al, 2014; Savic et al, 2017b). Despite these observations, individuals with SCI often have similar cholesterol, triglyceride and blood pressure levels as healthy able-bodied subjects (Bauman et al, 1992; Cardus, Ribas-Cardus and McTaggart, 1992a; Krum et al, 1992; Liang et al, 2007). Similarly, in the able-bodied population, traditional cardiovascular risk factors have been reported to account for only 27-41% of the cardioprotective benefits of exercise (Mora et al, 2007; Chomistek et al, 2011; Hamer et al, 2012). The data from *Chapter 3* represents one of the first to suggest that the strong link between physical inactivity (i.e. spinal cord injury) and CVD cannot be fully explained by an effect of inactivity on traditional cardiovascular risk factors. It has been proposed that the direct effects of physical (in)activity on the vasculature and the consequential structural and functional adaptations which occur, may contribute to the unexplained CVD risks associated with physical inactivity (Green et al, 2008). Collectively, this provides a strong rationale for 1) needing more accurate methods of CVD risk prediction in people with a SCI, and 2) appropriate interventions that

target the deconditioned vasculature as a way to try to minimise the process of accelerated CVD in people with SCI.

Findings from *Chapter 3* represent the first prospective study to support previous findings that traditional cardiovascular risk factors may underestimate the true CVD risk in people with SCI (Finnie et al, 2008; Sullivan et al, 2017). The results from this study illustrate that the discrimination of the FRS between groups of low- and high-risk for CVD is of reasonable strength and of comparable predictability as when used in able-bodied populations (Cook et al, 2012; DeFilippis et al, 2015). More specifically, individuals who were categorised into the high-risk group (FRS = >median) were recognised as being at 3.2 times greater risk for developing CVD than those in the low risk group. It must be acknowledged, however, that the median FRS for the cohort was only 1.36%. Traditionally, the FRS calculates a 10-year risk for CVD and automatically categorises individuals as low (<10%), medium (10-20%) or high risk (>20%). Although the study in *Chapter 3* only used a 5-year prediction model, a FRS of 1.36% would still suggest a negligible risk for CVD. This is in contrast to the results at 5 year follow up, which revealed that 19.5% of the study population developed a CV event. Surprisingly, in the below median 'low risk' group, 10 individuals developed an event. These data reinforce the significant underestimation of CVD risk in people with a SCI when using traditional cardiovascular risk factors alone.

Some studies suggest that certain individuals with SCI are at higher risk and greater predisposition for CVD based on their lesion characteristics (Groah et al, 2001). *Chapter 3* aimed to test this hypothesis and used separate univariate cox regression analysis to test for individual predictors of CVD. Level of injury and ASIA impairment had no influence on the risk for developing CVD. Similarly, Savic *et al.* demonstrated that CVD mortality rates were greatest in persons with paraplegia and functionally incomplete lesions, suggesting that the degree of autonomic deficiency *per se* has no direct effect on the development of CVD (Savic et al, 2017a). Possibly, the marked physical inactivity and life-long exposure to an extreme form of sedentary behaviour, independent of the level and completeness of lesion, may have a detrimental effect

on vascular health, accelerating the atherosclerotic processes, and contribute to developing premature CVD. Considering the strong associations between physical inactivity, impaired vascular structure and function and CVD risk, exploring the use of interventions to prevent extreme vascular deconditioning (e.g. exercise training, ES) seems warranted.

Collectively, the results from *Chapter 3* provide a compelling rationale for adjustment of current CVD risk prediction models for SCI to include novel risk factors (e.g. inflammatory markers, physical activity level) and/or the use of alternative screening methods. Studies in able bodied populations, have found higher levels of C-reactive protein to be associated with lower levels of physical activity (Kasapis and Thompson, 2005; Mora et al, 2006; Fischer et al, 2007). In accordance, there is a large body of evidence describing the associations between systemic inflammation and the development of atherosclerosis and coronary heart disease (Pearson et al, 2003). Furthermore, elevated circulating levels of plasma C-reactive protein are consistent with the presence of heart disease, hypertension (Goldstein et al, 2017), and increased CVD risk when assessed using the FRS in people with SCI (Finnie et al, 2008). Therefore, the effects of physical inactivity on systemic inflammatory markers combined with impaired vascular structure and function may partially explain the risk of CVD in lieu of abnormal traditional cardiovascular risk factors. These markers may be useful to improve risk prediction in SCI. At least, presence of a SCI should be considered as a risk factor importantly increasing CVD risk, independent of traditional CVD risk factors.

