Effect of Exercise in a Warm Environment on Circulating Neutrophils

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ABSTRACT

ERICA S. COOPER: Effect of Exercise in a Warm Environment on Circulating Neutrophils
(Under the direction of Robert McMurray)

The primary purpose of this investigation was to determine if exercise in the heat elicited a greater increase in the number of circulating neutrophils than exercise in a cool environment. The secondary purposes were to, 1) investigate the relationship between the change in core temperature ($T_{re}$) and the degree of neutrophilia and 2) validate a previously stated hypothesis that $T_{re}$ must increase to greater than 38°C to elicit a significant rise in neutrophilia. Cycling for 40 minutes at 65% VO$_2$peak caused neutrophilia, which was most evident two hours post-exercise. Moreover, exposure to the heat during exercise resulted in higher core temperatures and elicited a greater neutrophilia two hours post-exercise. No relationship was observed between a $T_{re}$ of 38°C and increases in the circulating neutrophil count. However, a $T_{re}$ of 38°C appears to contribute to exercise-induced neutrophilia since a greater exercise-induced $T_{re}$ occurred concomitantly with greater neutrophilia.
# TABLE OF CONTENTS

**LIST OF TABLES**..........................................................................................v

**LIST OF FIGURES**........................................................................................vi

Chapter

<table>
<thead>
<tr>
<th>I. BASIS FOR STUDY</th>
<th>.................................................................1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil Function</td>
<td>...................................................................1</td>
</tr>
<tr>
<td>Neutrophil Response to Exercise</td>
<td>................................................................2</td>
</tr>
<tr>
<td>Neutrophil Response to Heat Exposure</td>
<td>................................................................2</td>
</tr>
<tr>
<td>Neutrophil Response to Exercise in the Heat</td>
<td>..............................................3</td>
</tr>
<tr>
<td>Purpose</td>
<td>....................................................................3</td>
</tr>
<tr>
<td>Research Hypotheses</td>
<td>...................................................................4</td>
</tr>
<tr>
<td>Definition of Terms</td>
<td>....................................................................4</td>
</tr>
<tr>
<td>Assumptions</td>
<td>.....................................................................5</td>
</tr>
<tr>
<td>Delimitations</td>
<td>......................................................................5</td>
</tr>
<tr>
<td>Limitations</td>
<td>.......................................................................6</td>
</tr>
<tr>
<td>Significance of Study</td>
<td>...................................................................6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>II. REVIEW OF LITERATURE</th>
<th>.................................................................8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction and Basics of Immune Cells</td>
<td>..............................................8</td>
</tr>
<tr>
<td>The Role of Neutrophils</td>
<td>....................................................................10</td>
</tr>
<tr>
<td>Neutrophil Response to Exercise</td>
<td>.......................................................12</td>
</tr>
</tbody>
</table>
Neutrophil Response to Heat Exposure

Neutrophil Response to Exercise Combined with Heat Exposure

III. METHODOLOGY

Subjects

Instrumentation

Protocol

Procedures

IV. RESULTS

V. DISCUSSION

Circulating Neutrophil Count in a Hot and Cold Environment

Temperature Threshold

Limitations

Conclusions

Recommendations for Future Research

APPENDICES

Appendix A: Training History Form

Appendix B: Informed Consent Form

Appendix C: Maximal Exercise Session Data Collection Sheet

Appendix D: Experimental Trials Data Collection Sheet

REFERENCES

iv
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oxygen uptake, heart rate and rating of perceived exertion during exercise trials</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>Blood cell counts during and following exercise trials</td>
<td>35</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure

1. Exercise trial protocol diagram..........................................................29

2. Core temperature during exercise trials.................................................34

3. The circulating neutrophil count response to exercise in
   a warm environment..............................................................................34

4. Change in circulating neutrophil number during and
   following exercise in a cool and warm environment..............................36
CHAPTER I

BASIS FOR STUDY

Neutrophil Function

Activity of the human immune system is mediated by several types of leukocytes (white blood cells), including Granulocytes, Monocytes and Lymphocytes. Of these leukocytes, Granulocytes play a large role in the body’s immediate response to invading pathogens. Neutrophils are a subpopulation of granulocytes, comprising approximately 90% of the body’s circulating granulocyte concentration and up to 70% of the circulating leukocyte concentration (Mackinnon 1999). Neutrophils destroy pathogens such as viruses, bacteria and fungi by processes including adherence, chemotaxis, phagocytosis, oxidative burst, degranulation and microbial killing (Smith 1997). Additionally, research has associated circulating neutrophil number with tissue damage and inflammation (Pizza et al. 1995; Fielding et al. 1993). With regards to tissue damage, research indicates that neutrophils may be responsible for the degradation of damaged and necrotic muscle tissue and cellular debris, so that new contractile elements can be laid down and the muscle repaired (Tidball 1995; Teixeira et al. 2003).

Although neutrophils are key mediators of the immune response to repair and regenerate tissue, they are also associated with certain inflammatory conditions and the etiology of host tissue damage (Weiss 1989). Research suggests that the same reactive oxygen intermediates released by neutrophils that are responsible for the oxidative burst of
invading pathogens can also damage healthy muscle cells and connective tissues (Pizza et al. 2002; Frenette et al. 2002).

Neutrophil Response to Exercise

The neutrophil response to exercise has been summarized by Mackinnon (1999), and in general, the circulating neutrophil number increases during exercise and continues to do so throughout the first few hours of recovery. Many possible factors may be responsible for mediating the exercise-induced neutrophilia, including release from marginal pools, an increased cardiac output and an increase in the secretion of hormones, bioactive lipids and cytokines (Smith 1997). Of the aforementioned factors, many are secreted in response to stress, which is in turn, subject to mode, intensity and duration of the exercise (Kjaer 1989). Therefore, the pattern of exercise-induced neutrophilia depends on the mode, intensity and duration of activity (Mackinnon 1999).

Neutrophil Response to Heat Exposure

Heat exposure is another physiological stressor to the human body. Evidence shows that passive heat exposure can result in up to a 254% increase in the number of circulating neutrophils (Kappel et al. 1991). Like exercise-induced neutrophilia, research suggests that the mechanisms responsible for heat-induced neutrophilia include increased cardiac output and an increase in the secretion of stress-mediated hormones (Hoffman-Goetz & Pedersen 1994; Shephard 1998). Research implies, however, that the limiting variable controlling the heat-induced neutrophilia is core body temperature ($T_{re}$). Passive heating that fails to increase $T_{re}$ to at least 38°C may result in either little or no alteration of immune parameters (Severs et al. 1996).
Neutrophil Response to Exercise in the Heat

In addition to the role of exercise and heat exposure as individual physiological stressors, research suggests that these two factors have a synergistic stress response during exercise in the heat (Severs et al. 1996). Relative to thermoneutral exercise, exercise in the heat has been shown to result in higher circulating levels of stress-mediated hormones and increased cardiac output, both of which are potential mediating factors for neutrophilia (Shephard 1998). Although research widely recognizes the augmentation of the stress response during exercise in a warm environment, limited research has been conducted concerning the effect of exercise in the heat on immune function.

The limited evidence concerning the neutrophil response to exercise in the heat is equivocal. Some research suggests that there is little or no difference in the magnitude of neutrophilia following heated exercise as compared to thermoneutral exercise (McFarlin & Mitchell 2003, Laing et al. 2005), while other literature suggests that heated exercise results in a significantly larger circulating neutrophil number relative to more thermoneutral exercise (Niess et al. 2003, Mitchell et al. 2002). Differences in the conclusions of various research studies may be the result of differences in methodology including environmental factors and mode, intensity and duration of exercise. As a result, the exact extent to which exercise in the heat can modify neutrophilia is not well understood.

Purpose

The purposes of this study were to determine the combined effect of exercise and a warm environment on the number of circulating neutrophils post- and two hours post-exercise, and to investigate the relationship between the rise in $T_{re}$ and the degree of exercise-induced neutrophilia. The former purpose was investigated by comparing the number of
circulating neutrophils following exercise in a cool environment to the number of circulating neutrophils following exercise in a warm environment. The latter purpose was investigated by comparing $T_{re}$ to the circulating neutrophil count elicited by exercise in both conditions combined.

**Research Hypotheses**

It was hypothesized that:

1. Exercise in a warm environment would cause a rise in the circulating neutrophil count immediately and two hours post-exercise compared to resting levels.

2. Exercise in a warm environment would lead to a higher circulating neutrophil count immediately and two hours post-exercise when compared to exercise in a cool environment.

3. The change in $T_{re}$ would be positively correlated with the change in the circulating neutrophil count.

4. Exercise sessions in which $T_{re}$ did not exceed 38°C would not elicit a significant neutrophil response, but exercise sessions in which $T_{re}$ did exceed 38°C would elicit a significant neutrophil response.

**Definition of Terms**

1. **Leukocyte** – “Immune cells found in several lymphoid organs and tissues throughout the body and in the blood and lymph circulation” (Mackinnon 1999).

2. **Granulocyte** – “Large, granule-containing leukocytes, which are among the first cells to encounter and combat pathogens” (Mackinnon 1999). Types of granulocytes include eosinophils, basophils and neutrophils.
3. **Neutrophil** – A “phagocytic cell that kill[s] ingested microorganisms by releasing proteases and phospholipases from cytoplasmic granules and by generating toxic molecules such as oxygen radicals” (Mackinnon 1999).

4. **Neutrophilia** – Recruitment of neutrophils into circulation (Mackinnon 1999).

**Assumptions**

1. Subjects were free of infection for the three weeks preceding participation in this study.

2. Subjects refrained from vigorous exercise and alcohol consumption for the 24 hours preceding each exercise session, and caffeine consumption for the 12 hours preceding each exercise session.

3. Subjects had no prior incident of heat injury.

4. Subjects were not consuming anti-inflammatory medications (i.e.-NSAIDs).

5. Subjects had no fear of water, confined spaces, or blood draws.

**Delimitations**

1. Male subjects between the ages of 18 and 40 were be recruited.

2. Subjects were physically active, participating in at least 30 minutes of moderate physical activity at a minimum four times per week.

3. Subjects reported for each exercise session at least four hours post-prandial, having refrained from vigorous activity and alcohol consumption for the previous 24 hours, and caffeine consumption for the previous 12 hours.

4. The heat trial consisted of exposure to 39º C water.
5. Exercise occurred at 65% of peak oxygen uptake for 40 minutes.

Limitations

1. Results can be generalized only to healthy, recreationally active adult males between 18 and 40 years of age.

2. The subjects’ exercise history for the 24 hours preceding the exercise sessions was self-reported.

3. Although subjects were assumed to be free of infection during the study, and to have been so for three weeks prior to the study, some infection may have been unknown to the subjects, and could have resulted in perturbation of baseline neutrophil levels.

Significance of Study

Recent research continues to provide valuable information concerning the inflammatory response to both injurious damage and non-injurious perturbations in muscle tissue. These studies offer evidence to suggest that not only do neutrophils help to mediate the healing process, but that they also propagate tissue injury.

Individually, exercise and passive heat exposure result in neutrophilia. It is reasonable to hypothesize then that exercise combined with heat exposure would produce an additive effect on neutrophilia. However, the limited research concerning exercise in a heated environment offers equivocal evidence concerning neutrophilia, possibly due to differences in methodology (mode, intensity and duration of exercise). Therefore, it was necessary to attempt to validate the findings of previous research in order to gain a deeper understanding of the exact magnitude to which exercise in a heated environment affects the circulating neutrophil concentration.
The results of this study may have clinical and performance oriented implications. It is unknown if, and to what extent neutrophils mediate host-tissue repair, and to what extent neutrophils mediate host-tissue damage. However, if exercise combined with heat exposure is shown to elicit a larger neutrophilia than more thermoneutral exercise, exercise in the heat may exacerbate either host-tissue repair and/or damage. This may be of particular importance to individuals suffering from auto-immune or chronic inflammatory diseases, such as rheumatoid arthritis, lupus or obesity. These diseased individuals could either exacerbate or attenuate their symptoms by exercising in the heat. Additionally, individuals who regularly engage in vigorous physical activity in the heat, such as athletes, military personnel and firefighters, may either inhibit their ability to perform optimally following exercise in the heat due to the exacerbation of host tissue damage, or enhance the reparative process of damaged muscle tissue.
CHAPTER II

REVIEW OF LITERATURE

Introduction and Basics of Immune Cells

This chapter will review literature concerning the influence of exercise and heat on white blood cells, or leukocytes. The introduction will provide a brief overview of the immune system’s cells, while the following section will discuss the function of neutrophils, and their role in the immune system. Subsequent sections will discuss the neutrophil response to exercise, the neutrophil response to heat exposure, and finally, the neutrophil response to exercise combined with heat exposure.

