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THE EFFECT OF ACUTE EXERCISE ON MUCOSAL ASSOCIATED INVARIANT T-CELL ACTIVITY IN MODERATELY TRAINED YOUNG MALES

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A thesis defense submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Masters of Arts in the Department of Exercise and Sports Science (Exercise Physiology).

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ABSTRACT

Eli Joseph Danson: The Effect of Acute Exercise on Mucosal Associated Invariant T-Cell Activity in Moderately Trained Young Males
(Under the direction of Erik Hanson)

Purpose: To determine circulating mucosal associated invariant T (MAIT) cell number, frequency and activation and chemokine receptor expression in response to submaximal exercise. **Methods:** 20 young men performed a VO_2max test and then exercised for 40 minutes at 86% of ventilatory threshold (VT). Immune cells were isolated from blood samples obtained at rest and after 0h and 1h of recovery. **Results:** MAIT cell counts significantly increased by 92% (p=0.003) at 0h before returning to baseline at 1h. MAIT cells were preferentially mobilized, rising from 2.9% of T-cells at baseline to 4.5% at 0h and 4.6% at 1h (p=0.002). MAIT cell chemokine and activation marker expression was not affected by exercise. **Conclusion:** MAIT cells follow a biphasic response and are preferentially mobilized within the T cell subsets. MAIT cell numbers expressing activation and homing markers following exercise are higher but are driven by the exercise-stimulated lymphocytosis, rather than intrinsic cellular changes.

To James, Michael and Paxton. May what I do forever honor you.

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LIST OF ABBREVIATIONS

APC – Antigen Presenting Cell

AT – Adipose Tissue

β2m – β2-Microglobulin

CCR – CC Chemokine Receptor

CD – Cluster of Differentiation

CDR – Complementary Determining Region

CTL – Cytotoxic T-Lymphocyte

DXA – Dual Energy X-Ray Absorptometry

Gr – Granzyme

IFN – Interferon

IL – Interleukin

iNKT – Invariant Natural Killer T Cell

LDH – Lactate Dehydrogenase

MAIT – Mucosal Associated Invariant T

MHC – Major Histocompatability Complex

MR1 – MHC Related Protein-1

MS – Multiple Sclerosis

PMA – Phorbol 12-Myristate 13-Acetate

rRL-6-CH_2OH – 6-Hydroxymethyl-8-D-Ribityllumazine

RL-6-Me-7-OH – 7-Hydroxymethyl-6-Methyl-8-D-Ribityllumazine

RPE – Rating of Perceived Exertion

sIgA – Salivary Immunoglobulin A

TCR – T-Cell Receptor

T_h1 – T Helper 1

TNF – Tumor Necrosis Factor

URTI – Upper Respiratory Tract Infection

VT – Ventilatory Threshold

CHAPTER I

INTRODUCTION

The immune system utilizes a variety of cells to maintain a healthy homeostatic environment for all physiological systems. The first line of defense against any pathogen is the innate immune system which protects the body with physical barriers, phagocytic cells and natural killer cells (Treiner et al., 2003). The common mucosal system, another portion of the innate immune system, is made up of tissues which secrete mucosal immune antibodies such as secretory immunoglobulin A (sIgA) to protect vulnerable mucosal surfaces (Gleeson et al., 1999). While each component of the innate immune system has an individualized defense mechanism, each uses the same defense mechanism for all pathogens encountered and can respond to pathogens without previous exposure. Naïve cells of the acquired immune response, T-cells and B-cells, develop a pathogen specific response after encountering a pathogen for the first time. When the pathogen invades the organism at a later date, it is recognized by the now mature T-cells and B-cells triggering the release of a combination of cytokines and antibodies specifically designed to combat and kill the pathogen.

Certain immune cells display characteristics of both the innate and acquired immune systems. One of these cells, mucosal associated invariant T (MAIT) cells, are identified by the

evolutionarily conserved, semi-invariant Vα7.2-Jα33/12/20 T-cell receptor (TCR) which recognizes antigens presented on the major histocompatibility complex (MHC) class I-related (MR1) molecule (Tilloy et al., 1999, Gold et al., 2014). MR1-expressing hematopoietic cells are selected in the thymus triggering the development of the MAIT cell TCR (Martin et al., 2009). After thymic egress, MAIT cells express homing markers CCR5, CCR6, CCR9 and CXCR6 that direct MAIT cells towards mucosal tissue including the liver, gut, spleen and lungs (Dusseaux et al., 2011). MAIT cells in the lungs of mice with *Francisella tularensis*, a bacteria that causes pulmonary infection, had significantly higher expression of CCR5 compared to the other homing markers, highlighting the tissue specific characteristics these homing markers possess (Meierovics et al., 2013).

Once located in the appropriate/specific mucosal tissue, MAIT cells interact with commensal flora and B cells which initiates MAIT TCR adaptations and induces proliferation and ultimately accumulation in the mucosal tissue (Treiner et al., 2003; Martin et al., 2009; Koay et al., 2015). Illuminating the role of commensal flora in MAIT cell accumulation, germ free mice have significantly diminished MAIT cell numbers compared to wild type mice (Treiner et al., 2003). After interaction with commensal flora and B cells, MAIT cells recognize intermediates from the riboflavin biosynthetic pathway, such as 6-hydroxymethyl-8-D-ribityllumazine, bound to MR1 on antigen presenting cells (APCs) (Kjer-Nielsen et al., 2012). MAIT TCR recognition of MR1 with accompanying riboflavin ligand stimulates the upregulation of early activation marker CD69 which prompts the release of cytokines such as: interleukin (IL) 17, IL-2, interferon (IFN) γ, tumor necrosis factor (TNF) α, granzyme B and perforin (Le Bourhis et al., 2013). Thus, MAIT cells have the acquired characteristic of interacting with their environment to cause adaptations while also being able to combat a

pathogen without previous exposure, demonstrating properties of both the innate and acquired immune systems.

MAIT cell counts and function are affect by a number of different diseases. Individuals with irritable bowel diseases have reduced frequency of circulating MAIT cells with a significant increase within injured ileum tissue, along with higher secretion levels of the pro-inflammatory cytokine IL-17, compared to healthy controls (Serriari et al., 2014), though this finding has been recently debated (Hiejima et al., 2015). Similar to irritable bowel disease, obese individuals and diabetics possess lower MAIT cell numbers in the blood with a subsequent increase in the adipose tissue and higher IL-17 production (Magalhaes et al., 2015). Severe asthma reduces MAIT cell counts in both the blood and lung tissue (Hinks et al., 2015). The magnitude of MAIT cell loss was significantly correlated to clinical severity, indicating a potential relationship between MAIT cells and respiratory health.

MAIT cells have the ability to fight a range of bacterial and viral pathogens. The bacteria all have the riboflavin biosynthetic pathway and include *Mycobacterium tuberculosis*, E*scherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* (Ussher et al., 2014). In response to monocytes co-cultured with *Escherichia coli*, MAIT cells up regulate CD69 and secrete IFNγ (Le Bourhis et al., 2010). MAIT cells can also combat dengue, influenza A and hepatitis C viruses and do so through an IL-18, TCR-independent pathway. MAIT cells from individuals with the viruses listed above had higher CD69 and IFNγ expression (Loh et al., 2016, Wilgenburg et al., 2016).

While the effect of exercise on MAIT cells is just now being researched (Hanson et al., in review), many different types of immune cells have been shown to be significantly affected by exercise. Multiple studies have shown the amount of natural killer cells, cytotoxic non-MHC

restricted T-cells, cytotoxic MHC restricted T-cells, Naïve T-helper cells, eosinophils, granulocytes and monocytes increase in the venous blood following an acute bout of exercise, with the magnitude of response dependent on the duration and intensity of the exercise bout. (Hansen et al., 1991; Gabriel et al., 1992; Nieman et al., 1992; Bishop et al., 2014). Natural killer cells and $CD3^+$ (pan T cell marker) cells exhibit a biphasic response, where cell counts increase in the blood immediately after exercise and fall below baseline values during recovery, at high enough intensities (Nieman et al., 1992).

MAIT cells provide a potential novel link between the innate and acquired immune systems as well as the lymphocyte and mucosal immune response to exercise. With a role in all these systems, understanding MAIT cell response to exercise could uncover mechanistic reasoning behind discrepancies in the literature regarding the J-shaped curve. To our knowledge, there are no published studies detailing MAIT cell response to an acute bout of exercise. Preliminary data completed by Hanson and associates (2016) suggests that MAIT cell counts increase after a graded maximal exercise test, but the effect of moderate intensity exercise on MAIT cells immediately after and into recovery is not known (Hanson et al., 2016 unpublished). Thus, any finding in this thesis is novel and can give insight into MAIT cell counts and function after exercise.

Purpose

The purpose of this study is to determine the response of MAIT cell counts to an acute bout of moderate intensity exercise. A secondary purpose of this study is to determine the effects of an acute bout of moderate intensity exercise on MAIT cell activation (CD69) and tissue homing marker (CCR4, 5, 6) expression.

Research Questions

1. Do MAIT cell counts in the peripheral circulation change immediately and one hour after a 40-minute exercise bout at 90-98% ventilatory threshold (VT) on a cycle ergometer?
2. Is there increased activation of MAIT cells immediately and one hour after a 40-minute exercise bout at 90-98% VT on a cycle ergometer?
3. Does MAIT cell homing marker expression change immediately and one hour after a 40-minute exercise bout at 90-98% VT on a cycle ergometer?

Research Hypothesis

1. A 40-minute exercise bout at 90-98% VT on a cycle ergometer will cause an increase in MAIT cell counts immediately after exercise followed by a decline below baseline at one-hour post-exercise.
2. A 40-minute exercise bout at 90-98% VT on a cycle ergometer will cause decreased expression of CD69 in MAIT cells one-hour post-exercise
3. A 40-minute exercise bout at 90-98% VT on a cycle ergometer will cause an increase in CCR5 and CCR6 expression on MAIT cells immediately after exercise followed by a decrease in CCR5 and CCR6 expression on MAIT cells one-hour post-exercise.

Assumptions

1. Participants will follow pre-assessment guidelines.
2. Participants who say they are recreationally active will fit our inclusion criteria.
3. All Participants will answer the medical history questionnaire and physical activity readiness questionnaire truthfully.
4. All Participants will give maximal effort on the graded exercise test.

Delimitations

1. The sample will contain only young men, ages 18 to 35.
2. All Participants will be moderately active, partaking in 30 minutes of rigorous activity as defined by the American College of Sports Medicine, at least 3 times per week.
3. All Participants will have had no contraindications for exercise testing.
4. All Participants will be non-smokers.

Limitations

1. No females will be included in the study limiting the application of results to all individuals
2. Elite athletes will be excluded from the study
3. Totally sedentary individuals will be excluded from the study.

Significance of Study

MAIT cells have the ability to combat bacteria and viruses and appear to play a role in the defense against many diseases, including IBS, obesity, diabetes, tuberculosis and asthma. In response to bacterial and viral infections, MAIT cells release cytokines (TNFα, IFNγ, IL-17 and granzyme B which recruit additional immune cells and kill bacterially and virally infected cells. In many of these disease states, MAIT cell counts are lower than counts in aged matched, healthy individuals. Additionally, in diseased individuals, MAIT cells express higher levels of IL-17 that could trigger inflammation at the affected tissues. All previous research conducted on MAIT cells has been on individuals in a rested state, and the effect that exercise has on MAIT activity has not been elucidated. In this study, we seek to collect preliminary data to assess MAIT cell activity after exercise. Understanding how MAIT cells respond to moderate intensity exercise in

healthy males will allow future studies to observe MAIT cell response to exercise in diseased populations and determine the disease is altering MAIT cell response to exercise. Potential connections can also be made between MAIT cell response to exercise and mucosal health.

CHAPTER II

LITERATURE REVIEW

This review is divided into five sections. MAIT cell protein expression and development is covered in section 1. MAIT cell activation, cytokine profile, and cytotoxic capabilities will be discussed in section 2. MAIT cell response to bacterial and viral infection is discussed in section 3. The effect of a variety of diseases on MAIT cells will be summarized in section 4. Section 5 will review lymphocyte responses to varying levels of exercise.

Section 1: MAIT Cell Protein Expression and Development

MAIT cells express a semi-invariant T-cell receptor (TCR) Vα7.2-Jα33/12/20 on the cell membrane. The MAIT TCR was first characterized after a group of double negative (DN) T-cells had frequent expression of a novel Vα-Jα TCR combination that differed than that normally expressed by invariant natural killer T (iNKT) cells, another DN T-cell (Tilloy et al., 1999). The α chain of the new cells, across all 37 Participants, had a complementary determining region (CDR) 3 of constant length and high Vα and Jα homology to murine and bovine cells, demonstrating strong evolutionary conservation (Tilloy et al., 1999). Tilloy and associates were also able to sequence the Vβ chain of MAIT cells and found a heavy bias towards Vβ13 and Vβ2. While the Vβ region was skewed towards 13 and 2, the Jβ usage or βCDR3 length was not consistent between individuals. The restricted α chains and variable β chains lead Tilloy and colleagues to define MAIT cells as semi-invariant (Tilloy et al., 1999). Furthermore, there were a wide variety of TCR α and β chain combinations indicating that the MAIT TCR diversity most likely results from clonal expansion *in vivo* (Tilloy et al., 1999).

