THE EFFECT OF HIGH INTENSITY EXERCISE ON PH AND INFLAMMATORY BIOMARKERS

A thesis submitted to the Kent State University College and Graduate School of Education, Health, and Human Services in partial fulfillment of the requirements for the degree of Master of Science

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High-intensity and prolonged duration of exercise has been found to inflict acute inflammation and localized muscle injury by releasing lactic acid and increasing plasma concentrations of both pro- and anti-inflammatory cytokines. Previous studies had used blood sample analysis to investigate this effect. The purpose of this research study was to examine the effect of high intensity exercise on the inflammatory biomarkers IL-6 and IL-1β in male athletes using salivary analyses. A secondary purpose was to find a correlation between pH and each of the cytokines. Additionally, this study aimed to prove the accuracy of using saliva for inflammatory diagnosis in both current and future research studies. Nine conditioned male runners participated in an initial health questionnaire, a VO\textsubscript{2max} test, and a 60-minute run at 60% intensity, while maintaining at least 75% of their maximum heart rate. Saliva was collected at baseline, 30 min, 60 min, and 24 h post-trial run for the analysis of pH, IL-6, and IL-1β. No significant increases of IL-6 and IL-1β were observed. Additionally, there was no correlation found between pH and the biomarkers. The present study demonstrated an accuracy of using saliva for inflammatory diagnosis, producing a perfect standard curve and detectible data. The study also confirmed that the type of physical activity, frequency, duration, and intensity play a significant role in the acute inflammatory response.
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CHAPTER 1

INTRODUCTION

Physical activity is known for its various beneficial effects on the human body such as reducing cardiovascular risk factors (Mora et al., 2007) and improving blood and lipid profiles (Dunn et al., 1997). However, intense physical activity increases circulating inflammatory markers inducing acute inflammation, possibly leading to impaired exercise capacity (Bartziotou et al., 2007). Inflammation is defined as an innate system of cellular and humoral responses following injury in which the body attempts to restore the tissue to its pre-injury state (Ward, 2010). The acute phase of inflammation consists of increased blood flow and vascular permeability along with the accumulation of fluid, leukocytes, and inflammatory mediators such as cytokines (Feghali & Wright, 1997). If the acute inflammatory response becomes prolonged or excessive, leading to serious damage of tissues and organs (Ward, 2010). Lewis Thomas (1972) quoted “our arsenals for fighting off bacteria are so powerful, and involve so many different defense mechanisms, that we are more in danger from them than the invaders.”

Cytokines are the chief stimulators of the production of acute-phase proteins during the inflammatory processes (Gabay & Kushner, 1999). Inflammation-associated cytokines include interleukin-6 (IL-6), interleukin 1-β (IL-1β), tumor necrosis factor α (TNF-α) and others (Gabay & Kushner, 1999). Combinations of the cytokines can have additive, inhibitory, or synergistic effects, and the effects of cytokines on target cells may be inhibited or enhanced by other cytokines, hormones or circulating receptors (Gabay & Kushner, 1999). TNF-α is one of the most studied and central pro-inflammatory
cytokines. It targets immune cells to exert a wide array of actions including production of inflammatory chemokine and cytokines, promoting cellular growth, differentiation, and survival (Dhama et al., 2013). IL-6 is one of the main cytokines produced during acute inflammation by macrophages and monocytes at inflammatory sites (Gabay, 2006). The actions of IL-6 are to return the host to a homeostatic state to control the extent of tissue inflammatory responses (Gabay, 2006). Lastly, IL-1β is a prototypic pro-inflammatory cytokine that exerts pleiotropic effects on a variety of cells and plays a key role in acute and chronic inflammatory and autoimmune disorders (Ren & Torres, 2009). Together with chemokines, IL-1β promotes the infiltration of inflammatory and immunocompetent cells from circulation into the extravascular space and then into tissues where tissue remodeling is the end result of IL-1 induced inflammation (Dinarello, 2009).

High intensity exercise has been seen to substantially increase pro-inflammatory markers (Jee & Jin, 2012) Exercise-induced muscle damage triggers the immune system to play a role in the degeneration and regeneration of muscle and connective tissue (Peake, Nosaka & Suzuki, 2005). High intensity exercise is defined by the maximal rate of oxygen consumption (VO$_{2\max}$), which represents the highest amount of oxygen that an individual may consume under exercise conditions, representing the physical fitness of an individual (Williams, 2007). An individual is considered in above average to excellent condition according to the American College of Sports Medicine (ACSM) guidelines (2014) if their VO2max falls between 45-60%. Along with increases in inflammatory markers, intense exercise has been seen to increase blood and muscle lactate with a concomitant decrease in tissue pH (Robergs, Ghiasvand & Parker, 2004). pH stands for
potential of hydrogen, which is the measure of a solution’s hydrogen ion concentration (Cook, 2008). When blood pH levels shift to the side of excessive acidity or alkalinity, several clinical conditions can result such as inflammation, a compromised immune system, and cancer (Minich & Bland, 2007). Intense exercise can impact the extracellular pH for circulating leukocytes, and thus can rapidly alter the immune response in these cells (Kellum, Song & Li, 2004). When tissues are deprived of oxygen, lactic acid is produced and can lead to a degeneration of polymorphonuclear leukocytes (Menkin & Warner, 1936). A decrease in pH leads to an increase in inflammation, thus it is important to not only understand, but to control factors such as type of physical activity, frequency, duration, and intensity.

**Problem Statement**

High-intensity exercise leads to increased plasma concentrations of both pro-and anti-inflammatory cytokines (Friman & Ilback, 1998; Peake, Nosaka & Suzuki, 2005; Sloth, Sloth, Overgaard & Dalgas, 2013). If the acute inflammatory response becomes prolonged or excessive, it can lead to serious damage of tissues and organs (Ward, 2010). This muscle damage has the potential to modify leukocyte receptor expression and functional activity, leading to an infiltration of neutrophils and monocytes into damaged tissue (Tuan et al., 2007). These cells release proteolytic enzymes and reactive oxygen species to break down tissue fragments (Tuan et al. 2007).

High-intensity exercise also attributes to acute inflammation and localized muscle injury by releasing lactic acid and depleting the alkali reserve (Menkin & Warner, 1936). The hydrogen ion concentration drops pH levels to 6.7-6.5, which is a level too low for
polymorphonuclear cells to survive without injury (Menkin & Warner, 1936). If the acidity increases beyond a pH of 6.5, all types of leukocytes are injured (Menkin & Warner, 1936). There have been previous studies that have shown that exercise does not cause an anti-inflammatory or pro-inflammatory response (Markovitch, Tyrrell & Thompson, 2008; Pederson & Toft, 2000). Markovitch’s (2008) study showed that a single bout of moderate intensity walking did not lymphocyte and monocyte expression. Pederson’s (2000) study did not find significant changes in monocyte concentration after moderate activity. Although there have been findings that confirm that low or moderate intensity does not cause an increase in inflammatory biomarkers, it is important to conduct a study that will confirm that high intensity endurance exercise induces acute inflammation in athletes. This is particularly important in application, because it will show that the intensity, duration, and type of physical activity does make a drastic impact on the body.

There is no known study on the relationship between inflammatory markers, lactic acidosis, and high-intensity exercise using salivary analysis. Previous studies had measured inflammation by using blood for laboratory analysis (Gleeson, 2007; Jee & Jin, 2012; Tuan et al., 2007). Thus, a study needs to be conducted to confirm that duration and intensity of exercise does increase the acute inflammatory response through the collection and analysis of saliva.

**Purpose Statement**

The purpose of this research study was to examine the effect of high intensity exercise on pH and the inflammatory biomarkers IL-6, TNF-α, and IL-1β in male athletes
using salivary analyses. A secondary purpose was to find a relationship between pH and each of the cytokines. A third purpose was to prove the accuracy of using saliva for inflammatory diagnosis in both current and future research studies.

**Hypotheses**

1. Inflammatory biomarkers IL-6, TNF-α, and IL-1β will increase as a result of high-intensity endurance exercise.
2. As inflammation increases during the run, pH will decrease.

**Operational Definitions**

*Endurance runner*: an individual who possesses both muscular and cardiorespiratory endurance to perform. Muscular endurance is defined as the ability of a muscle or group of muscles to repeatedly develop or maintain force without fatiguing (Jones & Bartlett, 1983). Cardiorespiratory endurance is the ability of the cardiovascular and respiratory systems to deliver blood and oxygen to working muscles, which enables the muscles to perform continuous exercise (Jones & Bartlett, 1983).