The Immune System’s role is to defend the body from invading pathogens, foreign matter, or the body’s own malfunctions, such as the growth of tumor cells (Mackinnon 1999). This system is comprised of several different types of leukocytes and messenger molecules (cytokines), the actions and interactions of which mediate the overall immune response. Three types of leukocytes will be discussed in the following paragraphs: lymphocytes, monocytes and polymorphonuclear granulocytes.

Lymphocytes are produced in the body’s lymphoid tissue, and comprise 20-25% of all leukocytes. Lymphocytes primarily consist of the body’s adaptive immune cells, which require a few days to reach peak function following activation (Mackinnon 1999; Guyton 1979). These cells, based on “memory” from prior exposure to a pathogen, mediate the specific immune response to the same pathogen during subsequent infection (Mackinnon
Thus, the body *adapts* to previous antigen exposure in order to execute a swifter and more efficient immune response. Two major subpopulations of lymphocytes are “T” lymphocytes (T cells), and “B” lymphocytes (B cells). T cells are responsible for activating B cells, destroying tumor and virally infected cells, and secreting cytokines, which are further responsible for mediating the activity of other immune cells (Mackinnon 1999). B cells contain memory blueprints for antibodies that enable swifter antibody production for specific antigens. Upon T cell activation, B cells produce large quantities of antibodies for a specific antigen (Mackinnon 1999).

Monocytes, produced in the bone marrow, circulate in the blood and become macrophages when fixed within tissues. Monocytes/macrophages constitute 10-15% of leukocytes, and help to mediate the body’s innate, and therefore immediate, response to pathogens by processes including phagocytosis, destruction of infected cells, and secretion of lymphocyte-activating cytokines (Mackinnon 1999; Guyton 1979).

Polymorphonuclear granulocytes are characterized by a granular appearance and multiple nuclei (Mackinnon 1999; Guyton 1979). Like monocytes, granulocytes help to mediate the body’s innate immune response. There are three subdivisions of granulocytes, which include basophils, eosinophils and neutrophils. Basophils help mediate allergic and inflammatory reactions, while eosinophils are involved in fighting parasitic infections (Mackinnon 1999). Together, these two subpopulations comprise a small percentage of total granulocytes and a very small percentage of total leukocytes. Neutrophils, however, comprise the largest subpopulation of leukocytes, accounting for approximately 62% of total leukocytes, and approximately 90% of all granulocytes (Mackinnon 1999; Guyton 1979). Neutrophils are some of the first immune cells to
respond to infection, where they ingest and destroy invading pathogens by releasing free radicals, proteases and phospholipases that breakdown the pathogen. Additionally, neutrophils are the first immune cell to infiltrate damaged tissue and areas of inflammation, where it has been suggested that they help to break down necrotic tissue and cellular debris so that new contractile elements can be laid down, and the tissue repaired (Mackinnon 1999; Tidball 1995; Teixeira et al. 2003).

The purpose of the current study is to examine how the physiological stressors of heat and exercise affect the immune response. Although the overall immune response is mediated by all leukocyte subsets acting together, the current study will focus on circulating neutrophils as a means to begin understanding the overall immune response.

The Role of Neutrophils

In addition to being a part of the body’s first line of immune defense against invading pathogens, neutrophils provide the first immune response to areas of inflammation and tissue damage. Despite the rapid response, the role of neutrophils at sites of inflammation and tissue damage is less clear; research suggests that neutrophils act as mediators of both tissue repair and tissue damage.

Much research offers support for the role of neutrophils as mediators of tissue damage. Significant correlations between post-exercise neutrophilia and markers of tissue damage, including serum creatine kinase, serum myoglobin, and disruptions in myofibril structure, suggest a relationship between neutrophils and tissue damage (Fielding et al. 1993; Suzuki et al. 1999).

Pizza et al. (2005) found less tissue damage in mice with significantly lower neutrophil counts, relative to controls. At time points coinciding with neutrophil
depletion, mice exhibited a lower percentage of injured fibers, and attenuated force
deficits and oxidative damage following muscle injury. Likewise, Mishra et al. (1995)
found that administration of a non-steroidal anti-inflammatory drug (NSAID) following
exercise-induced injury in rabbits resulted in reductions in serum creatine kinase and the
number of injured fibers. Although neutrophil count was not measured directly, the
presumed NSAID-induced reduction in the inflammatory response, a large part of which
is comprised of neutrophils, suggests that mediators of the inflammatory response,
including neutrophils, could be responsible for exacerbating post-exercise muscle injury.

Ishchemia/repurfusion models also provide compelling evidence to suggest that
neutrophils play a role in mediating tissue damage. Depleting the blood’s circulating
neutrophil concentration prior to repurfusion in ischemic muscle tissue results in direct
attenuation of muscle damage, as well as indirect attenuation of muscle damage via
reductions in vascular damage (Romson et al. 1983; Korthius et al. 1988). Additionally,
administration of free radical scavengers to reduce the oxidative stress caused by
neutrophil-mediated free radical release resulted in less muscle damage relative to
controls (Jolly et al. 1984).

Several investigators have also suggested that neutrophils may mediate tissue
repair by breaking down damaged tissue and debris so that new contractile filaments can
be formed (Fielding et al. 1993; Teixeira et al. 2003; Tiidus 1998; Lowe et al. 1995; Jesse
et al. 1998). The number of investigations supporting this suggestion, however, is limited
(Teixeira et al. 2003; Jesse et al. 1998). Teixeira et al. (2003) provides some of the most
compelling evidence in support of the reparative function of neutrophils. In neutropenic
(neutrophil-depleted) male swiss mice injected with Bothrops asper snake venom,
myonecrosis and residual creatine kinase (CK) levels (index of muscle damage and damaged particles) did not differ from non-neutropenic mice 24 hours after infection, suggesting that neutrophils do not cause additional tissue damage. Additionally, the neutropenic mice exhibited impaired muscle fiber regeneration and CK synthesis, and prolonged presence of necrotic debris in the affected tissue, further suggesting that neutrophils mediate tissue repair. Jesse et al. (1998) suggest that the granulocytes and macrophages invading tissue following injury may have a role in activating satellite cells to begin the process of muscle repair and regeneration. This implication is based on the satellite cell expression of vascular cell adhesion molecule-1 (VCAM-1), and invading granulocytes and macrophage expression of the coreceptor specific for VCAM-1.

Neutrophil Response to Exercise

Research suggests that certain physiological stressors including exercise augment neutrophilia. It is widely recognized that neutrophils exhibit a biphasic response to exercise (Mackinnon 1999; Fairbarn et al. 1993; Nieman et al. 1995; Robson et al. 1999; Nieman et al. 1994; Gabriel et al. 1994). The number of circulating neutrophils increases throughout exercise, then displays a second marked increase which generally peaks two to three hours post-exercise. The magnitude and duration of the neutrophilia, however, is dependant on the interaction of the duration, intensity, and mode of exercise (Mackinnon 1999; Fairbarn et al. 1993).

In exercise of short duration, for example, Fairbarn et al. (1993) found a 32% increase in circulating neutrophils in six untrained males after twenty minutes of incremental cycling. Subjects cycled at workloads of 50, 100, 150 and 200 W (watts) for five-minute increments. Although VO$_2$ was not measured continuously during exercise,
the average intensity at the highest workload was ~76% VO$_{2\text{max}}$. In eight internationally competitive rowers, a six-minute VO$_{2\text{max}}$ test on a rowing ergometer resulted in a ~42% increase in circulating neutrophils. Two hours post-exercise, this concentration increased to ~179% above pre-exercise levels, and remained 86% elevated over pre-exercise levels four hours post-exercise (Nielsen et al. 1996). Although exercise duration was shorter in the Nielsen et al. study relative to the Fairburn et al. study, the resulting neutrophilia was larger, likely due to the higher exercise intensity.

In longer duration exercise, 22 male marathoners, treadmill running for 2.5 hr at 75% VO$_{2\text{max}}$, showed a significant elevation in circulating neutrophils over pre-exercise levels immediately post-exercise, 1.5, three and six hours post-exercise, with the number peaking three hours post-exercise. At each time point, increases approximated 186%, 243%, 260% and 175%, respectively (Nieman et al. 1995).

Robson et al. (1999) conducted a study comparing the neutrophil responses elicited by prolonged exercise at a lesser intensity and more brief exercise at a higher intensity. Eighteen male subjects (eight recreationally active, 10 club endurance trained athletes) cycled at 80% VO$_{2\text{max}}$ until fatigue (< one hr), and on a separate occasion, cycled for three hr (or to fatigue) at 55% VO$_{2\text{max}}$. Although both exercise sessions elicited significant increases in neutrophil count immediately post-exercise, one, 2.5 and five hr post-exercise, neutrophilia was significantly larger at all time points following cycling for three hr at 55% VO$_{2\text{max}}$. The authors concluded that longer duration exercise at a moderate intensity elicits a larger increase in the number of circulating neutrophils than does shorter duration, high intensity exercise.
Nieman et al. (1994) conducted a study comparing 45 minutes of exercise at high and low-intensities. Ten well-conditioned men ran on a treadmill for 45 minutes twice: once at 50% VO$_{2\text{max}}$, and once at 80% VO$_{2\text{max}}$. Exercise at 80% resulted in significantly larger neutrophil counts immediately post-exercise, one and 3.5 hours post-exercise. The authors conclude that the larger physiological stress induced by higher intensity exercise results in a larger number of circulating neutrophils.

Research suggests that extremely prolonged exercise elicits the largest increases in the circulating neutrophil concentration. In nine endurance-trained runners, an ultra-marathon (100 Km) run over the course of 7.5-10 hours, resulted in a 310% increase in neutrophils within 10-33 minutes of finishing the race. Three hours after the race, neutrophils were elevated 317% (Gabriel et al. 1994).

In addition to intensity and duration, mode of exercise can influence the degree of exercise-induced neutrophilia. Exercise with an eccentric component has been shown to elicit larger increases in post-exercise neutrophilia, perhaps due to a larger degree of muscle damage caused by eccentric exercise. In 10 runners, Pizza et al. (1995) found that 60 minutes of running at 70% VO$_{2\text{max}}$ at a –10% grade induced a significantly larger circulating neutrophil concentration post-exercise, relative to level running at the same intensity, for the same duration. At 1.5 hr and 12 hr into recovery, neutrophil number was 23% and 19% higher, respectively, for downhill running. The authors conclude that the larger neutrophilia likely reflects inflammation due to increased muscle damage elicited by eccentric muscle contraction during downhill running.
Taken as a whole, the results of these studies suggest that the degree of exercise-induced neutrophilia is the product of the interaction of exercise intensity, duration and mode.

**Neutrophil Response to Heat Exposure**

Passive heat exposure also elicits an increase in the circulating neutrophil concentration. Some investigators have suggested that the degree of neutrophilia is proportional to increases in core temperature above 38°C (Severs et al. 1996; Cross et al. 1996; Downing et al. 1988). As discussed below, however, the evidence supporting this suggestion is equivocal. Differences in methodologies of heat exposure, such as heat medium (water, environmental chamber), temperature and time of exposure, make it difficult to compare results and qualify the exact neutrophil response to passive heat exposure.