Subsequent research focused on the possibility of greater variation of the Jα and Vβ segments of pathogen-reactive MAIT TCRs. Building on to the findings of Tilloy and associates, Gold and colleagues detected that pathogen-reactive MAIT TCRs had either a Jα33, Jα 12 or Jα20 region (Gold et al., 2014). Additionally, bacteria such as *Salmonella typhimurium* activated MAIT cells with either the Jα33, Jα 12, or Jα 20 region while other bacteria such as *Candida albicans* only activated MAIT cells coding for Jα33 (Gold et al., 2014). Similar findings were made regarding the Vβ gene, the difference being there were 35 different Vβ genes found in pathogen-reactive MAIT cells, demonstrating greater Vβ diversity than first proposed in 1999. While there is some variability of MAIT TCR Jα and Vβ expression within an individual, a greater difference is found between individuals (Gold et al., 2014).

The MAIT TCR recognizes antigens derived from the riboflavin biosynthetic pathway presented on MR1. Elevated levels of folded MR1 with accompanying ligand were measured in cell culture medium RPMI-1640 which contains several vitamin supplements (Kjer-Nielsen et al., 2012). MR1 folding in the presence of folic acid was evaluated and a ligand with mass to charge ratio of 190.03 measured by negative mode electro-spray ionization time-of-flight mass spectrometry was discovered. This mass to charge ratio matched with 6-formyl pterin (6-FP) which was chemically synthesized and shown independently to cause folding of MR1. However, MR1 folded with 6-FP was not able to activate MAIT cells (Kjer-Nielsen et al., 2012). The supernatant of MAIT cells with *Salmonella typhimurium*, which activates MAIT cells, was analyzed and a ligand with a mass to charge ratio of 329.11 was found to complex with MR1 (Kjer-Nielsen et al., 2012). This mass to charge ratio matched up exactly with reduced 6-hydroxymethyl-8-D-ribityllumazine (rRL-6-CH_2OH), and nearly matched 7-hydroxy-6-methyl-8-D-ribityllumazine (RL-6-ME-7-OH) and its precursor 6, 7-dimethyl-8-D-ribitylumazine, all of which are metabolites of the riboflavin biosynthetic pathway (Kjer-Nielsen et al., 2012). All

three metabolites were chemically synthesized and rRL-6-CH_2OH was found to most strongly activate MAIT cells, measured by CD69 expression, TNFα and IFNγ levels (Kjer-Nielsen et al., 2012). Each of the ligands has a different orientation in the MR1 binding cavity, effecting their stimulatory capacity. The ribityl chain of the rRL-6-CH_2OH ligand emerges from the MR1 cavity, establishing hydrogen-bonds with both the CDR3 α and β loops of the MAIT TCR, making the bond between MR1 and the TCR more stable. RL-6-Me-7-OH does not have the ribityl chain binding to the CDR3 loops of the MAIT TCR which partially explains the different stimulatory capabilities of the different ligands (Lopez-Sagaseta et al., 2013).

MAIT cells have been shown to undergo a three-stage intrathymic developmental pathway, with an intra-thymic selection followed by peripheral expansion (Martin et al., 2009; Koay et al., 2016). MAIT cells develop in the thyums, where they undergo positive section and lineage commitment after interacting with MR1-expressing $CD4^+CD8^+$ double-positive thymocytes (Seach et al., 2013). To elucidate the role of MR1 in selecting MAIT cells, healthy fetal liver cells with MR1 expression were transferred into irradiated β2m-deficeint mice and transcripts of Vα19-Jα33 (the murine homolog to human MAIT TCR) were present. Conversely, when β2m deficient liver cells (MR1 expression in cells is dependent on the presence of (Brutkiewicz et al., 1995)) were transported into wild type mice, no vα19-Jα33 transcripts were present, illustrating the need of MR1 expression for MAIT cell selection (Treiner et al., 2003). Stage I MAIT cells have been defined as $CD27^-CD161^-$ as they are exclusive to the thymus and have minimal function (Koay et al., 2016). In the thymus, MAIT cells begin to display a naïve phenotype ($CD45^+$, $CD27^+$) and are also present in cord blood. This population of cells decreases in the blood with age and have been defined as stage II MAIT cells (Martin et al., 2009; Koay et al., 2016). While in the thymus, CD161 expression is low while MAIT cells in human cord blood have similar CD161 expression compared to adult blood MAIT cells, theoretically revealing that

MAIT cells acquire CD161 expression right before their exit from the thymus and remain naïve until birth (Martin et al., 2009).

After thymic egress, stage II MAIT cells interact with B cells, which causes their expansion and accumulation in the periphery. Martin and colleagues (2009) knocked out B cells in mice while maintaining MR1 expression which caused very low MAIT cell counts in the peripheral blood. After injection of splenocytes, which allowed for B cell counts to match wild type numbers, MAIT cell counts in the peripheral blood greatly increased, indicating that B cells are required for MAIT cell development/proliferation (Martin et al., 2009). Similarly, mice were genetically manipulated so that only macrophages and dendritic cells expressed β2m (Treiner et al., 2003). In these mice, there was no expression of Vα19-Jα33 cells in the lamina propria (Treiner et al., 2003). When β2m positive B cells were introduced into these mice, there was a significant increase in Vα19-Jα33 expression in the lamina propria, further demonstrating the role of B cells for MAIT cell peripheral expansion (Treiner et al., 2003). To demonstrate the role of B cells in human MAIT selection/expansion, Vα7.2-Jα33 transcripts within DN T cells was quantified in individuals with mutated Bruton tyrosine kinase which causes significantly fewer B cells in the blood. Significantly lower Vα7.2-Jα33 transcript levels were found in these Participants compared to healthy controls, confirming that B cells are involved in both human and mice MAIT cell selection and expansion (Treiner et al., 2003).

MAIT cells also interact with commensal flora which causes maturation and expansion outside the thymus. Germ free mice have significantly diminished levels of MAIT cells in the peripheral blood and mesenteric lymph nodes compared to wild type mice (Tilloy et al., 1999; Treiner et al., 2003; Le Bourhis et al., 2010). After germ free mice were fed a single strain of either *Enterobacter cloacae*, *Lactobacillus casei*, *Bacteroides thetaiotaomicron*, or

Bifidobacterium animalis, Vα19-Jα33 mRNA in the mesenteric lymph nodes increased to the level found in WT mice, further highlighting the role of commensal flora for MAIT cell expansion and accumulation in peripheral tissue (Le Bourhis et al., 2010). MAIT cells also interact with the transcription factor PLZF which triggers increases in CD161 expression and provides functionality to the MAIT cells (Koay et al., 2016). The findings from the studies reviewed above all support the theory of a three step intra-thymic developmental pathway and that different pathogen associated antigens and exogenous MR1-restricted epitopes presented by the commensal flora drive distinct donor-specific TCRβ clonotype expansions within the MAIT cell population (Gold et al., 2014). With the wide variety of Vβ genes seen in MAIT cells, it appears that MAIT TCRβ clonotypic expansion occurs in a manner similar to conventional T-cells.

Section 2: MAIT Cell Cytokine and Cytotoxic Profile.

MAIT cells secrete T_h1/T_h17 cytokines as well as cytotoxic chemicals. After stimulation with phorbol 12-myristate 13-acetate (PMA)/ionomycin, chemicals used to induce cytokine production, MAIT cells release high levels of IFNγ, IL-2 (both T_h1 cytokines), GrB and TNFα in amounts similar to memory CD8 T cells and IL-17 (a T_h17 cytokine) in amounts similar to CD4 T cells (Dusseaux et al., 2011). Interestingly, a higher percentage of MAIT cells released IL-17 after PMA/ionomycin stimulation compared to CD4 T cells. When stimulated with anti-CD3/CD28-coupled beads, IFNγ and IL-2 production decreased compared to PMA/ionomycin stimulation (Dusseaux et al., 2011).

In addition to activation through MAIT TCR interaction with MR1, MAIT cells *in vivo* are co-activated by IL-12 and IL-18. Stimulation of MAIT cells with IL-12 and IL-18 induced IFNγ expression and the presence of a MR1 blocking-antibody did not affect IFNγ production

(Ussher et al., 2014). In the liver, MAIT cells produced greater levels of T_h1/T_h17 cytokines after mitogen activation compared to blood MAIT cells, demonstrating the possible existence of MAIT cells with functionally distinct cytokine production in different tissues (Tang et al., 2013). Furthermore, both blood and liver MAIT cells had an increase in IFNγ, IL-17, TNFα and IL-2 production after incubation with IL-7 and stimulation with either bacteria or anti-CD3/CD28-coupled beads (Tang et al., 2013).

MAIT cells also display a unique cytotoxic profile. Unstimulated MAIT cells located in the blood of healthy Participants express GrA and GrK while 30% of MAIT cells express perforin and almost all lack GrB expression (Kurioka et al., 2015). Once activated, MAIT cells have decreased expression of GrA and GrK and increased expression of GrB and perforin. GrA and GrK in MAIT cells are located within cytotoxic granules and MAIT cells after activation degranulate as shown by a decrease in CD107α expression which could explain the decreased GrA and GrK expression (Kurioka et al., 2015). As GrA and GrK have been shown to activate monocytes to produce proinflammatory cytokines like IL-1β and TNFα, degranulation of resting MAIT cells may assist in the control of bacterial infection through phagocyte activation (Kurioka et al., 2015). Using anti-MR1 antibody or anti-IL-12 antibody inhibited GrB expression, indicating a similar intracellular pathway for GrB production when activated by MR1 or IL-12 (Kurioka et al., 2015).

Section 3: MAIT Cell Pathophysiological Response

One of the primary roles of MAIT cells in an organism is to combat bacterial infections. A complete list of bacteria that activate MAIT cells can be found in Table 1.

Activating Strains of Bacteria	Non-activating Strains of Bacteria	Activating Strains of Yeast
Eschericia coli *Pseudomonas aeroginosa* *Klebsiella pneumoniae* *Lactobacillus acidophilus* *Staphylococcus aureus* *Staphylococcus epidermidis* *Mycobacterium abscessus* *Mycobacterium tuberculosis* *Salmonella typhimurium* *Shigella dysenteriae* *Enterococcus faecalis*	*Streptococcus* group A *Listeria monocytogenes* *Salmonealla enterica Typhimurium*	*Candida albicans* *Candida glabrata* *Saccharomyces cerevisiae*

Table 1. Strains of bacteria and yeast that activate MAIT cells as well as strains of bacteria and viruses that do not activate MAIT cells. Adapted from Kjer-Nielsen et al., 2012 (Kjer-Nielsen et al., 2012; Le Bourhis et al., 2010; Gold et al., 2010)

In response to monocytes co-cultured with *Escherichia coli*, MAIT cells up regulate CD69 and secrete IFNγ (Le Bourhis et al., 2010). Ussher and colleagues (2014) determined that after a five hour culture with *E. coli*, introducing an anti-MR1 antibody blocked nearly all IFNγ expression. After a 20 hour culture, introduction of anti-MR1 antibody blocked 50% of IFNγ expression while anti-IL-12 and anti-IL-18 also blocked 50% of IFNγ expression, highlighting the kinetic order in which MAIT cells are activated by *E. coli*. MAIT cells also exhibit cytotoxic characteristics after activation by *E coli* (Le Bourhis et al., 2013). *E coli* cells were cultured with parental cell lines having either high MR1 expression or endogenous MR1 expression and MAIT cells were seeded on the MR1-presenting cells. Lactase dehydrogenase (LDH) from *E. coli* was measured after overnight incubation to show membrane permeation. In the culture supernatant with the parent cells with high MR1 expression there was increased levels of LDH (Le Bourhis et al., 2013). When more MAIT cells were added per parent cell cultured with *E. coli*, the amount of LDH in the culture supernatant increased (Le Bourhis et al., 2013). Additionally, in the culture supernatant with endogenous MR1 expressing parent cells, LDH release was limited. Furthermore, when anti-MR1 antibody was added to the culture, LDH levels reduced

significantly (Le Bourhis et al., 2013). Taken together, these results show the cytotoxic potential of MAIT cells in response to *E. coli* and that the cytotoxic response is MR1 dependent.

CD161 has been shown to modulate cytokine production in MAIT cells after culture with *E. coli*. When anti-CD161 antibody was added to a culture with MAIT cells and *E. coli*, CD69 expression was similar to the control conditions while upregulation of CD25 was reduced, indicating the cytotoxic potential of MAIT cells is not significantly modified by CD161 ligation (Le Bourhis et al., 2013). Conversely, in the presence of anti-CD161 antibody, MAIT cells released much lower levels of IFNγ, TNFα, and IL-12, illustrating the role CD161 has in modulating cytokine release in response to *E. coli* (Le Bourhis et al., 2013).

