Proximal Feed Artery Regulation of Skeletal Muscle Blood Flow during Exercise: The Paraplegic Model

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Assignment in partial fulfillment of the requirements for M.Phil. (Exercise Physiology) - Degree

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December 2003

Declaration

I, the undersigned, hereby declare that the work contained in this assignment is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

Abstract

The mechanisms of blood flow (BF) control to skeletal muscle during dynamic exercise are still not clearly understood. The paraplegic subject (P) has reduced sympathetic innervation to the lower limbs. The current study was designed to focus on the contribution of neural control, specifically the sympathetic nervous system (SNS), as part of the central vascular mechanism to skeletal muscle BF during dynamic exercise. Aims: We studied BF parameters in P vs. able-bodied subjects (AB) to determine whether the paraplegic can serve as a model for assessing the contribution of the SNS to changes in active vs. inactive muscle BF during exercise. Further questions addressed include: the influence of level of fitness on resting and exercise BF, how lesion level affects BF control in the paraplegic, the 'muscle pump' theory and its hypothesized role in exercise hyperemia and whether blood pooling occurs in the legs of paraplegics. Method: Noninvasive duplex Doppler studies of the large conduit arteries (brachial and common femoral) were performed on 10 elite paraplegic athletes (EP), 10 sedentary paraplegics (SP) en 10 sedentary able-bodied subjects (AB). The paraplegic groups were further subdivided by lesion level with T6 being the critical level. Tests were carried out at rest and after 2 bouts of arm ergometer exercise: a maximal incremental test and 3 minutes at 75% of maximal. Diameter, mean velocity, pulsatile index and blood flow were measured/calculated.

Results: Resting heart rate was significantly higher in the paraplegic groups (EP = 80 bpm ± 10, SP = 83 bpm ± 12) vs. the AB group (69 bpm ± 7), $p < 0.05$. Resting diameter in the common femoral artery (CFA) was similar in EP (5.93 mm ± 1.54) and SP (6.52 mm ± 0.95), but significantly lower than in AB (7.87 mm ± 1.38), $p < 0.05$. Similar resting pulsatile index (PI) in the CFA were contrary to that previously reported, casting doubt on venous blood pooling theories. Post-exercise values need to

be interpreted with caution in view of the large resting differences in CFA diameter. Percentage change values are therefore more appropriate. These differences were not statistically significant, but may suggest interesting trends. Large variability existed for most resting and post-exercise values. Conclusion: The paraplegic subject is an ideal model for the study of the influence of the SNS on blood supply to exercising skeletal muscle. The difference in CFA diameter at rest in the paraplegic vs. the AB group confirms previous results and is probably due to structural/non-physiological changes. Our observation that the BA and CFA diameters in EP and SP subjects do not differ significantly at rest, suggests that training does not have a spillover vasomotor effect on lower limb conduit arteries in paraplegia. Similar BF and PI values post-exercise in the SP and AB groups challenge the muscle pump theory. The SNS has an important role in the control of skeletal muscle blood flow – both at rest (vascular tone) and during exercise (redistribution). Suggestions for future research are made.

Abstrak

Die meganismes betrokke by die beheer van bloedvloei (BV) gedurende dinamiese oefening is nog onduidelik. Die parapleeg (P) het verminderde simpatiese innervasie na die onderste ledemate. Die huidige studie fokus op die bydrae van die simpatiese senuwee sisteem (SSS), as deel van die sentrale vaskulêre meganisme, tot skeletale spier BV tydens dinamiese oefening. Doelstellings: Ons het BV parameters in P vs. nie-gestremde proefpersone (kontrole) bestudeer om vas te stel of die parapleeg as model gebruik kan word om die bydrae van die SSS tot veranderings in die BV in aktiewe- en onaktiewe spiere gedurende oefening, te ondersoek. Verdere aspekte wat ondersoek is, sluit in: die invloed van fiksheidvlak ten opsigte van rustende en oefenings BV, of die verlammingsvlak by die parapleeg BV kontrole beïnvloed, die 'spierpomp-teorie' en sy hipotetiese rol in oefeninghiperremie, asook die vraag of bloedsaamstorting in die bene van parapleë plaasvind. Metode: Nie-indringende duplex Doppler studies van die groot geleidingsarteries (bragiaal [BA] en gemene femoral [CFA]) is by 10 elite paraplegiese atlete (EP), 10 sedentêre parapleë (SP) en 10 sedentêre nie-gestremde proefpersone (AB) uitgevoer. Die paraplegiese proefpersone is verder onderverdeel deur die vlak van T6 as kritiese verlammingsvlak te gebruik. Toetse is tydens rus en na 2 arm-ergometer oefeningsessies uitgevoer: een maksimale inkrementele toets en een van 75% van maksimum intensiteit. Deursnit, gemiddelde vloeispoed, pulsatiewe indeks en bloedvloei is gemeet en/of bereken. Resultate: Rustende hartspoed was beduidend hoër in die paraplegiese groepe (EP = 80 slae/minuut ± 10 en SP = 83 s/m ± 12) vs. die AB groep (69 s/m ± 7), $p < 0.05$. Rustende deursnit in die gemene femorale arterie (CFA) was dieselfde in EP (5.93 mm ± 1.54) en SP (6.52 mm ± 0.95), maar beduidend laer as in AB (7.87 mm ± 1.38), $p < 0.05$. Die feit dat rustende pulsatiewe indeks (PI) in die CFA dieselfde in albei

groepe was, laat twyfel ontstaan oor die veneuse bloedopdammings teorieë soos weergegee in die literatuur. Na-oefeningswaardes moet omsigtig evalueer word met inagneming van die groot rustende verskille in CFA deursnit. Persentasieverskil-waardes is dus meer toepaslik. Hierdie veskille was nie statisties beduidend nie, maar suggereer interessante tendense. Groot variasie het voorgekom vir beide rustende en na-oefenings waardes. Gevolgtrekking: Die parapleeg is 'n ideale model vir studies om die invloed van die SSS op bloedvloei aan aktiewe skeletale spier te bestudeer. Die verskil in rustende CFA deursnit in die parapleeg vs. die AB groep bevestig vorige resultate en is waarskynlik te wyte aan strukturele, nie-funksionele veranderinge. Ons bevindinge dat die BA en CFA deursneë nie beduidend verskil in die SP en EP groep gedurende rus nie, dui daarop dat gereëlde oefening nie 'n oorloop vasomotor effek op die onderste ledemate in die parapleeg het nie. Die feit dat daar geen verskil aangetoon kon word tussen BV en PI waardes na-oefening in die SP en AB groepe, betwis die spierpomp teorie.

Die studie toon dat die SSS 'n belangrike rol in die beheer van skeletale spier bloedvloei speel – beide met rus (vaskulêre tonus) en gedurende oefening (herdistribusie). Voorstelle vir toekomstige navorsing word gemaak.

Acknowledgements

My wife, Anél, and two children, Emma and Emile, deserve praise for allowing me to temporarily lay aside my fatherly duties to complete this thesis.

Professor Alan Scher, head of the Radiology Department at Tygerberg Hospital (TBH), for allowing the use of their duplex Doppler machine. Ms. J. Denby and Ms. S. Nelson, duplex sonographers at TBH, for their commitment while doing the Doppler measurements.

The Conradie Spinal Unit for supplying body straps and a table for the ergometer.

The University of Stellenbosch Research Sub-Committee B for financial assistance.

All the subjects who participated in this study, some of whom went to great lengths to participate in this trial.

The Almighty God who created us intricately and sustains us.

Professor Kathy Myburgh for her continued encouragement.

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List of abbreviations

SNS = sympathetic nervous system

BF = blood flow

EP = elite paraplegic

SP = sedentary paraplegic

AB = able-bodied controls

CFA = common femoral artery

BA = brachial artery

PI = pulsatile index

Vmean = mean velocity

Br/Fem = brachial-to-femoral ratio

HR = heart rate

CHAPTER 1

1. General introduction and study rationale

The supply competency of the vascular system determines the amount of blood made available to exercising muscle. Therefore, investigations of the control of the vascular system are important in the study of exercise physiology. The mechanisms of blood flow control to skeletal muscle during dynamic exercise are still not clearly understood (6, 13, 33, 48, 49, 53). Blood flow (BF) to a specific area is influenced by the quantity of muscle that is supplied, as well as the intensity of exercise of the specific muscle group (13, 53). Another factor that needs consideration is how the BF to inactive limbs influences the BF to active muscles and *visa versa*. On this specific issue very little documented data exist in the scientific literature (10, 13, 35, 53, 63).

The interacting mechanisms of BF control have been categorized as 1) central vascular mechanisms, 2) local vascular (muscle level) mechanisms and 3) the mechanical effects of muscle contraction itself (13, 20). The current study was designed to focus on the contribution of neural control, one of the central vascular mechanisms, to skeletal muscle BF.

The sympathetic nervous system (SNS), with spinal cord outflow from T1 to L2, is critical to the neural control of the vascular system. As part of the autonomic nervous system, the SNS regulates the function of numerous organs in the body, of which the vascular system is an important one (60). Some of the effects of the SNS are well described in the literature for their roles in preparing the body for an emergency ("fight-or-flight reaction"), namely: increase in heart rate, cutaneous and visceral vasoconstriction, skeletal muscle vasodilation and raised blood pressure (60). The

specific role of the SNS in regulating BF during exercise, however, is not clearly understood (5, 8, 13, 20, 32, 33, 46, 49, 52).

Current neuroanatomy and neurophysiology knowledge indicates that, similar to reactions to emergencies, the following SNS effects may influence cardiovascular changes during exercise (35, 60):

- Greatly increased cardiac function (inotropy),
- Increased heart rate (chronotropy),
- Alpha receptor stimulation (constriction), beta receptor stimulation (dilation) and cholinergic receptor stimulation (dilation) of the whole body vasculature, and therefore the muscle vascular bed, at rest and during exercise,
- Adrenal medulla stimulation (via neural input from T7 to T10) to increase circulating catecholamines.

The SNS is thus an important component of central vascular control and should be considered as critical in the regulation of BF to skeletal muscle. The SNS effects mentioned above are also influenced by a higher cerebral function, namely the limbic system. The limbic system is part of the emotional reaction response, which is mostly involuntary. It is localized in the middle of the cerebral hemispheres. This system, or its effects, cannot be measured quantitatively *in vivo* at present (60).

Animal- and single muscle (*in vitro*) experiments investigating exercise BF control are of limited significance to the exercising human as they do not allow for appropriate interaction of the whole nervous system and will therefore be reviewed in a limited way. Human studies on exercising individuals (*in vivo*) however, allow for more directly applicable conclusions to be made.

This study will use the paraplegic as a model to examine the SNS effect on exercising skeletal muscle BF. Paraplegia is defined as a condition where the function of the nervous system to the legs of an individual has been altered (3). The neurological level of paralysis in paraplegia is below T1 and in this study we only used complete post-traumatic paraplegics. The paraplegic subject has reduced sympathetic stimulation to the non-innervated vascular bed (below the level of injury), as well as absent motor- and sensory innervation of the paralysed skeletal muscles.

By comparing the BF in paraplegics to that in able-bodied subjects, it should be possible to probe the difference that non-innervation of the legs makes to BF (to both paralysed or non-paralysed muscle groups) at rest. Comparison of these two subject groups' differences in BF changes from rest to exercise will also probe the contribution of the SNS to BF during exercise to both active and inactive muscle groups. By including in the study design a group of sedentary paraplegics and a group of elite paraplegic athletes, the effect of fitness on resting and exercise BF values could be assessed. Furthermore, if the lesion level is at T6 or above, there is no adrenal gland innervation. The adrenal gland is the main origin of circulating catecholamines, adrenaline and noradrenaline. Therefore, comparing subjects with higher lesions to others with lower lesions, allows the study of the contribution of circulating catecholamines to exercise BF (3, 35, 44, 60).

Studies in wheelchair athletes may be considered a practical challenge because these subjects are wheelchair bound. They are however normally seated while exercising. Studying BF patterns to establish the role of the SNS during exercise in a stationary position is therefore not an unusual physiological challenge to these subjects (15).

We acknowledge from the outset that studying the influence of the SNS on skeletal muscle BF is a very complex field of research. This study therefore focused specifically on BF at the proximal feed/conductance artery level. The study design included several groups and subgroups and this should be taken into account when interpreting the results. Nevertheless, the study is a novel contribution to research in this field, especially considering that we chose to measure BF to both active and inactive limbs.

