IMPACT OF AN ACUTE BOUT OF SUBMAXIMAL AEROBIC EXERCISE ON CIRCULATING LEUKOCYTES IN INDIVIDUALS WITH SPINAL CORD INJURY

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Abstract

Experiencing a spinal cord injury (SCI) can be a traumatic and permanently life altering event. The inability to transmit neural impulses through the spinal cord to organs and tissues leads to widespread systemic impairments in sensory/motor, cardiovascular, endocrine and immune function. Exercise is generally known to lead to improvements in cardiometabolic measures such as obesity, cardiovascular function, and the promotion of a more anti-inflammatory cellular environment. The transient leukocytosis observed during acute exercise is indicative of immune function, contributing to long term immunomodulatory and anti-inflammatory changes resulting from chronic exercise. Though an abundance of able-bodied exercise leukocytosis literature exists, comprehensive leukocyte measures in response to acute exercise for individuals with SCI are limited.

Eight healthy, recreationally active adults with stable SCI (> 1 year) with injury below C3 were recruited to determine whether 30 minutes of submaximal aerobic exercise at 60% peak power output (PPO), in line with the updated SCI exercise guidelines, would lead to increases in circulating leukocytes. In a randomized crossover design, participants completed an exercise condition and a time-matched seated control condition separated by 7 days. Participants exercised for 30 minutes with blood draws at baseline, after 30 minutes of exercise, and 90 minutes after exercise. Blood draws during the seated control condition were taken at the same time points (baseline, 30 minutes, 120 minutes). Cells were quantified using multi-colour flow cytometry and analyzed using a linear mixed model.

Significant increases (condition X time interactions, $P \leq 0.05$) were observed for total CD3+ lymphocytes (19%), CD4+ T helper lymphocytes (16%), CD8+ T cytotoxic lymphocytes (24%), CD3+/CD56+ natural killer T (NKT) lymphocytes (31%), and CD56+ natural killer (NK) lymphocytes (63%) following 30 minutes of exercise. No changes were observed following exercise in CD19+ B lymphocytes, CD14+ classical monocytes, CD14+/CD16+ intermediate monocytes, CD16+ neutrophils or total CD45+ leukocytes. CD16+/CD14$_{dim}$ non-classical monocytes decreased significantly 90 minutes after exercise by 27%.

In conclusion, these preliminary data suggest that 30 minutes of acute submaximal aerobic exercise at 60% PPO is sufficient to increase most lymphocyte populations immediately following exercise yet is insufficient at inducing a general leukocytosis in individuals with SCI.

Lay Summary

Individuals with spinal cord injury (SCI) often have impaired immune function that can increase their risk of getting infections and chronic diseases. Exercise can boost the immune system but whether this occurs in people with SCI less understood. The purpose of this experiment was to see if a single 30-minute bout of arm cycling exercise would cause an increase in circulating immune cells, which are important for immune function, in people with SCI.

Eight individuals with SCI exercised for 30 minutes and immune cells were measured in blood samples taken before, after, and 90 minutes after exercise.

Most white blood cell populations increased after exercise, indicating that 30 minutes of arm cycling exercise can increase these cells in people with SCI. However, some white blood cells that are typically increased after exercise in able-bodied individuals did not increase suggesting that there may be some differences in exercise responses in SCI.

Preface

This concept of this study was conceived by Dr. Kathleen Martin Ginis and Dr. Jan van der Scheer to fill a significant disparity in the spinal cord injury and exercise literature through the use of a high-quality randomized crossover trial. Dr. Jonathan Little served as the supervisor, guiding research questions related to immune cells, data collection, data analysis, and the compilation of this thesis. The manuscript that will follow this thesis data will be overseen by supervisors and co-authors (Dr. Kathleen Martin Ginis, Dr. Jan van der Scheer, Dr. Jonathan Little). Garett Jackson performed the literature search and review, data acquisition, analysis and interpretation as well as the writing of this document. The study in this document is a registered clinical trial (NCT03955523) with human ethics approval granted by the University of British Columbia Clinical Research Ethics Board (H18-03191).

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Dedication

I dedicate this thesis to my family, my partner, the McLeod Lake Indian Band, and Janice Meier who initially sparked my interest in human physiology.

1.0 **Introduction**

1.1 **The Immune System**

The human immune system is an evolutionarily modified protective mechanism designed to prevent infections from various pathogens that threaten the homeostasis of its vertebrate host. The immune system can be broadly broken down into primary and secondary lines of defence. The primary defence consists of skin [1], tears [2], sweat [3], saliva [4] and mucous [5]. These initial barriers work to prevent infection from viruses, bacteria, and microbes that pose a risk to optimal physiological function. Should the pathogen breach the initial physical barriers, intervention from the cellular and humoral components [6] of the innate and adaptive immune system occurs by means of targeted inflammation. If the pathogen spreads beyond a reasonable threshold of containment, further action from these systems is required to terminate the threat and prevent overgrowth.

Threats that are not immediately deterred through the first line of defence and successfully infiltrate physical barriers are met with a second line of defence known as the innate immune system. The innate immune system is a highly conserved, protective germline encoded mechanism [7] that is passed down to successive vertebrate progeny, responding to specific carbohydrate, peptide or lipid signatures termed pathogen associated molecular patterns (PAMPs) via pattern recognition receptor (PRR) detection [8]. Through toll like receptor (TLR) expression and activation, responses to PAMP invasion by immune cells are acknowledged and countered. The innate phagocytic cluster of differentiation (CD)16+ neutrophil, CD56+ natural killer (NK) cell lymphocyte, hybrid CD3+/CD56+ natural killer T (NKT)

cell, CD14+ and CD16+ monocytes and dendritic cells respond to these activators, either by directly ingesting and destroying the pathogen [9], antigen presentation [10] or via cellular cytotoxicity leading to apoptosis (programmed cell death) [11, 12].

Presentation of antigens using the major histocompatibility complex (MHC) system is an elegant solution for displaying dangerous molecules to immune cells for assessment. Antigen fragments displayed on an antigen presenting cell (APC) using the MHCI complex are done so primarily in response to viral infiltration and are recognized by CD8+ T cytotoxic cells which in turn leads to cellular destruction if the APC is virally infected [13]. Conversely, antigens presented on MHCII complexes represent external threats taken in by the APC, and conveys to CD4+ T helper cells an intrusion has occurred, priming the subsequent immune response [13]. Because innate immune cells also serve as APCs, there is a clear relationship between both divisions of the immune system. Innate immunity is an integral component during the primary stages of pathogen infiltration as the cells of this system possess an inherited system for detecting dangerous, but very basic threats. The innate immune system can be described as cell-to-cell interaction in that once a PAMP is detected, appropriate actions are undertaken by each specific immune cell to terminate the threat.

Complimentary to the innate immune system, adaptive immunity serves to protect the host from pathogens albeit in a delayed, targeted and specific manner. As discussed previously, the innate immune system is germline encoded [7] to detect invaders that match hardwired descriptions of threat characteristics such as amino acid and carbohydrate signatures [14, 15] and to alert the adaptive immune system

through antigen presentation. The adaptive immune system, however, can protect against new and unique threats not passed to the progeny. Composed of T and B cell lymphocytes, the adaptive immune system is specific in pathogen recognition and attack, leaving a memory cell in place after initial encounters, which respond more rapidly upon re-exposure to the same or very similar pathogen [16]. CD3+ lymphocytes are split into two different types of T cell: the CD4+ T helper cell and the CD8+ T cytotoxic cell. T helper cells dictate the inflammatory process against invading pathogens by releasing a vast array of protein messengers termed cytokines, which shape the immune response, while T cytotoxic cells perform a more lethal role by destroying threats directly on a cell to cell basis [16]. CD19+ B cell lymphocytes possess an entirely different profession, it is their role to secrete antibodies that bind to antigens in the blood stream, allowing for recognition by other immune cells [17]. B cells may also become memory cells once they have been activated, leaving progeny for many years, in some cases protecting the host for the remainder of its lifetime [18]. Though often discussed as its own entity, the immune system is integrated with, modulates, and is modulated by alterations in the cardiovascular, endocrine, and nervous systems, which will be explored in the following sections.

1.2 **The Exercise Response**

Pathogens evoke an immediate and appropriate immune response, while events such as the physical stress of exercise may also evoke the mobilization and redistribution of leukocyte populations. Exercise is a physiological stressor that initiates many changes within circulating hormones [19] and cytokines [20] as well

as directly impacting cardiac output (hemodynamics) [21]. Exercise-induced increases in the circulating concentrations of hormones such as the catecholamines epinephrine and norepinephrine, as well as the glucocorticoid cortisol [22], exert strong influences over leukocyte recruitment and trafficking. Cytokines are small protein molecules [23] that can be released during exercise by various cells/tissues that shape the immune response to the exercise stimulus [24], including the subsequent inflammatory responses that follow. Cytokines secreted by macrophages, endothelial cells, immune cells, and even exercising skeletal muscle can be pro- or anti-inflammatory in nature. Though hormones are potent mediators leading to leukocytosis (the term used to describe an increased level of circulating immune cells), exercise also impacts immune cells by influencing leukocyte demargination (i.e., the redistribution of immune cells from spleen, vasculature, or other marginal pools). Hemodynamic changes due to an increase in cardiac output, blood pressure, and blood flow [21] causes friction upon the vascular walls termed shear stress. It is primarily through these changes in hemodynamics and shear stress that demarginates pools of adhered white blood cells allowing them to enter the peripheral circulation. These processes contribute to the demargination, mobilization, and subsequent margination and storage of many different leukocytes in response to exercise.

1.3 **Catecholamines**

Epinephrine is a catecholamine secreted by the adrenal glands during acute exercise and elevations in stress, which has a potent effect on the mobilization of leukocytes expressing beta 2 adrenergic receptors (β2AR) [25]. Researchers have

measured leukocyte responses in healthy men by intravenously infusing either sodium chloride placebo or epinephrine at 0.005 µg/kg/min. Following epinephrine infusion, significant increases in CD8+ T cytotoxic cells, CD3+/CD56+ NKT cells, CD56+ NK cells and CD16+/$CD14_{dim}$ monocytes were observed suggesting a relationship between epinephrine concentrations and the demargination and mobilization mainly consisting of lymphocyte subsets [25]. During acute exercise epinephrine can increase significantly, which is dependent on the intensity of work performed. Acute exercise can also lead to increased β2AR expression on various leukocytes, which would be expected to augment their response to an increase in epinephrine concentrations [26]. Direct experimental evidence for the role of epinephrine in mobilizing leukocytes comes from studies that implement pharmacological β2AR blockades during exercise, which have demonstrated decreased mobilization and trafficking of lymphocytes [27]. However, exercise-induced leukocytosis is not entirely prevented by pharmacological β2AR blockade, suggesting influences other than catecholamines contribute to immune cell mobilization [28]. Investigations also suggest that catecholamine administration appears to primarily influence the mobilization of the granular NK lymphocytes, which are stored in secondary lymphoid organs such as the spleen [28-30]. These findings suggest that lymphocyte populations respond strongly to exercise via adrenal hormones in a manner heavily influenced by β2AR stimulation.

1.4 **Proinflammatory Cytokines**

Cytokines released by cells during exercise direct specific immune cell responses and appear to shape the inflammatory landscape during recovery. Cytokines can be

classified as either pro- or anti-inflammatory, or in some cases both depending on the biological environment and their subsequent downstream influence [31]. Proinflammatory cytokines augment the inflammatory response and work to maintain a proinflammatory state within tissues. Following strenuous exercise, there may be a proinflammatory response likely arising from tissue damage [32]. This may be determined by an increase in the proinflammatory cytokines tumor necrosis factor alpha (TNFα) and Interleukin-1 beta (IL-1β) released into the peripheral circulation, primarily by activated macrophages [33, 34]. The degree of proinflammatory cytokine release is correlated with intensity and to an extent, duration, of the exercise bout with circulating TNFα and IL-1β concentrations increasing by up to two-fold [33]. The proinflammatory consequences of increased TNFα and IL-1β involves a cascade that includes increased adhesion molecule expression on vascular endothelial cells to mediate mobilization, chemoattraction, and extravasation of leukocytes with increases in nitric oxide that augment vascular permeability, thereby improving cellular transmigration [31]. The effects of proinflammatory cytokines are rapid and potent, allowing increased leukocyte trafficking, proliferation, and extravasation to potentially compromised tissues.

1.5 **Anti-Inflammatory Cytokines**

To counter the effects of inflammation, anti-inflammatory cytokines are secreted, thereby preventing uncontrolled inflammation that could lead to excessive and unnecessary tissue damage. Anti-inflammatory cytokines are designated as such by their ability to inhibit or counter the proinflammatory effects of TNFα or IL-1β [35]. The cytokines that typically exert anti-inflammatory effects include IL-1Ra, IL-4, IL-6,

IL-10, IL-11, IL-13, tumor growth factor beta (TGF-β) and are released by a number of different leukocytes and other cell types. IL-1Ra [36] and IL-10 [37] are regarded as exceptionally potent anti-inflammatory cytokines. IL-1Ra is released by macrophages and dendritic cells, which functions to inhibit IL-1α and IL-1β cellular activation at the receptor level [35]. IL-10 is also released by monocytes/macrophages [38]. One of the primary functions of Th2 lymphocytes is to inhibit cytokine production in monocytes/macrophages (self regulating) and neutrophils, leading to Th1 lymphocyte inhibition [35]. Th1 responses are regarded as proinflammatory in nature, while Th2 responses are regarded as favoring anti-inflammation [39]. The resulting inhibition of neutrophils is particularly important due to their potentially destructive nature if left unchecked [40]. Furthermore, IL-10 is often regarded as the most important anti-inflammatory cytokine due to its system-wide inhibition of proinflammatory cytokine synthesis by monocytes and macrophages [35]. Anti-inflammatory cytokines are undoubtedly important for the prevention of catastrophic, excessive inflammation and have been heavily implicated in the potential anti-inflammatory effects of exercise [35, 41-43].

1.5 **Glucocorticoids**

Glucocorticoids are steroid hormones with potent immunomodulatory effects that dynamically alter subsets of circulating leukocytes. The anti-inflammatory effects of glucocorticoids are exerted over most immune cells [44]. Cortisol is a glucocorticoid secreted by the adrenal glands as a result of the hypothalamic-pituitary-adrenal axis involving multiple organs and signalling pathways [45]. The release of cortisol in response to exercise is intensity dependent [46] and the increase has profound

immunomodulatory [43] as well as metabolic effects [47]. Cortisol plays an important role in antigen clearance through the opsonization (easier to phagocytose) of antigens, increased phagocytic activity by resident macrophages, and by potentiating interferon gamma (IFNγ) [48]. Cortisol also possesses the ability to reduce the adhesion molecule L (Ligand) - selectin via lipocortin 1 expression on neutrophils thereby preventing adhesion to vascular walls, subsequently impeding cellular transmigration [48]. Beyond exerting direct mechanical changes to adhesion molecules, cortisol is able to upregulate the function of anti-inflammatory cytokines IL-10 and TGF-β, and downregulate TNFα, IL-1β and IL-18 among others [48, 49]. In contrast, cortisol is also capable of activating certain proinflammatory processes through the secretion of macrophage migration inhibitory factor (MIF), leading to reduced immunosuppression and activation of proinflammatory responses in T cells and macrophages [48]. Overall, the increase in plasma cortisol with strenuous exercise is generally considered to blunt a potential uncontrolled proinflammatory response by both downregulation of proinflammatory and upregulation of anti-inflammatory cytokines.

1.6 **Shear Stress and Mechanical Mobilization**

Increased cardiac output and shear stress are potent mediators for the demargination of leukocytes from sites of vascular adhesion. The increase in blood flow during exercise exerts a friction on the endothelial vessel walls due to the cellular and humoral nature of blood, as well as the fluctuations in pressures within the arteries [50]. The effects of shear stress work to detach leukocytes that are adhered to the vascular lining thereby allowing these cells to enter the peripheral

circulation [51]. By pharmacologically blocking β2AR on various leukocytes using propranolol, Foster et al, determined that the demargination of leukocytes during higher intensity exercise was primarily dependent upon shear stress, with epinephrine signaling through β2AR playing a secondary role [27]. In a review by Cunningham, the importance of differences in shear stress are nicely summarized; when shear stress is low, promotion of leukocyte adhesion is increased, when it is high, there is an observable demargination and reduced adhesion leading to a proportional increase of leukocytes in the peripheral circulation, primarily comprised of neutrophils [52]. Shear stress observed during exercise exists on a spectrum or gradient, operating as a modulator of blood flow control and subsequent leukocyte demargination [50].

1.7 **Exercise as an Anti-Inflammatory Approach**

Though exercise is often proinflammatory in nature [32], the effects of regular exercise or physical activity are hypothesized to result in a cellular physiological environment that favours anti-inflammation [43]. Exercise is often used as an intervention in reducing obesity and improving cardiometabolic health, both of which are associated with chronic inflammation [53, 54]. In fact, the purported anti-inflammatory effects of exercise are believed to contribute to the widespread cardiometabolic benefits of regular physical activity [41, 42]. Some of the anti-inflammatory effects of exercise are viewed as adaptations to training whereas others are direct responses to each acute bout of exercise. Adaptations such as reductions in fat mass may lead to less proinflammatory cytokine TNFα and IL-6 release from adipose resident macrophages, thereby reducing systemic

inflammation [43]. Obesity has been shown to shift adipose resident macrophages towards a theorized proinflammatory M1 polarization [55]. A shift of macrophages from an M1 to M2 polarization is observed with chronic exercise, leading to a more anti-inflammatory state within adipose tissue [43]. Exercise may also shift immune cell profiles from Th1 to Th2, effectively altering the immune system environment [43]. Acutely, a single bout of exercise induces the secretion of cortisol, epinephrine, and IL-6 [43]. One of the most popular theories for the anti-inflammatory effects of exercise comes from the pioneering work from Pedersen and Febbraio [24, 56-58], who discovered that IL-6 is a “myokine” released from exercising skeletal muscle. When IL-6 is released as a myokine it is hypothesized to act as a systemic mediator that promotes IL-10 and IL-1Ra release, presumably from circulating monocytes or T cells, to promote an anti-inflammatory environment during recovery from exercise. As discussed above, the mobilization, demargination, and alteration in circulating leukocytes due to epinephrine, shear stress, cortisol, and cytokines in response to acute exercise is also crucial for dictating immune system responses. The anti-inflammatory effects of exercise are likely a valuable tool for reducing the chronic inflammation observed in conditions such as spinal cord injury (SCI) [59].