MAIT cells also play a role in the defense against *Mycobacterium tuberculosis* (Mtb), the bacteria which causes tuberculosis and remains a leading cause of infectious disease mortality worldwide (Gold et al., 2010). Mtb-infected A549, a human epithelial cell line that Mtb infects, cultured with MAIT cells resulted in significant IFNγ production by MAIT cells (Gold et al., 2010). When an anti-MR1 antibody was added to the culture, IFNγ production decreased significantly demonstrating the MR1-dependent mechanism for IFNγ release by MAIT cells when activated by Mtb cells. Interestingly, Gold and colleagues (2010) found that antibody concentrations lower than 0.05 μg/ml were able to block MAIT cell release of IFNγ, suggesting that low levels of MR1 expression are needed for MAIT cell recognition. This contradicts the results from Le Bourhis et al., 2013 in which only overexpressing MR1 cells cultured with *E. coli* activated MAIT cells. MAIT cell counts were lower in the blood of individuals with active tuberculosis compared to healthy individuals and trending towards lower MAIT cell counts in latent tuberculosis patients (Gold et al., 2010). While similar MAIT cells counts were found between the lungs and blood of active tuberculosis group, the production of TNFα was

significantly higher for lung MAIT cells, underlining the potential role bacteria can play in tissue specific MAIT cell function (Gold et al., 2010).

Work by Meierovics and colleagues (2013) provided a timeline of MAIT cell responses to bacteria *in vivo*, homing responses to bacteria, and the role of MAIT cells in the recruitment of additional lymphocytes in mice. Mice injected with a live vaccine strain of *Francisella tularensis*, a bacteria that causes pulmonary infection, had a significant increase of MAIT cells in lung tissue 7 days after infection with another significant increase observed 14 days after infection (Meierovics et al., 2013). Compared to the other tissues in the mice, the lungs displayed the highest abundance of MAIT cells, with high expression of CCR2, CXCR3, and CCR5, all of which are tissue homing markers (Meierovics et al., 2013). $MR1^-$ mice with *F. tularensis* had significantly higher bacteria counts and took longer to clear the bacteria out of the lungs compared to $MR1^+$ mice. Additionally, MAIT cells in $MR1^-$ mice had significantly less IFNγ, TNFα, and IL-17, a pro-inflammatory cytokine released (Meierovics et al., 2013; Dusseaux et al., 2011). Interestingly, Meierovics found significantly lower levels of TCRβCD4 and TCRβCD8 cells and significantly lower CD69 expression on the T cells present in $MR1^-$ mice, indicating MAIT cells play at least a partial role in the recruitment and activation of acquired immune cells *in vivo* (Meierovics et al., 2013).

The mechanism behind bacterial infection and interaction remains unclear in the literature. One proposal is that bacteria need to interact with the lysosomal compartment in order to activate MAIT cells. When *Shigella dysenteriae* invade a cell, they are able to interact with the lysosomal compartment and escape to the cytoplasm where their riboflavin derived ligand is able to interact with MR1 and present to the MAIT TCR (Le Bourhis et al., 2013). Conversely, MAIT cells were not activated by *Salmonealla enterica Typhimurium* infected epithelial cells which

invade a cell in a vacuole, preventing fusion with the lysosomal compartment (Le Bourhis et al., 2013). To further justify this claim, more research on bacterial invasion mechanisms and MAIT cell activation needs to be accomplished**.**

MAIT cells have recently been shown to be activated by certain viruses and play a role in their pathophysiology, however there is some conflicting literature. Wilgenburg et al., reported reduced MAIT cell counts in individuals with dengue fever, dengue haemorrhagic fever, influenza virus and hepatitis C virus (Wilgenburg et al., 2016). Wilgenburg and Loh et al. both have demonstrated *in vitro* that influenza A virus induces upregulation of IFNγ, CD69 and GrB by MAIT cells (Loh et al., 2016; Wilgenburg et al., 2016). *In vivo*, MAIT cells had increased CD38, another activation marker, and GrB expression in dengue fever patients, increased GrB expression in influenza patients and increased GrB upregulation in patients with prolonged hepatitis C infection (Wilgenburg et al., 2016).

As it is known that TCR activation is dependent on a metabolite from the riboflavin biosynthetic pathway, the mechanism behind viral activation of MAIT cells was unclear as viruses do not have the riboflavin biosynthetic pathway (Kjer-Nielson et al., 2012). The influenza A virus was described to be IL-18 dependent with limited impact by IL-12 and IL-15 (Loh et al., 2016; Wilgenburg et al., 2016). It has also been reported that dengue fever, dengue haemorrhagic fever and hepatitis C virus also activate MAIT cells through an IL-18 dependent manner, with IL-12 playing an important role in dengue fever and IL-15 playing an important role in hepatitis C virus activating MAIT cells (Wilgenburg et al., 2016). Blocking IL-18 in a culture including APCs with influenza virus and hepatitis C virus significantly decreased IFNγ secretion and CD69 expression. Reduced MAIT cell counts were found in patients with the viruses mentioned above (Wilgenburg et al., 2016). To determine whether MAIT cells possess

direct antiviral function, Wilgenburg et al. mixed the supernatant of activated MAIT cells with hepatitis C infected cells and found hepatitis C virus replication was potently suppressed while diluting the activated MAIT cell supernatant reduced the suppression of hepatitis C virus replication in a dose-dependent manner (Wilgenburg et al., 2016). One study has shown that murine MAIT cells were not responsive to viral infection (Le Bourhis et al., 2010). However, this discrepancy is likely due to the use of murine MAIT cells and the potential that human and murine MAIT cells secrete and response to cytokines differently.

Section 4: MAIT Cell Function in Disease States.

MAIT cell counts and function in the peripheral blood and select tissues are affected by a wide variety of pathological diseases. Individuals with asthma have very low MAIT cell counts in the blood, sputum, and biopsy specimens and MAIT cell counts were correlated to disease severity (Hinks et al., 2015). In healthy individuals, the range of circulating MAIT cells is 0.2-7.6% of CD3 cells with a median of 1.82% (Magalhaes et al., 2015). The median frequency of circulating MAIT cells of non-obese type II diabetic individuals is 0.27%, in obese type II diabetics is 0.10% and 0.05% in obese non-type II diabetics all of which were statistically lower than healthy individuals (Magalhaes et al., 2015). The frequency of circulating MAIT cells was negatively associated with individuals' BMI and positively associated with serum levels of adiponectin and insulin-sensitizing adipokines in severely obese individuals. Additionally, MAIT cell numbers are significantly higher in the omental adipose tissue than in the blood of obese individuals (Magalhaes et al., 2015). Five individuals who had undetectable levels of MAIT cells in the blood had detectable levels in the OM (Malgalhaes et al., 2015).

Obesity and diabetes also affect cytokine production by MAIT cells. After PMA/ionomycin stimulation, blood MAIT cells from type II diabetic individuals produced

significantly higher levels of IL-2, GrB, IL-17, IFNγ, and TNFα compared to healthy controls and obese individuals while obese type II diabetics produced higher levels of these cytokines compared to healthy controls (Magalhaes et al., 2015). Additionally**,** MAIT cells in the omental adipose tissue and subcutaneous adipose tissue of obese individuals produced more GrB and IL-17 while producing less TNFα compared to MAIT cells from the omental adipose tissue of healthy individuals. Likewise**,** there was a higher frequency of MAIT cells expressing IL-17 in obese patients compared to the frequency of IL-17 producing $CD4^+$, $CD8^+$, and double-negative T-cells in healthy controls (Malgalhaes et al., 2015). One explanation for the higher expression of these cytokines is MAIT cells in obese individuals have lower expression of Bcl-2, a protein which increases cellular strength and decreases cell death, and higher expression of Ki67, a proliferation marker (Magalhaes et al., 2015). With diminished Bcl-2 and enhanced Ki67 expression, MAIT cells might be more susceptible to cell death and to compensate for this increase their cytokine production and chronically proliferate to illicit a greater effect in their relatively shortened life span.

Remarkably, bariatric surgery in obese individuals shifts MAIT cell counts and function closer to what is observed in healthy controls. Three months after bariatric surgery, MAIT cell counts increased to the point where they were detectable in 12 individuals who had undetectable MAIT cells counts before surgery (Magalhaes et al., 2015). This increase is one possible reason for improved metabolic and inflammatory status after surgery, as the increase in MAIT cells is correlated with adiponectin, a hormone with anti-inflammatory properties (Magalhaes et al., 2015). Similarly, MAIT cells produced less GrB, IL-2 and IL-17 three months after surgery compared to before surgery. Six months after surgery, GrB production was comparable to healthy controls, IL-2 production was lower compared to healthy controls, and IL-17 production increased compared to healthy controls (Magalhaes et al., 2015).

Similar changes in MAIT cell counts and function are seen in individuals with irritable bowel diseases, though there is some disagreement. MAIT cell counts in the peripheral blood were significantly lower in individuals with Crohn's disease and ulcerative colitis (Serriari et al., 2014; Hiejima et al., 2015). While Serriari et al. reported MAIT cell counts to be significantly higher in ileal biopsies, jumping from 1.5% of $CD3^+$ cells to 6.6% of $CD3^+$ cells, Hiejima et al. reported lower MAIT cell counts in the inflamed tissue of individuals with Crohn's disease and ulcerative colitis (Serriari et al., 2014; Hiejima et al., 2015). One hypothesis for the discrepancy is that there are two subpopulations within the Crohn's disease group, one with normal MAIT cell counts and one with lower MAIT cell counts and the study conducted by Hiejima had more samples to study so they had a higher number of the low MAIT cell count group. MAIT cells in the blood of Crohn's disease and ulcerative colitis patients had higher expression of Ki67, IL-17, and IL-22 along with elevated expression of NKG2D and BTLA, co-stimulatory molecules of MAIT cells (Serriari et al., 2014). It has been hypothesized that the higher activation is due to the change in the mucosal barrier in these individuals which results in presentation of commensally-derived antigens by MR1 which do not take place in healthy physiological situations. Alternatively, identical ligands may be presented by healthy individuals and patients with irritable bowel disease, but the inflammatory milieu in the diseased state provides an additional signal either through co-stimulation or humoral factors causing MAIT cell activation (Serriari et al., 2014). Additionally, individuals with Chron's and ulcerative colitis had higher percentages of activated caspase-expressing MAIT cells, a molecule associated with the start of cell death (Hiejima et al., 2015). Finally, while the increased expression of IL-17 may contribute to the proinflammatory state in AT of obese individuals, the higher levels of IL-17 in irritable bowel disease individuals may be playing a protective role as blocking IL-17 receptors has been shown to exacerbate the irritable bowel diseases (Marwaha et al., 2012).

MAIT cells in individuals with multiple sclerosis (MS) display some of the same characteristics of MAIT cells in obese, diabetic, and irritable bowel disease individuals. No significant difference existed in total MAIT cell counts in the blood of healthy individuals and MS individuals (Illes et al., 2004). Conversely, Annibali and colleagues (2011) found there were higher $CD161^{+}CD8^{+}$ (higher cytokine producing) MAIT cell counts in the blood of individuals with MS compared to healthy donors (Annibali et al., 2011). This discrepancy could be attributed to the fact that $CD161^{+}CD8^{+}$ MAIT cells were not yet identified during Illes' study. MAIT cells were located in chronic inflammatory demyelinating polyneuropathy lesions, in infiltrated autopsy region of the central nervous system, cerebral spinal fluid samples, and peripheral nervous system lesions in MS individuals, demonstrating the hypothetical functional capacity of MAIT cells in the central nervous system (Illes et al., 2004). Similar to obesity, diabetes, and irritable bowel diseases, IL-17 production was increased in MS individuals compared to healthy controls (Annibali et al., 2011). Illes and colleagues speculated that a reason for increased MAIT cell counts in the lesions was due to the high number of B cells in inflammatory lesions (Illes et al., 2004). As described previously, MAIT cell expansion and accumulation is dependent on B cells, which justifies Illes hypothesis.

MAIT cells are also affected by the presence of tumors. In kidney tumors, 7 out of 8 individuals expressed the Vβ2 and Vβ13 mRNA while in brain tumors, 3 out of 6 patients expressed Vβ2 and Vβ13 mRNA (Peterfalvi et al., 2008). Additionally, different mRNA cytokine expression was discovered in the tumors, with kidney tumors expressing TNFα, IFNγ and IL-17 while brain tumors did not express the mRNA of IL-17 or IFNγ but did express TNFα (Peterfalvi et al., 2008). Furthermore, 7 out of 8 kidney tumors and 4 out of 6 brain tumors expressed MR1, revealing that there may be local activation by ligands occurring in the tumors which contribute to the cytokine environment and potential cytotoxicity of the MAIT cells

(Peterfalvi et al., 2008). In total, these results indicate tumors located in different tissues recruit functionally distinct MAIT cells which could provide mechanistic reasoning for varying inflammatory responses in tumors.