It is logical to assess skin microcirculation in people with SCI for a number of reasons. Firstly, the skin represents a valid model of generalised microvascular dysfunction and displays manifestations of global CVD risk earlier than in conduit arteries (Holowatz, Thompson-Torgerson and Kenney, 2008; Roustit and Cracowski, 2013). Secondly, a loss of muscle bulk concurrent with prolonged loading when seated and a loss of capillary network, means cutaneous and subcutaneous tissues receive an inadequate supply of blood, thus rendering them at an in increased risk for cell death

and pressure ulcers (Herrman et al, 1999). The data presented in *Chapters 4 and 5* examined the acute and chronic responses of gluteal and hamstring ES on cutaneous microcirculation in areas covering the active muscles. The results illustrated that cutaneous blood flow increased during acute ES in the gluteal and hamstring region (*Chapter 4*), whilst chronic improvement in microvascular function were only apparent in the gluteal area (*Chapter 5*). Assessment of microvascular function was performed by examining skin perfusion responses to local heating. The increases in gluteal CVC at the higher temperatures during local heating (39-42°C) suggests improved NO responsiveness of the cutaneous vascular smooth muscle. However, regional differences were observed with no adaptation in the hamstring region, whilst this latter finding is in agreement with a previous study which showed no effect of 8 weeks FES cycling on upper or lower limb cutaneous microvascular function (Van Duijnhoven et al, 2009).

Previous work has illustrated that cutaneous microvascular adaptations occur when exposed to regular sufficient increases in heat stress, hyperaemic shear stimuli or antioxidant supplementation (Green et al, 2010; Carter et al, 2014; Brunt et al, 2016a). Increases in skin and core temperature upregulate the expression of heat shock proteins, which are an essential cofactor for eNOS (Pritchard et al, 2001). The upregulation of heat shock proteins and their interaction with eNOS induces NO production and the subsequent vasodilation of cutaneous microvessels. The findings in *Chapter 5* propose that the ES intervention was sufficient for localised improvement in skin microcirculation of the posterior thigh. By only stimulating the hamstring and gluteal muscles, generation of (metabolic) heat may be insufficient for generalised adaptations. Possibly, the combined co-contraction of 3 muscle groups may increase the haemodynamic stimulus through the microvessels and potentially lead to larger adaptation.

Additional consideration should be paid to alternative methods of improving skin vascular function as well as the techniques used for measuring changes in skin blood flow. For example, clinical application of passive heat therapy has gained particular

interest in recent years due to its direct beneficial effects on microvascular function (Brunt et al, 2016a; Brunt et al, 2016b). Given that evidence suggests a sufficient increase in core/skin temperature coupled with exposure to hyperaemic shear stress is warranted for improved cutaneous microvascular function, this technique may be of particular interest for individuals with SCI although some caution is needed with regards to an elevated hyperthermic injury risk in spinal cord injured individuals. With regards to blood flow imaging techniques, the studies contained within this thesis use a very small sample of skin to examine changes in cutaneous blood flow. Comparing this to the sample area used for measuring changes in conduit artery blood flow, more explicit changes in vascular function may have been apparent if a larger area of skin was examined using other laser Doppler imaging methods.

Although gluteal and hamstring ES had little effect on chronic changes in cutaneous microvascular function, the acute effects observed in *Chapter 4* are significant, and potentially more relevant for the prevention of pressure ulcers in people with SCI. A major contributing factor to pressure ulcers is compromised blood flow and tissue ischemia induced by the occlusion of blood vessels from externally applied pressure (Reddy, Gill and Rochon, 2006). Similarly to *Chapter 4* of this thesis, studies have shown that gluteal ES induces a redistribution of pressure away from the ischial tuberosity, together with an increase in local tissue blood flow during muscle activation (Levine et al, 1989; Levine et al, 1990; Gyawali et al, 2011; Smit et al, 2013b). Combined with the acute increase in blood flow, repeated application of ES may improve the cutaneous capillary network and enhance the vascular capability and capacity to support a larger volume of blood. Collectively, better tissue perfusion will prevent cell death, tissue necrosis and the development of pressure ulcers. Another important point to consider is the effects of ES on the structural composition of the different layers within the skin. For example, a thicker and more resilient protective layer of epidermis may safeguard against cell deformation and occlusion during periods of prolonged pressure. Indeed, skin biopsy data has demonstrated that FES in spinal cord injured patients significantly increased thickness of the epidermis after 2 years of FES (Albertin et al, 2018a; Albertin et al, 2018b). In *Chapter*