Severs et al. (1996) conducted a study in which eleven male subjects sat in an environmental chamber for two three-hour sessions. Temperature during the thermoneutral trial was set at 23°C, while the heat exposure trial was 40°C, 30% RH (relative humidity). Exposure to the above temperatures elicited only modest alterations in $T_{re}$; an average 0.3°C decrease in the thermoneutral trial, and 0.7°C increase in the heat exposure trial. They found no significant increases in the circulating granulocyte concentration with passive heat exposure. Although this study measured total granulocytes, it is inferred from the lack of a significant increase in granulocytes that neutrophils did not increase during either trial. The authors attribute the lack of a significant difference between the heat exposure and thermoneutral trials to the modest
alterations in $T_{re}$, and concur with Kappel et al. (1991), that a threshold $T_{re}$ of 38°C must be met before significant increases in granulocytes can be elicited.

Studies involving heat exposure that causes $T_{re}$ to meet or exceed 38°C have elicited significant increases in the circulating neutrophil count. Cross et al. (1996) submerged nine moderately fit men in water to the mid-chest level for two 80-minute sessions. Water temperature for the sessions was either 23°C for the thermoneutral trial or 39°C for the heat exposure trial. $T_{re}$ during passive heat exposure peaked at ~38°C. Heat exposure granulocyte counts became significantly elevated over the thermoneutral condition after the 70th and 80th minutes of exposure. Granulocyte count did not change during the thermoneutral trial. Although neutrophils were not measured directly, it can be inferred that they rose significantly during heat exposure because they comprise ~90% of all granulocytes. These results, however, do not indicate what proportion of the increase in circulating granulocytes is attributable to an increase in neutrophils. Based on this data, the authors agreed with other investigators, in that a $T_{re}$ threshold of at least 38°C must be met to induce a significant neutrophilia.

Downing et al. (1988) offer additional evidence to suggest that heat exposure elicits a significant neutrophilia. Three healthy volunteer subjects sat with approximately 70% of their bodies submerged in 40-45°C water until $T_{re}$ reached 39.5°C. The circulating granulocyte concentration, again reflective of the circulating neutrophil concentration, increased a significant 39.5% by the time $T_{re}$ reached 39.5°C.

Research measuring neutrophils directly helps to quantify the proportional increases in individual granulocyte types in post-heat exposure granulocytosis. Kappel et al. (1991) conducted a study in which eight men were immersed to the neck
(sternoclavicular notch) for two hours in water, with one arm above the water. Water temperature was 34.5°C for the control trial, and 39.5°C for the heated trial. Blood samples were drawn before immersion, two hours after finishing immersion, and at time points coinciding with T_{re} of 38°C, 39°C, and 39.5°C. In the heat exposure trial, neutrophils were significantly elevated over pre-exposure values when T_{re} was 38°C and 39.5°C, as well as two hours after finishing immersion. These significant elevations measured 16%, 38% and 254%, respectively. At no point during the control trial were neutrophils significantly elevated over baseline values. The heat exposure neutrophil count was significantly elevated over control values at two hours post-immersion only, despite the return of T_{re} to baseline values in both trials at two hours post-immersion.

Kappel et al. (1998) conducted a similar follow-up study in which eight men again sat immersed in 39.5°C water for two hours, with one arm above the water. Immediately following two hours of hot water immersion, T_{re} had risen to 39.6°C, and circulating neutrophils had increased ~33%. Two hours following the cessation of immersion, circulating neutrophils had increased ~141% over baseline values, despite a fall in T_{re} to 37.4°C.

Taken as a whole, these results suggest that passive heat exposure elicits an increase in the circulating neutrophil count. As is the case for exercise-induced neutrophilia, quantification of the exact neutrophil response to passive heat exposure is difficult due to the differences in methodologies and results across research investigations.
Neutrophil Response to Exercise Combined With Heat Exposure

Like exercise and passive heat exposure studies, a variety of methodologies to induce exercise and heat stress have been employed. The results are equivocal concerning whether exercise and heat combined elicit a larger degree of neutrophilia than exercise or heat exposure separately. Additionally, the degree of neutrophilia following exercise and heat exposure varies across studies. The following section will first discuss the studies that found no significant effect of combined exercise and heat exposure on neutrophilia, and then discuss those studies that found a significant effect.

In 13 non-heat acclimatized endurance trained males, Liang et al. (2005) found no statistically significant difference between the neutrophilia induced by two hours of cycling at 62% VO$_{2\text{max}}$ in a hot environment and a thermoneutral environment (control). Subjects exercised in an environmental chamber at 30.3°C, 76% RH for the hot trial, and 20.4°C, 60% RH for the control trial. After 50 minutes of exercise, T$_{re}$ became significantly higher in the hot trial and remained so for the rest of the two-hour exercise session. After two hours of exercise, T$_{re}$ measured 38.7 and 38.1°C for the hot and cold trials, respectively. Despite the significant difference in T$_{re}$, neutrophil counts did not differ significantly immediately post-exercise or two hours post-exercise.

Likewise, a study conducted by McFarlin and Mitchell (2003) found no significant effect of a heated environment on exercise-induced neutrophilia. In this investigation, 10 men cycled in an environmental chamber for 60 minutes at 60% VO$_{2\text{peak}}$ twice, once at 38°C, 45% RH, and once at 8°C, 50% RH. T$_{re}$ exceeded 38°C in both trials, but became significantly different between trials after 45 minutes of exercise, at which points it measured ~38.3 and 38.7°C, respectively. Despite the significantly
different $T_{re}$, neutrophil counts did not differ between conditions at any point during exercise, or at 24 hours post-exercise.

Despite the results of the two aforementioned studies, research has also provided compelling evidence suggesting that exercise in the heat induces a larger degree of neutrophilia compared to thermoneutral exercise. For example, in a study by Niess et al. (2003), seven endurance-trained men completed two 60-minute treadmill runs at 90% of individual anaerobic threshold in an environmental chamber. The first trial (EX1) was performed in 18°C, 50% RH, while the second trial (EX2) was performed in 28°C, 50% RH. At the end of exercise, $T_{re}$ exceeded 38°C in both trials, but was significantly higher for EX2 at 39.8°C, than for EX1 at 38.7°C. The only time-point at which neutrophil counts were significantly different between EX1 and EX2 was three hours after exercise when EX2 exhibited an ~15% larger circulating neutrophil count. It is difficult to quantify these results, however, because the authors fail to explain if the subjects recovered outside of the environmental chamber in the laboratory’s ambient temperature, or remained seated in the chamber, exposed to the trial’s prescribed environmental conditions, following exercise. Additionally, the authors do not present $T_{re}$ data for the three hours following exercise. Nonetheless, the authors conclude that exercise in elevated ambient temperatures seems to elicit greater changes circulating neutrophils than exercise in a thermoneutral environment.

In another study, Mitchell et al. (2002) had 10 moderately trained men perform two bouts of cycling in an environmental chamber for 75 minutes at 55% $\text{VO}_{2\text{peak}}$. Conditions were 22°C, 30% RH and 38°C, 45% RH for the thermoneutral and hot trials, respectively. After 45 minutes of exercise, $T_{re}$ became significantly higher in the hot
trial, and remained so throughout the duration of exercise. In the hot trial, $T_{re}$ exceeded 38°C after 45 minutes and peaked at ~39°C, while $T_{re}$ in the cold trial peaked at ~37.7°C. While exercise in both environments elicited increases in the circulating neutrophil concentration over pre-exercise values immediately after, and two hours post-exercise, the count was ~37% higher in the hot trial, relative to the thermoneutral trial, at two hours post-exercise only. Again, the authors conclude that the stress imposed by exercise combined with heat exposure is additive, resulting in an additive effect on neutrophil mobilization.

In an investigation studying the effect of exercise combined with heat exposure on the circulating granulocyte count, Brenner et al. (1999) had seven moderately fit young men exercise on a cycle ergometer for 60 minutes at 55% VO$_{2peak}$ while immersed in water up to the shoulder. The first trial consisted of submersion in 18°C water, and the second trial in 35°C water. While $T_{re}$ was not significantly altered during exercise in 18°C water, it significantly increased 0.6°C after 30 minutes of exercise in 35°C water, and peaked at ~38.3°C after 60 minutes of exercise. In both hot and cold trials, the circulating granulocyte concentration increased significantly after 60 minutes of exercise. At 60 minutes, however, the circulating granulocyte concentration elicited by the hot trial was ~15% larger than that elicited by the cold trial. Although neutrophils were not measured directly, the granulocyte counts suggest that exercise in a warm environment may elicit a larger increase in circulating neutrophils than exercise in a cooler environment.

Cross et al. (1996) also measured the circulating granulocyte count in nine moderately-fit men following exercise for 40 minutes at 65% VO$_{2max}$ on two separate
occasions; once immersed to the mid-chest in 23°C water, and once in 39°C water. Following exercise, subjects remained sitting in the water for 40 minutes. $T_{re}$ progressively increased during exercise, peaking at $\sim$39.1°C ten minutes after the cessation of exercise in the hot trial, and 37.6°C immediately after the cessation of exercise in the cold trial. $T_{re}$ was significantly higher in the warm trial after 15 minutes of exercise, and remained so throughout the duration of exercise and the 40 minutes of exposure following exercise. This study measured granulocytes, rather than neutrophils, and found that the warm exercise trial resulted in an $\sim$19 to 25% larger circulating granulocyte concentration between minutes 15-25 of exercise, corresponding to the onset of the significant difference in $T_{re}$ between trials. Granulocyte count was again significantly larger by $\sim$57% during minutes 20-40 of the resting exposure following exercise, despite a decrease in $T_{re}$.

Similar to Cross et al. (1996), Rhind et al. (1999) conducted a study in which 10 recreationally active males cycled for 40 minutes at 65% VO$_{2\text{peak}}$ while immersed in water to the mid-chest, once in 39°C water, and once in 18°C water. Exercise in the warm water caused $T_{re}$ to significantly increase from 37.2°C to 39.3°C, while exercise in the cold water caused $T_{re}$ to increase only to 37.6°C. Exercise in the warm water resulted in significant increases in the circulating granulocyte concentration after 40 minutes of exercise ($\sim$34%), and two hours after exercise ceased ($\sim$65%). Although the significant increases in circulating granulocytes suggest significant increases in circulating neutrophils, the proportional increase in neutrophils is unknown, based on these results.

A shortcoming of the literature on these topics is the use of various methodologies of heat and exercise exposure throughout the studies. These differences elicit diverse
physiological responses, and therefore prevent the comparison of results. Among the aforementioned studies, differences in methodology include the type of environmental exposure (environmental chamber or water immersion), temperature and humidity of environmental exposure, percentage of body surface area exposed, the mode, intensity and duration of exercise, training status of subjects, and the timing of blood sampling. Additionally, the studies employing methodology closest to the current study (Cross et al. 1996; Rhind et al. 1999) measured total granulocytes rather than neutrophils, thus reinforcing the need for the current study.
CHAPTER III

METHODOLOGY

The purposes of this study were to determine the effect of exercise in a warm environment on the number of circulating neutrophils, and to investigate the relationship between $T_{re}$ and the degree of exercise-induced neutrophilia. The former purpose was investigated by comparing the circulating neutrophil count following exercise in a cool environment to the circulating neutrophil count following exercise in a warm environment. The latter purpose was investigated by comparing $T_{re}$ to the circulating neutrophil count elicited by exercise in both conditions combined. This chapter explains the methodology for this investigation by first describing the subject pool and the instrumentation used, followed by the protocol for the experiment and procedures for data analysis. Finally, the chapter will describe the statistical data analysis.

Subjects

Eight recreationally active male athletes between the ages of 18 and 40 years participated in this study. To be considered recreationally active for the purpose of this study, subjects must have engaged in at least 30 minutes of moderate physical activity at a minimum of 4 days per week. Exclusion criteria included: smoking, any history of major medical disease, any known infection within three weeks prior to participation in the study, any major orthopedic concerns, previous history of heat related maladies, or current use of anti-inflammatory drugs (i.e.- NSAIDs).
Instrumentation

The subject’s height was determined using a portable stadiometer (Perspectives Enterprises, Portage, MI). Body mass was measured by a mechanical scale (Detecto, Webb City, MO). Oxygen consumption during all trials was measured by a Parvo Medics TrueMax 2400 Metabolic System (Parvo Medics, Salt Lake City, UT) while the subject cycled on a mechanically braked cycle ergometer (Monark, Varberg, Sweden). The cycle ergometer was immersed in a 1,790-liter submersion tank. Core body temperature (T\textsubscript{re}) was monitored by a YSI rectal probe (YSI, Daytona, OH) connected to a thermistor thermometer (Cole-Parmer, Vernon Hill, Ill). Heart rate (HR) was monitored by a Polar telemetry system (Polar Electro Inc., Lake Success, NY). Blood pressure (BP) measurements were taken using a Diagnostix sphygmomanometer (American Diagnostic Corporation, Hauppauge, NY). Subjects reported ratings of perceived exertion (RPE) according to Borg’s original 6-20 RPE scale (Borg 1970).