CHAPTER 2

2. Literature Review

The discussion of previous research of the SNS control of skeletal muscle BF will be presented under the following headings:

1) The influence of the SNS on the control of resting muscle BF, and

2) The effect of the SNS on the control of BF during dynamic exercise.

2.1 SNS Control - Resting Blood Flow:

Previous studies have shown that skeletal muscle blood flow in humans at rest is lower than that of coronary- and visceral blood vessels (49). This was shown to be due to the high vasomotor tone via sympathetic nerve activity (predominantly alpha-receptors) causing vasoconstriction to the limbs (49). BF parameters in paraplegic subjects have been studied mainly at rest (23, 26, 27, 55, 58). These studies identified the paraplegic subject as a model to study the effect of the loss of tonic vasoconstrictor control mechanisms below the level of injury.

As early as 1959, authors have used animal models and have reported increases in BF to resting muscle ranging from 50-100% after *removal* of the SNS influence by surgical denervation or with alpha-adrenergic blockade (21, 40). These authors hypothesized that the vasodilation (and the resultant increase in BF) resulted only from the loss of innervation of the affected muscle. The contribution of sympathetic tone at rest (basal vascular resistance) was thus thought to be an important factor influencing resting muscle BF. Various BF parameters in the inactive lower limb muscles have since been investigated in paraplegic subjects (22, 27, 47, 55, 56).

A markedly smaller resting common femoral artery (CFA) diameter in paraplegic- vs. able-bodied subjects has been reported (27, 47, 55). However, the habitual activity levels of the different subjects in some of these studies were not reported. Such findings, indicating anatomical differences in the vascular system in paraplegics, were thought to indicate changes due to differences in nervous innervation (21, 56). It is unclear however, whether these differences signify functional physiological changes, in response to reduced demand at the time of measurement, or rather structural changes in response to reduced demand over time. Structural changes do occur in the paraplegic's lower limb vasculature after prolonged inactivity due to the0 paralysis and the associated muscle atrophy, which obviously reduces the muscle blood supply required for adequate perfusion (43, 56). This makes the interpretation of results more complex.

In contrast, it was shown that the resting brachial artery diameter is larger in paraplegic athletes vs. sedentary able-bodied subjects (controls) (56). That study did not include sedentary paraplegic subjects making the results difficult to interpret, since the upper body activity levels differed substantially between the two groups. In fact, no data exist on brachial arterial diameter in habitually active vs. inactive paraplegics. A further complication of the study (56) is that other researchers have shown higher cardiorespiratory fitness levels in wheelchair athletes vs. both sedentary paraplegics and sedentary able-bodied individuals (11). Wheelchair athletes may thus exhibit training- and/or paraplegia-induced differences in vessel diameter. While the diameter of blood vessels is an important factor that contributes to BF, various authors have reported that it is preferable to measure BF itself (8, 27, 32, 36, 37, and 55).

Resistance against arterial flow is another indicator of vascular tone that has been investigated (8, 27, 32, 36, 37, 55). The term used for this is pulsatile index (PI). PI is

calculated from various blood velocity measurements. This reflects the fact that velocity under constant conditions is in itself not constant and it therefore refers to the range of velocity measurements relative to the mean velocity. Hopman *et al.* (1996) showed an increased pulsatile index (PI) in the CFA of a paraplegic group vs. controls at rest (27), thereby suggesting a higher resistance in the paraplegic lower limb vasculature. This finding has not been confirmed by other studies. The above result, together with the diameter results mentioned previously (47), should be interpreted with caution, as long-term disturbed vascular innervation and neurohormonal changes could both cause structural, nonphysiological changes to the non-innervated lower limb vasculature.

2.2 SNS Control - Dynamic Exercise Blood Flow:

Although BF changes during exercise have been studied extensively (6, 13, 33, 48, 49, 53), an important issue that is still inconclusive is: what factors are *mechanisms regulating* skeletal muscle BF during exercise? Although it is widely accepted that the regulation is multi-factorial, the study of the paraplegic subject (athlete and sedentary) during exercise may help to further clarify specifically the contribution of the SNS to the regulation of BF to active and inactive limbs.

The sympathetic nervous system has been reported to play an important role in regulating skeletal muscle BF during exercise as it contributes to the milieu that mediates the nett decrease in vascular resistance (vasodilation), specifically to the exercising muscle (3, 13, 49, 50). As with any other organ, the blood supply to skeletal muscle depends primarily on two factors: the perfusion pressure at the feed artery level and the calibre (size) of the resistance vessels, mainly at the muscular

level, also referred to as vascular "tone" (58). Previous studies that investigated cardiovascular changes in paraplegics focused on perfusion pressure.

In able-bodied individuals, it is known that the extent to which BF increases to exercising skeletal muscle during maximal exercise can reach up to 20-fold that of resting BF (from 1.2 l/min total muscle BF to 20-25 l/min when exercising a large muscle group) (53). This increased perfusion pressure necessitates skeletal muscle vasodilation to accommodate the increased need. To avoid a drop in blood pressure with such a shunting of blood to the muscle, vasoconstriction must take place in inactive as well as active muscle. If this were not the case, a cardiac output of 60 l/min would be needed to maintain the blood pressure (48). In general, it is believed that vasodilation occurs in the feed artery to the active muscle whereas vasoconstriction occurs in the feed artery to the inactive muscle and possibly part of the capillary bed in the active muscle. The amount of vasodilation and/or vasoconstriction occurring at the proximal feed artery level (brachial- and common femoral artery) is still uncertain.

A recent review has challenged the existence of sympathetic vasodilation in human muscle during exercise altogether (33). Nevertheless, others have refuted these claims in well-controlled trials, showing the separate contribution of sympathetic alpha-1, alpha-2 and beta-2 receptors to the autonomic control of muscle BF at different times during exercise (5-9, 46). The two above-mentioned arguments focused mainly on the muscular level, but it is widely accepted that the same principles apply at the feed artery level.

Results have shown that a paradoxical problem exists in the study of paraplegic subjects when physiological changes during exercise are investigated. It centers on the

neurological level of injury, which is critical in the nervous innervation of skeletal muscle BF. Below the T6 level, the innervation of the adrenal medulla (the source of circulating catecholamines) is intact. In studies on subjects with a level T6 and above (to assess the withdrawal of circulating catecholamines from contributing to exercise hyperemia), a limitation occurs. The fall-out of the sympathetic drive to the heart (T2 to T5) dramatically reduces exercise capacity in the high paraplegic (23, 24, 29, 35 57, 60, 63). The lack of standardization of the lesion level amongst different studies complicates the interpretation of the literature (2). Moreover, the target innervation at the precise level of T6 has not been adequately and unambiguously defined, even in the neuro-physiological literature. However, it is accepted that a lesion level below T6 ensures adrenal medulla innervation (3, 35, 60).

Bidart and Maury (1973) found no increase in lower limb vascular tone in response to upper arm exercise in spinal cord injured (SCI) subjects with lesions above T7, although the authors admitted some unreliability in their data due to artefacts (3). A study where both high and low lesion levels are represented, should therefore provide an optimal model to evaluate the influence of lesion level, and therefore circulating catecholamines, on BF to the legs of paraplegics. However, even with such a study design, care should be taken to take into account the confounding effect of maximal cardiac capacity.

Pulsatile index (PI), an indication of vascular resistance, might shed light on the extent of vascular differences between the paraplegic and able-bodied subjects, as well as between two paraplegic groups. A decrease in the PI during exercise signifies functional vasodilation (27). This calculated value could therefore be used to interpret vascular adaptation as a long-term response to paraplegia and its adaptability in acute response to exercise. Hopman *et al.* (1996) only reported increased resting PI values

of the CFA of paraplegics in a study where 10 paraplegics were compared to 10 able-bodied individuals (27). However, measurements during or immediately post exercise could have indicated whether functional vasodilation had taken place. This might have shown whether or not the differences at rest were due to anatomical changes or not. No other studies have, to our knowledge, reported on PI immediately post-exercise.

The contribution of the skeletal muscle vasculature itself to muscle BF during exercise is also important. Normally, rhythmic contraction of smooth muscle in systemic arterial blood vessels takes place. This is due to the pulsating action of arterial BF in both active and inactive muscle groups that is also transferred to the essentially non-muscular venous system. The inactive muscles' vasculature has been reported to contribute significantly to increased BF at exercise onset and to maintaining BF during exercise (33, 46). The term "muscle pump" is used in the literature to describe this phenomenon. Although used quite broadly in the literature, its practical implication is still unclear. Two sites of action are proposed. Firstly, the intrinsic "pump" action of the vascular smooth muscle of the proximal and distal arterial vascular bed in inactive muscle is transferred to the venous system improving vascular return to the right atrium. This is affected by the loss of sympathetic innervation of the vascular bed below the lesion level in paraplegics. Secondly, the increased outflow caused by voluntary, low-intensity contraction of "inactive" skeletal muscle (e.g. the arm muscles while running) increases the available blood supply to the active muscle. This is non-existent in the paraplegic and negligible in the able-bodied subject if contraction of the inactive muscle is prevented. The proposed absence of the "muscle pump" in paraplegics, according to previous authors,

results in blood pooling in paraplegic lower limbs (19, 27, 35, 39). Whether or not this affects BF to the active limb has not been proven.

Another factor that affects skeletal muscle BF is muscle fibre type. Innervation of the vasculature has been shown to be different in individuals with different muscle fibre types (1), and therefore muscle fibre type may influence muscle BF regulation. In the current study (sessile arm exercise), the main exercising muscle is the deltoid muscle. Muscle fibre type in the deltoid muscle has been shown to be similar in wheelchair basketball players and active able-bodied controls (not arm trained) (61), and should therefore not influence the results in the current model.

2.3 Previous Experimental Designs – Exercise Blood Flow:

Many different experimental designs have been used previously and have contributed specific information in some cases and generally applicable principles in others. Although no perfect study design exists, it is worthwhile to highlight some complications that arise with some designs.

In 1959, dilation of the cat femoral artery was described as part of the response to a hindlimb tetanic contraction (21). This was not affected by chronic lumbar sympathectomy, thereby suggesting that a) the SNS does not influence exercise hyperemia, and b) other factors related to exercise do influence CFA dilation. But, more recently, CFA diameter was found to have *no* relationship to either resting BF or peak BF to lower limb during knee extensor exercise (47). However, in a third study, CFA diameter has been found to correlate with peak pulmonary oxygen uptake during cycle ergometry (34). The status of training/fitness might have influenced the results in this study. Lash (1994) found that trained rats have significantly greater functional dilation in their feed artery to the exercising *spinotrapezius* muscle vs. sedentary rats

(37). Although all four studies mentioned were ostensibly investigating the same principle (response of the feed artery size during exercise) the differences in study design (animal models vs. single muscle exercise vs. more complex exercise) led to different conclusions. Also, the latter study investigated a more distal type feed artery. It has been stated that large arteries and arterioles are similarly innervated (49), which would still make these results relevant, although it has to be remembered that local vasoactive substances play an important role in distal artery vasodilation.

Of most relevance to the current study is to review other studies of paraplegic subjects and to comment on differences in study design with this subgroup of studies on BF. In humans, Hopman *et al.* (1996) showed unchanged BF in the CFA during arm exercise in paraplegics (27). In these subjects, the CFA diameter did not increase or decrease during arm exercise. The exercise protocol consisted of arm ergometry at 50% of maximal effort, which might be a sub-optimal intensity for assessing the "spillover effect" of arm exercise. The term spillover refers to the ability of the body to, via the SNS, influence the inactive muscle vasculature directly or indirectly (e.g. via circulating catecholamines) to vasoconstrict in order to enhance the blood supply to the active muscle. A different study of the blood volume distribution changes during arm exercise in paraplegics vs. able-bodied controls showed that the loss of sympathetically induced vasoconstriction in the paraplegic group caused an inability to redistribute leg BF (24). In this study, EMG (electromyography) readings of the legs showed no activity in both the P and AB groups. This proves that voluntary and involuntary lower limb muscle contraction can be totally prevented even in the able-bodied individual, thus strengthening the study design and adding weight to the conclusions.