2.0 **Spinal Cord Trauma and Physiological Function**

SCI can be a devastating event in the lives of those impacted. Within SCI, injuries to the spinal cord can be divided into several distinctions with respect to the degree of autonomic nervous system (ANS) disruption: complete or incomplete, tetraplegia or paraplegia, and various degrees within. Depending on the area and degree of trauma, the American Spinal Cord Injury Association (ASIA) developed the ASIA Impairment Scale (AIS) in order to elucidate injury characteristics, however, this scale does not permit a determination of completeness. A complete injury to the spinal cord does not allow any afferent (sensory) or efferent (motor) nerve transduction through the site of injury [60]. An incomplete injury will allow some sensory, some motor, or a combination of both, however, with ongoing and often permanent impairments [60]. It is therefore not surprising that severe disruption is observed in cardiovascular function as innervation of various tissues is necessary for optimal function [61]. Additionally, damage to portions of the spinal cord also impact injury characteristics, such that paraplegia – damage to the thoracic, lumbar, or sacral spinal cord, and tetraplegia – damage to the cervical spinal cord, possess specific physiological consequences and prospective therapies [62].

A partial or complete loss of neural signaling to various tissues resulting from disrupted nervous system function poses an immense challenge to normal cardiovascular function. To maintain homeostasis and react to immediate changes during a physical response, the sympathetic nervous system (SNS) division of the ANS is responsible for increases in heart rate, as well as blood pressure, while activation of the parasympathetic system (PNS) division provides opposing control

to return to resting parameters. Residing at T1 to T4, sympathetic preganglionic neurons (SPNs) primarily located on the intermediolateral cell column ensure cardiac muscle regulation. SPNs lead to postganglionic neurons which deliver impulses to specific organs such as the heart, or peripheral vasculature. The vagus nerve (cranial nerve X) projects parasympathetic signals through pre- and then postganglionic neurons (PPN) which end at the heart, thereby reducing heart rate to resting values [63]. An impairment in the optimal functioning within the ANS, particularly the SNS manifests as different physiological symptoms that may not be consistent from one case to the next. Therefore, it is clear that each traumatic injury is unique and likely results in physiological heterogeneity that requires acknowledgement and consideration when describing SCI [64]. The unique nature of SCI led to the use of the AIS as a standardized tool to provide insight into injury severity and scope of the trauma aiming to enhance the effectiveness and direction of treatment following the injury. The use of the AIS, and various injury consequences such as cardiovascular complications and alterations, autonomic dysreflexia and immunodeficiency will be discussed.

2.1 **American Spinal Injury Association Impairment Scale**

Assessing a spinal cord injury is paramount in guiding acute and chronic care delivery for the injured patient. Upon arrival to emergency care, the patient must be clinically assessed to determine the severity and scope of the trauma. Depending on the location of the injury and state of the patient, early assessment may include diagnosis of tetraplegia or paraplegia [65]. Use of the standardized AIS outlines acute motor and sensory impairments [66]. The scale is capable of determining

disruption to skin dermatomes, muscle myotomes, sensory and motor function, as well as diagnosing the neurological level of injury (NLI) [65, 66]. During a standardized examination, testable patients have their motor and sensory functions graded. Motor functions are graded from 0 - 5 (0 being total paralysis, and 5 being fully functional) with sensory function graded as 0 - 2 (0 being absent, 2 being preserved) [65].

Following examination, patients are allocated into one of the following designations: A, B, C, D or E [67]. AIS-A is characterized as a complete injury, with no motor or sensory function below the sacral spine. AIS-B is regarded as an incomplete sensory injury, wherein some sensory function has been preserved, however no motor function below the NLI is present. AIS-C consists of an incomplete motor injury with some function existing below the NLI with less than a score of 3 regarding muscle grade for more than half of key muscle functionality. AIS-D shares similarities with C, except muscle grading for more than half of the key muscles is 3 or higher. Finally, AIS-E is designated if an individual with a prior SCI regains normal function from prior dysfunction. It is important to note that individuals who do not have an SCI are not provided with an AIS grade [65]. For specific information regarding appearance of the AIS grading scales, please refer to Figures A-6 and A-7 in Appendix A. This neurological assessment and AIS designation is critical in that it can be done in the absence of technology, and can be carried out shortly after injury following structural assessment to direct further diagnostic testing such as radiology, MRI and acute care [68].

2.2 **Cardiovascular Health**

Injury to the spinal cord leads to neural dysfunction that can manifest as increased risk for cardiovascular complications such as cardiovascular disease (CVD), coronary artery disease (CAD), orthostatic hypotension, and autonomic dysreflexia (AD). Though pulmonary and renal complications lead mortality rates for individuals with SCI, CVD is a prevalent contributor to premature death [69]. According to the American Heart Association (AHA), there are several health-related metrics that may lead to increased risk for cardiovascular disease: Body mass index (BMI), physical activity, blood glucose, total cholesterol, and blood pressure [70]. Exercise and improved dietary intake exerts many positive effects, reducing the impact of many of these risk variables which may counter the negative consequences of sedentary behaviour [71].

Spinal cord injuries often lead to orthostatic hypotension, described as a system wide reduction in blood pressure and heart rate pronounced when moving to an upright posture from a supine position [72]. For individuals with SCI, moving to an upright seated position may lead to a significant reduction in systolic blood pressure (SBP) which may cause light headedness and syncope. An inability to sympathetically constrict peripheral vasculature and increase heart rate leads to pooling in the extremities and organs when SNS activity is impaired [73]. Because of reduced SNS function and release of epinephrine, a hypersensitization in the arterial vasculature occurs likely due to a reduction in overall circulating catecholamine concentrations [74]. It is apparent that neural disruption impacts many facets of normal physiological function, not only with respect to neural signaling, but also its

influence over cardiovascular control and subsequent elevations in risk for developing CVD.

Sedentary behaviour is a major contributor to many chronic diseases [75] in which individuals with SCI may experience increased susceptibility. In a study involving 73 individuals with SCI, it was concluded that only 12% met the SCI-specific guidelines for physical activity, with up to 44% recording no activity at all [76]. Indeed, the SCI population possesses a high risk for developing CVD due to elevated rates of sedentary behaviour. Additionally, individuals with SCI experience greater risk for developing CVD as observed in a small cohort in Boston MA, USA. A 2011 investigation by Groah et al, followed 121 individuals all of which experienced SCI between C5 and T12, all of which had their injury for at least 1 year, and were observed over the course of 5 years [77]. To determine cardiometabolic health, participants completed an oral glucose tolerance test (OGTT) to determine glycemic control, as well as had triglycerides (TG), total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol results analyzed. Results of risk levels were determined using gender-specific Framingham risk scores (FRS). Findings indicated that high rates of obesity as measured using the BMI >25 were prevalent among individuals with paraplegia (57.5%) and tetraplegia (56.3%) [77]. FRS calculated for the total group resulted in ~90% being at low risk, ~8% were in the moderate risk, and ~2% at high risk [77]. Furthermore, duration of injury, and age were strongly associated with calculated FRS. Additionally, individuals with tetraplegia experienced significantly higher 2-hour glucose and 2-hour insulin levels, along with much lower SBP [77]. Obesity and lack of exercise are known to contribute greatly

to metabolic dysfunction [78], and even short bouts of exercise or leisure time activity performed regularly may reduce risk factors for cardiovascular disease [79]. Support through the use of social cognitive theory to deliver meaningful exercise education or enhanced programming may decrease sedentary lifestyles [80] and is a key element in exercise maintenance. Though the topic has been explored from a stance of risk factor analysis, it is important to note that the academic literature supporting the notion of specific exercise recommendations for individuals with SCI based on high quality sources is sparse as highlighted in a recent systematic review [81].

2.3 **Autonomic Dysreflexia**

SCI carries with it many potential complications that individuals and health care providers must always be prepared for. Individuals with SCI experience a higher risk for the development of respiratory, cardiovascular, bowel and urinary, spasticity, pain [82], osteoporosis and fracture complications [83]. Beyond these complications, AD presents specifically dangerous physiological challenges [84], in some cases even resulting in death [85, 86] . Location and scope of a spinal cord injury dictates the severity of disruption to physiological function. The higher the injury, the more devastating the results can be, leading to more serious secondary complications [83]. Injuries above T6 often lead to significant disruption and physiological sequelae. The spleen is innervated by nerves spanning anywhere from T6 to T11 [86, 87], and due to its ability to store large volumes of blood (~200 mL) [88], ultimately plays a substantial role in blood pressure responses. Injury at or above spinal nerve T6 leads to reduced or complete loss of sympathetic outflow to, and

control of the spleen [86]. Dysfunctional sympathetic nervous system control may lead to rapid elevations in blood pressure, bradycardia or tachycardia, headaches, altered vision, and sweating above the level of trauma [89].

AD is characterized by a signal in response to a noxious stimulus such as a full bladder or colon [90] sent to the spinal cord that does not reach the brain. This signal causes a potent release of adrenal hormones and thus widespread vasoconstriction below the site of injury [89]. As mentioned earlier with respect to injuries above spinal nerve T6, the spleen may become vasoconstricted, dramatically increasing blood pressure by rapidly expelling its reservoir. The rapid increase in blood pressure is sensed by baroreceptors above the site of injury, resulting in immediate countermeasures such as arterial vasodilation, and bradycardia [89]. Flushing of the skin and sweating only occurs above the level of injury due to the inability of inhibitory signals to reach the spinal cord [89] and continues until the initial stimulus is resolved. Since the above-mentioned immunomodulatory effects of exercise are influenced by catecholamines and shear stress, AD could impact how exercise effects leukocytosis, demargination, and immune function and is implicated in suppression of the immune system in individuals with high level SCI [91].

2.4 **Immunodeficiency in Spinal Cord Injury**

The complications of SCI not only exert effects over autonomic, motor, and cardiovascular function, but also contribute to alterations in immune response. Following a SCI, significant disruptions to the nervous system are observed, leading to altered immune function [49, 92, 93]. As discussed previously, injury at the T6

spinal nerve level is highly significant due to the direct blood based immune response originating from the spleen [92]. Innervation of the spleen is important for the recruitment of lymphocytes and monocytes, which respond primarily to epinephrine, as well as direct contact between nerve fibres and the leukocytes themselves [94]. Lymphocytes express high levels of β2ARs and appear to be sensitive to changes in epinephrine concentrations [95]. Acute increases in concentrations of epinephrine induces a temporary increase in lymphocyte cell counts within the circulation [49]. Norepinephrine and glucocorticoids are both released as a result of hypothalamic-pituitary-adrenal cross communication [45, 49]. Furthermore, sympathetic activation of adrenal tissues may be disrupted with damage to the spinal cord, reducing the effectiveness of this physiological response. As discussed earlier, the activation and mobilization of important lymphocytes into the peripheral circulation depend on mechanisms that SCI may impede. Dysfunctional secretion of cortisol, for example, leads to downregulation and/or upregulation of both pro- and anti-inflammatory genes and cytokines [96] which direct various immune responses. Overall reductions in natural killer cell activity have been documented in the SCI population [59, 97]. Additionally, wounds tend to heal slower, increasing risks of complications [98]. Therefore, balance or homeostasis within the immune system could become dysfunctional in some cases of SCI due, in large part, to significant SNS disruption.

2.5 **Exercise Recommendations for the SCI Population**

Exercise is known to reduce cardiovascular risk factors [99], improve cardiorespiratory fitness [100], lead to a more balanced inflammatory profile [43] and

improve overall physical performance in able bodied individuals. Additionally, individuals with SCI have demonstrated their elite level atheltic abilities by competing in endurance activities, such as wheelchair marathons and various events in the Paralympic Games. However, the impact of NLI and ANS disruption plays a role in performance with differences observed between athletes with paraplegia and tetraplegia [101]. Governments provide evidence informed exercise guideline recommendations for broad populations [102, 103], however these recommendations exclude specific prescription for individuals with SCI or other physical impairments [104]. For example, it has been recently determined that to potentially obtain the same degree of anti-inflammatory exercise benefit, individuals with SCI must work harder [105] which is at odds with the general held consensus that individuals with SCI can do less work for the same benefit as is discussed in the updated SCI exercise guidelines [104].

These recommendations require thorough literary analysis to ensure all individuals can partake in the guidelines safely, leading to improvements in overall health, ideally shaped by strong scientific evidence through rigorous exercise trials. Systematic reviews are required to collect and scrutinize said evidence, leading to informed decisions about exercise type and dosage. Though large in scope, it is not responsible to provide sweeping recommendations for all populations as some require specialized guidance based on specific evidence. Exercise recommendations for individuals with SCI should be based on evidence provided by strong randomized controlled trials (RCT) with a focus on the physiology of those

living with SCI. Additional exploration into responses based on the heterogeneous nature of spinal cord injuries is recommended.

Individuals with SCI face specific barriers when it comes to physical activity, due to various daily self care requirements [106], and physiological complications such as AD [89], pressure ulcers [107] and overuse injuries [104, 108] which must be taken into consideration when developing specific exercise guidelines. In fact, a 2017 review highlighted some of the SCI literature and its reliability for the determination of establishing exercise guidelines. The review highlights significant, high quality contributions made in the literature supporting favourable outcomes for cardiometabolic health and fitness for those with SCI [81]. The evidence provided allowed the guidelines to adopt two recommendations: a combination of aerobic and resistance exercise, as well as solely aerobic exercise with no resistance training. The evidence also pointed to a limited adverse event rate, suggesting that these exercise recommendations are indeed safe [81]. The review also highlights the need for high quality RCTs within older populations for aerobic plus resistance training, as well as aerobic only for older populations and for those with AIS-C and AIS-D [81].

The 2011 SCI exercise recommendations for Canada [109] were updated in 2018 [104] emphasizing research around cardiorespiratory fitness, power output, muscle strength, body composition and cardiovascular risk. For the intents and purposes of this thesis, the specific cardiometabolic health guideline of 30 minutes of moderate to vigorous intensity was chosen and explored using a randomized crossover design. Participants exercised for the 30-minute duration using an arm cycle ergometer at 60% of their peak power output. This component followed the guideline

provided suggesting that a minimum of 20 minutes of aerobic exercise should be completed if combined with strength training, or 30 minutes if improvements in cardiometabolic health are desired [81, 104].

3.0 **Leukocytes and the Exercise Response**

Leukocyte subpopulations are unique cells that have evolved to serve specific protective purposes. Cells of the innate immune system protect us on a basal level, recognizing antigens through means which have been passed down genetically. Neutrophils, basophils, eosinophils, monocytes, natural killer cells, natural killer T cells, macrophages and dendritic cells all reside within the innate arm of the immune system. Unlike the innate immune system, the adaptive immune system does not respond solely to primitive or “simple” threats. The adaptive immune system mounts specific attacks against select antigens that have overrun the innate immune system’s initial response. The cells belonging to the adaptive immune system are T cells (T helper, T cytotoxic) and B cells with a hybrid natural killer T cell illustrating the relationship between the innate and adaptive immune systems. Preceding most cells is a CD designation which denotes a protein complex located on the cellular surface that distinguishes leukocytes from one another and is used as a means for identifying different cell types using experimental techniques such as flow cytometry. This section will provide an overview of the cells themselves, their primary roles and functions, and what the response is during exercise in able-bodied individuals and those individuals with SCI.

3.1 **Neutrophils**

Belonging to the innate immune system, neutrophils are a large polymorphonuclear (PMN) myeloid granulocyte originating from the bone marrow. Neutrophils express CD16 and comprise ~60% of the circulating leukocytes making them the most abundant white blood cell [110]. Neutrophils contain many granules, hence the

namesake “granulocyte”. Neutrophils are potent phagocytes [111] that carry out many different roles during an immune response. The process of phagocytosis [112] is one of their primary roles. Upon activation or detection of a pathogen, neutrophils become asymmetrical, projecting pseudopodia which help the granulocyte move and engulf threats [111]. The granules contained within a neutrophil are filled with many different enzymes and proinflammatory proteins, which exist primarily for the breakdown of foreign cellular membranes [113, 114]. Neutrophils also produce neutrophil extracellular traps (NETs) primarily comprised of DNA chromatin which work to prevent the mobilization of viruses and bacteria [115]. NETs are excreted from the neutrophil directly, though surprisingly do not immediately precede cell death, or apoptosis of said neutrophil [115]. Neutrophils also possess the ability to secrete large amounts of reactive oxygen species (ROS) in a process termed “respiratory burst” that is particularly useful for exterminating pathogens within close proximity [110, 116]. Exercise generally causes an acute and rapid increase in circulating neutrophils, which is dependent on exercise intensity and duration.

3.1.1 **Able-bodied**

During exercise, the principal mechanism responsible for the demargination of neutrophils does not involve bacteria or viruses, but primarily through the influence of shear stress. In fact, the bulk of leukocytosis is made up of neutrophils [51]. During times of low shear stress, neutrophils remain adhered (marginated) to vascular walls primarily in the pulmonary vasculature [110]. During exercise, circulating neutrophil counts can increase from 2-5-fold over basal levels depending on intensity [110]. Neutrophils respond strongly to a stepwise treadmill running test

to volitional fatigue in young, healthy, endurance trained males significantly increasing from pre to post exercise [117]. In a study by Natale and colleagues, able-bodied individuals completed several exercise conditions with measurements of all leukocyte subsets. Neutrophils increased significantly after long (2 hours) submaximal exercise at ~60% VO_2max on a cycle ergometer [118]. Nieman saw similar results with an evident neutrophilia (prolonged increased neutrophil count) post exercise after walking for 45 minutes at 60% VO_2max [119]. Patlar and colleagues had participants exercise at 75% VO_2max for 20 minutes and found that there was no immediate increase in neutrophils, however 2 hours after exercise, neutrophil counts were significantly elevated [120]. High intensity exercise at 80% VO_2peak was also sufficient at inducing a neutrophilia 2 hours after cessation in healthy participants whereas 40% VO_2peak was not [121]. Incremental exercise appears to produce similar results. Moyna and colleagues examined neutrophils after participants exercised at 55%, 70% and 85% of their VO_2max for 6 minutes at each stage on a stationary cycle ergometer and observed significant increases in neutrophils occurring at each stage [122]. These findings suggest neutrophilia is intensity dependent and were corroborated by Quindry and colleagues with a similar stepwise protocol leading to increased neutrophil counts post exercise in an intensity-dependent manner [123].