*Volitional Exhaustion*: the point at which an individual cannot perform a muscular contraction and voluntarily terminates the contraction (Pitcher & Miles, 1997).
CHAPTER II
REVIEW OF LITERATURE

Inflammation

Inflammation and the Inflammatory Response

The inflammatory response, as defined by Peter Ward (2010), consists of an innate system of cellular and humoral responses following injury in which the body attempts to restore the tissue to its pre-injury state. Inflammation is characterized in the acute phase by increased blood flow and vascular permeability along with the accumulation of fluid, leukocytes, and inflammatory mediators such as cytokines (Feghali and Wright, 1997). According to Ward (2010), the acute inflammatory response consists of the release of water, salt, and proteins from the vascular compartment; activation of endothelial cells; adhesion between the leukocytes and the vascular endothelium; recruitment of leukocytes; activation of tissue macrophages; activation and aggregation of platelets; complement activation; clotting and fibrinolytic systems; and release of proteases and oxidants from phagocytic cells.

The acute phase response is marked by changes in the concentrations of plasma proteins known as the acute phase proteins, as well as behavioral, psychological, nutritional, and biochemical changes (Gabay & Kushner, 1999). The acute phase proteins are a set of plasma proteins whose concentrations increase (positive acute phase proteins) or decrease (negative acute phase proteins) by at least 25% in inflammatory disorders (Gabay & Kushner, 1999). Regardless of what has incited the response, there are several intravascular effects that accompany the acute inflammatory response. Upon activation of
the endothelial cells, pro-inflammatory cytokines and chemokines are released, which will chemotactically attract and activate polymorphonuclear (PMN) leukocytes (Ward, 2010). The endothelial cells then express tissue factor on luminal surfaces, which is a potent procoagulant that can lead to thrombus formation on the vascular surface. The reversible opening of endothelial cells’ tight junctions allows for the leak of protein and fluids from the vascular compartment into the extravascular compartment and can cause extensive edema, leading to increased hydrostatic pressure (Ward, 2010).

In many situations the acute inflammatory response becomes prolonged or excessive, leading to serious damage of tissues and organs (Ward, 2010). The acute response should be critical in the first few hours or days and then gradually decline unless the offending agent cannot be cleared by phagocytosis. Ward (2010) explains that resolution of the inflammation requires killing of bacteria and removal of debris. When tissue injury occurs in several organs, the response may involve regeneration in which an organ can rapidly replace damaged or destroyed cells to produce an outcome that resembles the original uninjured tissue (Ward, 2010).

All changes of the acute inflammatory response are reversible. The acute inflammatory response is a protective shield against damaged tissue to return tissue to its pre-damaged state which can be short or excessive due to the persistence of the damage-causing agent or the offending trigger (Ward, 2010). Lewis Thomas (1972) quoted, “our arsenals for fighting off bacteria are so powerful, and involve so many different defense mechanisms, that we are more in danger from them than the invaders.” Lewis refers to the dangers of continued inflammation. Although at first the body is eliciting an anti-
inflammatory effect to protect tissue from damage, prolonged exposure to acute phase proteins can lead to extensive tissue or organ injury, becoming pro-inflammatory (Ward, 2010). The chronic inflammatory response is defined by Ward (2010) as the nature of the inflammatory cells appearing in the tissues as opposed to the duration of the inflammatory response. Secreted polypeptides known as cytokines are divided into two groups: those involved in acute inflammation and those responsible for chronic inflammation.

Figure 1. Cytokines involved in acute and chronic inflammatory responses (Gabay & Kushner, 1999)

**Cytokines and Acute Phase Response**

Cytokines are intercellular signaling polypeptides with multiple sources, targets, and functions. They are the chief stimulators of the production of acute-phase proteins during inflammatory processes (Gabay & Kushner, 1999). Feghali and Wright (1997) identify cytokines as the major determinants of the make-up of the cellular infiltrate, state
of cellular activation, and systemic responses to inflammation. As pleiotropic molecules, they elicit their effects both locally and systemically in an autocrine or paracrine manner (p.13). Inflammation-associated cytokines include interleukin-6 (IL-6), interleukin 1-beta (IL-1 β), tumor necrosis factor alpha (TNF- α), interferon-γ, transforming growth factor β and interleukin-8 (Gabay & Kushner, 1999).

Gabay and Kushner (1999) have found that substantial acute-phase protein concentration changes are contributed to infection, trauma, surgery, burns, tissue infarction, immunologically mediated and crystal-induced inflammatory conditions, and advanced cancer (p. 1). Moderate changes in protein concentration follow strenuous exercise, heatstroke, and childbirth (Gabay & Kushner, 1999). Gabay and Kushner (1999) mention that cytokines operate both as a cascade and a network in the stimulation of the production of acute-phase proteins and can regulate the production of other cytokines and cytokine receptors. TNF-α is the main stimulator of interleukin-1 production in patients with rheumatoid arthritis; IL-1β may increase or decrease the expression of its own receptors; the IL-6 response to the injection of turpentine in mice requires IL-1β; and IL-6 inhibits the expression of TNF-α (Gabay & Kushner, 1999). Combinations of the cytokines can have additive, inhibitory, or synergistic effects.

**Role of TNF and Altering Factors**

Tumor necrosis factor (TNF) is one of the most studied and central pro-inflammatory cytokines (Gillett et al., 2010). It was discovered in 1984, identifying two forms: TNF-β from activated macrophages, and LT-α (also called TNF-α) from t-cells (Dhama et al., 2013). Dhama (2013) writes that most members of the TNF family are
expressed by immune cells and can target immune cells to exert a wide array of actions including production of inflammatory chemokine and cytokines, promoting cellular growth, differentiation, and survival (p. 2). TNF has been recognized as a key regulator of inflammatory responses, with its receptors active in mediating several autoimmune and inflammatory diseases (Watkins et al., 1995).

The TNF ligands are involved in a variety of inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease, ankylosing spondylitis, psoriasis, graves’ disease, SLE, diabetes, asthma, etc. (Croft et al., 2012). Tumor necrosis factor has been studied in its role in rheumatoid arthritis, in which several pro-inflammatory cytokines are produced within the inflamed joint cavity (Geiler et al., 2011). Because of their predicament in the inflammatory reaction cascade, tumor necrosis factor ligands are proven to be the most important cytokines in rheumatoid arthritis (Geiler et al., 2011). In a study by Hürlimann (2002), anti-tumor necrosis factor-α treatment improved endothelial function in patients with rheumatoid arthritis. TNF-α is an important mediator of systemic and vascular wall inflammation in both rheumatoid arthritis and atherosclerotic vascular disease. Hürlimann’s study (2002) proved that TNF-α mediated inflammatory changes play an important role in vascular dysfunction (p. 2186). Inflammatory conditions such as Crohn’s disease, inflammatory bowel disease and ulcerative colitis affect the lamina propria of the intestine and cause an overexpression and overproduction of TNF ligands (Sands et al., 2004). As studied by Gut (1997), the mucosal production of cytokines is increased in active inflammatory bowel disease and
has now been shown that the number of TNF-α producing lamina propria cells is
increased in Crohn’s disease, highly localized to the mucosa and intestinal lumen.

**Role of IL-6 and Altering Factors**

IL-6 is one of the main cytokines produced during acute inflammation produced
by macrophages and monocytes at inflammatory cites (Gabay, 2006). Gabay (2006)
explains that the actions of IL-6 are to return the host to a homeostatic state making it
clear that its role is to control the extent of tissue inflammatory responses. In chronic
diseases, IL-6 serves as an inducer of acute phase reactions but also is important in
eliciting cellular immune responses to affected cells and mucosal humoral responses
directed against infection (Ramsay et al., 2004). When its activity as a pro-inflammatory
cytokine persists, acute inflammation turns into chronic inflammation that includes
immune responses (Gabay, 2006) In chronic inflammation, IL-6 has a detrimental role
favoring mononuclear cell accumulation at the site of injury (Atreya et al., 2000).

IL-6 plays a role in several joint-related diseases. Serum levels of IL-6 increase
and induce plasmacytosis and hyperplasia of synovial cells in the joints of patients with
rheumatoid arthritis (RA) as a result of chronic inflammatory proliferation (Gabay,
2006). According to Yusof and Emery (2013) excess production of IL-6 has been found
in the synovial fluid and blood of RA patients and correlates with the disease activity and
joint destruction. Contributing to joint erosion, IL-6 causes bone resorption by inducing
osteoclast formation via the induction of receptor activator of nuclear factor kappa-B
ligand in synovial cells and cartilage degeneration by producing matrix
metalloproteinases in synovial cells and chondrocytes (Yusof & Emery, 2013). As a
pleiotropic cytokine, IL-6 is a suitable target in the treatment of RA because it induces the production of hepeiden, which is responsible for anaemia of chronic inflammation (Yusof & Emery, 2013).

In addition to the treatment of inflammatory joint diseases, IL-6 can be used as a biomarker to discriminate between multiple sclerosis and other inflammatory neurological diseases. According to Wullschleger (2013), elevated IL-6 levels have been found in the cerebral spinal fluid of those with central nervous system diseases. A study carried out by Xing and associates (1998), revealed an anti-inflammatory nature of IL-6 in both local and systemic acute inflammatory responses elicited by local lung or systemic exposure to endotoxin by using IL-6 gene knock-out mice. It was demonstrated that IL-6 is critically required to control the extent of local or systemic acute inflammatory responses, particularly to the level of pro-inflammatory cytokines in the local and systemic compartments. Xing (1998) found that in the endotoxic lung, the absence of IL-6 not only resulted in a more pronounced response of pro-inflammatory cytokines but also a greater extent of tissue neutrophilia. Xing’s findings suggest that in a normal host, one function of inducible IL-6 during acute responses is to suppress the level of pro-inflammatory cytokines without compromising the level of anti-inflammatory cytokines (Xing, 1998).