Cardiac output (CO) is a very important contributing factor to the BF at proximal feed artery level, as differences in CO affect vascular function. No differences in CO have, however, been shown during exercise in paraplegic subjects vs. able-bodied subjects using the CO_2 rebreathing method (12, 22, 25). Nevertheless, significantly higher heart rates were reported in the paraplegic groups, necessitated by a lower stroke volume (secondary to decreased venous return) to keep the cardiac output equal to ensure adequate blood supply (11, 12, 21, 25, 63). A complication preventing assessment of these studies in more detail is the fact that the lesion levels of the subjects varied in each study. In view of the above, a further issue that is unclear is how the fitness levels of paraplegics influence their resting heart rates and their heart rate responses to maximal exercise. Only with a study design that includes sedentary paraplegics and paraplegic athletes with well-defined performance and fitness criteria can we begin to unravel the complexities of the heart rate responses to exercise in paraplegic subjects.

A decrease of forearm muscle BF during leg exercise observed by Johnson and Rowell (1975) is consistent with the theory that vasoconstriction takes place in the inactive muscle group to enhance the BF to the exercising muscle group (31). Other authors have not confirmed this. In search of an understanding of what influences human BF changes during exercise, investigators have also studied the differences in BF between two legs during one-leg and two-leg exercise (34, 36, 52). Leg BF was found, in one study, to be lower bilaterally during two-leg exercise vs. one-leg exercise (36). Although it is recognized that two large muscle groups were being investigated, the physiological principle that was elucidated is that oxygen delivery to one muscle group may be limited if another muscle group exercises simultaneously. However, the studies used small sample sizes and results need to be verified by

further investigations. Furthermore, it might seem this study design is not relevant to the current study design (arm ergometry does not use large muscles), but relevance becomes clear if one considers that BF redistribution may be suboptimal in paraplegic subjects during exercise. The one-leg vs. two-leg exercise testing study design has been expanded to a more complicated design where such testing is done both pre- and post one-leg training. Saltin *et al.* (1976) recorded bilaterally equal leg BF in trained and previously non-trained subjects when doing two-legged exercise after one-leg training (52). This might imply a spillover effect of circulating factors or a direct sympathetic adjustment of muscle BF irrespective of training status.

Shenbergen *et al.* (1990) demonstrated a larger brachial artery diameter at rest in paraplegic subjects (P) vs. able-bodied (AB) controls, as well as a greater hyperemic response to exercise in the P vs. AB (56). They concluded that habitual activity structurally dilated the large conductance vessels and enhanced the capability to vasodilate resistance vessels acutely. However, this would be better tested in two paraplegic groups with different activity levels, because changes due to habitual activity are more comparable using two paraplegic groups.

Vascular compliance (ability to change conformability) at rest, which is a direct measurement of the ability of a blood vessel to change shape during exercise, and CFA diameter were compared in endurance-trained (ET) vs. sedentary (S) vs. paraplegic (P) subjects at rest (55). Significant differences (highest in ET vs. S vs. P, $p < 0.01$) were found for both parameters amongst all three groups. In contrast, shear stress (indicative of *flow* resistance) was higher in the CFA in P vs. ET and S suggests a non-physiological response to local BF requirements due to decreased muscle mass and chronic disuse.

2.4 Method of Blood Flow Measurement:

The method of BF measurement is very important and although it should be comparable between different studies, this may not be the case. Blood flow measurements, since first reported in 1628 (by calculation from heart rate measurements), have been regularly refined (28). Until recently, plethysmography has been used to measure "blood flow" (3, 25, 35, 45). This method, at best, measures blood volume changes rather than blood flow. The method involves the placing of electrocardiogram (ECG) electrodes at different levels along a limb and detection of pulse volume takes place. These were then reported as "exercise hyperemia" (increase in volume) rather than blood flow (volume/time) changes. Pulsed Doppler, traditionally used in many radiological diagnostic investigations, has recently been improved by the use of the duplex Doppler, a faster and more accurate method of measuring BF (28, 51, 55) (see methods for detail). This method has improved BF research possibilities, especially research into BF changes during exercise. Although new in South Africa, it is currently available in most large health institutions.

2.5 Summary of Previous Research:

Current literature provides contradictory information on possible mechanisms underlying BF changes to active and inactive muscle groups from rest to exercise. There seems to be a great deal of uncertainty regarding the role of the nervous system, and specifically the sympathetic nervous system (SNS), in the regulation of skeletal muscle blood flow. It is suggested that skeletal muscle BF control at rest is dominated by tonic sympathetic vasoconstrictor control. Although the dynamic control of BF during exercise is vastly complex, a contribution of the SNS via proximal feed arteries is suggested, but evidence is inconsistent or even contradictory.

A few questions are still unanswered from the current literature. What is the extent of the SNS contribution to BF changes during exercise? Does fitness play a role in the SNS response to exercise? More specifically, what is the large conduit artery response to dynamic exercise in active and inactive muscle? Will a comparison between paraplegic and able-bodied subjects aid us in understanding BF changes in the exercising athlete? More specifically, will the near-simultaneous measurement of the brachial- and common femoral artery BF parameters at rest and during dynamic exercise in paraplegics shed any light on whether active and inactive muscle BF is regulated differently?

To answer these questions, duplex Doppler studies of the brachial- and common femoral artery in paraplegic and able-bodied subjects will be investigated. The quantitative study of BF in both the brachial- and the common femoral arteries in the exercising paraplegic has not been done before.

2.6 Aims of Current Study:

The main aim of this study is to evaluate the paraplegic as a model for assessing the contribution of the SNS to changes in active vs. inactive muscle BF during exercise. Further issues to be addressed include:

- The influence of level of fitness on resting and exercise BF;
- The effect of lesion level on BF control in the paraplegic;
- The muscle pump theory and its hypothesized role in exercise hyperemia;
- Whether blood pooling occurs in the legs of paraplegics.

CHAPTER 3

3. Methods

3.1 Subjects:

Thirty subjects were randomly recruited, after consideration of time since injury and lesion level (for details see below). The paraplegic athletic subjects were invited from local wheelchair basketball clubs, while sedentary paraplegic outpatients and able-bodied staff and medical students were invited from the Conradie Spinal Unit, Cape Town. The wheelchair basketball athletes had all represented their country as part of the national team.

The subjects were:

1. 10 sedentary able-bodied (AB) subjects;
2. 10 sedentary paraplegics (SP);
3. 10 elite paraplegic athletes (EP).

Lesion levels in the paraplegic groups ranged from T3 to L1 with all the lesions being complete traumatic spinal cord injuries. The paraplegic groups were matched for time since injury and lesion level. The duration of paralysis in both the paraplegic groups was a minimum of two years.

Transport needs must be considered in research involving wheelchair bound individuals, as well as access to the testing facility. Only some of the subjects had access to their own transport, while others were dependent on provision of transport

by the investigator. The testing venue, Tygerberg Hospital, was thoroughly assessed with regards to accessibility and found to be adequate.

Written informed consent was obtained from each subject after a detailed explanation of the exercise protocol and all the procedures. The ethics committee of the University of Stellenbosch Research Sub-committee B gave permission for the study.

3.2 Experimental Protocol:

All the subjects had a full physical examination by a spinal unit doctor to verify the lesion level and to exclude any underlying cardiovascular abnormality or any spinal injury complications. Exclusion criteria were: the presence of decubitus ulcers, the presence of spasticity or the use of medication for spasticity, urinary tract abnormalities (infection or autonomic dysreflexia), heterotrophic ossification and any recent or current illness. Testing was done under standard conditions with no smoking or ingestion of caffeine or alcohol for 24 hours pre-test.

Measurements taken at rest included: height, weight, upper arm circumference, blood pressure and heart rate.

An incremental arm crank ergometer (ACE)-test was performed using a Monark ergometer (model 881 E, Varberg, Sweden). The ACE-test was chosen above wheelchair ergometry, as mechanical efficiency and reliability has been reported to be superior in the first mentioned (4, 41, 54, 57, 64). The subjects were all seated in a stationary/immovable chair/wheelchair, strapped around the chest to prevent any abdominal muscle, hip- or leg movement, while the ACE-axis was set at shoulder height for each subject (16, 35). Arm crank frequency was set at 50 rpm, which was previously reported to be the most reliable (41, 54) and the power output was read as

watts. The ACE was calibrated according to prescribed instructions using a 2 kg weight suspension.

The duration of the maximal ACE-test varied as exercise capacity amongst the subjects differed. The appropriate starting power output for each subject was first determined by an estimated bench press capacity (< 50 kg = 5 W, 50 kg = 10 W, 60 kg = 20 W, > 60 kg = 30 W). The investigator subjectively assessed the subject's comfort at this workload during a 5-minute warm-up period to finally determine the starting workload. During the incremental test to maximum the incremental increases were 5 or 10 watts every three minutes depending on the starting power output and fitness. The maximal exercise test thus consisted of a power output-based protocol, terminated by the inability of the subject to continue (fatigue) (41). A 10-minute rest period (post maximal test) was followed by a three minute ACE-test at 75% of achieved maximal power output (steady state).

Venous blood from the cubital vein was taken for lactate analysis and placed in grey top tubes containing sodium fluoride (vacutainer). The content of the tube was thoroughly mixed, put on ice and spun down in a refrigerated centrifuge. Plasma was drawn off and stored at –70 degrees Centigrade for later analysis. An automated lactate analyzer (1400 Sport, YSI Inc., Yellow Springs, Ohio, USA) was used for the analysis. Heart rate was monitored continuously during the ACE-test using a heart rate monitor (Accurex Plus, Polar Electro, Kempele, Finland).

Ultrasound measurements of the right brachial- and common femoral artery were performed using the duplex Doppler system (Toshiba SSA-340, Tochigi-Ken, Japan). This is a completely non-invasive procedure. The probe was held at an angle of 60 degrees to the blood vessel (insonation angle). This is the prescribed insonation angle

for blood flow measurements (18, 28). The site for measurement of each artery was marked after the resting measurements to improve the speed of post-exercise measurements. The location of the artery was confirmed by the use of colour flow tracing. In short, colour flow tracing enables the sonographer to differentiate between an artery and a vein by the signal produced by either vessel: red for an artery and blue for a vein. The duplex refers to the combination of B-mode images with a traditional pulsed Doppler trace (28). This enables the researcher to study *in vivo* changes much sooner after the end of exercise and with more accuracy than was previously possible with the conventional Doppler apparatus. These techniques have been standardized and have been found very reliable, with systematic error of less than 6 % (18).

Post-exercise, the duplex Doppler measurement of the brachial artery was taken first as this was the active limb. The common femoral artery measurement was taken within one minute of the exercise terminating in all the subjects. Values measured by the Doppler apparatus were maximal velocity (Vmax), minimal velocity (Vmin), mean velocity (Vmean) [all in meters/second (m/s)] and diameter (D) in millimeters (mm).

From these measurements the following values were calculated:

1) Pulsatile index (PI) = (Vmax-Vmin)/Vmean (27), and

2) Blood flow (BF) (ml/min) = $\pi D^2/4$ x Vmean (51).

3.3 Statistical Analysis

Results are presented as mean ± standard deviation. Analysis of variance (ANOVA) (Number Cruncher Statistical Systems, Kaysville, Utah, USA) was used to compare

the three main groups. For non-parametric data the Kruskal-Wallis One-Way ANOVA was used. The Newman-Keuls Multiple-Comparison Test was used as the post-hoc test. The paired T-test was used to compare intragroup lesion level differences. Statistical significance was set at $p < 0.05$. Spearman Rank correlation was used to correlate dependant variables ($r = 0.355$ for $n = 30$ and $r = 0.602$ for $n = 10$, $p < 0.05$).

CHAPTER 4

4. Results

4.1 Subject Characteristics:

All three groups had similar body weight (SP = 83 ± 11 kg, EP = 80 ± 6 kg and AB = 80 ± 4 kg) and height (SP = 174 ± 7cm, EP = 175 ± 12 cm and AB = 182 ± 11 cm). For a summary of the subject characteristics see Table 1.

Table 1. Subject characteristics.

	SP	EP	AB (controls)
Lesion level	T3 – L1	T4 – T12	
Age (years)	35 ± 10	26 ± 6	29 ± 7
Time since injury (years)	9 ± 6	11 ± 6	
Resting heart rate (bpm)	80 ± 10 *	83 ± 12 *	69 ± 7
Resting blood pressure (mmHg)	122 ± 16 (SBP) 83 ± 11 (DBP)	123 ± 14 (SBP) 80 ± 6 (DBP)	118 ± 8 (SBP) 80 ± 4 (DBP)
UAC (cm)	31 ± 4	32 ± 2	30 ± 3

Footnotes: SP = sedentary paraplegic, EP = elite paraplegic, AB = able-bodied subjects, bpm = beats per minute, UAC = upper arm circumference, SBP = systolic blood pressure, DBP = diastolic blood pressure. * Different from AB, $p < 0.05$ (ANOVA).