3.1.2 **SCI**

There are far fewer studies examining blood neutrophil counts after acute exercise in SCI, but the limited research does support a general exercise-induced neutrophilia in SCI participants. In a study by Klokker, individuals with SCI

participated in a 30-minute functional electrical stimulation (FES) protocol on a stationary cycle ergometer at their greatest manageable workload. There was a steady increase in neutrophils after 30 minutes, persisting for 2h following exercise [124]. Allgrove led a pilot intervention with individuals with SCI performing self paced wheelchair exercise for 1 hour on a 400m track with increases observed in neutrophils immediately after exercise, persisting for 1 hour after cessation [125]. Banno also observed similar increases in neutrophil count doubling after a half marathon completed by individuals with mid and high level cervical SCI [126]. Interestingly, a study by Kouda involving 20 minutes of arm cycle ergometry at 60% VO_2max in SCI and able-bodied individuals led to delayed neutrophilia 2 hours after exercise cessation [127]. The results of these studies imply that some variability in neutrophil response exists, however the response is dependent primarily on the intensity of exercise and likely related to the increase in shear stress and/or catecholamine response.

3.2 **Monocytes**

Monocytes are the sentinel cells of the peripheral circulation responsible for phagocytosis [111]. Monocytes can migrate into tissues to eventually become macrophages or can give rise to dendritic cells [128]. Expressing CD14, and to a smaller extent CD16 [129], monocytes are large, vesicle rich mononuclear cells originating in the bone marrow making up ~5-10% of circulating leukocytes [110, 128]. When not patrolling the peripheral circulation for pathogenic invaders, monocytes are stored primarily in the spleen and bone marrow, awaiting activation and recruitment [130, 131]. Around 90% of circulating monocytes are inflammatory

precursors to resident macrophages found inside the tissues which are consistently replaced [128, 131]. Once differentiated into a macrophage [132], their main role is to secrete cytokines to shape the immune response, as well as phagocytose pathogenic invaders that breach the epidermal barrier [128].

3.2.1 **Able-bodied**

Increases in monocytes during exercise may serve a protective mechanism (similar to neutrophils) as monocytes respond in an intensity dependent manner, resulting from a potentially threatening stimulus such as a predator. During exercise, there is a transitory increase in monocytes as seen in a study by da Silva Neves and colleagues. Participants completed a 350-kcal run on a treadmill at 80% VO_2max, wherein a significant monocytosis was observed during this effort, however, similar increases were not seen when participants exercised at 40% VO_2max, suggesting their intensity dependent nature [121]. Shinkai and colleagues observed a significant monocytosis following 60 minutes of continuous exercise on a cycle ergometer at 60% VO_2max in young healthy males [22]. Similar results were observed by Bieger and colleagues as a stepwise acceleration to exhaustion protocol on a treadmill evoked a significant monocytosis [133]. It appears that monocytes are intensity dependent, in that lower intensity exercise at 40% VO_2max does not increase monocyte counts, however, increases are observed during exercise at 60% VO_2max or greater.

3.2.2 **SCI**

The increase in blood monocytes in response to acute exercise in individuals with SCI appears to be blunted or absent. Kouda's intervention involving 20 minutes of

arm cycle ergometry at 60% VO_2max led to no changes in monocyte count after exercise, but the short duration of exercise is likely the reason for this [127]. Yamanaka and colleagues also did not see a monocytosis following 20 minutes of arm cycle ergometry at 60% VO_2max [97]. Though the short exercise duration in these two studies make firm conclusions difficult, a 2 hour protocol on an arm cycle ergometer at 60% VO_2max also did not evoke a monocytosis [134], suggesting individuals with SCI do not recruit monocytes at similar intensities when compared to able-bodied individuals, and that higher intensities are likely required.

3.3 **Natural Killer Cell and Natural Killer Cell T Lymphocyte**

Natural killer cells are a moderate sized granular cell comprising ~15% of total lymphocytes and are incredibly versatile leukocytes [135]. Expressing CD16 and CD56, NK cells are potent killers with an immunoregulatory capability of shaping immune responses as demonstrated by their ability of secreting pro and anti-inflammatory cytokines [136]. NK cells primarily respond to messages from T helper cells in the form of IFNα and IFNβ [136]. NK cells achieved their name due to their ability to “peer” into cells to determine whether the target has been compromised, and if so, may destroy the cell without being activated – unlike most leukocytes. The death of the target cell through apoptosis is important in preventing the synthesis of viral machinery and the subsequent spread of infectious virus [137]. Furthermore, NK cells carry out an important role in cancer by orchestrating innate and adaptive immune activity. NK cells are able to secrete cytokines that direct the immune response to prevent tumor growth, termed “immunosurveillance” [138] and can remain activated through an autocrine based positive feedback loop via the

secretion of IFNγ [139]. In fact, NK cells present a potential biological exploitation for pharmacological cancer interventions [138].

The natural killer T cell (NKT, a variant of a natural killer cell and a T lymphocyte) is a hybrid cell possessing characteristics of both the innate and acquired/adaptive immune systems. NKT cells much like traditional T cells originate and progress within the thymus [140] with evident expression of a T cell receptor (TCR) [141] and ability to recognize CD1d presented antigens of lipid origin on the outer membrane of APCs [142]. NKT cells are capable of producing and secreting IFNγ [143] illustrating some homogeneity when compared to NK cells. This cell illustrates the importance of a synergistic relationship between the innate and adaptive immune systems.

3.3.1 **Able-bodied**

NK cells appear to be highly responsive to varying intensities of exercise. Natale's group observed significant increases in NK cell count following moderate aerobic, maximal, and resistance training conditions suggesting they are highly sensitive to physiological perturbation [118]. Brenner and colleagues also observed a significant increase in NK cells during 2 hours of cycling at 60-65% VO_2max, maximum effort for 5 minutes on a cycle ergometer, as well as after acute resistance training [144]. Fry and colleagues observed a significant intensity-dependent increase in NK cells after an incremental protocol to exhaustion on a treadmill [145]. Hoffman-Goetz saw similar increases in NK cells after participants exercised on a stationary cycle ergometer at 65% VO_2max for 1 hour [146]. Pederson and colleagues observed significant increases in NK cell count following a 1 hour cycling protocol at 80%

VO_2max [147]. Vider and colleagues also observed a significant 200% increase in NK cells in young healthy males following a maximal incremental treadmill test [117]. Overall, blood NK cell numbers appear to consistently increase in response to many types of exercise in healthy, able-bodied individuals.

Very little work has been done to determine NKT cell responses during exercise. Natale's group observed an increase in NKT cells following low to moderate and high intensity cycling protocols [118]. Timmons also observed an increase in NKT cells by having two groups of participants complete two 30-minute exercise trials back to back on a cycle ergometer at 70% of their VO_2max while consuming either a carbohydrate enriched beverage with electrolytes, or electrolytes alone [148]. The groups were comprised of boys (~10 years of age), and men (~22 years of age). Men experienced a significant increase in NKT cell count following exercise, which was not observed in the younger group. The cell count dropped to baseline values 1 hour after exercise was terminated. This work suggests that NKT cells are susceptible to exercise induced perturbations in able-bodied adults, but the lack of research for this cellular response makes it difficult to arrive at a consensus.

3.3.2 **SCI**

Research on NK cell responses to exercise in SCI is less conclusive. Klokker observed significant increases in NK cells during FES training in individuals with SCI; interestingly, larger increases in NK cell concentration were observed in paraplegia vs tetraplegia [124]. During a wheelchair marathon completed by individuals with paraplegia, a significant increase in total leukocytes was observed, however NK cells counts were significantly lower post race suggesting that longer

duration exhaustive exercise may provoke differential effects [149]. Following a half marathon race, there was a significant increase in NK cells after cessation in individuals with paraplegia, but not tetraplegia [126]. The presence of β2AR on NK cells, and their response to sympathetic innervation [150, 151] may be responsible for the minimal changes in NK cells within this subpopulation of individuals with cervical SCI. Within the SCI population, increases in NK cells appear to correlate with intensity, likely highlighting the importance of shear stress, circulating catecholamine and cortisol concentrations. Additionally, no literature could be found investigating NKT cells during exercise in SCI using a variety of search terms and strategies.

3.4 **T Cell Lymphocytes – T Helper and T Cytotoxic Cells**

T cells are small lymphocytes that both promote and curtail the immune response to specific invaders. CD3+ T cells are comprised of CD4+ and CD8+ cells among other more obscure and far less numerous variations. CD4+ cells are known as T helper cells due to their ability to secrete various cytokines to enhance an immune response, thereby “helping” the proinflammatory response [110]. The T helper cell’s primary immune role is the recognition of foreign proteins and antigens displayed by APCs such as macrophages [34]. T helper cells are often further categorized into Th1 and Th2 cells based on different functional purposes. Th1 cells are proinflammatory in nature, secreting growth factors for other Th cells such as IL-2 and IFNγ, which serve to attract and activate other immune cells, notably NK cells and macrophages [34]. The Th1 response is said to be cell mediated due to the direct action on the effector cells themselves. Th2 CD4+ T cells are anti-

inflammatory in nature, as their primary role is to shape antibody responses by secretion of IL-4, 5, 6, and 10 [34]. CD8+ T cells are cytotoxic in nature, similar to NK cells, protecting against specific instead of general threats. The primary role of CD8+ T cells is to destroy target cells that present the CD8+ T cell's cognate antigen [34]. T cytotoxic cells bind to the target cell and either release granzymes into the virally infected cell, defragmenting its DNA, or by binding to an Fas receptor on the membrane surface, which signals the cell to undergo apoptosis [34]. When in a resting state, T cells primarily occupy the lymph nodes and the spleen [110], alluding to their reliance on sympathetic activation and catecholamine signaling [152]. It is therefore logical to assume that exercise will lead to T cell mobilization and redistribution due to increased sympathetic activation and elevated circulating catecholamines. Because of the common origin of T helper and T cytotoxic cells, both will be discussed simultaneously when applicable.

3.4.1 **Able-bodied**

During incremental exercise in able-bodied individuals, lymphocytes increase significantly in an intensity dependent manner. Fry and colleagues noted a significant reduction in CD4+ cells, but a significant increase in CD8+ cells during an incremental exercise protocol [145]. In individuals with high physical fitness, it appears that the same relative workload compared to unfit individuals evokes less of a response. Hong and colleagues compared the unfit and fit groups working at ~70% VO_2max for 20 minutes on a treadmill. Unfit individuals experienced a significant lymphocytosis of both CD4+ and CD8+ cells whereas the fit individuals saw no change [153]. Exercise intensity appears to be an important factor regarding

lymphocyte recruitment. In an incremental test until exhaustion, healthy young males experienced a two-fold increase in CD8 T cells, and a 47% increase in CD4 T cells [117]. Nieman et al compared exercise at 50% and 80% VO_2max and reported significant increases in both CD4 and CD8 lymphocytes following exercise at 80% VO_2max but not 50% VO_2max [154]. Though at a lesser workload of 60% VO_2max and a longer duration of 60 minutes, Shinkai and colleagues observed a significant increase in CD4 and CD8 cells [22]. Natale also observed increases in total CD3 lymphocytes, CD4 and CD8 T cells following both the 40% VO_2peak and maximal treadmill exercise protocols [118]. In contrast, Tvede and colleagues observed a decrease in total CD3 lymphocyte populations following 1 hour of cycling at 25, 50, 75% VO_2max, with no significant changes in CD8 cell counts in six healthy individuals [155]. Overall, the results are not entirely consistent but the majority of work in able-bodied individuals indicates an increase in total T cell counts occurs following acute aerobic exercise.

3.4.2 **SCI**

Acute exercise may lead to a moderate increase in total lymphocytes in individuals with SCI, particularly following extended exercise. Self paced wheelchair exercise sustained for 1 hour around a 400m track led to a 53% increase in total lymphocytes [125]. It is important to note that this increase is only reflective of a single trial with a small sample size and did not provide specific CD4 or CD8 T cell responses. Literature reporting specific values for CD4 or CD8 T cells during exercise in individuals with SCI remains largely missing.

3.5 **B Cells**

B cells are small lymphocytes of a common lymphoid progenitor lineage originating in the bone marrow. Expressing CD19, the primary role of B cells is to produce and secrete antibodies to detect antigens and pathogens that have passed both the first physical barrier, and the initial innate response [156]. B cells are often referred to as humoral cells because they secrete antibodies into plasma. Also functioning as an APC, B cells are important activators of T cells, initiating a specific adaptive immune response [156]. The antibodies secreted are so numerous that theoretically, B cells are capable of creating specific proteins to detect any possible pathogenic signature [156]. It is through the process of antigen detection that memory B cells are born. With incredibly long lifespans, these memory cells may secrete antibodies that protect the host for the remainder of its life [18].

3.5.1 **Able-bodied**

Lymphocyte populations change during exercise; however, this is primarily due to increases in CD56+ NK cells and CD8+ T cells (as discussed above). Nieman et al, observed a significant increase in B cells after 45 minutes of high intensity (80% VO_2max) exercise, but no changes were observed after moderate (50% VO_2max) exercise [154]. After cycling for 60 minutes at 60% VO_2max, Shinkai observed significant increases in CD19+ B cells immediately after exercise [22]. Natale's group also saw a significant increase in B cells only after exercise in the peak aerobic group at ~95% VO_2peak [118]. During an incremental test of 6 minutes at 55, 70 and 85% VO_2max on a cycle ergometer, Moyna observed a significantly higher B cell count at each stage compared to baseline [122]. Additionally, a

maximal incremental treadmill test to exhaustion in healthy males elicited a 43% increase in circulating B cells [117]. However, consistent increases in B cell mobilization does not seem reliable as seen in a study by Tvede, where there was no increase at 25, 50 or 75% VO_2max on a stationary cycle ergometer [155]. It appears that in able-bodied individuals, increases in B cells during exercise are not as reliable or consistent as that of NK cells, neutrophils, or monocytes suggesting a small or insignificant contribution to overall exercise-induced leukocytosis.

3.5.2 **SCI**

The impact of acute exercise on B cells in individuals with SCI appears to have not been studied as there were no studies located using a variety of search terms and strategies. Klokker and colleagues mention CD19 B cells in their methods, however specific values in the results of this paper were not provided [124]**.**

4.0 **Summary and Significance**

Overall, the literature reporting comprehensive leukocyte responses to exercise in individuals with SCI is limited. Some studies have explored specific immune cells noting some similar, and some potentially differential responses depending on the level of injury or in comparison to able-bodied individuals. There appear to be no studies that have comprehensively examined major leukocyte subpopulation responses (i.e., neutrophils, monocytes, NK cells, T cells, and B cells) in the same individuals. This lack of foundational information forces the researcher to extrapolate from the able-bodied population and/or rely on studies where responses were not consistent across exercise intensities. Furthermore, the differing methodology for cell staining and separation (e.g., flow cytometry vs. blood smears and use of different dye conjugates) make comparisons problematic. Specifically, conclusions regarding the impact of acute exercise on B cells and T cells are somewhat unreliable in the able-bodied literature, and extremely limited for those with SCI. This study will be the first, to our knowledge, to report complete, comprehensive values of all major leukocytes within the same group of individuals who perform an acute bout of exercise as well as a time-matched, non-exercise control condition. We utilized a randomized crossover design involving a seated control condition to control for any potential diurnal variation and employed an exercise bout that is modeled after a typical bout of physical activity in line with the recently updated international SCI physical activity guidelines [104]. Leukocytes were enumerated by flow cytometry with a commercially available 8-colour immunophenotyping kit that uses

recombinant engineered human antibodies resulting in highly accurate and reproducible data.

5.0 **Purpose**

The purpose of this thesis was to determine the impact of a single bout of submaximal aerobic exercise on leukocyte subpopulations in individuals with SCI. Specifically, I examined how 30 minutes of arm-crank cycle ergometry at 60% peak aerobic power output as per the updated SCI exercise guidelines influences circulating concentrations and proportions of total CD3+ T cells, CD3+/CD4+ T helper cells, CD3+/CD8+ T cytotoxic cells, CD3+/CD56+ NKT cells, CD14+/CD16- classical monocytes, CD14+/CD16+ intermediate monocytes, CD16+/CD14$_{dim}$ non-classical monocytes, CD16+ neutrophils, CD19+ B cells, CD3-/CD56+ NK cells, and total CD45+ leukocytes.

6.0 **Hypothesis**

It is hypothesized that a single bout of 30 minutes of acute, submaximal aerobic exercise carried out on an arm cycle ergometer will promote a general leukocytosis (increase in CD45+ Leukocytes and most individual immune cell types) in individuals with SCI.

7.0 **Methods**

7.1 **Participants**

Eight participants were recruited through advertisements within SCI groups in the province, and email correspondence with participants from previous trials who consented to being informed of future studies. Recruited individuals were recreationally active competing in wheelchair rugby, or similar sport of equivalent intensity 3 days per week, and all living with SCI for more than 1 year with injuries at or below the third cervical vertebrae. Participant SCIs ranged from AIS A-D, with a wide time since injury range of 6.4 to 35.1 years. All participants were required to meet specific criteria that required them to successfully provide multiple blood samples via venipuncture and be capable of completing maximal exercise as per American College of Sports Medicine (ACSM) guidelines with no existing metabolic conditions. Additionally, participants were asked to refrain from taking anti-inflammatory medications for 24 hours prior to testing, and female participants were excluded if they were pregnant. All participants were over the age of 18 years and provided written informed consent before participation. This was a registered clinical trial (NCT03955523) with human ethics approval through the University of British Columbia Clinical Research Ethics Board (H18-03191).

7.2 **Study Design**

Participants completed two experimental trials (exercise vs. seated control) in a randomized crossover design. Maximal aerobic power output was initially determined using an incremental exercise test to exhaustion on an electromagnetically braked wall-mounted Lode Angio CPET arm-crank cycle

ergometer (ACE; Groningen, The Netherlands) during a baseline testing and familiarization visit. This test was used to determine the power output, in Watts (W), for the exercise trial. Participants warmed up at a comfortable resistance and cadence for 5 minutes and performed subsequent continuous arm cranking at 55-65 revolutions per minute (rpm) for a maximum of 30 minutes. Starting at 0 W, the load would increase by 2W every minute for individuals with tetraplegia, and 10W per minute in those with paraplegia. Upon volitional fatigue, exhaustion or inability to maintain a 50-rpm cadence, 60% of the final load was recorded and served as the participant's 30-minute aerobic exercise resistance. Age, sex, injury level, and years with injury were recorded during an initial consultation using a questionnaire. Participant characteristics and familiarization trial details are provided in Table 1.

Table 1. Participant characteristics and familiarization trial results for SCI exercise study.