Blood samples were drawn by a trained technician using 10-mL syringes (Becton, Dickinson and Company, Franklin Lakes, NJ) attached to hypodermic needles (Kendall Healthcare Products, Covidien, Mansfield, MA), and transferred to three-mL K2-EDTA Vacutainers® (Becton, Dickinson and Company, Franklin Lakes, NJ) for storage prior to analysis.

Protocol

The protocol for this investigation required subjects to visit the Applied Physiology Laboratory on three separate occasions. The first session consisted of the subjects signing the informed consent form, providing subject characteristics, undergoing a physical examination and medical screening and performing a graded maximal exercise test on a cycle
ergometer to obtain peak oxygen uptake (VO$_{2peak}$). The subsequent two sessions consisted of submaximal cycling while immersed in water up to the xyphoid process. The submaximal exercise lasted 40 minutes at a workload designed to elicit an intensity of 65% VO$_{2peak}$. Blood samples were drawn pre-, post- and two hours-post exercise by a trained technician using standard venipuncture techniques. The circulating numbers of neutrophils were determined from whole blood samples.

**Session One.** Subjects arrived to the Applied Physiology Laboratory at least four hours post-prandial. Subjects then read the informed consent form. After having the opportunity to ask questions, subjects who agreed to participate in the study signed the informed consent form. Subjects then filled out a standard Applied Physiology Laboratory medical history form, and underwent a medical screening and physical examination. Next, subjects were asked to provide their age, and their physical characteristics were measured. Height was measured using a stadiometer as subjects stood flat on their feet without shoes, back and heels flush against the stadiometer, looking straight ahead. Body mass was measured using a mechanical scale as again subjects stood flat on both feet without shoes.

Subjects changed into appropriate underwater attire and strapped a heart rate monitor around their chests. After five minutes of seated rest, a two-minute resting VO$_2$ sample was obtained. Next, five minutes were allotted for subjects to warm-up on an ergometer on land at a self-selected pace, and five minutes to stretch prior to the graded maximal exercise test. Following the warm-up, subjects fastened a weighted belt around their waists to overcome buoyancy while immersed, and entered the water tank. Subjects had time to practice and become comfortable cycling on the cycle ergometer while immersed in 25º C water to the level of the xyphoid process. Water was added or removed as needed to insure the proper
level of immersion. Seat height and handle bar angle were adjusted for each subject and recorded for consistency during the subsequent exercise sessions.

Immediately after the seat and handle bars were adjusted, the graded maximal exercise test began with the subject pedaling at a rate of 40 rpm for five minutes. At the end of the first stage, the pedal rate was increased to 45 rpm and subjects rode at the new pedal rate for three minutes. Thereafter, pedal rate increased by five rpm every three minutes. Exercise intensity was monitored by continuous collection of VO\textsubscript{2} data, while HR and RPE measurements were collected during each minute of the test to monitor subject safety. The test was terminated when the subjects reached volitional fatigue. Following cessation of the test, subjects performed an active recovery at a self-selected pace on the cycle ergometer until HRs fell below 120 beats per minute (BPM). If HRs failed to fall below 120 bpm in three to five minutes, subjects stopped cycling and continued recovery lying down on a cot until HRs fell below 120 bpm. Subjects then left the Applied Physiology Laboratory when HRs were within 20 beats of the resting rate and blood pressures were within 10 mmHg of resting value. From this trial, pedal rates eliciting 65% of the subjects’ VO\textsubscript{2peak} were computed using the Karvonen method \((0.65 \times (\text{VO}_{2\text{peak}} - \text{VO}_{2\text{rest}}) + \text{VO}_{2\text{rest}}, \text{where VO}_{2\text{rest}} \text{is resting oxygen uptake})\).

**Sessions Two and Three.** The protocol for sessions two and three were the same, with the exception of the temperature of water in which the subjects were immersed: 25\(^\circ\) C (cool) or 39\(^\circ\) C (warm). At least two days following the previous exercise session, subjects again arrived to the Applied Physiology Laboratory at least four hours post-prandial; however, water could be consumed at the subjects’ discretion. Additionally, subjects abstained from vigorous activity and alcohol consumption for 24 hours prior to arrival, and
from caffeine consumption for 12 hours prior to arrival. Subjects were instructed to eat their typical pre-workout meal for the last meal consumed prior to arrival at the Applied Physiology Laboratory. The type and quantity of food consumed was recorded by the subjects, and the same meal was ingested as the last meal prior to the next exercise session. The arrival time to the Applied Physiology Laboratory was the same for sessions two and three. The water temperature during immersed exercise was randomly assigned and counterbalanced. For each session, subjects cycled for 40 minutes at a workload eliciting an intensity of 65% VO_{2peak}, while immersed in water up to the xyphoid process.

Subjects had their body mass measured using a mechanical scale while standing flat on their feet without shoes. Subjects changed into the same clothing worn for the graded maximal exercise test, self-inserted a rectal thermometer approximately 10 centimeters into the rectum, and strapped a heart rate monitor around their chests. Subjects then rested quietly for twenty minutes on land in an upright-seated position. Subjects were allowed to wear a shirt if they felt cold. At minute 10 of seated rest, a two-minute resting VO_{2} sample was collected. At minute 19 of rest, baseline HR and blood pressure measurements were taken. At the end of the rest period, a nine-mL blood sample was drawn. The blood sample was transferred from the syringe to three three-mL K2-EDTA treated Vacutainers® and stored on ice for later analysis.

Subjects were then escorted to the tank containing the cycle ergometer, and fastened a weighted belt around their waists. After the subjects were immersed up to the xyphoid process, a five-minute warm-up period began, during which the subjects cycled at a self-selected pace. Following the warm-up period, subjects began cycling at the workload estimated to elicit 65% of their VO_{2peak}. At minute three of exercise, the subjects’ VO_{2} was
monitored for two minutes to ensure that they were working at 65% VO$_{2peak}$. At this point in session two, if the subjects were not exercising at 65% VO$_{2peak}$, pedal rate was adjusted to elicit an intensity of 65% VO$_{2peak}$. Pedal rate was not adjusted again during session two. The magnitude and timing of any change in pedal rate was recorded to standardize the workload for session three. Thereafter, expired air was sampled for the last two minutes of each 10-minute interval (minutes 8-10, 18-20, 28-30, 38-40). Throughout the 40 minutes of exercise, the subjects’ HR and RPE were measured and recorded during the last 15 seconds of each five-minute interval in order to monitor subject safety (Figure 1).

$T_{re}$ was monitored minute-by-minute and recorded at five-minute intervals in order to avoid any large unexpected increases in core body temperature. Subjects had access to drinking water at their own discretion throughout exercise. The trial was stopped if a subject’s core temperature rose above 39°C (102°F), or if otherwise deemed necessary.

At the conclusion of the 40-minute cycling session, the subjects were helped out of the water tank and dried off. The participants were escorted to an upright chair where another nine-mL blood sample was drawn. The blood sample was transferred and divided equally into three K2-EDTA treated Vacutainers® and stored in an insulated ice container for later analysis. At this point, subjects were allowed to return to a land-based cycle ergometer for a cool down at a workload of their choice. The recovery on the cycle ergometer continued until subjects felt comfortable. Water was offered if subjects were thirsty.
After the cool down had been completed, subjects changed into a sweat suit to keep warm and remained in the Applied Physiology Laboratory for two hours. During this time, subjects were free to engage in general movement and participate in activities requiring minimal exertion (i.e.- reading). BP measurements were taken every five minutes during the first 20 minutes after the cessation of exercise to ensure subject safety. At the end of the two-hour period, final HR, BP and core temperature measurements were taken. Immediately following vital measurements, a third nine-mL blood sample was drawn, transferred to three K2-EDTA treated Vacutainers®, and placed on ice for storage and later analysis. Subjects then self-extracted the rectal thermometer and had a final body mass measurement taken, again using a mechanical scale while standing flat on their feet without shoes. Comparison of post-exercise and pre-exercise body mass measurements further allowed monitoring of subject safety, indicating if subjects were losing body mass due to water loss during the exercise sessions. Subjects then left the laboratory, providing core temperatures were below 38° C, and HRs and BPs had returned to within 20 bpm and 10 mmHg of resting values, respectively. If the vital measurements had not returned to resting values, subjects were
required to remain in the Applied Physiology Laboratory until the measurements decreased to within the aforementioned limits of resting values.

Procedures

**Hematocrit.** The UNC-Chapel Hill hospital’s core laboratory determined resting, pre-exercise and two hours post-exercise hematocrit (Hct) measurements using whole blood samples and the Advia 2120 Hematology system (Siemens Healthcare Diagnostics, Deerfield, IL).

**Hemoglobin.** The UNC-Chapel Hill hospital’s core laboratory determined resting, pre-exercise and two hours post-exercise hemoglobin (Hb) measurements using whole blood samples and the Advia 2120 Hematology system (Siemens Healthcare Diagnostics, Deerfield, IL).

**Plasma Volume Shift.** Plasma volume shifts were calculated using the method proposed by Dill and Costill (1974). Neutrophil counts were corrected for any shifts in plasma volume to ensure that any observed changes in neutrophilia were not due to hemoconcentration.

**Neutrophil Analysis.** The UNC-Chapel Hill hospital’s core laboratory performed a complete blood count with a differential using an Advia 2120 Hematology System (Siemens Healthcare Diagnostics, Deerfield, IL).

**Data Analysis.** Since all subjects did not complete the full duration of the warm trial, the VO\(_2\), HR, RPE and T\(_{re}\) data were reduced using minutes 10, 20, 30 and the subjects’ final minute, regardless of the exact minutes of exercise completed. Data analysis was performed using SPSS statistical software, version 15.0 (Chicago, Ill.). An \(\alpha\)-level was set *a priori* at 0.05 for all analyses.
For hypothesis one, analysis consisted of a one-way ANOVA to determine if the neutrophil count differed between measurements taken at rest, immediately post-exercise and two hours post-exercise during the exercise session conducted in a warm environment.

For hypothesis two, analysis consisted of a 2 X 3 (trials by time) ANOVA to determine if the change in neutrophil count from pre- to immediately post-exercise differed from pre- to two hours post-exercise both within and between the cool and warm environments. A Tukey HSD post-hoc test was used to determine between which comparisons significant differences occurred.

For hypothesis three, a Pearson-Product Moment Correlation determined if the change in T_{re} was positively correlated with the change in the circulating neutrophil count from pre- to post-exercise.

For hypothesis four, a 2 X 2 Chi Square Test of Independence determined if exercise sessions in which T_{re} exceeded 38° C elicited a significant neutrophil response, and if exercise sessions in which T_{re} did not exceed 38° C did not elicit a significant neutrophil response. A significant neutrophil response was defined as a change in the neutrophil count that was at least 5% greater than the change in plasma volume.
CHAPTER IV
RESULTS

The primary purpose of this investigation was to determine if exercise combined with heat exposure elicited a greater increase in the number of circulating neutrophils than exercise in a more thermoneutral (cool) environment. The secondary purposes were to, 1) investigate the relationship between the change in core temperature \( T_{re} \) and the degree of neutrophilia and 2) validate a previously stated hypothesis that the \( T_{re} \) must increase to greater than 38°C for a significant rise in neutrophilia to occur. This chapter will first discuss the subjects’ characteristics and general exercise responses during both trials. Thereafter, results will be presented by order of hypothesis.