5, no quantitative assessment of skin quality was performed and therefore it is difficult to know whether the intervention was sufficient to modify the different structural components. This warrants further studies to explore the structural adaptations of skin after ES and the clinical prevention of pressure ulcers. Taken together, although the skin responses to local heating did not significantly change, the repeated elevations in skin perfusion during ES (and redistribution of skin blood flow) and potential structural changes in the skin epidermis and/or skin microcirculation may provide beneficial effects. This should be the subject of future research.

A SCI results in a deleterious deconditioning of the conduit arteries in the inactive, paralysed limbs. For example, studies have demonstrated a 25% decrease at just 18 days after SCI (de Groot et al, 2006a), and a 30% reduction in vessel diameter in chronic SCI (De Groot et al, 2003; de Groot et al, 2004b; de Groot, Bleeker and Hopman, 2006). Similarly, models of extreme physical inactivity in able bodied humans such as unilateral lower limb immobilisation (Bleeker et al, 2005a), bed rest (Bleeker et al, 2005c) and leg casting (Sugawara et al, 2004) have all shown rapid reductions in vessel diameter. These findings suggest that vascular adaptations in the paralysed limbs of people with SCI are primarily a result of physical inactivity. In *Chapter 4* of this thesis, femoral artery blood flow increased during acute gluteal and hamstring ES. Changes in vessel structure occur secondary to chronic changes in blood flow and shear stress and are dependent on the release of NO from vascular endothelial cells (Langille and O'Donnell, 1986). This is supported by the significant increase in femoral artery diameter contained within *Chapter 5*. It is likely that the daily episodic increases in blood flow and arterial wall shear upregulate the expression or activity of vascular endothelial growth factors. Cell culture studies have demonstrated shear dependant elevations of eNOS mRNA, protein, NOS activity and NO production (Nadaud et al, 1996; Hyre, Unthank and Dalsing, 1998), which are influenced by the level of blood flow alteration (Tuttle et al, 2001). Subsequently, luminal expansion occurs and continues in a manner to homeostatically regulate arterial wall shear. Considering the direct relationship between blood flow and NO

production together with the potent anti-atherogenic properties of NO, the ES induced changes in femoral artery blood flow and diameter observed in *Chapters 4* and *5*, respectively, may upregulate NO bioavailability (Lam et al, 2006) and represent an important cardioprotective mechanism for people with SCI.

Although the study in *Chapter 5* did not include an able bodied control group for comparison of basal limb blood flow, previous studies have demonstrated resting blood flow in paraplegics is 28% lower compared to able-bodied subjects (Hopman, van Asten and Oeseburg, 1996). This may have important clinical implications considering reductions in limb perfusion and vascular conductance have been implicated in the pathogenesis of metabolic syndrome and may contribute to the development of CVD (Lind and Lithell, 1993). *Chapter 5* demonstrated an increase in basal femoral artery blood flow after 12 weeks of gluteal and hamstring ES. Similar observations have been reported in able bodied (Anton et al, 2006; Limberg et al, 2014) and spinal cord injured (Gerrits et al, 2001b; Thijssen et al, 2005) individuals after exercise intervention studies. The physiological mechanisms responsible for the increase in basal femoral blood flow are yet to be clarified, although several potential mechanisms can be speculated. Considering the close coupling between blood flow and muscle mass, it can be hypothesised that ES would result in improved blood flow by increasing leg muscle mass (and therefore demand for blood supply). Additionally, levels of circulating vasoconstrictors such as ET-1 and ANG II have been shown to contribute to the increased leg vascular resistance in spinal cord injured individuals (Thijssen et al, 2008c; Groothuis et al, 2010), which can be partly reversed with exercise training (Adams et al, 2005; Thijssen et al, 2007). Finally, enhanced vascular function at the arteriolar may contribute to increased basal femoral blood flow after chronic ES. Further studies are warranted to investigate the physiological mechanisms underlying changes in basal limb blood flow. Nonetheless, the findings presented in this thesis conclude that ES is an effective strategy for the treatment of reduced basal limb blood flow in persons with SCI.