	Paraplegia (n = 4; 3 male)	**Tetraplegia** (n = 4; 4 male)	**Total** (n = 8; 7 male)
Age (years)	42.2 ± 16.1	35.5 ± 5.32	38.9 ± 11.7
Injury Duration (years)	16.8 ± 12.7	16.43 ± 7.16	16.6 ± 9.6
Injury Level	T4 - L1	C5 - C7	C5 - L1
Incomplete	2	3	5
Complete	1	1	2
Unsure	1		1
Seated Resting BP (mmHg)			
Systolic	141.5 ± 23.1	103 ± 25.3	122.3 ± 30.4
Diastolic	74.3 ± 8.5	56 ± 13.2	65.13 ± 14.2
Heart Rate (bpm)			
Range of Max	182 – 199	102 – 131	102 – 199
Test Average	133.5 ± 7.3	99 ± 12.9	116.3 ± 20.9
RPE (6-20)			
Range of Max	18 – 20	17 – 19	17 – 20
Test Average	12.6 ± 1.4	12.9 ± 0.9	12.7 ± 1.1
Resistance (W)			
Range of Max	90 – 160	60	92.5 ± 39.6
Max Average	125 ± 29	60 ± 0	93 ± 40
60%	75 ± 17.3	36 ± 0	55.5 ± 23.7

Exercise descriptors for 30-minute familiarization trial. Values as mean ± SD. C, cervical vertebrae; T, thoracic vertebrae; L, lumbar vertebrae; BP, blood pressure; mmHg, millimeters of mercury; bpm, beat per minute; RPE, ratings of perceived exertion; W, watts.

7.3 **Experimental Trials**

On experimental trial days, participants arrived at the laboratory after an overnight (≥12 hour) fast. A researcher trained in phlebotomy obtained a blood sample by venipuncture from an antecubital vein using a 21-gauge needle and collected into an EDTA vacutainer. Prior to beginning the 30-minute bout of exercise, participants warmed up for 5 minutes at a self-selected cadence. During the exercise trial, participants completed 30 minutes of arm-crank exercise at 60% of the maximal power output achieved during the familiarization visit. Heart rate was monitored throughout exercise with a Polar heart rate monitor (Polar, Kempele, Finland) and ratings of perceived exertion (Borg Scale: 6-20) were assessed and recorded during the final 10 seconds of each stage of exercise. Immediately following exercise another blood sample was obtained by a second venipuncture. Participants rested quietly in a separate space and were allowed to read or watch a non-stimulating video such as “Planet Earth” or similar documentary for 90 minutes before a third and final venipuncture and subsequent blood sample was obtained.

7.4 **Control Protocol**

In the control conditions the participant sat quietly in their wheelchair in replace of the 30 minutes of exercise and blood samples were obtained at the same corresponding time points (0, 30 and 120 minutes) while the participant watched a documentary such as “Planet Earth”. To control for diurnal variation, all participants started both the exercise and control conditions at the same time between 9:00 am and 11:00 am on their respective days. Additionally, the participant remained in the same room as during the 90 minutes post exercise time frame to maintain consistent

laboratory settings. Blood samples were immediately transported to the wet lab on campus at room temperature for analysis by flow cytometry.

7.5 **Cell Labeling Protocol**

100 µl of EDTA whole blood was added to a 5 ml cell culture tube and stained with 10 µl of 8-colour immunophenotyping cocktail (130-120-640, Miltenyi Biotec, Auburn, California, USA) consisting of CD3 PE, CD4 Viobright 667, CD8 APC-Vio770, CD14 VioBlue, CD16 Viobright 515, CD19 PE-Vio770, CD45 VioGreen and CD56 VioBright 515 conjugated antibodies. 10µl of 7-AAD (130-111-568, Miltenyi Biotec, Auburn, California, USA) was added as a viability marker to separate dead from live cells. After adding the cocktail, the sample was vortexed (Thermo Fisher, MA, USA) at a low speed (~30% maximum) for 5 seconds to ensure adequate mixing and incubated at room temperature in the dark on a rocker (Benchmark Scientific, Edison, NJ, USA) for 10 minutes. After the first incubation, 2 ml of 1x RBC lysis buffer (130-094-183, Miltenyi Biotec, Auburn, California, USA) was added to the sample and was again vortexed (~30% maximum) for 5 seconds to ensure adequate mixing. The sample was incubated at room temperature in the dark on a rocker for 15 minutes. After the final incubation, the sample was analyzed immediately on a MacsQuant flow cytometer (Miltenyi Biotec, Auburn, California, USA). A secondary sample using CD45 APC-Vio770 (130-110-635, Miltenyi Biotec, Auburn, California, USA) was used to measure total leukocyte count. Both samples utilized identical incubation steps and duration, however 7-AAD was excluded and Propidium Iodide (130-093-233, Miltenyi Biotec, Auburn, California, USA) was added to the CD45

sample prior to flow cytometry analysis for dead cell exclusion. The procedures to determine cell counts were provided by Miltenyi and followed accordingly.

7.6 **FMO Controls and Automated Compensation**

On a testing day, the fluorescence minus one (FMO) protocol was completed using participant blood following the exact protocol for whole blood analysis. Appropriate quantities of each dye were added in an FMO format to ensure that gating during final analysis was accurate and objective. Additionally, all channels were compensated using Miltenyi's automated compensation with all dyes being identical to the ones found in the 8 colour immunophenotyping kit.

7.7 **Cell Analysis**

Flow cytometry data files were analyzed by MacsQuant Software (version 2.6, Miltenyi Biotec, Auburn, CA, USA). A hierarchical gating strategy was used to discern leukocyte subpopulations. First, cells were gated to determine single cells from doublet cells using Side Scatter Area (SSC-A) on the x-axis and Side Scatter Height (SSC-H) on the y-axis. Live cell analysis was used to determine dead cells staining positive for 7-AAD. Live cells were then morphologically analyzed using SSC-A and forward scatter area (FSC-A) to determine leukocyte populations. From the morphology gate, CD14+ monocytes were gated and separated using CD14 and SSC-A. These monocytes were then further interrogated to determine CD14+, CD14+/CD16+ and CD16+/CD14$_{dim}$ subpopulations. B cells were separated using CD19 against SSC-A. CD16+ neutrophils were isolated using CD16 against SSC-A and the entire plot was split into SSC-A high and SSC-A low. From SSC-A low, CD3+ lymphocytes, CD3+/CD56+ NKT cells and CD3-/CD56+ NK cells were

isolated by gating CD56 against CD3. CD4+ and CD8+ T cells were isolated after gating from CD3+ lymphocytes. For complete gating strategy, see Figure A-1(A – I).

7.8 **Statistical Analysis**

A linear mixed effect model (LMM) was performed to analyze the impact of acute exercise vs. sitting on leukocyte counts over time. Condition (exercise vs. sitting), time (T0, T1, T2), and their interactions were treated as fixed effects and participants as a random effect. As the main interest was to determine the direct impact of exercise on immune cell counts across time, significant interactions were followed up by Tukey's multiple comparison tests comparing timepoints within each condition separately. Main effects of time were followed up by Tukey's multiple comparison tests comparing timepoints with conditions collapsed. Prior to statistical testing normality and skewness were checked by visual inspection of Q-Q plots of the residuals and any variables deviating substantially were natural log transformed to better meet model assumptions. CD19+ B cells and CD14+/CD16+ monocytes were log transformed (Y = log(Y)) and successfully met visually assessed normality assumptions. Values for these data were reported as untransformed data. To determine potential outliers, data was screened using 1.5*IQR with follow up to determine if cells fell within clinical limits. LMM analyses were performed using GraphPad Prism (v 8.0.1) [157] with effect sizes calculated using the "effsize" package in R [158, 159]. Results are presented as means ± SD with Hedges G (*g*) effect sizes reported for pairwise comparisons, and significance established as $P \leq 0.05$ using a 2-tailed analysis.

8.0 **Results**

To quantify leukocyte responses to an exercise stimulus in individuals with SCI, we had participants perform a brief 30-minute submaximal aerobic exercise bout and analyzed blood samples at baseline (labeled in figures as T0 for both exercise and control conditions), following exercise or 30 minutes for control protocol (labeled in figures as T1 for both conditions), and 90 minutes following exercise or 120 minutes for control protocol (labeled in figures as T2 for both conditions) using multi-colour flow cytometry. Significant increases were observed in multiple cell phenotypes. Results will be discussed by cell type and described using a percentage change, *P* value, and Hedge's G (*g*) effect size. Specific cell counts shown for the seated control and exercise conditions are provided in Tables 2 and 3, respectively. Selected cell type responses to the exercise condition are shown in Figures 1 through 4 within the results. Responses categorized as autonomic complete and incomplete are located in Appendix A, Figures A-2 through A-5. All Hedge's G effect sizes (small effect = 0.2, medium effect = 0.5, large effect = 0.8) for both conditions are provided in Appendix B, Tables B-1 and B-2.

8.1 **Ratings of Perceived Exertion During Exercise**

Ratings of perceived exertion (RPE) using the 6-20 Borg scale were recorded during the last 10 seconds of every minute during the 30-minute exercise trial in all eight participants. A group average RPE of 13.4 ± 1.7 was recorded. Individuals with paraplegia reported an average RPE of 13.7 ± 1.8, while individuals with tetraplegia reported an average RPE of 13.1 ± 1.6.

8.2 **Lymphocytes**

A significant time X condition interaction was found for total CD3+ lymphocytes (P = 0.003). Post-hoc tests revealed that total CD3+ lymphocytes increased 19% from baseline to post exercise with a small effect size (P = 0.004, g = 0.319, Figure 1A). For CD4+ T helper cells, a subset of CD3+ lymphocytes, a significant time X interaction was also detected (P = 0.007). Post-hoc tests indicated a 16% increase from baseline to post exercise with a small effect size (P = 0.007, g = 0.286, Figure 1C). For the final subset of CD3+ lymphocytes, CD8+ T cytotoxic cells, a significant time X condition interaction was also found (P = 0.002). An increase of 24% from baseline to post exercise was detected in post-hoc testing, with a small effect size (P = 0.008, g = 0.329, Figure 1E). A significant time X condition interaction for CD3+/CD56+ NKT cells was also found (P = 0.003). CD3+/CD56+ NKT cells increased by 31% from baseline to post exercise with a small effect size (P = 0.003, g = 0.419, Figure 2C). For CD19+ B cells there was a main effect of time (P = 0.01), and when conditions were collapsed, a significant change of 17% was observed from baseline (T0) to the final time point (T2; P = 0.008, g = 0.374) with a small effect size. Of the lymphocyte subpopulations, the largest exercise-induced increase was observed in CD56+ NK cells with a time X condition interaction (P < 0.001) which increased from baseline to post exercise by 63% with a large effect size within the post-hoc pairwise comparison (P < 0.001, g = 0.905, Figure 2E). During the seated protocol, there was a significant time X condition interaction observed in total CD3+ lymphocytes, which increased 19% from baseline to 120 minutes (P = 0.004, g = 0.407), and 20% from 30 minutes to 120 minutes (P = 0.002, g = 0.415, Figure

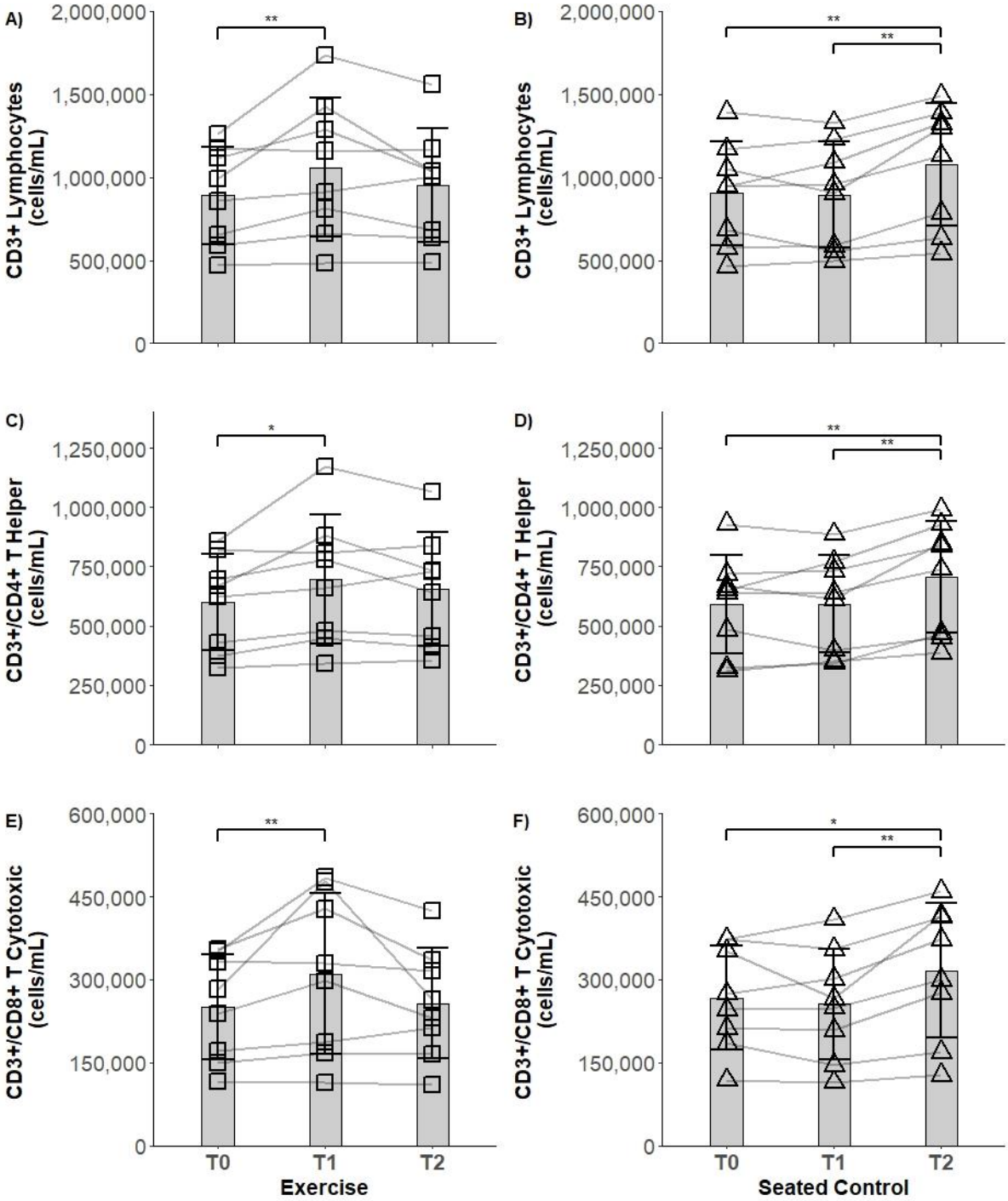

Figure 1. Cellular responses for total lymphocytes, T helper cells, and T cytotoxic cells during 30-minutes of submaximal aerobic exercise on an arm cycle ergometer (left), and during a seated control condition (right) in individuals with SCI. T0 = Baseline, T1 = Post exercise/30 minutes control, T2 = 90 mins post exercise/120 mins control. * $P \leq 0.05$, ** $P \leq 0.01$ (Tukey's post-hoc test).

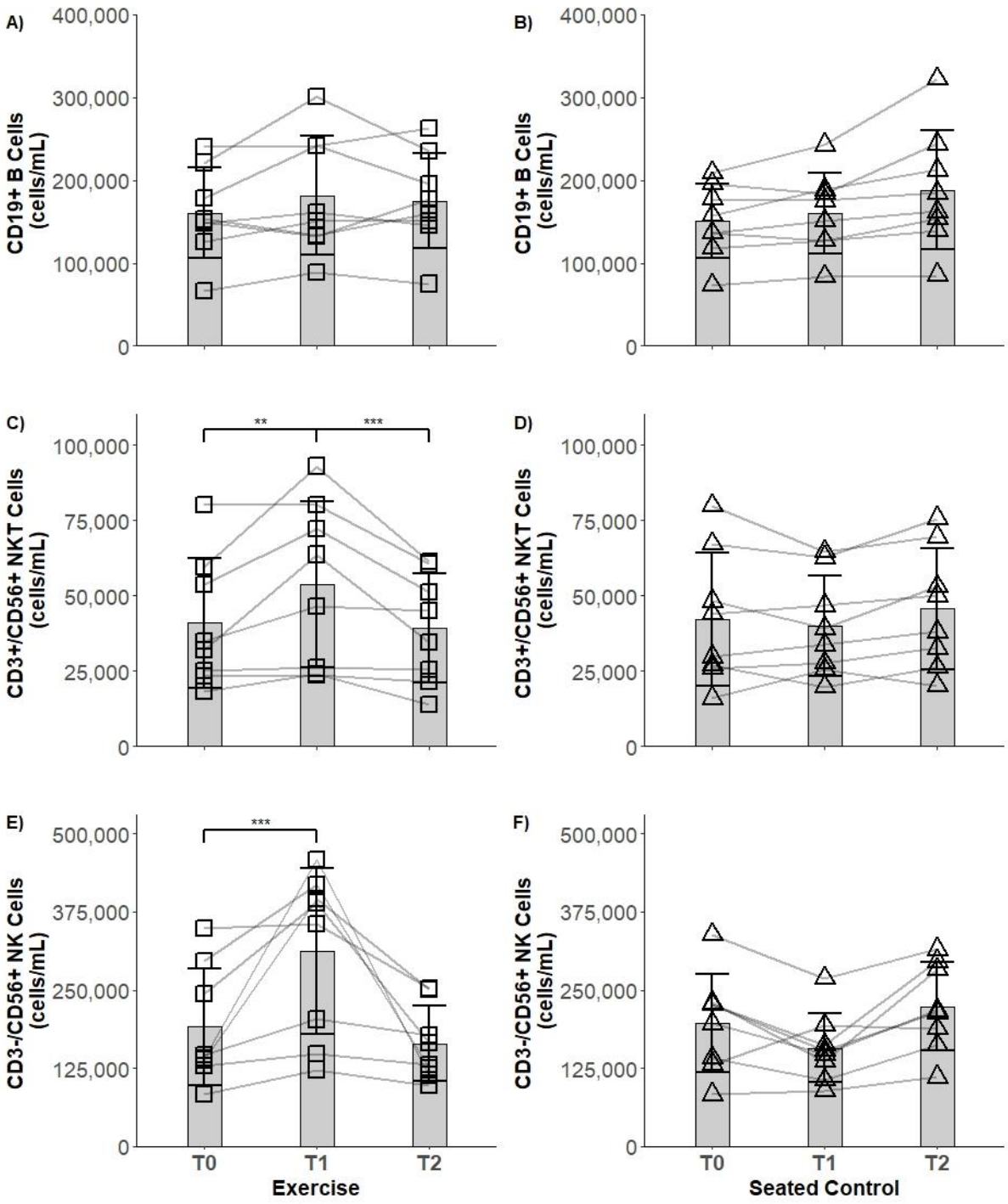

Figure 2. Cellular responses for B cells, NKT cells, and NK cells during 30-minutes of submaximal aerobic exercise on an arm cycle ergometer (left), and during a seated control condition (right) in individuals with SCI. T0 = Baseline, T1 = Post exercise/30 minutes control, T2 = 90 mins post exercise/120 mins control. ** $P \leq 0.01$, *** $P \leq 0.001$ (Tukey's post-hoc test).