**Role of IL-1B and Altering Factors**

As defined by Ren and Torres (2009), interleukin-1 α (IL-1α) and β (IL-1β) are prototypic pro-inflammatory cytokines that exert pleiotrophic effects on a variety of cells and play key roles in acute and chronic inflammatory and autoimmune disorders. IL-1β
signals through IL-1RI type one receptor, upon which a second receptor termed IL-1 receptor accessory protein gets recruited at the cell membrane to form a high affinity binding receptor complex leading to intracellular signaling (Ren & Torres, 2009). In a normal organism, IL-1β has homeostatic functions such as the regulation of feeding, sleep, and temperature (Dinarello, 1996). The release of IL-1β is administered by keratinocytes, fibroblasts, synoviocytes, endothelial, neuronal, immune cells such as macrophages and mast cells, and glial cells such as Schwann cells, microglia and astrocytes (Watkins et al., 1995). Together with chemokines, IL-1β promotes the infiltration of inflammatory and immunocompetent cells from circulation into the extravascular space and then into tissues where tissue remodeling is the end result of IL-1 induced inflammation (Dinarello, 2009). IL-1β is also an angiogenic factor and plays a role in tumor metastasis and blood vessel formation (Dinarello, 2009). IL-1β is particular involved in neuropathic pain, which arises from dysfunction of the nervous system.

Overproduction of IL-1β is implicated in the pathophysiological changes that occur with different diseases such as rheumatoid arthritis, neuropathic pain, inflammatory bowel disease, osteoarthritis, vascular disease, multiple sclerosis, and Alzheimer’s disease (Dinarello, 1996; Braddock & Quinn, 2004; and Dinarello, 2004). The pro-inflammatory cytokines, including IL-1β, are contributory to the pain state, especially low back pain that arises from nerve injury. In degenerate and herniated human intervertebral discs, IL-1β expression is higher than in non-degenerative intervertebral disc controls (LeMaitre & Hoyland, 2007). In various animal models of neuropathic pain, IL-1β expression is increased in the injured sciatic nerve, dorsal root ganglion, and spinal
cord (Rotshenker et al., 1992). In a study conducted by Sommer (1999), it was seen that neutralizing antibodies reduced both thermal hyperalgesia and mechanical allodynia, suggesting a role for the upregulated IL-1β in the induction of neuropathic pain.

**How to Measure Inflammatory Biomarkers**

In biomedical research, the most commonly used method for measuring the concentration of inflammatory markers is enzyme-linked immunosorbant assay (ELISA) (Barbour & Cauley, 2013). This method is especially useful for low-abundance markers such as cytokines. ELISA uses an antibody sandwich technique with one antibody to specifically detect the cytokine or receptor of interest that is fixed to a plastic well, while the second antibody is linked to an enzyme that acts as an amplification factor to enable calorimetric or chemiluminescent detection and quantitation (Barbour & Cauley, 2013).

There are methodological limitations that can coincide with using ELISA to quantify inflammatory marker concentrations. First, for very low-abundance markers, such as TNF-α, the ELISA can require a relatively large volume of serum for analysis. Second, the cost of markers can add up and be prohibitive for researchers who lack adequate funds to conduct such measurements (Barbour & Cauley, 2013).

Another measurement tool are multiplex arrays, which have the ability to estimate levels of several inflammatory markers in one assay (Pearson et al., 2003). Compared to ELISA, they require smaller sample volume and are less expensive and more time efficient. This technique is based on flow cytometry technology, using microscopic beads with several predefined colors, representing different cytokines (Pearson et al., 2003).
pH and the Acid Base Equilibrium

What is the Definition of Systemic pH?

The term pH stands for potential of hydrogen, which is the measure of a solution’s hydrogen ion concentration (Cook, 2008). Cook (2008) explains that our blood and most of our tissues need to remain balanced in the neutral to slightly alkaline zone for optimum health (p. 25). Bowels should be slightly acidic. Urine needs to be between neutral and slightly acidic. Saliva tends to fluctuate more between acid, alkaline, and neutral. pH is a measure of acidity or alkalinity on a scale of zero to fourteen, with zero as extremely acidic, while 14 is extremely alkaline. A pH of seven is neutral, meaning there is complete balance between acid and base.

Overall, keeping your blood slightly alkaline at around 7.365 is the primary goal, since blood nourishes tissues, organs, and organ systems (Cook, 2008). At a cellular level, every cell has a small optimum range within which it performs its many functions.

How is pH Measured?

There are various tests that can help determine whether the body is acidic, including saliva, urine, and blood tests. Saliva testing gives a general range of the saliva’s pH and over time can provide a broad picture of a person’s overall state of acid-alkaline balance. Salimetrics Company suggests that saliva is an ideal testing fluid because samples can be collected in a minimally invasive, convenient and repeated manner. Proper saliva collecting and handling procedures are necessary for accurate results. Levels of analytes in saliva do not remain static, and concentrations may change in response to a number of influences. Sample collection must be made first thing in the
morning, prior to eating or drinking, when saliva is the most potent (Cook, 2008). In order to maintain consistency in the type of sample collected, Salimetrics advises using the unstimulated, whole saliva that pools on the floor of the mouth, collected by the passive drool technique. However, for those individuals such as children who would have difficulty with a passive drool technique, the use of an absorbent device can be placed in the mouth (Salimetrics).

Absorbent devices may collect localized saliva rather than whole saliva, affecting analyte results. Salimetrics advises recording the amount of time necessary to collect the desired volume of saliva. The assay results can be multiplied by the flow rate in order to express the results as a secretion rate. If an absorbent device, such as an oral swab is used to collect the saliva for determination of an analyte that is flow rate sensitive, the volume of saliva collected can be determined by weighing the device along with the storage tube before and after collection (Salimetrics). Ideally, saliva pH should be between 7.0 and 7.4 (Cook, 2008).

Urine testing also uses pH paper and although more accurate than saliva testing, it should only be used as a general guideline (Cook, 2008). A two-inch or longer strip of pH paper must be placed into the urine stream or dipped into a collected paper cup, first thing in the morning (Cook, 2008). Lastly, blood testing is the most accurate way to evaluate pH level, but will only give a reading of blood’s acid-alkaline balance at a specific moment and thus cannot be used to determine whether blood pH levels trend toward acidity (Cook, 2008).
Definition of Acidosis

Although it is uncommon, blood pH levels can shift to the side of excessive acidity or alkalinity, leading to several clinical symptoms. Minich and Bland (2007) mention that acidosis can lead to symptoms of lethargy, progressing to stupor and coma, while alkalosis can lead to nervous conditions such as cramps, muscle spasms, irritability, and hyperexcitability. Maintenance of constant hydrogen ion concentration (pH) is crucial for health. Enzymatic reactions have a pH optimum that is critical for allowing maximum enzyme activity (Rosner, 2009). Thus, changes in pH will effect enzyme activity and thereby disrupt critical metabolic pathways. Secondly, Rosner (2009) argues that the tertiary structure of a protein changes when pH rises or falls. Structural changes in proteins can disrupt its function and even accelerate its degradation (Rosner, 2008). Cells are also are very sensitive to changes in hydrogen ion concentration and require a precise maintenance of pH in the blood and extracellular fluid (Arnett, 2003). Blood pH is buffered by plasma proteins, histidine residues of hemoglobin, and by the carbon dioxide-bicarbonate system (Arnett, 2003).

Termed metabolic acidosis, Arnett (2003) explains that the addition of hydrogen results in a decrease in pH and a reduction of bicarbonate levels without substantial alteration in carbon dioxide concentration. Excess hydrogen ions and waste anions, must be excreted by the kidneys to produce acidified urine (Arnett, 2003). Systemic acidosis may result from high-protein diets, ingestion of inorganic acids, and declining renal, respiratory and vascular function with advancing age (Arnett, 2003). Acidosis can arise locally at tissue level as a result of reduced vascular supply due to infections,
inflammation, tumors, wounds, diabetes, and aging (Arnett, 2003). At a cellular level, a basic action of many growth factors and cytokines is to stimulate rapid hydrogen efflux from cells as a result of increased cellular metabolism (Arnett, 2003).

