FACTORS AFFECTING MILITARY PHYSICAL PERFORMANCE: EFFECTS OF MORPHOLOGY, PHYSIOLOGICAL CAPACITY, INFLAMMATION AND HEAT

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M.Sc., University of Ottawa 2017

Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
in partial fulfilment of the requirements
for the Doctorate in Philosophy - Human Kinetics

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THESIS ABSTRACT

This thesis work was undertaken to investigate the effects of internal (i.e. morphology, physiological capacity, stress and inflammatory cytokines) and external (i.e. heat exposure) factors on military physical performance in members of the Canadian Armed Forces (CAF). By gaining insight into how these factors affect military physical performance, training and intervention strategies could be better tailored for optimizing performance. Both morphology and physiological capacity have previously been recognized to affect performance on certain military physical performance tasks. However, the effect of such factors on the newly implemented Common Military Task Fitness Evaluation (CMTFE), the current physical employment standard for the CAF, has not been determined. Stress and inflammatory cytokines have also been shown to affect physical performance in the general population, but there is no data available on levels of stress and inflammatory cytokines in the CAF, or their potential effect on military physical performance. Recently, the CAF have also implemented a loaded march, followed by an assessment of military physical performance (FORCEcombat), as a part of the physical performance testing for all members of the Canadian Army. However, there is a lack of research currently available on how factors like heat exposure can affect thermoregulatory and cardiovascular response, as well as performance on a loaded march, and the following FORCEcombat test. In order to provide key information about the requirements and delivery of the military physical performance tests currently used in the CAF, my thesis focused on four main parts to better understand the importance of some of the internal and external factors known to affect physical performance.

Firstly, my thesis assessed CAF members’ morphological and physiological characteristics that may affect overall performance on the CMTFE. In Chapter 2, results showed that both characteristics of morphology and physiological capacity separated the top and bottom performers.
Even though a difference in morphology was observed between top and bottom performers, performance on the CMTFE was mainly dependent on aerobic capacity and measures of strength, rather than morphology. Aerobic capacity explained ~36% of variability in performance among women, and ~32% variability in performance among men. Core strength also had a significant effect on performance in both groups, however, men relied more on upper body strength than did women. Apart from showing that physiological capacity, rather than morphology was the main component affecting performance outcome on the CMTFE, it was also concluded that, unlike physical performance tests used by the U.S. Armed Forces (i.e. push-ups, sit-ups, mile run), no body mass bias exists against larger individuals performing the CMTFE.

Chapter 3 and 4 focused on describing levels of stress and inflammatory cytokines among CAF members, and their effect on military physical performance. Stress exposure is known to induce an increase in the production of stress and inflammatory cytokines, and an increase in levels of inflammatory cytokines have been shown to be associated with a decrease in physical performance in general population. Members of the armed forces are susceptible to high stress exposure, but no current data exist on basal levels of stress and inflammatory cytokines in a military population. We therefore performed a descriptive analysis of levels of stress and inflammatory cytokines among CAF members. The results from this analysis showed a generally low detection rate of most of the inflammatory cytokines measured in our military population. However, we did observe a higher detection rate for IFN-γ, TNF-α, IL-2, IL-5, IL-8, IL-17α, IL-23 and IL-31 with increasing age. Adiponectin levels were higher in women compared to men (5.81 (3.52-13.19) µg/ml vs 16.71 (7.68-25.32) µg/ml), whereas IL-18 levels were higher in men compared to women (89.25 (84.03-94.48) vs 75.91 (69.70-82.13) pg/ml). Increasing age was associated with higher basal levels of C-Reactive Protein (CRP), Adiponectin, IL-18 and IL-2, and
we also found a significant positive correlation between body fat percentage (BF%) and CRP levels. Following the outcomes of the descriptive study, the associations between levels of stress and inflammatory cytokines and military physical performance were elucidated. Using multiple linear regression, controlling for covariates such as age, sex and BF%, a significant negative association was observed between CRP levels and Total Performance on the CMTFE (p=0.01), picking and digging performance (p=0.04), aerobic capacity (p=0.05) and plank time (p<0.01) among CAF members.

Finally, in Chapter 5, the effect of heat exposure on the capacity to perform a task oriented military test was quantified. Many CAF members are subject to a significant heat exposure on a daily basis, and studies from the general population have shown that heat exposure can have a detrimental effect on physical performance. As mentioned previously, The Canadian Army have recently implemented a test of loaded march performance, as a part of their occupational physical performance testing. The loaded march will be performed while wearing military personal protective equipment (PPE) and a daypack (~35 kg), and all members of the Canadian Army will be required to perform this test. Due to this need for mass testing, a large part of the loaded march performance assessments will be required to take place during the summer months. Temperature and relative humidity (RH) can reach high levels on several of the Canadian Army bases located around Canada, during the summer months. However, there is currently limited research available assessing the thermoregulatory and cardiovascular responses to performing a loaded march in the heat while wearing military PPE. Consequently, a study was designed to determine the thermoregulatory and cardiovascular responses to a loaded march (60 min, 5.17 km*h⁻¹, ~35 kg external load) at normal temperature (21°C, 50% RH) and in the heat (30°C, 50% RH). This study also aimed to quantify the effect of heat exposure and previous experience on loaded march and
military physical performance (FORCEcombat). Ten participants experienced with loaded march (military reservists), and ten participants inexperienced with loaded march (civilians) were recruited for this study. The results showed that whereas nine out of ten participants in the experienced group completed the loaded march in the heat, only five of the ten participants in the inexperienced group were able to do the same. Performing the loaded march in the heat while wearing military PPE led to a state of uncompensable heat stress, for both the experienced and the inexperienced group. Both groups showed a continuous increase in core temperature and heat storage (0.025°C/min and 0.02°C/min mean increase in core temp, 8.7 kj/min and 6.7 kj/min mean increase in heat storage, for the inexperienced and experienced group respectively) throughout the heat trial. Apart from the difference in completion rate on the loaded march, experienced participants also had a lower heart rate (134.2±11.9 vs 143.1±8.9 bpm, p≤0.05), perceived exertion (10.2±1.4 vs 13.0±0.9, p≤0.05), thermal comfort (1.9±0.5 vs 2.4±0.4, p≤0.05), and FORCEcombat completion time (662±133 vs 530±49 sec, p≤0.05) compared to the inexperienced participants.

The overall results from this thesis show that physiological capacity, inflammatory cytokines, heat exposure and previous experience, all have an effect on military physical performance. It was found that physiological capacity rather than morphology, was the superior predictor of performance on the CMTFE: inflammatory cytokines are present in CAF members and CRP levels increased with increasing age, CRP levels were negatively associated with military physical performance, performing a loaded march in the heat while wearing military PPE exposed both experienced and inexperienced participants to a state of uncompensable heat stress and decreased performance on the FORCEcombat test, and that previous experience has a positive effect on loaded march completion rate and FORCEcombat performance.
I would like to start by thanking my amazing wife Jennifer Peddie, for all the love and support she has given me throughout the process of conducting my PhD thesis. She has always been positive, supportive and helped me out when I was struggling with motivation. She has also given me great feedback and help in my struggles with the English language.

I would also like to thank my thesis supervisor, Dr. Francois Haman. Conducting this thesis work would not have been possible without him. Through my masters and PhD thesis, he has given me tremendous help, support and guidance, and put me back on track when I have been steering in the wrong direction. Dr. Haman has given me every opportunity to work on the research I have been interested in, and given me the opportunity to work with excellent researchers in the field of physiology and thermal physiology. My thesis co-supervisor Dr. Michael Spivock has been a tremendous help facilitating access to military databases and participants, and he has been a great help with statistical methodology and the process of putting this PhD thesis together.

My friends and family has given me enormous support, and has always been available to help when I have needed advice, or for social events when I needed a break from the PhD work. I would also like to give a special thanks to my brothers-in-law; Daniel Gray and John Peddie, and my friend Jackson Rigg, for their help and feedback on my thesis work. I also want to extend my sincerest thank you to my parents Kari and Gudbrand Tingelstad, which throughout my life have given my amazing support and love, who have always encouraged me to pursue my interests and done everything they could to make that possible.

I also want to thank Dr. Denis Blondin, for help and guidance he has given me in my PhD work, and everything he has thought me about using experimental equipment. He has also been a great help and guidance in the work of putting this PhD thesis together. I would also like to thank Brian Kheo and Eric Verdon for all their help with data collection and other lab work.

Lastly, I would like to thank Dr. Tara Reilly, Daniel Theoret and all the other researchers at The Canadian Forces Morale and Welfare Services – Personnel Support Program for all their help with data collection, participant and equipment availability, and input on study conceptualization.
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“Success is not final, failure is not fatal: it is the courage to continue that counts”

Sir Winston Churchill (1908-1965)
CHAPTER 1 - INTRODUCTION
GENERAL INTRODUCTION

Members of the Canadian Armed Forces (CAF) are expected to obey orders and carry out their military duties, even in highly challenging and stressful situations (The Canadian Armed Forces, 2011). To be able to achieve this, CAF members are required to meet a minimum physical employment standard (PES), allowing them to carry out demanding occupational tasks and cope with multiple stressors (The Canadian Armed Forces, 2011). However, there is currently limited information available concerning the effects of internal (i.e. morphology, physiology, stress and inflammatory cytokines) and external (i.e. heat exposure) factors on military physical performance, and how these factors can affect performance outcome. Studies from the civilian population have shown that physiological capacity, morphology, stress and inflammatory cytokines, and heat exposure can have an effect on physical performance (Barrett & Manning, 2004; Cesari et al., 2004; Ingjer, 1991; Nybo et al., 2011; O’Hearn et al., 2016; Peterson et al., 2015; Thompson, 2017). However, due to large differences in the tasks performed, population type, and expectations put on CAF members, results from the general population cannot be readily transferred and applied to a military population. By increasing the knowledge on factors affecting military physical performance, strategies can be developed to limit or enhance the effect of said factors, to increase military physical performance, and potentially lower psychological and physiological stress exposure. This chapter will focus on introducing the current knowledge related to internal and external factors that can affect physical performance, identifying gaps in the literature, and presenting the specific aims for how this thesis will attempt to fill these gaps.
MILITARY PHYSICAL PERFORMANCE TESTING

In today’s occupational environment, several jobs require their employees to possess a minimal physiological capacity to be able to meet occupational demands. Police officers (Anderson et al., 2001), firefighters (Williams-Bell et al., 2009), correctional officers (Jamnik et al., 2010), and members of the armed forces (Pandorf et al., 2003; Rayson et al., 2000) are only a few of the occupations subjected to occupational physical performance testing. While some of these occupations have recently implemented occupational physical performance standards and testing, the armed forces has a long history of physical performance testing (East, 2013). Members of the CAF are required to perform a variety of tasks, such as digging ditches and foxholes, building protective shelters with sandbags, performing soldier rescues, and stretcher carry. In addition they perform essential military tasks, like lifting and handling of military supplies (ammunition boxes, water cans etc.), loading and unloading trucks, and other types of material handling. Due to the variety of physically demanding tasks, members of the armed forces are required to possess a minimal physiological capacity to carry out their duties.

The occupational physical performance testing performed in the CAF, has undergone a transition throughout the years. From the mid 1960’s to the 1980’s, the land forces were required to perform a return 10-mile march, carrying supplies, with an overnight stay in between (Reilly 2010). In the mid 1980’s, the Battle Efficiency Test was implemented as the occupational performance test for the CAF. It consists of a 2x10 mile march, as previously described, but also scaling a six-foot wall, jumping an eight-foot ditch, and carrying a soldier 200m. This test was followed by an Indoor Standardized Obstacle Course based on the work of Jetté et al (1989). This 19-item test included testing the soldiers’ abilities of running, scaling walls, crawling, pulling, lifting, pushing and carrying.
In the 1980’s the CAF implemented the Universality of Service principle, stating that: “all members of the CAF are soldiers of arms first, no matter the occupation held” (Spivock et al., 2011). The Universality of Service principle means that all members of the armed forces are required to possess a minimum physiological capability to perform basic skills that can be expected by any CAF member. Thus, simple tests of military physical fitness were insufficient to assess military personnel’s ability to “perform general duties and common defence and security duties in addition to their military occupation or occupational specifications” (Reilly et al., 2012).

Between 1983 and 1988, based on the Universality of Service principle, the Environmental Chiefs of Staff identified five common military tasks, which were later accepted by the Armed Forces Council and the Defence Management Committee as the bona fide minimum requirements all CAF members could be expected to possess (Table 1).

**Table 1**: The five common military tasks and the minimum requirements for a passing grade

<table>
<thead>
<tr>
<th>Task</th>
<th>Performance Criteria</th>
<th>Males and Females 34 years and younger</th>
<th>Males and Females 35 years and older</th>
</tr>
</thead>
<tbody>
<tr>
<td>Land Evacuation</td>
<td>900 sec</td>
<td>1180 sec</td>
<td></td>
</tr>
<tr>
<td>Sea Evacuation</td>
<td>210 sec</td>
<td>277 sec</td>
<td></td>
</tr>
<tr>
<td>Low/High Crawl</td>
<td>140 sec</td>
<td>185 sec</td>
<td></td>
</tr>
<tr>
<td>Sandbag Carry</td>
<td>12 sand bags</td>
<td>9 sandbags</td>
<td></td>
</tr>
<tr>
<td>Entrenchment Dig</td>
<td>510 sec</td>
<td>673 sec</td>
<td></td>
</tr>
</tbody>
</table>

(Spivock et al., 2011)
Due to the logistical difficulty and time consuming process of having all CAF members perform the five common military task tests every year, the CAF adopted the Canadian Standardized Test of Fitness, 3rd edition (Canada, 1987), as a predictor of successful task completion (predicted VO$_{2\text{max}}$, sit-ups, push-ups and hand grip strength). The predicted VO$_{2\text{max}}$ test was later replaced by a 20-meter shuttle run test, as it was found to be more cost effective, while still providing an accurate estimation of successful task performance. This set of tests (CF EXPRES) served as the CAF’s physical performance tests up until 2010. To be able to adapt to the changing face of modern warfare, the process of developing a new PES began in June 2010. Due to recent incidents in other occupations, the new PES was also required to be in line with the Canadian legal and human rights context of employment standard, while also being able to hold up in a court of law. After three years of extensive literature review to determine essential components of the military occupation (Spivock et al., 2011), consultation with expert subject matter, and extensive field testing (Reilly et al., 2013), the new PES was deemed ready to be implemented.

The new standard consists of a minimum performance on six common military tasks: escape to cover, pickets and wire carry, picking and digging, sandbag fortification, stretcher carry and vehicle extrication, and was named the Common Military Task Fitness Evaluation (CMTFE) (Reilly et al., 2013). Although a set of more field expedient tests have been developed for mass testing, the CMTFE is still the main standard all CAF members are held to, as a part of the universality of service principle. All CAF members, whether you are a soldier in the field, or a desk clerk, are held to these standards, and their physical performance is tested annually (Spivock et al., 2011). CAF members possessing a good physiological capacity will perform better on the PES testing, and be able to perform occupational tasks and duties at a higher standard.
When compared to research into factors affecting sports and athletic performance, limited research is available on factors affecting military physical performance. For example, it is known that cross country skiing and long distance running, in addition to good technique, requires a high aerobic capacity (Ingjer, 1991; Sandbakk & Holmberg, 2014; Thompson, 2017), and that maximal leg strength and the ability to develop force quickly are a good predictors of sprinting and jumping performance (Bissas & Havenetidis, 2008; Wisløff et al., 2004).

Attempts have been made to explain factors affecting certain tasks related to military physical performance in the past. During the development of the new PES for the British Royal Army, Rayson et al. (2000), investigated the relationship between performance on the criterion tasks (single lift ammunition box, carry 2x20 kg can, repetitive lift and 10 m carry of ammunition box, loaded march 12.8 km in 120 min), physical fitness and morphology. A sample of British Army soldiers were tested, both on the criterion tasks and a battery of physical fitness tests (static strength, dynamic strength, muscular endurance and aerobic fitness). Measures of morphology (e.g. stature, body mass, percent body fat (%BF) and lean body mass (LBM)) were also recorded. Multiple linear regression analysis showed that although sex had an influence on performance outcome (men performing better than women), most of the variance in performance of the single lift task could be explained by measures of upper body and dynamic lift strength, as well as lean body mass. This was also the case for both the carry task and the repeated lift and carry task, where measures of upper body and dynamic lift strength were the primary factors explaining the majority of the variance in performance outcome. Performance on the loaded march task was in large part explained by aerobic endurance and aerobic power. The issue with applying the outcomes of this study to the current tests used by the CAF is that the PES used by the British Royal Army does not contain the same criterion tasks, nor the same physical performance tests as the current PES for
the CAF does. Whereas the CAF assess their members ability to build a sandbag fortification, perform an escape to cover maneuver, perform a picking and digging task, perform pickets and wire carry, vehicle extrication and stretcher carry, the British Royal Army uses a single box lifting task, a carry task, a repetitive lift and carry task and a loaded march (Rayson, 1998). There have also been studies looking at factors explaining performance outcome on a single military task, where Nottrodt and Celentano (1987) found that both LBM and dynamic lift strength were significant predictors of single lift performance (male $r^2=0.82$, female $r^2=0.80$) (lifting ammunition container onto truck bed). Pytel and Kamon (1981) found that dynamic lift strength and sex could explain 94.1% of performance outcome in a single lift task. Other studies have been able to develop non-sex specific prediction equations for performance on single military tasks. Both Beckett and Hodgdon (1987), and Mello et al. (1995) developed sex-independent equations, predicting performance outcome on repetitive lift and carry tasks. Beckett and Hodgdon (1987) found that LBM and run time (1.5 mile run) were the best predictors of carry performance ($r^2=0.72$) (carrying a box, variable weight, as many times as possible in two 5-min bouts, 1 min rest). Mello et al. (1995) found that depending on the speed of the lift and carry task (1 or 4 lifts/min), performance outcome could be predicted by upper body anaerobic capacity ($r^2=0.82$, 1 lift/min), or stature, lean arm mass and LBM ($r^2=0.88$, 4 lift/min).

The outcome of these previous studies cannot be directly applied to the new set of physical performance tests developed for the CAF. Neither of the tasks assessed in these studies are included in the CMTFE, calling for an up-to-date assessment of which factors of physiological capacity (e.g. strength, aerobic capacity) or morphology (e.g. height, weight, body composition) can affect performance outcome on the CMTFE. From previous research, it is known that body composition, strength and aerobic capacity have an effect on physical performance, both athletic
and military, but due to the specificity and complexity of the CMTFE, containing tasks with widely different demands (Figure 1.1), it is not known which of these factors can best predict, and have the biggest effect on performance outcome. Thus, investigating the effects of physiological capacity and morphology on performance outcome on CMTFE would help CAF members discover where they need to focus their training to improve performance both on the CMTFE, but also in performing day-to-day military tasks.

**Figure 1.1.** Work (J) performed over horizontal distance covered during loaded condition for each of the CMTFE tasks.

**STRESS AND INFLAMMATORY CYTOKINES**

As mentioned in the previous section, although not completely quantified, several factors of physiological capacity and morphology have been recognized to affect both athletic and military physical performance. However, a mounting body of evidence suggests that other less obvious
factors, like stress and inflammatory cytokines, could also potentially affect military physical performance. Exposure to any type of stressor, physical, psychological or emotional, elicits a stress response to bring the body back to homeostasis (Tsigos et al., 2016). The neuroendocrine stress response activates the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis, initiating the secretion of catecholamines (adrenaline and noradrenaline) from the adrenal medulla, and the secretion of stress hormones, like glucocorticoids, from the adrenal cortex (Johnson et al., 1992; Tsigos & Chrousos, 2002).

A stress response is considered to be either acute or chronic. An acute stress response is characterized by a short-term elevation in catecholamine and glucocorticoid secretion, preparing the organism for a ‘fight-or-flight’ response, and as the stressor is removed, catecholamine and glucocorticoid levels return to normal (Black, 1994; Romero & Butler, 2007). The main stress hormone is the glucocorticoid cortisol, which increases several fold during an acute stress response (Foster & Wulff, 2005; Kirschbaum et al., 1995; Kirschbaum et al., 1992; van Eck et al., 1996).

The acute elevation in cortisol levels experienced during acute stress serves many important purposes, such as increasing blood glucose levels by breaking down amino acids in the liver, and proteins and fat stores in other tissues, to free up energy reserves for a fight-or-flight response (Berdanier, 1987; Dickerson & Kemeny, 2004).

Chronic stress, on the other hand, is an ongoing process of constant exposure to a single or multiple stressors, leading to a prolonged elevation in cortisol levels (Miller et al., 2007; Schulz et al., 1998). Constant elevation in cortisol levels, as seen during episodes of chronic stress, will in the long term have detrimental effects on the human organism, causing tissue breakdown and disturbance of homeostasis in other regulatory systems (Dickerson & Kemeny, 2004). It has also been shown that stress can have a negative effect on cognitive performance (Lieberman et al.,
mood state (Lieberman et al., 2002; van Eck et al., 1998), and stress is known to be a major contributor to the onset of depression (Lloyd, 1980; Pine et al., 2002). Chronic stress may also have an effect on disease susceptibility. A study by Cohen et al. (2012) found that major, stressful life events increased the susceptibility to a viral infection (rhino-virus = common cold). This happens through a suppression of the immune system caused by corticoid receptor resistance. Stress and variations in cortisol levels, seem to be closely linked to the immune system and the inflammatory response (Cohen et al., 2012; Steptoe et al., 2007). A number of studies have reported that chronic stress can induce development of low-grade systemic inflammation, which in turn upregulates the production of pro-inflammatory cytokines (Black, 2002, 2003; Black & Garbutt, 2002).

Cytokines are a large group of proteins known to activate, mediate and regulate innate and adaptive immunity (Abbas et al., 2014). They are also responsible for the release of acute phase proteins (e.g., C-reactive protein (CRP)), another group of proteins known to be potent mediators of inflammation (Gabay & Kushner, 1999). Cytokines serve multiple purposes when it comes to initiating, mediating and regulating the inflammatory process. However, the actions of many cytokines can be both pleiotropic (one cytokine having multiple effects on several cell types), antagonistic (one cytokine inhibiting the action of another) and redundant (a number of cytokines having the same or overlapping effects on the same cell type) (Abbas et al., 2014). Cytokines also exert different effects depending on concentration level. Interleukin-1 (IL-1) functions as a mediator of local inflammation at low concentration levels, whereas at high concentrations, IL-1 can enter the blood stream and exert systemic endocrine effects (Abbas et al., 2014). Certain cytokines are known to be pro-inflammatory, upregulating the inflammatory response (e.g. IL-1, Tumor Necrosis Factor-α (TNF-α), Interleukin-18 (IL-18)), whereas others are anti-inflammatory,
down regulating the inflammatory response (e.g., Interleukin-4, Interleukin-10) (Cavaillon, 2001). Other cytokines can be both pro and anti-inflammatory, depending on the receptor the ligand binds to (e.g. Interleukin-6 (IL-6) will exert anti-inflammatory actions when bound to the membrane-bound IL-6 receptor, and pro-inflammatory actions when bound to the soluble IL-6 receptor) (Scheller et al., 2011).

Inflammation and pro-inflammatory cytokines also play a role in all stages of atherosclerotic disease (Libby, 2006; Libby et al., 2002), while many cancers have been found to arise from sites of infection or inflammation (Coussens & Werb, 2002). Additionally, several studies have shown both a correlative and causative relationship between inflammation and insulin resistance (Shoelson et al., 2007; Shoelson et al., 2006; Xu et al., 2003). Outside the effect of acute and chronic stress, there have also been other factors shown to affect levels of stress and inflammatory cytokines. On the other hand, it is well recognized that several diseases, such as atherosclerosis (Hansson 2005; Ross 1999), inflammatory bowel disease (Strober et al., 2007), as well as cuts and injuries (Koh & DiPietro, 2011), elicits a stress and inflammatory response, but factors like age and obesity can further exacerbate the production stress and inflammatory cytokines.

Following the “wear-and-tear” hypothesis of lifelong stress exposure, levels of stress hormones seem to increase with age. Over the last decades several studies in the general population have reported higher cortisol levels in the elderly population, and age has been shown to be strongly associated with cortisol levels (Halbreich et al., 1984; Seeman et al., 2001; Van Cauter et al., 1996). High levels of stress and increased cortisol secretion are some of the confounding factors of the onset of obesity and type 2 diabetes (Björntorp, 1997). Plasma levels of several pro and anti-inflammatory cytokines also seem to correlate with increasing age in the general
population. C-reactive protein, IL-1β, IL-6, and IL-18, all potent pro-inflammatory cytokines, have been reported to be higher in the elderly population (Ferrucci et al., 2005; Ong et al., 2013; Woloshin & Schwartz, 2005). Elevated levels of inflammatory cytokines, like CRP, TNF-α and IL-6 are associated with the onset of obesity, and they serve as predictors of an increased risk of developing cardiovascular disease and type 2 diabetes, while high levels of IL-18 have been associated with a greater risk of mortality in patients suffering from coronary heart and artery disease (Bastard et al., 2006; Blake & Ridker, 2002; Hu et al., 2004; Koenig et al., 1999; Ridker et al., 2000; Yudkin et al., 1999). Levels of the anti-inflammatory cytokine adiponectin also seem to increase with age (Adamczak et al., 2005; Cnop et al., 2003; Obata et al., 2013). Although an underlying state of low-grade systemic inflammation cannot be disregarded as a cause of the upregulation of adiponectin, it has been more commonly suggested that a decrease in renal function with increasing age, and thereby lower adiponectin filtration, is the cause of this observed elevation, but the exact reason has yet to be determined (Adamczak et al., 2005; Isobe et al., 2005).

There have also been reports of sex-related differences in levels of stress and inflammatory. Sex differences have been observed in several studies, where higher values of CRP have been reported in women compared to men (Marques-Vidal et al., 2011; Rojo-Martinez et al., 2013), while other studies have found evidence of higher levels of cortisol and IL-6 in men compared to women (Marques-Vidal et al., 2011; Van Cauter et al., 1996).

While high levels of stress can have a detrimental effect on both cognitive and mental health (Lieberman et al., 2005; Lieberman et al., 2002; McEwen & Sapolsky, 1995), but it can also have a severe negative effect on a person’s physiological state. Chronic stress has been associated with an increased risk of developing several critical diseases and complications, like

Apart from having negative effects on physical health and well-being, inflammation and inflammatory cytokines have also been suggested to have detrimental effects on physical performance. Several studies have reported a negative association between levels of inflammatory cytokines and physical performance and physical functioning, especially in the elderly population (Oliveira et al., 2008; Pereira et al., 2013; Taaffe et al., 2000). The InCHIANTI study (Cesari et al., 2004), investigated the association between physical performance and serum levels of IL-6, CRP, IL-10 and IL-1 receptor antagonist (IL-1RA) in persons 65 years and older. Results from this study showed significant inverse relationship between CRP (r = -0.162), IL-6 (r = -0.251), IL-1 Receptor Antagonist (r = -0.127) and physical performance (p<0.05). The inflammatory cytokine IL-18 has also been suggested to be associated with functional decline and physical performance. Both Thomas et al. (2009), and Frayling et al. (2007) found evidence of a negative association between IL-18 levels and physical functioning (e.g. muscle strength, gait speed, get-up-and-go test) and walking speed in persons 60-85 years.

Negative associations between inflammatory cytokines and physical performance are not exclusive to the elderly population. A recent study by Peterson et al. (2015) containing data from six different studies, and with participants ranging in age from 22 to 89 years, showed negative correlations between the inflammatory cytokines IL-6 (r = -0.22), TNF-α (r = -0.10), and both VO₂peak and walking speed (p<0.05). This association was also observed with several other inflammatory, coagulation and endothelia function markers. A negative effect of IL-6 on physical performance was also observed in study by Robson-Ansley et al. (2004). They observed an increase in treadmill 10 km run time and increased sensation of fatigue after an injection of a low
dose recombinant human IL-6. Apart from the activation of the immune and inflammatory system, stress itself has been shown to have a negative effect on physical performance. van der Does et al. (2015), studied the effect of psychosocial stress in female Dutch floorball players, and found that more psychosocial stress, and less psychosocial recovery led to a decrease in physical performance on a modified agility test.