1B) both with small effect sizes. CD4+ T helper cells increased 19% with a time X condition interaction from baseline to 120 minutes (P = 0.001, g = 0.438), and remained 19% above baseline from 30 to 120 minutes (P = 0.002, g = 0.4, Figure 1D) with small effect sizes. CD8+ T cytotoxic cells increased 19% with a time X condition interaction from baseline to 120 minutes (P = 0.031, g = 0.295) and 24% from 30 to 120 minutes (P = 0.008, g = 0.424, Figure 1F). No significant changes during the seated condition were observed in CD19+ B cells (Figure 2B), CD3+/CD56+ NKT (Figure 2D), CD56+ NK (Figure 2F) populations. Additionally, no significant time X condition interactions were observed between baseline and 30 minutes during the seated condition.

8.3 **Monocytes**

A main effect of time was found for CD14+ classical monocytes (P = 0.018), which increased 15% from baseline to the final measurement when conditions were collapsed (P = 0.015, g = 0.384). CD16+/CD14$_{dim}$ non-classical monocytes showed a significant condition X time interaction (P = 0.005), with post-hoc tests indicating a significant decrease of 27% from post exercise to 90 minutes post exercise with a large effect size (P = 0.017, g = 0.804, Figure 3E). During the seated control condition, a 40% increase was observed in CD16+/CD14$_{dim}$ non-classical monocytes from 30 to 120 minutes (P = 0.033, g = 1.055 Figure 3F) with a large effect size. CD14+/CD16+ intermediate monocytes showed no significant interactions (P = 0.072, Figure 3C, 3D) or main effects of time (P = 0.274).

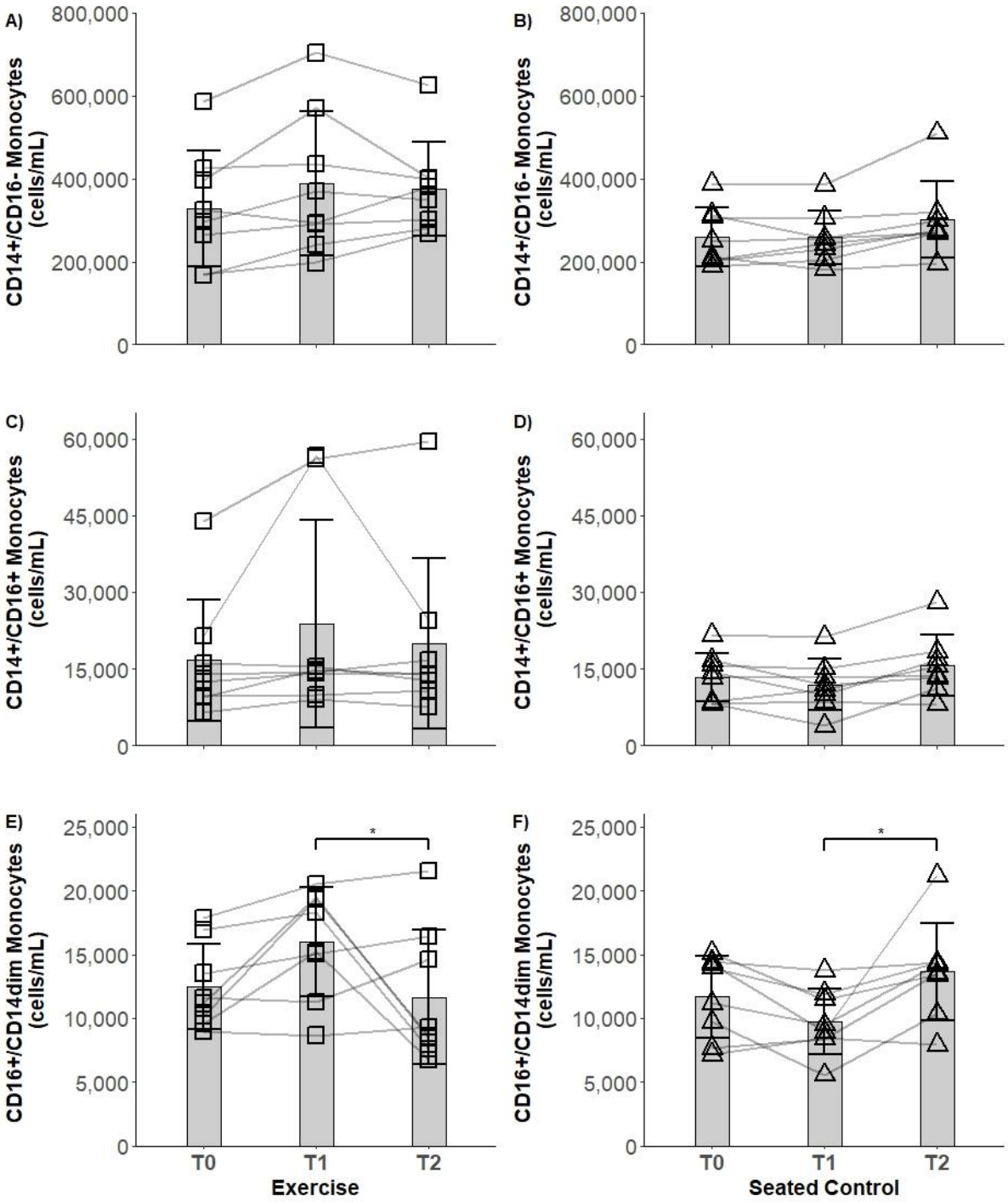

Figure 3. Cellular responses for classical monocytes, intermediate monocytes, and non-classical monocytes during 30-minutes of submaximal aerobic exercise on an arm cycle ergometer (left), and during a seated control condition (right) in individuals with SCI. T0 = Baseline, T1 = Post exercise/30 minutes control, T2 = 90 mins post exercise/120 mins control. * $P \leq 0.05$ (Tukey's post-hoc test).

8.4 **Granulocytes and Total Leukocytes**

CD16+ neutrophils showed no time X condition interactions (*P* = 0.317, Figure 4A, 4B) but a main effect of time was detected (*P* = 0.011). Post hoc testing revealed a 26% increase from baseline to the final measurement when exercise and seated control conditions were collapsed with a medium effect size (*P* = 0.008, *g* = 0.664).

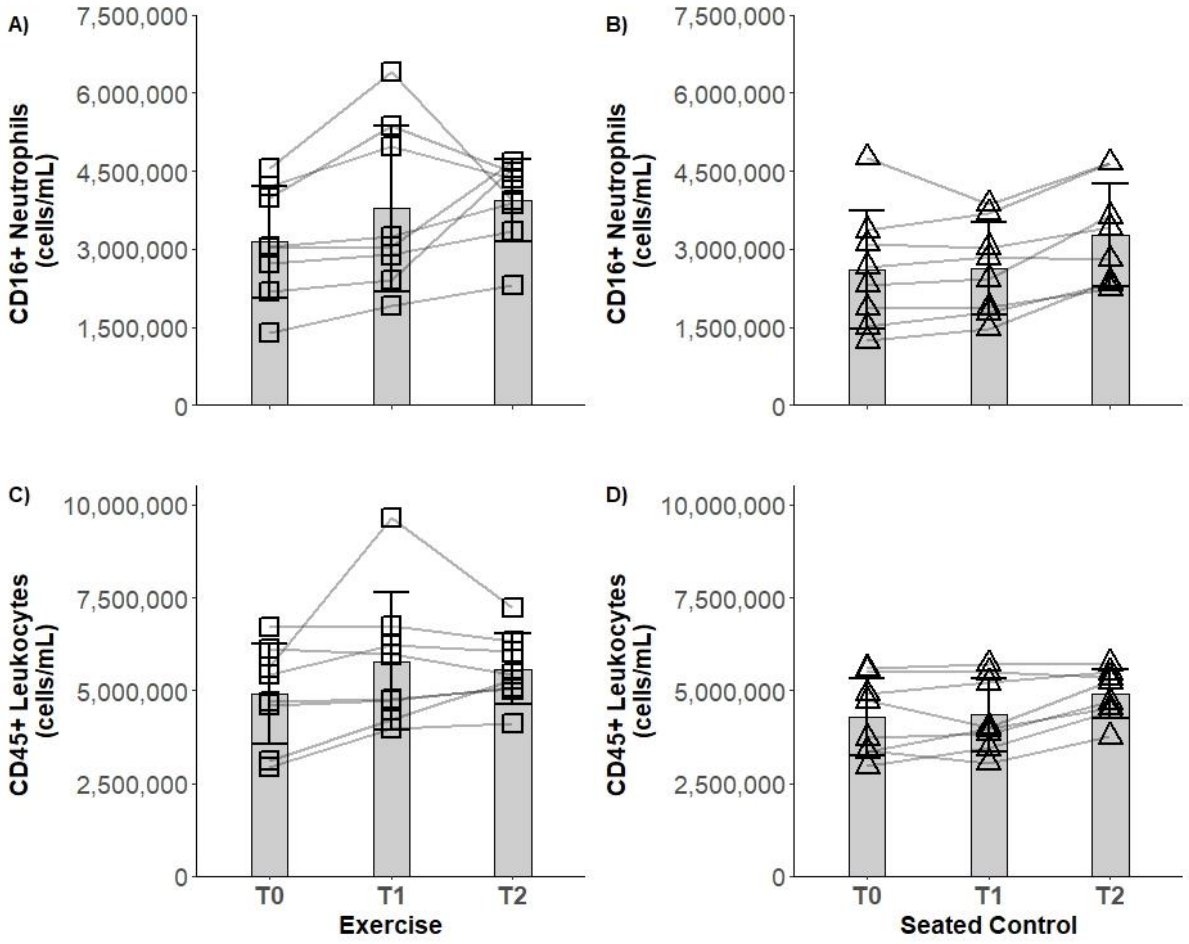

Figure 4. Cellular responses for neutrophils and total leukocytes during 30-minutes of submaximal aerobic exercise on an arm cycle ergometer (left), and during a seated control condition (right) in individuals with SCI. T0 = Baseline, T1 = Post exercise/30 minutes control, T2 = 90 mins post exercise/120 mins control.

Though there was no significant time X condition interaction (*P* = 0.111, Figure 4C, 4D), a significant main effect of time for total CD45+ leukocytes was observed (*P* = 0.039). Post hoc testing revealed a 14% increase from baseline to 120 minutes (*P* = 0.037, *g* = 0.533) with a medium effect size when groups were collapsed. Additionally, there was a main effect of group (*P* = 0.005) for CD45+ leukocytes with the exercise condition possessing higher overall values.

As an exploratory analyses, participants were separated into presumed injury categories of autonomic complete and incomplete based on the ability to achieve a maximal HR of <130 bpm (complete) or >130 bpm (incomplete) to help ascertain if sympathetic innervation may have impacted the observed immune cell responses to acute submaximal aerobic exercise. These data are shown in Appendix A, figures A-2 through A-5. Based on this proposed classification scheme [160, 161], all participants with tetraplegia were categorized as autonomic complete achieving a maximal HR of 102 – 131 bpm during the familiarization trial whereas all individuals with paraplegia were categorized as autonomic incomplete achieving a maximal HR of 182 – 199 (Table 1). However, it should be noted that for individuals with tetraplegia, a lower intensity incremental ramp (2W/min) was chosen. All 4 individuals with tetraplegia were successfully able to complete the entire 30-minute maximum duration allotted for the familiarization protocol, and therefore may not represent true maximal HR values. None of the 4 individuals with paraplegia were able to endure the full length of the trial due to volitional fatigue, suggesting accurate maximal HR values were recorded during their test with a 10W/min incremental resistance increase. Although the sample size did not permit separating these

groups for formal statistical analyses, it can be seen in Appendix A that there did appear to be potential differences in responses between the autonomic complete (tetraplegia) and incomplete (paraplegia) for NK cells (Figure A-3E) and non-classical monocytes (A-4E). The planned analyses of plasma catecholamines may help further support this mechanism of sympathetically mediated immune responses to exercise for these cells in individuals with SCI.

9.0 **Discussion**

Individuals with SCI face myriad secondary health consequences of which immune system dysregulation remains a priority. This is the first study, at the time of writing to our knowledge, providing comprehensive leukocyte responses to acute submaximal aerobic exercise in individuals with varying spinal cord injuries (e.g. paraplegia, tetraplegia, complete or incomplete). Such comprehensive leukocyte responses to aerobic exercise in line with the SCI physical activity guidelines have been largely unreported in the literature. The data obtained in this thesis provides detailed leukocyte subset responses to a standard, and achievable bout of exercise in individuals with SCI and may help guide further investigations to explore unique mechanisms regarding specific cell responses and functions, leading to improved understanding of how exercise impacts the immune system in individuals with SCI.

9.1 **Acute exercise increases NK cells in SCI**

In this study, we obtained cell counts for all major immune cell types at baseline (T0), upon exercise completion or the 30-minute time point in seated control (T1), and 90 minutes after exercise termination or 120 minutes of seated control (T2). Using flow cytometry, we were able to characterize and measure 11 cell phenotypes for subsequent analysis as seen in Tables 2 and 3. Of the leukocyte measures obtained, the largest increase and effect size was observed in NK cells following acute submaximal aerobic exercise (Figure 2E). NK cells respond strongly to catecholamine release and signaling, as well as sympathetic innervation [22, 30] both of which are increased during exercise. Our findings coincide with observations made by Klokker and colleagues in which participants with SCI exercised for 30

minutes while experiencing FES. NK cells increased significantly in the paraplegia and tetraplegia group, however individuals with paraplegia experienced a greater response corresponding with observations of greater plasma catecholamine concentrations [124]. NK cell recruitment appears to be highly responsive to extremes of both aerobic and anaerobic exercise in the able-bodied population – in that very high [117], short duration and moderate, long duration exercise both promote significantly higher cell numbers in the peripheral circulation in able bodied individuals [118, 144]. Additional meta-analysis suggests that NK cell recruitment appears to peak around 30 minutes into exercise, with smaller increases seen as exercise duration is prolonged [162]. Thus, it appears that an exercise duration of ~30 minutes may be sufficient for recruiting NK cells into the peripheral circulation for individuals with SCI as observed in our study.

9.2 **Acute exercise increases T cells in SCI**

Thirty minutes of submaximal aerobic exercise increased circulating CD3+ lymphocytes (Figure 1A) of which CD4+ T helper (Figure 1C), CD8+ T cytotoxic (Figure 1E) and CD3+/CD56+ NKT cell (Figure 2C) counts were significantly greater than baseline values immediately following exercise. Lymphocytes express both alpha and beta adrenergic receptors thereby prompting circulating cell numbers to be impacted by fluctuations in plasma catecholamine concentrations [163-165]. NKT lymphocytes share an innate lineage as they recognize MHCI receptor antigens acting as a bridge between innate and adaptive immunity [12]. It is possible that these cells enter the peripheral circulation upon exercise as a defense mechanism given their connection to the innate immune system. Plasma catecholamine

concentrations are elevated during exercise in those without cervical spinal cord injury [97], and this augmentation coupled with increased sympathetic stimulation may lead to greater numbers of CD4+ T cells entering the peripheral circulation. The significant exercise-induced increases in CD4+ and CD8+ in our study coincide with other studies, albeit in able bodied individuals. Similar to NK cells, CD4+ and CD8+ T cells also increase in relation to intensity and duration of exercise. Maximal incremental treadmill tests lead to significant elevations of CD4+ and CD8+ T cells [117]. Exercise at a brief, higher intensity (80% VO_2max) elicits a lymphocytosis, whereas short durations of 50% VO_2max do not [154]. Alternatively, longer durations of ~60 minutes at lesser intensity leads to increases in circulating lymphocytes [22]. Interestingly, fitness level appears to influence recruitment of lymphocytes. Exercising for 20 minutes 70% VO_2max leads to a lymphocytosis in unfit individuals for both CD4 and CD8 T cells, but not in fit individuals suggesting long term adaptations to exercise may be important for influencing exercise induced lymphocytosis [153]. Our increases of 16% and 24% for CD4 and CD8 T cells, respectively, resulted from exercising at 60% peak power output, similar to intensities in many prior studies [22]. The 31% increase seen in CD3+/CD56+ NKT cells was similarly observed during an exercise trial involving back to back 30-minute aerobic efforts at 70% VO_2max wherein a significant increase was observed in men but not adolescent boys [148]. It would appear, again, that 30 minutes of submaximal aerobic exercise may be sufficient for promoting a lymphocytosis in individuals with SCI. Further research exploring the relationship between CD4+ T helper, CD8+ T cytotoxic and CD3+/CD56+ NKT cell recruitment and catecholamine

responses to exercise in individuals with SCI compared to the able-bodied population is warranted.

9.3 **Non-classical proinflammatory CD16+/CD14$_{dim}$ monocytes decrease during recovery from acute exercise in SCI**

In contrast to the general increase in lymphocytes, monocytes did not increase following exercise in the current study. The monocyte subpopulation consisting of a CD16+/CD14$_{dim}$ phenotype displayed a significant reduction from post exercise to 90 minutes post exercise. Monocytes are separated into three distinct phenotypes, classical (CD14+/CD16-), intermediate (CD14+/CD16+) and non-classical or proinflammatory (CD16+/CD14$_{dim}$) [129]. TNFα is a cytokine released from monocytes [166] though the specific origin with respect to the three monocyte subpopulations remains contentious [129]. The 27% decrease observed between exercise cessation and our final measure as seen in Figure 3E illustrates that this monocyte subset responded strongly during the following 90 minutes after submaximal aerobic work. Exercise has been shown to favourably alter monocyte microRNAs, of which several being potentially linked to atherosclerosis and vascular disruption [167]. Additionally, Radom-Aizik and colleagues observed a doubling of monocytes into the circulation, albeit in young, able-bodied individuals and while using a sprint style protocol at ~80% of participant VO_2max [167]. Monocytes as a whole appear to increase during high intensity exercise, but not at lower intensities suggesting a reliance on some combination of shear stress, catecholamines, and sympathetic innervation [118]. Step wise increases in intensity also lead to a transitory monocytosis [133], as do prolonged bouts of submaximal work [118].