All the paraplegic subjects were completely paralysed below the mentioned level.

For the maximal incremental exercise test, starting power output was similar amongst all the groups (SP = 14 ± 6 W, EP = 14 ± 7 W and AB = 18 ± 6 W). Maximum workloads, an indication of power, were significantly different (SP = 39 ± 15 W vs. EP = 51 ± 16 W and AB = 60 ± 16 W) ($p < 0.05$). There was also a significant

difference between the groups in the total workload change from start to exhaustion (SP < EP & AB, $p < 0.05$) (see Fig. 1). This was used as an indication of endurance.

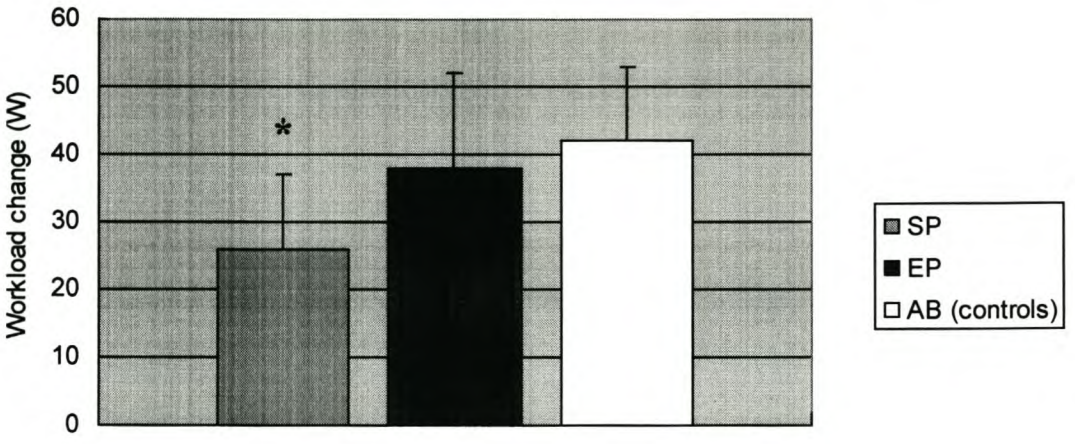

Figure 1. Endurance capacity (maximum workload – starting workload) during incremental arm crank ergometer (ACE)-test.

Footnotes: W = watts. * SP < EP and AB, $p < 0.05$. See Table 1 for abbreviations.

The results of all the blood flow parameters will firstly be presented in two sections by dividing the results into resting- and post-exercise data. This will then be followed by the results after dividing the paraplegic groups by lesion level.

4.2 Resting values:

Resting heart rate differed significantly between the paraplegic and the able-bodied subjects ($p < 0.05$) (see Table 1). All the measured and calculated resting brachial artery values (diameter, pulsatile index, mean velocity and blood flow) were similar between the groups (see Table 2). There was a positive correlation between the brachial artery diameter at rest and UAC in all the groups ($r = 0.42$, $p < 0.05$, $n = 30$).

Table 2. **Characteristics of the blood supply (Brachial Artery) to the upper limb at rest.**

Brachial Artery:	**SP**	**EP**	**AB**
Diameter (mm)	4.0 ± 0.8	4.1 ± 0.7	3.7 ± 0.3
Pulsatile index	4.2 ± 1.2	4.2 ± 0.9	3.4 ± 1.4
Vmean (m/s)	0.21 ± 0.09	0.23 ± 0.03	0.30 ± 0.12
Blood flow (ml/min)	260 ± 130	297 ± 75	279 ± 115

Footnote: Vmean = mean velocity, mm = millimeter, m/s = meter per second, ml/min = millimeter per minute.

In the lower limb PI and Vmean were similar in all the groups (see Table 3). Resting diameter however was significantly lower in EP and SP vs. AB subjects (see Fig. 2).

Table 3. **Characteristics of the blood supply (CFA) to the lower limbs at rest.**

CFA:	**SP**	**EP**	**AB**
Pulsatile index (PI)	5.4 ± 1.8	4.8 ± 1.4	5.3 ± 1.7
Vmean (m/s)	0.16 ± 0.07	0.21 ± 0.11	0.16 ± 0.06

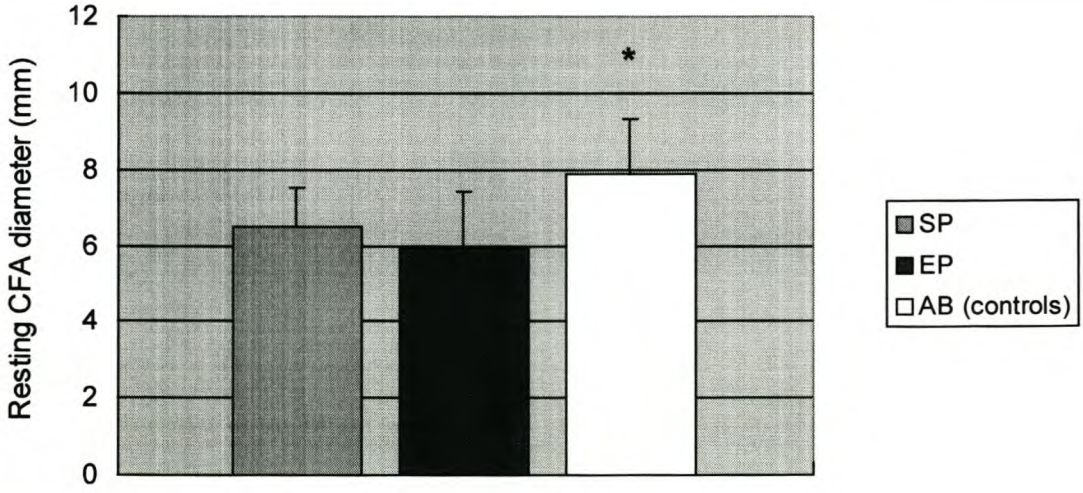

Figure 2. **Structural characteristics of the common femoral artery (CFA) at rest in the sedentary paraplegic (SP), elite paraplegic (EP) and able-bodied subject (AB).**

Footnote: * AB > SP & EP, $p < 0.01$.

Despite above, the resting blood flow (BF) in the CFA was not significantly different, although a trend was observed for SP to be lower than AB, $p = 0.15$ (see Fig. 3). Throughout the study there was large variability in the BF values.

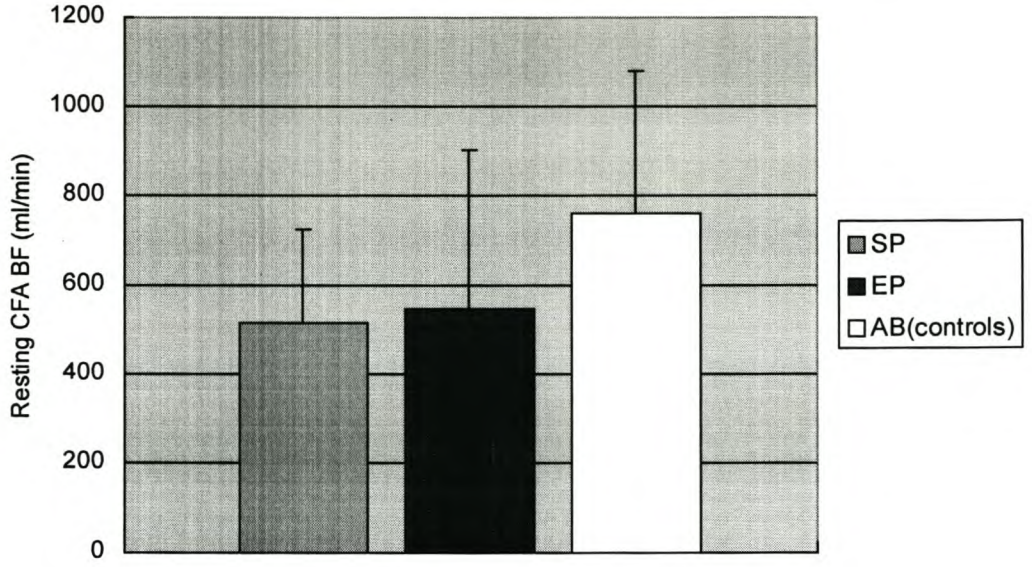

Figure 3. Resting common femoral artery (CFA) blood flow (BF) in sedentary (SP)-, elite paraplegics (EP) and able-bodied controls (AB).

We calculated the ratio of brachial-to-femoral BF to determine whether the relative BF in innervated to non-innervated arteries was similarly variable. The resting brachial-to-femoral artery ratios (Br/Fem) for Vmean and PI values (see Table 4) were not significantly different amongst the three groups. The resting Br/Fem diameter however differed significantly between SP and EP vs. AB ($p < 0.01$) (see Fig. 4), indicating that in AB there is a greater capacity to regulate vessel size differently between upper and lower limb at rest.

Table 4. Resting brachial-to-femoral artery ratios for mean velocity (Vmean) and pulsatile index (PI).

	SP	EP	AB
Vmean	1.41 ± 0.55	1.29 ± 0.80	1.75 ± 0.67
PI	0.80 ± 0.19	0.94 ± 0.36	0.65 ± 0.21

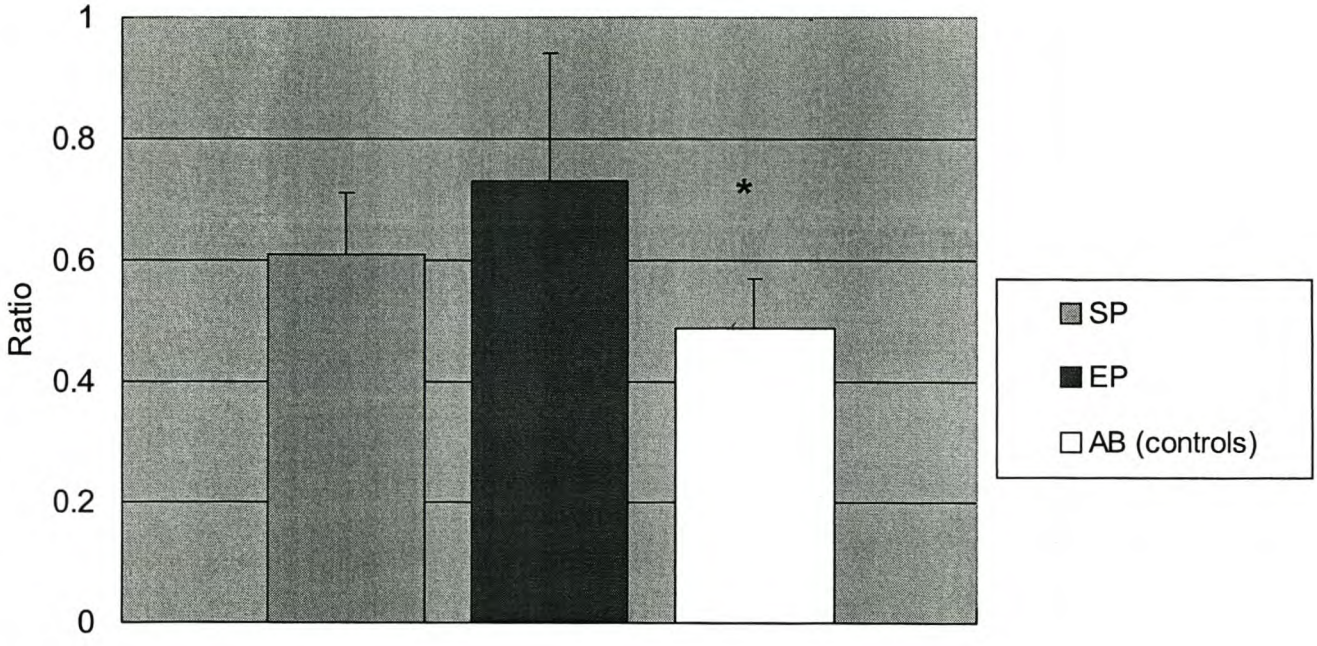

Figure 4. Resting brachial-to-femoral (Br/Fem) diameter ratio.

Footnote: * AB < SP and EP, $p < 0.01$.

Despite the structural differences indicated above, the brachial-to-femoral (Br/Fem) BF ratio was not significantly different ($p = 0.5$) (see Fig. 5).