The acute pressure reducing effects of gluteal muscle contractions during ES are relatively well documented and acknowledged (Liu et al, 2006; van Londen et al, 2008). However, temporary reductions in pressure fail to address the intrinsic risk factors associated with pressure ulcers such as muscle atrophy. Very few studies have examined changes in sitting pressure under resting conditions after a prolonged period of daily ES. In *Chapter 5,* daily gluteal and hamstring ES reduced sitting pressures, and increased limb volume suggesting ES was effective at inducing muscle hypertrophy. Due to the impaired sensation below the level of their lesion, persons with SCI do not feel discomfort or pain during prolonged sitting and are unaware of the necessity to relieve pressure. This exposes them to significant vulnerability for developing pressure ulcers. Therefore, improving tissue health and its tolerance for deformity remain a key priority for people with SCI. The formation of pressure ulcers can occur at the dermis, usually as a result of excessive friction or compromised dermal integrity and then progress towards the deeper layers of tissue. However, muscle is considered to be more susceptible to tissue degradation from mechanical loading than dermal layers, consequently resulting in severe full thickness deep tissue pressure ulcers (Kosiak, 1961; Bouten et al, 2003). Previous studies support the findings in this thesis and have documented evidence of muscle hypertrophy (Baldi et al, 1998; Bogie, Wang and Triolo, 2006) and reduced interface sitting pressure (Bogie, Wang and Triolo, 2006) after prolonged ES. It should be noted that the ES methods differ between these previous studies and the work contained within this thesis, where the latter is the first to demonstrate the effectiveness of home based ES therapy on sitting pressure and limb volume. ES discussed above involve complex equipment such as FES cycle ergometers and/or invasive procedures to implant electrodes under the gluteal muscle. The finding of a drop in sitting pressure in the current study is potentially clinically relevant since high sitting interface pressure has been linked to the incidence of pressure ulcers and the location has shown to coincide with the location of peak interface pressure (Brienza et al, 2001). In conclusion, the findings in *Chapter 5* illustrate the favourable effects of ES on tissue tolerance and may therefore represent a clinically relevant tool for people for safeguarding against pressure ulcers in people with SCI.

Chapter 5 demonstrated that ES using a wearable clothing garment can be successfully incorporated into the daily routine of people with SCI. The feasibility feedback data identified a number of strengths associated with the garment. Firstly, the majority of participants who took part in the study chose to wear the ES garment during the night whilst sleeping and indicated that the ES induced muscle activations did not affect their sleep quality. This is an important consideration when making daily ES accessible and feasible for individuals with SCI, particularly for those individuals who have less time to commit during working hours. To ensure long term daily ES is feasible and to guarantee ongoing commitment from an individual, the method of applying the ES must be a simple and efficient process. This is another strength of the ES garment used in *Chapter 5*, in which all participants who completed the intervention indicated that applying the ES garment took on average 10 minutes or less. Although only one participant who completed the intervention decided to wear the shorts during the day, they indicated that doing so did not affect their daily living activities and the shorts could be worn under regular clothing. However, two participants dropped out of the study and comments were raised concerning the clumsy design of the garment and wires connecting to the stimulator. Therefore, the current ES intervention is not perfect for everyone and future research concerning other practical home-based ES interventions is warranted, e.g., methods that incorporate the use of wireless technology.

6.4. Implications

The experimental studies described in this thesis relay a number of important health messages which have added to the understanding of CVD risk prediction and how ES impacts markers of vascular health and risk factors for pressure ulcers in people with SCI. Accordingly, these findings have the potential to impact clinical practice and subsequently improve early diagnosis and prevention of CVD and pressure ulcers in this vulnerable population.