Interestingly, aerobic submaximal exercise performed in individuals with SCI does not promote the same result. In fact, shorter durations of 20 minutes [97, 127], and longer durations of 2 hours did not induce an overall monocytosis in individuals with SCI [134]. Though we observed a significant increase with a main effect of time for CD14+ monocytes (P = 0.018), we did not detect a time X condition interaction (Figure 3A, 3B), indicating that the increase in CD14+ monocytes from T0 to T2 was likely a diurnal effect. Overall, the results suggest that submaximal aerobic exercise does not reliably elicit a monocytosis for total or classical CD14+ cells but is sufficient at leading to decreased numbers of circulating peripheral non-classical CD16+/CD14$_{dim}$ monocytes in individuals with SCI in the time following exercise cessation. Given that SCI is associated with chronic low-grade inflammation [59, 168], it is possible that the significant reduction of the more proinflammatory CD16+/CD14$_{dim}$ monocytes from the circulation and possibly into marginal pools may be beneficial for the SCI population although this notion requires further investigation.

9.4 **B cells do not appear to be affected by exercise in SCI**

B cells increased as a main effect of time over the course of 120 minutes in our SCI participants (P = 0.01) with no significant time X condition interactions (Figure 2A, 2B). Similar to other leukocytes, B cells appear to respond to both high exercise intensities and prolonged submaximal aerobic exercise in the able-bodied population. Studies have produced fairly consistent meaningful increases at intensities of ≥ 70% VO_2max [117] but not generally at intensities of 50% VO_2max or lower during a brief, or sustained effort [154]. B cell numbers increase strongly

during very high intensity work in which significant increases were observed during brief exercise sessions at ~ 95% VO_2max [118]. Longer durations of cycling for 60 minutes at 60% VO_2max have led to increases in circulating B cells [22] suggesting duration is an appreciable influence for their recruitment and mobilization when performed at ≥ 50% VO_2max. Moyna and colleagues observed an increase in B cells following each stage of an 18 minute (6 min per stage) duration stepwise cycling protocol at 55%, 70% and 85% VO_2max [122] suggesting that even a short duration at 6 minutes of 55% VO_2max is sufficient to increase B cells in the peripheral circulation in healthy, able bodied individuals. Contentiously, Tvede and colleagues failed to observe a significant increase in B cells in untrained healthy males following 1 hour of exercise on a cycle ergometer at 25%, 50% and 75% VO_2max [155]. Lymphocytes in general express β2ARs [169] and thus one would expect a response to exercise in relation to the release of catecholamines. The lack of exercise induced B cell recruitment in our study is difficult to explain as we observed considerable increases in CD56+ NK cells, CD4+ T cells and CD8+ T cells, which are presumably recruited by similar catecholamine-mediated mechanism(s). Mechanistically, the primary function of B cells is antibody secretion, and this component may not be paramount during physical exertion versus a bacterial or viral pathogen challenge. Immune function in individuals with SCI is known to be suppressed, persisting into the chronic injury stage due to nerve damage and subsequent autonomic disruption [59, 93, 170]. Observations of lower epinephrine and norepinephrine levels during exercise have been recorded in individuals with tetraplegia [97, 171], which make up half of our participant study population. More

intriguing is that in many cases, individuals experiencing a cervical injury may still possess some level of descending sympathetic nervous system control [172] reinforcing the need to measure catecholamine concentrations and immune cell recruitment concurrently to characterize unique cases and individual variability. Unfortunately, due to the COVID-19 situation we were unable to perform the planned catecholamine assays for this study at the time of thesis submission. In summary, it appears that 30 minutes of submaximal aerobic exercise on an arm cycle ergometer was insufficient for recruiting B cells into the peripheral circulation within the SCI sample in this study.

9.5 **Acute exercise does not appear to increase neutrophils in SCI**

Though a significant main effect of time was observed in CD16+ neutrophils (P = 0.01), there was no time X condition interaction (Figure 4A, 4B) suggesting that the exercise intensity was inadequate for neutrophil vascular demargination. Similarly, a main effect of time (P = 0.039) was observed for total CD45+ leukocytes which is expected given that neutrophils comprise the bulk of total CD45+ leukocytes [51] and significantly increased over time in our trial. Because neutrophils primarily reside at rest on the vascular endothelium, they are readily demarginated and may enter the peripheral circulation rapidly [110]. In the able-bodied population, neutrophils respond to a wide variety of exercise protocols, intensities and durations. For instance, neutrophils increased following a 2 hour cycle ergometer protocol at 60% VO_2max, brief durations at ~95% VO_2max [118], and maximal incremental treadmill tests [117]. Neutrophils were also reported to be increased following a moderate 45 minute exercise bout at 60% VO_2max [119], with a delayed neutrophilia

2 hours after a 20-minute 75% VO_2max test [120]. Observable increases in neutrophils were also recorded after stepwise protocols starting at moderate intensities [122, 123]. Many studies in SCI have replicated similar results. During upright cycling using FES, neutrophils have been observed to increase following a 30-minute cycling protocol, remaining elevated for 2 hours after exercise termination [124]. Neutrophils also increased during a shorter protocol of 20 minutes at 60% [127]. Persisting neutrophilia was observed after 1 hour of self-paced wheel chair exercise around a 400m track in individuals with SCI [125]. Longer durations have also led to significant increases in circulating neutrophils as observed after a wheelchair half marathon [126]. The lack of neutrophil response in our exercise trial can possibly be explained by two factors: shear stress and exercise intensity. Neutrophils at rest are adhered to vascular endothelium and the detectable cells in the peripheral circulation represents a fraction of the total neutrophil pool. The total pool is vast, as exercise has been shown increase circulating neutrophils by 2-5 fold [110]. Resting neutrophils “roll” along the arterial walls via adhesion molecules L-selectin and P (Platelet and endothelial) -selectin glycoprotein ligand – 1 (PSGL-1) [173] thereby eluding hematological analysis. During sufficient exercise intensities, increased cardiac output leads to subsequent increases in shear stress which overcome the bond strength of these adhesion molecules [173] and the neutrophil enters the peripheral circulation. In individuals with SCI, cardiac output is further reduced due to a lower end diastolic volume (EDV) from a subsequent reduction in sympathetic innervation below the site of injury [174]. Additionally, heart rate correlates strongly with the level of injury [172], depending on the remaining

sympathetic function with overall reductions experienced in this population. Lower absolute exercise intensity and reductions in engaged skeletal muscle mass may lead to lower cardiac output when compared to that of able-bodied individuals [61]. Thus, it is likely that the degree of exercise intensity and lack of sufficient cardiac output required to increase shear stress to initiate neutrophil demargination was not satisfied, therefore, no significant exercise induced increase was observed.

9.6 **Diurnal effects seen during the seated control condition.**

Interestingly, significant increases were observed during the seated control protocol in CD3+ lymphocyte (Figure 1B), CD4+ T helper (Figure 1D), CD8+ T cytotoxic (Figure 1F) and CD16+/CD14$_{dim}$ monocytes (Figure 3F). Many of the measures achieved significance from the baseline to final measurement such as CD3+, CD4+, CD8+ and from the 30-minute measurement to the final measurement including CD3+, CD4+, CD8+, CD16+/CD14$_{dim}$. It should be noted that no significant difference was observed in any cellular measure from baseline to the 30-minute time point, indicating that, in general, 2 hours of seated control (and continued fasting) led to increases in circulating concentrations of several different leukocytes. Diurnal variation appears to be the logical explanation for these phenomena. Participants sat quietly for 120 minutes while measures were taken, in the absence of physical activity or food intake and yet increases were observed over the course of 2 hours. A study in which hematological parameters were observed over the course of 24 hours in able-bodied individuals demonstrated a steady increase in neutrophils and total leukocytes from 9am until ~8pm and ~10pm, respectively [175], though the participants were provided meals throughout the day whereas our participants

remained fasted. Total leukocytes have also been observed to increase steadily from 8:30am until 11:00am alluding to some level of diurnal influence [176]. Similar gradual increases were observed in total leukocyte count from 9:00am until ~12:00pm [177] further reinforcing the gradual increase in leukocytes throughout the day from influences not related to exercise. It is unlikely that this diurnal increase is influenced by catecholamines as there are not emergent trends in plasma epinephrine or norepinephrine when measurements are made throughout the day, although less is known regarding diurnal activity in SCI [178]. The circadian clock genes Per1 and Per2 are the primary clock genes in human leukocytes. Importantly, Per1 is strongly associated with the oscillations observed in diurnal leukocyte variation in healthy adults [179]. In individuals with cervical SCI, there exists disruption to clock gene expression specifically when compared to able bodied individuals [180] which may account for some of the observations established in our study that appear at odds with previous literature on diurnal variation in neutrophils, lymphocytes and monocytes in volunteers without SCI. Future studies should explore clock gene variance with relation to circadian rhythms in individuals with varying chronic spinal cord injuries to identify whether these patterns are disrupted and whether they influence immune function. These findings of changes in several leukocytes following 2 hours of sitting in the morning may highlight the importance of seated control conditions in exercise immunology studies.

10.0 **Conclusion**

10.1 **Strengths and Limitations**

Our study utilized a randomized crossover design in which we tested the exercise hypothesis against the same set of individuals across a seated control condition where blood samples were collected at matched timepoints. This allowed us to determine changes relative to the individual, and not against the able-bodied population in which extensive evidence can be found. We did not recruit individuals with specific injury characteristics such as cervical or thoracic injury only, instead we obtained a heterogenous, recreationally active population to determine whether exercise alters immune responses in this diverse group as a whole. Furthermore, participants exercised in a supervised and controlled clinical setting designed around SCI access to ensure exercise requirements were met, while cells were analyzed immediately following blood draws ensuring accurate cell staining and subsequent quantification.

Though our study provides thorough exercise response data, it is only carried out on 8 individuals with varying degrees of SCI. Studies in the SCI literature often report smaller sample sizes, so it is difficult to generalize to a population when each injury and set of physiological disruptions are unique. We chose 60% peak power output as our aerobic exercise resistance due to evidence guided cardiometabolic recommendations [81] for this population and this exercise intensity and duration is in line with the most recent SCI activity guidelines [104]. However, much of the exercise immunology literature reports using ~70% VO_2max and often exercise is of longer duration (e.g., 60 min). Measuring a comprehensive leukocyte panel as we

did in response to various intensities and/or durations of exercise could have provided more mechanistic insight into what factors drive cell-specific responses. Additionally, a lack of catecholamine measures reduces the ability for us to correlate our observed cellular responses with existing sympathetic activity. Furthermore, our participants were not homogenous, as individuals possessed a wide range of spinal cord injury level, level of completeness, as well as basal level of fitness. Because we recruited recreationally active individuals, it is likely these individuals specifically did not experience chronic inflammation to the same degree as a sedentary individual. It is therefore likely that our results are more relevant for active populations that are accustomed to regular exercise. We also did not include an able-bodied control group which may limit the ability to draw direct comparisons. This decision was made due to striking differences in physiology, as all SCIs differ and are largely heterogenous. Appropriate exercise prescription becomes difficult given the degree of functional skeletal muscle use, combined with determinations for which parameters are to be used to select able-bodied participants (e.g., how to match for relative fitness, habitual activity levels, familiarity with arm cycle ergometry, etc.). Finally, it may serve future investigations to stratify individuals into groups and carry out a fully powered study analyzing differences and similarities within NLI, injury completeness, catecholamine responses, and exercise induced changes in peripheral leukocyte populations.

10.2 **Future Directions**

Further study into exercise-induced leukocytosis in SCI should focus on the potential for specific changes to impact immune function (e.g., ability to fight off pathogens) or

influence chronic low-grade inflammation. This study also reported a previously unidentified decrease in proinflammatory CD16+/CD14$_{dim}$ monocytes following moderate exercise in SCI and the functional consequences of this should be explored. It is possible the significant decrease in CD16+/CD14$_{dim}$ monocytes could help reduce chronic low-grade inflammation over time, in that a reduction in circulating concentrations of this monocyte subset following exercise could be viewed as promoting a more anti-inflammatory environment, which may be considered as ideal in someone with chronic inflammation. Future research should also determine the extent of T-regulatory behavior and its impact on promoting a cellular environment that favours anti-inflammation. Furthermore, a large scale, fully powered study would be needed to properly compare the effects of exercise on circulating leukocytes stratified by different SCI categories such as AIS scores, injury location, and injury severity with a long-term chronic exercise program. Finally, this data should be considered for future exercise-based recommendations and characterization of cellular responses for exercise interventions, and the potential to impact the immunodepression experienced by those with SCI.

10.3 **Final Remarks**

Acute, submaximal aerobic arm crank ergometer exercise at 60% peak power output for 30 minutes in our participants appears sufficient to increase important circulating peripheral leukocyte subpopulations, including total CD3+ T cells, CD4+ T helper, CD8+ T cytotoxic, CD3+/CD56+ NKT, and CD56+ NK cells. However, this moderate bout of exercise appeared insufficient to cause general leukocytosis, nor did it impact circulating neutrophil counts beyond a main effect of time. The

outcomes of our pilot study suggest that specific immune cell phenotypes are highly variable and respond differently to submaximal aerobic exercise. Additionally, we have contributed measures for cells in the same group of participants that are either nonexistent or underreported in the SCI exercise literature including CD4+ T helper, CD8+ T cytotoxic, CD3+/CD56+ NKT and CD16+/$CD14_{dim}$ monocytes. With the reporting of these results, we anticipate new directions of cellular responses to exercise in SCI to be explored, such as mechanistic functions and cell effector functions to better understand and treat immunodeficiency in this unique population.

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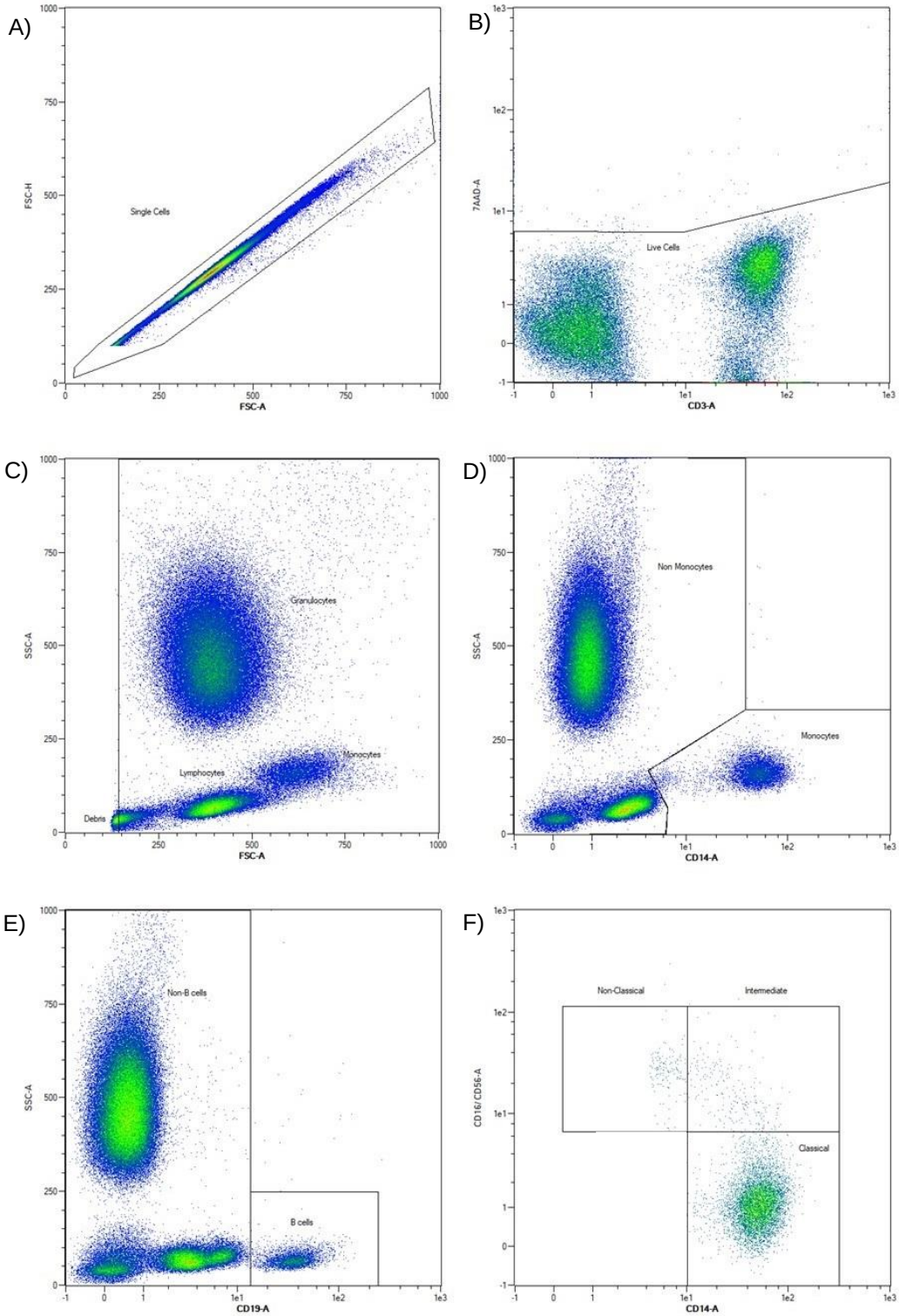
Appendices