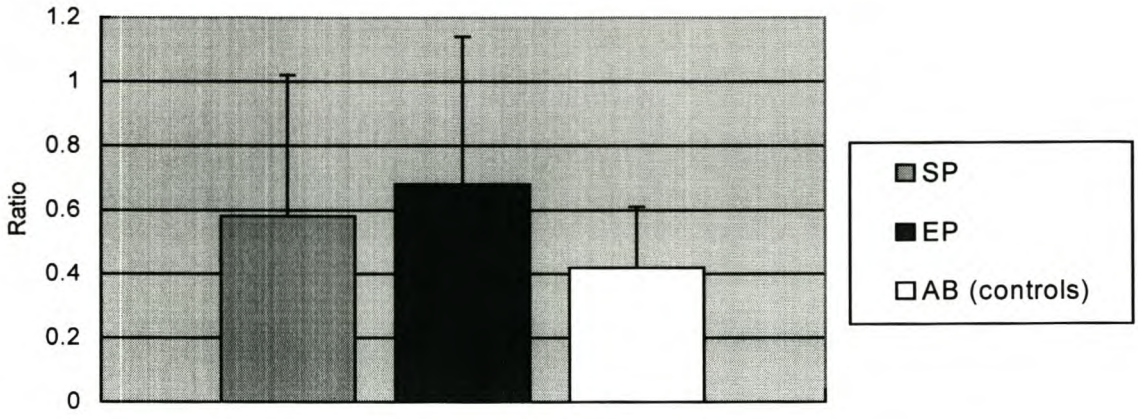

Figure 5. **Resting brachial-to-femoral (Br/Fem) blood flow (BF) ratios.**

Large standard deviations were observed in both groups. Above results showed AB to have smaller variation in the resting brachial-to-femoral BF ratio values. There was no correlation between any of the subject characteristics (i.e. upper arm circumference) and resting Br/Fem diameter or BF ratios.

4.3 Post-Exercise Test:

Heart rate (HR) at the end of maximal exercise differed significantly ($p < 0.01$) between both the paraplegic groups vs. the AB group (SP = 163 ± 25 beats/min, EP = 172 ± 16 beats/min vs. AB = 144 ± 18 beats/min). The HR reached during the subsequent submaximal exercise test at 75 % of maximal workload was also significantly different (SP = 147 ± 25 beats/min, EP = 155 ± 21 beats/min, AB = 128 ± 15 beats/min) ($p < 0.01$). The increase in HR from resting to maximal exercise (HR reserve) however was similar amongst the groups (SP = 83 ± 28 beats/min, EP = 90 ± 19 beats/min, AB = 75 ± 21 beats/min). If expressed as a percentage of the maximal HR, the mean HR reserve was almost identical between the groups (SP = 50 ± 11%,

EP = 52 ± 8%, AB = 51 ± 9%). Mean lactate values (post-maximal exercise) were similar amongst the groups (SP = 7.6 ± 1.6 mmol/l, EP = 9.2 ± 2.8 mmol/l, AB = 8.4 ± 1.6 mmol/l).

Maximal and submaximal PI, diameter and BF were similar amongst the groups for the brachial artery, but brachial artery (BA) Vmean at the end of maximal exercise was significantly less in the SP (0.55 ± 0.22 m/s) vs. EP (0.80 ± 0.26 m/s) and AB (0.80 ± 0.23 m/s) ($p < 0.05$). However, if these values were standardised against the workload change, the significance disappeared (SP = 0.015 ± 0.008 m/s/W, EP = 0.016 ± 0.007 m/s/W and AB = 0.012 ± 0.007 m/s/W). Standardising the maximal brachial Vmean against maximal watts achieved, irrespective of starting watts, gave the same result (SP = 0.015 ± 0.006 m/s/W, EP = 0.016 ± 0.005 m/s/W and AB = 0.013 ± 0.005 m/s/W).

The maximal CFA diameter was highly significantly smaller in the two paraplegic groups ($p < 0.001$) (see Fig. 6). The maximal exercise values for PI and Vmean (see Table 5) and BF (see Fig. 7) were not significantly different between the groups.

Table 5. Maximal CFA mean velocity (Vmean) and pulsatile index (PI).

	SP	EP	AB
Vmean (m/s)	0.21 ± 0.09	0.25 ± 0.14	0.20 ± 0.05
PI	6.3 ± 2.4	6.9 ± 2.5	6.6 ± 1.0

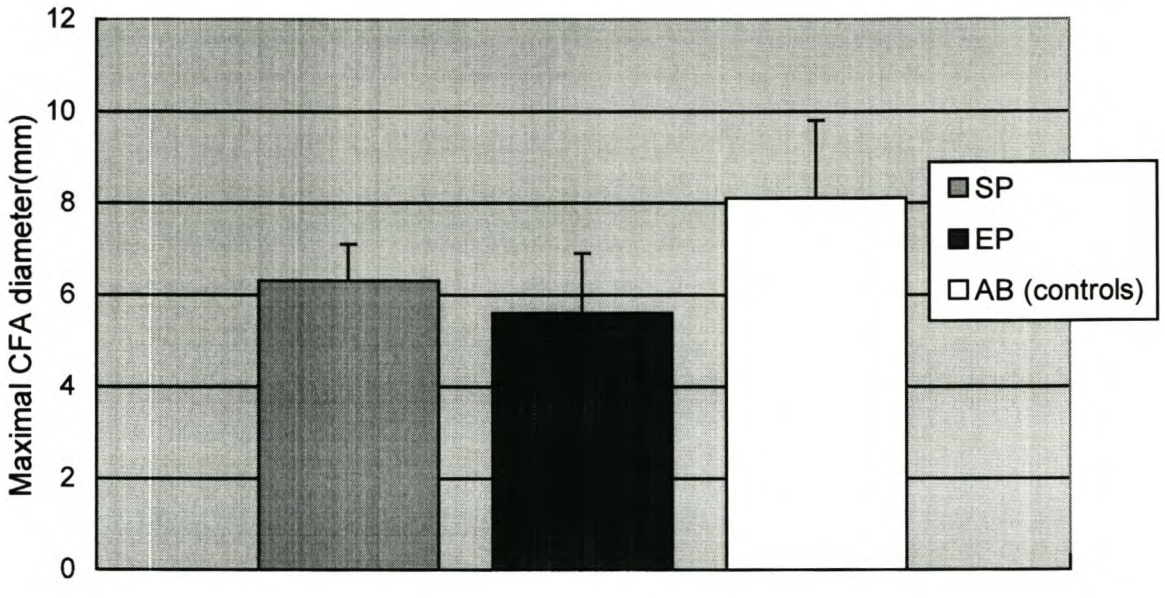

Figure 6. Common femoral artery (CFA) diameter immediately after maximal exercise.

Footnote: * SP and EP < AB, $p < 0.001$.

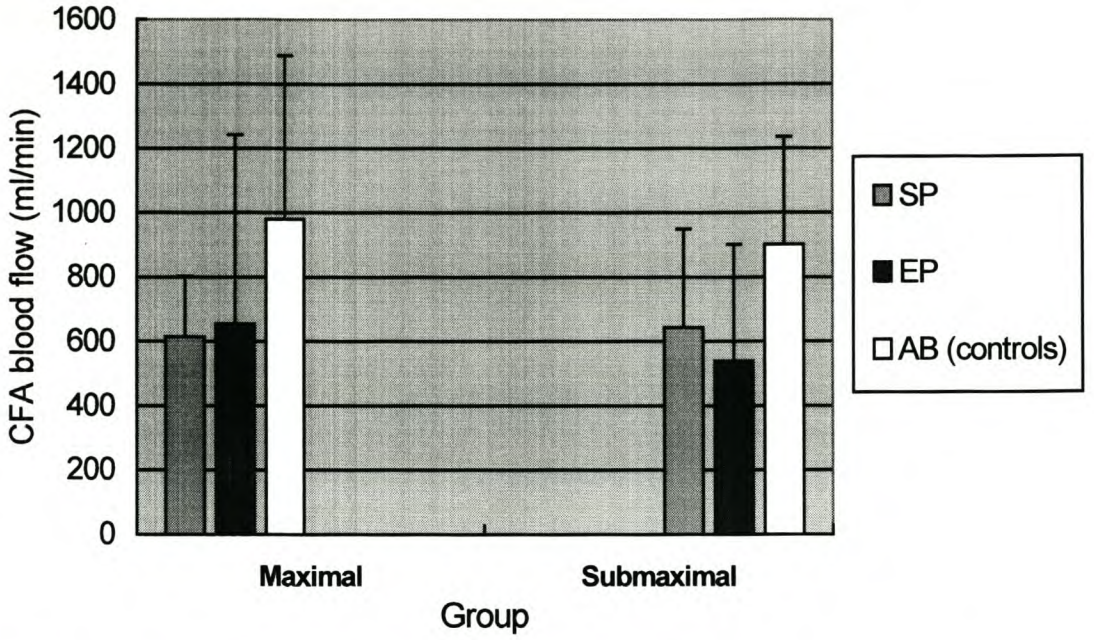

Figure 7. Maximal and submaximal common femoral artery (CFA) blood flow (BF).

The practical significance of the absolute maximal values for BA and CFA diameters is of limited value since the resting values were so different. Hence, the dynamic changes in these values from rest to post-exercise were compared. The *changes* in the different parameters from rest to either post-maximal or post-submaximal exercise test were however not statistically significantly different, except for maximal BA diameter, amongst the groups for both BA (see Table 6) and CFA (see Table 7).

Table 6. Change in diameter (D), pulsatile index (PI), mean velocity (Vmean) and BF from rest to maximal and submaximal exercise in the brachial artery (BA).

	SP	EP	AB
D (mm) (max)	0.66 ± 0.41	0.34 ± 0.45 *	0.88 ± 0.50
D (mm) (submax)	0.52 ± 0.34	0.32 ± 0.48	0.60 ± 0.78
PI (max)	-1.9 ± 1.6	-2.7 ± 1.0	-1.9 ± 1.4
PI (submax)	-1.7 ± 1.5	-2.5 ± 0.9	-1.7 ± 1.4
Vmean (max) (m/s)	0.34 ± 0.17	0.58 ± 0.28	0.51 ± 0.28
Vmean (submax) (m/s)	0.27 ± 0.18	0.43 ± 0.15	0.34 ± 0.21
BF (max) (ml/min)	730 ± 474	970 ± 580	1036 ± 520
BF (submax) (ml/min)	512 ± 270	723 ± 424	665 ± 530

* EP < SP and AB, $p < 0.05$.

Table 7. Change in pulsatile index (PI), diameter (D), mean velocity (Vmean) and BF from rest to maximal and submaximal exercise in the common femoral artery (CFA).

	SP	EP	AB
PI (max)	0.9 ± 3.5	2.1 ± 2.2	1.3 ± 1.5
PI (submax)	0.3 ± 2.5	2.4 ± 3.0	0.5 ± 1.6
D (max) (mm)	-0.20 ± 0.29	-0.31 ± 0.73	0.18 ± 1.02
D (submax) (mm)	-0.10 ± 0.45	-0.06 ± 0.48	0.40 ± 1.18
Vmean (max) (m/s)	0.05 ± 0.08	0.03 ± 0.14	0.03 ± 0.06
Vmean (submax) (m/s)	0.03 ± 0.07	-0.02 ± 0.09	0.01 ± 0.05
BF (max) (ml/min)	98 ± 209	61 ± 487	219 ± 323
BF (submax) (ml/min)	127 ± 282	-54 ± 315	141 ± 192

As was reported with the resting measurements, the brachial-to-femoral artery *ratios* for both the post-exercise values (maximal and submaximal) and rest to post-exercise change values for Vmean, diameter, PI and BF were calculated (see Table 8). There were however no statistically significant differences demonstrated amongst the groups with these calculated parameters.

Table 8. Change in pulsatile index (PI), diameter, mean velocity (Vmean) and blood flow (BF) when the brachial-to-femoral artery (Br/Fem) ratio is considered.

	SP	EP	AB
PI (max)	5.1 ± 16.8	2.6 ± 6.9	-1.2 ± 1.5
PI (submax)	-0.2 ± 1.7	-0.4 ± 3.7	2.6 ± 8.6
Diameter (max)	0.7 ± 7.1	0.5 ± 3.9	-2.0 ± 5.1
Diameter (submax)	-0.4 ± 2.2	-0.7 ± 2.4	-3.7 ± 13.6
Vmean (max)	5.1 ± 6.8	11.6 ± 17.6	15.6 ± 28.2
Vmean (submax)	-9.2 ± 21.8	-3.4 ± 9.5	6.0 ± 22.3
BF (max)	5.2 ± 7.0	-41.4 ± 131.5	16.4 ± 31.1
BF (submax)	7.5 ± 27.6	-8.6 ± 14.9	-0.2 ± 8.2

Percentage change, which is subject specific was calculated in each artery for Vmean, diameter and PI (see Table 9 and 10) and BF (see Fig 8a and 8b).