Of clinical relevance, MAIT cells counts are unaffected by certain chemotherapy treatments. Individuals undergoing 6 cycles of anthracycline containing chemotherapy for breast cancer had minimal decreases in MAIT cells after the third cycle of treatment and remained stable 1 month after the sixth cycle (Dusseaux et al., 2011). By comparison, conventional memory CD8 T cells significantly decreased in number by the sixth cycle compared to baseline before slightly recovering after chemotherapy. Additionally, all conventional CD4 and CD8 T cells remained decreased after treatment (Dusseaux et al., 2011). Mechanistically, Dusseaux proposed that MAIT cell survival after anthracycline containing chemotherapy was attributed to high expression of ABCB1, a multidrug efflux protein that effluxes anthracycline (Dusseaux et al., 2011).

Section 5: Lymphocyte Response to Exercise.

There is a general consensus among exercise immunologists that lymphocytes exhibit a biphasic response to exercise and the magnitude of lymphocyte response to exercise is relative to exercise duration and intensity. In response to high intensity, short duration bouts of exercise, there is a lymphocytosis observed immediately after exercise sometimes followed by a lymphopenia during recovery. In 7 healthy, recreationally active men running at maximum speed at varying distances (1.7 km, 4.8 km, 10.5 km), there was a 105.8%, 90.5% and 84.1% increase in total lymphocytes immediately after exercise followed by a 32-39% decrease in lymphocytes below baseline values for all running conditions (Hansen et al., 1991). Similar results were seen in 10 healthy recreationally active men completing a modified-Wingate cycle test with the

majority of the lymphocytosis increase resulting from a 176% increase in NK cells (Nieman et al., 1992). Lymphopenia was observed 1-hour post-test because of a sharp decrease in $CD4^+$ T cells and a moderate decrease in $CD8^+$ T cells and NK cells (Nieman et al., 1992). Preliminary data completed by Hanson and colleagues (2016) suggests that MAIT cell counts increase after a graded maximal exercise test (Hanson et al., 2016 unpublished).

It has also been established that lymphocytes exhibit a biphasic response in response to moderate intensity, endurance exercise. 14 healthy individuals performed a five minute warm up on a cycle ergometer at 50% of anaerobic threshold followed by cycling to exhaustion at 100% anaerobic threshold (Gabriel et al., 1992). During the first 10 minutes of exercise, lymphocyte counts had increased by 47% followed by an additional 10% increase between 10 minutes and the end of the exercise trial. The majority of the increase in lymphocyte counts after 10 minutes of exercise, similar to Nieman (1992), could be attributed to a large (+229%) increase in NK cell counts (Gabriel et al., 1992). NK cell counts did not significantly increase throughout the remainder of the exercise trial. Naïve cytotoxic T cells and non-MHC restricted T cells also increased after the moderate intensity endurance bout while mature cytotoxic cells and naïve helper cells did not change (Gabriel et al., 1992). These results suggest that high intensity exercise is needed to trigger cytotoxic T cell increases. A similar lymphocytosis was observed in nine, non-elite male cyclists completed a 60 minute exercise bout on a cycle ergometer at 75% VO_2peak while lymphopenia was observed 2 hours into recovery (Bishop et al., 2014). A summary of additional lymphocyte responses to exercise can be found in Table 2.

The primary reason for lymphocytosis after exercise is due to increased catecholamines and specifically epinephrine in peripheral blood. After a maximal exercise test, epinephrine increased 2-fold in 9 healthy, recreationally active males. In this subject group, β-adrenergic

receptors were measured and NK cells had the greatest concentration of β-adrenergic receptors, followed by cytotoxic T cells, while helper T cells had the lowest (Maisel et al., 1990)**.** After exercise, NK cells were the only lymphocyte subset to have a significant increase in β-adrenergic receptors (Maisel et al., 1990). Work done by Kruger and colleagues in mice models demonstrated that introducing a β-adrenergic receptor antagonist before exercise blocked any changes in lymphocyte tissue redistribution (Kruger et al., 2008). Specifically, before administering the antagonist, there was significant increase after exercise in lymphocytes in the blood and lungs of the mice while a significant decrease in lymphocytes from the spleen, all of which was not observed after the antagonist was administered (Kruger et al., 2008).

Author	Participants	Time and Intensity	Pre Exercise T-cell counts (10^9)	Immediately After Exercise T-cell counts	1-h After Exercise T-Cell Counts
Vider 2001	19 endurance trained athletes	VO_{2max} test	1.30 ± 0.35	2.09 ± 0.63 *	1.13 ± 0.24 *
Nieman 1992	10 recreationally active men	30 second Wingate test	1.89 ± 0.18	2.00 ± 0.20 *	1.24 ± 0.15 *
Nieman 1994	10 well-conditioned men	45 minutes at 80% VO_{2max}	1.70 ± 0.18	2.60 ± 0.27 *	0.95 ± 0.10
Nieman 1994	10 well-conditioned men	45 minutes at 50% VO_2max	1.72 ± 0.14	1.78 ± 0.19 *	1.16 ± 0.08
Nieman 1994	10 well-conditioned men	45 minutes at 60% VO_2max	1.50 ± 0.06	1.63 ± 0.07 *	1.43 ± 0.09

Table 2. Summary of biphasic response of lymphocytes to varying exercise bouts. * denotes $p < 0.05$

Summary

MAIT cells are defined by the semi-invariant T-cell receptor (TCR) Vα7.2-Jα33/12/20 which recognizes metabolites from the riboflavin biosynthetic pathway bound to the nonpolymorphic class Ib major histocompatibility complex (MHC)-related molecule 1 (MR1).

MAIT cells primarily fight bacterial infection through the release of T helper 1 (T_h1) and T_h17 cytokines as well as perforin and a collection of granzymes (Gr). MAIT cell counts and function are also affected in the blood and tissue of individuals with different diseases. Lymphocytes exhibit a biphasic response to exercise, with the magnitude of response dependent on intensity and duration of the exercise bout. NK cells account for a significant portion of the lymphocytosis while CD4 and CD8 cells account for a large portion of the lymphopenia.

There has been no published research looking at the effects of exercise on MAIT cells. Preliminary data completed by Hanson and associates suggests that MAIT cell counts increase after a graded maximal exercise test (Hanson et al., 2016 unpublished). Understanding MAIT cell response to exercise could help answer many of the discrepancies found in exercise immunology literature because they share characteristics of NK cells, cytotoxic T cells, helper T cells, and are located within the common mucosal system.

CHAPTER III

METHODOLOGY

Subjects

20 males, ages 18-35 completed the study. All participants were recreationally active having participated in vigorous activity outlined by the American College of Sports Medicine for 30 minutes at least three times per week (CDC: General Physical Activities). All participants were non-smokers. Exclusion criteria included: any contraindications for exercise testing, a body mass index greater than 30 and consumption of therapeutic corticosteroid medication. The study was approved by the University of North Carolina at Chapel Hill's institutional review board. Participants signed an informed consent form prior to participation in the study.

Study Design

In visit one, participants filed out a medical history questionnaire, physical activity readiness questionnaire (PAR-Q) and informed consent was obtained. Participants had body composition measured via dual energy X-ray absorptiometry (DXA) (Hologic Inc., Bedford, MA, USA; Apex Software Version 3.3), underwent a 12-lead resting electrocardiogram (EKG) (GE CASE Cardiosoft V. 6.6 ECG diagnostic system; General Electric, Palatine, IL, USA) and a familiarization session was also completed

During visit two, participants completed a graded exercise test to determine ventilatory threshold (VT) and VO_2max. Participants reported back to the lab approximately 3 to 7 days after the first visit. During visit three, participants had a cannula inserted into the arm where blood

was drawn. After the collection of resting blood samples, participants completed a moderate intensity exercise trial lasting 40 minutes at 86% VT. Additional blood samples were obtained immediately after and one hour after the exercise trial.

Immune cells were isolated via density gradient centrifugation and treated with an antibody cocktail and analyzed via flow cytometry to determine MAIT cell count, activation levels, and expression of homing markers. Plasma was isolated from remaining whole blood and frozen at -80C for future analysis. Summary of study design found in Figure 1.

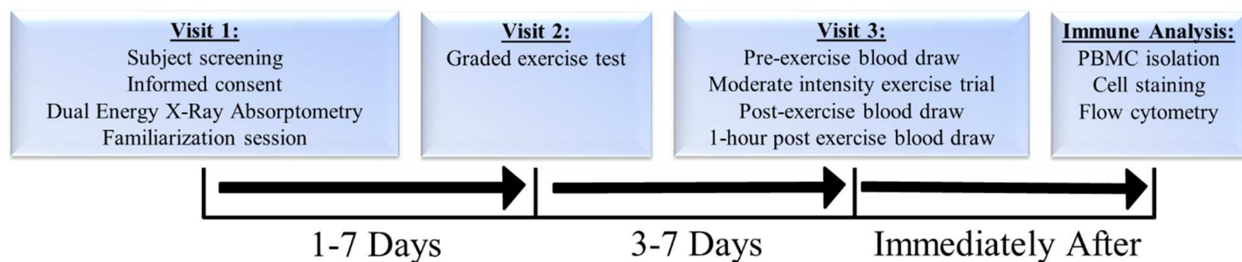


Figure 1. Summary of study design.

Body Composition Assessment

Participants reported to the lab and confirmed with researchers that pre-assessment guidelines were followed. Participants height (Perspectives Enterprises, Portage, MI, USA) and body mass (Tanita Corp, Tokyo, Japan) were measured. Participants were centered on the DXA table in the supine position and removed any metal or jewelry prior to the scan. A full body DXA scan was taken where fat mass (FM), fat free mass (FFM), and bone mineral content (BMC) were measured.

Familiarization Session

Participants completed the familiarization session on an electro-magnetic breaking cycle ergometer (Lode, Groningen, Netherlands). Cycle ergometer seat height was adjusted to ensure proper cycling mechanics. Participants were fitted with a heart rate monitor (Polar FT1, Polar USA, Port Washington, NY, USA) for heart rate telemetry and a respiratory mask for gas

collection. Oxygen uptake and pulmonary ventilation were continuously recorded by a Parvo Medics' TrueOne 2400 metabolic cart (Parvo Medics, Sandy, UT, USA). Participants began the test by sitting quietly on the cycle ergometer for three minutes while the researchers collected resting metabolic data. Participants began with a two minute warm up period on the cycle ergometer at no resistance. At the end of the warm up, participants started a two minute stage at 50 watts of resistance. Every two minutes, resistance was increased by 50 watts. This continued until the end of the 250 watt stage. At this point, stages were one-minute-long and resistance was increased by 30 watts. Participants cycled until 70% heart rate reserve was reached determined by the Karvonen formula (Karvonen et al., 1957) during the familiarization session.

Graded Exercise Test

During visit two, participants completed a graded exercise test on an electro-magnetic breaking cycle ergometer (Lode, Groningen, Netherlands). The graded exercise test protocol used in the study was the same protocol used in Kerry Martin's thesis (Martin 2016). Subject's height (Perspectives Enterprises, Portage, MI, USA) and body mass (Tanita Corp, Tokyo, Japan) was measured and seat height was adjusted ensure proper cycling mechanics. Participants sat for five minutes after which resting heart rate and blood pressure (Omron Healthcare, Lake Forest, IL, USA) were obtained. Participants were fitted with a heart rate monitor for heart rate telemetry and a respiratory mask for gas collection**.** Heart rate was logged every 30 seconds and rating of perceived exertion (RPE) was logged 30 second before the end of each stage during the exercise test on a data collection sheet (Appendix A1). Oxygen uptake and pulmonary ventilation were continuously recorded by a Parvo Medics' TrueOne 2400 metabolic cart (Parvo Medics, Sandy, UT, USA).

The protocol for the graded exercise test matched the familiarization protocol with the only difference that termination of the test was determined by the participant reaching volitional

fatigue and signaling to stop the test, if the VO_2 value plateaued or decreased with an increase in exercise intensity, or if an abnormal participant response to the test was observed. At the conclusion of the graded exercise test, participants were moved from the cycle ergometer to a chair where their feet were propped on a second chair. Three minutes after terminating the exercise test, one finger was cleaned by two alcohol swabs, dried with a gauze pad, and pricked with a lancet. A single drop of blood was pooled on the finger and wiped away. A second drop of blood was pooled on the finger and drawn onto a test strip in a Lactate Plus Analyzer (Nova Biomedical, Waltham, MA, USA). After lactate was measured, participants were moved back to the cycle ergometer to complete a two minute cool down at minimal resistance (~ 25 Watts). Criteria established for VO_2max was that set forth by Howley et al. (Howley et al., 1995).

VT was determined by adjusting the graph plotting the ventilatory equivalent of O_2 (V_E/VO_2) and the ventilatory equivalent for CO2 (V_E/VCO_2) from the Parvo Medic program as described by Gaskill et al (Gaskill et al., 2001).

Blood Draws

Participants reported back to the laboratory 3-7 days later after visit two and were overnight fasted. Participants confirmed with researchers that pre-assessment guidelines were followed. Participants had a peripheral venous catheter indwelling venous catheter (BD Bioscience, Franklin Lanes, NJ, USA) inserted into the arm and secured with Tegederm™ dressing (BD Bioscience, Franklin Lanes, NJ, USA). Approximately 24 mL of blood was drawn into blood collection tubes containing EDTA (BD Bioscience, Franklin Lanes, NJ, USA). The blood collection tube were inverted three times and placed on ice for the duration of the visit. Sterile saline was flushed through the catheter to ensure that clotting did not occur in the line. Blood was drawn through catheter at 0H and 1H following same procedure described above. The participant sat in the lab for 1 hour after the exercise test consuming water *ad libitum* and

limiting their movements. After the third blood draw, the cannula was removed and pressure was directly applied to the puncture site for 1 minute after which a Band-Aid was placed.