Eight recreationally active males with an average age of 24.0 ± 1.7 (SD) years participated in this investigation. They were 180.4 ± 6.1 cm tall and had a body mass of 78.3 ± 6.1 kg. The average Body Mass Index (BMI) measured 24.05 ± 1.92 kg/m\(^2\). Their \( VO_2\)peak was 52.8 ± 3.7 mL/kg/min, while their absolute \( VO_2\)peak was 4.1 ± 0.4 L/min. Together, the \( VO_2\)peak and BMI scores indicate that the subjects were of a normal body weight and in good cardiovascular condition.

All subjects completed the cool exercise trial. Of the eight subjects, three subjects failed to complete the warm exercise trial; two subjects stopped exercise due to volitional fatigue, one each after 30 and 35 minutes, and a third subject stopped exercise after 30 minutes due to surpassing the core temperature threshold established for subject safety.
Values for VO\textsubscript{2}, HR and RPE during each trial are presented in Table 1. On average, subjects exercised at 33.2 ± 2.7 and 34.7 ± 3.2 mL/kg/min in the cool and warm trials, respectively. The average VO\textsubscript{2}’s did not significantly differ from each other or from the average VO\textsubscript{2} estimated to elicit exercise at 65% of the subjects’ maximal capacity. Ventilation and the respiratory exchange ratio (RER) increased over resting values throughout exercise in both trials, but did not differ between trials during exercise. Relative to the cool trial, T\text{re} became significantly higher than in the warm trial after 20 minutes and remained so for the duration of exercise (Figure 2).

Table 1. Means ± SD oxygen uptake (VO\textsubscript{2}), heart rate (HR) and rating of perceived exertion (RPE) responses during the cool (C) and warm (W) trials (n = 8). * p ≤ 0.02, † p ≤ 0.011; warm vs. cool trial.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>VO\textsubscript{2} (mL/kg/min)</th>
<th>HR (bpm)</th>
<th>RPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>W</td>
<td>C</td>
</tr>
<tr>
<td>Rest</td>
<td>4.4 ± 1.2</td>
<td>4.2 ± 0.6</td>
<td>66.1 ± 4.5</td>
</tr>
<tr>
<td>10</td>
<td>31.4 ± 3.7</td>
<td>33.0 ± 4.4</td>
<td>130.7 ± 19.6</td>
</tr>
<tr>
<td>20</td>
<td>33.3 ± 2.9</td>
<td>35.5 ± 2.7</td>
<td>138.5 ± 21.3</td>
</tr>
<tr>
<td>30</td>
<td>33.6 ± 4.0</td>
<td>34.5 ± 4.1</td>
<td>142.4 ± 19.7</td>
</tr>
<tr>
<td>Final</td>
<td>34.3 ± 3.6</td>
<td>35.6 ± 5.8</td>
<td>141.9 ± 8.8</td>
</tr>
</tbody>
</table>

Hypothesis one stated that exercise in a warm environment would cause a rise in the circulating neutrophil count immediately and two hours post-exercise, compared to resting levels. The results of a one-way, repeated measures ANOVA showed a significant effect of time on the circulating neutrophil count during exercise (Figure 3). Post-hoc analysis showed that the circulating neutrophil count was significantly elevated over resting values immediately post-exercise and at two hours post-exercise (p ≤ 0.002). At two hours post-exercise, the circulating neutrophil count was also significantly elevated over immediately post-exercise values (p = 0.004).
Figure 2. Means ± SD \( T_c \) in both the cool and warm trials during rest, at minutes 10, 20, 30 and during the final minute of exercise. * \( p \leq 0.003 \) warm vs. cool at same time.

Figure 3. Means ± SD circulating neutrophil count response to exercise in a warm environment. Pre-exercise (pre), immediately post-exercise (post) and two hours post exercise (2 Hr Post). Values are corrected for shifts in plasma volume. * \( p \leq 0.002 \) vs. pre-exercise; ‡ \( p = 0.004 \), vs. post-exercise.
Hypothesis two stated that exercise in a warm environment would lead to a higher circulating neutrophil count immediately and two hours post-exercise when compared to exercise in a more thermoneutral environment (Figure 4 and Table 2). A two-by-two trial-by-change from rest ANOVA revealed significant main and interaction effects for environmental temperature and time during exercise. Tukey HSD post-hoc analysis showed that the change in the circulating neutrophil count from pre- to post-exercise did not differ between the two trials (p = 0.05). In a significant time-by-trial interaction effect, the neutrophil count increased two hours post-exercise in both trials, but the change in the neutrophil count from pre- to two hours post-exercise was significantly larger in the warm trial (p = 0.05). Additionally, WBC counts did not differ between trials; however, the percentage of WBC’s comprised by neutrophils was significantly greater in the warm trial at two hours post-exercise (p ≤ 0.019).

Table 2. Means ± SD circulating total white blood cell (WBC) and neutrophil counts during cool (C) and warm (W) exercise trials. Values corrected for shifts in plasma volume. * p ≤ 0.019 between trials.

<table>
<thead>
<tr>
<th>Circulating Blood Parameter</th>
<th>Pre-Exercise</th>
<th>Immediately Post-Exercise</th>
<th>2 Hours Post-Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>W</td>
<td>C</td>
</tr>
<tr>
<td>WBC (x 10^3 cells/µL)</td>
<td>5.22 ± 0.94</td>
<td>5.30 ± 1.13</td>
<td>6.00 ± 1.05</td>
</tr>
<tr>
<td>Neutrophils (x 10^3 cells/µL)</td>
<td>2.76 ± 0.79</td>
<td>2.86 ± 0.84</td>
<td>3.25 ± 0.75</td>
</tr>
<tr>
<td>Neut/WBC (%)</td>
<td>52.6 ± 6.4</td>
<td>53.5 ± 4.9</td>
<td>57.0 ± 6.6</td>
</tr>
</tbody>
</table>

Hypothesis three stated that the change in $T_{re}$ would be positively correlated with the change in the circulating neutrophil count from pre- to post-exercise. The results of a
Pearson-Product Moment correlation indicated that the change in $T_{re}$ was not correlated with the change in the circulating neutrophil count ($r = 0.052; p = 0.850$).

Hypothesis four stated that exercise sessions in which $T_{re}$ did not exceed $38^\circ C$ would not elicit a significant neutrophil response, but exercise sessions in which $T_{re}$ did exceed $38^\circ C$ would elicit a significant response. Any increase in the neutrophil count that was at least five percent greater than the change in plasma volume was considered significant. The circulating neutrophil count increased significantly during each exercise session, regardless of $T_{re}$. Immediately post-exercise, the counts increased $0.552 \pm 0.456$ and $0.518 \pm 0.231 \times 10^3$ cells for trials in which $T_{re}$ did not exceed $38^\circ C$ and trials in which $T_{re}$ exceeded $38^\circ C$, respectively. Two hours post-exercise, the neutrophil counts had increased $1.486 \pm 0.909$...

**Figure 4.** Means ± SD change in the circulating neutrophil count from pre-exercise to post-exercise (Post - Pre) and pre-exercise to two hours post-exercise (2 Hr Post - Pre) in cool and warm environments. Values corrected for shifts in plasma volume. * $p = 0.05$ vs. pre-post in both conditions; ‡ $p = 0.05$ vs. all other comparisons.
and $2.977 \pm 1.361 \times 10^3$ cells for trials in which $T_{re}$ did not exceed 38°C and trials in which $T_{re}$ exceeded 38°C, respectively. Although a two-by-two Chi Square test of Independence showed that there was no relationship between a $T_{re}$ of 38°C and the degree of neutrophilia ($p = 1.00$), further analysis of an independent t-test revealed a significantly larger increase in neutrophils at two hours post-exercise for sessions in which $T_{re}$ exceeded 38°C ($p = 0.04$).
CHAPTER V
DISCUSSION

Research suggests that neutrophils could be responsible for causing both host tissue damage and repair (Suzuki et al. 1999; Pizza et al. 2005; Jesse et al. 1998; Teixeira et al. 2003). The exact function of neutrophils may be increasingly important during situations in which the circulating neutrophil count is augmented, and therefore the likelihood of neutrophil-mediated tissue damage or repair increased. Certain physiological stressors are known to elicit increases in this circulating immune parameter; two of these stressors are exercise and passive heat exposure. The purpose of this investigation was to determine if exercise combined with heat exposure had an additive effect on augmenting the circulating neutrophil count. Subjects cycled at 65% VO₂peak for 40 minutes while immersed in water up to the xyphoid process twice, once in 25°C and once in 39°C. During exercise, the monitored physiological parameters reacted as anticipated. Exercise intensity as quantified by VO₂ did not differ between trials; however, significantly higher HR and RPE values were observed during the warm trial, perhaps due in part to larger thermoregulatory demands during the warm trial. Tre was also significantly higher in the warm trial due to the subjects’ inability to dissipate heat as effectively as in the cool trial.

Circulating Neutrophil Count in a Hot and Cool Environment

Previous literature has shown that WBC’s respond biphasically to exercise in both cool and warm environments (Mitchell et al. 2002; Cross et al. 1996; Rhind et al. 1999; Niess
et al. 2003). This response is characterized by an initial increase during exercise, followed by a secondary, larger increase two hours post-exercise. The WBC counts in the present study also increased during exercise, and exhibited a further increase two hours post-exercise. The increases in the WBC count were attributable almost entirely to increases in circulating neutrophils. Exercise in the warm environment also elicited a neutrophil response post and two hours post-exercise, but the two hours post-exercise response was significantly greater in the warm environment. Similarly to the current study, Cross et al. (1996) found no difference between cool and warm conditions in the increase in circulating granulocytes from pre- to post-exercise; however, the increase in circulating granulocytes was found to be significantly higher in the warm trial at 40 minutes post-exercise. Because neutrophils comprise ~92% of total granulocytes, these results are suggestive of neutrophil responses. Mitchell et al. (2002) and Niess et al. (2003) found that the circulating neutrophil count was significantly higher in the warm trial only at two and three hours post-exercise, respectively. Furthermore, the magnitude of increase in circulating neutrophils from baseline to two hours post-exercise for both the warm and cool trials in the current study are similar to those reported by Rhind et al. (1999) who found differences of ~1.6 and ~3.0 x 10^3 granulocytic cells/µL for a cool and warm trial, respectively.

A large body of research shows that exercise in the heat places greater physiological demands on the human body than exercise in a more thermoneutral environment. Several of these exacerbated physiological demands could be responsible for eliciting a larger neutrophilia when exercising in the heat. One manifestation of the greater physiological demand is an increased cardiac output required to meet both metabolic and thermoregulatory requirements (Rowell 1974). While cardiac output was not measured in the current study,
the significantly higher HR without a concomitant increase in VO\textsubscript{2} during the warm trial indirectly suggests that cardiac output may have been higher, relative to the cool trial. As suggested by McCarthy and Dale (1988) and Niess et al. (2003), a greater cardiac output could result in greater blood flow through the lungs, therefore inducing greater shear forces throughout the pulmonary and systemic vasculature. Exacerbated shear forces could lead to increased neutrophil efflux from the lungs and endothelium into circulation. The primary effect of cardiac output on neutrophilia would be expected immediately post-exercise, because cardiac output decreases towards resting levels shortly following the cessation of exercise.

Exercise in a warm environment also elicits greater neuroendocrine activity than exercise of the same intensity and duration performed in a cooler environment (Cross et al. 1996; Rhind et al. 1999; Niess et al. 2003; Mitchell et al. 2002). Although the stress hormones epinephrine, norepinephrine and cortisol were not measured in the current study, results of similar research make it is reasonable to suspect that their concentrations were higher in the warm trial (Rhind et al. 1999; Cross et al. 1996). Rhind et al. (1999) found that immediately post-exercise of the same intensity and duration performed in similar environmental conditions to the current study, concentrations of both epinephrine and norepinephrine were significantly greater in the warm trial. Considering the short half-life of catecholamines (~2.5 minutes), this suggests that most of their influence on neutrophilia is evident in the first of the biphasic responses. Thirty minutes into recovery, Rhind et al. (1999) also found that the cortisol concentration was significantly greater in the warm trial. Considering the lag time between exercise and peak cortisol concentrations (~30 minutes), this result suggests that much of cortisol’s influence on neutrophilia is evident in the second
of the biphasic responses. Additionally, Rhind et al. (1999) found that cortisol levels were significantly positively associated with total circulating granulocytes ($R^2 = 0.136, p = 0.004$), indirectly suggesting a relationship between cortisol concentrations and the circulating neutrophil count. Cross et al. (1996), using exercise of the same intensity and duration performed in similar environmental conditions to the current study, found significantly larger cortisol concentrations during and 40 minutes post-exercise during the warm trial. Exercise in the heat has also been shown to strengthen the correlation between the circulating leukocyte count and cortisol concentrations (Severs et al. 1996).