The armed forces is an occupational group susceptible to high stress exposure (Pflanz & Sonnek, 2002; Pflanz & Ogle, 2006; Spielberger & Reheiser, 1994). In the Universality of Service charter it is stated that military service men and women are expected to perform their military duties under both physically and mentally stressful situations (The Canadian Armed Forces, 2011). Members of the armed forces experience stress in many forms, such as pressure to perform their duties, prolonged military deployment, the strive for promotion, and fear of relocation, among others (Bray, 2009; Bray et al., 2010). A study by Pflanz & Sonnek (2002), reported that 26% of members of the U.S. Armed Forces suffered from significant work stress, while another 15% reported experiencing significant work related emotional stress.

Members of the armed forces are also highly susceptible to physical stress exposure (Morgan III et al., 2000). The day-to-day schedule for many members of the armed forces consists of repeated lifts and carry, loading and unloading supplies and ammunition boxes from trucks and other modes of transport, performing extended loaded march trials either to prepare for deployment or during deployment. Post-Traumatic Stress Disorder (PTSD) is common among many of the soldiers returning from deployment, and results from studies conducted on veterans of the British Armed Forces indicated that 15-31% of combat veterans show symptoms of PTSD (Kulka, 1988; Weiss et al., 1992), while numbers in the general population in the U.K. have been estimated to be in the 2-3% range (Wessely & Deahl, 2003). It has also been shown that people suffering from
PTSD have an increased production of pro-inflammatory cytokines compared to healthy controls (Baker et al., 2003; Gola et al., 2013).

The potential for high stress exposure puts members of the armed forces at a greater risk of possessing elevated levels of stress hormones and pro-inflammatory cytokines, which in turn could have a negative effect on physical and cognitive performance. High levels of stress and inflammatory cytokines could also increase the risk of developing cardiovascular disease, obesity and type 2 diabetes. There are currently no studies investigating the occurrence of stress and inflammatory cytokines in a military population, how they change with increases in age and adiposity, and differences between men and women. The negative effect of stress and inflammatory cytokines on physical performance could also mean that members of the armed forces suffering from high stress levels are at a disadvantage when it comes to performing occupational tasks, and performing their annual physical employment tests. A descriptive analysis, documenting levels of stress and inflammatory cytokines, as well as their association with age, sex and adiposity, would yield important information about the current physiological state of the CAF. Stress and inflammatory cytokines have also been shown to be involved in the development of several diseases, and to affect cognitive and physical performance. However, the relationship between levels of stress and inflammatory cytokines and physical performance on a variety of military physical performance tests has not been elucidated. Thus, an investigation into the association between stress and inflammatory cytokines and military physical performance in CAF members would help quantify the extent of this issue.
THE EFFECTS OF HEAT EXPOSURE ON PHYSICAL PERFORMANCE

Environmental factors, like cold, heat, altitude etc., are another constraint that could have severe effects on military physical performance. A large part of military training and day-to-day work takes place outside (Epstein et al., 2012), exposing members of the armed forces to different climates on a daily basis. For soldiers serving in the army, loaded march is frequently performed outdoors, both as training and as a mode of transport. Loaded march is an essential component of a soldier’s duties, where it is expected that all soldiers be able to march a given distance carrying a battle load in the form of helmet, fragmentation vest, tactical vest, weapon and a backpack, and remain battle ready at the completion of the march (Reilly, 2010; van Dijk, 2009). The current physical employment tests used by the CAF do not contain an element assessing loaded march performance. Therefore, The Canadian Army has recently implemented a test of loaded march performance, which will be performed annually by all members of the Canadian Army.

The factors affecting loaded march performance and energy cost have been well studied. It has been established that energy cost of walking while carrying a load is higher compared to unloaded walking, and an increase in external load is associated with an increase in total energy cost (Abe et al., 2008; Epstein et al., 1988; Keren et al., 1981; Lyons et al., 2005; Quesada et al., 2000; Schertzer & Riemer, 2014). A study by Bastien et al. (2005), found that the relative energy cost (W*kg⁻¹ body mass + external weight carried) is not influenced by the load carried, and remains the same for external loads from 0-75% of body mass. Even though relative energy cost does not seem to be affected by an increase in external load, studies have suggested that maximal exercise capacity is affected by the total external load carried. Studies by Raven et al. (1977) and De Maio et al. (2009), estimated that for every 1 kg of external load added to the participant, maximal exercise capacity is reduced by 1% .
Walking speed is also a crucial component for determining the energy cost of load carriage. As with unloaded walking, the energy cost of a loaded march follows a U-shaped curve, where energy expenditure decreases with increasing walking speed, until an optimal speed is reached (Figure 1.2) (Abe et al., 2008; Bastien et al., 2005). The duration of the loaded march can also have an effect on total energy cost. Epstein et al. (1988) found that carrying a load of 25 kg, led to a constant energy expenditure during a 120 min march. However, if the external load was increased to 40 kg, energy expenditure, measured in the form of %VO₂\text{max}, increased from 52.1% after 20 min to 56.2% after 120 min.
A few studies have been conducted looking at physiological and morphological factors affecting loaded march performance. Studies by Dziados et al. (1987) and Mello et al. (1988), found strong correlations between loaded march performance and measures of lower body strength, especially hamstring strength (r = ~0.55). Aerobic capacity (VO₂max) showed only a weak correlation. A more recent study by Rayson et al. (2000), found that predictors of loaded march performance were dependent on the weight of the load carried. In their study, loaded march performance (carrying 25 kg over 12.6 km), was predicted by measures of absolute VO₂max (l/min⁻¹) and body mass. On the same distance, carrying 15 kg and 10 kg, performance on a multistage fitness test (shuttle run) was the best predictor of loaded march performance. As a part of the loaded march, CAF members are required to wear PPE, which is often heavy and cumbersome. A recent study by De Maio et al. (2009), showed that wearing military PPE (helmet and Kevlar plate vest ~10 kg) led to a decrease in aerobic capacity and treadmill time to exhaustion, and had a diminishing effect on strength and functional field test performance.

As mentioned previously, environmental factors can have an effect on physical performance, and soldiers performing a loaded march outside will be highly exposed to the environment. Previous research has shown that the terrain a loaded march is performed on can affect the energy cost of the loaded march (Haisman & Goldman, 1974; Soule & Goldman, 1972), and that performing a loaded march at altitude can cause an increase in heart rate and energy cost, compared to performing a loaded marched at sea level (Chatterjee et al., 2017). However, heat exposure might be the environmental factor with the largest effect, and the one to which members of the armed forces will be most frequently exposed. Recent climate changes have led to more cases of extreme weather and higher ambient temperatures (Lucas et al., 2014; Stocker, 2014), and a large part of military training and operations take place outside, often in extreme environmental
conditions (Epstein et al., 2012). Several CAF operations over the last decades have taken place in extreme environments, and with the increase in humanitarian and environmental crises seen around the world, there is a high probability that more future military operations will be carried out in extreme environments.

Figure 1.3: Relationship between dry and evaporative heat loss with increasing ambient temperature. From Sawka and Pandolf (2001) reproduced from Nielsen (1938).

Humans are a tropical species, better suited to deal with high ambient temperatures compared to extreme cold (Lee, 1964; Yousef, 1987). Whereas the human organism is ill equipped to limit heat loss to the environment in the cold, it has several mechanisms to increase heat loss and maintain thermal homeostasis in the heat (Piantadosi, 2003). The human body is continuously
gaining and losing heat through radiative and dry convective heat transfer between the body and the environment, and in the need for an upregulation in heat loss capacity, evaporative heat loss can be activated (Höppe, 1993; Yousef, 1987). At low to moderate ambient temperatures, most of the heat transfer happens through convection (Havenith, 1999; Sawka & Pandolf, 2001), and small amounts through radiation and conduction, depending on the circumstance (Havenith, 1999). Upregulation in dry convective heat loss is facilitated through an increase in vasodilation of peripheral blood vessels, to increase skin blood flow (Charkoudian, 2003; Kenney & Johnson, 1992). This increases the flow of heat from the core to the periphery, where it can be dissipated to the environment. As the ambient temperature increases, the temperature gradient between the skin and the environment is reduced, decreasing the capacity of dry heat loss (Figure 1.3). To maintain thermal balance, the production and secretion of sweat from sweat glands on the skin surface are activated, which increases skin wettedness (Ingram & Mount, 1975). The difference in water vapour gradient between the skin surface and the environment facilitates heat loss through evaporation (Candas et al., 1979; Gagge & Gonzalez, 2010). With increasing ambient temperature, a greater amount of heat is lost through evaporation (Ingram & Mount, 1975). As ambient temperatures rise to a level equal to skin temperature, heat can no longer be dissipated through convection, and with external temperature higher than skin temperature the body is gaining heat through convection, rendering evaporative heat loss as the only mechanism left for heat dissipation (Sawka & Pandolf, 2001; Yousef, 1987).

Exercising in a hot environment has been shown to have a negative effect on physical performance, (Nybo & Nielsen, 2001a; Nybo et al., 2011; O’Hearn et al., 2016), and can under certain circumstance lead to heat exhaustion and heat illnesses (Armstrong et al., 2007; Bouchama & Knochel 2002; Wendt et al., 2007). Several factors affect the degree of performance decrease
during exercise in the heat, and the occurrence and degree of heat illness one can expect to experience. Ambient temperature, humidity, heat acclimation status, aerobic capacity, hydration level, exercise intensity and duration are all factors that will affect performance outcome and risk of heat illness (Coris et al., 2004; Gisolfi, 1973; Nadel et al., 1974; Sawka & Pandolf, 2001; Sawka et al., 1983).

Exercising in a dry environment with a low relative humidity allows evaporative heat loss to remain effective, even at high ambient temperatures. Compensable heat stress is defined as an environment where required evaporation ($E_{\text{req}}$) is smaller than maximal evaporative capacity ($E_{\text{max}}$), total heat loss is equal to heat gain, and core temperature remains in steady state (Cheung et al., 2000; Latzka et al., 1998). Studies have found that during exercise in compensable heat, core temperatures as high as 40-41°C can be tolerated, before heat exhaustion occurs (Pugh et al., 1967; Sawka et al., 1979). Core temperatures reaching such levels can by no means be called safe, but compared to uncompensable heat exposure, higher core temperatures can be tolerated during compensable heat stress. Exercise intensity will also affect performance and time to exhaustion, where a higher intensity will lead to a higher heat production and therefore a higher $E_{\text{req}}$ (Montain et al., 1994).

Heat acclimation and high aerobic capacity are both factors that can significantly improve thermoregulatory responses, physical performance, and time to exhaustion during exercise in the heat (Nielsen et al., 1993; Sawka et al., 2010; Shvartz et al., 1977). A study by Sawka et al. (2010), found that 7 days of heat acclimation (49°C, 20% relative humidity) improved completion rate on a 100 min treadmill walk at the specific ambient temperature from 0% on day one, to 96% on day seven.
Hydration level is another factor playing an important role in the ability to tolerate heat stress. Dehydration of 3% body mass or larger, have repeatedly been shown to affect physical performance (Barr, 1999; Cheuvront et al., 2003; Sawka, 1992). During exercise in the heat, a decrease in water loss as small as 2% of body mass, can have a negative effect on heat tolerance and performance (Cheung & McLellan, 1997, 1998; Maughan, 2003; Sawka et al., 1992).

Along with dehydration, exercising in a hot and humid environment poses the toughest cardiovascular and thermoregulatory challenge on the human organism. The high relative humidity decreases the water vapour pressure gradient between the air and skin, and combined with the high ambient temperature and heat production from exercise, a state of uncompensable heat stress can be experienced. Uncompensable heat stress occurs when $E_{\text{req}}$ is exceeding $E_{\text{max}}$ and the body’s heat loss ability is no longer in balance with heat gain (Givoni & Goldman, 1972; Kraning & Gonzalez, 1991; Sawka & Pandolf, 2001). This leads to an upregulation in heat storage and a continuous increase in core temperature until heat exhaustion occurs. As mentioned previously, relatively high core temperatures can be tolerated during compensable heat stress. However, during exercise in uncompensable heat, heat exhaustion can occur at significantly lower core temperatures (between 38 and 40°C) (Montain et al., 1994; Sawka et al., 1992). The reduced ability for evaporative heat loss causes the body to increase vasodilation of peripheral blood vessels to facilitate heat transport from the core to the periphery, and dry heat exchange between the skin and environment (Charkoudian, 2003; Sawka & Pandolf, 2001), further increasing cardiovascular strain. In extreme conditions, skin blood flow can reach levels as high 8 l/min (~60% of cardiac output) (Charkoudian, 2010; Johnson & Proppe, 2010; Rowell, 2011), and it has been suggested that the extreme cardiovascular stress causes the reduction in heat tolerance time and early onset heat exhaustion (Cheuvront et al., 2010; Périard et al., 2011; Sawka & Pandolf, 2001).
Wearing PPE, like firefighting gear, nuclear biological and chemical (NBC) protective gear, and military protective equipment during exercise in the heat can cause a state of uncompensable heat stress, due to the decrease in heat loss capacity (Cheung et al., 2000). The uncompensable heat stress causes heat exhaustion to occur at lower ambient temperatures and relative humidity compared to wearing normal clothing (Cheung et al., 2000). The properties of the PPE allows for limited or no evaporative heat loss, and the added weight of the PPE leads an increase in metabolic heat production, further disrupting the heat balance equilibrium (Duggan, 1988). Calculations done by Cheung et al. (2000), showed that for a 75 kg male with a VO$_2$max of 50 ml*kg$^{-1}$*min$^{-1}$ wearing the Canadian Armed Forces NBC protective suit, light exercise of 1.0 VO$_2$ L/min at 30°C (10% RH) was sufficient to induce a state of uncompensable heat stress. Based on their calculations, E$_{req}$ would be equal to 135 W/m$^2$, however E$_{max}$ was limited to 95 W/m$^2$. The NBC is not the only type of PPE shown to induce a state of uncompensable heat stress at relatively low ambient temperatures. Similar results have been found for people wearing firefighting equipment (Cheung et al., 2010; Selkirk & McLellan, 2004), football players wearing helmet and pads (Armstrong et al., 2010; Fowkes Godek et al., 2004), and people wearing military body armour (Larsen et al., 2012; Stewart et al., 2014).

Extensive literature exists regarding factors affecting loaded march performance and the effect of heat exposure on physical performance. However, the combined effects of a loaded march carrying a heavy external load while wearing military PPE and heat exposure has not been well studied. Both leadership and soldiers serving in the armed forces, carrying heavy external loads and performing operations in extreme environmental temperatures would greatly benefit from research that can help determine upper limits for environmental temperature, humidity, duration
and intensity rendering it safe to perform heavy loaded march while wearing military PPE, without risking heat exhaustion and heat illness.

**THE EFFECT OF PREVIOUS EXPERIENCE ON TASK PERFORMANCE**

Previous experience is an undervalued factor that might have a larger effect on performance outcome than people realize. The effect of previous experience in solving a specific task is a topic that has received limited attention, but there are a few publications supporting the perception of the beneficial effect of having previous experience on performance outcome. A study by Knecthle et al. (2012) found that training volume and previous experience were the main factors predicting performance for long distance inline skating, whereas anthropometric characteristics had no effect on performance. Pacing strategies and rate of perceived exertion (RPE) on cycling time trials does also seem to be positively affected by previous experience. Mauger et al. (2009), compared two groups of experienced cyclists on their pacing strategies on a 4 km time trial. A total of four trials were performed for each group, one group received full distance feedback, and the other no feedback on distance. On the first time trial, the group with no distance feedback was significantly slower than the feedback group. From trial two through four, receiving no external feedback and relying only on previous experience, the group without feedback closed the gap and improved their pacing strategy and completion time. On the fourth trial, no difference in completion time was observed between the feedback and no feedback group.

Micklewright et al. (2010), suggests that cyclists rely on a combination of previous experience and sensory feedback to determine optimal pacing strategies and the RPE they can tolerate. They explained this using the multilevel theories of cognition, where the cyclist would be using experience-derived mental representations from previous attempts on the same task, which will determine how he/she plans and adjusts RPE and pacing strategies.
Seifert et al. (2013), compared the ice climbing performance of two groups of university students, one with previous climbing experience and one without. Their results showed that although the experienced group didn’t have ice climbing experience, but were familiar with rock climbing, the experienced group had better climbing movement fluency and movement coordination tendencies compared to the inexperienced group, and performed better on the ice climbing task. It has also been shown that players with previous experience with Australian Football performed better on a hand pass task, compared to inexperienced participants, although previous experience had no effect on learning and improvement over time (Panchuk et al., 2013).

Based on this limited research, it seems evident that previous experience is beneficial for performance outcome. However, more research is needed to quantify these effects. The previous studies were also not perfectly controlled studies, meaning other factors than experience level could affect performance outcome. With the potential effect of previous experience on performance outcome, the loaded march physical performance test, recently implemented for all members of the Canadian Army, could potentially pose a bias against less experienced individuals. Based on the results from aforementioned studies, soldiers performing regular loaded march could possess an advantage when it comes to pacing strategies, RPE and overall performance, compared to equally fit truck drivers and desk clerks. Therefore, more research is necessary to determine the effect of previous experience on loaded march performance, and to determine if a bias exists against less experienced individuals.

Clearly, many questions are still unanswered concerning the effects of internal and external factors on military physical performance. In this context, my thesis will focus on determining the effects of a specific set of internal and external factors, known to affect physical performance in
the general population. To achieve this overarching objective, I devised four studies with specific aims directed at determining the effects of morphology, physiological capacity, stress and inflammatory cytokines, previous experience, and heat exposure on military physical performance.

**SPECIFIC AIMS**

In Chapter 2, the effect of physiological capacity and morphology on military physical performance was assessed by investigating which morphological and/or physiological characteristics could be used to explain overall performance outcome on the CMTFE. A detailed analysis of which physiological and morphological characteristics separated top and bottom ranked performers on the CMTFE was also performed. The physical employment standard currently used by the U.S. Armed Force has been under scrutiny for being biased against larger individuals. Therefore, the potential existence of a body mass bias against larger individuals performing the CMTFE was also elucidated in Chapter 2.

Chapter 3 of this thesis sought to determine the occurrence of stress and inflammatory cytokines among CAF members, by describing basal blood plasma levels of cortisol and cytokines, both inflammatory and non-inflammatory, in regular CAF members. The association between sex, age, adiposity and levels of circulating cortisol and cytokines was also examined, and compares to known trends in the general population.

In Chapter 4, the association between inflammatory cytokines and military physical performance was elucidated, by assessing the relationship between levels of cortisol, inflammatory cytokines and military physical performance in the CAF.
Chapter 5 sought to determine the effects of heat exposure and previous experience on loaded march performance. This was achieved by investigating the thermoregulatory and cardiovascular responses to performing a loaded march in the heat, as well as the effects of heat exposure on cost of locomotion and military physical performance. Chapter 5 also sought to determine how previous experience could affect load march and military physical performance.

HYPOTHESES

Chapter 2: Based on the fact that the CMTFE is set of military specific tasks, rather than a measure of general physical fitness, it was hypothesised that physiological capacity, rather than morphology would be the best predictor of performance outcome on the CMTFE. Unlike the tests employed by the U.S. Armed Force, no body mass bias against larger individuals performing the CMTFE was expected to exist.

Chapter 3: The high risk of stress exposure makes members of the armed forces more likely to possess high levels of stress and inflammatory cytokines. Therefore, it was hypothesized that CAF members would have a significant presence of stress and inflammatory cytokines, and that levels would increase with an increase in age and adiposity.

Chapter 4: With basis in current literature, it was hypothesized that high levels of stress and inflammatory cytokines would be inversely related to military physical performance.

Chapter 5: It was also hypothesized that heat exposure would have a significant negative effect on loaded march performance, and on performance of the FORCENeutral combat test. Previous experience was also expected to affect performance outcome. Thus, it was hypothesised that
participants with previous loaded march experience would perform better than inexperienced participants on the loaded march and the FORCEcombat test.

The manuscripts included in this thesis have been published, or are in the process of being published in peer-reviewed journals. However, to increase comprehensibility of the final thesis, each chapter has been modified to fit the thesis format.
CHAPTER 2
Explaining Performance on Military Tasks in the Canadian Armed Forces: The Importance of Physiological Capacity and Morphology

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Published in Military Medicine 188(11): e1623-e1629 (Appendix I)

Author contributions: All authors contributed to the conception and design of the experiments. HCT performed the analysis and interpretation of data, and writing of the manuscript. All authors contributed to the critical revising of the manuscript, and approved the final version of the manuscript.
ABSTRACT

Several occupations apply physical performance tests to confirm that their employees meet the minimum physical employment standards. Knowledge about factors affecting performance outcome on these physical performance tests could provide valuable information concerning mode of training. The main purpose of this study was to determine which physiological and/or morphological characteristics could explain overall performance outcome on the CMTFE, used to measure CAF members’ occupational physical performance. Measures of morphology (height, weight, body composition) and physiological capacity (grip strength, shuttle run distance, plank time etc.), together with performance on six common military tasks were recorded from female (n=127) and male (n=294) CAF members. Results showed large differences in both morphology and physiological capacity between top and bottom performers in both the male and female group. Despite large differences in morphology, multiple linear regression analyses showed that measures of upper body strength and aerobic capacity could explain a large part of the performance variability in both the male and female group. This study showed that total performance on the CMTFE is dependent on physiological capacity rather than morphology.
INTRODUCTION

Several occupations require a minimum physiological capacity to efficiently carry out and meet occupational task requirements. First responders are required to perform physical performance tests to assess their occupational suitability and physiological capacity to perform occupational tasks (Anderson et al., 2001; Michaelides et al., 2011; Michaelides et al., 2008). Analysis of the effects of morphology and physiological capacity on such tests can provide valuable information about physical requirements and training regimen needed to improve performance. A recent study by Michaelides et al. (2008), showed that firefighting performance could be predicted based on a battery of fitness parameters, where upper and lower body muscular power, muscular endurance and body fat percentage (BF%), together with flexibility could explain 55% of variation in performance on a firefighting ability test. The armed force is another occupation using physical performance tests to determine physical readiness and the occupational suitability of their members. Although the armed forces consists of a variety of different elements and occupations, all members of the CAF are held to the Universality of Service Principle, stating that independent of individual occupation, CAF members are soldiers first. This means that all CAF members, being a soldier in the field or working behind a desk, have to possess the physiological capacity to perform essential common military tasks, such as trench digging, soldier rescue and evacuation, building sandbag fortifications, and all CAF members could be called upon to perform these tasks at a moment’s notice. To measure the physiological capacity and occupational suitability of CAF members’, a new physical employment standard (PES) was established in 2013. The new PES consists of minimum physical performance requirements tested on six common military tasks, designed to represent situations which all CAF members could be expected to encounter (Mattie et al., 2013). Though nearly 80 000 service men and women in the CAF are held to this standard annually, a detailed analysis of the physiological and morphological
differences between the elite and lower ranked performers on these military specific tasks has not yet been established. Analyzing which physiological and morphological characteristics are separating top and bottom performers on the CMTFE would yield a better platform for exercise prescription to help CAF members excel in their work and on their PES tests.

Therefore, the purpose of this study was to investigate which physiological and/or morphological characteristics could be used to explain overall performance outcome on the CMTFE. A detailed analysis of which physiological and morphological characteristics separate elite and lower ranked performers were also performed. Previous research has criticised the U.S. Armed Force physical fitness tests for being biased against larger individual with a large body mass, and not just a large body fat mass (Vanderburgh, 2008). However, it remains unknown if a body mass bias also exists against larger individuals when performing the CMTFE. Because of the novelty of theses physical performance tests, and lack of relevant research on such a complex battery of military physical performance tests, our hypotheses are based on components of the CMTFE, with four out of six tasks requiring lifting and load carriage, and two tasks requiring a displacement of body mass over a certain distance. Based on previous research investigating factors influencing such tasks (Beckett & Hodgdon, 1987), we hypothesize that overall performance on the CMTFE will be dependent on lean body mass and aerobic capacity.
METHODOLOGY

Participants

The analyses conducted in this study were secondary analysis of previously collected data from male and female CAF members, collected during the development of the new physical employment standards for the CAF. The participants ranged from 19 to 39 years of age. Women (n=127) had a mean height of 163±6 cm (mean±SD) and a mean weight of 68±13 kg, while the mean height and weight of the men (n=294) were 176±6 cm and 86±15 kg. All testing was performed between May 1st and October 1st 2012, on seven military bases across Canada. All data was collected with the approval of the Defence Research and Development Canada’s Human Research Ethics Board.

Measurements of Morphology

Measurements of height, body mass, abdominal circumferences (hip and waist) and body composition were collected during the initial study. Height was measured using a Seca 213 Portable stadiometer (Seca Industries, Hanover, Maryland, USA), while hip and waist circumference data were collected using a standard anthropometric measuring tape. Body mass was measured using a standardized and calibrated professional grade digital weighing scale (Health-o-meter, Alsip, Illinois, USA). Body composition was estimated through bioelectrical impedance analysis, using the InBody 520 (BioSpace Technologies, Los Angeles, USA), previously validated against the gold standard measure Dual-Energy X-ray Absorptiometry (Anderson et al., 2012). The body composition analysis gave an estimate of lean body mass (LBM) and fat mass (BFM). Body fat % was calculated by dividing BFM with total body mass, whereas BMI was calculated by dividing body mass by height squared (weight (kg)/height²( m)).
Measurements of Physiological Characteristics

As the goal of the initial study was to identify simple field-expedient tests to reflect the demands and predict performance on the more complex CMTFE, participants performed a variety of tests of physiological capacity. To estimate aerobic capacity, a multi stage 20-meter shuttle run test was used. This test has previously been validated and found to accurately represent a measure of aerobic capacity (Palczka et al., 1987; Ramsbottom et al., 1988), and the procedures for the tests are described in the CF EXPRES Operations Manual (Director of Fitness - Canadian Armed Forces, 2010). The level attained and the number of stages completed on that level, was recorded and converted into total distance covered. To determine upper body strength, grip strength was measured. Grip strength has been found to be a valid predictor of total upper body strength in young adults (Wind et al., 2010), and was measured using a Smedly Analog hand dynamometer (Smedly TTM, Tokyo, Japan). The procedures for grip strength measurement can be found in the CF EXPRES Operations Manual (Director of Fitness - Canadian Armed Forces, 2010). Following standard protocol, grip strength was measured twice for both left and right hand, and the highest recorded measurement for each hand was summed and used as the measure of total grip strength. Core strength was estimated, measuring front plank time (sec). Having forearms and toes placed on the ground, participants were asked to lift their hips off the ground and maintain a neutral spine. The test was terminated when the participant failed to maintain a neutral spine. Detailed description of the procedures for performing front plank can be found elsewhere (Parkhouse & Ball, 2011).
The CAF Common Military Task Fitness Evaluation (reproduced from Reilly et al. (2012))

The six task simulations of the CMTFE were derived via subject matter expert consultation and detailed physiological analysis. Brief descriptions of each of the six tasks included in the CMTFE are found in Table 2.1, and a more detailed description can be found in Appendix III.

Table 2.1: Brief description of the six military specific tasks constituting the CMTFE used by the CAF.

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<th>Task</th>
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<tbody>
<tr>
<td><strong>Sandbag Fortification (SBF)</strong></td>
<td>Participants were required to lift 60 (20 kg) sandbags from a stacked pallet and transfer (without throwing) the bag 125 cm, up onto a 91.5 cm platform.</td>
<td>Time to completion (sec)</td>
</tr>
<tr>
<td><strong>Escape to cover (E2C)</strong></td>
<td>Each participant was required to run 10 m, make a 180° turn, stop and take a knee for 7 s, run 50 m, low crawl for 10 m and finish the task with a 30 m run. This task was performed wearing full fighting order (Boots, helmet, weapon, etc. = 25kg)</td>
<td>Time to completion (sec)</td>
</tr>
<tr>
<td><strong>Picking and Digging (P&amp;D)</strong></td>
<td><em>Picking</em>: While using a sledgehammer (4 kg), each participant was required to move a standardized weight (22 kg) 400 cm along a track on a picking simulator. <em>Digging</em>: Using a shovel, each participant was asked to transfer 180 L of river rocks into a 0.18 m³ box; at 60 s work to 30 s rest intervals.</td>
<td>Time to completion (sec)</td>
</tr>
<tr>
<td><strong>Pickets and Wire Carry (P&amp;W)</strong></td>
<td>Participants had to walk while carrying 23 pieces of equipment (ranging from 5.5 kg to 16 kg) from a drop zone to various perimeter points to complete the task.</td>
<td>Time to completion (sec)</td>
</tr>
<tr>
<td><strong>Stretcher Carry (SC)</strong></td>
<td>Participants were asked to lift (from the ground) and carry a 43 kg deadlift bar (half the weight of a casualty weighing 86 kg) 25 m, setting down the bar and taking a 10 s pause, before completing a second 25 m lift and carry. Each participant then had to lift, from the ground, a 21.5 kg EZ Curl bar (quarter of a casualty weight of 86 kg) up and slide it onto a 91.5 cm tall platform. The test was performed with increasing casualty weight until maximal performance was reached.</td>
<td>Maximal weighted lifted and carried (kg)</td>
</tr>
</tbody>
</table>
**Vehicle Extrications (VE)**

Participants were asked to drag an 86 kg mannequin 80 cm across a simulated vehicle front seat (at a height of 76 cm), remove and carry it 5 m, and then carry the torso (help has arrived to lift the legs) of the mannequin another 5 m where it was lifted and placed on a platform at a height of 76 cm. This task was performed continuously.

| Maximal weighted lifted and carried (kg) |

---

**Development of Composite Scores**

CAF members are required to pass all six military tasks, and failing one tasks leads to a failed physical performance test. Therefore, a composite score including the results from all six military specific tasks was developed, as a measure of total performance. Since males and females are held to the same PES, the total study cohort was given a percentile score for each task, based on their performance, where the top performer was given a score of 100, and the bottom performer was given a score of 1. The scores for each of the six CMTFE tasks were then summed to give a total performance score out of 600.