Moderate Intensity Exercise Trial

Participants were fitted with a heart rate monitor and a respiratory mask connected to the metabolic cart. The protocol used was modified from Bishop et al (2014). Participants began a warm up period consisting of two minutes at 25 watts followed by two minutes at 50 watts at a self-selected cadence. At the end of the warm up, resistance will be increased to the wattage at which 90% VT occurred. If participant Wattage was set to be greater than 150 Watts, subjects were also given 30 seconds at 100 Watts to avoid a resistance increase of greater than 100 Watts. Participants cycled at this resistance until 86% VT was reached for two time points on the Parvo cart set at five second average calculation. At this point, participants cycled for 10 minutes while oxygen uptake and pulmonary ventilation were monitored to keep participants between 90-98% VT. Respiratory gases were collected for the first 10 minutes of the trial followed by additional five minute collections taken at 20 and 35 minutes. Resistance was adjusted so that the subject remained within the 5% of the desired VT (Figure 2). Participants cycled without the respiratory mask from minutes 10-20 and 25-35 of the trial. Resistance was not altered while the mask was off. Participants were be allowed to drink water *ad libitum* while not wearing the respiratory mask during the trial.

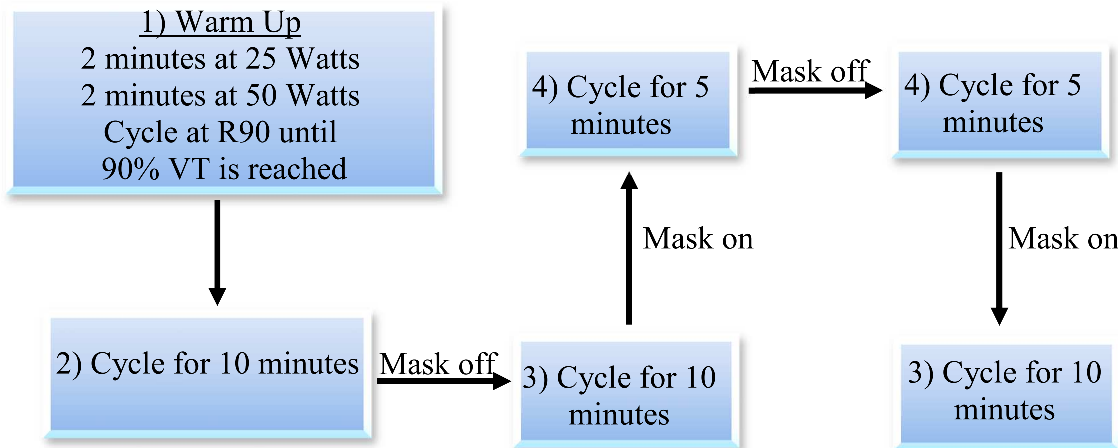


Figure 2. Protocol for moderate intensity exercise trial.

Blood Analysis

Peripheral bound mononuclear cells (PBMCs) were isolated using SepMate^{TM}-50 (Stemcell, Vancouver, BC Canada) as specified by the manufacture (Appendix A3). PBMCs were used for immune cell phenotyping to conduct analysis of MAIT cell profile.

MAIT cell phenotyping was prepared by direct immunofluorescence labeling of cell surfaces with mouse anti-human monoclonal antibodies. Cells were analyzed using flow cytometry (BD Biosciences, CA USA) and FlowJo CE software (Figure 3). Antibodies used included CD3 (APC-Cy7), CD4 (BV510), CD8 (AF700), CD14 (PE-Dazzle594), CD45 (PerCP-Cy5.5), CD66 (APC), CD69 (AF488), CD161 (BV605), TCR Vα7.2 (PE), CCR5 (BV421), CCR6 (BV650), CCR4 (PE-Cy7) all of which were from Biolegend except CD66 which was ordered from Nexus. Titration was run on all antibodies to determine optimal concentrations. Appropriate fluorescence minus one (FMO) controls were run. CD14 and CD66 was used in combination during gating to ensure desired lymphocyte population does not consist of monocytes or neutrophils. AbC™ Total Antibody Compensation Bead Kit was used for CD69, CCR4, CCR5 and CCR6 compensation controls (Thermo Fisher Scientific, NC USA).

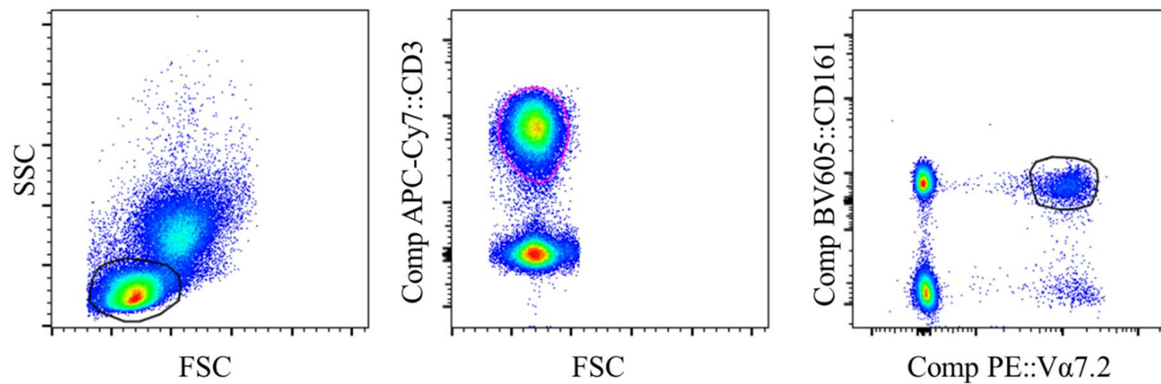


Figure 3. Gating strategy for MAIT cell identification. Side scatter-Forward scatter plot was used to select lymphocytes. T cells were identified using $CD3^+$ cells. MAIT cells were identified as $V\alpha7.2^+CD161^+$ cells. Additional gates were then used to look at CD69, CCR4, 5 and 6 expression within MAIT cells and $CD8^+V\alpha7.2\ CD161^+$ cells.

Statistical Analyses

Data collected was analyzed with SPSS Statistics version 21.0 (SPSS, IN., Durham, NC, USA). The α level was set *a priori* for all statistical procedures at $\alpha=0.05$. Descriptive statistics were used to summarize subject characteristics and values were reported as mean ± SD. A one-way, repeated measures ANOVA was used to compare MAIT cell counts, activation marker expression and homing marker expression between three separate time points: pre-exercise, immediately after exercise, one hour after exercise. Bonferroni post-hoc analysis was used. Additionally, a Pearson product-moment correlation coefficient was calculated comparing MAIT cell counts, activation markers, and homing marker expression.

CHAPTER IV

RESULTS

Participants

20 young, healthy males with moderate aerobic fitness completed the study (Table 3). The 40 minute acute bout was completed with an average power output of 152 (30) Watts and at 63.5% (5.3) of VO_2max and 86% (18.0) of ventilatory threshold.

Table 3. Participant characteristics (n=20).

	Average (SD)	Range
Age (y)	22 (4)	18 - 34
Height (cm)	181.1 (5.4)	169.6 - 190.3
Mass (kg)	78.2 (9.6)	61.2 - 92.5
Fat Mass (kg)	13.6 (4.3)	9.2 - 23.0
Lean Mass (kg)	60.7 (8.3)	46.5 - 74.4
Body Fat (%)	17.6 (4.8)	11.3 - 27.8
VO_2max (ml/kg/min)	51.3 (9.9)	38.2 - 67.1
Ventilatory Threshold (L/min)	2.6 (0.6)	2.0 - 3.9
% VT during moderate intensity trial (%)	86.0 (18.0)	61.3 - 130

Mean (SD)

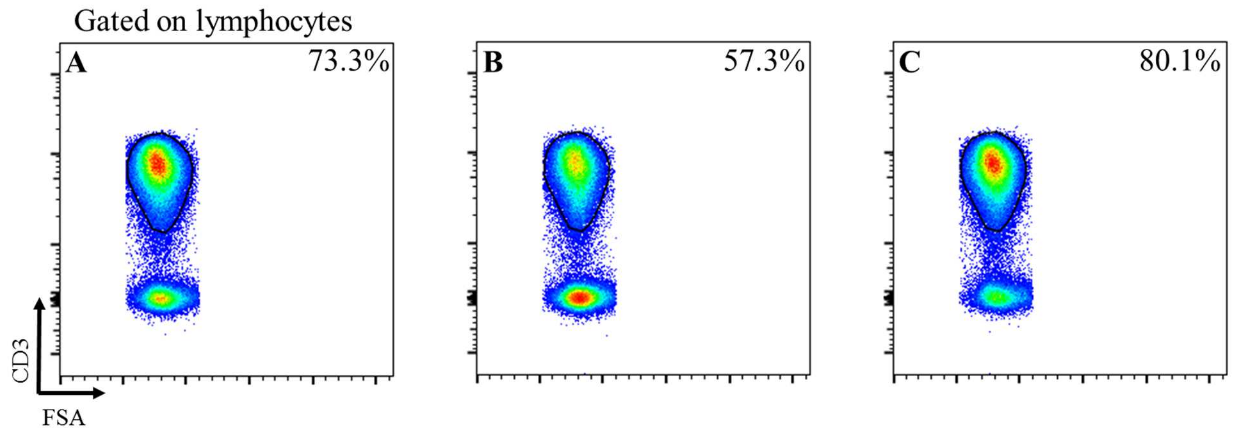
Changes in lymphocytes and conventional T-cells

Acute exercise increased lymphocyte counts by 44% (33.9) from baseline to 0h ($p < 0.001$) before decreasing significantly below baseline values at 1h by -33.7% (19.3) ($p < 0.001$ Table 4).

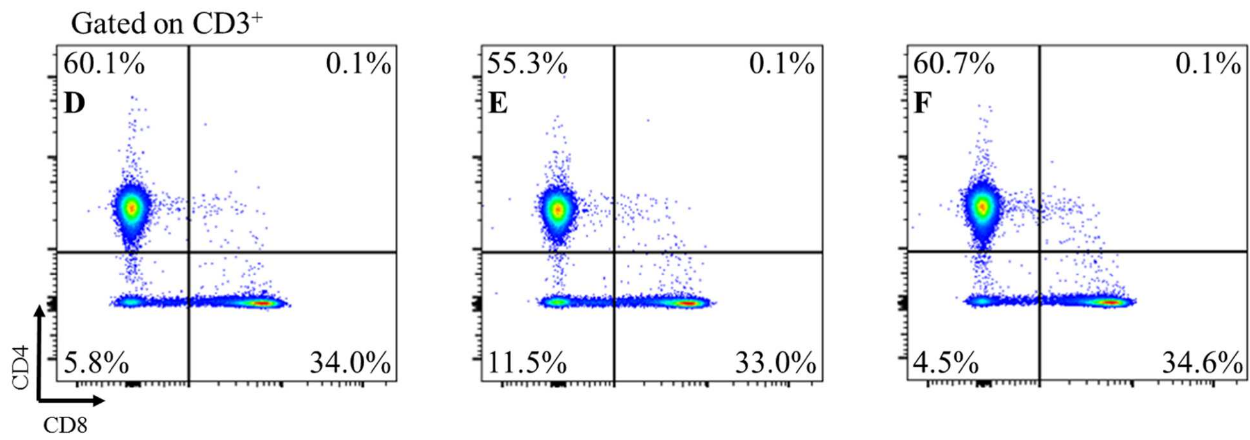
Table 4. Complete blood count completed on whole blood			
	Baseline	Immediately Post	One-Hour Post
Red blood cells (x10^6 cells/µl)	5.1 (0.4)	5.5 (0.4) ^{α}	5.2 (0.4)
White blood cells (x10^3 cells/µl)	6.5 (1.6)	8.7 (2.1) ^{α}	7.4 (2.4)
Lymphocytes (x10^3 cells/µl)	1.9 (0.6)	2.7 (0.7) ^{α}	1.3 (0.3) ^{α}
Monocytes (x10^3 cells/µl)	0.5 (0.3)	0.8 (0.3) ^{α}	0.4 (0.2)
Granulocytes (x10^3 cells/µl)	4.2 (1.5)	5.4 (1.7) ^{α}	5.6 (2.3) ^{α}
Hemoglobin (g/dl)	14.3 (1.0)	15.8 (1.0) ^{α}	14.7 (1.1)
Hematocrit (%)	45.1 (2.8)	49.1 (3.0) ^{α}	45.7 (3.3)
Platelets (x10^3 platelets/µl)	223.3 (52.5)	280.5 (69.1) ^{α}	207.2 (51.2) ^{α}