The mechanisms by which these hormones elicit increases in the circulating neutrophil count vary. Boxer et al. (1980) proposed that epinephrine’s primary mechanism of action may be related to stimulating beta-adrenergic receptors on the endothelial lining of blood vessels to release cyclic-AMP, which then may decrease the adhesion of granulocytes to the endothelium and result in granulocyte egress into circulation. Research suggests that action of norepinephrine is two-fold; first, it may increase neutrophil demargination from the spleen and lymph glands, and second, in increasing cardiac contractility, it may also increase cardiac output, and therefore demargination of neutrophils located in stores of the lungs and endothelium (Brenner et al. 1998; Shephard 2002). Nakagawa et al. (1998) suggest that cortisol elicits granulocyte egress from marginal and bone marrow stores, possibly by altering adhesion molecules on both the endothelium and granulocytes.

Finally, relative to exercise in a cool environment, the increased physical discomfort associated with exercise in a warm environment could induce greater mental stress that feeds back to augment circulating catecholamine and cortisol concentrations, and therefore
neutrophil demargination from the marginal stores as previously discussed (Niess et al. 2003; Shephard & Sidney 1975).

Although the exact cause of the neutrophilia during exercise in cool and warm environments cannot be determined from the present study, a large body of research offers several possible mechanisms. It may be concluded however, that 40 minutes of exercise at 65% VO$_2$peak performed in 39°C water produces a perturbation of the immune system sufficient to cause neutrophilia post- and two hours post-exercise. Furthermore, exercise in a warm environment causes a greater perturbation in the immune system than exercise of the same duration and intensity performed in a cooler environment.

**Temperature Threshold**

Kappel et al. (1991) suggested that a $T_{re}$ of 38°C had to be exceeded for significant increases in neutrophilia to occur. The researchers based this theory on results obtained by passively increasing $T_{re}$ via whole body immersion in warm water, not on exercise induced increases in $T_{re}$. Although several investigators support this threshold theory (Cross et al. 1996; Brenner et al. 1999; Severs et al. 1996; Downing et al. 1988), the present study found no relationship between the exercise-induced changes in $T_{re}$ and circulating neutrophil count. Rhind et al. (1999) and Brenner et al. (1999) similarly found no significant associations between $T_{re}$ and the circulating neutrophil count. Furthermore, a Chi-Square Test of Independence in the current investigation showed no relationship between the proposed $T_{re}$ threshold of 38°C and significant increases in the circulating neutrophil count. This result is attributable to the fact that, regardless of $T_{re}$, the circulating neutrophil count increased significantly during the exercise sessions. Results of an independent t-test however, show that exercise sessions in which $T_{re}$ exceeded 38°C elicited a significantly greater peak
circulating neutrophil count than exercise sessions in which $T_{re}$ did not exceed 38°C. In agreement with the present findings, Mitchell et al. (2002) noted that when exercise $T_{re}$ peaked at 39°C, the rise in circulating neutrophils was greater than when $T_{re}$ peaked at 37.7°C. Similarly, Rhind et al. (1999) and Cross et al. (1996) found that the increase in circulating granulocytes was greater when exercise $T_{re}$ surpassed 39°C, than when $T_{re}$ peaked at ~37.6°C.

The results of the current investigation suggest that $T_{re}$ is not the primary controller of neutrophilia during exercise; several additional mechanisms may be working together to determine the circulating neutrophil count. The present findings also show that exercise-induced neutrophilia is not based on a 38°C $T_{re}$ threshold. However, $T_{re}$ appears to influence the circulating neutrophil count since a greater exercise-induced $T_{re}$ occurred concomitantly with greater neutrophilia.

**Limitations**

One limitation of this investigation is the small sample size of eight subjects. This sample size however, is comparable with similar studies that have employed samples of between seven and ten subjects (Niess et al. 2003; Cross et al. 1996; Mitchell et al. 2002). Additionally, blood cell counts for seven of the eight subjects responded similarly between trials. A second limitation stems from three of eight subjects failing to complete exercise during the warm trial. Examination of their individual results shows that despite the abbreviated exercise time, their responses were similar to subjects who completed the exercise.
Conclusions

Exercise for 40 minutes at 65% VO\textsubscript{2}peak causes neutrophilia, which is most evident two hours post-exercise. Moreover, exposure to heat during the exercise results in higher core temperatures and elicits a greater neutrophilia two hours post-exercise. Based on these findings, hypothesis one was accepted and hypothesis two was partially accepted. Although no relationship was observed between a T\textsubscript{re} of 38°C and increases in the circulating neutrophil count, T\textsubscript{re} appears to contribute to exercise-induced neutrophilia since a greater exercise-induced T\textsubscript{re} occurred concomitantly with greater neutrophilia. Considering these results, hypotheses three and four were rejected.

Recommendations for Future Research

Although these results suggest that exercise in a warm environment may elicit greater immune system perturbations than exercise in a cooler environment, no conclusions may be made concerning the effect of exercise in any temperatures other than 25°C or 39°C, or during exercise of any other intensity or duration. To further elucidate the combined environmental and exercise effects on the circulating neutrophil count, future studies should employ various environmental conditions during exercise of the same duration and intensity, or the same environmental condition during exercise of various durations and intensities. In the same manner, the current results can be generalized only to men between the ages of 18 and 40 years. Additional research is needed to discover if this combination of exercise and environmental condition elicits the same neutrophil responses in pediatric, geriatric or female populations.

Future endeavors may also benefit from using a larger subject pool. In doing so, the statistical power increases; however, investigators should be sure to select subjects who are
likely able to complete the protocol. Although heat acclimatization prior to participation could lead to altered physiological and neutrophil responses during testing, it could help subjects avoid premature cessation of exercise and eliminate any confounding variable that may be the result of different levels of heat acclimatization across the subject pool.

Measuring neutrophil responses elicited by resting exposure to several environmental temperatures in the same subjects could help to distinguish between the temperature and exercise effects and further examine the mechanisms contributing to the neutrophilia. Measuring stress-mediated hormones, such as epinephrine, norepinephrine and cortisol, would also provide data to examine relationships between the potential modifiers of exercise-induced neutrophilia and the circulating neutrophil count. Finally, an additional practice session prior to participation in the exercise trials may help to eliminate any additional stress the subjects experience during the first trial due to unfamiliarity with the feeling of cycling in water. In attenuating a stress that could potentially influence stress-mediated hormones, the possibility of an order effect is reduced.

These results carry implications for both clinical and performance-oriented populations. Exercise in the heat appears to exacerbate the immune response as manifested by the circulating neutrophil count. The question for future research is whether the augmented circulating neutrophil response is protective of or harmful to damaged and healthy host tissue. Clinically, individuals suffering from chronic inflammatory diseases such as rheumatoid arthritis may need to exercise caution with respect to environmental conditions during exercise if a larger circulating neutrophil count predisposes them to exacerbated tissue injury. Similarly, if the augmented neutrophil count exacerbates tissue damage and prolongs tissue repair, individuals chronically engaged in physical activity in the
heat may need to alter their training regimen to allow for a longer recovery. On the other hand, if augmenting the neutrophil response results in more efficient tissue repair, these individuals may consider exercise in the heat as a means to attenuate disease effects or accelerate recovery following muscle damage.
APPENDIX A:

Training History Form

Training History Form

Are you currently involved in a regular exercise program? ___yes___no

Do you regularly walk or run one or more miles **continuously**? ___yes___no
How often per week? __________

Do you participate in other forms of regular aerobic exercise? ___yes___no
If yes, average number of miles covered per workout or day: ___miles___

Do you regularly lift weights? ___yes___no
____ # times/wk

Do you consider yourself: (please circle)

sedentary   lightly active   moderately active   highly active

How long (years) have you been consistently training?

Overall, how many days are you active each week?

Besides those listed, are there any other types of activities that you participate in on a regular basis? How much time do you spend each week in these activities?

Please describe your average typical weekly workout schedule over the last six months (i.e. types of workouts/amount per day)

When was the last time you had an injury that stopped you from exercising for more than three days, and what was the injury?

How long were you injured?
APPENDIX B:

Informed Consent Form

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects age 18-40
Biomedical Form

________________________________________________________________________

IRB Study # 07-1827
Consent Form Version Date: 12.5.2007

Title of Study: The Effect of Exercise in a Warm Environment on Tumor Necrosis Factor-α Concentration and Circulating Neutrophil Count

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________________________________________________________________________

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.
What is the purpose of this study?

**Background:** Stress, either physical or mental, has been shown to alter immune function. Two individual stressors that are known to alter immune function include exercise and heat exposure. While many studies have examined the effects of heat and exercise individually on immune function, little research has been devoted to exploring the combined effect of exercise and heat on immune function. Any disruption of immune function due to exercise in the heat may be of particular importance to coaches, athletes, physicians, and individuals whose professions require activity in warm environments (firefighters, military personnel). The current study will focus on the effect of exercise in the heat on two markers of immune function: Tumor Necrosis Factor-α (TNF-α) and Neutrophils.

The purpose of this research study is two fold; 1) to determine how exercise in the heat effects the TNF-α concentration in the blood and 2) to determine how exercise in the heat effects the number of circulating neutrophils in the blood.

Are there any reasons you should not be in this study?

You should not be in this study if you are female, have a history of heat related problems, heart disease, diabetes or diseases affecting your lungs (such as asthma), are not between the ages of 18-40, have an orthopedic injury that would hinder your ability to exercise, are not comfortable with water, have claustrophobic tendencies, if you are currently on anti-inflammatory medications, or if you are not comfortable with blood draws. Also, you should not be in this study if you have had an infection within the past three weeks.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately eight people in this research study.

How long will your part in this study last?

Your participation in the study will consist of three sessions. The first session will take approximately 1.5 hours. The second and third sessions will take approximately 3.5 hours. The first session will be used to conduct a physical exam, to determine your peak aerobic capacity, and to establish the exercise intensity for the following two sessions. The two exercise sessions will involve sub-maximal exercise. They will be separated by at least 24 hours, and must occur within 2 weeks of the first test session.

What will happen if you take part in the study?

You will be asked to complete three exercise sessions. Each session will consist of approximately 40 minutes of actual exercise. The first session will be used...
consent, weight and height measurements, to complete a physical exam, and to complete a
peak exercise test.

You will arrive to the Applied Physiology Laboratory for the first appointment having
refrained from alcohol consumption for the past 24 hours. Immediately after arriving to the
lab you will read and sign the informed consent form. Once you have agreed to participate
and sign the consent form, you will fill out a medical history form and have your weight and
height measured. You may elect not to answer any of the questions on the medical history
form; however, this may result in your exclusion from the study. You will complete the
standard Applied Physiology Laboratory medical screening history and undergo a physical
examination administered by Dr. McMurray.