**Statistical Analysis**

All of the morphology, physiological capacity and performance measures were analyzed for normality before any statistical analyses were performed. An independent sample t-test with equal variance was applied to determine significant differences in morphology, physiological capacity and performance features between top and bottom performers on the CMTFE. An analysis of covariance among independent variables was performed, and covariates with the lowest association with the dependent variable were excluded from further analysis. Step-wise multiple
regression analyses were used to determine the ability of the morphological and physiological variables to explain overall performance outcome on the CMTFE. The male and female samples were analyzed separately. Results were presented as mean±SD and the 95% confidence interval, and significance level was set to p≤0.05. All statistical analyses were conducted using SPSS Statistics 17.0.
RESULTS

Task Complexity and Physiological Strain

To address the physiological strain on the participants, total work done against gravity and loaded distance covered for each task was calculated and plotted in Figure 2.1. Work performed was calculated by multiplying the external load with the displacement against gravity, while loaded distance was defined as horizontal distance covered walking or running with an external load.

![Figure 2.1:](image)

**Figure 2.1:** Work (J) performed over horizontal distance covered during loaded condition for each of the CMTFE tasks.

Differences between top and bottom performers

Based on their composite score ranking, the male and female group of CAF members were each split into quintiles, and the top and bottom performance quintiles were compared for differences in physiological capacity and morphology (Table 2.2 and 2.3). In the male group, a
70.7% differences in total performance scores were observed between the top and bottom quintile (p<0.01) (**Table 2.2**). Relatively large differences in morphology were observed between the top and bottom male performers, as well as a small yet significant difference in height (3.3%, p<0.01). The participants in the top quintile had a lower fat mass (62.4%, p<0.01), higher lean body mass (12.7%, p<0.01), and a lower BMI (9.7%, p<0.01) compared to the bottom performers. The top male performers also had better aerobic capacity, with a 51.9% (p<0.01) difference in shuttle run distance compared to the bottom quintile. Differences in upper body and core strength were also observed (20.3% difference in grip strength, p<0.001; and 60.6% difference in plank time, p<0.001) between the top and bottom quintiles. Interestingly, the only measure not different between the top and bottom quintile was body mass (3.2%, p=0.45). Similar to what was observed in the male group, the female CAF members in the top performance quintile had a superior total performance score (134.2%, p<0.01) compared to the bottom quintile (**Table 2.3**). The top female performers also had significantly better aerobic capacity (65.8% difference in shuttle run distance, p<0.01), and core strength (56.0% difference in plank time (p<0.01) compared to the bottom quintile. However, unlike the male group, there was no difference in grip strength (10.9%, p=0.22) between top and bottom performers in the female group. The top female performers were also taller (3.6%, p<0.01), had more lean body mass (9.8%, p<0.01), lower fat mass (65.3%, p<0.01), and a lower BMI (18.0%, p<0.01). As in the male group there was no significant difference in body mass between top and bottom female performers (11.2%, p=0.09).
Table 2.2: Comparison of morphology and physiological capacity between top and bottom performance quintiles in the male group

<table>
<thead>
<tr>
<th>Top</th>
<th>Mean ± SD</th>
<th>95% CI.</th>
<th>Bottom</th>
<th>Mean ± SD</th>
<th>95% CI.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>27 ± 5</td>
<td>[25, 28]</td>
<td>31 ± 6</td>
<td>[29, 32]</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178 ± 6</td>
<td>[176, 181]</td>
<td>173 ± 7</td>
<td>[171, 175]</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>82 ± 11</td>
<td>[79, 86]</td>
<td>85 ± 19</td>
<td>[79, 91]</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8 ± 3.0</td>
<td>[24.9, 26.7]</td>
<td>28.4 ± 6.0</td>
<td>[26.6, 30.2]</td>
<td>0.01*</td>
<td></td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>12.8 ± 6.5</td>
<td>[10.8, 14.8]</td>
<td>24.4 ± 14.5</td>
<td>[19.8, 28.9]</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td>Lean Body Mass (kg)</td>
<td>69.6 ± 6.5</td>
<td>[67.6, 71.5]</td>
<td>61.2 ± 12.3</td>
<td>[57.4, 65.0]</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td>Body Fat%</td>
<td>25.8 ± 3.0</td>
<td>[24.9, 26.7]</td>
<td>28.6 ± 7.4</td>
<td>[26.3, 30.9]</td>
<td>0.01*</td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>0.87 ± 0.06</td>
<td>[0.86, 0.89]</td>
<td>0.92 ± 0.07</td>
<td>[0.88, 0.90]</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td>Waist Circ. (cm)</td>
<td>87.9 ± 8.0</td>
<td>[85.4, 90.3]</td>
<td>96.6 ± 15.3</td>
<td>[91.9, 101.3]</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td>Shuttle Run Dist. (m)</td>
<td>1707 ± 335</td>
<td>[1604, 1809]</td>
<td>1004 ± 412</td>
<td>[874, 1133]</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td>Grip Strength (kg)</td>
<td>120 ± 25</td>
<td>[113, 128]</td>
<td>98 ± 20</td>
<td>[92, 105]</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td>Plank time (sec)</td>
<td>166 ± 86</td>
<td>[139, 192]</td>
<td>89 ± 44</td>
<td>[75, 102]</td>
<td>0.00*</td>
<td></td>
</tr>
<tr>
<td>Performance Score</td>
<td>504 ± 28</td>
<td>[495, 513]</td>
<td>241 ± 55</td>
<td>[224, 257]</td>
<td>0.00*</td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference (p≤0.05)
Table 2.3: Comparison of morphology and physiological capacity between top and bottom performance quintiles in the female group

<table>
<thead>
<tr>
<th></th>
<th>Top</th>
<th>Bottom</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>31 ± 5</td>
<td>31 ± 6</td>
<td>0.93</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167 ± 5</td>
<td>161 ± 6</td>
<td>0.00*</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>64 ± 5</td>
<td>72 ± 17</td>
<td>0.09</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.0 ± 0.9</td>
<td>27.5 ± 5.4</td>
<td>0.00*</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>12.8 ± 3.0</td>
<td>25.2 ± 11.3</td>
<td>0.00*</td>
</tr>
<tr>
<td>Lean Body Mass (kg)</td>
<td>51.4 ± 3.5</td>
<td>46.6 ± 6.3</td>
<td>0.01*</td>
</tr>
<tr>
<td>Body Fat %</td>
<td>23.0 ± 0.9</td>
<td>27.5 ± 5.4</td>
<td>0.00*</td>
</tr>
<tr>
<td>WHR</td>
<td>0.82 ± 0.06</td>
<td>0.85 ± 0.07</td>
<td>0.11</td>
</tr>
<tr>
<td>Waist Circ. (cm)</td>
<td>80.1 ± 5.1</td>
<td>89.2 ± 14.3</td>
<td>0.02*</td>
</tr>
<tr>
<td>Shuttle Run Dist. (m)</td>
<td>1366 ± 224</td>
<td>689 ± 302</td>
<td>0.00*</td>
</tr>
<tr>
<td>Grip Strength (kg)</td>
<td>82 ± 19</td>
<td>74 ± 20</td>
<td>0.22</td>
</tr>
<tr>
<td>Plank time (sec)</td>
<td>156 ± 45</td>
<td>88 ± 82</td>
<td>0.01*</td>
</tr>
<tr>
<td>Performance Score</td>
<td>274 ± 49</td>
<td>54 ± 15</td>
<td>0.00*</td>
</tr>
</tbody>
</table>

* Significant difference (p≤0.05)
**Multiple Regression Analysis**

Step-wise multiple regression analysis was used to determine the factors best predicting performance outcome on the CMTFE (Table 2.4). The results from the analyses showed that a model, containing shuttle run distance, height and plank time explained 51.9% of the variance in total performance in the female group (Table 2.4A). In the male group, a regression model containing Shuttle Run Dist., Lean Body Mass, Grip Strength and Plank Time explained 61.2% of the variance in total performance outcome (Table 2.4B). Body mass was not included as a predictor of performance in either the male or female group.
**Table 2.4:** Female (A) and Male (B) Regression Model of predictors for Total Performance Score

<table>
<thead>
<tr>
<th>Variable</th>
<th>R²</th>
<th>Partial R²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>0.519</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><em>Shuttle Run Dist. (m)</em></td>
<td>0.363</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><em>Height (cm)</em></td>
<td>0.109</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><em>Plank Time (sec)</em></td>
<td>0.047</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>0.612</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><em>Shuttle Run Dist. (m)</em></td>
<td>0.322</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><em>Lean Body Mass (kg)</em></td>
<td>0.212</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><em>Grip Strength (kg)</em></td>
<td>0.043</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td><em>Plank Time (sec)</em></td>
<td>0.035</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>
DISCUSSION

The purpose of this study was to determine which measures of physiological capacity and/or morphology could help explain overall performance outcome on the CMTFE. We also attempted to establish whether a body mass bias against larger individuals performing the CMTFE was present. Whereas previous studies have investigated the effect of morphology and physiological capacity on single task performance, this is the first study to investigate their effect on total performance on a battery of complex military physical performance tests. Differences in morphology and physiological capacity were observed between the top and bottom performers, with aerobic capacity, stature, lean body-, and fat mass being significantly different between the top and bottom performance quintiles in both the male and female group. Although comparisons of differences in morphology and physiological capacity between elite level and lower ranked counterparts have been well studied in athletes (Bale et al., 1985; Sánchez-Muñoz et al., 2007; Tanda & Knechtle, 2015), such comparisons have not previously been made in military cohort. Even with large differences in morphology between top and bottom performers, the outcome of the step wise multiple linear regression analysis showed that total performance is dependent on trainable factors of physiological capacity rather than morphology. Body mass was not found to be a separating factor between top and bottom performers in either the male or female group, nor was it a significant predictor of performance outcome. Thus, no body mass bias against larger individuals seem to exist on the CMTFE test.

Factors predicting performance on the CMTFE

The six military specific task simulations forming the CMTFE, are widely different in nature and have different physical demands. As seen in Figure 2.1, each distinct task had widely different demands for total work and loaded distance covered, requiring different physical features
for optimal performance. As mentioned previously, studies on athletic performance have shown that body size and morphology can have a large effect on performance (Bale et al., 1985; Sánchez-Muñoz et al., 2007; Tanda & Knechtle, 2015). Morphological differences between the top and bottom performance quintiles were also observed on the CMTFE, with the participants in the top quintile being taller and having more lean body mass and lower fat mass compared to the bottom quintile, in both the male and female group. Despite the difference in morphological characteristics, morphology was not a determining factor for performance outcome. Shuttle run distance and plank time explained ~40% of the variability in performance in the female group, while shuttle run distance and plank time, together with grip strength explained ~40% of the variability in performance in the male group. These results show that total performance on the CMTFE is based on trainable features of physical performance like aerobic capacity, core-, and upper body strength. However, the morphological features, height and lean body mass were responsible for a certain part of the variability in performance in the female and male group respectively. In the female group, ~10% of performance could be explained by differences in height. This was likely caused by the standardized height objects had to be lifted to, and three out of the six tasks requiring vertical displacement of a certain weight to a pre-determined height. With females having shorter stature than men, more of the lifting phase is spent using upper body strength to reach the target height. Studies have shown a more pronounced difference in strength in the upper body compared to lower body between males and females (Bishop et al., 1987; Miller et al., 1993), and a positive correlation between box lifting performance and stature in females (Stevenson et al., 1996). In the male group, ~21% of the variability could be explained by differences in lean body mass. This is likely caused by the heavier weights lifted in the male group, requiring more lean body mass. The male group lifted significantly heavier weights compared to
the female group (data not presented) in the VE and SC task. Lean body mass is correlated with strength and lifting capacity (Horvat et al., 2003; Maughan et al., 1983), and could thereby explain more of the variability in total performance outcome in the male group. Apart from performing better on the SC and VE tasks, which require a maximal load to be lifted, the male group also performed better on the picking and digging task. In this task, performance is based on the speed a picking simulator can be moved and speed to dig gravel, where also upper body strength and lean body mass plays an important role.

Results showed that aerobic capacity (shuttle run distance) was the main predictor of performance outcome in both the male and female group, which corresponds well with four out of six tasks being endurance based. Females relied more on aerobic capacity than males, with ~36% of performance outcome being explained by shuttle run distance, and no observed difference in grip strength between the top and bottom quintiles (Table 2.3). The male performers, although shuttle run distance explained ~32% of the performance outcome, relied more on total and upper body strength for performance, where differences in grip strength and lean body mass explained another ~25% of performance outcome. Lean body mass is correlated with muscle strength (Horvat et al., 2003; Maughan et al., 1983), and can be improved through exercise and strength training (West & Phillips, 2012).

Even with the large difference in total work performed and loaded distance covered between the six CMTFE tasks (Figure 2.1), ~60% and ~52% of the variability in total performance for the male and female group respectively, could be explained by linear regression models containing simple measures of aerobic capacity, muscular strength, stature and lean body mass. Although performed on a battery of separate military tasks, similar results have been observed by Rayson et al. (2000). This group found that shuttle run distance and lean body mass, together with
dynamic strength were the best predictors of performance on a multitude of military specific performance tests. Out of nine different lift and carry tasks, shuttle run distance and performance on an incremental lift machine were among the best predictors of performance in six tasks, while lean body mass and upright pull was among the best predictors of performance in four of the tasks.

**No body mass bias against performance outcome**

The physical fitness tests applied in some military services, especially the U.S. Armed Force, have been under scrutiny for being biased against individuals with a larger body mass. Studies have shown a strong negative relationship between performance outcome on the versions of sit-ups, push-ups and 2-mile run test used in the U.S. Armed Forces and total body mass (Vanderburgh, 2008), leaving individuals with a higher body mass at a disadvantage. Research has shown that performance on military related tasks, due to a large requirement of lifting and carrying capacity, is independent of total body mass and more related to lean body mass and lean body mass to dead mass ratio (dead mass = body mass + external weight) (Bilzon *et al.*, 2002). The results from our study showed that no body mass bias against larger individuals exist on total performance of the CMTFE. No difference in body mass was observed between the top and bottom performance quintiles in either the male of female group, and body mass was not included in the step wise multiple regression analysis of performance predictors for either the male of female group. This indicates that heavier individuals, known to perform well on task specific military operations, are not at a disadvantage when performing the CMTFE tests.

**Limitations**

There were a few potential limitations to this study that should be addressed. Leg strength was not measured, and with four out of six tasks including an element of lifting, leg strength could
potentially have helped explain more of the variability in performance outcome, further improving the prediction models. The current analysis was performed on previously collected data, and therefore, we did not have the option to add a measure of leg strength to the database. It is suggested that future studies investigating factors affecting military performance include a measure of leg strength. During the performance testing, instructors observing task performance noticed a widely different set of techniques applied to perform the different tasks, depending on body size. Performing a biomechanical analysis of the different techniques used could potentially add further detail about the effect of morphology and physiological capacity on performance outcome.

CONCLUSION

Through a thorough analysis of the factors separating the top and bottom performers on the CMTFE, it was discovered that most of the variability in total performance could be explained by measures of aerobic capacity and muscular strength. Apart from height having a small effect on performance in the female group, morphology did not have an effect on performance of the CMTFE. We also found that the total score on the six military tests used for the CMTFE does not carry a body mass bias and does not discriminate against larger individuals. Based on these results we conclude that total performance on the CMTFE is dependent on trainable factors of physiological capacity, like aerobic capacity and muscular strength, whereas morphology have little or no effect on performance.

Acknowledgments The authors would like to acknowledge the members of the Canadian Armed Forces Directorate of Fitness - Human Performance Research and Development team who collected the original data for this study, as well as for their support in the development of the current manuscript.
CHAPTER 3
Levels of Circulating Cortisol and Cytokines in Members of The Canadian Armed Forces: Associations With Age, Sex and Morphology

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²Department of Biochemistry Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario Canada
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Accepted for publication in Applied Physiology, Nutrition and Metabolism (published online December 04, 2017) (APPENDIX II)

Author contributions: All authors contributed to the conception and design of the Experiments. HCT, LF and VT performed the analysis and interpretation of data. HCT wrote the manuscript. All authors contributed to the critical revising of the manuscript, and approved the final version of the manuscript.
ABSTRACT

The purpose of this study was to assess blood levels of cortisol and cytokines (inflammatory and non-inflammatory) in regular CAF members, and examine the associations between sex, age and adiposity and circulating levels of cortisol as well as pro- and anti-inflammatory cytokines. As part of a larger ranging project, 331 blood samples were collected from a representative population of the total CAF, which included officers and non-commissioned women and men from the Air Force, Navy and Army. The blood samples were analyzed for levels of cortisol, C-Reactive Protein, adiponectin and 20 cytokines (that included interleukins, interferons and tumor necrosis factors). Higher levels of adiponectin were found in women compared to men (median and IQR; 16.71 (7.68-25.32) vs 5.81 (3.52-13.19) µg/ml), and higher levels of IL-18 in men compared to women (89.25 (84.03-94.48) vs 75.91 (69.70-82.13) pg/ml). An association between age and levels of stress and inflammatory cytokines was observed, with CRP, IL-18, IL-2 and adiponectin all increasing with increasing age. However, contrary to trends seen in the general population, cortisol levels decreased with increasing age. Levels of CRP and IL-18 increased with an increase in adiposity, while adiponectin levels decreased. Most importantly, at the entire cohort level, a low detection rate for most of the cytokines was observed, with 17 out of 22 cytokines having a detection below 10%. In this CAF population, although an association between age and inflammatory cytokines was observed, both sex and adiposity had a small effect on levels of cortisol and cytokines.
INTRODUCTION

Circulating levels of cortisol and cytokines can be strong indicators of the physiological state of an individual. While it is well accepted that elevated blood cortisol levels are generally indicative of chronic stress (Miller et al., 2007), elevated levels of specific cytokines (inflammatory and non-inflammatory) are associated either with induction, maintenance or regulation of an immune response and could possibly indicate the presence of acute or chronic diseases (Fichtlscherer et al., 2000; Libby et al., 2002; Tilg et al., 1992). For example, high levels of circulating CRP, IL-6 and IL-1 are indicators of ongoing inflammation and have been associated with an increased risk of developing cardiovascular disease (Anker & von Haehling, 2004; Blake & Ridker, 2002; Libby et al., 2002). Cytokines have also been suggested to play a role in the development of other diseases, such as type 2 diabetes (Duncan et al., 2003), chronic obstructive pulmonary disease (Gan et al., 2004), and osteoarthritis (Spector et al., 1997). Several studies have looked at basal levels of cytokines in the general population (Marques-Vidal et al., 2011; Stowe et al., 2010; Woloshin & Schwartz, 2005), but no such data is currently available for CAF members, who can be exposed to a variety of extreme conditions as part of their daily activities. In this context, this study was the first to assess basal blood levels of cortisol and cytokines, both inflammatory and non-inflammatory, in regular CAF members. In addition, the association between circulating cortisol and cytokine levels and sex, age and adiposity was determined and compared to known trends in the general population. It was hypothesized that CAF members would have a significant presence of stress and inflammatory cytokines, and that levels would increase with age and adiposity. This assessment will provide an important insight to the physiological state of the CAF and will serve as an important reference for future assessment in this population.
METHODOLOGY

Participants

A total of 331 CAF members were included in this analysis, which was performed on previously collected data. Females and older CAF members were deliberately oversampled to reach sufficient statistical power, allowing predictive relationships for specific demographic groups to be determined. All participants were pre-screened prior to taking part in the study, where resting blood pressure and heart rate was recorded, and participants completed the PAR-Q (American College of Sports Medicine, 1997) and the Risk Stratification Questionnaire (American College of Sports Medicine, 2010). Participants having any medical condition or restrictions limiting their ability to perform any of the physical performance tasks were excluded from the study. In addition, participants identified as “high risk” according to the Risk Stratification Questionnaire, as well as pregnant women were excluded from participating in the study. The study was conducted following the guidelines of the Helsinki Declaration and received ethics approval from Defence Research and Development Canada’s Human Research Ethics Board and The University of Ottawa Research Ethics Board.

Body Composition Measurements

Hip and waist circumference data were collected using a standard anthropometric measuring tape. Body mass was measured using a standardized and calibrated professional grade digital weighing scale (Health-o-meter, Alsip, Illinois, USA), while height was measured using a Seca 213 Portable stadiometer (Seca Industries, Hanover, Maryland, USA). BMI was calculated based on height and weight measurements (weight (kg)/height² (m)). Body composition was estimated through bioelectrical impedance analysis, using the InBody 520 (BioSpace
Technologies, Los Angeles, USA), previously validated against the gold standard measure Dual-Energy X-ray Absorptiometry (Anderson et al., 2012). Based on the analysis of body composition, an estimate of body fat percentage was calculated.

**Blood Sample Collection**

Participants were instructed not to drink caffeinated beverages during the two hours prior to testing and not to exercise or consume alcoholic beverages for six hours prior to testing. Blood samples were taken in the morning by a registered nurse. Blood samples were collected in 6 mL EDTA tubes and immediately placed on ice until centrifugation at 2428 x g. Plasma was separated in 2 mL aliquots and stored in a -80°C freezer until analysis.

**Blood Sample Analysis**

All blood samples analyzed were measured for levels of Cortisol, Adiponectin, C-reactive Protein (CRP), Interferon gamma (INF-γ), Tumor necrosis factor alpha (TNF-α), Granulocyte macrophage colony-stimulating factor (GM-CSF), Interleukin 1 beta (IL-1β), Interleukin 2 (IL-2), Interleukin 4 (IL-4), Interleukin 5 (IL-5), Interleukin 6 (IL-6), Interleukin 8 (IL-8), Interleukin 9 (IL-9), Interleukin 10 (IL-10), Interleukin 12p70 (IL-12p70), Interleukin 13 (IL-13), Interleukin 17a (IL-17a), Interleukin 18 (IL-18), Interleukin 21 (IL-21), Interleukin 22 (IL-22), Interleukin 23 (IL-23), Interleukin 27 (IL-27), Interleukin 31 (IL-31). Cortisol was measured using a Parameter Cortisol Assay (R&D Systems, Minneapolis, MN, USA), while CRP and adiponectin were measured using a Human C-Reactive Protein ELISA or the Human Adiponectin Platinum Sandwich ELISA kit (Affymetrix eBiosciences, San Diego CA USA). All assays were performed as instructed by the manufacturer. Each plate had a standard curve in duplicate and all plates were read at 450 nm in the ELISA plate reader FilterMax F5 Multimode microplate reader (Molecular
The SoftMax Pro 6.2.2 (Molecular Devices, Sunnyvale CA) software was also used to determine the levels of cytokine in the unknown using the standard curve found on each plate. Levels of IFN-γ, TNF-α, GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-17a, IL-18, IL-21, IL-22, IL-23, IL-27 and IL-31 were determined using the Procarta Plex Multiplex Immunoassay (Affymetrix eBiosciences, San Diego CA USA). Samples were prepared and used as instructed by the manufacturer of the kits. Standard curves were run on each plate together with unknowns. The plates were read on the Luminex MAGPIX instrument (Affymetrix eBiosciences, San Diego CA USA) and the cytokine levels for each unknown were extrapolated from the standard curves using the Procarta Plex analyst software with either a 4 or 5 parameter logistic curve of the standards. Cortisol and cytokine levels in plasma were determined as described previously (Cowan et al., 2012; Imbeault et al., 2012).

**Data/Statistical Analysis**

Differences in morphology between men and women were assessed with an independent sample t-test, whereas morphological differences between age groups were determined by one-way ANOVA. Of the 24 cytokines measured, 16 of these had less than 10% of values above the lower limit of detection (LOD). We therefore decided to treat these as dichotomous variables, detectable or non-detectable. To determine differences in percentage detectable values between sexes, age and BMI categories, cross tab analysis with a Chi-square test was used. Where significant difference between age and/or BMI categories were observed, a multinomial logistic regression was used to establish where the significant difference occurred. The cytokines IFN-γ, IL-2 and IL-8 all had a detection rate of values above LOD of more than 40%, and were analyzed as continuous markers together with CRP, cortisol, adiponectin and IL-18. Continuous markers are reported as median and interquartile range (IQR), for the total sample, separated by sex, age.
and BMI categories, except for IL-18, which is presented as mean and 95% confidence interval. Apart from IL-18, neither cortisol, nor any of the other cytokines were normally distributed. Therefore, a Mann Whitney-U test was used to determine potential differences between men and women. A Kruskal-Wallis test with a pair-wise Bonferroni post hoc test was used to determine significant difference between age and BMI categories for CRP, cortisol, adiponectin, IL-2, IL-8 and IFN-γ. Interleukin 18 was normally distributed, thus an independent sample t-test was used to determine potential differences between men and women, while a one-way ANOVA with a Bonferroni post hoc test was used to determine differences between age and BMI categories. To determine the independent relationship between cortisol and cytokines and measures of morphology, cortisol and cytokine values were log10 transformed, before performing a linear regression analyses. A p<0.05 was considered statistically significant. Data was analyzed using IBM SPSS Statistics version 23.0 (IBM SPSS Statistics for Windows, Version 23.0, IBM Corp, Armonk, NY).
RESULTS

Participant Characteristics

Participant characteristics are found in Table 3.1. We did observe certain differences in morphology between men and women, with women being slightly older than men, and they also had a higher body fat percentage (all p-values<0.01). Men tended to be taller, and had a higher body mass and waist circumference, and also a higher waist to hip ratio compared to women (all p-values<0.01). Only small differences in morphology were observed between age groups where CAF members in the 19-29-year age group were taller compared to members in the 30-39 (p=0.01) and 40-49 (p=0.02) year age group. They also had a lower body fat percentage compared to all the other age groups (all p-values<0.01), and a larger lean body mass than CAF members in the 30-39 and 40-49 year age groups (both p-values<0.01). We also observed a lower waist-to-hip ratio in the 19-29 year age group compared to the 50-59 year age group (p=0.02). Apart from this, no other differences in morphology between age groups were observed.
Table 3.1: Descriptive statistics of sample population, stratified by sex and age groups.