Mean (SD). ^{α} = $p < 0.05$ compared to baseline

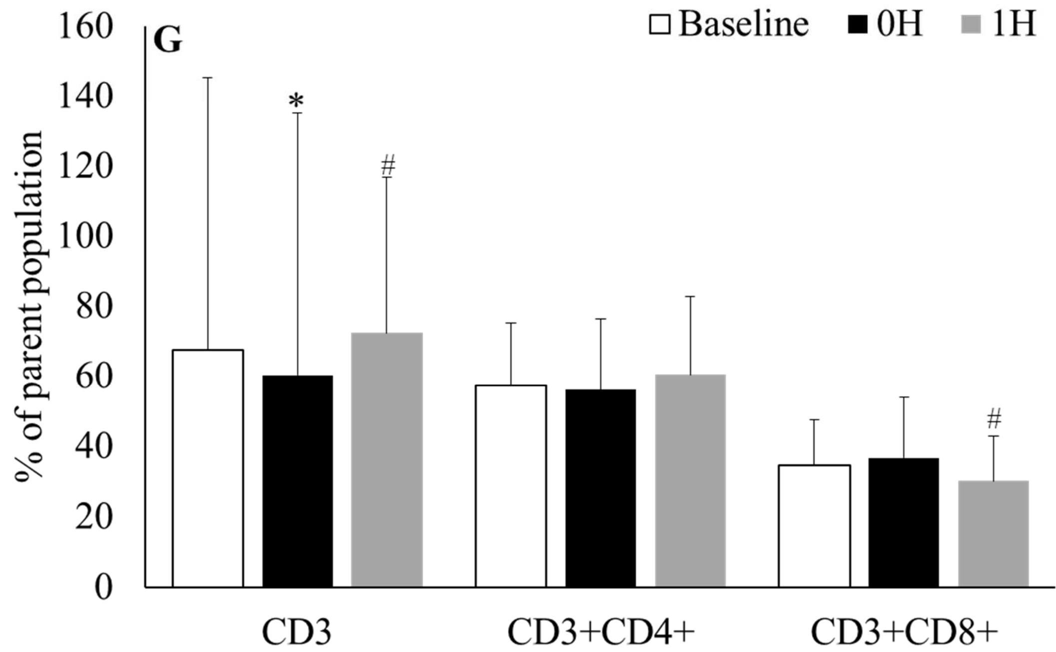
The percent of lymphocytes that were $CD3^+$ cells decreased from baseline to 0h ($p <0.001$), increased from 0h to 1h ($p = 0.028$) and increased from baseline to 1h ($p=0.001$; Figure 4A, 4B, 4C, 4G). $CD3^+$ T-cell counts significantly increased by 27% (25.6) from baseline to 0h ($p < 0.001$) and significantly decreased by 22.1% (19.5) from baseline to 1h ($p = 0.001$; Figure 4H). $CD4^+$ and $CD8^+$ sub-populations were determined at all three time points by gating on $CD3^+$ cells (Figure 4D, 4E, 4F). $CD8^+$ cells comprised a significantly smaller percent of all $CD3^+$ cells at 1h compared to baseline ($p = 0.021$; Figure 4G) Moderate intensity acute exercise caused a 32.8% (30.8) increase in $CD3^+CD8^+$ cytotoxic T-lymphocyte (CTL) counts from baseline to 0h ($p < 0.001$) followed by a significant decrease below baseline values at 1h by 32.4% (23.7) ($p = 0.008$; Figure 4H). $CD3^+CD4^+$ helper T-cell counts significantly increased by 21.8% (28.2) from baseline to 0h ($p < 0.001$) and significantly decreased by 15.9% (29.2) from baseline to 1h ($p = 0.035$; Figure 4H).



Gated on lymphocytes
A
73.3%
B
57.3%
C
80.1%
CD3
FSA



Gated on CD3+
60.1%
0.1%
D
5.8%
34.0%
55.3%
0.1%
E
11.5%
33.0%
60.7%
0.1%
F
4.5%
34.6%
CD4
CD8



G
Baseline
0H
1H
% of parent population
160
140
120
100
80
60
40
20
0
*
#
#
CD3
CD3+CD4+
CD3+CD8+

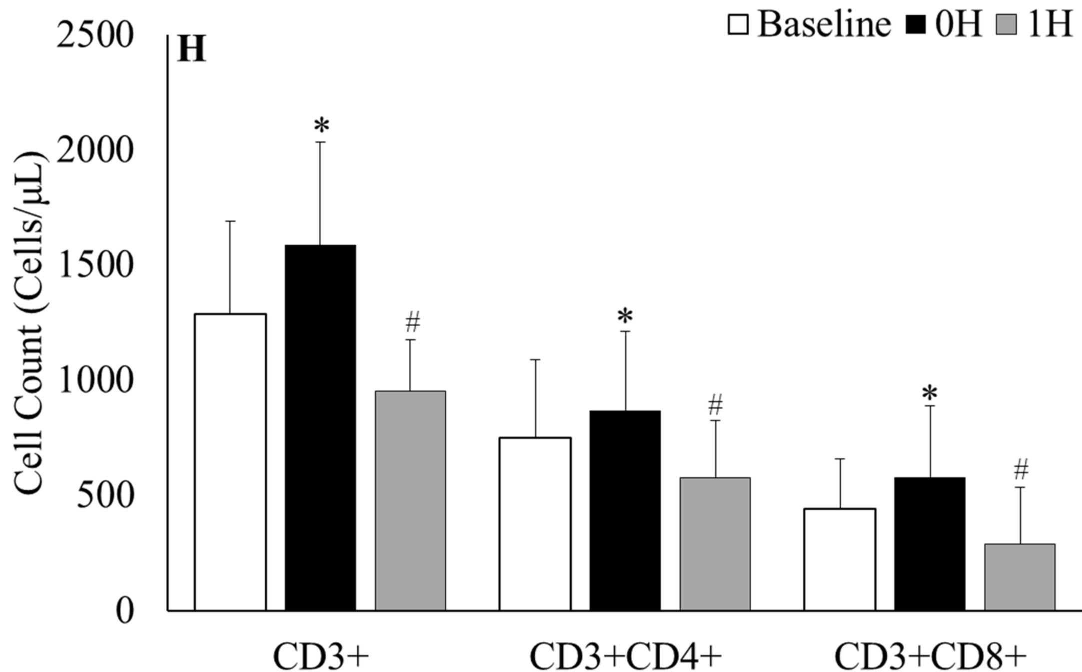
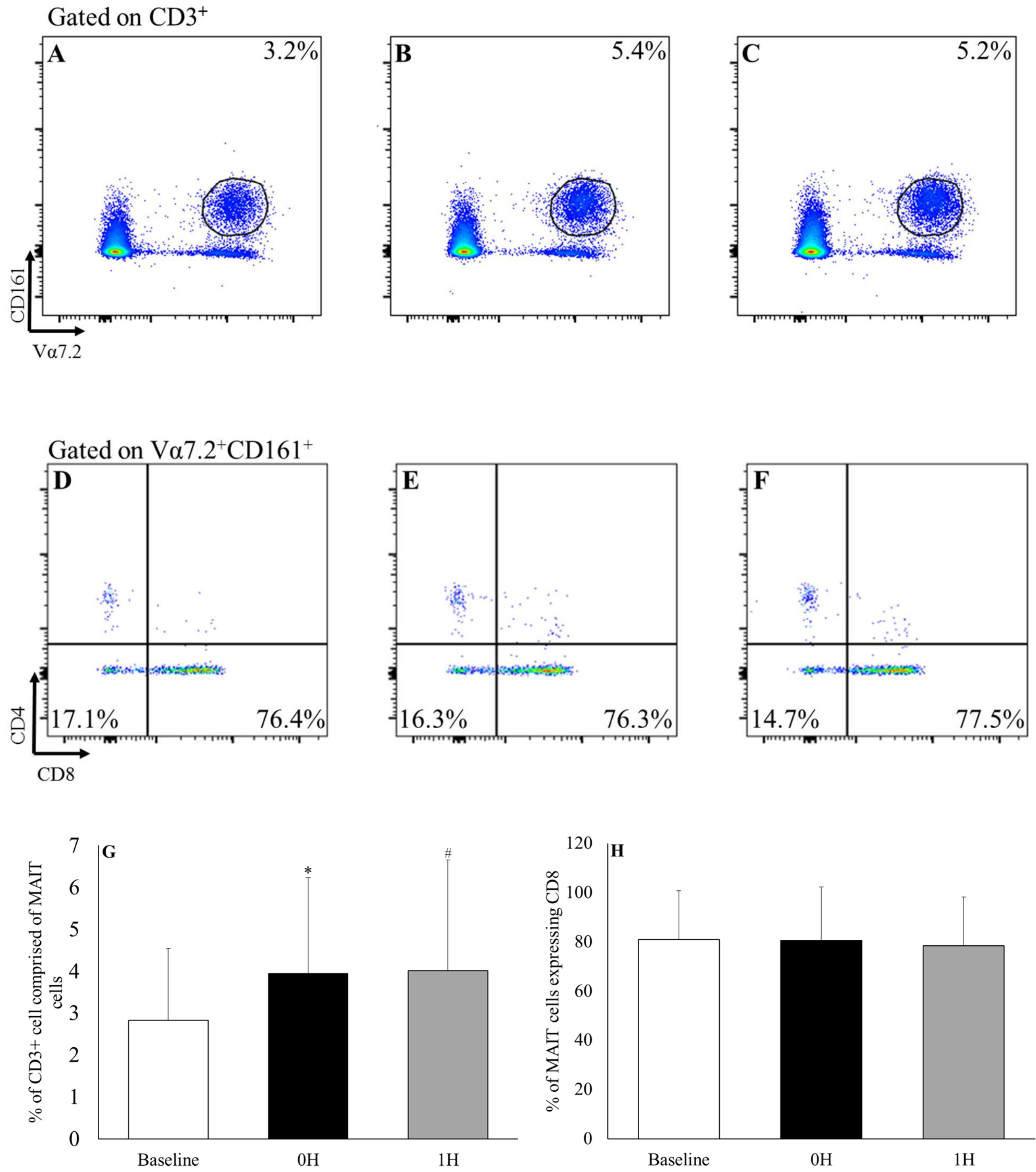


Figure 4. $CD3^+$ T-cells gated on lymphocytes at **A)** baseline, **B)** 0h and **C)** 1h after sub-maximal aerobic exercise. $CD4^+$ and $CD8^+$ T-cells gated on $CD3^+$ T-cells at **D)** baseline, **E) 0**h and **F)** 1h after sub-maximal aerobic exercise. Representative FACS plots were selected from the participant with percent of T-cells closest to mean baseline value for the respective population while the 0h and 1h plots are from the same participant. **G)** Percent change in classic T-cell populations, **H)** Absolute classic T-cell counts; * = $p < 0.05$ from all time points, # = $p < 0.05$ from baseline

Changes in MAIT cells

MAIT cells were defined in this study as $CD3^+V\alpha7.2^+CD161^+$. There was a significant increase in the percent of T-cells that were MAIT cells from baseline to 0h ($p = 0.002$) and the increase was maintained at 1h ($p = 0.03$) (Figure 5A, 5B 5C, and 5G). MAIT cell counts increased by 91.5% (100.1) from baseline to 0h ($p = 0.003$) and returned to baseline values at 1h (Figure 5I). MAIT cell subpopulations were predominately $CD8^+$, accounting for 80.9% (19.8), 80.6% (21.7) and 78.4% (19.8) of all MAIT cells at baseline, 0h and 1h with no significant changes in percentage over time (Figure 5D, 5E, 5F and 5H). Moderate intensity acute exercise caused an increase in

CD8^{+} MAIT cell (84.1% (70.6); p = 0.002) and DN MAIT cell (185.7% (474.7); p = 0.036) counts from baseline to 0h before returning to baseline values at 1h (Figure 5I).



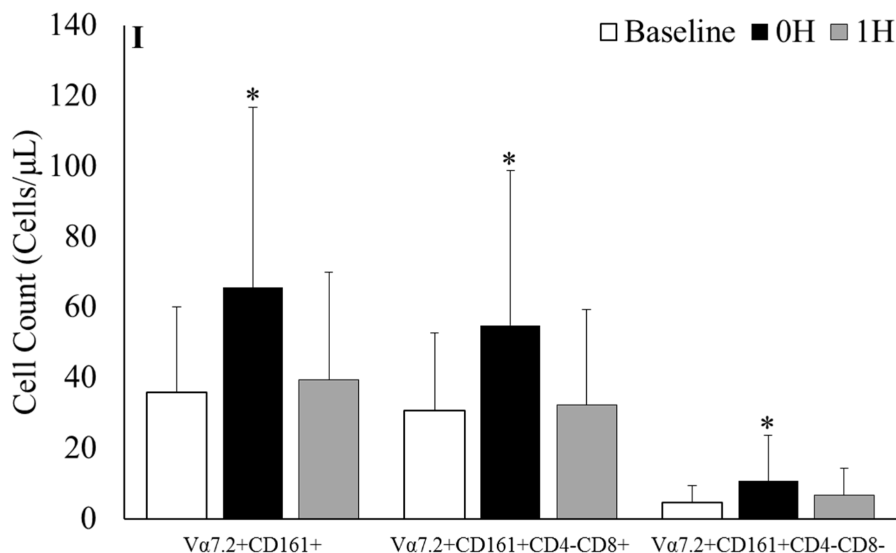


Figure 5. MAIT cells gated on CD3^{+} T-cells at **A)** baseline, **B)** 0h and **C)** 1h after sub-maximal aerobic exercise. CD4^{+} and CD8^{+} gated on MAIT cells at **D)** baseline, **E)** 0h and **F)** 1h after sub-maximal aerobic exercise. Representative FACS plots were selected from the participant with percent of MAIT cells closest to mean baseline value for the respective population while the 0h and 1h plots are from the same participant. **I)** Absolute MAIT cell counts and the respective subpopulations before and after acute exercise; * = $p < 0.05$ from all time points.