**Acidosis and Disease**

Thus, an acid-base imbalance can lead to a plethora of systemic problems and disease. Arnett (2003) explains that the direct effects of pH on bone resorption were discovered when it was found that rising ambient carbon dioxide levels caused osteoclasts to excavate characteristic resorption pits. In order to investigate whether this effect was due to changes in hydrogen concentrations, osteoclasts were cultured overnight in low bicarbonate media without carbon dioxide, using an artificial buffering system to maintain a range of pH values between 6.8 and 7.4. Osteoclasts were observed to be almost inactive at pH 7.4, which corresponds to physiological or blood pH, but resorption formation increased steeply as pH was reduced (Arnett & Dempster, 1986). Osteoclasts resorb bone efficiently even at pH 6.3-6.4, although their survival may be somewhat reduced (Murrills et al., 1993). Short-term experiments using physiological carbon dioxide-bicarbonate buffering indicated that osteoclasts are particularly sensitive to pH changes at approximately pH 7.1, such that pH reductions of only a few hundredths of a unit caused a doubling of resorption pit formation; below a pH of 7.0, the stimulatory effect begins to plateau (Arnett & Spowage, 1996).

Several possibilities exist for the mechanism by which extracellular acidification activates and stimulates osteoclasts to resorb bone. A low ambient pH will favor resorption simply by reducing the gradient against which osteoclasts must pump
hydrogen ions across the border (Arnett, 2003). It is possible that a pH receptor on the osteoclast cell membrane could be of the acid-sensing ion channel type (Arnett, 2003). Acidosis in vitro induces the activity of the vacuolar-type hydrogen-ATPase in osteoclast membranes within minutes; this enzyme is thought to be primarily responsible for pumping hydrogen ions out of the osteoclast into the extracellular resorption compartment in order to dissolve hydroxyapatite (Arnett, 2003). As far as dietary implications on bone resorption, a study with human volunteers showed that increasing the dietary acid load without altering overall protein intake resulted in increases in urinary calcium and collagen C-telopeptide excretion, suggesting increased bone resorption (Arnett, 2003). Given the great sensitivity of osteoclasts to ambient pH, it seems likely that even slight chronic acidosis could be sufficient to cause appreciable bone loss over time (Arnett, 2003).

In addition to acidity affecting bone cells, loss of substantial amounts of bicarbonate can lead to serious metabolic acidosis in patients with gastrointestinal disease (Rosner, 2009). The gastrointestinal (GI) tract has a key role in total acid-base balance. On a typical day, large amounts of hydrogen and bicarbonate traverse the specialized epithelia of the various gut segments (Rosner, 2009). Under normal circumstances, only a small amount of alkali is lost in the stool per day, which is easily regenerated by net renal acid excretion, regulated to maintain aid-base balance (Rosner, 2009). Metabolic alkalosis can lead to vomiting and nasogastric suction, while metabolic acidosis can lead to cholera, congenital chloridorrhea, pancreatic drainage, jejunostomy or ileostomy drainage, and short bowel (Rosner, 2009). For acid-base and electrolyte abnormalities to
occur in diarrheal states, the volume of enteric fluid that is lost must be large in order to overwhelm the ability of the kidney to maintain acid-base balance (Rosner, 2009). Various disease states lead to varying amounts of sodium, chloride, and bicarbonate losses. In the setting of biliary or pancreatic fistulas or external drains, there may be massive loss of bicarbonate rich fluids leading to both metabolic acidosis, as well as volume depletion which helps to maintain the acid-base abnormality (Rosner, 2009).

An acid-base imbalance can also lead to increased cancer risk, particularly induced by select dietary factors (Forrest, 2012). Lower pH levels in the extracellular space promote the invasive and metastatic potential of cancer cells (Forrest, 2012). Extracellular acidity is generated by tumor cells because of an increased hydrogen and lactic acid production due to a net-acid diet (Forrest, 2012). Most fruits and vegetables are net-base producing foods since the metabolized products are organic anion precursors such as citrate, succinate, and conjugate bases of carboxylic acids (Forrest, 2012). The final metabolite of these precursors is bicarbonate anion. Sulfur-containing amino acids, methionine and cysteine, typically found in meats, eggs, and dairy products, are oxidized into sulfuric acid, which is ultimately net-acid producing (Forrest, 2012). Although there are no studies showing a direct link between diet-induced acidosis and cancer, acid-base disequilibrium has been shown to modulate molecular activity including adrenal glucocorticoid, insulin growth factor, and adipocyte cytokine signaling, dysregulated cellular metabolism, and osteoclast activation, which may serve as intermediary or downstream effectors of carcinogenesis or tumor production (Forrest, 2012).
Lastly, metabolic acidosis has found to be associated with chronic kidney disease, in a study administered by John Hopkins University in 2004. There was little knowledge regarding the relationship of acidosis and inflammation with hypoalbuminemia and different glomerular filtration rates (GFR). Using data from the National Health and Nutrition Examination Survey III (NHANES III), a large cross-sectional study to examine the effect of acidosis and inflammation on moderate to severe GFR reductions and hypoalbuminemia (Forrest, 2012). The results of Forrest’s study (2012) showed that while hypoalbuminemia is associated with reduced GFR as well as with dietary protein and caloric intake, much of this association is caused by associated factors, such as the presence of proteinuria, reduced sodium bicarbonate, and inflammation. Thus, the nutritional status of patients with low GFR rates should be monitored.

**pH and Inflammation**

Extracellular pH for circulating leukocytes is easily altered and the changes in pH can rapidly alter the immune response in these cells (Kellum, Song, & Li, 2004). A characteristic feature of the inflammatory locus is local acidosis, which is attributed to the local increase of lactic-acid production by the anaerobic glycolytic activity of infiltrated neutrophils and to the presence of short-chain, fatty acid by-products of bacterial metabolism (Kellum, Song, & Li, 2004). As found in a study administered by Menkin (1934), the cellular picture in an area of inflammation is apparently a function of the local hydrogen ion concentration. The usual cytological sequence in the development of acute inflammatory reaction consists of an initial infiltration of polymorphonuclear cells that are replaced by macrophages (Menkin & Warner, 1936). This change is correlated by the
development of a local acidosis at the site of inflammation (Menkin & Warner, 1936). Menkin and Warner (1936) explain that in the initial phase, when the polymorphonuclear cells predominate, the pH of the exudate is within an alkaline range of about 7.3-7.4. With the progress of the inflammatory reaction the pH can drop to 6.7-6.5, in which polymorphonuclear cells seem unable to survive without injury (Menkin & Warner, 1936). If the acidity increases beyond a pH of 6.5, all types of leukocytes are injured and frank pus results (Menkin & Warner, 1936).

This finding supports the deduction that pH depends on the rate of glycolytic activity and on the available local alkali reserve (Menkin & Warner, 1936). With a fall in pH, there is a concomitant reduction in the local alkali reserve as measured by the carbon dioxide content (Menkin & Warner, 1936). If the exudate remains alkaline throughout the period of the acute reaction, the carbon dioxide content fails to be reduced; on the other hand, if a frankly purulent exudate develops with a pH below 6.5, the carbon dioxide content may fall to about eight percent volume or less (Menkin & Warner, 1936).

Discovered by Warburg, Posener, and Negelein (1924) when tissues are deprived of oxygen, lactic acid is produced from glucose through the process of glycolysis. The tissues most susceptible to high rates of glycolysis include malignant tissue, normal retina and embryonic tissue (Warburg, Posener, & Negelein, 1924). With interference in normal oxidative reactions, there is a possibility that an anaerobic type of glycolytic activity may result (Menkin & Warner, 1936). With the impaired fluid exchange in an area of injury an anaerobic type of glycolytic respiration might prevail and this would manifest itself in a conversion of sugar into lactic acid and a true lactic acid acidosis results at the site of
inflammation (Menkin & Warner, 1936). In the examination of extracellular acidosis in dendritic cells and pro-inflammatory response, Martinez and colleagues (2007) found that interstitial acidification (pH of 5.5-7.0) is commonly associated with the development of inflammatory reactions against pathogens in peripheral tissues.

Not only are inflammatory reactions against pathogens associated with acidity, but autoimmune processes such as rheumatoid arthritis and asthma are associated with the development of acidic microenvironments in injured tissue (Martinez et al., 2007). The pH of synovial fluid of compromised joints in patients with rheumatoid arthritis is acidic and acidosis appears to correlate with both synovial fluid leukocytosis and joint destruction (Martinez et al., 2007). Acidosis also develops in the tumor microenvironment and studies have found values of extracellular pH ranging from 5.8-7.4 both in human and rodent malignant tissues (Martinez et al., 2007). Ultimately it was found that extracellular acidosis is a hallmark of inflammatory processes resulting from a number of factors (Martinez et al., 2007). The factors include the massive infiltration of leukocytes and the production of protons during the activation of the respiratory burst, the accumulation of by-products of microbial metabolism, and tissue hypoxia resulting in the stimulation of glycolysis in the ischemic tissue and the subsequent accumulation of lactate (Martinez et al., 2007).