<table>
<thead>
<tr>
<th></th>
<th>Total (N=331)</th>
<th>Men (N=208)</th>
<th>Women (N=123)</th>
<th>19-29 (N=122)</th>
<th>30-39 (N=81)</th>
<th>40-49 (N=86)</th>
<th>50-59 (N=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>35.5 ± 10.4</td>
<td>34.2 ± 11.0</td>
<td>37.7 ± 9.1*</td>
<td>24.4 ± 2.8</td>
<td>34.4 ± 2.8†</td>
<td>44.0 ± 2.9†</td>
<td>52.4 ± 2.1</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>171 ± 9</td>
<td>176 ± 7</td>
<td>163 ± 6*</td>
<td>173 ± 8</td>
<td>169 ± 9</td>
<td>170 ± 9</td>
<td>173 ± 8</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>80.4 ± 16.0</td>
<td>86.7 ± 14.7</td>
<td>69.4 ± 11.8*</td>
<td>80.7 ± 14.7</td>
<td>78.4 ± 16.4</td>
<td>80.4 ± 17.5</td>
<td>83.0 ± 15.6</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>27.3 ± 4.2</td>
<td>27.9 ± 4.2</td>
<td>26.2 ± 4.0*</td>
<td>26.8 ± 4.1</td>
<td>27.2 ± 4.1</td>
<td>27.8 ± 4.3</td>
<td>27.7 ± 4.2</td>
</tr>
<tr>
<td><strong>BF%</strong></td>
<td>24.8 ± 9.1</td>
<td>21.7 ± 8.3</td>
<td>30.2 ± 8.0*</td>
<td>20.6 ± 8.9</td>
<td>25.9 ± 8.7†</td>
<td>28.2 ± 8.2†</td>
<td>27.3 ± 7.9†</td>
</tr>
<tr>
<td><strong>LBM (kg)</strong></td>
<td>60.0 ± 11.7</td>
<td>67.1 ± 8.1</td>
<td>47.8 ± 5.1*</td>
<td>63.7 ± 10.7</td>
<td>57.6 ± 11.8†</td>
<td>57.4 ± 12.6†</td>
<td>59.9 ± 10.3</td>
</tr>
<tr>
<td><strong>WHR</strong></td>
<td>0.88 ± 0.07</td>
<td>0.90 ± 0.07</td>
<td>0.84 ± 0.07*</td>
<td>0.87 ± 0.06</td>
<td>0.88 ± 0.9</td>
<td>0.89 ± 0.08</td>
<td>0.91 ± 0.07†</td>
</tr>
</tbody>
</table>

* Significant difference between men and women (p<0.05)
† Significantly different from 19-29-year age group (p<0.05)

BMI, body mass index; WC, waist circumference; BF%, body fat percentage; LBM, lean body mass; WHR, waist to hip ratio.
Detection rate of cortisol and cytokines.

Of the 24 markers measured, only seven had a detection rate higher than 10% above the LOD (CRP, cortisol, adiponectin and IL-18 had 100%, while IFN-γ, IL-2 and IL-8 had 44%, 42% and 53% detection rate respectively). The detection rate above LOD for each cytokine are found in Table 3.2. Results from multinomial logistic regression showed no difference in detection rate between men and women for any of the cytokines measured. However, there was an observed association between age and detection rate for several cytokines. People in the older age categories were more likely to have detectable values of IFN-γ (40-49 years, odd ratio (OR)=2.34, 95%CI:1.32-4.13, and 50-59 years, OR=4.26, 95%CI:2.02-8.97), IL-8 (40-49 years, OR=1.84, 95%CI: 1.05-3.21, and 50-59 years, OR=2.52, 95%CI: 1.21-5.25) and TNF-α (40-49 years, OR=5.14, 95%CI: 1.08-26.25, and 50-59 years, OR=10.00, 95%CI: 1.93-51.71) compared to people in the 19-29 year age category. People in the 50-59 year age category were also more likely to have detectable values of IL-5 (OR= 3.87, 95%CI:1.31-11.43), IL-9 (OR=8.11, 95%CI: 1.51-45.54), IL-17a (OR=5.51, 95%CI: 1.69-17.93), IL-23 (OR=3.06, 95%CI:1.15-8.15) and IL-31 (OR=5.36, 95%CI: 1.22-23.51) compared to the 19-29 year age category. No difference in detection rate of cytokines was observed between the BMI categories; normal, overweight and obese.
Table 3.2: Percentage of cytokine values above LOD, stratified by sex age and BMI categories.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>(pg/mL)</th>
<th>LOD Total</th>
<th>Men</th>
<th>Women</th>
<th>Age (years)</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=331)</td>
<td>(N=208)</td>
<td>(N=122)</td>
<td>(N=86)</td>
<td>(N=42)</td>
<td>Normal (BF% 18.6)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>(7.18)</td>
<td>44</td>
<td>46</td>
<td>41</td>
<td>32</td>
<td>41</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>(17.46)</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>TNF-α</td>
<td>(6.03)</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>IL-1β</td>
<td>(2.20)</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>IL-2</td>
<td>(5.25)</td>
<td>42</td>
<td>38</td>
<td>48</td>
<td>39</td>
<td>42</td>
</tr>
<tr>
<td>IL-4</td>
<td>(9.77)</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>IL-5</td>
<td>(5.42)</td>
<td>8</td>
<td>7</td>
<td>9</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>IL-6</td>
<td>(7.62)</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>IL-8</td>
<td>(3.22)</td>
<td>53</td>
<td>45</td>
<td>50</td>
<td>45</td>
<td>52</td>
</tr>
<tr>
<td>IL-9</td>
<td>(11.87)</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>IL-10</td>
<td>(1.66)</td>
<td>9</td>
<td>7</td>
<td>12</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>(4.69)</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>IL-13</td>
<td>(1.56)</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>IL-17a</td>
<td>(3.61)</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>IL-21</td>
<td>(17.99)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>IL-22</td>
<td>(41.97)</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>IL-23</td>
<td>(18.60)</td>
<td>5</td>
<td>9</td>
<td>10</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>IL-27</td>
<td>(18.99)</td>
<td>9</td>
<td>8</td>
<td>11</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>IL-31</td>
<td>(17.04)</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

*significantly more likely to have detectable values of cytokines compared to first category (age = 19-29), Adiponectin, C-reactive protein (CRP), cortisol and IL-18 all had 100% detection rate above lower limit of detection (LOD). BF%, body fat percentage.
Absolute levels of cortisol and cytokines.

The median and IQR for each of the cytokines with a detection rate above 10%, are found in Table 3.3 and Table 3.4. No significant difference in CRP, cortisol, IL-2, IL-8 or IFN-γ could be observed between men and women. Significantly higher adiponectin levels (U=7103.5, p>0.01) were observed in women compared to men, while men had higher levels of IL-18 (U=10239.0, p>0.01) compared to women. Between the age categories 19-29, 30-39, 40-49 and 50-59 no differences in levels of CRP, adiponectin, IL-18 or IL-8 were observed. Cortisol levels were highest in the youngest age category, and decreased with increasing age. The opposite was found for IFN-γ, where median cytokine levels were significantly higher in the 40-49 (p=0.04) and 50-59 (p<0.01) year age category compared to the 19-29 year category. Levels of IL-2 were also significantly higher among people in the 50-59 year age category compared to people in the 19-29 year category (p=0.01).

Cortisol, IFN-γ, IL-2 and IL-8 levels were not different between BMI categories (Table 3.4). People characterized as obese based on their BMI category, had significantly higher CRP values compared to both the normal and overweight (p=0.03 and p<0.01 respectively). People in the obese BMI category also had higher IL-18 levels, compared to people in the normal weight category (p=0.01), but there was no difference between overweight and obese (Table 3.3). Adiponectin levels showed an inverse relationship with BMI categories, where people classified as obese had significantly lower adiponectin levels compared to both the normal and overweight.
Table 3.3: Median and inter quartile range (IRQ) for CRP, Cortisol, Adiponectin and IL-18, for sex, age and BMI categories

<table>
<thead>
<tr>
<th>CRP</th>
<th>Cortisol</th>
<th>Adiponectin</th>
<th>IL-18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>Median</td>
<td>Median</td>
<td>Mean</td>
</tr>
<tr>
<td>(µg/ml)</td>
<td>(ng/ml)</td>
<td>(µg/ml)</td>
<td>(pg/ml)</td>
</tr>
<tr>
<td>(IQR)</td>
<td>(IQR)</td>
<td>(IQR)</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------</td>
<td>-------------</td>
<td>----------------</td>
</tr>
<tr>
<td>All subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>332</td>
<td>3.14</td>
<td>23.54</td>
<td>8.41</td>
</tr>
<tr>
<td>(1.25-7.73)</td>
<td>(14.91-59.17)</td>
<td>(3.98-20.00)</td>
<td>(80.26-88.36)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>209</td>
<td>25.08</td>
<td>5.81</td>
</tr>
<tr>
<td></td>
<td>(1.16-7.64)</td>
<td>(15.29-58.05)</td>
<td>(3.52-13.19)</td>
</tr>
<tr>
<td>Women</td>
<td>123</td>
<td>22.60</td>
<td>16.71*</td>
</tr>
<tr>
<td></td>
<td>(1.39-7.96)</td>
<td>(14.58-60.32)</td>
<td>(7.68-25.32)</td>
</tr>
<tr>
<td>Age category</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-29</td>
<td>122</td>
<td>32.08</td>
<td>7.46</td>
</tr>
<tr>
<td></td>
<td>(0.86-7.30)</td>
<td>(19.19-76.36)</td>
<td>(3.90-15.38)</td>
</tr>
<tr>
<td>30-39</td>
<td>81</td>
<td>30.24</td>
<td>6.11</td>
</tr>
<tr>
<td></td>
<td>(1.47-12.97)</td>
<td>(16.78-90.68)</td>
<td>(3.60-20.49)</td>
</tr>
<tr>
<td>40-49</td>
<td>86</td>
<td>20.22†</td>
<td>14.29</td>
</tr>
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<td></td>
<td>(1.60-6.97)</td>
<td>(14.53-36.07)</td>
<td>(5.34-22.58)</td>
</tr>
<tr>
<td>50-59</td>
<td>42</td>
<td>16.03‡‡</td>
<td>9.45</td>
</tr>
<tr>
<td></td>
<td>(1.45-7.14)</td>
<td>(10.62-21.86)</td>
<td>(4.58-16.81)</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>105</td>
<td>24.28</td>
<td>13.03</td>
</tr>
<tr>
<td></td>
<td>(0.79-5.25)</td>
<td>(14.38-24.28)</td>
<td>(5.59-24.77)</td>
</tr>
<tr>
<td>Overweight</td>
<td>145</td>
<td>21.87</td>
<td>7.64</td>
</tr>
<tr>
<td></td>
<td>(1.50-7.67)</td>
<td>(14.26-58.53)</td>
<td>(3.93-18.00)</td>
</tr>
<tr>
<td>Obese</td>
<td>81</td>
<td>26.61</td>
<td>6.37†‡‡</td>
</tr>
<tr>
<td></td>
<td>(2.35-11.60)</td>
<td>(17.09-73.70)</td>
<td>(3.60-15.20)</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; IL-18, interleukin 18; N, number of subjects; BMI, body mass index.*significant difference between men and women, † significantly different from 19-29 years and normal BMI, ‡ significantly different from 30-39 years and overweight.
Table 3.4: Median and inter quartile range (IRQ) for IFN-γ, IL-2 and IL-8, for sex, age and BMI categories.

<table>
<thead>
<tr>
<th></th>
<th>IFN-γ</th>
<th></th>
<th>IL-2</th>
<th></th>
<th>IL-8</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Median (pg/ml)</td>
<td>N</td>
<td>Median (pg/ml)</td>
<td>N</td>
<td>Median (pg/ml)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(IQR)</td>
<td></td>
<td>(IQR)</td>
<td></td>
<td>(IQR)</td>
</tr>
<tr>
<td>All subjects</td>
<td>146</td>
<td>10.14 (8.29-12.20)</td>
<td>139</td>
<td>6.43 (5.36-7.83)</td>
<td>176</td>
<td>4.47 (3.80-6.40)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>96</td>
<td>10.14 (8.54-12.17)</td>
<td>80</td>
<td>6.13 (5.36-7.68)</td>
<td>115</td>
<td>4.47 (3.80-6.37)</td>
</tr>
<tr>
<td>Women</td>
<td>50</td>
<td>10.14 (7.94-12.30)</td>
<td>59</td>
<td>6.66 (5.36-9.83)</td>
<td>61</td>
<td>4.47 (3.72-6.45)</td>
</tr>
<tr>
<td>Age category</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td>33</td>
<td>10.00 (8.12-12.67)</td>
<td>34</td>
<td>6.13 (5.36-7.41)</td>
<td>42</td>
<td>4.69 (3.76-6.45)</td>
</tr>
<tr>
<td>40-49</td>
<td>45</td>
<td>10.73 (8.24-12.08)</td>
<td>33</td>
<td>7.31 (6.00-9.83)</td>
<td>51</td>
<td>4.15 (3.67-6.15)</td>
</tr>
<tr>
<td>50-59</td>
<td>28</td>
<td>9.84 (8.65-11.95)</td>
<td>25</td>
<td>7.31 (6.00-10.09)</td>
<td>28</td>
<td>4.77 (3.91-6.99)</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>42</td>
<td>10.73 (7.68-13.22)</td>
<td>47</td>
<td>6.55 (5.36-7.83)</td>
<td>51</td>
<td>4.30 (3.80-6.45)</td>
</tr>
<tr>
<td>Overweight</td>
<td>61</td>
<td>9.84 (8.54-11.50)</td>
<td>63</td>
<td>6.00 (5.36-7.71)</td>
<td>80</td>
<td>4.47 (3.70-6.08)</td>
</tr>
<tr>
<td>Obese</td>
<td>42</td>
<td>11.04 (8.52-13.43)</td>
<td>29</td>
<td>7.07 (6.00-9.83)</td>
<td>45</td>
<td>5.02 (3.91-6.46)</td>
</tr>
</tbody>
</table>

IL-2, interleukin 2; IL-8, interleukin 8; IFN-γ, interferon gamma; N, number of subjects; BMI, body mass index;
*significant difference between men and women, † significantly different from 19-29 years and normal BMI, ‡ significantly different from 30-39 years and overweight
The independent relationship between log10-transformed cortisol, cytokines and measures of sex, age, and adiposity was assessed using linear regression analysis (Table 3.5). An association between sex and IL-18 and adiponectin levels was observed, where being a man was positively associated with higher levels of IL-18, while an inverse association was observed between being a man and adiponectin levels. None of the other log10-transformed cytokines were associated with sex. The association between age and levels of cortisol and cytokines were more pronounced after the log10 transformation. The results from the linear regression showed that levels of CRP, adiponectin, IL-18 and IL-2 increased with increasing age. The decrease in cortisol levels with increasing age, seen in Table 3.3, was confirmed by the results from the linear regression analysis. We also observed an association between adiposity and cytokine levels, where an increase in BMI was associated with higher levels of both IL-18 and CRP. BMI was negatively associated with adiponectin levels, where we observed an inverse relationship between BMI and adiponectin. Body fat percentage was only associated with CRP levels, showing an increase in CRP levels with a higher body fat percentage (Table 3.5). Waist-to-hip ratio was associated with CRP, adiponectin and IL-18 levels, and higher waist-to-hip ratio corresponded with to higher levels of CRP and IL-18, and lower adiponectin levels (Table 3.5).
Table 3.5: Linear regression analysis of log transformed cytokines and measures of age, sex and morphology.

<table>
<thead>
<tr>
<th></th>
<th>CRP</th>
<th>Cortisol</th>
<th>Adiponectin</th>
<th>IL-18</th>
<th>IFN-gamma</th>
<th>IL-2</th>
<th>IL-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (yes vs no)</td>
<td>-0.094</td>
<td>0.030</td>
<td>-0.305</td>
<td>13.342</td>
<td>-0.023</td>
<td>-0.050</td>
<td>-0.026</td>
</tr>
<tr>
<td>Age</td>
<td>0.008</td>
<td>-0.009</td>
<td>0.005</td>
<td>0.443</td>
<td>0.001</td>
<td>0.004</td>
<td>0.001</td>
</tr>
<tr>
<td>Body Fat %</td>
<td>0.026</td>
<td>-0.002</td>
<td>0.003</td>
<td>0.035</td>
<td>-0.001</td>
<td>0.003</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>0.041</td>
<td>0.003</td>
<td>-0.019</td>
<td>1.151</td>
<td>0.001</td>
<td>0.004</td>
<td>0.002</td>
</tr>
<tr>
<td>WHR</td>
<td>1.396</td>
<td>-0.239</td>
<td>-1.378</td>
<td>89.807</td>
<td>-0.339</td>
<td>-0.329</td>
<td>-0.355</td>
</tr>
</tbody>
</table>

Results are expressed as slope and (standard error). CRP, C-reactive protein; IL-2, interleukin 2; IL-8, interleukin 8; IL-18, interleukin 18; IFN-γ, interferon gamma; BMI, body mass index. *p<0.05, **p<0.01.
DISCUSSION

This study is the first to describe basal levels of circulating cortisol and cytokines (inflammatory and non-inflammatory) in a large military population. This represents 0.3% of regular CAF members and includes both officers and non-commissioned members from the Air Force, Navy and Army. Results from this analysis showed lower levels of adiponectin and higher levels of IL-18 in men compared to women, but no other sex differences in levels of cortisol or cytokines were observed. In contrast, levels of cytokines were significantly associated with age, where CRP, IL-18, IL-2 and adiponectin all increased with increasing age. Finally, a number of key differences in changes in cortisol and cytokines as a function of age and adiposity were observed between CAF members and the general population. This assessment of basal cortisol and cytokine levels in a military population, and the association between age, sex and adiposity and these levels, will serve as a foundation for future research into how levels of cortisol and cytokines (inflammatory and non-inflammatory) can affect the health and performance of CAF members and other armed forces worldwide.

Association between sex and cortisol and cytokine levels

Levels of cortisol and cytokines have been shown to differ between men and women in the general population, with higher adiponectin and CRP levels observed in women compared to men (Cartier et al., 2009; Cnop et al., 2003), and higher cortisol production in men compared to women (Purnell et al., 2004). Here, in CAF members, we found no sex differences in detection rate of any of the cytokines measured between men and women (Table 3.2). However, adiponectin levels were lower and IL-18 levels higher in men compared to women (Table 3.3), and these trends were confirmed by the results from the linear regression analysis (Table 3.5). There was no association between sex and levels of cortisol or any of the other cytokines measured. Few studies have
investigated the association between sex and detection rate of 24 unique cytokines (inflammatory and non-inflammatory) all in one study, but Marquez-Vidal et al. (2011), assessed detection rate and levels of CRP, IL-6, IL-1β and TNF-α in the Swiss population. This study reported a higher detection rate for IL-1β in women compared to men, and a higher IL-6 detection rate in men compared to women (Marques-Vidal et al., 2011). Contrary to their findings, no difference in detection rate of either IL-1β or IL-6 was observed between men and women in our military population. Lower adiponectin levels in men compared to women seen in this study are in line with previous reports from the general population (Cnop et al., 2003; Isobe et al., 2005; Obata et al., 2013). Reasons for this difference in adiponectin levels between men and women have not been fully established, but Cnop et al. (2003), suggested that both the number of adipocytes and their size are different in men compared to women, which could be potential factors determining adiponectin production. Higher IL-18 levels observed in men compared to women, have also been seen in studies from the general population (Moriwaki et al., 2003; Yamagami et al., 2005). There has also been several reports of differences in levels of cortisol and CRP between men and women (Abdullah et al., 2007; Khera et al., 2009; Larsson et al., 2009; Purnell et al., 2004), and an effect of male and female sex hormones on production of IL-2 and IFN-γ (Bouman et al., 2004; Giron-Gonzalez et al., 2000), but neither cortisol, CRP, IL-2 or IFN-γ levels were different between men and women in this military cohort. The origin for the difference in levels of cytokines between men and women is largely unknown. Differences in adiposity have been suggested as one way to explain the difference (Cartier et al., 2009), while also sex hormones, like estrogen and testosterone, have been shown to promote and suppress cytokine production (D'Agostino et al., 1999). Although adiposity level was significantly different between men and women in this population (Table 3.1), the overall effect of adiposity on the levels of several of the cytokines
measured was small, with no association between adiposity and cortisol, IL-2, IL-8 and IFN-γ (Table 3.5). We could speculate that the small effect of adiposity on cortisol and cytokine levels could be part the reason for an absence of a difference in these cytokines between men and women in the CAF.

**Effect of age on levels of cortisol and cytokines**

An increase in cytokine levels are observed in humans as they age (Ferrucci et al., 2005; Marques-Vidal et al., 2011; Van Cauter et al., 1996). Current measurements of inflammatory and non-inflammatory cytokines in the CAF confirm this finding. We observed that people in the oldest age group (50-59 years) had a higher occurrence of detectable values of IFN-γ, TNF-α, IL-2, IL-5, IL-8, IL-9, IL-17a, IL-21 and IL-31 compared to people in the youngest age group (19-29 years) (Table 3.2). CAF members in the 40-49 year age group had a higher occurrence of detectable values of IFN-γ, TNF-α and IL-8 compared to people in the youngest age group (19-29). The effect of age on cytokine levels were further supported by the results from the linear regression analysis (Table 3.5), showing a significant association between age and CRP, adiponectin, IL-18 and IL-2. This trend is coherent with previous reports from the general population, which reported an increase in levels of inflammatory cytokines with increasing age (Cnop et al., 2003; Ferrucci et al., 2005; Hung et al., 2005; Marques-Vidal et al., 2011; Obata et al., 2013; Sakata-Kaneko et al., 2000). The reason for the increase in inflammatory cytokines with increasing age is not well understood, but an “oxidative stress hypothesis” has been proposed (Yu & Yang, 1996). With increasing age, according to this hypothesis, there is an ongoing up regulation in the production of reactive oxygen species, as well as reactive nitrogen and reactive lipid species from metabolism. This, coupled with a decrease in the production of anti-oxidants, induces an inflammatory milieu and an up regulation in the production of inflammatory cytokines (Yu & Yang, 1996). Whereas
level of unique inflammatory and non-inflammatory cytokine levels followed the same trend as seen in the general population, cortisol levels exhibited an opposite trend, and we observed a decrease in cortisol levels with increasing age. The median cortisol levels were lower in the two oldest age groups compared to the youngest (Table 3.3), and the results from the linear regression analysis showed a significant decrease in cortisol levels with increase in age (Table 3.5). These observations are contradicting results seen in the general population, where studies have shown that cortisol levels tend to increase with increasing age (Purnell et al., 2004; Van Cauter et al., 1996). Although not confirmed, some researchers have related this increase to the “wear-and-tear hypothesis”, suggesting the increase in cortisol with increasing age is caused by a life-long repeated stress exposures and fluctuations in cortisol levels (Van Cauter et al., 1996). More research is need to explain this decrease in cortisol levels with increase in age, but although controversial, some studies have reported a diminished cortisol production in people suffering from post-traumatic stress disorder (Boscarino, 1996; Horn et al., 2014; Yehuda et al., 1991). On the contrary, the decrease in cortisol levels could also be attributed to lower stress exposure with increasing age, or better coping mechanisms for stress exposure, offsetting the increase in cortisol levels. Investigating the cause of the decrease in cortisol levels with increasing age is an interesting topic for future studies.

Effects of adiposity on levels of cortisol and cytokines

It is generally understood that obesity is an underlying state of low grade chronic inflammation (D'Agostino et al., 1999; Mortensen et al., 2009; Nijhuis et al., 2009; Panagiotakos et al., 2005), which is in turn linked with an upregulation in the production of inflammatory cytokines. However, in our study results only partially support these findings. Detection rates were not affected by adiposity, with no difference in detection rates between BMI categories for any of
the inflammatory and non-inflammatory cytokines measured (Table 3.2). However, the levels of some specific cytokines covaried with changes in the level of adiposity. We report that adiponectin levels decreased with increasing BMI categories (Table 3.4), and the results from the linear regression analysis showed a significant negative association between adiponectin levels and BMI and waist-to-hip ratio. Levels of CRP, IL-18 and IL-2 were higher in the obese BMI category compared to normal (Table 3.3 and 3.4). The association between BMI and CRP and IL-18 remained significant after the linear regression analysis, but the association between BMI and IL-2 were no longer significant. Total body fat percentage was only significantly associated with CRP levels, while waist to hip ratio, a good descriptor of central obesity, was associated with both CRP, adiponectin and IL-18 (Table 3.5). Levels of cortisol, IFN-γ, and IL-8 were not different between BMI categories. The negative association between adiposity and adiponectin levels seen in this military population are similar to what has been found in the general population (Cnop et al., 2003; Isobe et al., 2005; Obata et al., 2013). The increase in IL-18 and CRP with higher levels of adiposity are also in line with trends seen in the general population (Hung et al., 2005; Kim et al., 2006; Marques-Vidal et al., 2011; Obata et al., 2013). Although the association between obesity and inflammation has been well established (D’Agostino et al., 1999; Mortensen et al., 2009; Nijhuis et al., 2009; Panagiotakos et al., 2005), the origin of the increase in production of cytokines with increased adiposity remains to be confirmed. Trayhurn and Wood (2004) suggested that the increased production of inflammatory cytokines could originate from organs other than adipose tissue, like the liver, either directly or through secondary messengers produced by adipose tissue, or through production of inflammatory cytokines by the white adipose tissue itself, or a combination of the three. A conformation of the pathway dictating the increase in cytokine production with increases in obesity is still missing, but potential causes are hypoxia in adipose
tissue, caused by expansion of adipocytes, inducing a state of inflammation (Trayhurn, 2013; Trayhurn & Wood, 2004), or an imbalance in the leptin (pro-inflammatory) and adiponectin (anti-inflammatory) ratio (de Heredia et al., 2012). The larger effect of central adiposity, rather than general adiposity, on the production of inflammatory cytokines has also been reported previously (Hermsdorff et al., 2011; Lapice et al., 2009). Visceral adipose tissue has direct access to portal vein, meaning inflammatory markers produced in this tissue will be carried to central organs and the liver where they can have an immediate effect on the production of other inflammatory mediators, like CRP (Shoelson et al., 2007). Analyzing absolute levels of cortisol and cytokines may provide substantial information about the physiological state of a person or population, but as described in the following section, care needs to be taken before drawing finite conclusion based on a single blood sample analyzed for levels of cytokines.

**Study limitation and cautions when measuring cytokine levels**

The data presented in this study provides some unique and valuable information about basal levels of cortisol, inflammatory and non-inflammatory cytokines in a representative sample of the CAF. Although we were able to determine the association between sex, age and adiposity and cortisol and cytokine levels, previous research have shown that factors like fasting status (Esposito et al., 2002; Faris et al., 2012), ethnicity (Stowe et al., 2010), medication (Endres et al., 1996; Hannestad et al., 2011), and psychosocial stress (Coussons-Read et al., 2007; Maes et al., 1998) may also have an effect on cytokine levels. Due to this assessment being a secondary analysis of previously collected data, we were unable to collect this information and use it in the analysis.

Investigating the detection rates of numerous cytokines can provide important information to determine how these factors are related to morphology and physiological status of a population. However, it is important to keep in mind that immunity and inflammation are complex processes,
with immune and non-immune cells producing cytokines under different and unique stimuli. Cytokines are pleiotropic (Kishimoto, 2006), and can be antagonistic, or synergistic when in the presence of other cytokines. They also may act in an autocrine and paracrine fashion, and their effects are mediated by their concentration (Tracey & Cerami, 1994). The overall effect may be pro- or anti-inflammatory depending on the microenvironment that they are in (Scheller et al., 2011).

With previous research showing a potential relationship between stress and inflammation and chronic diseases, such as obesity, type 2 diabetes and cardiovascular disease (Black, 2006; Black & Garbutt, 2002; Wellen & Hotamisligil, 2005), it is tempting to draw diagnostic conclusions based on a single blood sample. Arbitrary thresholds for the levels of certain inflammatory markers (e.g. CRP) and disease risk have been developed (Ridker, 2003), but comparing values between labs have been shown to be problematic, due to differences in analysis techniques (Tarrant, 2010; Zhou et al., 2010). Comparing absolute values between studies are difficult, due to the fact that type of sample (saliva, plasma, serum, etc.), the laboratory performing the analysis, the type of analysis kit used, difference in kit lot number and the standard curves developed, all can affect the measured values of cytokines (Tarrant, 2010; Zhou et al., 2010). Determining serum levels of cytokines are further complicated by complex interactions between the cytokines and other factors in the blood stream. As an example; TNF-α can be measured based on its bioactivity, or immunoreactivity (ELISA), but due to interactions with TNF binding proteins (TNF-BPs), the properties of the cytokine can be altered (Tracey & Cerami, 1994). It’s been shown that some TNF-BPs have the ability to neutralize the bioactivity of TNF-α, while still being detected by ELISA (Tracey & Cerami, 1994). It has also been shown that TNF-α - TNF-BP complexes can have a higher bioactivity compared to the single TNF-α molecule (Tracey &
Cerami, 1994). The origin of TNF-α release can also affect its bioactivity. TNF-α produced in the brain can cause severe anorexia without protein loss, while peripherally produced TNF-α causes less severe anorexia, but induced a large protein catabolism (Tracey et al., 1990). This, together with the fact that half-life of different cytokines can vary from minutes and hours (TNF-α 6-20 min (Tracey et al., 1986), CRP 19 hours (Vigushin et al., 1993)), to days and weeks (IL-8) (Remick, 2005), further complicates using a single blood sample analyzed for levels of cytokines for performing a diagnostic procedure. Still, large amounts of information can be deducted from descriptive studies of levels of cortisol and cytokines in different populations, but care must be taken before drawing conclusions when no other measurements are taken or variables controlled for. Reporting detection rates and the LOD can provide the information needed for future studies to make comparisons with the findings reported in a specific study.