Table 9. Percentage change in pulsatile index (PI), diameter and mean velocity (Vmean) from rest to maximal and submaximal exercise in the brachial artery (BA).

	SP	EP	AB
PI (max)	-47 ± 31 %	-64 ± 11 %	-64 ± 11 %
PI (submax)	-40 ± 30 %	-59 ± 10 %	-40 ± 28 %
Diameter (max)	18 ± 13 %	9 ± 13 %	24 ± 15 %
Diameter (submax)	14 ± 10 %	8 ± 14 %	17 ± 23 %
Vmean (max)	170 ± 106 %	263 ± 144 %	243 ± 174 %
Vmean (submax)	136 ± 101 %	197 ± 82 %	166 ± 137 %

Table 10. Percentage change in pulsatile index (PI), diameter and mean velocity (Vmean) from rest to maximal and submaximal exercise in the common femoral artery (CFA).

	SP	EP	AB
PI (max)	35 ± 85 %	48 ± 55 %	34 ± 46 %
PI (submax)	20 ± 56 %	48 ± 48 %	15 ± 26 %
Diameter (max)	-3 ± 5 %	-4 ± 10 %	2 ± 13 %
Diameter (submax)	-1 ± 7 %	-1 ± 9 %	6 ± 16 %
Vmean (max)	49 ± 90 %	24 ± 58 %	31 ± 52 %
Vmean (submax)	44 ± 73 %	-5 ± 36 %	18 ± 48 %

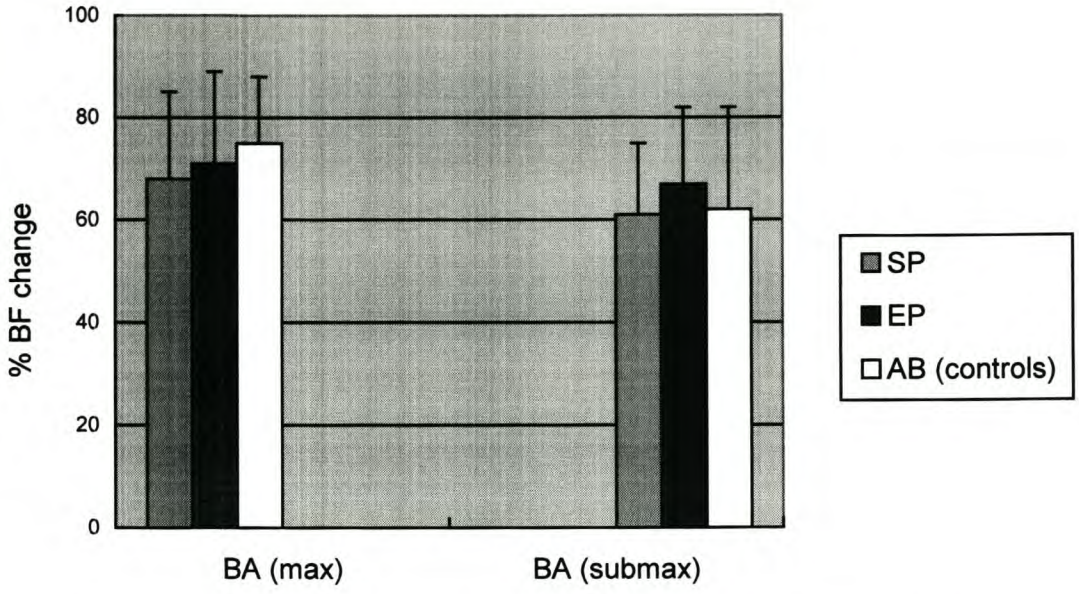

Figure 8a. Percentage change in blood flow (BF) in the brachial artery (BA) at maximal and submaximal exercise intensity.

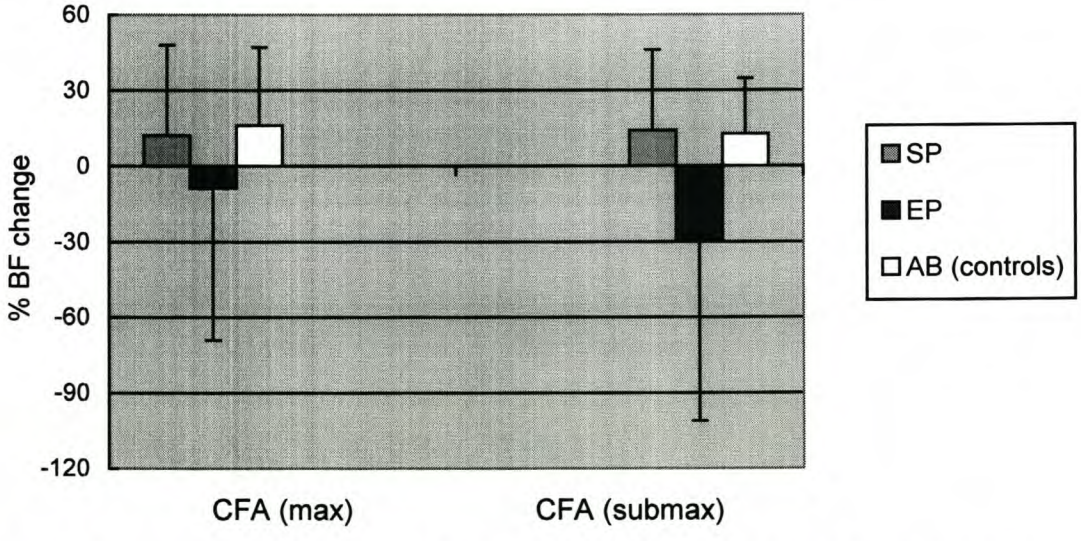

Figure 8b. Percentage change in blood flow (BF) in the common femoral artery (CFA) at maximal and submaximal exercise intensity.

No statistically significant differences were identified when using the percentage change. Changes in BF were very variable between subjects, but only in the EP was

the CFA mean percentage change negative (see Fig. 8a and 8b). Large standard deviations however occurred.

The percentage change in BF during maximal and submaximal exercise in all the groups had a significant negative correlation to the percentage change in PI for both the CFA ($r = -0.69$, $p < 0.05$, $n = 30$) and BA ($r = -0.57$, $p < 0.05$, $n = 30$). Separation of the groups did not change this phenomenon or the p-value of the correlations.

A critical question however that remains is whether and how the lesion level affected these results.

4.4 Lesion Level:

The level of T6 was used to divide the paraplegic subjects into subgroups (T6 and above = high lesion level, below T6 = low lesion level). All the parameters were compared amongst the four groups using a non-parametric statistical analysis (Kruskal Wallis). The SP group had 4 subjects with high lesion levels and 6 with low lesion levels, while the EP group had 5 subjects with high lesion levels and 5 with low levels. When lesion level was considered, the characteristics of the subjects showed no new statistically significant information (except that previously observed in the three groups). Therefore, only selected data are presented below.

There were no differences in the resting HR or post-exercise HR responses between the paraplegic groups regardless of level (see Table 11). A notable exception was the maximal HR achieved of a T3-level SP subject who only reached 121 beats/minute. This was expected as the sympathetic innervation to the heart is compromised above T4.

The resting and post-exercise vascular parameters did not differ statistically when lesion level was considered, although certain trends were identifiable in the diameter and BF parameters (see Fig. 9 and 10).

Table 11. Resting heart rate (HR), maximal HR and HR reserve (all in beats per minute) for high (T6 and above) and low (below T6) lesion level paraplegic subjects.

	Resting HR (bpm)	HR max (bpm)	HR reserve (bpm)
High level SP	74 ± 12	158 ± 33	83 ± 44
Low level SP	83 ± 9	166 ± 20	83 ± 16
High level EP	74 ± 14	180 ± 21	106 ± 8
Low level EP	83 ± 13	174 ± 18	91 ± 23

Footnote: If T3 paraplegic was removed, the resting HR (70 ± 10) and HR max (170 ± 28) was not significantly different for high-level SP.

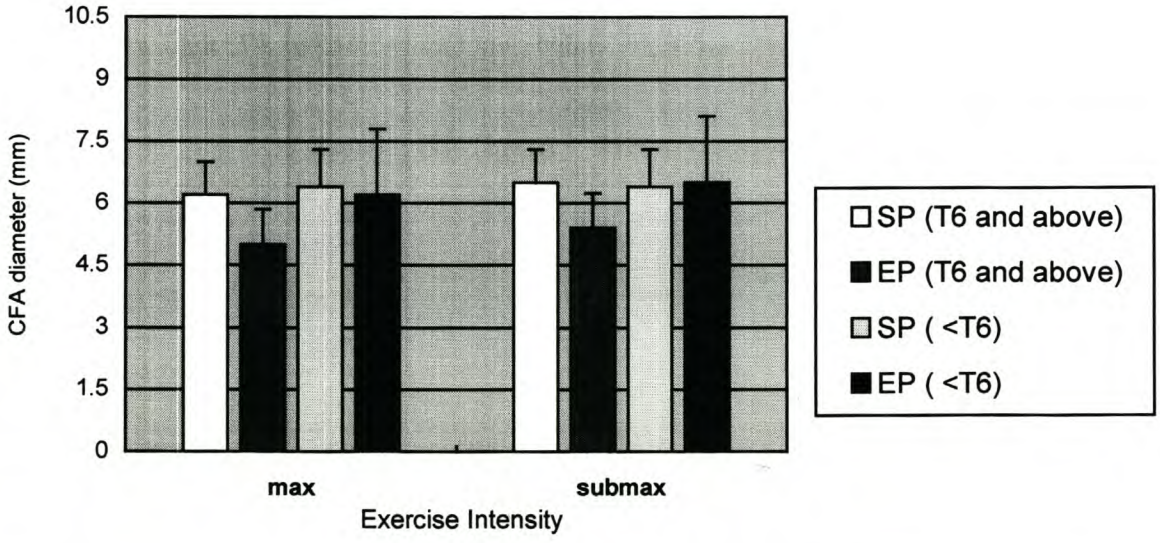

Figure 9. Common femoral artery (CFA) diameter after maximal (max) and submaximal (submax) exercise for high- (T6 and above) and low- (below T6) level paraplegic subjects.

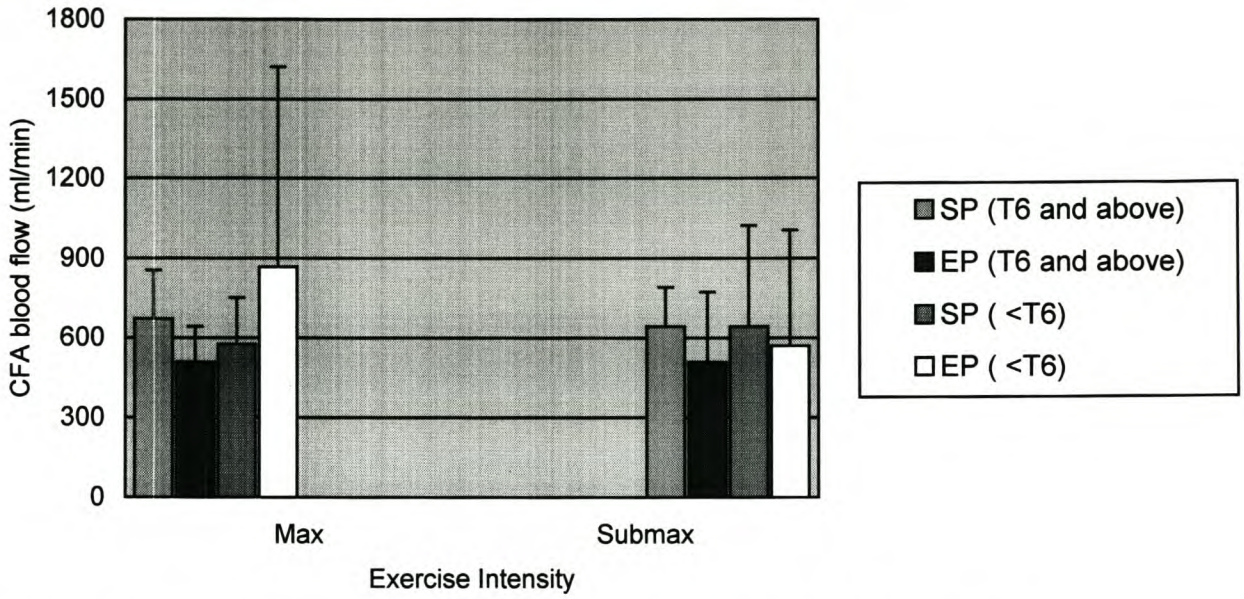

Figure 10. Common femoral artery (CFA) blood flow (BF) at maximal (max) and submaximal (submax) exercise intensity for high- (T6 and above) and low- (below T6) level paraplegic subjects.