Within the CTL population, MAIT cells significantly increased from 6.8% (4.7) at baseline to 8.8% (5.4) at 0h ($p = 0.008$) and again from baseline to 10.3% (6.9) at 1h ($p = 0.026$, Figure 6). There was no difference in MAIT cell counts when gated as CD3^{+}CD8^{+}Vα7.2^{+}CD161^{+} compared to CD3^{+}Vα7.2^{+}CD161^{+}CD8^{+} (data not shown).

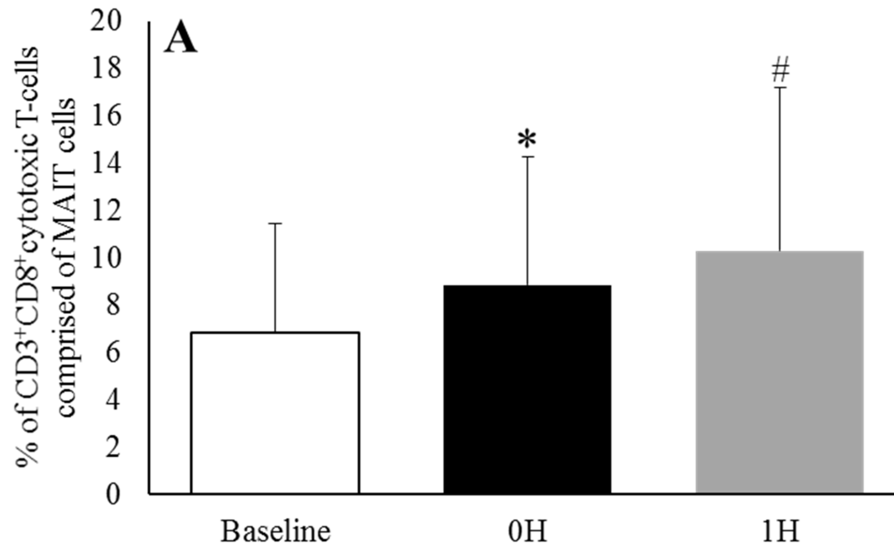


Figure 6. Percent of $CD3^{+}CD8^{+}$ cytotoxic T-cells comprised of MAIT cells. MAIT cells were gated on $CD3^{+}CD8^{+}$ cells. * = $p < 0.05$ from baseline to 0h, # = $p < 0.05$ from baseline to 1h

Chemokine receptor and activation marker alterations

At baseline, 37.3% (25.8) of MAIT cells expressed the chemokine receptor CCR4 (Figure 7A), 81.3% (31.2) of MAIT cells expressed CCR5 (Figure 7B) and 31.6% (31.2) of MAIT cells expressed CCR6 (Figure 7C) and these did not change with exercise (data not shown). $CCR5^{+}$ MAIT cell counts significantly increased by 86.8% (100.7) from baseline to 0h ($p = 0.004$) and returned to baseline at 1h (Table 5). $CCR6^{+}$ MAIT cells showed a trend towards increasing at 0h but did not reach statistical significance ($p=0.081$; Table 5). There was no significant difference between the percent of all MAIT cells and $CD8^{+}$ MAIT cells expressing the chemokine receptors (Table 5).

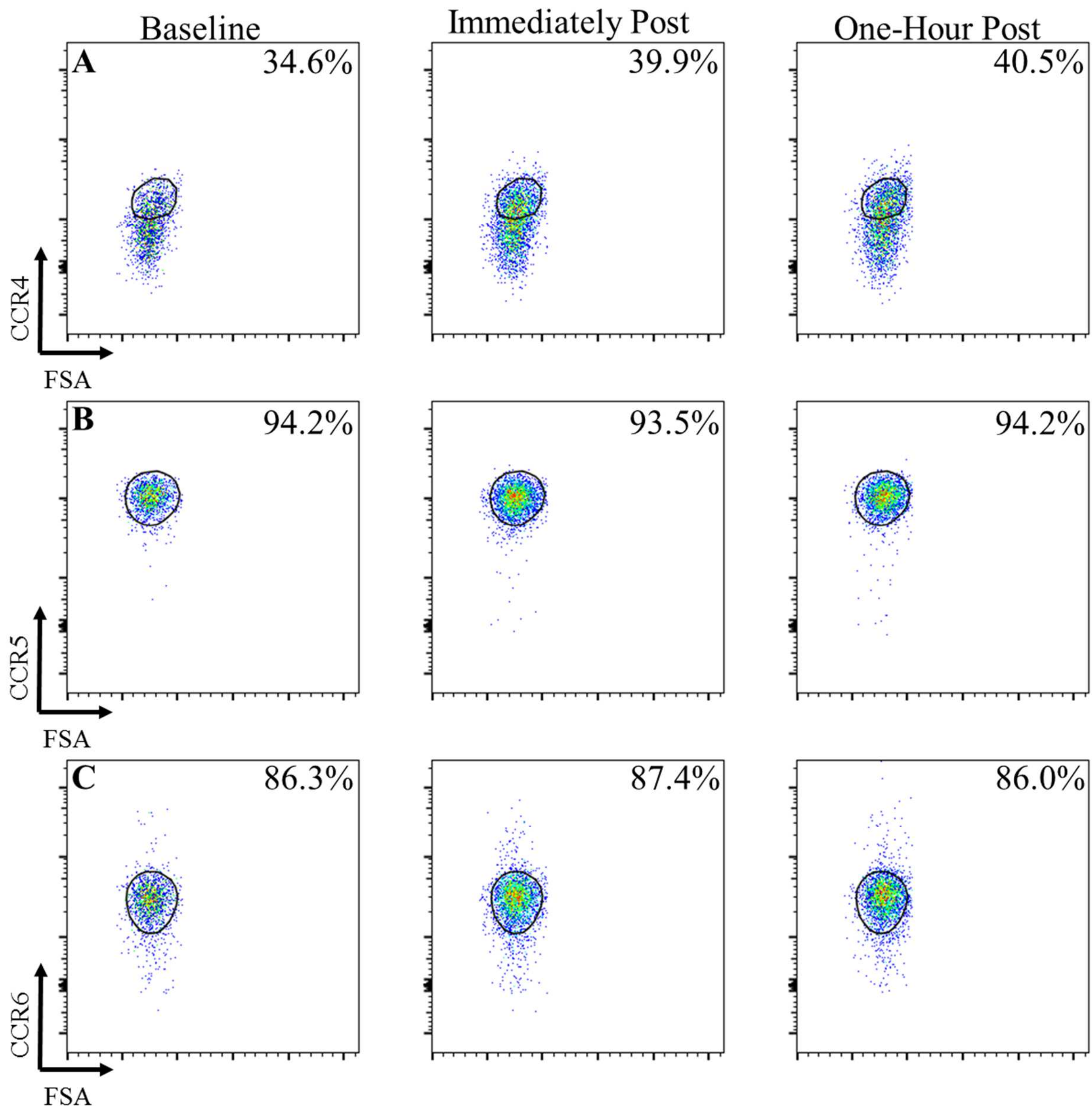


Figure 7. Chemokine receptors gated on MAIT cells at baseline, 0h and 1h for **A)** CCR4, **B)** CCR5 and **C)** CCR6. Representative FACS plots were selected from the participant with percent of MAIT cells closest to mean baseline value for the respective population while the 0h and 1h plots are from the same participant.

Table 5. Chemokine and activation marker cell counts during submaximal exercise.

	Baseline	Immediately Post	One-Hour Post
Chemokine Receptors (cells/µL)			
$V\alpha7.2^{+}CD161^{+}CCR4^{+}$	13.7 (12.4)	25.3 (28.6)	15.6 (17.5)
$V\alpha7.2^{+}CD161^{+}CCR5^{+}$	28.5 (24.6)	56.6 (54.6) $^{\alpha}$	30.1 (25.6)
$V\alpha7.2^{+}CD161^{+}CCR6^{+}$	12.6 (18.5)	26.5 (41.2)	14.2 (18.4)
$V\alpha7.2^{+}CD161^{+}CD8^{+}CCR4^{+}$	12.1 (10.2)	22.1 (22.1) $^{\alpha}$	14.0 (15.1)
$V\alpha7.2^{+}CD161^{+}CD8^{+}CCR5^{+}$	25.4 (21.9)	48.8 (46.7) $^{\alpha}$	25.3 (21.3)
$V\alpha7.2^{+}CD161^{+}CD8^{+}CCR6^{+}$	11.8 (14.7)	22.0 (30.9)	12.0 (14.8)
Activation Marker (cells/µL)			
$V\alpha7.2^{+}CD161^{+}CD69^{+}$	2.2 (2.4)	4.5 (5.8) $^{\alpha}$	2.3 (2.7)
$V\alpha7.2^{+}CD161^{+}CD8^{+}CD69^{+}$	1.9 (2.1)	2.9 (3.4)	1.7 (1.9)

Mean (SD) $^{\alpha}$ = $p < 0.05$ compared to baseline

Because of the high percentage of MAIT cells expressing CCR5 and CCR6 (ceiling effect), mean fluorescence intensities were measured. Mean fluorescence intensities for CCR4, CCR5 and CCR6 did not change over any time point (Figure 8).

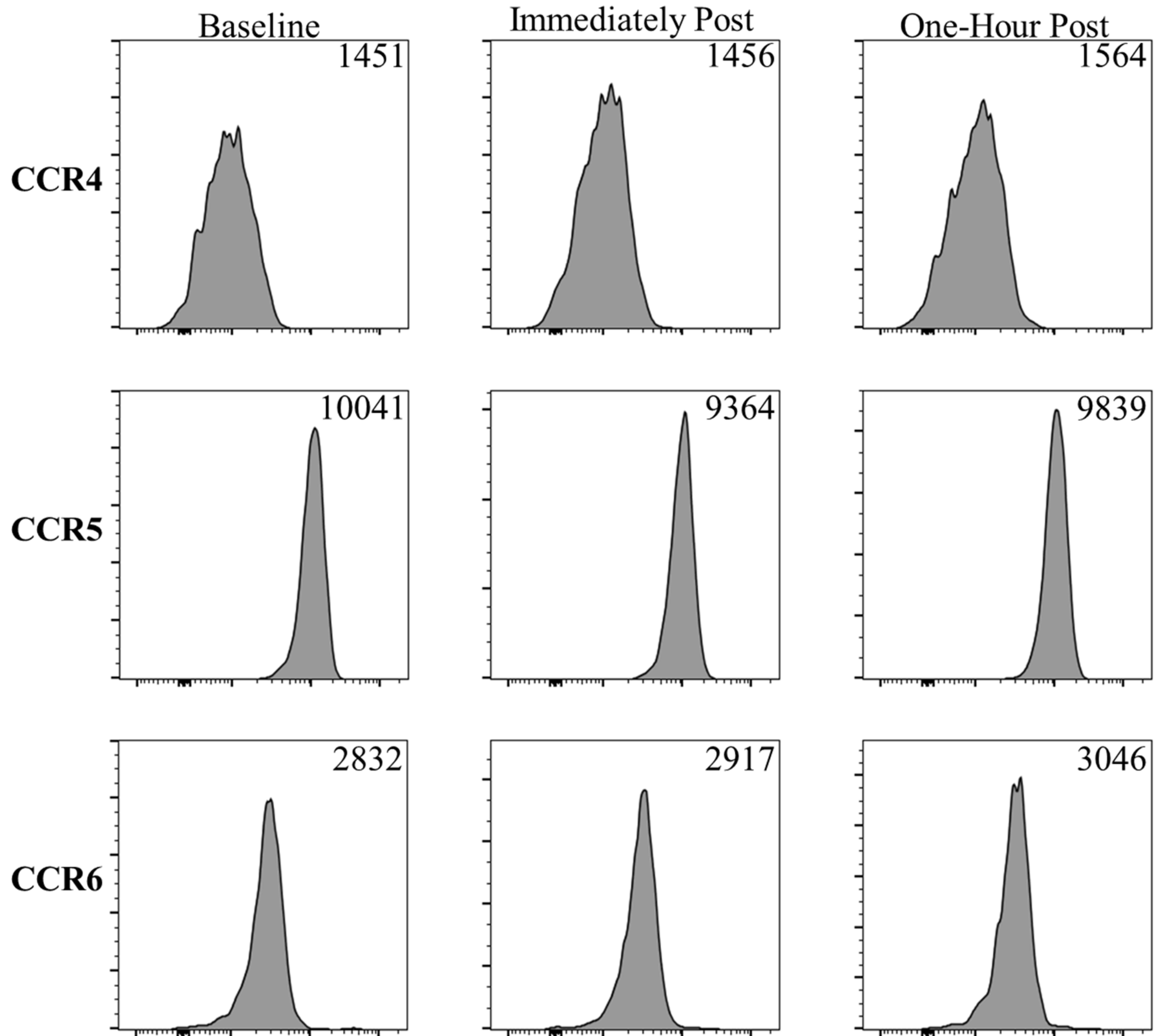


Figure 8. Mean fluorescence intensities of chemokine receptors CCR4, CCR5 and CCR6 at baseline, 0h and 1h after exercise. MFI histograms were determined from FACS plots in Figure 7.