Next, you will be given the opportunity to practice cycling on a stationary bike, while also
taking time to stretch and warm up before the exercise test. You will then strap a battery
operated heart rate monitor around your chest. Before beginning the test, you will practice
breathing through a mouthpiece apparatus. When comfortable, you will move to a different
cycle ergometer that is immersed into chest high water of a cool temperature (25° Celsius,
77° Fahrenheit). You will climb down large no-slip stairs into the tank with assistance.
Before entering the tank, you will change into appropriate underwater attire: your own
athletic/swimming trunks and no shirt. You may wear shoes (water, kayaking, sandals,
runtime or tennis shoes) or perform the underwater cycling barefoot. Your clothing and shoe
choice must remain consistent throughout all 3 exercise trials. You will then sit on the cycle
ergometer for two minutes while your oxygen consumption is measured. After two minutes,
the graded peak cycling test will begin. The first stage of the test will last 5 minutes, and you
will cycle at 40 rpm. At the end of the five-minute stage, the pedal rate will increase to 50
rpm. You will cycle at the new pedal rate for three minutes. From then on, you will increase
your pedal rate by 5 rpm every three minutes until you are too tired to continue. At this point,
the exercise test will be stopped. You will recover by cycling at a very low pedal rate until
your heart rate drops below 120 beats per minute. If your heart rate does not drop below 120
beats per minute within 3-5 minutes, you will stop cycling, be escorted out of the tank, dried
off, and continue recovery lying down on a cot until your heart rate does decline below 120
beats per minute. Once your heat rate has slowed to within 20 beats of your resting rate, and
blood pressure has returned to within 10 mmHg of your resting pressure, the next exercise
trial will be scheduled, and you will be released. If you become uncomfortable at any point,
you may stop the test. The test may also be stopped if the situation becomes unsafe or if any
member of the research team decides to stop the test.

The procedures for the experimental trial and the control trial are the same, except for the
water temperature in which you will be immersed. In the control trial, you will cycle while
immersed in cool water (25° Celsius; 77° Fahrenheit) and in the experimental trial, you will
cycle while immersed in warm water (39° Celsius; 102° Fahrenheit). The temperature of
water for the warm trial is lower than the temperature of most hot tubs. Water temperature
for the exercise sessions will be randomly assigned. For 24 hours leading up the trials, you
must abstain from exercise and alcohol consumption. Approximately 4 hours prior to
arriving at the Applied Physiology Laboratory, you will eat a meal typical to what you might
eat prior to a regular workout. After this meal you will not be allowed to consume any food
until the end of the trial. Please avoid alcohol for 24 hours and caffeinated drinks for a minimum of 4 hours before the exercise trials. During the trial, you will be allowed to drink water at your discretion.

After arriving at the Applied Physiology Laboratory for exercise trials 2 and 3, you will change into appropriate attire (athletic/swimming shorts and no shirt) and self-insert a battery operated rectal thermometer. The rectal thermometer consists of a small circular electric temperature sensor (approximately the size of a small button) connected to a wire. The other end of the wire will be connected to a thermistor thermometer, allowing the core temperature readout to be seen. The rectal thermometer will allow for the accurate and continuous monitoring of internal temperature changes during exercise and will ensure your safety during the exercise sessions. Battery powered skin thermisters will be attached to your mid-thigh and chest to monitor changes in skin temperature. Then, you will rest quietly, sitting in an upright position for twenty minutes. At minute 19 of the rest period, your heart rate and blood pressure will be measured. At the end of the 20 minutes of rest, a trained individual will use a sterile technique to draw a 10 ml (~2 teaspoons) blood sample. Your blood sample will be labeled with your subject identification number, and stored for analysis.

After the first blood draw, you will enter the water tank. You will be immersed in water to the mid-chest level during exercise. When you are fully submerged, you will begin a 5-minute warm-up period, cycling at a self-selected pace. During this time, a member of the research team will ensure that you are comfortable with the cycle ergometer, headset and mouthpiece apparatus used to measure oxygen consumption, and water submersion.

After the warm-up, you will begin the exercise test. You will cycle for 40 minutes at 65% of your peak exercise capacity, while submerged up to the mid-chest in water. After four-minutes of exercise, your expired air will be monitored for one minute to ensure that you are exercising at the 65% of your peak capacity. If you are not exercising at the correct intensity, pedal rate will be adjusted as needed. Pedal rates in the second session will be exactly the same as the first session. Your expired air will be sampled for the last 4 minutes of each ten-minute interval (minutes 6-10, 16-20, 26-30, 36-40). Throughout the 40 minutes of exercise, your heart rate and rating of perceived exertion will be recorded during the last 15 seconds of each five-minute interval. Also, your internal temperature (via rectal thermometer) and skin temperature (via skin thermisters) will be monitored every minute and recorded at five-minute intervals to ensure your safety. During the exercise test, you will have access to water at any point. Again, if you become uncomfortable at any point, or if the research team deems in necessary, the test may be stopped.

After 40 minutes, you will stop cycling and your headset and mouthpiece apparatus will be removed. You will be assisted walking up the stairs and out of the water tank, and dried off. A research assistant will escort you to an upright chair where again, a trained individual will use a sterilized procedure to obtain another 10 ml (~2 teaspoons) blood sample. You will then move to a stationary bicycle located in room air to cool down at a self-selected pace until you are comfortable. Again, you may drink water at any time.
After the cool down period, you will put on a sweat suit (provided by the Applied Physiology Laboratory) to keep warm. You will remain in the laboratory for two hours, during which you may walk around the laboratory, use the bathroom, read or study. Magazines will be made available for you to read during this time. After two hours, a trained individual will take a third 10 ml (~2 teaspoons) blood sample using a sterile procedure. The location of puncture will be cleaned and bandaged. You will schedule the next experimental trial and ask any questions you may have. You will be allowed to leave the Applied Physiology Laboratory when your heart rate is within 20 beats/minute of your resting rate, your blood pressure is within 10 mmHg of your resting blood pressure, and your core temperature drops below 38° C (100° F).

**What are the possible benefits from being in this study?**
Research is designed to benefit society by gaining new knowledge. Upon completion of this study, athletes, coaches, physicians, and individuals engaged in activity in heat will have a greater understanding of the immune system’s response to activity in the heat, specifically, how exercise in the heat may or may not increase the risk of illness. You will also have the benefit of knowing your sub-maximal and peak aerobic capacity and heart rates, which may be valuable for your training; however, you may not benefit directly from participating in this study.

**What are the possible risks or discomforts involved with being in this study?**
There should be no risk of psychological harm from completing the research; therefore there should be no emotional distress.

You will be asked to complete very strenuous exercise bouts during participation within this study. When completing strenuous exercise some common side effects may include, muscle soreness, fatigue. As you are accustomed to regular exercise the muscle soreness and fatigue should be minimal. Uncommonly, strenuous exercise has also been associated with the risk of heart attack or stroke, as there is a 1 in 10,000 chance that a healthy subject may experience an adverse event during aerobic fitness testing. However, the following precautions are in place to minimize the risk including: close supervision, pulse rate monitoring, periodic blood pressure monitoring, pre-physical examinations and extensive medical screening. The supervising faculty (Dr. McMurray) and the exercise research test assistants are trained in exercise stress testing, cardio-pulmonary resuscitation, and the use of an automated defibrillator device. Dr. McMurray, PhD., has maintained his certification with the American College of Sports Medicine (originally certified in 1982), which ensures current knowledge/expertise in exercise testing special populations, including older adults and those with disease processes. Supplemental oxygen is available in the lab along with a telephone with posted emergency call instructions for notifying campus security to arrange medical transport for the subject to UNC hospital. Further, a fully equipped Sports Medicine Rehabilitation facility is located 100 yards away.

You will also be asked to provide blood samples during the experimental trials. The blood draws will be conducted by a trained individual, Dr. McMurray, from the University of North Carolina-Chapel Hill Applied Physiology Laboratory. All standard safety and sterile procedures will be used during all blood draws. If minor bruising does develop as a result of
the blood draws, ice and compression will be applied to the area. Also, the total amount of blood drawn (approximately 30 ml) is small and should have no adverse effects.

Upon entering and exiting the water tank, there is a small risk of slipping or falling. The water tank in which the cycle will be immersed is equipped with large no-slip steps that provide easy access for you. You will also be provided assistance to aid your balance as you enter and exit the tank. Absorbent towels will cover the floor area surrounding the water tank to prevent accidental slips or falls. Additionally, the tank provides easy access in the event that the investigators need access to you during your exercise sessions. Although the water comes up only to your chest, there is a small risk of accidental submersion. In order to minimize this risk, a flotation device will be available while you are in the water. Also, once the cycle ergometer is in place in the tank, there is little space for you to move around the tank, reducing the potential for submersion.

There is the minor risk of developing a degree of temperature related stress, including heat cramps, heat exhaustion, or heat stroke. Constant monitoring of core temperature via a battery operated rectal thermometer, which is the most reliable method of monitoring core temperature, minimizes this risk. Additionally, studies involving exercise combined with heat exposure have been performed in UNC Chapel Hill’s Applied Physiology Laboratory since the mid-1980s with no adverse effects reported. You will be carefully monitored during all exercise sessions, and testing will stop if core temperature exceeds 39°C (102°F).

In the case that your core temperature does rise above 39°C (102°F) during exercise, immediate action will be taken in order to cool you off. You will be escorted out of the tank, dried off, and guided to a separate area of the Applied Physiology Laboratory. Once completely dry, all uncovered areas of your skin will be sprayed with cool water, while a fan blows air across your body. Evaporative cooling will be increased and your internal body temperature will begin to go down. This method of cooling is regularly used to treat heat related illnesses and has been shown to cause rapid drops in internal body temperatures. Another member of the team will also apply ice to your neck in order to augment the cooling process. The entire cooling protocol will continue until your internal temperature drops to within one degree of your resting temperature. Monitoring your body temperature during exercise and use of these cooling protocols in case your internal temperature exceeds 39°C (102°F) will help to avoid any large increases in internal temperature.

Although rectal thermometry is considered a safe method of internal temperature measurement, it has been associated with a small increase in the risk of infection. In order to combat this risk, all thermometers will be stored in a disinfected area of the laboratory, and cleaned with an alcohol solution following every use. Taking these steps to sterilize the thermometers should negate any increased risk of infection.

If you fall, strain a muscle, or in any way injure yourself during the exercise testing process, first aid will be provided.

Although no radioactive materials are being used for this study, the UNC Chapel Hill Applied Physiology Laboratory, where you will be performing exercise tests, houses
radioactive materials. The amount of radioactive material is very small and should pose no health threat to you. All radioactive material is stored in a contained biochemistry section of the Applied Physiology Laboratory, and is kept in compliance with the UNC Office of Environmental, Health & Safety regulations.

In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers.

**What if we learn about new findings or information during the study?**
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

**How will your privacy be protected?**
No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

Upon agreement to participate in the study, each participant will be assigned an ID number. All data will be stored by ID number in a computer that is password protected. Only the researchers and the faculty adviser will have access to this information. Once you have completed all three trials, your name, telephone number, and email address will be destroyed. The data will be kept by ID with no other identifiers. All data collection sheets and questionnaires used for data collection will be kept in a locked filing cabinet located in room 25B Fetzer Gym (Dr. McMurray’s office). After every trial, a research team member will transfer all data to the computer.

**What will happen if you are injured by this research?**
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from being in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. The University of North Carolina at Chapel Hill has not set aside funds to pay you for any such reactions or injuries, or for the related medical care. However, by signing this form, you do not give up any of your legal rights.

**What if you want to stop before your part in the study is complete?**
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

**Will you receive anything for being in this study?**
You will be allowed to retain a copy of your personal test data to aid you in planning your training. Otherwise, you will not be compensated for participation in this study.

**Will it cost you anything to be in this study?**
It will not cost you anything to take part in this study. If you enroll in this study, you will have to pay for your own transportation. Parking behind Fetzer Gym will be provided for those coming from off campus.

**What if you are a UNC student?**
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

**What if you are a UNC employee?**
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

**Who is sponsoring this study?**
There is no outside funding for this study, as the study will be sponsored by the University of North Carolina - Chapel Hill Applied Physiology Laboratory. The researchers do not have a direct financial interest in the final results of the study.