Consistent with these results, it has been shown that exposure of human neutrophils to low pH values results in the production of platelet-activating factor, a strong inflammatory stimulus, as well as activating the alternative pathway of complement (Martinez et al., 2007). Another study was conducted to find the effect of
lactic acid on mononuclear cell secretion of pro-inflammatory cytokines in response to group B streptococci (GBS) by Steele, Augustine, and Hill (1997). Because lactic acid is commonly found in association with elevated concentrations of TNF-α in the cerebrospinal fluid during meningeal infections and lactic acidosis accompanies septic shock, it was hypothesized that elevated concentrations of lactic acid might modulate TNF-α production induced by GBS (Steele, Augustine & Hill, 1997). The production of the pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, by human mononuclear cell preparations was examined, after exposure to various concentrations of lactate in the presence or absence of GBS (Steele, Augustine & Hill, 1997). It was found that physiologic concentrations of lactate showed a slight stimulatory effect on TNF-α production (Steele, Augustine & Hill, 1997). Lactate alone or in combination with GBS significantly enhances mononuclear cell secretion of pro-inflammatory cytokines (Steele, Augustine & Hill, 1997).

The environment in which pro-inflammatory cells function is often glucose-poor and acidic, and contains an abundance of lactic acid or lactate, in which macrophages are able to carry out their varied functions (Steele, Augustine & Hill, 1997). Many cells are required to function in lactate-rich or anaerobic conditions, and because pro-inflammatory cells must be able to rapidly produce large quantities or energy for metabolic needs, a lactate-rich environment provides substrate for energy production (Steele, Augustine & Hill, 1997). Along with TNF-α, IL-1β is thought to be one of the principal cytokines responsible for induction of fever, changes in leukocyte numbers, and synthesis of acute-phase reactants following acute-bacterial infection (Steele, Augustine
Monocyte and macrophage secretion of IL-1β and IL-6 were also affected by increasing lactate concentrations with additional exposure to GBS (Steele, Augustine & Hill, 1997).

**High-Intensity Exercise**

**Definition of High-Intensity Exercise**

Exercise intensity refers to how much energy is expended when exercising (Stradley, 2004). It is expressed as a percentage of maximal oxygen consumption (VO₂max). Exercise is divided into three intensity levels, low, moderate, and high, which are measured by metabolic equivalents (METs). High-intensity exercise is characterized by a MET that is greater than 6.0 (Vehrs, 2011). A high-intensity workout is defined as exercises that push an individual’s heart rate up to 75% of its maximum or more (Stradley, 2004). A high level of effort characterizes this type of exercise during a relatively brief workout, as opposed to a low or moderate intensity for a longer duration (Jones, 1973). High-intensity training is taxing on the neuromuscular system, because an individual can only perform for so long at such high intensity before creating a chronic recovery deficit that causes results to diminish (Fitzgerald, 2013). High-intensity training is so effective, because it increases the resting metabolic rate for the following 24 hours due to excess post-exercise oxygen consumption, and may improve maximal oxygen consumption (Fitzgerald, 2013).

**VO₂max Definition and Measurement**

According to Yoon, Kravitz, and Robergs (2007), the maximal rate of oxygen consumption (VO₂max) is one of the most commonly measured parameters in basic and
applied physiological sciences. The maximal oxygen uptake represents the highest amount of oxygen that an individual may consume under exercise conditions (Williams, 2007). When oxygen does not increase with an increase in workload, the maximum oxygen uptake has been reached (Williams, 2007). \( \text{VO}_{2\text{max}} \) can also be measured by volitional exhaustion, which is defined as the point in which an individual can no longer perform a muscular contraction and voluntarily terminates the contraction (Pitcher & Miles, 1997). \( \text{VO}_{2\text{max}} \) represents the physical fitness of an individual because it measures the maximum capacity at which oxygen can be transported and used and is also known as maximal aerobic capacity (Yoon, Kravitz & Robergs, 2007). \( \text{VO}_{2\text{max}} \) is expressed either as an absolute rate in liters of oxygen per minute or as a relative rate in millimeters per kilogram of bodyweight per minute; the latter being used to compare the performance of endurance sport athletes (Yoon, Kravitz & Robergs, 2007).

Accurately measuring \( \text{VO}_{2\text{max}} \) involves a physical effort sufficient in duration and intensity in order to fully tax the aerobic energy system (Sloth, Overgaard & Dalgas, 2013). Clinical or athletic measurement involves a graded exercise test, usually on a treadmill or cycle ergometer, in which exercise intensity is progressively increased while measuring ventilation and oxygen and carbon dioxide concentration of inhaled and exhaled air (Sloth, Overgaard & Dalgas, 2013). Measuring \( \text{VO}_{2\text{max}} \) on individuals who are not considered healthy subjects can be dangerous because any problems with the cardiovascular or respiratory system will be exacerbated in clinically ill patients (Sloth, Overgaard & Dalgas, 2013).
Levels of VO\textsubscript{2max} vary among individuals. Values are typically 40-60\% higher in men than in women (Sloth, Overgaard & Dalgas, 2013). The average untrained healthy male will have a VO\textsubscript{2max} of 35-40 mL/(kg/min) while an untrained healthy female will score 27-31 mL/(kg/min) (Sloth, Overgaard & Dalgas, 2013). These scores can improve with training and decrease with age although the degree of trainability can vary: conditioning may double VO\textsubscript{2max} in some and will only marginally improve it in others (Billat & Koralsztein, 1996). Endurance performance sports athletes typically have higher VO\textsubscript{2} maxima with elite male and female runners consuming up to 85 and 77 mL/(kg/min) respectively (Billat & Koralsztein, 1996). The American College of Sports Medicine (ACSM) has established VO\textsubscript{2max} norms for men and women. The norming chart divides the VO\textsubscript{2max} norms by age and places that score into a percentile.

The factors affecting VO\textsubscript{2max} are divided into supply and demand factors. Supply is the transport of oxygen from the lungs to the mitochondria while demand is the rate at which mitochondria can reduce oxygen in the process of oxidative phosphorylation (Billat & Koralsztein, 1996). Variables that may affect VO\textsubscript{2max} include age, gender, fitness and training, changes in altitude, and action of the ventilatory muscles (Billat & Koralsztein, 1996). VO\textsubscript{2max} determinants include cardiac output, pulmonary diffusion capacity, oxygen carrying capacity, and other peripheral limitations like muscle diffusion capacity, mitochondrial enzymes and capillary density (Billat & Koralsztein, 1996). Because the body works as a system, if one of these factors is performing poorly, then the whole system loses its normal capacity to function properly (Billat & Koralsztein, 1996).
Running and its Relationship to Inflammation

Physical activity is known for its various beneficial effects including reduction of cardiovascular risk factors and improvement of blood and lipid profiles (Jee & Jin, 2012). Intense exercise may induce inflammatory reactions and immune disturbances with an increase in circulating inflammatory biomarkers (Tuan et al., 2007). After exercise-induced muscle damage, the immune system plays a role in the degeneration and regeneration process of surrounding connective tissue (Peake, Nosaka & Suzuki, 2005). An intense treadmill exercise can induce a high percentage of lymphocyte apoptosis, suggesting that strenuous exercise can augment cell sensitivity to death induction (Tuan et al., 2007). Engaging in moderate activity may enhance immune function above sedentary levels and improve both endothelium function and inflammatory responses (Jee & Jin, 2012). However, excessive amounts of prolonged, high-intensity exercise may impair immune function by increasing circulating inflammatory markers and altering the state of endothelium for further induction into the pathogenic state (Tuan et al., 2007). In addition, chronic inflammation and endothelial dysfunction may lead to impaired exercise capacity (Jee & Jin, 2012).

Exercise induces an array of systemic inflammatory and anti-inflammatory reactions. Immediately after eccentric exercise, anti-inflammatory cytokines are released into systemic circulation. Within one day after exercise, neutrophils are replaced in damaged muscle while pro-inflammatory cytokines are produced in muscle (Peake, Nosaka & Suzuki, 2005). These responses are significant for the acute-phase of inflammation in order to remove damaged muscle fragments (Peake, Nosaka & Suzuki,
Muscle damage that results from lengthening contractions attracts leukocytes to the site of injury (Peake, Nosaka & Suzuki, 2005). Within several hours, neutrophils invade skeletal muscle and remain present up to 24 hours after exercise, whereas macrophages are present in muscle from 24 hours to 14 days after exercise (Peake, Nosaka & Suzuki, 2005). Neutrophils and macrophages contribute to the degradation of damaged muscle tissue by releasing nitrogen and reactive oxygen species and may also produce pro-inflammatory cytokines (Peake, Nosaka & Suzuki, 2005). The cytokines can remain within skeletal muscle up to five days after exercise, but normally return to pre-exercise values within three to four hours (Gleeson, 2007).