CONCLUSION

This descriptive study was conducted to provide the foundation for future studies on levels of cortisol and cytokines in the CAF. Our results showed a small effect of sex on levels of cortisol and cytokines, and apart from a decrease in cortisol levels with increase in age, there was a general trend towards an increase in cytokine levels with increasing age. A higher BMI was associated with increased cytokine levels, but lower adiponectin levels. The results from the linear regression analysis suggest a larger effect of central, rather than general adiposity on cytokine levels. These results will serve as a foundation for future research into how levels of cortisol and cytokines can affect the health and performance of CAF members and may be used as comparison for the other armed forces across the world.
Acknowledgments The authors would like to acknowledge the members of the Canadian Armed Forces Directorate of Fitness - Human Performance Research and Development team who collected the original data for this study, as well as for their support in the development of the current manuscript.
CHAPTER 4
Stress and Inflammatory Cytokines in the Canadian Armed Forces:
Associations Between Basal Levels and Military Physical Performance

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Submitted to Applied Physiology, Nutrition and Metabolism

Author contributions: All authors contributed to the conception and design of the Experiments. HCT, LF and VT performed the analysis and interpretation of data. HCT wrote the manuscript. All authors contributed to the critical revising of the manuscript, and approved the final version of the manuscript.
ABSTRACT

The purpose of this study was to determine if an association exists between basal levels of stress and inflammatory cytokines and military physical performance among CAF members. Research has shown that such an association seem to exist in the general population, but no previous study has investigated the association between stress and inflammatory cytokines and physical performance in a military population, susceptible to high stress exposure. Blood samples were collected from 219 CAF members (86 women and 133 men), and analyzed for levels of cortisol, C-reactive protein (CRP), adiponectin, IL-18, IL-2, and IFN-γ. Participants’ grip strength, aerobic capacity, and CMTFE performance was also assessed, and composite score for total performance was computed for each individual, based on rank scores from the six CMTFE tasks. Multiple linear regression analyses, controlling for age, sex and body fat, were used to determine the association between stress and inflammatory cytokines and military physical performance. Results showed that an increase in CRP values was associated with a decrease in total performance (p=0.01), picking and digging time (p=0.04), aerobic capacity (p=0.05) and plank time (p<0.01). The results from this study showed that high basal levels of CRP were associated with a decrease in military physical performance, suggesting high levels of inflammatory cytokines could be another factor that can potentially hamper military physical performance.
INTRODUCTION

The following chapter of this thesis will combine aspects studied in Chapter 2 and 3. In Chapter 2, the effect of morphology, and physiological capacity on predicting performance outcome on the CMTFE was evaluated as well as which characteristics of morphology and physiological capacity separate top and bottom performers on the CMTFE. In Chapter 3, the presence and detection rate of various stress and inflammatory cytokines in CAF members was established. In this chapter, we investigated the potential association between basal levels of stress and inflammatory cytokines and the performance outcome on the CMTFE.

Over the last decades, research has documented evidence of an association between increased levels of stress and pro-inflammatory cytokines and a decline in physical performance. For example, elevated levels of IL-1β, TNF-α, IL-6 and C-reactive protein (CRP) have been linked to functional decline and a decrease in general physical performance (Cesari et al., 2004; Oliveira et al., 2008; Schaap et al., 2009; Schaap et al., 2006; Taaffe et al., 2000). An increase in levels of circulating serum IL-6 have been associated with a decline in handgrip strength and muscle power, independent of age, sex, and BMI (Barbieri et al., 2003; Blain et al., 2012). Brinkley et al (2009) found that both CRP and IL-6 levels were negatively associated with grip strength and performance on a Short Physical Performance Battery, and the association was again age independent. Some studies have also suggested that IL-18 may be associated with a decrease in physical performance in the elderly population (Frayling et al., 2007). Although the majority of these studies were conducted in the elderly population, similar relationships have also been observed in a younger population and in athletes. A study by Robson-Ansley et al. (2004), found that an acute administration of a dose of the cytokine rhIL-6 (recombinant human IL-6) led to decrease in aerobic performance and an increased sensation of fatigue in a group of trained runners.
during a treadmill time trial. A study investigating stress level and its effect on physical performance in semi-elite Dutch floorball players, found that high levels of perceived psychological stress was associated with an increased sensation of fatigue and a decline in aerobic capacity (van der Does et al., 2015).

Members of the armed forces are at an increased risk of stress exposure (CareerCast.com, 2015, 2016, 2017). The everyday life of a soldier often entails strive for promotion, concern about potential relocation, deployment in combat operations, as well as physical and environmental stress stemming from occupational tasks (Pflanz & Sonnek, 2002). Studies by Pflanz and Sonnek (2002), and Pflanz et al. (2006), showed that as many as 26% of U.S. Air Force service men and women reported to suffer from work related stress, and that 15% reported that work stress was causing them significant emotional stress.

In Chapter 3 of this thesis, levels of stress and inflammatory cytokines in CAF members were investigated, and the presence of both stress and inflammatory cytokines among CAF members was confirmed, as well as trends towards an increase in inflammatory cytokines with increasing age. Based on these findings and the current literature, CAF members suffering from high stress levels could be fighting a hidden enemy, in the form of high levels of stress and pro-inflammatory cytokines, which could potentially impede and diminish physical performance capacity. Therefore, the purpose of this study was to determine if an association exist between basal levels of cortisol and inflammatory cytokines, and military physical performance in a representative sample of the CAF. It was hypothesised, based on previous research and results seen in the general population, that high levels of stress and inflammatory cytokines would be negatively associated with military physical performance.
METHODOLOGY

Participants

The following analysis was performed on previously recorded data, collected during the development of the new physical employment standard for the CAF. In order to assess the association between levels of cortisol, cytokines and physical performance in a military population, blood draws were collected from a representative sample of CAF members (n=219). All participants were pre-screened prior to taking part in the study, where resting blood pressure and heart rate was recorded, and participants completed the PAR-Q (American College of Sports Medicine, 1997) and the Risk Stratification Questionnaire (ACSM, 2010). Participants having any medical conditions or restrictions limiting their ability to perform physical tasks were excluded from the study. In addition, participants identified as “high risk” according to the Risk Stratification Questionnaire, and pregnant women were excluded from participating in the study. The study was conducted following the guidelines of the Helsinki Declaration and received ethics approval from Defence Research and Development Canada’s Human Research Ethics Board and The University of Ottawa Research Ethics Board.

Morphology Data

Height was measured using a Seca 213 Portable stadiometer (Seca Industries, Hanover, Maryland, USA). Hip and waist circumferences were measured using a standard measuring tape. Body mass was measured using a standardized and calibrated professional grade digital weighing scale (Health-o-meter, Alsip, Illinois, USA). Body composition was estimated through bioelectrical impedance analysis, using the InBody 520 (BioSpace Technologies, Los Angeles, USA), previously validated against a gold standard measure (Anderson et al., 2012). The body
composition analysis gave an estimate of lean body mass (LBM) and fat mass, and based on these estimates body fat percentage (BF%) was calculated. BMI was calculated by dividing body mass by height squared (body mass (kg)/height²( m)).

**Blood Sample Collection**

Participants were instructed to avoid consuming caffeinated beverages during the 2 hours prior to testing, and not to exercise or consume alcoholic beverages less than 6 hours prior to testing. Blood draws were performed in the morning by a registered nurse. All blood samples were collected before any exercise testing was performed. Blood samples were collected in 6 mL EDTA tubes and immediately placed on ice until centrifugation at 2428 x g. Plasma was separated in 2 mL aliquots and stored in a -80°C freezer until analysis.

**Blood Sample Analysis**

All blood samples were analyzed for levels of Cortisol, Adiponectin, CRR, INF-γ, TNF-α, IL-1β, IL-2, IL-6, IL-8, and IL-18. These markers were selected based on a high detection rate in the current population, and previous studies showing an association between these markers and physical performance in the general population (Brinkley *et al.*, 2009, Cesari *et al.*, 2004, Taaffe *et al.*, 2000). Cortisol was measured using a Parameter Cortisol Assay (R&D Systems, Minneapolis, MN, USA), while CRP and adiponectin were measured using a Human C-Reactive Protein ELISA or the Human Adiponectin Platinum Sandwich ELISA kit (Affymetrix eBiosciences, San Diego CA USA). All assays were performed as instructed by the manufacturer. Each plate had a standard curve in duplicate and all plates were read at 450 nm in the Elisa plate reader FilterMax F5 Multimode microplate reader (Molecular Devices, Sunnyvale CA). The SoftMax Pro 6.2.2 (Molecular Devices, Sunnyvale CA) software was used to determine the levels
of cytokine in the unknown, using the standard curve found on each plate. The levels of IFN-γ, TNF-α, IL-1β, IL-2, IL-6, IL-8, IL-18 were determined using the Procarta Plex Multiplex Immunoassay (Affymetrix eBiosciences, San Diego CA USA). Samples were prepared and used as instructed by the manufacturer of the kits. Standard curves were ran on each plate together with unknowns. The plates were read on the luminex MAGPIX instrument (Affymetrix eBiosciences, San Diego CA USA) and the cytokine levels for each unknown were extrapolated from the standard curves using the Procarta Plex analyst software with either a four or a five parameter logistic curve of the standards. Same procedures for determining cortisol and cytokine levels in plasma have been used in previous publications (Cowan et al., 2012; Imbeault et al., 2012).

**Physiological Capacity and Military Physical Performance Measures**

Military physical performance was measured on six military specific tasks. These tasks were sandbag fortification (lifting and moving sandbags), escape to cover (obstacle course resembling an escape to cover under fire task), picking and digging (separate picking and digging simulators), pickets and wire carry (resembling carrying equipment for a fence erection), stretcher carry and vehicle extrication (resembling extracting a wounded service member from a disabled car). A detailed description of each task can be found in Appendix III. Performance on sandbag fortification, escape to cover, picking and digging and pickets and wire carry was measured as time to complete the task. For stretcher carry and vehicle extrication performance was measured as maximal load lifted and carried. Total performance was calculated by ranking each individual score, before giving it a percentile score (best performance = 100, lowest performance = 1) based on rank order. Thereafter, the sum of the rank scores for each participant were calculated and used as an indicator for total performance. To estimate aerobic capacity, a multi stage 20-meter shuttle run test was used. This test has previously been validated and found to accurately represent a
measure of aerobic capacity (Paliczka et al., 1987; Ramsbottom et al., 1988), and the procedures for the tests are described in the CF EXPRES Operations Manual (Director of Fitness - Canadian Armed Forces, 2010). The level attained and the number of stages completed on that level was recorded, and converted into total distance covered (m). Grip strength was used as a measure of upper body strength. Grip strength has been found to be a valid predictor of total upper body strength in young adults (Wind et al., 2010), and was measured using a Smedly Analog hand dynamometer (Smedly TTM, Tokyo, Japan). The procedures for grip strength measurement are found in the CF EXPRES Operations Manual (Director of Fitness - Canadian Armed Forces, 2010). Following standard protocol, grip strength was measured twice for both left and right hand, and the highest recorded measurement for each hand was summed and used as the measure of grip strength. Core strength was estimated by measuring front plank time (seconds). Having forearms and toes place on the ground, participants were asked to lift their hips of the ground and maintain a neutral spine. The test was terminated when the participant failed to maintain a neutral spine. Detailed description of procedures are found elsewhere (Parkhouse & Ball, 2011).

**Statistical Analysis**

Due to non-normal distribution, all cytokine values were log10 transformed before any statistical analyses were performed. As men and women are held to the same physical employment standard and perform the same physical performance test, male and female data was combined into one cohort. Multiple linear regression analysis was used to determine the association between cytokine levels on military physical performance. With age, sex and BF% being covariates of both cytokine levels and performance (Åstrand, 1956; Forouhi et al., 2001; Khera et al., 2009; Roubenoff et al., 1998; Saito et al., 2003; Samson et al., 2000), the regression model was adjusted for these factors. Results from the linear regression analysis were presented as slope and standard
error. All statistical analyses were performed using SPSS 23.0 (IBM SPSS Statistics for Windows, Version 23.0, IBM Corp, Armonk, NY).
RESULTS

Performance data and blood samples from a total of 219 CAF members were made available for this analysis. A detailed description of participant characteristics are found in Table 4.1. The results from the blood sample analysis showed a low detection rate of IL-1β, IL-6 and TNF-α. Only 4%, 4% and 2% of men, and 5%, 6% and 3% of women had measurable values of IL-6, TNF-α and IL-1β respectively. Due to the low rate of measurable values, these inflammatory cytokines were excluded from further analysis.

Table 4.1: Participants’ descriptive statistics. Data presented as mean±sd.

<table>
<thead>
<tr>
<th></th>
<th>Total (n=219)</th>
<th>Women (n=86)</th>
<th>Men (n=133)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.5±10.1</td>
<td>37.4±8.7</td>
<td>35.9±10.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.6±8.9</td>
<td>162.6±5.8</td>
<td>175.8±6.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.1±15.8</td>
<td>69.0±11.8</td>
<td>85.7±14.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1±4.4</td>
<td>26.1±4.0</td>
<td>27.7±4.1</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>24.6±8.9</td>
<td>29.4±8.2</td>
<td>21.6±8.0</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>90.6±11.8</td>
<td>84.8±10.0</td>
<td>94.2±11.4</td>
</tr>
<tr>
<td>Cortisol (ng/ml)</td>
<td>38.87±34.55</td>
<td>38.85±38.91</td>
<td>38.89±31.60</td>
</tr>
<tr>
<td>CRP (µg/ml)</td>
<td>7.10±9.10</td>
<td>7.07±9.10</td>
<td>7.11±9.14</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>12.67±9.94</td>
<td>17.90±10.12</td>
<td>9.24±8.20</td>
</tr>
<tr>
<td>IL-18 (pg/ml)</td>
<td>85.64±37.30</td>
<td>79.58±34.18</td>
<td>89.60±38.82</td>
</tr>
<tr>
<td>IL-2 (pg/ml)</td>
<td>6.50±4.20</td>
<td>7.42±6.33</td>
<td>5.89±1.48</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>6.43±23.57</td>
<td>9.25±37.29</td>
<td>4.59±2.70</td>
</tr>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>9.42±6.96</td>
<td>10.47±10.65</td>
<td>8.73±2.98</td>
</tr>
</tbody>
</table>

Results from the multiple linear regression analyses are found in Table 4.2. Higher CRP levels were associated with a decrease in military physical performance. A negative association
was observed between CRP levels and; aerobic capacity (p=0.05), plank time (p<0.01), picking and digging performance (p=0.04), and total performance on the CMTFE (p=0.01). A negative association between adiponectin levels and grip strength (p<0.01) was observed, however, higher adiponectin levels were also associated with an increase in plank time (p=0.01). Interleukin 2 was found to be positively associated with grip strength (p=0.03), where higher IL-2 levels indicated better grip strength. There were no observed association between cortisol, IL-18, IL-8, IFN-γ or any of the physical performance measures recorded.
Table 4.2: Linear regression results, adjusted for age, sex and body fat percentage, showing the effect of cortisol and inflammatory markers on military physical performance.

<table>
<thead>
<tr>
<th>Total Performance Score</th>
<th>E2C (sec)</th>
<th>P&amp;D (sec)</th>
<th>P&amp;W (sec)</th>
<th>SC (kg)</th>
<th>VE (kg)</th>
<th>SBF (sec)</th>
<th>GS (kg)</th>
<th>AC (m)</th>
<th>PT (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortisol</strong></td>
<td>6.23</td>
<td>0.13</td>
<td>0.70</td>
<td>7.08</td>
<td>-4.23</td>
<td>-1.05</td>
<td>0.50</td>
<td>-1.96</td>
<td>-41.63</td>
</tr>
<tr>
<td></td>
<td>(15.25)</td>
<td>(1.44)</td>
<td>(36.95)</td>
<td>(8.60)</td>
<td>(6.21)</td>
<td>(3.70)</td>
<td>(9.01)</td>
<td>(2.97)</td>
<td>(55.85)</td>
</tr>
<tr>
<td><strong>CRP</strong></td>
<td>-23.0</td>
<td>0.42</td>
<td>45.75</td>
<td>3.72</td>
<td>-6.84</td>
<td>-2.56</td>
<td>5.75</td>
<td>-2.74</td>
<td>-71.81</td>
</tr>
<tr>
<td></td>
<td>(8.88)*</td>
<td>(0.88)</td>
<td>(22.05)*</td>
<td>(5.37)</td>
<td>(3.75)</td>
<td>(2.92)</td>
<td>(5.54)</td>
<td>(1.92)</td>
<td>(36.27)*</td>
</tr>
<tr>
<td><strong>Adiponectin</strong></td>
<td>-0.98</td>
<td>-0.18</td>
<td>-25.50</td>
<td>-7.11</td>
<td>-3.91</td>
<td>-1.76</td>
<td>-8.19</td>
<td>-8.27</td>
<td>-5.35</td>
</tr>
<tr>
<td></td>
<td>(15.78)</td>
<td>(1.39)</td>
<td>(37.90)</td>
<td>(8.67)</td>
<td>(6.14)</td>
<td>(3.71)</td>
<td>(9.33)</td>
<td>(3.00)*</td>
<td>(56.60)</td>
</tr>
<tr>
<td><strong>IL-18</strong></td>
<td>0.06</td>
<td>-0.02</td>
<td>0.22</td>
<td>-0.07</td>
<td>0.01</td>
<td>0.01</td>
<td>-0.12</td>
<td>0.03</td>
<td>-1.09</td>
</tr>
<tr>
<td></td>
<td>(0.15)</td>
<td>(0.01)</td>
<td>(0.37)</td>
<td>(0.09)</td>
<td>(0.06)</td>
<td>(0.04)</td>
<td>(0.09)</td>
<td>(0.03)</td>
<td>(0.58)</td>
</tr>
<tr>
<td><strong>IL-2</strong></td>
<td>16.33</td>
<td>1.74</td>
<td>-140.96</td>
<td>50.18</td>
<td>-20.27</td>
<td>0.56</td>
<td>12.21</td>
<td>20.83</td>
<td>225.88</td>
</tr>
<tr>
<td></td>
<td>(54.70)</td>
<td>(5.70)</td>
<td>(127.54)</td>
<td>(27.80)</td>
<td>(17.08)</td>
<td>(12.84)</td>
<td>(28.80)</td>
<td>(9.48)*</td>
<td>(184.80)</td>
</tr>
<tr>
<td><strong>IL-8</strong></td>
<td>2.40</td>
<td>-0.48</td>
<td>-75.95</td>
<td>24.07</td>
<td>-5.30</td>
<td>3.45</td>
<td>-9.00</td>
<td>-2.89</td>
<td>149.09</td>
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<tr>
<td></td>
<td>(30.87)</td>
<td>(3.05)</td>
<td>(83.14)</td>
<td>(17.30)</td>
<td>(10.89)</td>
<td>(7.52)</td>
<td>(17.10)</td>
<td>(6.42)</td>
<td>(109.64)</td>
</tr>
<tr>
<td><strong>IFN-γ</strong></td>
<td>7.93</td>
<td>2.05</td>
<td>-112.21</td>
<td>24.71</td>
<td>-16.71</td>
<td>-3.29</td>
<td>-6.33</td>
<td>5.55</td>
<td>57.95</td>
</tr>
<tr>
<td></td>
<td>(53.48)</td>
<td>(5.14)</td>
<td>(134.06)</td>
<td>(28.72)</td>
<td>(16.20)</td>
<td>(12.47)</td>
<td>(27.01)</td>
<td>(11.65)</td>
<td>(240.33)</td>
</tr>
</tbody>
</table>

Results are presented as slope and std error. *p>0.05, ** p>0.01. TPS, total performance score; E2C, escape to cover; P&D, picking and digging; SC, stretcher carry; VE, vehicle extrication; SBF, sandbag fortification; GS, grip strength; AC, aerobic capacity; PT, plank time; CRP, C-reactive protein; IL-18, interleukin 18; IL-2, interleukin 2; IL-8, interleukin 8; IFN-γ, interferon gamma.
DISCUSSION

Members of the Canadian Armed Forces rely on high physiological capacity to perform their occupational duties; however, the high stress exposure associated with being a member of the armed forces could potentially have a negative effect on military physical performance. This study is the first to investigate if an association exists between basal levels of stress and pro-inflammatory cytokines and military physical performance. Results showed a negative association between CRP levels and military physical performance, where high levels of CRP were associated with a decline in total performance, picking and digging time, plank time and aerobic capacity. Higher adiponectin levels were associated with lower grip strength, but improved performance on the front plank task. Based on these results, a link between levels of inflammatory cytokines and military physical performance seems evident, and this could potentially mean that members of the armed forces suffering from high levels of stress and inflammatory cytokines might be at a disadvantage when it comes to performing military tasks and physical performance tests.

C-Reactive Protein and Military Physical Performance

C-reactive protein is an acute phase protein, produced by the liver during the initial stage of an inflammatory reaction, and is a potent marker for the presence of an inflammatory reaction (Baumann & Gauldie, 1994; Macintyre et al., 1982). Previous studies in the elderly population have suggested a negative association between CRP levels and physical performance, and similar results were observed in this study. The results from the multiple linear regression analyses showed a negative association between CRP levels and aerobic capacity, plank time, picking and digging performance and total performance (Table 4.2). This is the first time an association has been shown between inflammatory cytokines and physical performance in a military population, but studies from the general population have found similar results. Taaffe et al. (2000), found that
higher CRP levels were associated with poorer grip strength, and they also observed a trend towards lower CRP levels in people with faster walking speeds. A cross-sectional study conducted by Norman et al. (2014), showed an independent inverse relationship between CRP levels and handgrip strength. The inverse relationship between CRP and grip strength did not reach significance in our cohort (p=0.156), but CRP levels were found to be inversely related to performance on the picking and digging task, plank time and aerobic capacity. The results from this study seems to clearly indicate a negative association between CRP levels, independent of age, sex and adiposity, and military physical performance.

**Adiponectin and Military Physical Performance**

The adipokine adiponectin plays a crucial role in glucose regulation and lipid metabolism (Diez & Iglesias, 2003), and is considered to have anti-inflammatory effects (Ouchi & Walsh, 2007; Villarreal-Molina & Antuna-Puente, 2012; Wolf et al., 2004). High plasma concentrations of adiponectin have also been associated with a lower BMI and lower waist circumference (Matsubara et al., 2002; Yang et al., 2001). Based on the relationship between adiponectin, BMI and waist circumference, we expected, if any, a positive effect of adiponectin on physical performance in this military population. Instead, an inverse relationship between adiponectin levels and grip strength was observed (Table 4.2), suggesting high adiponectin levels to be negatively associated with physical performance. However, previous research have found similar results in studies in an elderly population, where a significant inverse relationship between physical performance, muscle strength and adiponectin levels have been shown (Bucci et al., 2013; Huang et al., 2015). It is therefore likely that the negative relationship between adiponectin and physical performance could be explained by an inverse relationship between adiponectin levels and body mass, more specifically lean body mass (Esposito et al., 2003; Yang et al., 2001), rather
than a direct negative effect of adiponectin on physical performance. As previously described, adiponectin levels increase with a decrease in BMI and body mass, and this has been associated with a decrease in lean body mass in the elderly population (Bucci et al., 2013; Huang et al., 2015). Unpublished data from our lab showed a negative correlation between adiponectin levels and lean body mass in a military population, which could explain the inverse relationship found between adiponectin levels and grip strength in this study. Participants with higher adiponectin levels also recorded a better plank time, compared to participants with lower adiponectin levels. This could also be explained by an inverse relationship between adiponectin levels and body mass. Previous studies have shown a negative correlation between plank time and body mass, where people with a higher body mass (i.e. lower adiponectin levels) had a lower plank time (Ervin et al., 2014). Based on these results, it seems that rather than having a direct effect on physical performance, changes in adiponectin level could serve a marker of change in body composition, which could again affect physical performance.

The results from this study are not able to determine the specific cause of the association between inflammatory cytokines and military physical performance. If one were to speculate, it is known that mental and physical stress exposure itself can affect physical performance (van der Does et al., 2015), and clear evidence exists of a connection between stress level, immune system activation and release of inflammatory cytokines (Ader et al., 1995; Dantzer & Kelley, 1989; Elenkov & Chrousos, 1999; Glaser & Kiecolt-Glaser, 2005). It is also known that inflammation and pro-inflammatory cytokines play a role in muscle wasting, and high levels of cytokines like TNF-α and IL-1β measured in muscle tissue, have been associated with muscle wasting and a decrease in muscle mass (Anker et al., 1999; Visser et al., 2002). Previous studies have also shown a negative association between plasma levels of CRP and the synthesis rate of myosin heavy chain
in muscle (Toth et al., 2005). It is therefore possible that high levels of pro-inflammatory cytokines in the circulating plasma could be a marker of an ongoing inflammatory reaction in the muscle, rather than high cytokine levels in circulating plasma having a direct negative effect on physical performance. These are only speculations of potential pathways linking levels of inflammatory cytokines and physical performance, and more research is required to clarify the cause and effect of inflammatory cytokines on physical performance. However, based on these results, strategies to decrease inflammation and levels of inflammatory cytokines, could be beneficial to improve military physical performance. Several anti-inflammatory drugs are available (e.g. corticosteroids, Aspirin), which have been found to decrease levels of pro-inflammatory cytokines (Barnes et al., 1993; Morris et al., 2009). Regular physical exercise (Gleeson et al., 2011), and sufficient sleep (Mullington et al., 2010), has also been shown to be beneficial to decrease levels of pro-inflammatory cytokines. This study was not designed to answer such questions, but potential anti-inflammatory strategies and their effect on military physical performance should be elucidated in future studies.

**Limitations**

The models used in this study to determine the association between cortisol and cytokine levels and military physical performance were adjusted for age, sex and adiposity, which are all known to affect cortisol and cytokine levels. Other factors like; fasting status (Esposito et al., 2002; Faris et al., 2012; Klasing, 1988), ethnicity (Stowe et al., 2010) and medication (Endres et al., 1996; Hannestad et al., 2011), can also affect cortisol and cytokine levels, but due to this being a secondary analysis of previously collected data, we were unable to control for these variables. There are also several factors affecting the rate of transcription, translation and breakdown of cytokines (e.g. exercise (Kasapis & Thompson, 2005; Ostrowski et al., 1999; Pedersen, 2000),
acute stress exposure (Maes et al., 1998; Steptoe et al., 2007), as well as their circadian fluctuation (Born et al., 1997; Lange et al., 2010), and differences in half life. CRP’s half life is \( \sim 19 \) hours (Pepys & Hirschfield, 2003), whereas IFN-gamma have a half-life of only a few minutes (Lortat-Jacob et al., 1996). Certain cell types contain cytokines prepackaged in vesicles, which can be released immediately upon stimulation (Sheshachalam et al., 2014). Therefore, cytokine levels measured in blood samples collected in the morning might not be a perfect representation of plasma values later in the day. To follow up on this, a controlled study should be performed comparing physical performance between people with normal and high cytokine levels, as well as collecting blood samples pre and post exercise. Catecholamine levels (adrenaline and nor adrenaline) should also be assessed, since the pro-inflammatory effects of stress are in a large grade related to an upregulation of SNS activity and increased release of catecholamines (Vale, 2005).

**CONCLUSION**

This study is the first to assess the association between basal levels of cortisol, inflammatory cytokines and physical performance in a military population. There was no observed association between cortisol, IL-18, IL-8, IFN-\( \gamma \) and any of the outcomes of the military physical performance test. However, CRP levels were negatively associated with total military physical performance, aerobic capacity, plank time and picking and digging performance. Even though more research is required to confirm these results, the association between CRP levels and military physical performance seems clear. The potential negative effect of pro-inflammatory cytokines on military physical performance could a part of the explanation of why certain CAF members might struggle to meet the physical employment standard. Based on these results, developing strategies
to decrease inflammation in CAF members with high levels of pro-inflammatory cytokines, could help to improve military physical performance.