The research undertaken in this thesis contextualises previous observations that a SCI results in increased risk for CVD in the absence of abnormal traditional cardiovascular risk factors. These findings reinforce that clinical assessment and diagnosis of CVD should be interpreted with caution when using well established risk prediction calculators such as the FRS. Additionally, *Chapter 3* indicates that CVD risk is independent of SCI characteristics such as level and ASIA impairment, but instead may be related to the pre-injury level of physical inactivity. Collectively, understanding these factors will enable physicians to prescribe appropriate interventions earlier than they previously would have, even when traditional CVD risk factors suggest a low to moderate risk.

Improvement in traditional cardiovascular risk factors alone cannot account for the magnitude of CVD risk reduction and reduced mortality associated with exercise training (Dimmeler and Zeiher, 2003; Mora et al, 2007). Independent from known risk factors, it is postulated that the effects of repeated exposure to haemodynamic stimuli during muscle contractions, and the subsequent functional and structural adaptations that occur in the vessel, contribute to the 'unexplained' cardioprotective effects of exercise. Therefore, adaptations that occur as a consequence of repeated haemodynamic stimulation within the arteries supplying the active muscles during ES may contribute to reducing atherosclerotic risk in conduit arteries. Furthermore, reduced basal limb blood flow has been associated with metabolic syndrome and regarded as a major precursor to coronary atherosclerotic diseases (Julius et al, 1992; Lind and Lithell, 1993). Although bloods were not collected in any of the studies within this thesis, the increased blood flow both during ES and at rest may improve peripheral glucose uptake and clearance of atherogenic lipids. Thus, ES seems a potentially clinically relevant strategy that may assist in the prevention of atherosclerosis, but also type 2 diabetes mellitus, and accordingly reduce the prevalence of cardiometabolic mortality in spinal cord injured individuals.

Keeping with the observation of improved blood circulation, the application of ES activates the physiological muscle pump, thereby facilitating venous return and

preventing venous pooling in the lower limbs. As such, the ES induced contractions of the leg muscles may prevent the drop in systolic blood pressure that occurs in individuals with SCI during orthostatic hypotension. Furthermore, the prevalence of DVTs amongst spinal cord injured individuals is exceptionally high (*Chapter 3*) and represents an important risk for pulmonary embolisms. The improved venous circulation and reduction in blood stasis in people with SCI during ES may therefore offer greater protection against DVT and prevent mortality resulting from pulmonary embolisms. This provocative idea requires further follow-up and may also warrant the application of ES at the calf muscles.

Pressure ulcers are a common problem and a significant concern for individuals with SCI. They interfere with daily living activities, occupational duties, and in severe cases, can be life threatening. The work contained in this thesis demonstrates that gluteal and hamstring ES significantly improves several physiological risk factors pertaining to the development of pressure ulcers. Consequently, this has important implications for the individual and health care providers. The cost of treating a single pressure ulcer in the UK varies from £1214 to £14108 (Dealey, Posnett and Walker, 2012). In comparison, the costs of applying home based ES are relatively low, and primarily relate to the device itself (~£2000). Considering that 85% of individuals with a SCI are expected to develop a pressure ulcer during their lifetime, it seems logical that health care providers invest in techniques to prevent the occurrence of pressure ulcers rather than wait for a pressure ulcer to occur and treat them. Not only would this translate into a considerable saving, it would also release more beds and allow more time for healthcare staff to treat other patients. For the individual with a SCI, the process of healing a pressure ulcer can cause distress and ultimately be psychologically damaging (Kruger et al, 2013). A complete relief of pressure is required for the healing of a pressure ulcer, meaning individuals have to spend weeks without sitting, lying in bed in an uncomfortable prone position and only being able to move around on a branchard. This has a dramatic impact on daily family life, working life, and most of all, their independence and self-efficacy (Kruger et al, 2013). Taken together, if home based ES is an effective intervention for pressure ulcer

prevention, then this may translate to favourable physiological and psychological health benefits for individuals with SCI.