The concentration of inflammatory markers is known to bioactively change in response to exercise intensity, duration, recruited muscle mass, and endurance capacity (Jee & Jin, 2012). It has been reported that the regular performance of around two hours of moderate exercise per day is associated with a 2% reduction in risk of picking up upper respiratory tract infection compared with a sedentary lifestyle (Gleeson, 2007). There is a 100-500% increase in the risk of picking up an infection in the weeks following a competitive ultra-endurance running event (Gleeson, 2007). Downhill running and eccentric cycling at high intensities, greater than 75% VO$_{2\text{max}}$, stimulate a greater increase in the plasma concentrations of IL-6 and IL-1 that other forms of moderate intensity exercise such as downhill running (Peake, Nosaka & Suzuki, 2012). Thus, the release of inflammatory markers is more likely related to the intensity and duration of exercise as well as increased core temperature rather than muscle damage (Peake, Nosaka & Suzuki, 2012).
At a cellular level, there several events that occur as a result of exercise. Functional capacities of leukocytes may be decreased by repeated bouts of intense and prolonged exercise, which could be due to increased levels of stress hormones during exercise and entry into the circulation of less mat leukocytes from the bone marrow (Gleeson, 2007). Additionally, there is an increased production of reactive oxygen species, which can impair immune cells because of an excess of free radicals (Gleeson, 2007). TNF-α, IL-6, and hs-C-reactive protein (CRP) are known to be involved in the classic symptoms of inflammation and vigorous exercise has been shown to substantially increase these markers (Jee & Jin, 2012). IL-6 increases the most during exercise and the elevated systemic levels of this inflammatory biomarker could be the mechanism by which exercise provides protection against the development of chronic disease (Gleeson, 2007). Circulating anti-inflammatory cytokines respond to local production of pro-inflammatory cytokines and restrict systemic inflammation (Peake, Nosaka & Suzuki, 2012).

**Running and its Relationship to pH**

During intense exercise the increase in blood and muscle lactate and the coincident decrease in pH in tissues has traditionally been explained by the production of lactic acid (Robergs, Ghiasvand & Parker, 2004). Acidosis is explained by the production of lactic acid, causing the release of a proton of hydrogen and leaving the final product to be the acid salt lactate, a process termed lactic acidosis (Robergs, Ghiasvand & Parker, 2004). When there is a rapid increase in the production of lactic acid, the free hydrogen can be buffered by bicarbonate causing the non-metabolic production of carbon dioxide
(Robergs, Ghiasvand & Parker, 2004). In turn, the developing acidosis and raised blood carbon dioxide levels stimulate an increased rate of ventilation causing the temporal relationship between the lactate and ventilator thresholds (Robergs, Ghiasvand & Parker, 2004).

The term lactic acid can be traced back to the pioneer work of Meyerhof and Hill (1922) who demonstrated that lactic acid was produced as a side reaction to glycolysis in the absence of oxygen. Hill (1922) quantified the energy release from glucose conversion to lactic acid and proposed that glucose oxidation in times of limited oxygen availability, as well as when the energetic demands of muscle contraction exceeded that from oxidation involving oxygen, can supply a rapid and high amount of energy to fuel muscle contraction. Margaria and colleagues (1920) demonstrated that lactic acid concentration in the blood is noncomitant with changes in blood pH. Researchers measured muscle pH, lactate, and pyruvate during exercise and recovery from different intensities of exhaustive exercise. Plots of the sum of lactate and pyruvate to muscle pH revealed a linear relationship between the two variables (Margaria et al., 1920).

The production of lactic acid occurs when oxygen is not supplied fast enough to meet the needs of the muscle cells during fatiguing contractions (Karp, 2012). Karp (2012) explains that lactate production maintains the ratio of certain biochemical molecules, supporting the continued ability of glycolysis to keep working. Lactate is used as a fuel by the heart to make glucose by the liver and to convert back to glycogen so that glucose and glycogen can be used as fuels by muscles for high intensity to continue (Karp, 2012). At slower running speeds, lactate is removed from the muscles as quickly
as it is produced; but at faster speeds there is a greater reliance on anaerobic glycolysis for energy, and aerobic metabolism cannot keep up with the production of pyruvate from glycolysis (Karp, 2012). Pyruvate is converted into lactate and lactate removal starts lagging behind lactate production.

The lactate threshold is the fastest pace above which lactate production begins to exceed its removal, with lactate concentrations increasing exponentially (Karp, 2012). The lactate threshold is measured during a VO$_{2\text{max}}$ test and demonstrates a relationship between ventilation and respiratory gas samples: as speed increases, there is a greater reliance on oxygen-independent metabolism which increases the amount of carbon dioxide produced and stimulates ventilation to expire the carbon dioxide (Karp, 2012). The better the athlete’s endurance, the higher the percentage the lactate threshold occurs in relation to VO$_{2\text{max}}$ (Karp, 2012). Training the lactate threshold increases the speed at which lactate accumulates and acidosis occurs, which will enable the athletes to run at a higher percentage of VO$_{2\text{max}}$ for a longer time (Karp, 2012).
**Figure 2.** Alterations in gas exchange which result from exercising at work rates above the anaerobic threshold (Karp, 2012).

It is now evident that exercise above the anaerobic threshold results in an altered oxygen uptake kinetics with a delay in oxygen uptake and an increase in oxygen deficit and debt (Wasserman, Whipp, Koyal & Beaver, 1973). As previously mentioned, Hill and Meyerhof demonstrated the relationship between oxygen supply and lactic acid transport. If the local circulation is adequate for the work rate being performed, all of the energy requirements may be supplied by ATPs generated by aerobic mechanisms. However, if the number of muscle units needed to contract exceeds the oxygen delivery and exhausts the oxygen stores, the oxygen level with drop to critical levels in the muscles. This will prevent ATP generation at the adequate rate needed for muscle contraction (Wasserman, Whipp, Koyal & Beaver, 1973). Thus, this results in anaerobic metabolism...
glycolysis to sustain the availability of ATP (Wasserman, Whipp, Koyal & Beaver, 1973).

Because of lactic acid’s low pH, it will be more than 99% dissociated and buffered predominately by the bicarbonate system. Carbon dioxide can then be readily exhaled into the atmosphere, thereby preventing an accumulation of lactic acid in the body tissues (Wasserman, Whipp, Koyal & Beaver, 1973). The decrease in local tissue and blood bicarbonate results in a component of respiratory compensation for the metabolic acidosis (Wasserman, Whipp, Koyal & Beaver, 1973). It is important to note that for work rates below 50-60% of the VO\textsubscript{2max}, muscle lactate does not increase (Davis, 1985). For activities with durations between 30 and 60 minutes, investigators have reported that blood lactate concentrations between three and five mM can be sustained (Davis, 1985). This demonstrates that shorter duration endurance events can be performed above the anaerobic threshold (Davis, 1985).

**Measurement Techniques for Inflammatory Markers**

**Definition of Saliva and its Reflection of Systemic Disease**

Saliva is produced by multiple salivary glands lying beneath the oral mucosa with a production of almost 600 mL of serous and mucinous saliva a day (Lawrence, 2002). Saliva contains minerals, electrolytes, buffers, enzymes and enzyme inhibitors, growth factors and cytokines, immunoglobulins, mucins and other glycoproteins (Lawrence, 2002). Components within saliva not only protect the integrity of oral tissues, but also provide clues to local and systemic diseases and conditions (Lawrence, 2002). Several types of inflammatory biomarkers associated with both oral diseases, as well as systemic
diseases, have been detected in saliva, such as IL-6, IL-6, IL-1β, TNF-α, and matrix metalloproteinases (Rathnayake et al., 2013).

The molecular composition of saliva reflects the tissue fluid levels of therapeutic, hormonal, immunological, and toxicological molecules (Zimmerman, Park & Wong, 2007). These fluids provide sources for assessment and monitoring of systemic health and disease states, exposure to environmental and job-related toxins, and the use of abusive or therapeutic drugs (Zimmerman, Park & Wong, 2007). Dental health professionals first used the use of saliva as a diagnostic tool in its detection of dental carries by measuring its buffering capacity and bacterial content (Lawrence, 2002). Now, saliva is increasingly being used as an investigational aid in the diagnosis of systemic diseases that affect the function of the salivary glands and the composition of saliva, such as Sjögren’s syndrome, alcoholic cirrhosis, cystic fibrosis, sarcoidosis, diabetes mellitus and diseases of the adrenal cortex (Lawrence, 2002). Additionally, the onset and severity of the infectious diseases can be determined by monitoring the presence of antibodies to the microorganisms found in saliva and the oral cavity (Lawrence, 2002).

An increasing number of specific molecular markers for different diseases are being identified by salivary analysis (Rathnayake et al., 2013). Interaction among salivary enzymes that promote the adhesion and colonization of mucosal surfaces by respiratory pathogens may explain the potential role of oral bacteria in the pathogenesis of respiratory infections (Lawrence, 2002). Another example of saliva and systemic health is evident in breast cancer research, where salivary testing for markers of the disease is studied for potential use in conjunction with mammography (Lawrence, 2002). Pilot
studies have indicated that the saliva test for oncogene is reliable and potentially useful in early detection and follow-up screening for breast cancer (Lawrence, 2002). In endocrinology, the ease of collecting saliva is simplifying serial measurements of hormone levels and their diurnal variations (Lawrence, 2002). Lastly, whole saliva may replace blood as a monitoring medium for nutritional deficiencies in elderly people (Lawrence, 2002). Older adults who suffer from malnutrition may also exhibit signs of an impaired immune response. Reduced resistance of oral tissues to disease often leads to increased colonization by oral pathogens and to sustained oral infections (Lawrence, 2002). Clinical signs of malnutrition and compromised immune systems appear first in the oral cavity, with dimeric sIgA, the predominant immunoglobulin in saliva acting as the first line of defense against microbial invasion (Lawrence, 2002).