The greatest variability occurred in the low-level EP group. There was no correlation between BF and maximal work performed in this group ($r = 0.42$, $n = 10$).

To negate the influence of the smaller resting CFA diameter in the paraplegic groups, it is imperative to compare the percentage change that occurred in both the vessels (see Table 12 and 13).

Table 12. Percentage diameter change in the CFA and BA for high and low lesion levels at maximal and submaximal exercise intensity.

	CFA (max)	CFA (submax)	BA (max)	BA (submax)
SP (≥T6)	-6 ± 4 %	-1 ± 8 %	11 ± 7 %	11 ± 5 %
EP (≥T6)	-7 ± 8 %	9 ± 7 %	16 ± 3 %	14 ± 17 %
SP (<T6)	-1 ± 4 %	-1 ± 8 %	22 ± 14 %	16 ± 14 %
EP (<T6)	-2 ± 11 %	-2 ± 7 %	10 ± 17 %	6 ± 16 %

Table 13. Percentage BF changes in CFA and BA for high and low lesion levels at maximal and submaximal exercise intensity.

	CFA (max)	CFA (submax)	BA (max)	BA (submax)
SP (≥T6)	4 ± 30 %	-2 ± 18 %	201 ± 87 %	108 ± 32 %
EP (≥T6)	-4 ± 48 %	3 ± 51 %	334 ± 226 %	256 ± 126 %
SP (<T6)	69 ± 124 %	65 ± 72 %	331 ± 204 %	272 ± 152 %
EP (<T6)	32 ± 57 %	14 ± 31 %	367 ± 268 %	264 ± 239 %

It is clear from above results that large variability again occurred in the diameter and blood flow changes in both the large conductance vessels. When the brachial-to-femoral ratio (Br/Fem) was calculated, no remarkable differences were identified when lesion level was taken into account.

Duration of Lesion: The paraplegic subjects were empirically divided into two categories with the cutoff at ten years duration. No definite cutoff for stabilization of physiological indices in paraplegia exists in the literature. No significant differences were found for any of the measured indices by separating the subjects into two categories of 1) 10 years and above, and 2) below 10 years duration of paraplegia (or even if any other elapsed time frame was used).

Chapter 5

5. Discussion:

No clarity exists on the role of the sympathetic nervous system (SNS) in regulating blood flow during exercise (1, 6). Authors even challenge the notion that the SNS is in fact involved in exercise induced BF regulation (17, 33). The current study had certain clearly identifiable strengths and weaknesses. To our knowledge, no data exist comparing BF in *upper and lower* limb conduit/proximal feed arteries in athletes. In the current study we measured near-simultaneous BF parameters at rest and immediately after exercise in the brachial and common femoral artery in paraplegic and able-bodied subjects in search of clarifying the role of the SNS in BF regulation. The majority of this discussion will focus on the extent to which we could achieve this objective.

Secondly, we aimed to investigate two influences of the SNS, namely:

- The direct sympathetic control of the vascular system, which is reduced below the level of injury in all paraplegics, by comparing them to able-bodied subjects;

- The effect of catecholamines, the SNS's main circulating messengers, was assessed indirectly by comparing paraplegic subjects with different lesion levels. The level of T6 was the distinguishing level as it was previously identified as the critical level of adrenal innervation (35). By doing this, the subject numbers became relatively small, but still compare well to subject numbers in previous studies using paraplegic subjects (2, 12, 19, 23, 25, 27, 35, 55, 62, 63).

Thirdly, it is important to remember that the magnitude of the BF change in response to exercise is influenced by the size of the active muscle group (53). An important

issue in the current study design is the fact that the upper extremity muscle group was the active muscle group during exercise. This is a relatively small muscle group compared to the leg muscle group. Therefore, the magnitude of the BF changes during upper arm exercise would be smaller than would be the case if active lower extremity muscles were studied. The advantage of this model is that cardiac output is unlikely to be a limiting factor and that this model therefore truly investigates the capacity of the conduit vessels to supply an area with high demand by, either localized changes alone, or by also acutely adjusting characteristics in an opposing area with low demand. A disadvantage of the paraplegic model is that the leg vasculature in chronic paraplegia may undergo structural changes due to inactivity. Therefore BF values from rest to post-exercise must be interpreted very carefully. For theoretical purposes, it is worth mentioning here that a type of spinal cord injury occurs where the arms are paralysed while the legs remain innervated and functioning. This is described as a central cord syndrome and is usually the result of a cervical hyperextension injury (60). The available active muscle size to be studied in such a subject would thus be larger. Studying subjects with this kind of neurology could be very interesting. However, these injuries usually occur in elderly patients (50 – 70 years), which would be problematic due to poor maximal cardiac capacity.

Resting Values: Our study supports previous studies that found resting HR was significantly higher in both the paraplegic groups vs. the AB (controls) (22, 25, 63). The raised HR at rest in both paraplegic groups confirms the paraplegic's ability to increase cardiac frequency (intact cardiac innervation). The reason for the raised resting HR is thought to be a compensatory mechanism secondary to a reduced stroke volume (SV) to maintain the required cardiac output (CO). The reduced SV is due to the reduced venous return (12, 22, 25 63). This in itself suggests that the resting

sympathetic control (tonic vasoconstriction) in the lower limbs, necessary to keep the SV at an adequate level, is reduced or absent. Stroke volume is greatly influenced by both venous return and cardiac ejection fraction. However, the fact that cardiac function and proximal arterial supply was equal in all the subjects (similar resting blood pressures and limb BF), further strengthens the argument that it is a reduced SV that is different in paraplegics and that this is due to reduced sympathetic vasoconstrictor control of the lower limbs. Therefore, we are indeed able to say that our model probes the influence of the SNS on at least some parameters of the cardiovascular system.

The lower limb conduit vessel (CFA) diameter at rest was significantly smaller in the paraplegic than in the able-bodied group. The smaller vessel size however does not reduce the vasoconstrictor ability of a blood vessel. In the paraplegic, the smaller CFA diameter is accepted as being a pure anatomical/structural adaptation to inactivity (paralysis) (22, 56). A more significant finding of our study is that the much smaller resting CFA diameter in the paraplegic groups did not automatically translate into lower BF values. The *mean* and standard deviation for resting BF values in the CFA however do suggest lower BF in some of the paraplegic subjects (see Fig. 3). This might be due to the reduced muscle mass in the paraplegic groups. Quadriceps circumference is notoriously difficult to compare (especially in the paraplegic population), which precluded us from commenting on a correlation between muscle mass and CFA blood flow.

The similar resting brachial artery (BA) values demonstrated no obviously important differences. The similar resting BA diameter however, are contrary to Shenberger *et al.* (1990) who reported a larger brachial artery diameter in paraplegics athletes vs. AB sedentary subjects (56). This study however compared active paraplegics to

sedentary able-bodied subjects and is therefore not a scientifically sound design. Our results of similar BA values in sedentary paraplegics vs. the paraplegic athletes might suggest that resting vascular tone in the active muscle group is not influenced by fitness level. However, a further interesting observation was that the resting BA diameter had a positive correlation with upper arm circumference (UAC), but that UAC did not differ amongst any of the groups. This proves that the caliber of the feed artery is influenced by the size/amount of muscle supplied.

The brachial-to-femoral (Br/Fem) ratio was calculated to indirectly assess the relative contribution of the upper- and lower limb vasculature to venous return and to determine the flexibility of the cardiovascular system to preferentially redistribute the blood supply during exercise The higher the ratio, the larger the blood flow in the brachial artery relative to the common femoral artery. We found that, at rest, the common femoral artery blood flow was larger than that of the brachial artery despite the lack of demand for BF to the lower limb. The smaller resting Br/Fem ratios for diameter and blood flow (see Fig. 4 and 5) in able-bodied subjects vs. EP and SP suggest that the lower limb BF in the AB is satisfying the demand of a greater muscle mass, even at rest, and that it makes up a greater proportion of the overall cardiac output than in the paraplegic group. This might suggest that a relative sympathetic vasoconstriction in the legs of the able-bodied subject contributes to the available blood supply to the arms. These values should however be interpreted with caution as differences in resting CFA diameter (non-physiological) and mean resting CFA blood flow between the paraplegic and able-bodied groups (see Fig. 2 and 3) do contribute to Br/Fem differences.

The resting pulsatile index (PI) value, however, is an indication of vascular resistance and therefore more physiologically significant, given its highly dynamic nature. The

PI values were similar in all three groups at rest. This is contrary to results previously reported by Hopman *et al.* (1996), who showed a larger PI in the paraplegic subjects (27). This, together with the similar resting CFA BF values, challenges the theory of venous blood pooling. The mechanisms involved need some consideration: pulsatile index (PI) is influenced by arterial conformability (ability to vasoconstrict or –dilate) and venous return (22, 25, 37, 63). Both these factors are reduced in the paraplegic subject. It is my opinion that an inability of the paraplegic to vasoconstrict arteries below the level of injury (due to the sympathectomy), and a reduced venous return due to the non-innervated muscle mass may in fact be balancing each other out, resulting in similar PI measurements between the paraplegic groups and the AB controls at rest.

In summary, despite differences in endurance capacity and training status, the cardiovascular parameters measured in this study were similar in the SP and EP groups. Both paraplegic groups however differed in their resting HR from the AB controls. Characteristics of the upper arm blood supply did not differ between paraplegic and AB controls, but the paraplegic subjects have a reduced CFA diameter. Nevertheless, our BF and Br/Fem ratio results confirm previous studies. Similar PI values in our study however contradict previously reported differences in PI values between paraplegics and AB controls. No *quantitative* conclusions can thus be made from the resting measurements concerning the role of the SNS in BF to the limbs at rest, but the consistent finding of a raised resting HR in the paraplegic groups confirms its role in maintaining sufficient vascular tone for normal venous return. Fitness does not seem to affect resting vascular tone.

Post-exercise Values: An adequate intensity and duration of exercise was achieved during this experiment. This is confirmed by the similar HR reserve values in all three

groups, even if expressed as a percentage of maximal HR. The high lactate values, with relatively small standard deviations, are further proof of adequate exercise intensity necessary to assess exercise-induced changes in BF in all the subjects.

The absolute values for post-exercise measurements need to be interpreted with caution due to the lower *resting* CFA values in the paraplegic groups (especially in the resting diameter). Nevertheless, the results clearly indicate that the mean velocity of BF to the upper limb was less in the sedentary paraplegic group. This could be satisfactorily explained as being a result of a decreased demand for supply because of a lower work output in this group and was unlikely to have anything to do with the SNS. Redistribution of BF from the inactive muscle (lower limb) to the active muscle (arm) occurred to a certain extent in all the groups: PI (= vascular resistance) decreased in the BA, but increased in the CFA, although not significantly; diameter increased in the BA, but decreased in the CFA (see Table 6 and 7), although again not significantly.

Paraplegic post-exercise CFA diameter- and absolute *change* in diameter values is misleading as they follow on vastly smaller resting values. The percentage change values however, which allow for inter subject variability and are not influenced by the magnitude of the resting values, are more appropriate to interpret. There was still large variability in most results in all three groups. No previous authors have commented on possible reasons for the large variability. I will suggest some possible reasons for the large variability in the results at the end of this discussion under "Reasons for variability" (see page 57).

The percentage change values were not significantly different between any of the groups for measured or calculated parameters. A closer inspection of the percentage

change in vessel diameter however suggests an interesting trend. All the groups responded similarly in the upper limbs, but a negative *mean* percentage change in the CFA diameter occurred in both paraplegic groups during maximal and submaximal exercise, while a positive *mean* percentage change occurred in the AB group (see Table 10). CFA diameter percentage changes signify that the lower limb conduit vessel either increased in size (positive) or decreased in size (negative) while the upper limb was exercising. Given the large variability in response in all three groups with some subjects having positive change and others negative change, indicates that the classification of the subjects was not the main influence.