**Acknowledgments** The authors would like to acknowledge the members of the Canadian Armed Forces Directorate of Fitness - Human Performance Research and Development team who collected the original data for this study, as well as for their support in the development of the current manuscript.
Thermoregulation and Cost of Locomotion During Loaded March: The Effects of Heat Exposure and Previous Experience

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Author contributions: HCT, TR and FH contributed to the conception and design of the Experiments. HCT, BK and EV performed the data collection. HCT analyzed and interpreted the data, and wrote the manuscript. All authors contributed to the critical revising of the manuscript, and approved the final version of the manuscript.
ABSTRACT

The purpose of this study was to investigate the thermal and cardiovascular responses to performing a loaded march in the heat, and its effect on cost of locomotion and military physical performance in participants experienced and inexperienced with loaded march. Twenty healthy male participants were recruited for this study, ten civilians (inexperienced with loaded march) and ten military reservist (experienced with loaded march). All participants were asked to perform a 60 min loaded march while wearing military personal protective equipment, weighing ~35kg, at NORMAL temperature (21°C, 50%RH) and in the HEAT (30°C, 50%RH). Following the loaded march, maximal performance on a military physical performance test (FORCEcombat) was assessed. Results showed that nine out ten Experienced participants were able to complete the loaded march in the HEAT, and only five out of ten Inexperienced participants completed the 60 min loaded march in the HEAT. Performing the loaded march in the HEAT caused both the Inexperienced and Experienced participants to experience a state of uncompensable heat stress, with a mean rate of increase in core temperature of 0.025°C/min and 0.02°C/min, for Inexperienced and Experienced participants respectively. Mean heart rate (143.1±8.9 vs 134.2±11.9 bpm) and RPE (13.0±0.9 vs 10.2±1.4) was higher in the Inexperienced compared to the Experienced group. The Experienced group also had better completion times on the FORCEcombat test compared the Inexperienced group, following both the loaded march at NORMAL temperature (487±47 sec vs 574±106 sec) and in the HEAT (530±49 sec vs 662±133 sec). This study showed that both heat exposure and previous experience had a significant effect on loaded march and FORCEcombat performance.
INTRODUCTION

Performing high intensity exercise in a hot and humid environment has been shown to cause hyperthermia and to severely reduce exercise performance (Galloway & Maughan, 1997b; Marino et al., 2000; Tatterson et al., 2000). Wearing personal protective equipment (PPE) can further lower the intensity level and environmental temperature threshold for the onset of hyperthermia. The protective design and limited water permeability of the PPE, causes a reduction in maximal evaporative capacity (Caldwell et al., 2011; Nunneley, 1986). Exercising in the heat while wearing PPE, creates a state of uncompensable heat stress (Cheuvront et al., 2008; Havenith & Vrijkotte, 1994), where core temperature keeps rising until heat exhaustion occurs (Sawka & Pandolf, 2001). Uncompensable heat stress occurs when the requirement for evaporative heat loss is larger than the maximal capacity for evaporative heat loss from the skin surface (Cheuvront et al., 2010; Périard et al., 2011; Sawka & Pandolf, 2001). Whereas relatively high core temperatures can be tolerated during compensable heat stress (Kenefick & Sawka, 2007; Sawka & Pandolf, 2001), heat exhaustion can occur at much lower core temperatures during exercise in uncompensable heat (Montain et al., 1994; Sawka et al., 1992). Montain et al. (1994), observed that soldier walking on a treadmill (heat production equal to ~625 W) with an ambient temperature of 35°C (50% RH) wearing a full protective clothing ensemble, suffered from heat exhaustion at core temperatures as low as 38.5±0.5°C. Heat exhaustion occurring at lower core temperatures during uncompensable heat is believed to be caused by elevated cardiovascular strain, due to an increase in blood flow to working skeletal muscle, vasodilation of peripheral blood vessels and increased skin blood flow to facilitate heat loss (Cheuvront et al., 2010; Périard et al., 2011; Sawka & Pandolf, 2001).
Occupations requiring employees to perform moderate to high intensity physical tasks, wearing PPE (i.e. fire fighter, football player), are all in danger of exposing the workers to uncompensable heat stress, (Armstrong et al., 2007; Paull & Rosenthal, 1987). Continued exposure to uncompensable heat stress can lead to heat exhaustion and heat illness. Members of The Canadian Army are another group of employees required to perform daily physical tasks at moderate to high intensity, and they are also required perform annual physical performance testing, both while wearing PPE. The Canadian Army has recently implemented a loaded march (5km, wearing military PPE weighing 35 kg) physical performance test for all of its members. A large part of military testing and training takes place outside, and an increasing number of military operations are taking place in extreme environmental conditions (Epstein et al., 2012). The recent climate changes have also led to more cases of extreme weather and higher environmental temperatures (Lucas et al., 2014; Stocker, 2014). Performing a loaded march while wearing military PPE during summer months, could potentially expose members of The Canadian Army to a state of uncompensable heat stress. However, no study to date has examined the cardiovascular and thermoregulatory response to performing a 60 min loaded march in a hot and humid environment while wearing military PPE.

A number of studies have suggested that previous task experience can have a beneficial effect on performance outcome and improve the tactics used to solve the task (Mauger et al., 2009; Micklewright et al., 2010; Seifert et al., 2013). The loaded march physical performance test is required to be performed by all members of the Canadian Army, ranging from soldiers in the field, to truck drivers and desk clerks. Thus, soldiers with extensive loaded march experience might be at an advantage when it comes to performing the loaded march test, and Canadian Army members
with limited experience might struggle to meet the same performance standards as the experienced members.

Therefore, the purpose of this study was to investigate the thermoregulatory and cardiovascular responses to performing a loaded march in the heat, as well as the effects of heat exposure on cost of locomotion and military physical performance. More specifically, ten inexperienced and ten experienced men performed a loaded march at NORMAL temperature (21°C, 50% relative humidity (RH)) and in the HEAT (30°C, 50% RH), where the effects of heat exposure and experience level on thermoregulatory and cardiovascular responses, cost of locomotion and loaded march performance were assessed. Following the loaded march, an assessment of performance on a military physical performance/urban operations simulations test (FORCEcombat) was performed, where the effects of heat exposure and previous experience on performance outcome, was investigated.

Based on results from previous studies showing a negative effect of heat exposure on physical performance (Nybo & Nielsen, 2001b; Nybo et al., 2011; O’Hearn et al., 2016), it was hypothesised that performing a loaded march in the heat would have a negative effect on thermoregulatory and cardiovascular responses, and on FORCEcombat performance. It was also hypothesised that participants with previous experience would perform better on the loaded march, have a lower cost of locomotion, and better FORCEcombat performance compared to inexperienced participants.
METHODOLOGY

Participants:

A total of 20, young (19-35 years), healthy male participants were recruited for this study. Ten participants were recruited from the civilian population, and had limited or no previous experience with loaded march (Inexperienced group), and ten were reservist CAF members, recruited from the Cameron Highlanders of Ottawa, Regiment of the CAF, and had extensive previous experience with loaded march (Experienced group). Participants with previous history of hypertension and cardiovascular disease, and/or current back pain or previous history of back pain were excluded from the study. Ethics approval for this study was received from The University of Ottawa Research Ethics Board, and the study was conducted following the guidelines of the Helsinki Declaration.

Preliminary Session

Participants recruited for this study were asked to partake in a preliminary session prior to any experimental data collection. During the preliminary session written consent was obtained from each participant and they also filled out the Par-Q & You health questionnaire (American College of Sports Medicine, 1997) and the AHA/ACSM Health/Fitness Pre-participation Screening Questionnaire (Balady et al., 1998). The participants’ height (Seca 217 Stadiometer, Seca, Hamburg, Germany) and weight (Sartorius Combics 2, Sartorius AG, Goettingen, Germany) were recorded, as well as an estimate of body composition, using bioelectrical impedance analysis (InBody 520, InBody USA). Bioelectrical impedance analysis has previously been validated against the gold standard for body composition measurements (Anderson et al., 2012). A measurement of maximal oxygen consumption was also performed, using a metabolic cart and an
modified CSEP incremental stepwise treadmill protocol (i.e. 1 min incremental stages until exhaustion).

**Experimental Procedures**

This study consisted of three experimental session; an unloaded march at NORMAL temperature (21°C, 50% RH), a loaded march at NORMAL temperature and a loaded march in the HEAT (30°C, 50% RH). For the loaded march at NORMAL temperature and in the HEAT, participants wore military PPE and a day pack, equal to a total external load of ~35 kg. The unloaded march was used to familiarize participants with the equipment and procedures of the trial, and to rule out any difference between the participant groups when performing a regular unloaded march. The unloaded march was performed on the first experimental visit to the lab, whereas the order of the loaded march in NORMAL temperature and HEAT was randomized. All experimental sessions were separated by a minimum of four days to avoid any effect of fatigue. Participants were asked not to perform strenuous physical activity 24 hours prior to the experimental session, and abstain from alcohol and caffeine consumption a minimum of 6 hours prior to testing. Participants were also encouraged to drink a minimum of 500 ml of water the night before and the morning of the experimental session, and arrive in a fasted state. All experimental sessions took place in the morning between 7:00 and 11:00 am. Upon arrival participants ingested a telemetric pill (Jonah™ Ingestible Core Temperature Capsule, Philips, NV, USA), used to measure core temperature. Nude weight and equipment weight was then recorded (Sartorius Combics 2, Sartorius AG, Goettingen, Germany), before participants were equipped with a heart rate monitor (Garmin Forerunner 310xt, Canton of Schaffhausen, Switzerland) and iButtons (Thermocron iBUTTONS® model DS1922H, Maxim Integrated, CA, USA) on 12 skin sites to measure mean skin temperature. Following the equipment placement, participants donned the
standardized military uniform and PPE for battle order (total 35 kg). Participants then entered a climate controlled chamber (3.3 m length x 2.3 m width x 2.3 m height), containing a pre calibrated treadmill (True 850, True Fitness Technology, St. Louis, MO, USA). Inside the climate chamber, a facemask connected to a Field Metabolic System (FMS Field Metabolic System, Sable Systems International, Las Vegas, NV) was fitted on the participants, for the measurement of oxygen consumption and carbon dioxide production. Next, participants stepped on to the treadmill for a period of standing baseline data collection. Standing energy expenditure, skin ($T_{\text{skin}}$) and core temperature ($T_{\text{core}}$), as well as heart rate (HR) was recorded for a ten min period. At the end of baseline period, participants reported their rate of perceived exertion (RPE) and thermal comfort level (PMV scale). Participants were then asked to straddle the treadmill, while the correct speed (5.17 km/h) and grade (1%) of the treadmill was set, and the loaded march was initiated. Oxygen consumption and carbon dioxide production, HR and $T_{\text{skin}}$ were measured continuously. $T_{\text{core}}$ was recorded every 5 min, while thermal comfort and RPE was recorded every ten min. The trial lasted 60 min or until participants’ voluntary termination. Following the completion of the loaded march, the facemask was removed and the participant stepped out of the climate chamber.

Within five min of termination of the loaded march, participants performed a military physical performance test (FORCEcombat), designed to simulate the demands of urban operations (Gagnon et al., 2016; Reilly et al., 2016). This test consisted of four military physical performance tasks (20-meter rushes, sand bag lifts, loaded shuttle and a sandbag drag), performed in a continuous manner. The test of maximal performance on the FORCEcombat test took place outside the environmental chamber, at room temperature. Following completion of FORCEcombat, all experimental equipment was removed from the participant, and a post measurement of nude body and equipment weight was performed.
**Loaded March Clothing and Equipment**

During all the experimental sessions, participants wore personal cotton socks and a cotton t-shirt, together with the standardized military uniform (CADPAT) and military walking boots. The weight of socks, t-shirt, boots and uniform was approximately 3 kg (small differences in uniform and boot sizes). For the loaded march (NORMAL and HEAT), in addition to the socks, t-shirt, boots and uniform, participants wore the CAF Battle Order, consisting of a fragmentation vest (7 kg), a tactical vest (10 kg), C7 Colt replica rubber rifle (3.7 kg), a helmet (1.6 kg) and a day pack (10 kg), for a total external load of ~35.3 kg.

**Total External Work Performed and Load to Body Mass Ratio**

Total external work performed while walking on the treadmill was calculated based on body mass, external load and vertical distance covered, using the following equation (Robertson *et al.*, 2013)

\[
\text{Total Work (kJ)} = \left( \frac{\% \text{ incline}}{100} \times \text{DIST}_{\text{total}} \right) \times (BM + EXT_{\text{load}}) \times 0.009806
\]

where \(\% \text{ incline}\) is the incline of the treadmill in %, \(\text{DIST}_{\text{total}}\) is total distance walked in meters, BM is body mass in kg, \(EXT_{\text{load}}\) is external load carried in kg, and 0.009806 is the conversion factor from kgm to kJ. External load to body mass ratio was calculated by dividing total external load carried by body mass.

**Skin, Core and Body Temperature measurement**

Wireless temperature sensors (Thermocron iBUTTONS® model DS1922H, Maxim Integrated, CA, USA) located on 12 sites; forehead, upper back, lower back, abdominal area,
quadriceps, hamstrings, front calf, back calf, chest, biceps, forearm, and hand were used to measure $T_{skin}$. Mean skin temperature ($\overline{T}_{skin}$) was calculated using the following skin site weightings: head 7%, hand 4%, upper back 9.5%, chest 9.5%, lower back 9.5%, biceps 9%, forearm 7%, abdominals 9.5%, quadriceps 9.5%, hamstring 9.5%, front calf 8.5% and back calf 7.5% (Du Bois D, 1916; Hardy et al., 1938). $T_{core}$ was measured using a telemetric pill (Jonah™ Ingestible Core Temperature Capsule, Philips, NV, USA), and the signal from the telemetric pill was received, monitored and recorded on a Vital Sense Integrated Physiological Monitor (VitalSense, Philips, NV, USA). Body temperature ($T_{body}$) was calculated based on measurements of $\overline{T}_{skin}$ and $T_{core}$ using the equation from Ely et al. (2009)

$$T_{body} = 0.8(T_{core}) + 0.2(\overline{T}_{skin})$$

**Rate of heat storage**

To determine heat storage and change in rate of heat storage, total body heat content was calculated with the formula developed by Tucker et al. (2006)

$$Qc = T_{body} \times m \times 3.47$$

where $m$ is body mass in kg and 3.47 is a constant measured in kJ*°C^{-1}*kg^{-1}.

Rate of heat storage was then be calculated based on the change in heat content over time (Tucker et al., 2006)

$$Qs = Qc,T1 - Qc,T2$$

where T1 is the initial time point, and T2 is the final time point.
Physiological Strain Index

The physiological strain index was calculated using the equation developed by Moran et al. (1998)

\[
\text{PSI} = 5(T_{\text{core}t} - T_{\text{core}0})x(39.5 - T_{\text{core}0})^{-1} + 5(HRt - HR0)x(180 - HR0)^{-1}
\]

where \(T_{\text{core}0}\) is resting core temperature, and \(T_{\text{core}t}\) is final core temperature. \(HR0\) is resting heart rate, and \(HRt\) is final heart rate.

Energy Expenditure and Fuel selection

A pull-trough open circuit field metabolic system (FMS Field Metabolic System, Sable Systems International, Las Vegas, NV) together with The Life Wind flow meter (Polycontrols Inc, QC, Canada) was used to determine the participants’ oxygen consumption (\(VO_2\)) and carbon dioxide production (\(VCO_2\)). Based on the \(VO_2\) and \(VCO_2\) measurements, whole body energy expenditure (EE) was calculated using the equations developed by Livesey and Elia (1988), and reported in kJ·min\(^{-1}\).

Loaded March Exercise Intensity

The exercise intensity of performing the loaded march was determined based on the participant’s \(\%VO_2\text{max}\). The following equation was used to calculate \(\%VO_2\text{max}\)

\[
\%VO_2\text{max} = \frac{\text{mean}VO_2 \text{ during march}}{VO_2\text{max}} \times 100
\]
**Cost of locomotion**

Cost of locomotion \((J/kg^{1}*m^{1})\) was calculated based on the subtraction method (Rubenson *et al.*, 2007)

\[
\text{Cost of Locomotion} = \frac{(\text{EE}_{\text{loc}} - \text{EE}_{\text{stand}})}{(\text{BW} + \text{EW})/\text{Speed}}
\]

where \(\text{EE}_{\text{loc}}\) is energy expenditure during locomotion in J/min, \(\text{EE}_{\text{stand}}\) is standing energy expenditure in J/min, \(\text{BW}\) is body mass in kg, \(\text{EW}\) is external load in kg, and speed is treadmill speed in m*s\(^{-1}\).

**Heart Rate, RPE and Thermal Comfort**

Heart rate (HR) was measured using a Garmin Forerunner 310x (Garmin Ltd., Canton of Schaffhausen, Switzerland). The Garmin Forerunner 310x collected multiple samples per min, which were averaged and presented as the mean of a five min segment. The Borg scale (Borg, 1982) was used to assess participants RPE, and a modified PMV scale (Matzarakis *et al.*, 1999) was used to determine the participants perceived thermal comfort. Participants were asked to rate their RPE and thermal comfort level at the end of the baseline period, and every ten min during the march.

**FORCEcombat**

To measure the participants’ military physical performance following the loaded march, total time to complete the FORCEcombat test was recorded. FORCEcombat consists of four military physical performance tasks: 20 meter rushes with a drop to prone position every 10 meter (4x), sandbag lifts (thirty 20 kg sandbags lifted to a height of 1 meter), an intermittent loaded shuttle carrying a 20kg sandbag (participants perform five 40 meter loaded shuttles, intermitted by a
40 meter unloaded shuttle) and a 20 meter sandbag drag (pull load was equal to 330 newton). A detailed description of the procedures of the FORCEcombat test are found in Appendix IV. All tasks were performed continuously, in the order described above, and total time (sec) to complete all four tasks were recorded as the performance measure. Due to previous pilot work on the FORCEcombat test, showing an approximate 10% improvement from the first to second attempt, and no significant improvement from the second to third attempt (Reilly et al., 2016), FORCEcombat results recorded following the unloaded march was not included in the data analysis.

**Statistical Analysis**

Before performing any statistical analysis, all samples were tested for normal distribution. To compare changes over time and the effect of condition (NORMAL and HEAT), a two-way repeated measures ANOVA was used, with an LSD post-hoc test to determine where significant differences occurred. An independent sample t-test was used to determine effects of experience level. Difference in mean values for the total duration of the trial was compared for the effect of condition and experience level using a two-way ANOVA, with paired and independent sample t-test post-hoc tests to determine where significant differences occurred. Results were presented as mean±SD, and all statistical analyses were performed using SPSS 17.0 (IBM SPSS Statistics, Armonk, NY, USA).
RESULTS

Participant characteristics for the Inexperienced and Experienced group are found in Table 5.1. Apart from the difference in previous loaded march experience, there were no differences in participant characteristics between the Inexperienced and Experienced group. There was also no difference in total external work performed, loaded march intensity (%VO\textsubscript{2max}) or external load to body mass ratio (Table 5.2) Performing the unloaded march, there were no differences in cardiovascular and thermoregulatory responses, or cost of locomotion between the Inexperienced and the Experienced group. The mean environmental temperature (°C) and RH (%) for each experimental condition, for the Inexperienced and Experienced group are found in Table 5.3.

Table 5.1: Participant characteristics for the Inexperienced and Experienced group.

<table>
<thead>
<tr>
<th></th>
<th>Inexperienced</th>
<th>Experienced</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age</td>
<td>26±3</td>
<td>23±5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>183.4±8.4</td>
<td>178.3±4.1</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>80.9±9.4</td>
<td>78.6±13.2</td>
</tr>
<tr>
<td>BMI (kg*m\textsuperscript{2})</td>
<td>24.2±3.2</td>
<td>24.7±3.4</td>
</tr>
<tr>
<td>BSA (m\textsuperscript{2})</td>
<td>1.59±0.14</td>
<td>1.54±0.19</td>
</tr>
<tr>
<td>Lean Body Mass</td>
<td>70.5±7.5</td>
<td>66.8±7.4</td>
</tr>
<tr>
<td>Body Fat %</td>
<td>12.1±7.3</td>
<td>14.1±6.2</td>
</tr>
<tr>
<td>VO\textsubscript{2max} (ml*min\textsuperscript{-1}*kg\textsuperscript{-1})</td>
<td>49.1±4.3</td>
<td>49.2±4.9</td>
</tr>
</tbody>
</table>
Table 5.2: Total external work performed (kJ), loaded march intensity (%VO$_2$max) and external load to body mass ratio for the Inexperienced and Experienced group during the unloaded and loaded march in the NORMAL and HEAT condition.

<table>
<thead>
<tr>
<th></th>
<th>Total Work (kJ)</th>
<th>%VO$_2$max (%)</th>
<th>External Load/Body mass ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>INEXP</td>
<td>EXP</td>
<td>INEXP</td>
</tr>
<tr>
<td>Unloaded (21°C 50% RH)</td>
<td>42.9±5.0</td>
<td>41.3±6.7</td>
<td>30.0±3.3</td>
</tr>
<tr>
<td>Loaded (21°C 50% RH)</td>
<td>58.7±4.9</td>
<td>57.8±6.9</td>
<td>42.9±4.5</td>
</tr>
<tr>
<td>Loaded Heat (30°C 50% RH)</td>
<td>58.8±0.5</td>
<td>57.5±6.5</td>
<td>43.7±5.0</td>
</tr>
</tbody>
</table>

Table 5.3: Environmental temperature (°C) and relative humidity (%) for the Inexperienced and Experienced group during an unloaded march, and loaded march wearing military PPE at NORMAL temperature and in the HEAT.

<table>
<thead>
<tr>
<th></th>
<th>Unloaded</th>
<th>NORMAL</th>
<th>HEAT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inexperienced</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>21.3±0.7</td>
<td>20.9±0.3</td>
<td>29.8±0.1</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>46.2±3.2</td>
<td>49.2±2.6</td>
<td>46.8±1.2</td>
</tr>
<tr>
<td><strong>Experienced</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>21.1±0.6</td>
<td>21.0±0.2</td>
<td>29.8±0.2</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>47.7±4.5</td>
<td>48.3±3.2</td>
<td>45.4±2.9</td>
</tr>
</tbody>
</table>

Completion Rate

The completion rates for the unloaded and loaded march for both the Inexperienced and the Experienced group are found in Figure 5.1. All participants in both groups completed the unloaded march at NORMAL temperature. Ten out ten participants completed the loaded march
at NORMAL temperature in the Experienced group, and nine out of ten participants completed the loaded march in the HEAT. The one participant unable to complete the loaded march in the HEAT, asked to terminate the trial after 40 min due to gastric distress. In the Inexperienced group, eight out of ten participants were able to complete the loaded march at NORMAL temperature, and only five participants were able to complete the full 60 min of loaded march in the HEAT. One participants asked to stop the trial after 25 min in the NORMAL condition, due to neck pain and migraine symptoms from the external load, and he did not attempt the loaded march in the HEAT. The data from this participant was not included in the data analysis. Four other participants in the Inexperienced group requested to stop the trial before 60 min in the HEAT condition, due to exhaustion and sever discomfort.
Figure 5.1: Completion rate on the unloaded march and loaded march in NORMAL and HEAT condition for the Inexperienced and Experienced group.

Give the inability of a large number of participants to continue the trial beyond 30 min in the Inexperienced group, and 40 min in the Experienced group, statistical analysis comparing the effect of time and condition was performed only up until 30 min in the Inexperienced group, and 40 min in the Experienced group.

Cardiovascular and Thermal Responses

The HR responses to a loaded march in NORMAL and HEAT are found in Figure 5.2A and B, for the Inexperienced and Experienced group respectively. A main effect of time and
condition was observed in the Experienced group for HR, as well as an interaction between time and condition. In this group, a continuously steeper increase in HR over time was observed in the HEAT condition compared to NORMAL, from 20 to 40 min. A main effect of time and condition for HR was also observed in the Inexperienced group, with HR increasing over time and being higher in HEAT compared to NORMAL. However, there was no interaction between the two. Mean HR for the total duration of trial (Figure 5.2C) was significantly higher in HEAT compared to NORMAL, in both the Inexperienced (8%) and the Experienced group (7%). Although there was no difference in HR between the Inexperienced and Experienced group at any specific time point, mean HR was 4.4%, and 5.6% higher in the Inexperienced compared to the Experienced group, in the NORMAL and HEAT condition respectively (Figure 5.2C).

Change in $T_{\text{core}}$ over time are seen in Figure 5.2D and E, for the Inexperienced and Experienced group respectively. A main effect of time and an interaction between time and condition was observed in both the Inexperienced and the Experienced group, but there was no main effect of condition. Increase in $T_{\text{core}}$ was significantly steeper in HEAT compared to NORMAL in both the Inexperienced and the Experienced group. Mean $T_{\text{core}}$ for the duration of the loaded march was not different between conditions (NORMAL and HEAT) in either the Inexperienced (p=0.096), or the Experienced group (p=0.122), and there was no effect of experience level on $T_{\text{core}}$ (p=0.709 and p=0.719, NORMAL and HEAT) (Figure 5.2F).

A main effect of time and condition was observed for $T_{\text{skin}}$, $T_{\text{body}}$ and rate of heat storage, in both the Inexperienced and the Experienced group (Figure 5.2G and H, Figure 5.2J and K, and Figure 5.2M and N respectively). There was also a significant interaction between time and condition for $T_{\text{skin}}$ and $T_{\text{body}}$, where a steeper increase in $T_{\text{skin}}$ and $T_{\text{body}}$ was observed over time in the HEAT condition compared to NORMAL, for both the Inexperienced and the Experienced
group. $\overline{T}_{\text{skin}}$ for the total duration of the loaded march was 3.7% and 5.5% higher in HEAT compared to NORMAL, in the Inexperienced and the Experienced group respectively (Figure 5.2I). Except for a significantly lower $\overline{T}_{\text{skin}}$ at 15 and 20 min in the Experienced group, no other differences in $\overline{T}_{\text{skin}}$ was observed between the Inexperienced and the Experienced group. Mean $T_{\text{body}}$ was significantly higher in HEAT compared to NORMAL, in both the Inexperienced and the Experienced group (0.4°C higher in HEAT compared to NORMAL in both groups) (Figure 5.2L). Rate of heat storage peaked at 15 min in the NORMAL and HEAT condition, in both the Inexperienced and Experienced group. In the NORMAL condition, rate of heat storage decreased following the peak at the 15 min and approached zero (Figure 5.2M and N). In the HEAT condition, the decrease in rate of heat storage following the peak at 15 min was attenuated, and rate of heat storage remained elevated for the duration of the trial in both the Inexperienced and the Experienced group. Rate of heat storage was significantly higher in HEAT compared to NORMAL at all time points in the Inexperienced group. In the Experienced group however, rate of heat storage was higher in HEAT compared to the NORMAL condition, only at 30 and 35 min mark. Mean rate of heat storage for the total duration of the loaded march was significantly higher in the HEAT compared to NORMAL, in both the Inexperienced (3.5 fold increase) and the Experienced group (0.75 fold increase) (Figure 5.2O). However, there was no effect of experience level on $\overline{T}_{\text{skin}}$, $T_{\text{body}}$ or rate of heat storage, in either the NORMAL or HEAT condition.
Figure 5.2: Cardiovascular and thermal responses for Inexperienced and Experienced participants performing a loaded march in NORMAL and HEAT condition. * significantly different from previous time point, # significant difference between NORMAL and HEAT, Φ significant difference between Inexperienced and Experienced. p≤0.05.
Physiological Strain Index

There was limited or no physiological strain associated with performing the unloaded march in either the Inexperienced or the Experienced group (Figure 5.3). However, performing a loaded march in the NORMAL condition caused a moderate physiological strain for participants in both the Inexperienced and the Experienced group, and when performing the loaded march in the HEAT, both groups reached the high physiological strain zone. Experience level had no effect on Physiological Strain Index, and there was no difference between the Inexperienced and the Experienced group in either condition (NORMAL p=0.123, and HEAT, p=0.519).

Figure 5.3: Calculated physiological strain index at the end of an unloaded march and loaded march in NORMAL and HEAT for Inexperienced and Experienced participants. # significantly different from Unloaded, ## significantly different from Loaded. p≤0.05.
Water Loss

Relative water loss was affected by condition, and was significantly higher in HEAT compared to NORMAL, in both the Inexperienced and Experienced group (18% and 17% respectively) (Figure 5.4). However, there was no difference in relative water loss between the Inexperienced and the Experienced group in either the NORMAL (p=0.645) or HEAT (p=0.840) condition.