**Advantage of Using Saliva as Means for Analysis**

Tests using saliva as a diagnostic tool have made its way into an array of clinical and research areas such as virology, immunology, microbiology, endocrinology, epidemiology and forensics (Lawrence, 2002). In the past, serum has been the fluid most often used in disease diagnosis; however, saliva has many advantages over both serum and urine (Lawrence, 2002). In the past decade, the potential for the use of saliva for the detection of oral cancer has been laid out by the analysis of genetic changes in the cellular compartment (Zimmerman, Park & Wong, 2007). However, low target concentrations, the subtleness of genetic changes and heterogeneity of early events in cancer development and progression have as yet prevented the translation of these findings to concepts for early noninvasive detection of oral cancer (Zimmerman, Park &
Wong, 2007). Thus, the genomic variation in saliva has mainly been applied for the genetic banking for pharmacogenomics and epidemiologic studies, and for a variety of identity testing situations (Zimmerman, Park & Wong, 2007).

Salivary testing presents a list of positive opportunities for researchers and health care professionals. While being easily accessible in a noninvasive manner, saliva has the advantage that the background of normal material and inhibitory substances is much lower and less complex than in blood (Zimmerman, Park & Wong, 2007). Salivary tests for antibodies, unconjugated steroid hormones, environmental toxins, tobacco and certain drugs are sufficiently sensitive to accurately reflect the blood concentrations of these substances (Lawrence, 2002). Saliva is relatively easy to collect in sufficient quantities for analysis, and the costs of storage and shipping tend to be lower than serum and urine (Lawrence, 2002). There are however, a variety of factors that may influence the salivary flow and its physiologic characteristics, including circadian rhythms and activities such as exercise, which must be taken into account when saliva is used as a diagnostic tool (Lawrence, 2002). For health care professionals, saliva tests are safer than blood tests, which are more likely to result in exposure to HIV or hepatitis (Lawrence, 2002). For the patient, the noninvasive salivary collection techniques allow for reduced anxiety and discomfort, simplifying collection of serial samples for monitoring health and disease states over time (Lawrence, 2002). Additionally, results of analysis of mRNA biomarkers in cancer detection showed that analysis of saliva, may in certain instances, not only be less invasive, but also provide a better value than blood (Zimmerman, Park & Wong, 2007).
CHAPTER III

METHODS

Introduction

The purpose of this research study was to examine the effect of high intensity exercise on the inflammatory biomarkers IL-6, IL-1β, and TNF-α in male athletes using salivary analyses. A secondary purpose was to find a correlation between pH and the markers. Lastly, the study aimed to prove the accuracy of using saliva for inflammatory diagnosis in both current and future research studies. This study was a pretest/posttest experimental design with repeated measures at four checkpoints: 0 h, 30 min, 60 min, and 24 h. Each participant served as his own control.

Subjects and Recruitment

All recruited subjects were required to be male, between the ages of 18 and 60, have a history of endurance running, and able to run at 60% of their VO\(_{2\text{max}}\) for one hour. Participants were required to be above the 60\(^{th}\) percentile for their age group according to the American College of Sports Medicine (ACSM) norming chart, which divides VO\(_{2\text{max}}\) norms by age. Females were excluded from the study due to menstruation and risk of pregnancy, which would endanger their safety in the trial. The study also excluded individuals with a presence of infectious, inflammatory, immune, and cardiac disease, use medication or supplementation that alter immune status, or were ill on test day.

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VO\(_{2\text{max}}\) assessment occurred on a Techno gym brand treadmill equipped to a Parvomedics true max 2400 metabolic cart, while the participant breathed into a secured facemask. Measurements were recorded using the Parvomedics true one metabolic system software OUSW 4.3.4. Participants completed the maximal exercise test using a client-selected pace with 3% progressive grade every three minutes until volitional exhaustion was reached. Volitional exhaustion is defined as the moment in which an individual’s muscles are so fatigued that he voluntarily ends a muscle contraction. When the participants reached this point of exhaustion, they jumped both feet laterally on the treadmill to mark the end of the VO\(_{2\text{max}}\) assessment. Participants were asked to continue
their normal daily physical activities without any intense exercise one week prior to test day.

**Procedure and Salivary Collection**

The official test day for participants occurred at Kent State University’s exercise physiology lab. Subjects could not consume any food or beverages, other than water, two hours prior to the test run and could not be sick for test day. Participants were required to run at 60% of their assessed VO$_{2\text{max}}$ and maintain 75% of their maximum heart rate, wearing a heart rate monitor throughout the 60-minute run. To establish the 60% intensity value, the individual’s VO$_{2\text{max}}$ score was simply multiplied by 0.6. The test began with the participant first breathing into a facemask attached to a metabolic cart for three minutes. This gathered baseline data prior to initiating the run. With the facemask still attached, the participant began the run at a client-selected pace. The researcher or assistant adjusted the pace until the metabolic levels suggested that 60% of VO$_{2\text{max}}$ had been reached. At this time, the researcher removed the facemask so that the run would be more comfortable for the participant. VO$_2$ levels were assessed every 15 minutes to ensure the stability of 60% of VO$_{2\text{max}}$ until the participant reached 60 minutes, which was the completion of the trial.

Salivary collection occurred at four stages: 0 minutes, 30 minutes, 1 hour, and 24 hours post-completion for analysis of pH, IL-6, TNF-α, and IL-1β. Collection of the 0 min, 30 min and 60 min saliva samples were gathered in the lab during the run of the trial by the researcher. Participants were required to meet with the researcher at the exercise physiology lab 24-hours post run for the final saliva sample. Collection of samples was
by unstimulated passive drool, in which the head was tilted forward, allowing the saliva to pool on the floor of the mouth and into a paper cup. Saliva was collected via pipette and stored in a cryovial until analysis. For proper assaying, the following volumes were collected: 135 µL of IL-6 and 50 µL of IL-1β. Samples had to refrigerated within 30 minutes of collection and were stored at -20°C in a so-low ultra-low freezer environmental equipment (Cincinnati, Ohio) within four hours of collection. Frozen samples were thawed completely, vortexed and centrifuged at 1500 x g (at 3000 rpm) for 15 minutes to remove mucins and other particulate matter.

**Salivary Analysis**

Phinex Diagnostic’s wide-range pH test strips were used to measure systemic acidity in the samples. Concentrations of IL-6 and IL-1 β were measured using Salimetrics enzyme-linked immunoabsorbent assay (ELISA) kits according to manufacturer’s instructions (Salimetrics, 2014). The inflammatory cytokine TNF-α did not have an ELISA analysis kit for saliva samples, and thus had to be dropped from the study from the original proposal. The ELISAs were performed using a microplate reader at a dual wavelength of 450 and 630 nm. The intra-assay precisions of the measurement for IL-6 were provided by the manufacturer and were reported to have a high control range of 74.48 pg/mL ± 18.62 and a low control range of 10.36pg/mL ± 4.14. The intra-assay precisions presented by the manufacturers for IL-1β were reported to have a high control range of 158.34 pg/mL ± 39.59 and a low control range of 27.06 pg/mL ± 10.82.
Statistical Analysis

Statistical analysis of the data was performed by the Statistical Package for Social Sciences (SPSS) Version 20. Baseline characteristics of age, height, weight, BMI, VO$_2$max, and running history are expressed as means with standard deviation for all participants. According to Salimetrics, inter-assay % coefficients of variations (CV) of less than 15% were generally acceptable. Intra-assay % CVs should be less than 10. This guideline was followed in order to include data from participants if the duplicates were differentiating (Moss DW, 1970). The standard curve for both IL-6 and IL-1β had a value of 1.0, proving the accuracy of the Salimetrics ELISA kits. To determine the changes in inflammatory marker concentrations and pH for each checkpoint, 0 min, 30 min, 60 min, and 24-hours, repeated measures analysis of variance test (ANOVA) was performed. Pearson correlation analysis was performed to examine the relationship between the markers and the checkpoints, as well as pH and the checkpoints. The results were considered significant at $p \leq 0.05$. 
CHAPTER IV

JOURNAL ARTICLE

Introduction

Physical activity is known for its various beneficial effects on the human body such as reducing cardiovascular risk factors (Mora et al., 2007) and improving blood and lipid profiles (Dunn et al., 1997). However, intense physical activity increases circulating inflammatory markers inducing acute inflammation, possibly leading to impaired exercise capacity (Bartziotou et al., 2007). Inflammation is defined as an innate system of cellular and humoral responses following injury in which the body attempts to restore the tissue to its pre-injury state (Ward, 2010). The acute phase of inflammation consists of increased blood flow and vascular permeability along with the accumulation of fluid, leukocytes, and inflammatory mediators such as cytokines (Feghali & Wright, 1997). In many situations, the acute inflammatory response becomes prolonged or excessive, leading to serious damage of tissues and organs (Ward, 2010).