Johnson and Rowell (31) found arm BF to be increased during leg exercise in AB subjects. With arm exercise in the AB group, femoral BF would thus be expected to increase. It was further expected that the 'contribution' of the leg blood supply to boost the active arm muscle blood supply (31, 36, 53) in the AB control group (as indicated by CFA diameter and BF changes) would be greater than in the paraplegic groups, but this was not the case. Percentage change in BF was in fact similar in the AB and SP groups. The fact that circulating blood volume availability from the paraplegic lower limb is less than that of the AB might be a complicating factor in the interpretation of these results.

Muscle pump: The interpretation of the BF values in relation to the PI values enables us to comment on the contribution of the muscle pump. Whereas the BF values (in ml/min) give us an amount of flow over time, the PI values give us an indication of vascular resistance. Together, these two values give us a better indication of whether vasoconstriction or vasodilation has taken place during exercise, thereby enabling us to comment on the contribution of the lower limb vasculature to the upper limb blood supply. The measured/calculated values do not allow any conclusive interpretations to

be made. However, the mean percentage changes in the PI and BF values reveal interesting trends. The SP mean percentage changes of both PI and BF in both blood vessels (see Table 9 & 10, Fig. 8a & 8b) are almost exactly equal to the AB values. This implies that the non-innervated lower limb of the SP did not alter the supply to the active arm muscle groups. This challenges the muscle pump-theory (19, 27, 33, 35, 46, 53) in part. If an active muscle vascular bed pump mechanism existed, even in the inactive muscle group's vascular bed, the BA changes would be expected to be larger in the AB group vs. the paraplegic group. This was not the case. It is however recognized that, in our experiment, the measurements were taken post-exercise. The fact that the Doppler measurements cannot be done during exercise makes commenting on the contribution of the muscle pump at the onset of exercise impossible. The muscle pump has been advocated as being important in contributing to blood supply at the onset of exercise as well as maintaining adequate blood supply during exercise (33, 46). The contribution of the muscle pump during exercise, according to our findings, seems unlikely. The measurements taken directly post-exercise reflect changes occurring during exercise. These results showed no difference between the paraplegic and able-bodied groups in the current study.

The significant negative correlation between the changes in the percentage BF and percentage PI in all the groups (see Fig. 8a and 8b) confirms the indirect relationship that vascular resistance has to blood flow as reported by numerous authors (5-9, 46, 53). The fact that neither of the groups differed from each other in these values makes it unlikely that paraplegia is a major influence.

Influence of fitness: The comparison of the two paraplegic groups (a very fit elite athlete group and a sedentary group) was used to test the hypothesis that fitness level influences BF parameters. As mentioned earlier in the discussion, the significant

difference in the comparative amount of work performed by these two groups is proof that the fitness levels were indeed different. Surprisingly, none of the BF parameters differed significantly between the two paraplegic groups. There appears however to be a "diverging nature" in the BF responses to maximal exercise in the two groups. I use the term "diverging nature" to indicate an observation that the BF *changes* (brachial vs. common femoral) in the two paraplegic groups differ after maximal exercise. This trend is seen best when interpreting the means of the percentage BF changes in the separate arteries (see Fig. 8a and 8b): the direction of change in the CFA (reduction) of the EP group seems more pronounced than in the SP group, while the difference in the BA change (increase) is minimal in the EP vs. SP group. This can be seen clearly if the both arteries changes are charted together (see Fig. 11 and 12 below). This might suggest that the change in the lower limb conduit artery during maximal exercise is more pronounced in the EP vs. the SP. This again might imply that fitness sensitizes the vascular system to be more responsive to the changing blood supply demands during exercise. The mechanism responsible for the increase in the sensitivity of the vascular system might be the increase in neural feedback (muscle memory) via the SNS due to training The measured maximal values might however have been influenced by the different maximal exercise workloads achieved by the two paraplegic groups. This problem was overcome by the use of the steady state (75% of maximal exercise) submaximal test, which equalizes effort. This produced consistently parallel results to the maximal values in all the measurements (see Fig. 8a and 8b) and therefore strengthens the above argument.

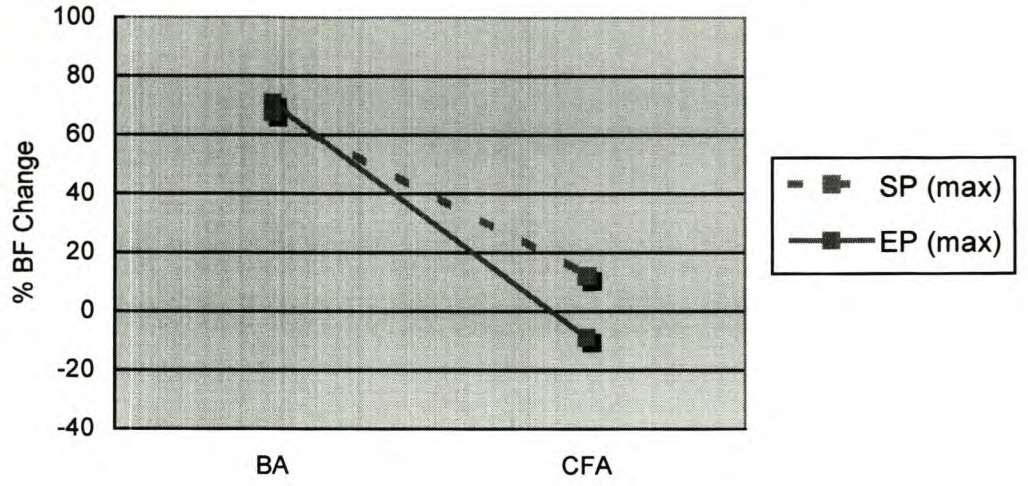

Figure 11. Mean percentage BF changes in the brachial artery (BA) and common femoral artery (CFA) after maximal exercise.

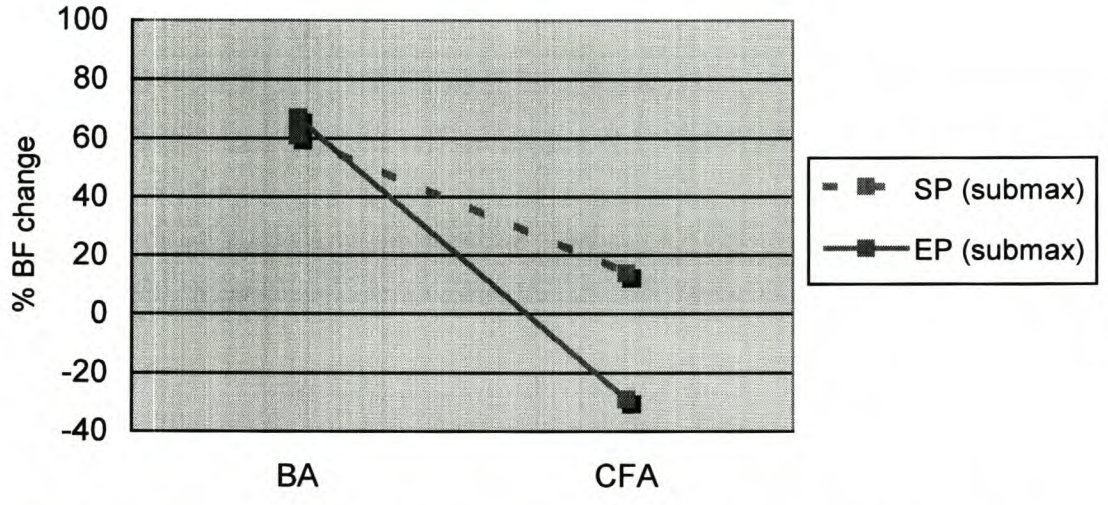

Figure 12. Mean percentage BF changes in the brachial artery (BA) and common femoral artery (CFA) after submaximal exercise.

Lesion level: Another important factor that was addressed in our experiment was the evaluation of the effect of lesion level on BF parameters to establish the role of circulating catecholamines during exercise. The level of T6 was used as the critical level, as a level of below T6 meant that the adrenal gland was innervated. Although the subject numbers are small when the two paraplegic groups were divided into

lesion levels, these numbers however still compare well with those of previous studies (27, 35, 54, 55, 56, 63).

A single T3 lesion level paraplegic in the SP group, having reduced cardiac innervation, was unable to register an appropriate tachycardic response to exercise. If this subject is removed from the high level SP group, then both high level groups tended to have a higher maximal HR and HR reserve. This finding could imply that the loss of adrenal gland innervation in both high level subject groups (T6 and above) does not prohibit the subjects from achieving an adequate HR response to maximal exercise. The mean resting HR in these two groups (high level paraplegics) is actually lower than that of their low-lesion level counterparts, which argues that the loss of adrenal gland innervation (and therefore circulating catecholamines) does affect resting HR.

The percentage diameter changes in both vessels were similar for different lesion levels, which would imply that adrenal gland innervation (and therefore circulating catecholamines) does not affect conduit artery caliber. The percentage BF changes of the high level subjects (non-innervated adrenal gland) (see Table 13) however, showed a definite trend toward smaller BF changes in their lower limb vessel response to exercise. This suggests that the loss of circulating catecholamines reduces the BF changes in the CFA during exercise, while not affecting the BF changes in the brachial artery. This may effectively reduce the BF contribution of the inactive muscle vasculature relative to the BF of the active muscle and suggests that circulating catecholamines play an important role in redistributing BF during exercise away from areas with lower demand. In contrast, locally acting agents are possibly more important in increasing BF to the active limb.

Reasons for variability: The reasons for the large variability in the measurements remain unclear. Two possible factors need consideration:

1) The use of the duplex Doppler method for "dynamic" blood flow measurements requires that measurements need to be done with quickly in order to minimize any delay in detecting BF parameters post-exercise. This creates the possibility of inconsistent post-exercise readings. The fact that, in our experiment, large variability occurred in all the groups, even in the resting values where speed of measurement does not play a role, makes the likelihood of human error less likely. The duplex Doppler method is a very useful addition to future research on exercise blood flow changes, but future studies should address the repeatability of the technique in the hands of each ultrasonographer in order to reduce the variability.

2) Physiologically the role of the limbic system, an integral part of the SNS, must be considered. Its influence on the SNS may play a role in the variability in measured BF parameters. Currently there is no scientifically reliable method to quantitatively measure limbic function (60).

3) A further issue is the influence of the absolute quantity of work performed by each subject. In the current study we chose to investigate the responses to an *intensity* of exercise (maximal and submaximal), but the workload itself differed for each subject. This approach is useful to determine potential limitations in the paraplegic subject. However, since limitations to brachial artery supply did not seem to be apparent, a different approach might be to determine a difference in response to an absolute submaximal change between two specific workloads.

Future: In the light of the above, further research is needed to clarify existing unresolved mechanisms. The paraplegic model can make a valuable contribution to

improve the knowledge of the influence of the sympathetic nervous system to exercise blood flow changes. A study comparing T5 paraplegic subjects to controls would be beneficial as cardiac innervation is preserved in these subjects, while adrenal gland innervation is absent. This will make it possible to assess the contribution of circulating catecholamines to skeletal BF during exercise more accurately. Adequate subject numbers could remain a challenge in the South African setting. Sport participation by spinal cord injured patients is currently increasing and this would make future research more viable.

Psychoanalytical testing, together with BF measurements, might shed some light on the influence of the limbic system, as part of the SNS, on exercise BF. To our knowledge, this has not been done.

Chapter 6

6. Summary:

The loss of lower limb nervous innervation in paraplegia makes the paraplegic an ideal model for the study of the influence of the sympathetic nervous system on blood supply to exercising skeletal muscle.

Our experiment confirms previous findings of smaller resting common femoral artery diameter and a raised resting heart rate in paraplegics vs. able-bodied subjects. The raised resting heart rate in the paraplegic groups is thought to be a compensatory mechanism for the absent tonic vasoconstriction in the lower limbs. Our finding of equal resting pulsatile index (PI) challenges previously reported increased PI in paraplegics vs. able-bodied subjects. This, together with similar resting blood flow measurements in all the groups, challenges the hypothesis that blood pooling occurs in the legs of paraplegics.

Post-maximal and submaximal exercise measurements and calculations suggest that:

- The loss of sympathetic innervation to the legs seems to reduce the contribution of the inactive lower limb to the blood supply of the active muscle group,

- The lower limb muscle pump does not contribute to blood flow changes during upper limb exercise,

- Fitness might sensitize the SNS spillover effect on BF distribution during exercise in paraplegic subjects,

- Circulating catecholamines might play a role in exercise BF changes.

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