Figure 5.4: Relative water loss (ml/min) for Inexperienced and Experienced participants during a loaded march in a NORMAL and HEAT condition. # significant difference between NORMAL and HEAT. p≤0.05.
RPE and Thermal Comfort

Rate of perceived exertion was not different between the NORMAL and HEAT condition in neither the Inexperienced (p=0.067), nor the Experienced group (p=0.097) (Figure 5.5A). However, the Experienced group reported a 26% lower RPE in the NORMAL condition and a 25% lower RPE in the HEAT condition, compared to the Inexperienced group.

Thermal comfort scores were significantly higher in the HEAT compared to the NORMAL condition, in both the Inexperienced and the Experienced group (34% and 47% respectively) (Figure 5.5B), meaning participants were less comfortable performing the loaded march in the HEAT compared to the NORMAL condition. Participants in the Experienced group also reported lower thermal comfort scores in both the NORMAL and HEAT condition, compared to the Inexperienced group (38% and 56% lower in NORMAL and HEAT respectively).
Figure 5.5: Mean rate of perceived exertion (RPE) (A) and mean thermal comfort scores (B) for the Inexperienced and Experienced group during a loaded march in a NORMAL and HEAT condition. # significant difference between NORMAL and HEAT, Φ significant difference between Inexperienced and Experienced group, p≤0.05.
Cost of Locomotion

Cost of locomotion over time, and mean cost of locomotion for each condition is presented in Figure 5.6A, B and C. Cost of locomotion increased over time in the NORMAL and HEAT condition for both the Inexperienced and the Experienced group. In the Inexperienced group, the increase in cost of locomotion occurred in the initial 10 min before plateauing in the NORMAL condition, however this increase was prolonged until the 20 min mark in the HEAT condition. The initial increase in cost of locomotion during the first 10 min was also observed in the Experienced group, however, the increase in cost of locomotion in the HEAT continued until the 40 min mark. Mean cost of locomotion was also significantly higher in HEAT compared to NORMAL in the Experienced group (6.6%) (Figure 5.6C). No difference in mean cost of locomotion between NORMAL and HEAT was observed in the Inexperienced group (p=0.086), and there was no difference in mean cost of locomotion between the Inexperienced and the Experienced group in either the NORMAL (p=0.680) or HEAT (p=0.727) condition.
Figure 5.6: Cost of locomotion for the Inexperienced (A) and Inexperienced (B) group during a loaded march in a NORMAL and HEAT condition, and mean cost of locomotion values (C) for the total duration of the trial for both groups in a NORMAL and HEAT condition. * significantly different from previous time point, # significant difference between NORMAL and HEAT, Φ significant difference between Inexperienced and Experienced. p≤0.05.

FORCEcombat Performance

During the loaded march in the NORMAL condition, one of the Experienced participants injured his shoulder, which severely affected his performance time on the FORCEcombat test. Therefore, his results were excluded from the analysis. Completion time on the FORCEcombat test was significantly affected by both condition and experience level (Figure 5.7A). Completion
time was significantly higher in the HEAT compared to the NORMAL condition, where the Inexperienced group increased completion time by 15% (88 sec), and the Experienced group increased completion time by 9% (43 sec). The interindividual variability in change in FORCEcombat performance between the NORMAL and HEAT condition was larger in the Inexperienced compared to the Experienced group (Figure 5.7B). In the Inexperienced group, several participants had a large increase completion time (some by over 150 sec), whereas others actually performed better in HEAT compared to NORMAL. In the Experienced group on the other hand, most of the participants only showed a small increase in completion time going from NORMAL to HEAT. There was also a difference in FORCEcombat completion time between the Experienced and the Inexperienced group, where the Experienced group completed the FORCEcombat test significantly faster than the Inexperienced group, in both conditions (88 sec, or 10.7% faster in NORMAL, and 132 sec, or 14.3% faster in the HEAT condition) (Figure 5.7A).
Figure 5.7: FORCEcombat completion times following a loaded march in a NORMAL and HEAT condition for Inexperienced and Experienced participants (A). Change in FORCEcombat completion time between NORMAL and HEAT condition (B). # significant difference between NORMAL and HEAT, Φ significant difference between Inexperienced and Experienced. p≤0.05.
DISCUSSION

This study is the first to assess cardiovascular and thermoregulatory responses to performing a 60 min loaded march in a hot and humid environment, and the effects of previous experience on loaded march and military physical performance. Results showed that heat exposure had a negative effect on completion rate, thermal and cardiovascular responses and FORCEcombat performance. Performing a loaded march at 30°C (50% RH) while wearing military PPE, led to a state of uncompensable heat stress for participants in both the Experienced and Inexperienced group. On the other hand, previous experience had a positive effect on loaded march completion rate and FORCEcombat performance. The Experienced group had a higher completion rate (90% vs 50%), lower mean HR (134.2±11.9 vs 143.1±8.9 bpm), lower RPE (10.2±1.4 vs 13.0±0.9) and lower thermal comfort scores (1.9±0.5 vs 2.4±0.4) on the loaded march in the HEAT, compared to the Inexperienced group. Performing a loaded march in the HEAT prior to performing the FORCEcombat test also led to a significant increase in FORCEcombat completion times for both the Experienced (8.8%) and the Inexperienced (15.3%) group. Based on these results is seems clear that heat exposure has a negative effect on cardiovascular and thermoregulatory responses, and can causes a state of uncompensable heat stress in participants performing a loaded march while wearing military PPE. Heat exposure also lead to a decrease in in FORCEcombat performance. However, participants with previous experienced had better heat tolerance, lower cardiovascular stress, and performed better on the FORCEcombat test following the loaded march, compared to Inexperienced participants.
Cardiovascular and Thermal Responses to Loaded March in the Heat

Personal protective equipment is designed to shield the human body from external bodily harm. The properties of the PPE causes a reduction in heat loss ability, due to the impermeable material the equipment is constructed in. Studies have reported a reduced heat loss ability in participants wearing different types of PPE, such as in soldiers wearing nuclear, biological and chemical protective ensemble (Cheung et al., 2000), fire fighters (Barr et al., 2010) and football players wearing pads and helmets (Armstrong et al., 2010). The reduced heat loss ability can cause a state of uncompensable heat stress, depending on the intensity of the activity and environmental temperature (Sawka & Pandolf, 2001). Based on the continuous increase in HR, $\bar{T}_{\text{skin}}$, $T_{\text{core}}$ and $T_{\text{body}}$, observed in this study, the results suggests that performing a loaded march in the HEAT (30°C 50%RH) while wearing military PPE, exposes an individual to uncompensable heat stress. Uncompensable heat stress is also likely to be one of the main causes for several participants in the Inexperienced group being unable to complete the loaded march in the HEAT. The reduced ability for evaporative heat loss caused by the PPE, would lead to an upregulation in skin blood flow, to facilitate dry heat loss from the skin, which further exacerbates the cardiovascular strain caused by performing the loaded march. During uncompensable heat stress, the increase in cardiovascular strain, rather than $T_{\text{core}}$, is believed to be the cause of participants inability to continue exercise (Cheuvront et al., 2010; Périard et al., 2011; Sawka et al., 2010).

Core temperature did not reach critical levels in this trial, however, the physiological strain index scores suggests that performing a loaded march in the HEAT exposes participants to significant physiological strain. When performing the loaded march at NORMAL temperature, participants only reached a moderate physiological strain (Figure 5.3). Increasing environmental temperature from 21°C to 30°C, pushed participants in both the Inexperienced and the Experienced
group into the high physiological strain category. Based on these results it seems evident that performing a loaded march in the heat while wearing military PPE exposed participants to an uncompensable heat stress and a high physiological strain, which could have a significant effect on CAF members continued physiological capacity and operational readiness.

Cost of Locomotion

The effects of heat exposure on energy expenditure during exercise has been controversial, with some studies claiming an increase in energy expenditure (Arngrímsson et al., 2003; Consolazio et al., 1963; Dimri et al., 1980; Fink et al., 1975), other a reduction (Brouha et al., 1961; Petersen & Vejby-Christensen, 1973; Williams et al., 1962; Young et al., 1985), or no effect of heat exposure on energy expenditure (Febbraio et al., 1994; Nelson et al., 1948). The results observed in the Inexperienced group in this study, seems to support the latter notion. Even though there was an increase over time in both conditions, no difference in cost of locomotion was observed between the NORMAL and HEAT condition in this group (Figure 5.6A and C). In the Experienced group on the other hand, cost of locomotion also increased over time in both NORMAL and HEAT, however, except from the 35 min mark, cost of locomotion was significantly higher in HEAT compared to NORMAL from 15 min all the way until 40 min (Figure 5.6B). This also led to the mean cost of locomotion being significantly higher in HEAT compared to NORMAL, in the Experienced group (Figure 5.6C). The mechanisms behind an increase in cost of locomotion in the heat have not been confirmed, but a reduction in walking economy due to fatigue has been suggested as a potential reason (Fink et al., 1975). Participants RPE was not different between the NORMAL and HEAT condition, but this study did not include a direct measure of participants’ fatigue. Therefore, future studies should include measurements of biomechanical parameters and/or measures of fatigue, to determine how heat exposure can affect
walking economy and efficiency. On the other hand, several researchers have attributed the increase in cost of locomotion, at least in part, to either increased cardiovascular strain, dehydration or a Q10 effect (Barr, 1999; Kampmann & Bröde, 2015). Thus, it is possible that the increase in relative water loss observed in the HEAT compared to the NORMAL condition could have caused a more rapid dehydration, and an increase in cost of locomotion. However, there was no difference in relative water loss between the Experienced and the Inexperienced group, suggesting a similar increase in cost of locomotion should have occurred in this group as well. Instead, cost of locomotion was not different between the NORMAL and HEAT condition in the Inexperienced group. The absence of an increase in cost of locomotion in the heat in the Inexperienced group could have been caused by an increase in anaerobic energy expenditure, with aerobic energy expenditure remaining unchanged. Previous studies have reported higher muscle lactate concentrations (Young et al., 1985), and increased anaerobic energy expenditure during exercise in the heat (Dimri et al., 1980). Mean HR was higher in the Inexperienced group compared to the Experienced, which could suggest they relied more on anaerobic energy pathways to sustain the exercise intensity. Without measuring Excessive Post-Exercise Oxygen Consumption, the contribution of anaerobic processes cannot be confirmed.

The Effect of Previous Experience on Completion Rate, Thermoregulatory and Cardiovascular Responses

The effect of previous experience on performance outcome is a topic that has been given limited attention throughout the years, but a few studies have been conducted, suggesting a beneficial effect of previous experience on performance and task solving ability (Mauger et al., 2009; Micklewright et al., 2010; Seifert et al., 2013). The results from this study support this idea. Previous loaded march experience was the only characteristic separating the two participant groups
in this study, and there was no difference in total work performed, loaded march intensity, or external load to body mass ratio between the two groups (Table 5.2). However, only eight out of ten and five out of ten participants in the Inexperienced group were able to complete the loaded march in the NORMAL and HEAT condition respectively. In the Experienced group on the other hand, ten out of ten participants completed the loaded march at NORMAL temperature, and nine out of ten completed the loaded march in the HEAT. The one participant unable to complete the loaded march in the HEAT in the Experienced group, was suffering from gastric distress, which is a common incident during high intensity exercise in the heat (Neufer et al., 1989; van Nieuwenhoven et al., 2000). Thermoregulatory responses (\(\bar{T}_{\text{skin}}, T_{\text{core}}, \text{and } T_{\text{body}}\)) were not affected by experience level. However, participants in the Experienced group had a significantly lower mean HR during the loaded march, in both the NORMAL and HEAT condition, compared to the Inexperienced group. Participants in the Experienced group also reported significantly lower RPE and thermal comfort scores in both the NORMAL and HEAT condition. Total external work performed, loaded march intensity and external load to body mass ratio (Table 5.2) were not different between the two groups, and could not have caused the difference in HR, RPE and thermal comforts scores. There was also no difference in aerobic capacity, body mass, or body composition between the two groups. Based on these results it seem that having previous loaded march experience lead to a lower mean HR, RPE and Thermal Comfort Scores during loaded march, both in a NORMAL and HEAT condition. Limited research exists on the effect of previous experience on task performance, but studies have suggested that previous experienced improved both pacing strategies and RPE in cycling time trials (Mauger et al., 2009; Micklewright et al., 2010), and that previous experienced had a positive effect on climbing performance while performing ice climbing (Seifert et al., 2013). Micklewright et al. (2010), suggested that
participants with previous task experience were able to draw upon schematic mental representations from previous attempts performing the specific task, together with mental feedback, which can affect RPE, but also potentially the tactic used to solve the task. Although his is an interesting theory for potentially explaining the difference observed between the Experienced and the Inexperienced group, more research is needed to determine the specific effects of previous experience on loaded march performance observed in this study.

**FORCEcombat**

For a large number of CAF members, loaded march is a frequently used mode of transportation. Following the loaded march to the objective, soldiers need to maintain combat readiness, and be able to perform combat duties. Therefore, this study sought to determine how performing a loaded march in the HEAT, compared to NORMAL temperature, affects performance on the FORCEcombat test, performed immediately after the loaded march. The results showed that heat exposure led to a significant increase in FORCEcombat completion time, in both the Inexperienced and Experienced group (Figure 5.7A). These findings are in accordance with previous publication showing that heat exposure can have a negative effect on exercise performance (Ely et al., 2007; Galloway & Maughan, 1997a; Gonzalez-Alonso & Calbet, 2003; MacDougall et al., 1974). There are multiple factors that could have contributed to the increase in completion time. A high cardiovascular strain, prior to and during FORCEcombat (Cheuvront et al., 2010; Périard et al., 2011; Sawka & Pandolf, 2001), dehydration (Cheung & McLellan, 1997, 1998; Maughan, 2003; Sawka, 1992), and an elevated core temperature prior to starting FORCEcombat (O’Hearn et al., 2016) are all factors that could contribute to a decrease in exercise performance and increase in FORCEcombat completion time. It was also shown that participants in the Experienced group had significantly lower completion times compared to participants in the
Inexperienced group (Figure 5.7A). The participants in the Experienced group had, apart from previous loaded march experience, also previous experience with urban operation exercises, but none of the participants in either group had previously been exposed to this specific test used in this study. The results from this study confirmed the negative effect of heat exposure on physical performance, and showed that previous experience had a positive effect on FORCEcombat performance.

CONCLUSION

After assessing the effects of heat exposure and previous experience on loaded march and FORCEcombat performance, the results from this study showed that high environmental temperature (30°C, 50%RH) had a negative effect on thermoregulatory and cardiovascular responses during a loaded march. The high environmental temperature also caused a state of uncompensable heat stress, and reduced performance on the FORCEcombat test. On the other hand, previous experience had a beneficial effect on the ability to complete a loaded march in the HEAT, and on FORCEcombat performance. Based on these results CAF/military officers should take environmental temperature and humidity into considerations when deciding which days to schedule loaded march testing and training. The effect of previous experience also show that CAF members with limited loaded march experience might be at a disadvantage when it comes to performing a loaded march at NORMAL temperature and in the HEAT, and this could effect the outcome of a loaded march physical employment test.
CHAPTER 6 - GENERAL DISCUSSION AND CONCLUSIONS
THESIS OVERVIEW

This doctoral thesis work was undertaken to investigate the effects of morphology, physiological capacity, stress and inflammatory cytokines, previous experience and heat exposure on military physical performance. Members of the CAF are expected to obey orders and carry out their military duties in any situation, no matter how challenging or stressful (The Canadian Armed Forces, 2011). To be able to carry out demanding occupational tasks and cope with multiple stressors, CAF members are required to meet a minimum PES (The Canadian Armed Forces, 2011). Physical performance assessments are performed annually for all CAF members, but limited research has been conducted investigating factors affecting military physical performance. Thus, investigating the effects of the previously mentioned factors on military physical performance would not only help increase CAF members ability to perform occupational tasks, but the outcomes could also be used as a tool to help guide CAF physical training. To accomplish this, four studies were conducted, and incorporated into this doctoral thesis. In the first study (Chapter 2), I examined differences in morphology and physiological capacity between the top and bottom performers on the CMTFE, and which of these factors could be used to predict overall performance outcome. The results showed that large differences in physiological capacity and morphology exist between the top and bottom performers on the CMTFE. However, measures of physiological capacity (aerobic capacity), rather than morphology were the best predictors of performance outcome on the CMTFE (Chapter 2). In Chapter 3, the occurrence of stress and inflammatory cytokines in the CAF was investigated. This was accomplished by determining the detection rate of 19 inflammatory cytokines, as well as performing a descriptive analysis of basal levels of cortisol, CRP, adiponectin, IFN-γ, IL-2, IL-8 and IL-18. The association between levels of stress and inflammatory cytokines and age, sex and adiposity was also determined. The results
from this descriptive analysis showed that stress and inflammatory cytokines are present among CAF members, and that both detection rate and basal levels tends to increase with increasing age (Chapter 3). Following the description of basal levels of stress and inflammatory cytokines among CAF members in Chapter 3, the association between stress and inflammatory cytokines and military physical performance was determined in Chapter 4. Using multiple linear regression analysis, it was determined that a negative association exists between basal levels of the cytokine CRP and aerobic capacity, picking and digging performance, and total performance on the CMTFE (Chapter 4). The Canadian Army has recently implemented a loaded march test, followed by an assessment of FORCEcombat performance as a part of their physical performance testing. To facilitate annual loaded march testing for all its members, a large part of the testing and training would be required to take place during the summer months, where temperature and relative humidity can be very high. However, there is currently limited research available investigating the effect of heat exposure on thermal and cardiovascular responses during a loaded march. Thus, the effects of heat exposure and previous experience on loaded march and FORCEcombat performance, were investigated in Chapter 5. The results from this study showed that heat exposure (30°C, 50% RH) had a negative effect on cardiovascular and thermal responses when performing a loaded march while wearing military PPE, and led to state of uncompensable heat stress for both experienced and inexperienced participants. Both participant groups also experienced a decrease in FORCEcombat performance, following the loaded march in the HEAT compared to NORMAL temperature. On the other hand, previous experience had a positive effect on the ability to complete the loaded march in both the NORMAL and HEAT condition, and participants with previous experience performed better on the FORCEcombat test, compared to inexperienced participants.
Overall, this thesis has discovered several factors affecting military physical performance, some expected, other less obvious. As expected, it was found that aerobic capacity was the best predictor of performance on the CMTFE, CRP levels were negatively associated with military physical performance, and that heat exposure had a negative effect on loaded march and FORCEcombat performance. On the other hand, this thesis has also uncovered less expected outcomes. It was hypothesised that previous experience would have a beneficial effect on military physical performance, which was confirmed by the results in Chapter 5. However, the large difference in both loaded march and FORCEcombat performance between experienced and inexperienced participants was somewhat unexpected. The following sections contains a more detailed discussion of the main outcomes from thesis work.

**THE EFFECTS OF PHYSIOLOGICAL CAPACITY AND MORPHOLOGY ON MILITARY PHYSICAL PERFORMANCE**

The objective of the first study of this thesis (Chapter 2) was to quantify which factors of physiological capacity and/or morphology separate the top and bottom performers on the CMTFE, and which of these factors could be used to predict performance outcome. By comparing the top and bottom performance quintiles, a large difference in physiological capacity and morphology was observed for both men and women (Table 2.2 and 2.3). It is interesting to notice that the top performers in the male group had a lower age, smaller waist-to-hip ratio, and better upper body strength compared to the bottom performers, but neither of these factors were different between top and bottom performers in the female group. The absence of a difference in age between the top and bottom performers in the female group could potentially be explained by women experiencing a smaller decline in physical performance until about the age of 55. Whereas men
have a more gradual decline in physiological capacity over the adult age range, women experience relatively small declines until the age of 55, after which a more rapid decline in physiological capacity occurs (Samson et al., 2000). These results would also suggest that women rely less on upper body strength than men do, to perform the CMTFE. This was supported by the linear regression analysis, showing aerobic capacity, height and core strength were the best predictors of total CMTFE performance in the female group (Table 2.4). Men on the other hand, relied more on lean body mass and upper body strength, with these factors explaining a combined 25% of performance variability. However, aerobic capacity was the factor explaining the largest part of the variability in total CMTFE performance, for both men and women. Aerobic capacity explained ~37% of variability in CMTFE performance in the female group, and ~32% in the male group (Table 2.4). These results confirm the importance of good aerobic capacity for overall performance on the CMTFE. As seen in Figure 2.1, the six tasks constituting the CMTFE require either a large distance to be covered or a large work to be performed, and in accordance with previous research, this study showed the importance of aerobic capacity for task solving and physical functioning (Fleg et al., 2000; Kaminsky et al., 2013). Aerobic capacity and muscular strength have also been shown to be good predictors of performance outcome on similar tests of military physical performance (Mello et al., 1995; Nottrodt & Celentano, 1987; Rayson et al., 2000). Even though large differences in morphology were observed between the top and bottom performers on the CMTFE, morphology played a negligible role in predicting performance outcome. The only factors of morphology found to have an effect on performance outcome were height in the female group, predicting ~11%, and LBM in the male group, predicting ~21% of performance variability (Table 2.4). The effect of height on total performance in the female group was likely caused by its effect on performance on the sandbag fortification task. In this task,
participants had to lift sandbags (20 kg) to a height of 90 cm. Personal communications from the research assistants responsible for the data collection confirmed that especially shorter women struggled to get the 20kg sandbag to a height of 90cm. Still, height only predicted ~11% of variability in performance outcome, and cannot be considered a key predictor of performance on the CMTFE.

The physical performance test used by the U.S. Armed Forces has been under scrutiny for placing penalties on individuals with a larger body mass (Vanderburgh, 2008; Vanderburgh & Crowder, 2006). Nonetheless, research has shown that larger individuals perform better on work related fitness tasks, like carrying loads, lifting and material handling (Vanderburgh, 2008). The results from Chapter 2 showed that no body mass bias seems to exist against larger individuals performing the CMTFE. There was no difference in body mass between the top and bottom performers in neither the male, nor female group (Table 2.2 and 2.3), and body mass was not included as a predictor of total performance. In fact, a larger lean body mass was found to be beneficial for performance in the male group (Table 2.4).

The results from Chapter 2 showed that physiological capacity, and more specifically aerobic capacity, was the main predictor of performance on the CMTFE. Therefore, improving aerobic capacity seems to be the most beneficial for improving to total performance on the CMTFE. Unlike the physical performance tests used by the U.S. Armed Forces, no body mass bias against larger individuals performing the CMTFE was observed.
THE PRESENCE AND BASAL LEVELS OF STRESS AND INFLAMMATORY CYTOKINES IN THE CAF

During the collection of physical performance data for the development of the new PES for the CAF in 2012, blood samples were also collected from 331 CAF members. Samples were collected from a representative sample of the CAF, and analysing these blood samples allowed us to investigate the occurrence and basal levels of stress and inflammatory cytokines in this population (Chapter 3). In general, a low detection rate was observed for most of the inflammatory cytokines measured. Only CRP, adiponectin, cortisol, IFN-γ, IL-2, IL-8 and IL-18 had a detection rate above 10% (Table 3.2). Factors like sex, age and adiposity have previously been shown to affect levels of stress and inflammatory cytokines in the general population (Bastard et al., 2006; Cartier et al., 2009; Ferrucci et al., 2005; Isobe et al., 2005; Lapice et al., 2009; Ong et al., 2013; Pruijm et al., 2013; Woloshin & Schwartz, 2005). However, there was no difference in detection rate between men and women, or between BMI categories in the CAF. There was, however, an increase in detection rate of several inflammatory cytokines (IFN-γ, TNF-α, IL-2, IL-5, IL-8, IL-9, IL-17a, IL23, IL-21) with increasing age (Table 3.2). Due to the generally low detection rate of most of the inflammatory cytokines measured, only CRP, cortisol adiponectin, IL-18, IFN-γ, IL-2 and IL-8 were included for further analysis. Basal levels for each of these cytokines were reported for the total sample, men and women respectively, as well as for age groups and BMI categories (Table 3.3 and 3.4). We also examined the association between cytokine levels and sex, age and BMI categories in the CAF population (Table 3.5). Based on the results from the linear regression analysis, higher IL-18 and lower adiponectin levels were observed in men compared to women. There was also a positive association between age and levels of CRP, adiponectin, IL-18 and IL-2, suggesting an increase in levels of inflammatory cytokines with increasing age. Cortisol, on the
other hand, was negatively associated with age, suggesting a decrease in this stress hormone with increasing age. This is contradictory to results seen in the general population, where cortisol levels have been found to increase with increasing age (Purnell et al., 2004; Van Cauter et al., 1996). The increase in cortisol levels with increasing age has often been attributed to the wear and tear hypothesis (McEwen, 1998), caused by a life long exposure to multiple stressors. Based on the results from the linear regression, a positive association between measures of obesity and basal levels of CRP and IL-18 was also observed, and an inverse association between adiponectin levels and measures of body fat. Both of these outcomes are in accordance with results from the general population, where higher levels of body fat have been shown to be associated with higher levels of CRP and IL-18 (Ferrucci et al., 2005; Hung et al., 2005; Marques-Vidal et al., 2011), and inversely related to adiponectin levels (Cnop et al., 2003).

Although levels of stress and inflammatory cytokines have been described in several samples of the general population (Marques-Vidal et al., 2011; Stowe et al., 2010; Woloshin & Schwartz, 2005), this is to our knowledge, the first attempt at describing basal levels of stress and inflammatory cytokines in a military population. The results from this descriptive analysis will serves as a foundation for future research into levels of stress and inflammatory cytokines, and their implications on CAF members’ physiological state. Even though the detection rate of most of the inflammatory cytokines measured was low, the presence of stress and inflammatory cytokines among CAF members in undeniable.
ASSOCIATION BETWEEN STRESS AND INFLAMMATORY CYTOKINES AND MILITARY PHYSICAL PERFORMANCE

Following the determination of basal levels of stress and inflammatory cytokines among CAF members in Chapter 3, the association between cytokine levels and military physical performance was investigated in Chapter 4. A number of studies have previously reported an association between levels of stress and inflammatory cytokines and physical performance in the general population (Barbieri et al., 2003; Blain et al., 2012; Cesari et al., 2004; Oliveira et al., 2008; Peterson et al., 2015; Schaap et al., 2009; Schaap et al., 2006; Taaffe et al., 2000; van der Does et al., 2015), but the presence of such an association in a military population has not previously been investigated. After running multiple linear regression analyses, controlling for sex, age and adiposity, a significant negative association between CRP levels and aerobic capacity, picking and digging performance, plank time and total performance on the CTMFE was observed (Table 4.2). It was also found that adiponectin levels were negatively associated with grip strength, but positively associated with plank time (Table 4.2). These results are the first evidence of the potential negative effect of high levels of inflammation and inflammatory cytokines on military physical performance.

Studies from the general population have shown that a negative association seem to exist between CRP levels and overall physical performance (Brinkley et al., 2009; Cesari et al., 2004) and grip strength (Cesari et al., 2004; Taaffe et al., 2000). These results were all observed in samples of older participants, but the results from Chapter 4 are in line with these observations from the general population. The association between high levels of inflammatory cytokines and physical performance seems clear, however, the cause of the negative association between physical performance and inflammatory cytokines remains to be confirmed. Researchers have suggested...
that inflammatory cytokines might accelerate changes in body composition related to the aging process, such as increase in adiposity and decrease in muscle mass (Ferrucci et al., 2005). This is unlikely to be the cause in this military population, where the mean age was 35.5±10.4 years, and the oldest participant was 59 years old. A more plausible explanation would be that an increase in inflammatory cytokines could have a negative effect on muscle mass, and cause an upregulation in protein catabolism. Both human (Anker et al., 1999; Visser et al., 2002) and animal models (García-Martínez et al., 1993; Tsujinaka et al., 1995; Zoico & Roubenoff, 2002) have shown that inflammatory cytokines can have a diminishing effect on muscle mass. Cytokines are also known to play a role in regulation of protein metabolism (Zoico & Roubenoff, 2002). Toth et al. (2005), observed an inverse relationship between myosin heavy chain protein synthesis and plasma levels of CRP, as well as the muscle expression of TNF-α. However, the effects of inflammatory cytokines on muscle fibre wasting was measured locally, warranting caution about drawing conclusions on intramuscular effects based on measurement of systemic circulating values. It is possible a spillover of locally produced cytokines from muscle to the blood stream could occur (Späte & Schulze, 2004), but until concrete evidence exist of an association between circulating cytokines levels and muscle wasting, this will only be speculation. It is also possible a subclinical disease could be the cause of the association between higher cytokine levels and a decrease in physical performance. Rather than a direct effect of cytokines on physical performance and muscle mass loss, the cytokines serve as a marker of an ongoing subclinical disease (i.e. hypothyroidism, diabetes, rheumatoid arthritis), which is the actual cause of the decrease in performance and muscle mass (Brennan et al., 2006). Although there are several potential ways the inverse association between levels of inflammatory cytokines and physical performance could be explained, the fact remains that due the variety of roles inflammatory cytokines play in immunoregulation, the
inflammatory process, substrate availability, protein metabolism and hematopoiesis, it is difficult to pinpoint a single mechanism responsible for their effects on physical performance (Kishimoto, 2006; Van Snick, 1990; Zoico & Roubenoff, 2002). The cause of the negative association between levels of stress and inflammatory cytokines and physical performance are currently not understood, and more research is required to determine the mechanisms involved. However, the results from this study, combined with the results from the general population, confirms the negative association between levels of inflammatory cytokines and physical performance.