$CD69^+$ MAIT cells comprised 7.3% (7.4), 7.2% (7.3) and 7.4% (7.5) of MAIT cells at baseline, 0h and 1h respectively (Figure 9). There was a significant increase from baseline to 0h

in CD69$^+$ MAIT cell counts by 89.7% (117.9) (p = 0.031) before returning to baseline values during recovery (Table 5).

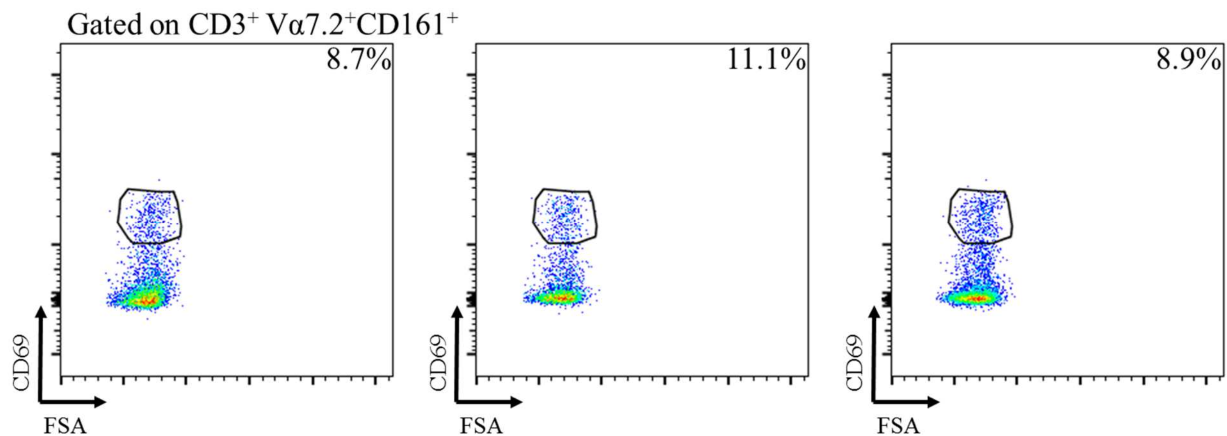


Figure 9. CD69$^+$ cells gated on MAIT cells at **A)** baseline, **B)** 0h and **C)** 1h after sub-maximal aerobic exercise. Representative FACS plots were selected from the participant with percent of CD69$^+$ MAIT cells closest to mean baseline value for the respective population while the 0h and 1h plots are from the same participant.

Body composition and cardiopulmonary correlations with MAIT cells

There were no significant correlations between body composition values and MAIT cell count changes in response to acute moderate intensity aerobic exercise. Similarly, there were no correlations between any exercise testing value, including VO_2max, and changes in MAIT cell counts and chemokine expression (data not shown).

CHAPTER V

DISCUSSION

The purpose of this study was to examine changes in Vα7.2^{+}CD161^{+} MAIT cell counts and chemokine expression (CCR4, CCR5, CCR6) and activation markers (CD69) with acute moderate intensity aerobic exercise. We report for the first time that MAIT cells and their subpopulations (CD8+ and DN) significantly increase in number immediately after exercise and follow a biphasic response by returning to baseline values after 1h of recovery. Additionally, absolute number of MAIT cells that expressed CCR4, CCR5 or CD69 followed the biphasic response after exercise whereas exercise-induced changes in CCR6 were less pronounced. Another novel finding within this study was MAIT cells are preferentially mobilized within the total T cell and also the CTL populations, demonstrating significant increases in the proportion of cells at 0h and 1h after exercise. This suggests that MAIT cells are rapidly mobilized within the exercise-induced lymphocytosis but may migrate/undergo egress more slowly in the 1h after exercise, which is consistent with the constant chemokine expression levels. While it is unclear if the transient rise in MAIT cell numbers over time results in functional change or tissue accumulation, it supports previous work and raises the possibility that exercise has the potential to heighten mucosal immunity in patient populations presenting with low MAIT cell counts.

MAIT cells comprised 2.8% of all T cells at rest, which supports our and others previous work (Serrari et al., 2015; Magalhaes et al., 2015; Hanson unpublished), but increases to 3.9% and 4.0% in response to a 40-minute exercise bout at 90-98% ventilatory threshold at 0h and 1h

respectively. Additionally, MAIT cell counts follow a biphasic response with counts at 1h matching baseline values while $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ cell counts drop below baseline values during recovery. Several possibilities may exist for the higher MAIT cell percentage despite the decline in absolute cell number at 1h. The heightened percentage of MAIT cells within the $CD3^+$ population could be due to decreased blood flow to mucosal tissue. It is well established that during moderate intensity exercise blood flow to the small intestine and kidney are reduced to facilitate oxygen delivery to active tissue (van Wijck et al., 2012; Tidgren et al., 1991). Accordingly, with less access to their residential tissue, MAIT cells would remain in the blood longer and account for the increased percentage immediately after exercise.

An additional hypothesis for the kinetic difference between MAIT cells and other $CD3^+$ subpopulations could be due to the relative expression of adhesion proteins on MAIT cells. The high responsiveness of natural killer cells to exercise has been reported to potentially be due to modulation of adhesion molecules such as CD44 by exercise-induced catecholamine release (Nagao et al., 2000). Both murine MAIT cells and human MAIT cells have been shown to express CD44 (Martin et al., 2009; Sugimoto et al., 2015). Alternately, IL-18 stimulated MAIT cells upregulate very late antigen-4, an integrin that mediates T-cell migration through its interaction with vascular cell adhesion molecule-1 (Chiba et al., 2017). As such, $CD44^+$ MAIT cell and other adhesion marker response to acute aerobic exercise is a potential target for future research to determine the mechanism behind elevated MAIT cell proportions after exercise.

The increase in MAIT cell number was largely expected, considering the increased % of MAIT cells and the overall lymphocytosis. The MAIT cell count increase of 72% with submaximal exercise we observed was lower than the reported 116% increase in MAIT cells following maximal exercise to fatigue (Hanson et al., *unpublished data)*. The difference in reported values between the studies can be attributed to alterations in lymphocyte changes after exercise, as maximal

exercise induced lymphocytosis that was several fold (137% vs. 35%) greater than the current study, despite larger changes in MAIT cell % (Fig 5). This difference in MAIT cell count changes at different exercise intensities supports previous literature which suggests that exercise intensity plays a key role in determining the extent of lymphocyte response to exercise (Nieman, 1992).

Our preliminary evidence indicates MAIT cell numbers increase with exercise (Hanson unpublished), but it is unclear if these cells undergo any functional changes. MAIT cells migrate to specific mucosa via expression of certain chemokines and we wanted to determine if acute exercise would increase chemokine expression. Increased $CCR4^+$ MAIT cell counts and $CCR5^+$ MAIT cell counts were observed after exercise with a tendency for elevated CCR6 MAIT cell counts. However, contrary to our hypothesis, there was no change in the frequency of CCR5 MAIT cells or CCR5 antigen density. The chemokine expression for $CCR5^+$ MAIT cells in the current study (81.3%) was in line to previous work (Saeidi et al., 2015), while the percent of MAIT cells expressing $CCR6^+$ (31.6%) was below previously reported work (Saeidi et al., 2015; Cosgrove et al., 2013). To investigate a possible ceiling effect, MFI was examined in these cells with no change in chemokine antigen expression with exercise. In healthy Individuals, chemokine expression and MAIT cell function may be optional and it is only with co-infections such as HIV/TB that decreased $CCR6^+$ expression on MAIT cells is observed (Saeidi et al., 2015). Additionally, moderate intensity exercise may be insufficient to alter chemokine expression or one-hour after exercise is likely too soon to observe increases in new cell surface proteins.

Like the chemokine receptor MAIT cell population, $CD69^+$ MAIT cell response to exercise was of particular interest due to their ability to combat a wide range of pathogens and the array of cytokines released when upregulated including TNFα, granzyme B and perforin (Dussuex et al., 2011; Kurioka et al., 2014). $CD69^+$ has been shown to be upregulated on MAIT cells as an activation marker when cultured with bacteria and *in vivo* in certain disease states (Le Bourhis et

al., 2010; Wilgenburg et al., 2016). While our $CD69^+$ MAIT cell numbers increased after exercise, there was no change in the percentage of MAIT cells expressing CD69 or the total amount of CD69 being expressed, confirming previous work reported no change in CD69 expression in lymphocyte subpopulations (Green et al., 2003; Millard et al., 2013). The lack of stimulation precludes us from determining whether MAIT cell activation may increase after moderate intensity exercise.

It is also important to note that the percent of MAIT cells remains elevated while the total percentage of CTLs decreases during recovery (Figure 4). Previous literature reporting $CD3^+CD8^+$ T-cell responses to exercise have failed to take into account MAIT cell changes within the population. $CD3^+CD8^+V\alpha7.2^+CD161^+$ make up approximately 10% of all CTLs (Dusseaux, Martin et al. 2011, Walker, Kang et al. 2012, Cosgrove, Ussher et al. 2013, Fergusson, Huhn et al. 2016). While we report a slightly lower percentage at baseline (6.8%), increased proportions of MAIT cells within the CTL population at 0h and 1h after acute moderate intensity exercise contradicts our initial findings (Hanson et al unpublished). Previous research has found that $CD8^+$ cells with high tissue-migrating potential are preferentially mobilized during exercise (Campbell et al., 2009; Simpson et al., 2007). In particular, Campbell et al. identified $CD45RA^-$ $CD8^+$ cells, which mature MAIT cells express, as a subpopulation which is elevated after exercise (Campbell et al., 2009). Other cells within the CTL population may migrate into tissues which would explain the increase from 8.8% to 10.3% from 0h to 1h after moderate intensity exercise (Figure 6).

Previously, we proposed that failing to account for MAIT cells within CTL analyses would underestimate cytotoxic function but this error was consistent pre- and post-exercise in the absence of changes in MAIT cell proportions within CTLs (Hanson et al, *unpublished data*). In the current study, CTL function would still be underestimated and this would be exacerbated with exercise. Because we did not include makers for CTL subpopulations or cytokine production, we are unable to determine the exact implications of these findings. However, moving forward, CTL analyses

should be aware of this issue and future studies should be designed to provide more direct evidence of these effects now that it has been identified in multiple studies.

While MAIT cell chemokine and activation expression appears to be unaffected by moderate intensity aerobic exercise in a healthy population, these markers have been shown to be affected in diseased populations. Individuals with asthma, HIV, obesity and diabetes have all been shown to have decreased MAIT cell counts (Hinks et al., 2015; Cosgrove et al., 2013; Magalhaes et al., 2015). Furthermore, CCR6 and CD69 expression on MAIT cells has been altered in individuals affected with HIV and dengue virus (Cosgrove et al., 2013; Wilgenburg et al., 2016). Regular moderate intensity exercise may be a means to boost delivery of MAIT cells in these populations and the altered environmental factors associated with those diseases may contribute to chemokine alterations caused by exercise.

There were some limitations involved in this study. One limitation was the choice to investigate MAIT cell response in men only and this was done because there is some evidence that estrogen status may alter immune response (Tiidus et al., 2008). Another potential limitation to the study was the allowance of varying exercise modes to count towards moderate intensity exercise. Some participants participated exclusively in resistance training and were unable to maintain the Wattage selected throughout the moderate intensity exercise bout. However, these participants stayed within the desired VT range throughout the bout as determined by the VO_2max test.

In conclusion, $V\alpha7.2^{+}CD161^{+}$ MAIT cells increase in frequency relative to total T cells and the cell numbers follow the biphasic response following 40-minute acute exercise bout at 90-98% ventilatory threshold. The absolute number of MAIT cells expressing homing marker CCR5 and activation marker CD69 increased after exercise but there is no change in the frequency or the expression level of either marker. Additionally, MAIT cells make up a greater proportion of $CD8^{+}$

cells immediately and one-hour after exercise, highlighting preferential mobilization within this population for potential pathogenic targets. These finding support our previous work but also expand the MAIT cell and exercise field a collection of results from this study introduce MAIT cell response to acute sub-maximal exercise and provide new hypotheses/support to further investigate the understanding of how MAIT cells respond to exercise in healthy individuals.

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