**What if you have questions about this study?**
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

**What if you have questions about your rights as a research subject?**
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

Title of Study: The Effect of Exercise in a Warm Environment on Tumor Necrosis Factor-α Concentration and Circulating Neutrophil Count

Principal Investigators: Mark P. Berry and Erica S. Cooper

Subject’s Agreement:
I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.
Signature of Research Subject

Printed Name of Research Subject

Signature of Person Obtaining Consent

Printed Name of Person Obtaining Consent

Date

Date

Date
APPENDIX C:
Maximal Exercise Session Data Collection Sheet

SUBJECT_________ DATE__________

Orientation Session

Prior to Subject Arrival
1. Fill tank with water
2. Adjust temperature to 25 °C.
   Time: _________ Initial Water Temperature: _________
3. Set up metabolic system (calibrate, mouthpiece, etc)

Informed Consent
1. Inform participant of the experimental protocol
2. Make participant aware of the possible risks
3. Sign informed consent

Participant Compliance Questions
1. Did the participant perform strenuous physical activity for 24 hours prior to VO_{peak} testing:
   Y    N
2. Did the participant eat, smoke or consume alcohol within 4 hours prior to testing, or ingest caffeine within 12 hours prior to testing:
   Y    N

Forms
1. Training history form
2. Medical history form

Examinations ______
1. Medical Examination
2. 12 Lead EKG
3. Physical Examination

Physical Characteristics
1. Sex _________
2. Age _________ yrs
3. Height _________ cm
4. Weight _________ kg

Before VO_{peak} Protocol
1. Have the subject change into the proper attire (athletic shorts, no shirt, and foot apparel of their choice)
   ATTIRE: ____________________________________________________________
2. Fit polar heart rate (HR) monitor to participant
3. Make sure polar heart rate monitor picks up signal
4. Place RPE scale near cycle ergometer/explain RPE to participant
5. Resting HR: ______________
6. Resting BP: ______________
7. Record resting oxygen consumption for 2 minutes (as the participant sits next to tank) – headpiece (NOT mouthpiece) is mandatory for all MAX tests

Warm Up
1. 5 minutes of cycling on a land based cycle at self selected pace (subject may also stretch as needed)
2. Fit cycle ergometer located within the tank to the participant - record seat position using flexible tape (line tape up from black cross bar on back of seat to the clear piece of tape on the cycle)
   Seat height: _______ notches  Foot straps: __________
3. Measure height from the floor to the subject's xyphoid process as they are seated on the cycle
   Height from floor of tank to xyphoid process: ________ cm
4. Adjust water height to reach subjects xyphoid process

**VO_2peak Protocol**

1. Stage 1: 40 rev for 5 minutes
   Min 1→HR _______; RPE __________
   Min 2→HR _______; RPE __________
   Min 3→HR _______; RPE __________
   Min 4→HR _______; RPE __________
   Min 5→HR _______; RPE __________

2. Stage 2: 45 rev for 3 minutes
   Min 6→HR _______; RPE __________
   Min 7→HR _______; RPE __________
   Min 8→HR _______; RPE __________

3. Stage 3: 50 rev for 3 minutes
   Min 9→HR _______; RPE __________
   Min 10→HR _______; RPE __________
   Min 11→HR _______; RPE __________

4. Stage 4: 55 rev for 3 minutes
   Min 12→HR _______; RPE __________
   Min 13→HR _______; RPE __________
   Min 14→HR _______; RPE __________

5. Stage 5: 60 rev for 3 minutes
   Min 15→HR _______; RPE __________
   Min 16→HR _______; RPE __________
   Min 17→HR _______; RPE __________

6. Stage 6: 65 rev for 3 minutes
   Min 18→HR _______; RPE __________
   Min 19→HR _______; RPE __________
   Min 20→HR _______; RPE __________

7. Stage 7: 70 rev for 3 minutes
   Min 21→HR _______; RPE __________
   Min 22→HR _______; RPE __________
   Min 23→HR _______; RPE __________

8. Increase workload until volitional fatigue → add more stages if necessary (record to right)

**Recovery**

1. Escort subject out of the tank- dry before leaving the tank area
2. Begin active recovery on land based cycle ergometer at self selected pace
3. Continue active recovery until HR drops below 120 bpm
4. If heart rate does not drop below 120 bpm within 5 minutes, subject will rest supine until heart rate reaches appropriate level
5. Measure temperature of water at the end of MAX test
   End Water Temperature: __________
Prior to Participant Exiting
1. Ensure subjects heart rate is within 20 bpm of resting value
2. Ensure subjects blood pressure is within 10 mmHg of resting values

Criteria for valid VO$_{2peak}$ Test
1. Did the participant have a maximal RER equal to or greater than? 1.1 RER = __________
2. Did the participant reach age predicted maximal HR (220-age ± 5%)? HR$_{max}$ = __________
3. Did the participant have a RPE equal to or greater than 18? RPE = __________
4. Plateau of VO$_2$ with increase in revolutions/minute __________

Power Output Estimation for Experimental Trials
1. VO$_{2peak}$ = __________
2. Peak Revolutions/Minute = __________
3. $(0.65 \times (VO_{2peak} - VO_{2rest})) + VO_{2rest}$
4. Revolutions/Minute that corresponds to 65% VO$_{2peak}$ = __________
APPENDIX D:
Experimental Trials Data Collection Sheet

SUBJECT_________ Exercise Trial
DATE_________ I          II
TIME_________ warm    cold
(circle)

Special Notes
1. Session I must start at least two days after determination of \( \text{VO}_{2\text{peak}} \)
2. Sessions I-II must be spaced at least 24 hours apart, at or about the same time each day

Prior to Subject Arrival
1. Set up cycle ergometer within the tank to previously recorded seat notch: _________
2. Fill tank with water to appropriate height: _________ (based on orientation session measurement)
3. Adjust water to appropriate temperature. Record Time and Temperature.
4. Set up metabolic system (calibrate, mouthpiece, etc)
5. Set up blood supplies (take out equipment, label Vacutainer® tubes)
6. Set up and CALIBRATE rectal thermometer and skin thermister equipment
7. Fill ice bucket
8. Stop watch, RPE sign, metronome, towels, floatation device,

Participant Compliance Questions
3. Did the participant abstain from exercise and alcohol consumption for 24 hours prior to trial? Y N N/A
4. Did the participant perform vigorous activity the day prior to the trial? Y N N/A
5. Has the participant been sick in the 3 weeks period prior to testing? Y N N/A
6. Did the participant eat in the 4 hours leading up to the trial, or consumed caffeine in the 12 hours leading up to the trial? Y N
7. Has the participant taken any NSAIDS in the past 24 hours? Y N
8. Last meal consumed prior to arrival:

Before Starting Exercise Protocols for Sessions II-IV
8. Have the subject change into the same attire as max test
9. Measure and record subject’s body mass
10. Have the subject insert the rectal thermometer
11. Fit polar heart rate (HR) monitor to participant
12. Make sure polar heart rate monitor picks up signal
13. Secure skin thermisters to the head, arm, mid-chest and mid-thigh of the subject
14. Place RPE scale near cycle ergometer

Exercise Protocol for Experimental Trials I and II
1. The participant will rest in an upright chair for 20 minutes
2. Early in the rest period the subject will be fitted with the headset and mouthpiece
3. At minute 19 of the rest period blood pressure and heart rate will be measured
4. At minute 20 of the rest period obtain 10-mL of venous blood using the standard Venipuncture technique
5. Placed blood into a sterile K2 - EDTA (purple top) Vacutainer® tube
6. Place tube on ice immediately
7. Obtain a two-minute resting VO$_2$ sample from the subject
8. Escort the subject to the water tank
9. 5 minute warm up of a self selected pace
10. Begin the 40 minutes of cycling at the previously determined workload that elicits 65% of VO$_{2peak}$
    a. 65% VO$_{2peak}$: __________
    b. RPM @ 65% VO$_{2peak}$: __________
    c. At the three-minute mark of exercise, obtain VO$_2$ to ensure subject is working at 65% of VO$_{2peak}$
    d. Note if any changes are made to pedal rate: ________________
11. During the 40 minutes of cycling
    a. Min 0 → HR; $T_{re}$; $T_H$; $T_A$; $T_C$; $T_T$
    b. Minute 3 → Begin monitoring VO$_2$. Record any change above.
    c. Minute 5 → HR; RPE; $T_{re}$; $T_H$; $T_A$; $T_C$; $T_T$
    d. Minutes 8-10 → VO$_2$ measurement (remove mouthpiece after sampling)
    e. Minute 10 → HR; RPE; $T_{re}$; $T_H$; $T_A$; $T_C$; $T_T$
    f. Minute 15 → HR; RPE; $T_{re}$; $T_H$; $T_A$; $T_C$; $T_T$
    g. Minutes 18-20 → VO$_2$ measurement (remove mouthpiece after sampling)
    h. Minute 20 → HR; RPE; $T_{re}$; $T_H$; $T_A$; $T_C$; $T_T$
    i. Minute 25 → HR; RPE; $T_{re}$; $T_H$; $T_A$; $T_C$; $T_T$
    j. Minutes 28-30 → VO$_2$ measurement (remove mouthpiece after sampling)
    k. Minute 30 → HR; RPE; $T_{re}$; $T_H$; $T_A$; $T_C$; $T_T$
    l. Minute 35 → HR; RPE; $T_{re}$; $T_H$; $T_A$; $T_C$; $T_T$
    m. Minutes 38-40 → VO$_2$ measurement (remove mouthpiece after sampling)
    n. Minute 40 → HR; RPE; $T_{re}$; $T_H$; $T_A$; $T_C$; $T_T$
    o. Note: If core temperature rises above 39°C the trial must be stopped

**Recovery**
6. Escort subject out of the tank- dry before leaving the tank area
7. Obtain 10-mL of venous blood using the standard Venipuncture technique
8. Placed blood into a sterile K$_2$-EDTA (purple top) Vacutainer® tube
9. Place tube on ice immediately
10. Begin active recovery on land based cycle ergometer at self selected pace
11. Measure and record BP during recovery at:
    Min 0
    Min 5
    Min 10
    Min 15
    Min 20
12. Continue active recovery until HR drops below 120 bpm
13. If heart rate does not drop below 120 bpm within 5 minutes, subject will rest supine until heart rate reaches appropriate level
14. Measure and record temperature of water at the end of experimental trial
15. Subject allowed to get up from supine position when HR declines below 120
16. Escort the subject to the bathroom where they will change into a sweat suit
17. The subject will then remain in the APL for two hours
18. At the end of the two hour period obtain 10-mL of venous blood using the standard Venipuncture technique
19. Placed blood into a sterile K$_2$-EDTA (purple top) Vacutainer® tube
20. Place tube on ice immediately
21. End of the two-hour period measure $T_{re}$
    HR
    BP
    Body Mass
22. Remove skin thermisters
23. Escort the subject to the bathroom where he will remove the rectal thermometer
24. Subject will be allowed to leave the APL when heart rate is within 20 bpm of resting value; blood pressure is within 10 mmHg of resting values, and core temp. is < 38°C
25. Schedule next experimental trial if necessary
   Trial Date: __________
| TIME  | H₂O Temp/Time | Body Mass | Blood Sample | BP  | HR  | RPE  | T_Re | T_N | T_A | T_C | T_T |
|-------|---------------|-----------|--------------|-----|-----|------|------|-----|-----|-----|-----|-----|
| Pre   | N             |           |              |     |     |      |      |     |     |     |     |     |
| Rest (Sitting) |   |           |              |     |     |      |      |     |     |     |     |     |
| 0     |               |           |              |     |     |      |      |     |     |     |     |     |
| 5     |               |           |              |     |     |      |      |     |     |     |     |     |
| 10    |               |           |              |     |     |      |      |     |     |     |     |     |
| 15    |               |           |              |     |     |      |      |     |     |     |     |     |
| 20    |               |           |              |     |     |      |      |     |     |     |     |     |
| 25    |               |           |              |     |     |      |      |     |     |     |     |     |
| 30    |               |           |              |     |     |      |      |     |     |     |     |     |
| 35    |               |           |              |     |     |      |      |     |     |     |     |     |
| 40    |               |           |              |     |     |      |      |     |     |     |     |     |
| Immediately Post | |           |              |     |     |      |      |     |     |     |     |     |
| 5 min Post |       |           |              |     |     |      |      |     |     |     |     |     |
| 10 min Post |      |           |              |     |     |      |      |     |     |     |     |     |
| 15 min Post |     |           |              |     |     |      |      |     |     |     |     |     |
| 20 min Post |    |           |              |     |     |      |      |     |     |     |     |     |
| 2 hr Post |      |           |              |     |     |      |      |     |     |     |     |     |

COMMENTS:
REFERENCES