NEGATIVE EFFECT OF HEAT EXPOSURE ON LOADED MARCH AND MILITARY PHYSICAL PERFORMANCE

In Chapter 5, the effect of high environmental temperature and humidity on loaded march and military physical performance was investigated. The CAF has recently implemented an assessment of loaded march performance as a part of the PES, for all members of the Canadian Army. However, there is limited information available on the effects of high temperatures and humidity on cardiovascular and thermoregulatory responses to performing a loaded march while wearing military PPE. We therefore assessed the cardiovascular and thermal responses to performing a loaded march at NORMAL temperature compared to the HEAT, in participants Experienced and Inexperienced with loaded march. Experienced participants performed better on the loaded march compared to Inexperienced participants, and ten out of ten Experienced participants were able to complete the full 60 min of the loaded march at NORMAL temperature. In the Inexperienced group, only eight out of ten participants were able to complete the loaded march at NORMAL temperature (Figure 5.1). One participant requested to stop the trial after 25 min, due to the external load causing severe neck discomfort and migraine symptoms, and this
participant did not attempt the loaded march in the HEAT. A second participant was unable to continue past the 55 min mark of the loaded march at NORMAL temperature, and requested to terminate the trial due to exhaustion. Increasing the environmental temperature from 21°C to 30°C did not severely affect the Experienced group’s ability to complete the loaded march, where nine out of ten participants completed the full 60 min. The one participant unable to continue past the 45 min mark suffered from gastric distress, which is a common incident during high intensity exercise in the HEAT (Neufer et al., 1989; van Nieuwenhoven et al., 2000). In the Inexperienced group, however, the increase in environmental temperature rendered four of the nine participants unable to complete the loaded march (Figure 5.1). The inability of the Inexperienced participants to complete the loaded march in the heat, and the difference in completion rate between the two groups was a surprising discovery. Previous research has shown that heat exhaustion is more common in older, unfit individuals (Kenney, 1997; Pandolf, 1997), and that disease state can further shorten the time to exhaustion (Addonizio & Susman, 1984; Bourdon & Canini, 1995; Smith et al., 1995). However, both participant groups consisted of healthy, young and fit individuals, and there was no difference in age between participant groups. Participants were also screened for any disease that could affect heat tolerance. The inability of participants in the Inexperienced group to complete the loaded march in the HEAT could be in part related to a higher cardiovascular strain exhibited by these participants, compared to Experienced group. Mean heart rate for Inexperienced group was 143.1±8.9 (74% of HRmax) during the loaded march in the HEAT, which was 5.6% higher than the Experienced group. Several studies have suggested that the increase in cardiovascular strain, rather than core temperature, is the main cause of exhaustion during exercise in uncompensable heat stress (Cheuvront et al., 2010; Nybo & Nielsen, 2001a; Nybo et al., 2011; Périard et al., 2011; Sawka & Pandolf, 2001). Even though there was a
difference in cardiovascular strain and completion rate between the Inexperienced and Experienced group, performing the loaded march wearing military PPE in the HEAT, led to both participant groups experiencing a state of uncompensable heat stress. Figure 5.2 shows the continuous increase in core temperature, skin temperature and heat storage in both groups during the loaded march in the HEAT. The large number of Inexperienced participants unable to complete the loaded march, and both the Experienced and the Inexperienced group experiencing uncompensable heat stress at 30°C (50% RH), suggest this is not a safe environmental condition for performing loaded march while wearing military PPE. Thus, future research should focus on finding a safe environmental temperature for performing loaded march while wearing military PPE.

Cost of locomotion during the loaded march was not affected by temperature in the Inexperienced group, and there was no difference in cost of locomotion between the Inexperienced and the Experienced group in either the HEAT or NORMAL condition. In the Experienced group on the other hand, cost of locomotion was significantly higher in the HEAT compared to NORMAL temperature (Table 5.6C). Previous studies have argued both for (Arngrímsson et al., 2003; Consolazio et al., 1963; Dimri et al., 1980) and against (Febbraio et al., 1994; Nelson et al., 1948) a change in energy expenditure during exercise in the heat. The results from this study seem to lend support to both of these notions. The increase in cost of locomotion in the HEAT, observed in the Experienced group, could be explained by elevated cardiovascular strain caused by a combination of dehydration and increased skin blood flow (Barr, 1999), but it is also possible that the increase in cost of locomotion could have been caused by a Q10 effect (Kampmann & Bröde, 2015). The absence of an increase in cost of locomotion in the Inexperienced group is a phenomenon more intricate to explain. Previous research has shown a higher anaerobic
metabolism during exercise in the heat (Dimri et al., 1980), and it is possible that the inexperienced participants relied more on anaerobic processes for energy metabolism during the loaded march in the HEAT. This would not affect oxygen consumption during the loaded march, and would only be possible to detect as an increase in post exercise oxygen consumption. Neither the purpose or the design of the current study allowed us to determine the cause of the difference, and absence of a difference in cost of locomotion between the NORMAL and HEAT condition for the Experienced and Inexperienced group respectively. Therefore, future studies should work to examine potential causes for the difference in cost of locomotion between the NORMAL and HEAT condition in the Experienced group, and why a similar result was not seen in the Inexperienced group.

This study also investigated how performing a loaded march at NORMAL temperature and in the HEAT prior to a military physical performance test (FORCEcombat), would affect the outcome of the physical performance test. Previous studies have shown that exposure to high ambient temperatures both during (Nybo & Nielsen, 2001a; Nybo et al., 2011), and prior to exercise (O’Hearn et al., 2016), can diminish exercise performance and the results from this study lends support to this notion. Performing a loaded march in the HEAT just prior to the FORCEcombat test, led to an elevation in core temperature pre FORCEcombat (Figure 5.2), and a significant decrease in performance, in both the Experienced and the Inexperienced group (Figure 5.7). FORCEcombat completion time increased by 9% in the Experienced group and 15% in the Inexperienced group, following the loaded march in HEAT compared to NORMAL temperature. These results show, that performing a loaded march in the HEAT not only had a negative effect on cardiovascular and thermoregulatory responses during the loaded march, but also led to a reduction in FORCEcombat performance, following the loaded march. This suggests
that CAF members performing a loaded march as a mode of transportation to the front line of battle, in environments of high environmental temperature (>30°C) and humidity (>50%RH), could suffer a reduction in military physical performance, which could affect battle readiness. Future research should look into potential strategies to reduce the effect of heat exposure on both cardiovascular and thermoregulatory responses during a loaded march, but also its effect on military physical performance following the loaded march. Using cooling garments underneath the PPE (Arngrímsson et al., 2004; Smolander et al., 2004; Webster et al., 2005), and performing activities at lower exercise intensity (Montain et al., 1994), have been shown beneficial to reduce heat stress and increase heat tolerance time. However, more research is required to find safe environmental temperatures where the loaded march and FORCEcombat test can be performed without participants experiencing heat exhaustion and large reductions in physical performance.

POSITIVE EFFECT OF PREVIOUS EXPERIENCE ON LOADED MARCH AND MILITARY PHYSICAL PERFORMANCE

The recently implemented loaded march test, followed by FORCEcombat, is required to be performed by all members of the Canadian Army, from actively serving soldiers in the field with extensive loaded march and combat experience, to truck drivers and desk clerks with limited or no previous experience. Studies from the general population have suggested a beneficial effect of previous experience on performance (Knechtle et al., 2012; Mauger et al., 2009; Micklewright et al., 2010; Seifert et al., 2013). This suggests that members of The Canadian Army, inexperienced with loaded march, might be at a disadvantage compared to members with extensive loaded march experience. Thus, the effect of previous experience on military physical performance
was investigated in Chapter 5 of this thesis. After comparing two groups of participants, with no other discernible difference than previous loaded march experience (Table 5.2 and 5.3), a significant difference in loaded march completion rate, and FORCÉcombat performance was observed, between the Experienced and the Inexperienced group. Whereas, ten out of ten participants in the Experienced group completed the loaded march at NORMAL temperature, only eight out ten participants in the Inexperienced group were able to do the same. The difference between the two groups were even more staggering in the HEAT condition. Nine out ten participants completed the loaded march in the HEAT in Experienced group, but only five out of ten participants in the Inexperienced group were able to complete the loaded march in the HEAT (Figure 5.1). Mean HR, RPE and thermal comfort scores were also significantly lower in the Experienced compared to the Inexperienced group, during the loaded march at NORMAL temperature and in the HEAT. The Experienced group also had a better completion time on the FORCÉcombat test following the loaded march at NORMAL temperature and in the HEAT (Figure 5.7A). The results from this study are the first to show the beneficial effects of previous experience on the ability to perform loaded march, and on a military physical performance test. Even though previous experience seems beneficial for performance outcome, and other studies have found similar results (Mauger et al., 2009; Micklewright et al., 2010; Seifert et al., 2013), the mechanism behind the beneficial effects of previous experience remains to be elucidated. Based on personal experience and communications, we had hypothesised that participants with previous experience would be more accustomed to walking with a load, and therefore more efficient, but there was no difference in cost of locomotion between the Experienced and Inexperienced group, in the NORMAL condition, or in the HEAT (Figure 5.6A, B and C). Both Mauger et al. (2009), and Micklewright et al. (2010), suggested that participants with previous
experience are able to use experience derived schematic mental representations of similar experiences, and on a cognitive conscious level interpret performance feedback, to improve pacing strategies and RPE. On the other hand, Seifert et al. (2013), reported that previous rock climbing experience, although it being a different sport and different equipment being used, had a positive influence on ice climbing technique and performance. Both of these theories could potentially explain some of the performance variability seen between the two participant groups in this study.

It has also been shown that members of the armed forces possess a greater resilience (the ability to grow and thrive in the face of challenges, and be able to bounce back from adversity) (Simmons and Yonder, 2013, U.S. Army 2011), allowing them to push themselves further, and carry out tasks in stressful conditions. This could also be a part of the reason why the experienced participants, being military reservists, had a higher completion percentage, and better completion time on the FORCEcombat task. However, more research is needed to determine the specific mechanism, giving participants with previous experience an advantage on both loaded march and FORCEcombat. Still, the results clearly showed that participants in the Experienced group performed better on the loaded march and the FORCEcombat test, and had a lower mean HR, RPE and thermal comfort scores compared to participants in the Inexperienced group. The large difference in loaded march and FORCEcombat performance should be noted by CAF leadership and officers, and be taken into consideration when it comes to determining standards and expectations for using loaded march and the FORCEcombat test as a part of the PES for the Canadian Army.
OVERARCHING CONCLUSIONS

This doctoral thesis work was undertaken to examine internal and external factors affecting military physical performance, and how they can affect performance outcome. Due to a high reliance on physical performance to carry out occupational tasks and meet minimum physical employment standards, determining factors affecting military physical performance would be of great benefit for members of the Canadian Armed Forces. Experimental evidence showed that aerobic capacity was the best predictor of total performance on the CMTFE, and that high levels of inflammatory cytokines were negatively associated with military physical performance. Furthermore, this thesis showed that heat exposure had a negative effect on loaded march and FORCEcombat performance, and that performing a loaded march while wearing military PPE at 30°C can expose CAF members to a state of uncompensable heat stress. It was also found that previous experience was beneficial for loaded march and FORCEcombat performance. Several questions still remain unanswered concerning the specific mechanism behind some of the observed outcomes from this thesis, and future research is required to expand on these findings. However, the results from this thesis work will hopefully increase awareness about the positive effects of physiological capacity and previous experience, and the negative effects of inflammatory cytokines and heat exposure, on military physical performance. Based on this work, strategies and interventions can be developed to increase military physical performance, and decrease exposure to factors potentially harmful to military physical performance.
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CHAPTER 8 – APPENDICES
APPENDIX I: Final published version of Chapter 2
Due to copyright policies of the respective publishers, the final published version of Chapter 2 can be found using the following link:

https://academic.oup.com/milmed/article/181/11-12/e1623/4158529
APPENDIX II: Final version of Chapter 3 accepted for publication
Due to copyright policies of the respective publishers, the final published version of Chapter 3 can be found using the following link:

APPENDIX III: Detailed Descriptions of CMTFE Procedures
Detailed Descriptions of CMTFE Procedures

**Sandbag Fortification (SBF).** Performance was measured as time spent to transfer 60 sandbags (20kg each) stacked in six layers of ten, from a pallet (120cm x 120 cm x 15 cm) on to a box (height 91.5cm) placed 125cm away from the pallet. Sandbags had to be lifted up and placed on the box, throwing them was not allowed. A timer was started as the first sandbag was lifted off the pallet and stopped as the last sandbag touched the platform.

**Escape to Cover (E2C).** Performance on the E2C task was measured as time spent to complete an agility course created to replicate an escape to cover manoeuver. From a starting line, the participant sprinted 10 meters to the first cone before taking a knee. A seven second pause was ordered, while the evaluator performed a loud countdown, knee touching the ground at all time. After seven seconds, the evaluator shouted “clear” and the participant rose and performed a 50 meter straight run. A cone marked the end of the 50 meter run, where the participant made a 180° turn. The participant assumed a prone position, before completing a 10 meter leopard crawl under low-crawl hurdles (45 cm high) for 10 meters. As the 10 meter crawl was completed, the participant got up and ran the last 30 meters to the finish line. This task was performed wearing a helmet, a fragmentation vest with plates and carrying a 3.7kg C7 rubber rifle, for a total weight of 15kg. The total length of the course was 100 meters, and the participant’s time to completion (in sec) was used as the performance measure.

**Picking and Digging (P&D).** The P&D task was comprised of two separate exercises picking and digging, were performance was based on the sum of picking time and digging time combined. The task was created to simulate a pair-wise picking and digging performance were the picker would loosen the dirt for a period of time, before the digger would excavate the dirt. Based on this the picking task consisted of 30 sec picking bouts with a 60 sec pause in-between, while the digging task consisted of 60 sec digging bouts with 30 sec pauses in-between. The picking task starts with the participant straddling a picking simulator holding a sledge hammer (2.7 kg). As the evaluator gave the ‘GO’ signal, the participant started swinging the sledgehammer at the simulator (22 kg) using a picking motion. The length of the simulator was one meter, so as the participant reached the end they are instructed to ‘SWITHC’, when he or she would turn around 180° and continue the picking from the opposite side. The test was terminated as the participant reached a picking distance of four meters. Performance on the task was based on time to completion, with pause time subtracted. The digging task was performed on a digging simulator, comprised of two boxes (50x35x50cm), one empty, the other filled with 180 liters of river rock sized gravel (~1.9 to 3 cm). The test was initiated by a ‘GO’ signal given by the evaluator, were the participant started shovelling gravel from the full box into the empty. As all the gravel was moved from the full to the empty box, the test was terminated, and time to complete the task, minus the pauses were calculated and used as the performance measure.

**Pickets and Wire carry (P&W).** This task was designed to test the participants lift and carry capability during a fence erection, and was performed around a marked course. Weight bars and
a kettle bell simulating pickets and wire, were carried back and forth along the marked course and picked up and put down at different check points. Two weight bars weighing 5.5 kg each was picked up and dropped off six times and was carried for a total distance of 292 meters (25m x 2, 27m x 4, 32m x 2, 35m x 2), a single weight bar weighing 6.6 kg was picked up and dropped off four times and carried for 208 meters (26m x 2, 27m x 2, 35m x 2, 32m x 1), while a kettle bell weighing 16 kg was picked up and dropped off four times and carried for 210 meters (35m x 6). Total unloaded transportation distance between lift and carries was 678 meters. Due to safety reasons carrying pickets and wire, participants where only allowed to walk during the lift and carry task, but were permitted to run during the unloaded transportation phase. Total distance covered was 1388 meters, and the performance measure was time (sec) to complete the course.

**Stretcher Carry (ST).** ST was performed using a deadlift bar, simulating a two-person stretcher carry (50% of casualty weight), which was carried 25 meters back and forth a long a marked course. After carrying the bar 25 meters, the bar was lowered and a 10 sec pause was ordered. Stopping or setting down the bar was not permitted outside of the prescribed rest period. As the 50 meter walk holding the deadlift bar was completed, the participant was instructed to lift an EZ Curl bar on to a box (91.5 cm) simulating a four man stretcher carry lift onto the back of a pickup (25% of casualty weight). Performance on the task was determined by the maximal casualty weight the participant was able to carry around the track and lift onto the box. The test was performed with increasing casualty weight until maximal performance was reached, a total of no more than five trials.

**Vehicle Extrication (VE).** Vehicle extraction was designed to simulate the extraction of a casualty from a Ford Ranger pickup truck. The test started with the participant pulling a mannequin off a bench (80 cm long), before lifting it and performing a backward walking casualty drag for 5 meter around a marked course. At the 5 meter mark, removable legs were detached from the mannequin (equal to 40% of casualty weight) and the participant proceeded to carry the mannequin for another 5 meters before lifting it back onto the box (height 72.6 cm) it was picked up from. Performance on the task was determined by the maximal casualty weight they were able to extricate.
APPENDIX IV: Detailed Descriptions of FORCEcombat Procedures
**Detailed description of FORCEcombat procedures**

The FORCE combat circuit consists of four military tasks, performed in a continuous manner. The four tasks are: 20 meter rushes, sandbag lifts, intermittent loaded shuttles and a sandbag drag. The course is constructed so that the participant can easy and quickly transition between the tasks. A timer is started at the commencement of the 20 metre rushes and stopped once the sandbag drag is completed, and the performance measures is total time to complete the four tasks. Participants will be wearing battle order minus the day pack (military boots and uniform (including cotton undershirt and socks), fragmentation vest, tactical vest, helmet and a Colt 7 replica rubber rifle, for a total external load of ~25 kg) when performing the FORCEcombat test. Prior to starting the FORCEcombat circuit, each participants is given a detailed walkthrough of how to perform each component of the circuit. The measurement of maximal performance on the FORCEcombat will start within 5 min of completion of the loaded march.

**20-meter rushes**

The participant starts by lying face down on the floor in the prone position, holding the replica rifle in their hands. The participant lies facing the opposite end, with their shoulders and hands behind the start line. The evaluator counts down from three to one, before giving the “GO” signal. On the “GO” signal the participants will get up off the floor and run the 10 meters to the first marked line on the floor, 10 meters away. The participant will have to touch the line with their foot, step back, and get down into a prone position, with both hands on the replica rifle. As the evaluator confirms that both hands are on the replica rifle, the “UP” signal is given, and the participants gets up and runs to the next red line on the floor, 10 meters away. Here the participant again touches the line, steps back, and gets into the prone position, with two hands on rifle. Once this position is confirmed by the evaluator, the participant again gets the “UP” signal, gets up, turns around and runs back toward the starting line. As the participant reaches the red line 10 meters away, they will again touch the line with their foot, get into the prone position and follow the same procedure as previously described. The 20-meter rushes task continues until the participant has covered 80 meters, 4x20 meter rushes (Figure S1).
**Figure S1:** Depiction of what the 20 meter rushes course would look like (reproduced from The Force Program Operations Manual (2014)).

**Sandbag Lifts**

Immediately following the completion of the 20 meter rushes, the participant will sling the replica rifle onto their back, and move over to sandbag lift station. When setting up the FORCEcombat test, using marking tape, a shape equal to what seen in **Figure S2**, is drawn on a wall. Two sandbags are place on the floor, 125 cm apart, right on top of the red lines marked on the floor. Each sandbag weighs 20 kg each, and the participant has to use two hands, one on each side of the sandbag when they are lifting. The participant is informed not to use the handle on top of the sandbag prior to starting the FORCEcombat course. The participant must immediately start the sandbag lifts, lifting the first sandbag 100 cm, to touch the horizontal line drawn on the wall. Once the participant taps the redline with the sandbag, the evaluator counts the sandbag, and the participant can drop the sandbag, and shuttle to the side and pick up the next sandbag. The participant keep shuttling side to side, each time picking up a sandbag, and lifting it to tap the red line, before dropping it. This motion is repeated until 30 sandbags have been lifted. The evaluator is counting each sandbag, and monitoring that each sandbag touches the red line. In cases where the participants loses control of the sandbag with one hand, and the bag ends up vertically, the whole bag needs to be lifted above the red line. Touching the red line with a bag hanging vertically will not be approved.
Intermittent Loaded Shuttle

Following the completion of the 30 sandbag lifts, the participants return to the same course used for the 20 meter shuttle. At the starting line, the participant will pick up a sandbag (20 kg), and carry it around the marked course (40 meters). The participant will walk the 20 meter course, red line to red line, carrying the sandbag in their hands, on their shoulder, or using the handle on top of the sandbag. At the end of the course (20 meters) the participant will turn around a pylon placed on the red line, and walk the 20 meters back to the starting line. The participants is prohibited from running while carrying the sandbag. Once the participants crosses the starting line, the participant drops the sandbag, turns around, and completes the same course without the sandbag (unloaded). During the unloaded part the participant is allowed to run. After completing the course unloaded, the participant crosses the starting line (on the way back), picks up the sandbag, and starts another loaded shuttle. The participants completes 5 loaded shuttles, intermitted by 5 unloaded shuttles, for a total of 400 meters. A depiction of the intermittent loaded shuttle task can be seen in Figure S3.
Sandbag Drag Protocol

Following the completion of the last unloaded shuttle, the participant must immediately start the sandbag drag. The sandbag drag will be setup according to what is depicted in Figure S4A and B. The total force required to drag the sandbags on the floor should be equal to 330 newton (~100 kg). The total weight of the drag must be adjusted depending on friction of the floor surface to match 330 newton. The participant starts by picking up the “carry” sandbag, with an underhand grip, cradling the sandbag. The participant precedes to start pulling the sandbags as fast as the can down the 20 meter course, walking backwards. The evaluator are required to guide the participant, to make sure they stay on straight course and don’t walk into anything. The participants continue the drag until the front tip of the sandbag drag (not the participant holding the sandbag, or the sandbag held by the participant) crosses the finish line, as seen in Figure S4B. This marks the spot where the evaluator stops his timer, and the total time to complete the FORCEcombat course is recorded.
Figure S4A and B: Depiction of what the sandbag drag setup (A) and course (B) would look like (reproduced from The Force Program Operations Manual (2014)).

References:
APPENDIX V: Ethics Approval Notice for Thesis Project
To: Mr. Hans Christian Tingelstad, Principal Investigator  
Catherine Paquet, Director, Research Grants and Ethics

4 July 2016

Ref: ORM approval of use and protocol of scientific instruments and physical testing used for Long Duration Loaded March: The Effects of Previous Experience and Heat Exposure on Mechanical Efficiency (File #H05-16-13).

This is to confirm that the Office of Risk Management (ORM) has conducted a detailed review of the methodologies for the physical testing and apparatus involved with Long Duration Loaded March: The Effects Of Previous Experience And Heat Exposure On Mechanical Efficiency (File #H05-16-13) and to inform you of our conclusions.

Applicability of the Regulated Health Professionals Act

As interpreted by external counsel (BLG opinion letter dated 14 Feb 08) and subsequently reviewed and affirmed by internal counsel (Louis Benoit), the Registered Health Professionals Act (RHPA) is applicable only to the provision of health care services. Their opinion is that the RHPA does not apply to research on human subjects of the nature undertaken by professors, staff and students of the University of Ottawa, essentially because these do not involve caring, treating or advising the study participants with respect to their individual health.

However, this is not to suggest that these research activities are without regulatory guidance. The Ontario Occupational Health and Safety Act, provides the broadest standards in its “General Duty” clause. This clause requires the University to take all reasonable precautions to ensure the safety of the study participants.

ORM has regulatory oversight responsibilities for all activities of the University, including teaching and research, under approximately 180 Acts and regulations including the Occupational Health and Safety Act. Professionals from within ORM are assigned responsibility for regulatory compliance for each of the faculties, including Health Sciences.

ORM’s role is to ensure that appropriate precautions are taken for the health and safety of all of the participants. A detailed review of the methodologies and techniques used for the physical testing and apparatus involved with Long Duration Loaded March: The Effects of Previous Experience and Heat Exposure on Mechanical Efficiency was conducted by ORM.

The processes and equipment used are as follow:

- Physical Testing involving equipment such as Field Metabolic System and Bioelectrical Impedance Analysis
1. A series of trials on a calibrated treadmill in an enclosed temperature and humidity controlled metabolic chamber with monitoring of key vital signs through the use of telemetric pills, skin capsules, surface electromyography (EMG), wireless heart rate monitor and a face mask connected to the Field Metabolic System (FMS).

ORM has established with the PI that:

1) The participants will be screened thoroughly to remove any participants that may have a more significant risk factor or elevated discomfort from this protocol such as smokers, persons with a previous history of hyper tension or cardiovascular disease, current or previous back pain, a previous history of medical conditions that may impede cardiovascular performance (such as Atrial Fibrillation). In addition, only participants meeting the pre-established criteria for age, height, weight and VO2max (to be measure prior to commencing study) will be accepted into the study.

2) The participants will be given a detailed description and demonstration of the equipment to be used and the procedures of the study prior to signing an informed consent form.

3) The participants will be thoroughly evaluated prior to commencing the trial, using a Par-Q and American Health Association Physical Activity Readiness Questionnaire as well as an assessment of the body composition and sub maximal leg strength. This will help to identify any potential risk factors relating to health or fitness for this trial that may not have been flagged in the initial screening.

4) A Canadian Armed Forces (CAF) standard fitness test will be administered by either a CAF trainer contracted in for this trial or the PI will personally become qualified to administer this test through training with the CAF.

5) Since this trial involves the consumption of an isocaloric meal, food allergies will also be flagged and either screened out or accommodated with an alternative option.

6) The Principal Investigator (PI) will administer or apply the following sensors or data collection devices: a telemetric pill, skin capsules (using medical tape), wireless heart rate monitor, EMG electrodes and fit the participant with a face mask for the Field Metabolic System. He will be assisted by Research Assistant, Mr. Kyle Gordon with the data collection and equipment preparation.

7) Participants will be continuously monitored throughout the study, not only through the parameters described above, but also visually through one of several windows into the metabolic chamber. Participants can speak to the experimenters and ask for assistance or indicate any issues that may arise at any time during the experiment.

8) The PI, Mr. Tingelstad has a master’s degree in Human Kinetics and has conducted extensive research with identical or similar equipment for a period of 5 years over three studies with the exception of the EMG sensors which will be a novel experience. Mr. Tingelstad has completed 5 hours of training for the use and placement of EMG sensors with Dr. Graham here at the University.

9) All of the sensors are single use with the exception of the heart rate monitor, which will be disinfected with disinfectant spray after use, before being washed in a washing machine.
Conclusion

The ORM has therefore concluded that 1) the methodologies and techniques used for the physical testing and apparatus involved with Long Duration Loaded March: The Effects Of Previous Experience And Heat Exposure On Mechanical Efficiency are appropriate; 2) the personnel charged with the instruction of the participants, conducting the evaluations and carrying out the physiological monitoring have the necessary skills and training to do so properly. This experimental procedure is considered to be of low risk to the participants.

The ORM hereby grants approval for the methodologies and techniques used for the physical testing and apparatus involved with Long Duration Loaded March: The Effects of Previous Experience and Heat Exposure on Mechanical Efficiency by Mr. Tingelstad, assisted by Mr. Gordon. In the event of any modifications to the process, ORM will re-assess and provide specific written approval.