Appendix A - Figures



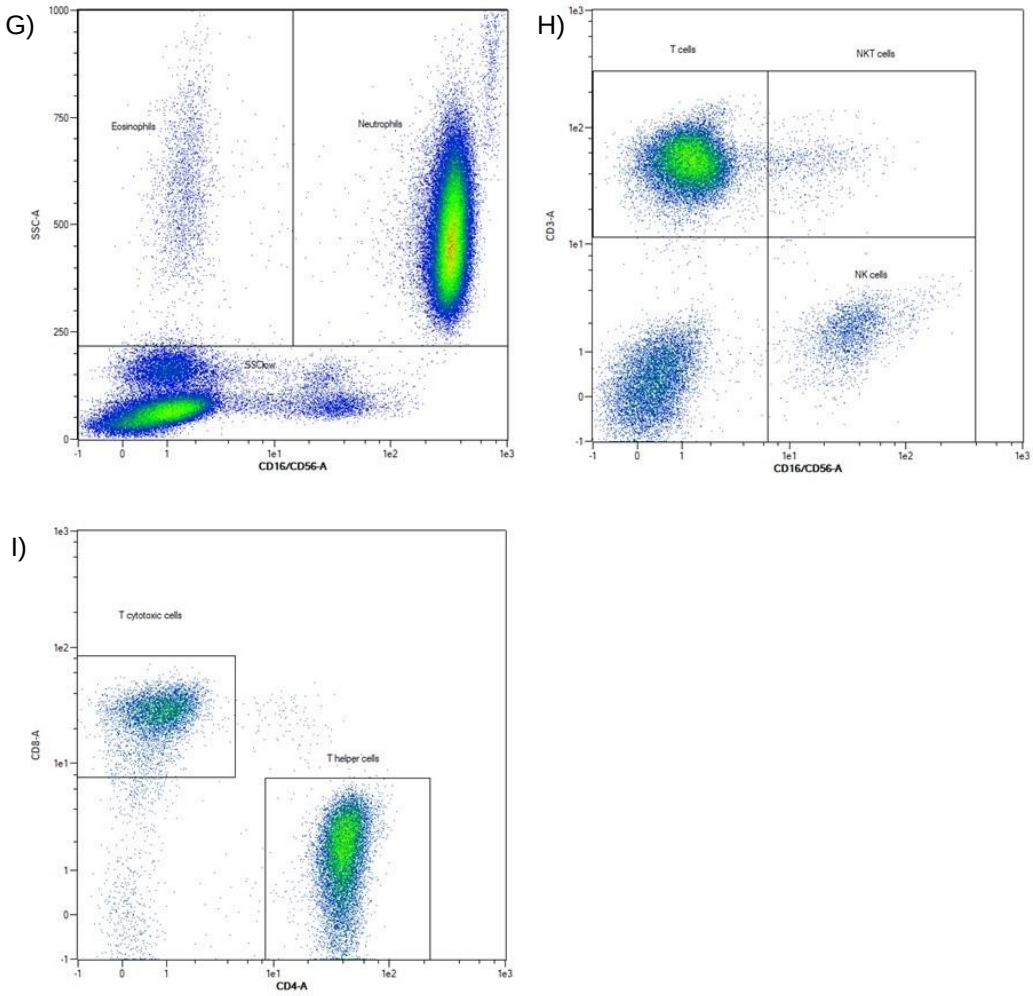


Figure A-1. Hierarchical gating strategy for separation of human peripheral leukocytes. ***A)*** Single cells are separated from doublet, or adhered cells and sent to the dead cell exclusion gate. ***B)*** Dead cells are determined by positive staining for 7-AAD and excluded from analysis sending live cells to the morphology gate. ***C)*** Cell morphology is plotted, and cell populations determined by plotting forward scatter against side scatter. ***D)*** Monocytes are separated using CD14 against side scatter from live cells in the morphology gate. ***E)*** B cells are separated using CD19 against side scatter from live cells in the morphology gate. ***F)*** Classical, intermediate, and non-classical monocytes are determined by the presence of CD16 on CD14 monocytes using CD14 against CD16 from monocytes in the total monocyte gate. ***G)*** Neutrophils are plotted using CD16 vs side scatter and separated from the low side scatter cells (SSCLow) from the non monocyte gate. ***H)*** Total lymphocytes, NK cells and NKT cells are determined by plotting CD56 against CD3 from cells in the SSCLow gate. ***I)*** T cell are finally gated using CD4 against CD8 to determine T helper, and T cytotoxic cells.

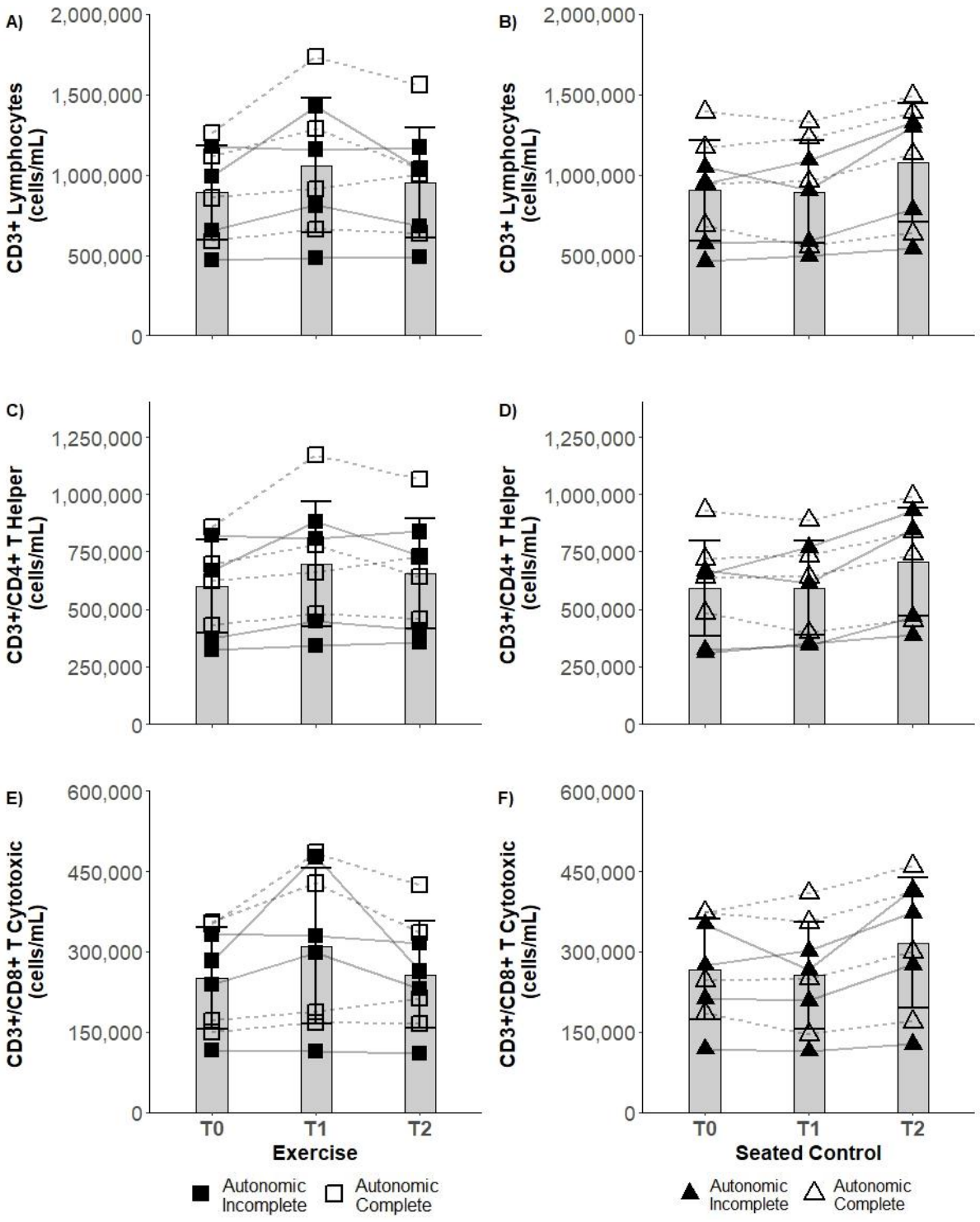


Figure A-2*.* Cellular responses for total lymphocytes, T helper cells, and T cytotoxic cells during 30-minutes of submaximal aerobic exercise on an arm cycle ergometer (left), and during a seated control condition (right) in individuals with SCI separated by autonomic completeness. T0 = Baseline, T1 = Post exercise/30 minutes control, T2 = 90 mins post exercise/120 mins control.

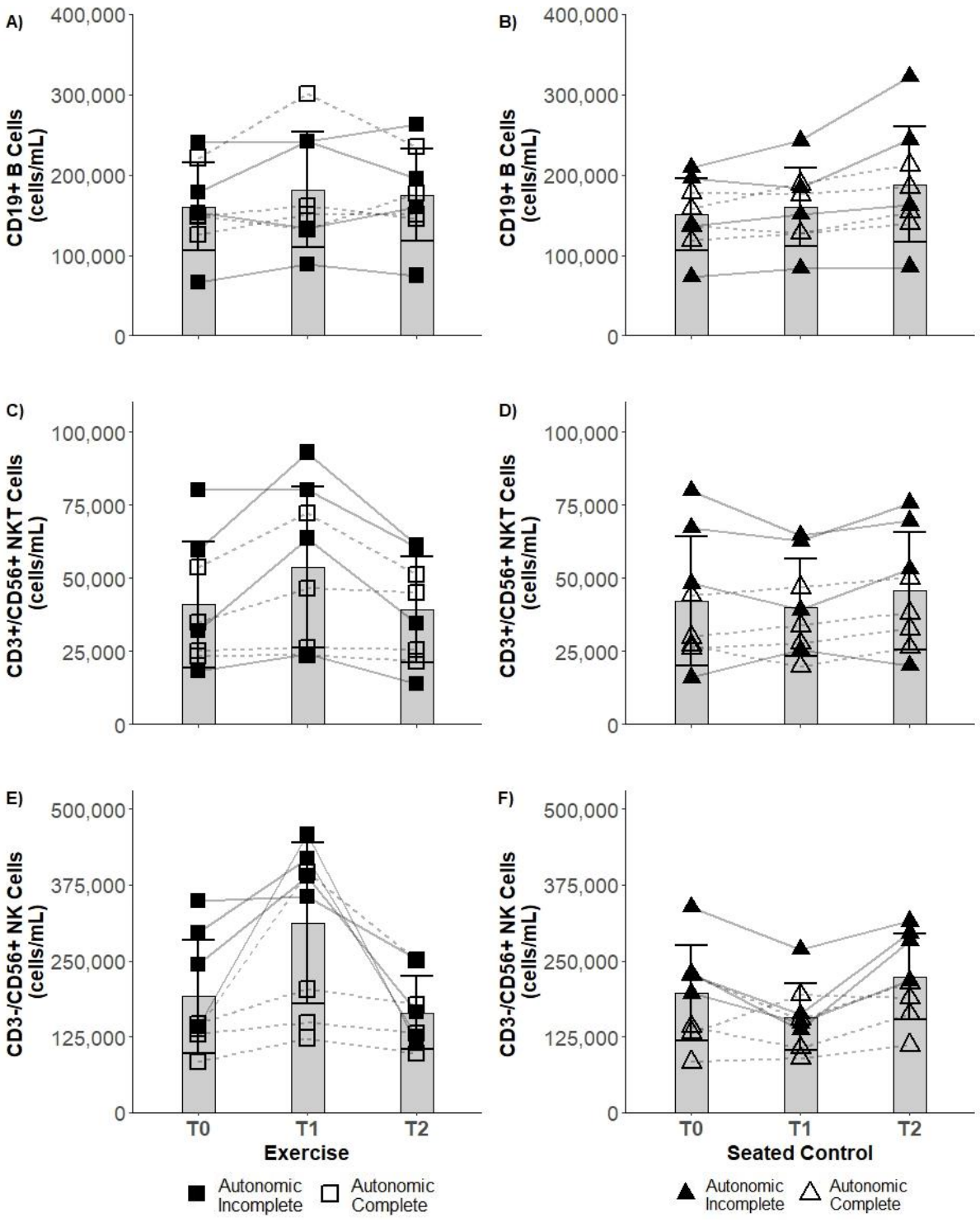


Figure A-3. Cellular responses for classical monocytes, intermediate monocytes, and non-classical monocytes during 30-minutes of submaximal aerobic exercise on an arm cycle ergometer (left), and during a seated control condition (right) in individuals with SCI separated by autonomic completeness. T0 = Baseline, T1 = Post exercise/30 minutes control, T2 = 90 mins post exercise/120 mins control.

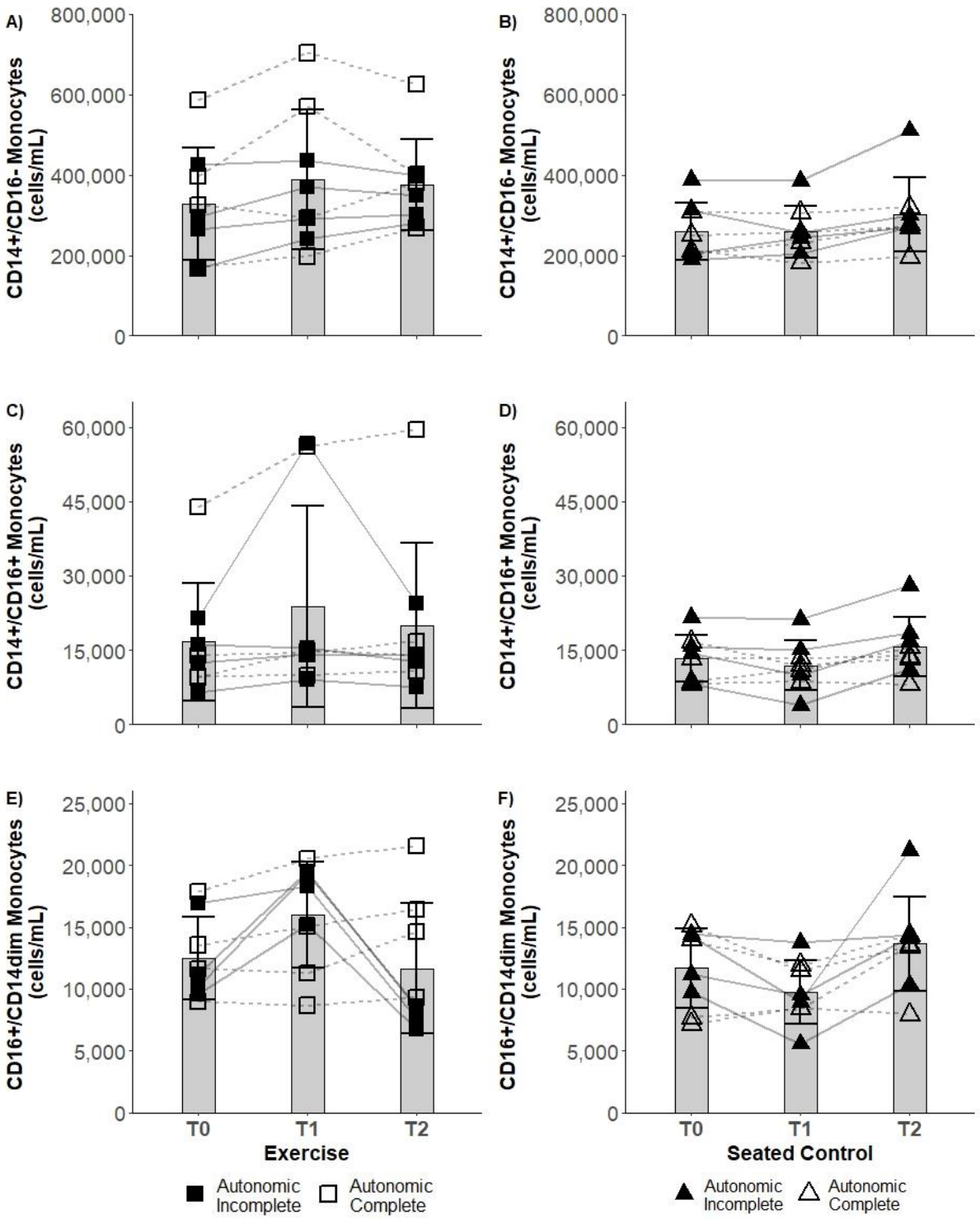


Figure A-4. Cellular responses for neutrophils and total leukocytes during 30-minutes of submaximal aerobic exercise on an arm cycle ergometer (left), and during a seated control condition (right) in individuals with SCI separated by autonomic completeness. T0 = Baseline, T1 = Post exercise/30 minutes control, T2 = 90 mins post exercise/120 mins control.