Chapter 5 is the first study to comprehensively explore the effects of daily low-level ES on vascular health and pressure ulcer risk factors using a non-invasive wearable ES clothing device, requiring no supervision of staff and the application under home-based environment. Despite the numerous benefits of ES presented within this thesis, if stimulation is discontinued, the vasculature and muscles revert back to their former wasted, deconditioned morphology. Therefore, ongoing commitment and motivation from the subject is essential and should be a priority when considering treatment interventions of this kind. This also highlights the need for simple, easy to adjust equipment as long-term use seems warranted to fully benefit from ES. The ES shorts used in the studies within this thesis are of a major benefit compared to other methods as they allow ES to be applied with the least amount of intrusion into the patients daily activities. The shorts are portable and can travel with the individual, be worn under normal clothing and whilst sleeping. Furthermore, they are non-invasive, cost relatively little, and the integrated electrodes allow consistent and efficient electrode placement. Increasing the accessibility and user friendliness of ES methods with devices such as these may therefore improve the likelihood of spinal cord injured individuals using them on a regular basis, allowing them to benefit from the many health improvements associated with ES.

6.5. Methodological Considerations and Limitations

There several notable strengths in the methodology of the work contained within this thesis. Firstly, the use of custom-designed edge-detection and wall tracking software for the analysis of femoral artery structural and functional characteristics (*Chapter 4 and 5*) is largely independent of researcher bias. Previous work has demonstrated that using this semi-automated technique, diameter calculations can be reproduced far more accurately than manual methods with an intra-observer coefficient of variation of 6.7% (Woodman et al, 2001). Thus, the accuracy and validity of femoral artery measures was maximised.

Secondly, never before has ES been applied for prolonged periods (12 weeks, 6 hr/day), using a wearable clothing garment that can be applied without supervision and in a home based environment. This thesis provides new insights into the effects of a novel way of applying low level ES in people with SCI. The average level of ES used for acute stimulation in *Chapter 4,* and chronic stimulation in *Chapter 5,* was 75mA and 90mA, respectively. This level of stimulation (and therefore risks and direct impacts on the skin) is considerably lower in comparison to other studies that have reported using stimulation levels of up to 150mA. The high intensity stimulation used in other studies induces marked movement of the limbs and can only be applied for short durations due to muscle fatigue and energy source depletion. This type of ES also requires continuous supervision of trained staff. The significant benefit of the low level ES used throughout this thesis is that it can be applied for long durations, and because of the limited limb movement, can be applied during the day and night with minimal disturbance to day to day activities. Furthermore, only the gluteal and hamstrings were stimulated, which was considered as an important point in the methodological design of the studies. As mentioned previously, making ES accessible and user friendly with the least amount of intrusion into daily life is essential in ensuring commitment and motivation from the individual. Stimulating the quadriceps as well would have limited the usability of the shorts whilst seated in the wheelchair as this would have caused significant flexion at the knee. Therefore, the intervention was designed for minimal interference with daily activities.

Methodological rigor was further achieved with the adherence of strict inclusion and exclusion criteria throughout the experimental chapters. In *Chapter 3*, the inclusion of individuals with all forms of SCI injury (i.e. traumatic and non-traumatic; ASIA A, B, C and D), who were recruited from 8 different SCI rehabilitation centres throughout the Netherlands, suggests that the results are a true and valid representation of the SCI population and can be confidently generalised and applied to other spinal cord injured individuals.

Despite the methodological strengths of this thesis, there are some apparent limitations that should be addressed. The main limitation in *Chapter 3* is the relatively small sample size and CVD incidence. Epidemiology and mortality studies in general use much larger sample sizes to ensure the results can be extrapolated and applied to the wider population. Therefore, the results should be interpreted with caution until confirmed using a larger sample size. In *Chapter 5*, one limitation concerns the method of assessing muscle mass. Initially, the research project had planned to quantify muscle mass using MRI or DEXA which, are considered as being the most accurate methods of assessment. However, due to numerous factors out of the researcher's control (facility and equipment availability), quantifying lower limb muscle mass using these techniques were not possible. Although access to ultrasound was available, learning a new muscle imaging technique and acquiring the accuracy required to obtain reliable data at such short notice was also not a feasible option. Regardless of such setbacks, the anthropometric measurements and calculation of limb volume used in *Chapter 5* has been previously validated in people with SCI. All assessment sites were marked on the skin, anatomical landmarks were noted and digital photographs taken as a reference to ensure accurate re-assessment post intervention.