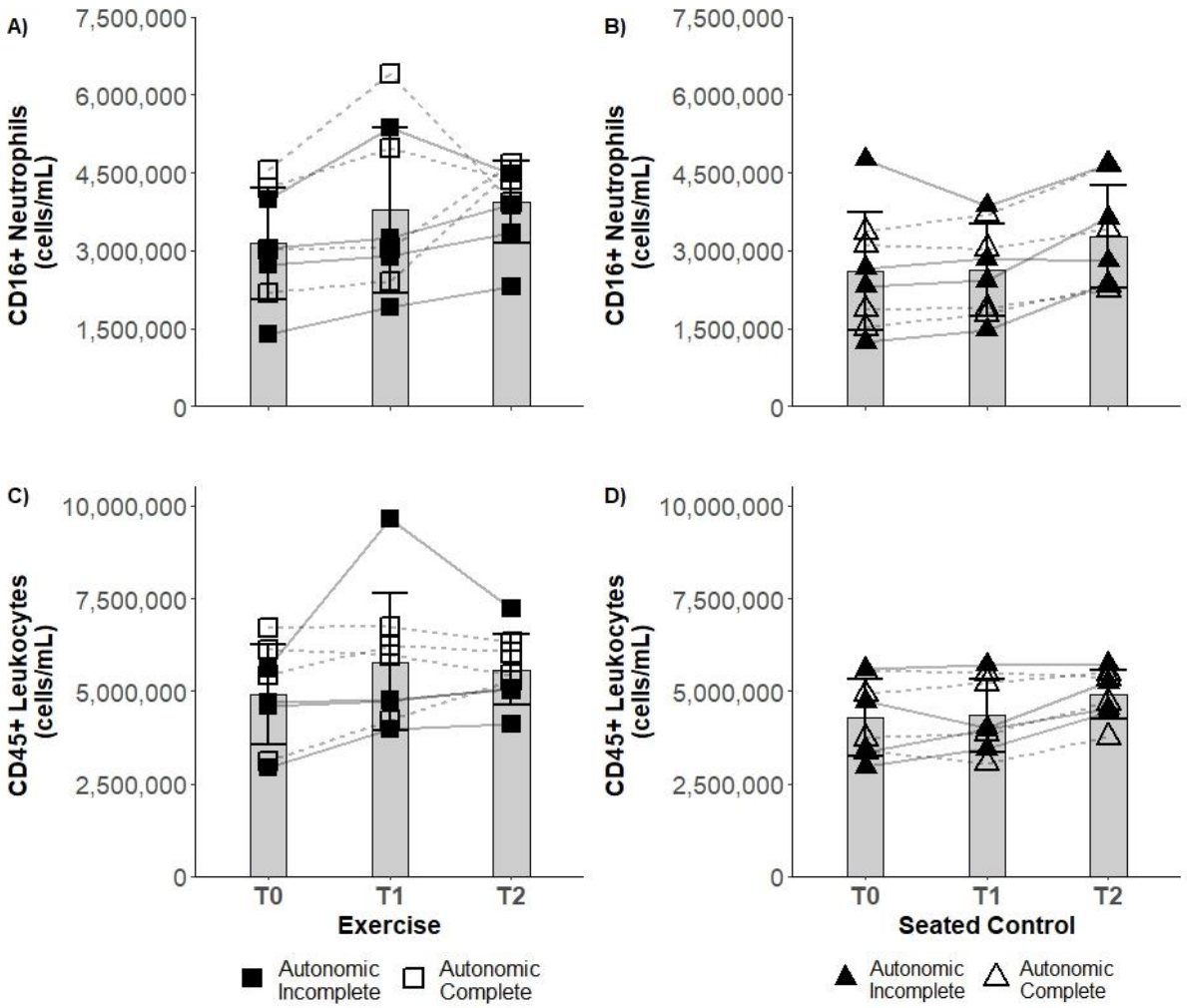
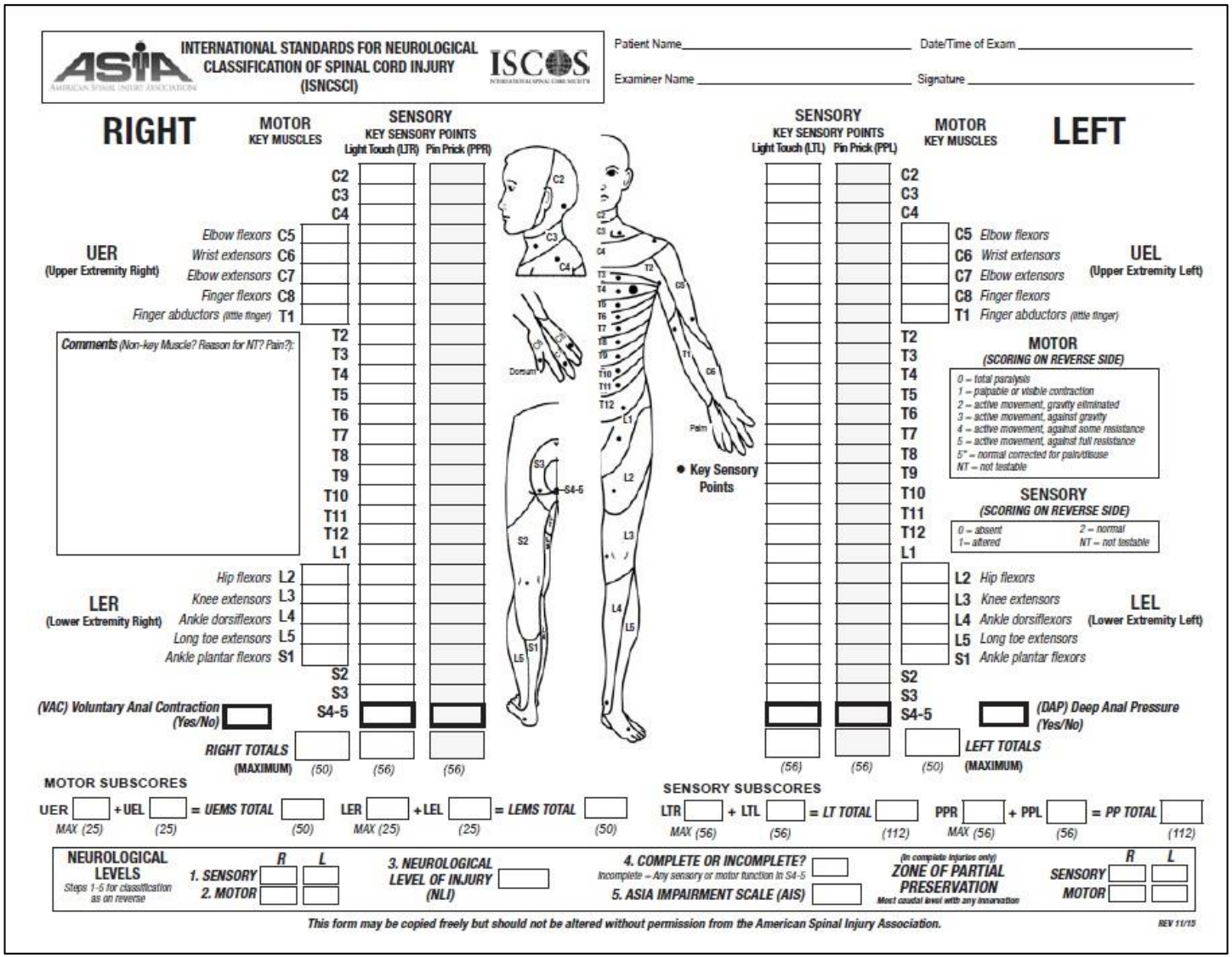


Figure A-5. Cellular responses for neutrophils and total leukocytes during 30-minutes of submaximal aerobic exercise on an arm cycle ergometer (left), and during a seated control condition (right) in individuals with SCI separated by autonomic completeness. T0 = Baseline, T1 = Post exercise/30 minutes control, T2 = 90 mins post exercise/120 mins control.



INTERNATIONAL STANDARDS FOR NEUROLOGICAL CLASSIFICATION OF SPINAL CORD INJURY (ISNCSCI)

Patient Name ____ Date/Time of Exam ____

Examiner Name ____ Signature ____

RIGHT | MOTOR KEY MUSCLES | SENSORY KEY SENSORY POINTS Light Touch (LTR) Pin Prick (PPR) | LEFT | SENSORY KEY SENSORY POINTS Light Touch (LTL) Pin Prick (PPL) | MOTOR KEY MUSCLES

UER (Upper Extremity Right): Elbow flexors C5; Wrist extensors C6; Elbow extensors C7; Finger flexors C8; Finger abductors (little finger) T1

UEL (Upper Extremity Left): C5 Elbow flexors; C6 Wrist extensors; C7 Elbow extensors; C8 Finger flexors; T1 Finger abductors (little finger)

Comments (Non-key Muscle? Reason for NT? Pain?):

MOTOR (SCORING ON REVERSE SIDE)
0 = total paralysis
1 = palpable or visible contraction
2 = active movement, gravity eliminated
3 = active movement, against gravity
4 = active movement, against some resistance
5 = active movement, against full resistance
5* = normal corrected for pain/disuse
NT = not testable

SENSORY (SCORING ON REVERSE SIDE)
0 = absent; 2 = normal
1 = altered; NT = not testable

LER (Lower Extremity Right): Hip flexors L2; Knee extensors L3; Ankle dorsiflexors L4; Long toe extensors L5; Ankle plantar flexors S1

LEL (Lower Extremity Left): L2 Hip flexors; L3 Knee extensors; L4 Ankle dorsiflexors; L5 Long toe extensors; S1 Ankle plantar flexors

C2 C3 C4 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12 L1 S2 S3 S4-5

(VAC) Voluntary Anal Contraction (Yes/No)

(DAP) Deep Anal Pressure (Yes/No)

RIGHT TOTALS (MAXIMUM) (50) (56) (56)

LEFT TOTALS (MAXIMUM) (56) (56) (50)

● Key Sensory Points

MOTOR SUBSCORES: UER ___ (MAX 25) + UEL ___ (25) = UEMS TOTAL ___ (50); LER ___ (MAX 25) + LEL ___ (25) = LEMS TOTAL ___ (50)

SENSORY SUBSCORES: LTR ___ (MAX 56) + LTL ___ (56) = LT TOTAL ___ (112); PPR ___ (MAX 56) + PPL ___ (56) = PP TOTAL ___ (112)

NEUROLOGICAL LEVELS (Steps 1-5 for classification as on reverse): 1. SENSORY R L; 2. MOTOR R L

3. NEUROLOGICAL LEVEL OF INJURY (NLI)

4. COMPLETE OR INCOMPLETE? (Incomplete – Any sensory or motor function in S4-5)

5. ASIA IMPAIRMENT SCALE (AIS)

(In complete injuries only) ZONE OF PARTIAL PRESERVATION (Most caudal level with any innervation): SENSORY R L; MOTOR R L

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Figure A-6. Page 1 of the American Spinal Injury Association work sheet.

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 = (normal) active movement, full ROM against gravity and full resistance in a functional muscle position expected from an otherwise unimpaired person

5* = (normal) active movement, full ROM against gravity and sufficient resistance to be considered normal if identified inhibiting factors (i.e. pain, disuse) were not present

NT = not testable (i.e. due to immobilization, severe pain such that the patient cannot be graded, amputation of limb, or contracture of > 50% of the normal ROM)

Sensory Grading

0 = Absent

1 = Altered, either decreased/impaired sensation or hypersensitivity

2 = Normal

NT = Not testable

When to Test Non-Key Muscles:

In a patient with an apparent AIS B classification, non-key muscle functions more than 3 levels below the motor level on each side should be tested to most accurately classify the injury (differentiate between AIS B and C).

Movement	Root level
Shoulder: Flexion, extension, abduction, adduction, internal and external rotation **Elbow:** Supination	C5
Elbow: Pronation **Wrist:** Flexion	C6
Finger: Flexion at proximal joint, extension. **Thumb:** Flexion, extension and abduction in plane of thumb	C7
Finger: Flexion at MCP joint **Thumb:** Opposition, adduction and abduction perpendicular to palm	C8
Finger: Abduction of the index finger	T1
Hip: Adduction	L2
Hip: External rotation	L3
Hip: Extension, abduction, internal rotation **Knee:** Flexion **Ankle:** Inversion and eversion **Toe:** MP and IP extension	L4
Hallux and Toe: DIP and PIP flexion and abduction	L5
Hallux: Adduction	S1

ASIA Impairment Scale (AIS)

A = Complete. No sensory or motor function is preserved in the sacral segments S4-5.

B = Sensory Incomplete. Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-5 (light touch or pin prick at S4-5 or deep anal pressure) 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 at the most caudal sacral segments for voluntary anal contraction (VAC) OR the patient meets the criteria for sensory incomplete status (sensory function preserved at the most caudal sacral segments (S4-S5) by LT, PP or DAP), and has some sparing of motor function more than three levels below the ipsilateral motor level on either side of the body.
(This includes key or non-key muscle functions to determine motor incomplete status.) For AIS C – less than half of key muscle functions below the single NLI have a muscle grade ≥ 3.

D = Motor Incomplete. Motor incomplete status as defined above, with at least half (half or more) of key muscle functions below the single NLI having a muscle grade ≥ 3.

E = Normal. If sensation and motor function as tested with the ISNCSCI are graded as normal in all segments, and the patient had prior deficits, then the AIS grade is E. Someone without an initial SCI does not receive an AIS grade.

Using ND: To document the sensory, motor and NLI levels, the ASIA Impairment Scale grade, and/or the zone of partial preservation (ZPP) when they are unable to be determined based on the examination results.

ASIA
AMERICAN SPINAL INJURY ASSOCIATION

INTERNATIONAL STANDARDS FOR NEUROLOGICAL CLASSIFICATION OF SPINAL CORD INJURY

ISCOS
INTERNATIONAL SPINAL CORD SOCIETY

Steps in Classification

The following order is recommended for determining the classification of individuals with SCI.

1. Determine sensory levels for right and left sides.
The sensory level is the most caudal, intact dermatome for both pin prick and light touch sensation.

2. Determine motor levels for right and left sides.
Defined by the lowest key muscle function that has a grade of at least 3 (on supine testing), providing the key muscle functions represented by segments above that level are judged to be intact (graded as a 5).
Note: in regions where there is no myotome to test, the motor level is presumed to be the same as the sensory level, if testable motor function above that level is also normal.

3. Determine the neurological level of injury (NLI)
This refers to the most caudal segment of the cord with intact sensation and antigravity (3 or more) muscle function strength, provided that there is normal (intact) sensory and motor function rostrally respectively.
The NLI is the most cephalad of the sensory and motor levels determined in steps 1 and 2.

4. Determine whether the injury is Complete or Incomplete.
(i.e. absence or presence of sacral sparing)
*If voluntary anal contraction = **No** AND all S4-5 sensory scores = **0** AND deep anal pressure = **No**, then injury is **Complete.***
*Otherwise, injury is **Incomplete.***

5. Determine ASIA Impairment Scale (AIS) Grade:

Is injury Complete? **If YES, AIS=A** and can record ZPP (lowest dermatome or myotome on each side with some preservation)

NO ↓

Is injury Motor Complete? **If YES, AIS=B**

NO ↓ (No=voluntary anal contraction OR motor function more than three levels below the motor level on a given side, if the patient has sensory incomplete classification)

Are at least half (half or more) of the key muscles below the neurological level of injury graded 3 or better?

NO ↓ **AIS=C**

YES ↓ **AIS=D**

If sensation and motor function is normal in all segments, AIS=E
Note: AIS E is used in follow-up testing when an individual with a documented SCI has recovered normal function. If at initial testing no deficits are found, the individual is neurologically intact; the ASIA Impairment Scale does not apply.

Figure A-7. Page 2 of the American Spinal Injury Association work sheet.

Appendix B - Data Tables

Table B-1. Hedge's G effect sizes over the course of the two-hour seated control.

		Seated Control Effect Sizes		
Cell	**Stain**	**T0 to T1**	**T0 to T2**	**T1 to T2**
Lymphocyte	CD3+	0.025	0.407	0.415
T Helper	CD3+/CD4+	0.005	0.438	0.400
T Cytotoxic	CD3+/CD8+	0.096	0.295	0.424
Natural Killer T cell	CD3+/CD56+	0.079	0.136	0.233
Classical Monocyte	CD14+/CD16-	0.004	0.429	0.336
Intermediate Monocyte	CD14+/CD16+	0.264	0.355	**0.579**
Non-Classical Monocyte	CD16+/CD14$_{dim}$	**0.578**	0.491	**1.055**
Neutrophil	CD16+	0.015	**0.534**	**0.585**
B Cell	CD19+	0.173	0.369	0.215
Natural Killer Cell	CD56+	0.471	0.308	**0.895**
Leukocyte	CD45+	0.058	0.480	0.439

T0, Baseline; T1, 30 minutes after baseline; T2, 120 minutes after baseline. 0.2, small effect; 0.5, medium effect; 0.8, large effect. Medium and large effect sizes are bolded.

Table B-2. Hedge's G effect sizes over the course of the exercise intervention.

		Exercise Effect Sizes		
Cell	**Stain**	**T0 to T1**	**T0 to T2**	**T1 to T2**
Lymphocyte	CD3+	0.319	0.159	0.219
T Helper	CD3+/CD4+	0.286	0.192	0.135
T Cytotoxic	CD3+/CD8+	0.329	0.065	0.320
Natural Killer T cell	CD3+/CD56+	0.419	0.070	0.381
Classical Monocyte	CD14+/CD16-	0.290	0.270	0.054
Intermediate Monocyte	CD14+/CD16+	0.003	0.069	0.120
Non-Classical Monocyte	CD16+/CD14$_{dim}$	**0.801**	0.156	**0.804**
Neutrophil	CD16+	0.260	**0.732**	0.106
B Cell	CD19+	0.261	0.219	0.082
Natural Killer Cell	CD56+	**0.905**	0.295	**1.213**
Leukocyte	CD45+	0.458	0.474	0.068

T0, Baseline; T1, 30 minutes after baseline (post exercise); T2, 120 minutes after baseline (90 minutes after post exercise). 0.2, small effect; 0.5, medium effect; 0.8, large effect. Medium and large effect sizes are bolded.

Table B-3. Cellular response to two-hour seated control.

		(cells/mL) x 10^3		
Cell	**Stain**	**T0**	**T1**	**T2**
Lymphocyte	CD3+	903 ± 313	894 ± 319	1075 ± 368 [1,2]
T Helper	CD3+/CD4+	591 ± 209	592 ± 206	706 ± 237 [1,2]
T Cytotoxic	CD3+/CD8+	267 ± 94	256 ± 100	317 ± 121 [1,2]
Natural Killer T Cell	CD3+/CD56+	42 ± 22	40 ± 17	46 ± 20
Classical Monocyte	CD14+/CD16-	258 ± 71	258 ± 63	301 ± 92
Intermediate Monocyte	CD14+/CD16+	13 ± 5	12 ± 5	16 ± 6
Non-Classical Monocyte	CD16+/$CD14_{dim}$	12 ± 3	10 ± 3	14 ± 4 [2]
Neutrophil	CD16+	2600 ± 1143	2622 ± 891	3265 ± 996
B Cell	CD19+	151 ± 44	160 ± 49	188 ± 72
Natural Killer Cell	CD3-/CD56+	197 ± 79	157 ± 56	223 ± 71
Leukocyte	CD45+	4272 ± 1043	4339 ± 934	4901 ± 661

Leukocyte count at baseline (T0), 30 minutes (T1), and 120 minutes (T2) during sitting control protocol. Values (cells/mL) displayed as mean ± SD. Significant interaction effects with significant Tukey post-hoc tests ($P < 0.05$) specified as: [1]different from baseline; [2]different than 30 minutes. Total leukocytes, classical monocytes, neutrophils and B cells were all significantly increased ($P < 0.05$) from baseline to 120 minutes (Tukey's post hoc test with conditions collapsed) following a main effect of time ($P < 0.05$).

Table B-4. *Cellular response to 30 minutes of acute submaximal aerobic exercise on an arm cycle ergometer.*

		(cells/mL) x 10^3		
Cell	**Stain**	**T0**	**T1**	**T2**
Lymphocyte	CD3+	889 ± 294	1058 ± 418 [1]	953 ± 343 [2]
T Helper	CD3+/CD4+	599 ± 202	696 ± 271 [1]	654 ± 239 [2]
T Cytotoxic	CD3+/CD8+	250 ± 95	311 ± 145 [1]	257 ± 100 [2]
Natural Killer T Cell	CD3+/CD56+	41 ± 22	54 ± 28 [1]	39 ± 18 [2]
Classical Monocyte	CD14+/CD16-	329 ± 140	388 ± 174	376 ± 114
Intermediate Monocyte	CD14+/CD16+	17 ± 12	24 ± 20	20 ± 17
Non-Classical Monocyte	CD16+/$CD14_{dim}$	12 ± 3	16 ± 4	12 ± 5 [2]
Neutrophil	CD16+	3140 ± 1073	3780 ± 1599	3940 ± 797
B Cell	CD19+	160 ± 54	181 ± 72	175 ± 58
Natural Killer Cell	CD3-/CD56+	191 ± 94	312 ± 133 [1]	164 ± 60 [2]
Leukocyte	CD45+	4905 ± 1349	5781 ± 1853	5567 ± 947

Leukocyte count at baseline (T0), post exercise (T1), and 90 minutes post exercise (T2). Values (cells/mL) displayed as mean ± SD. Significant interaction effects ($P < 0.05$) with significant Tukey's post-hoc tests ($P < 0.05$) specified as: [1]different than baseline; [2]different than post exercise. Total leukocytes, classical monocytes, neutrophils and B cells were all significantly increased ($P < 0.05$) from baseline to 120 minutes (Tukey's post hoc test with conditions collapsed) following a main effect of time ($P < 0.05$).