Another limitation is that the ES shorts used in this study are not suitable for persons with flaccid paralysis like in cauda equine syndrome. When the somatic nerves in the skeletal muscles become severed the muscle becomes flaccid and cannot contract when using the small electrodes and low level of ES as adopted in this thesis. A final limitation is the modest sample size and a lack of a control group in the intervention study. Nonetheless, the sample size used was appropriate considering the complex nature of a SCI and the commitment warranted from participants to complete these intervention studies. In addition, clear statistical differences and medium to large effects were found. Therefore, it is not expected that the main findings will change if more individuals were to be included in the intervention study.

6.6. Future Recommendations

The work presented in this thesis has established a number of interesting and potentially clinically relevant findings regarding CVD risk prediction and the effects of practical home based low-level ES on vascular health and risk factors for pressure ulcers. With that in mind, several areas for future research have emerged that could further expand on the current findings and better inform clinical staff and spinal cord injured individuals about the best practices for CVD and pressure ulcer prevention.

Firstly, *Chapter 3* identified that traditional cardiovascular risk factors and the FRS significantly underestimate the risk of CVD in SCI. However, considering a higher FRS corresponds with increased occurrence of CVD, it might first be logical to adjust/lower current cut off values for traditional cardiovascular risk factors in spinal cord injury. This would result in an estimated CVD risk output that is higher than in able bodied individuals despite similar traditional cardiovascular risk profiles. Alternatively, using a constant to shift the FRS score upwards due to the presence of a spinal cord injury could also be a possibility to better reflect the increased risk of CVD in this population. Similarly, adding additional co-variates into the original FRS such as physical activity levels and biochemical markers of inflammation may also increase the sensitivity and accuracy of such risk algorithms for CVD prediction in the spinal cord injured population. Furthermore, some evidence in able bodied populations support the use of early pharmaceutical interventions to lower the risk of CVD in those with completely 'normal' risk factor profiles (Thanassoulis et al, 2016). It might be that prescribing spinal cord injured individuals with pharmaceutical interventions as a standard operating procedure may slow the development of CVD in this high-risk population.

Chapter 5 provides convincing evidence that low level home based ES is effective for reducing the risk of pressure ulcers and inducing structural modifications in the vessels supplying the active muscles. However, the current study was designed as a pilot, and should be expanded on using longitudinal randomised control trials (RCT). A study design comparing two groups of spinal cord injured individuals, an ES

intervention group and control group who would take normal pressure ulcer preventative measures (i.e. weight shifting, cushions etc.), with a longitudinal follow up period of 3-4 years should be used. This will give definitive answers as to whether low level home based ES is effective for preventing pressure ulcers in people with SCI. Additionally, for the intervention in *Chapter 5,* participants were asked to use the ES every day. Whether a lower dose of ES can yield the same or similar results should also be considered as well as using more reliable methods of measuring muscle mass such as the 'gold standard' MRI or DEXA.

It was mentioned that the increase in basal limb blood flow may be an effective mechanism for improved glucose uptake and clearance of atherogenic lipids. Therefore, collecting bloods and performing biochemical analysis before and after ES will help better understand this hypothesis. Biochemical analysis will also add to the findings in *Chapter 3* and help to understand whether traditional cardiovascular risk factors (cholesterol) remain unchanged in the presence of arterial remodelling. This will help to strengthen the argument that changes in arterial structure and function contribute to the unexplained CVD risk with physical inactivity.

After SCI, the paralysed muscles become more easily fatigued due to loss of oxidative capacity and a switch from slow fatigue type 1 into fast fatiguing type 2. Consequently, this rapid fatigue is one of the many factors limiting prolonged clinical effectiveness of ES. Considering some of the consequences of compete muscle denervation can be reversed with ES (Ashley et al, 2008), it would be interesting to perform histological examination such as fibre type, capillary density and cross sectional area of the stimulated muscles in response to the low level home based ES protocol used in this study.

Although the shorts used in this study were effective for daily application of home based ES, and regardless of the positive feedback, there are obvious design flaws that could be altered to make them more practical. For example, the wires running from the shorts to the simulator make the device 'clumsy' and at times an inconvenience.

Therefore, the development of software/hardware to wirelessly electrically stimulate the muscles will make devices such as this one far more attractive. Finally, this particular design of shorts meant the gluteal and hamstrings had to be stimulated at the same level. Given the gluteal muscle is much larger and can tolerate a higher intensity, the device could be far more effective at inducing pressure changes and gluteal muscle mass if the intensity could be specified for each individual electrode.

Chapter 7
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