Cytokines are the chief stimulators of the production of acute-phase proteins during the inflammatory processes (Gabay & Kushner, 1999). Inflammation-associated cytokines include interleukin-6 (IL-6), interleukin 1-β (IL-1β), tumor necrosis factor α (TNF-α) and others (Gabay & Kushner, 1999). Combinations of the cytokines can have additive, inhibitory, or synergistic effects, and the effects of cytokines on target cells may be inhibited or enhanced by other cytokines, hormones or circulating receptors (Gabay & Kushner, 1999).
Intense exercise leads to increased plasma concentrations of both pro-and anti-inflammatory cytokines (Friman & Ilback, 1998; Peake, Nosaka & Suzuki, 2005; Sloth, Sloth, Overgaard & Dalgas, 2013). If the acute inflammatory response becomes prolonged or excessive, it can lead to serious damage of tissues and organs (Ward, 2010). This muscle damage has the potential to modify leukocyte receptor expression and functional activity, leading to an infiltration of neutrophils and monocytes into damaged tissue (Tuan et al., 2007). These cells release proteolytic enzymes and reactive oxygen species to break down tissue fragments (Tuan et al. 2007).

Intense exercise also attributes to acute inflammation and localized muscle injury by releasing lactic acid and depleting the alkali reserve (Menkin & Warner, 1936). The hydrogen ion concentration drops pH levels to 6.7-6.5, which is a level too low for polymorphonuclear cells to survive without injury (Menkin & Warner, 1936). If the acidity increases beyond a pH of 6.5, all types of leukocytes are injured (Menkin & Warner, 1936). There have been previous studies that have shown that exercise does not cause an anti-inflammatory or pro-inflammatory response (Markovitch, Tyrrell & Thompson, 2008; Pederson & Toft, 2000). Markovitch’s (2008) study showed that a single bout of moderate intensity walking did not lymphocyte and monocyte expression. Pederson’s (2000) study did not find significant changes in monocyte concentration after moderate activity. Although there have been findings that confirm that low or moderate intensity does not cause an increase in inflammatory biomarkers, it is important to conduct a study that will confirm that high intensity endurance exercise induces acute inflammation in athletes. This is particularly important in application, because it will
show that the intensity, duration, and type of physical activity does make a drastic impact on the body.

There is no known study on the relationship between inflammatory markers, lactic acidosis, and high-intensity exercise using salivary analysis. Previous studies had measured inflammation by using blood for laboratory analysis (Gleeson, 2007; Jee & Jin, 2012; Tuan et al., 2007). Thus, a study needs to be conducted to confirm that duration and intensity of exercise does increase the acute inflammatory response through the collection and analysis of saliva. The purpose of this research study was to examine the effect of moderate intensity exercise on pH and the inflammatory biomarkers IL-6 and IL-1β in male athletes using salivary analyses. A secondary purpose was to find a relationship between pH and each of the cytokines. A third purpose was to prove the accuracy of using saliva for inflammatory diagnosis in both current and future research studies. It was hypothesized that IL-6 and IL-1β would increase as a result of the exercise, and that as inflammation increased, the pH would decrease.

Methods

Introduction

The purpose of this research study was to examine the effect of moderate intensity exercise on the inflammatory biomarkers IL-6, IL-1β, and TNF-α in male athletes using salivary analyses. A secondary purpose was to find a correlation between pH and the markers. Lastly, the study aimed to prove the accuracy of using saliva for inflammatory diagnosis in both current and future research studies. This study was a pretest/posttest
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and 24-hours, repeated measures analysis of variance test (ANOVA) was performed. Pearson correlation analysis was performed to examine the relationship between the markers and the checkpoints, as well as pH and the checkpoints. The results were considered significant at \( p \leq 0.05 \).

**Results**

In order to observe changes of pH and inflammatory markers during prolonged high intensity exercise, pH, IL-1β, and IL-6 counts were analyzed from the saliva collected at 0 h, 30 min, 60 min, and 24 h. Baseline characteristics of age, weight, height, BMI, running history, and VO\(_{2\text{max}}\) are shown in Table 1. Participant speeds ranged from 5.5 to 7.2 mph for 60 minutes based on 60\% of VO\(_{2\text{max}}\). Fourteen trained male runners volunteered to participate in this study. Over the course of the study, four participants withdrew due to injuries and one participant did not complete the trial run. A total of nine runners completed the entirety of the study.

Table 1. *Baseline characteristics of age, height, weight, BMI, VO\(_{2\text{max}}\), and running history (n=9)*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.7</td>
<td>9.51</td>
</tr>
<tr>
<td>Height (inches)</td>
<td>70.4</td>
<td>1.34</td>
</tr>
<tr>
<td>Weight (kilograms)</td>
<td>75.9</td>
<td>6.83</td>
</tr>
<tr>
<td>BMI</td>
<td>23.7</td>
<td>1.57</td>
</tr>
<tr>
<td>VO(_{2\text{max}}) (ml/kg/min)</td>
<td>55.9</td>
<td>5.12</td>
</tr>
<tr>
<td>Running history (years)</td>
<td>11.67</td>
<td>5.69</td>
</tr>
</tbody>
</table>
Effect of High-Intensity Endurance Exercise on IL-1β and IL-6

No significance was found between IL-1β and the high-intensity run, $p=0.568$. IL-6 concentration was not affected by the high-intensity run, without significance, $p=0.399$.

As shown in Table 2, the only increase in inflammatory concentration was seen between the 30 and 60 min mark.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Checkpoint</th>
<th>n</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1B*</td>
<td>0 h</td>
<td>5</td>
<td>13.2</td>
<td>13.69</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>7</td>
<td>8.6</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>6</td>
<td>12.8</td>
<td>10.37</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>6</td>
<td>5</td>
<td>3.24</td>
</tr>
<tr>
<td>IL-6**</td>
<td>0 h</td>
<td>8</td>
<td>317.5087</td>
<td>211.13</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>9</td>
<td>198.2422</td>
<td>148.83</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>9</td>
<td>243.8667</td>
<td>172.9</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>8</td>
<td>218.8475</td>
<td>188.94</td>
</tr>
</tbody>
</table>

Note: *$p= 0.399$, **$p=0.568$

Effect of High-Intensity Endurance Exercise on pH

There was no correlation found between pH and the inflammatory markers.

Baseline salivary pH level of 0 h averaged at $7.3 \pm 0.63$. pH at the 30 min time mark averaged $7.37 \pm 0.59$. Mean pH at 1 h was $7.23 \pm 0.62$, and at the 24 h was $7.01 \pm 0.75$. 

Figure 3. Correlation between pH and IL-6 concentrations at 0 h
*Note:* n=5 (data was non-detectible for 4 participants at 0 h)

Figure 4. Correlation between pH and IL-6 concentration at 30 min.
*Note:* n=7 (data was non-detectible for 2 participants at 30 min)
Figure 5. Correlation between pH and IL-6 at 60 min.
*Note:* n=5 (data was non-detectible for 4 participants at 60 min)

Figure 6. Correlation between pH and IL-6 at 24 h.
*Note:* n=6 (data was non-detectible for 3 participants at 24 h)
Figure 7. Correlation between pH and IL-1B at 0 h
Note: n=8 (data for 1 participant was non-detectible at 0 h)

Figure 8. Correlation between pH and IL-1B at 30 min
Note: n=9
Figure 9. Correlation between pH and IL-1B at 60 min  
Note: n=9

Figure 10. Correlation between pH and IL-1B at 60 min  
Note: n=8 (data for 1 participant was non-detectible at 60 min)
Discussion

The purpose of this research study was to examine the effect of a high-intensity exercise on inflammatory markers during and after a one hour run at 60% of VO_{2max}. Additionally, the study aimed to find a relationship between inflammation and pH. Concentrations of the analytes were measured at four different checkpoints: 0 h, 30 min, 60 min, and 24 h. Results of this study showed no significant difference in salivary concentrations of pH, IL-6, and IL-1β. There also was no relationship between pH and each of the inflammatory markers. Thus, both hypotheses were rejected. Saliva was used as a testing medium, whereas blood was commonly used in previous studies testing similar cytokines during exercise (Tuan et al, 2007; Jee & Jin, 2012).

IL-1β and IL-6 were both detectible in the saliva samples and produced detectible changes, however the changes were not significant. IL-1β samples had a wide range of 4.63-678.30 pg/mL. At the 0 h, the minimum reading was 8.91 pg/mL and the maximum was 678.30 pg/mL. At 30 min, the concentrations produced a range of 50.92-420.30 pg/mL. A range of 14.91-521.50 pg/mL was seen at 60 min, and a range of 4.63-558.35 at 24 h. Based on a 15% CV, data from eight of the nine participants was analyzed at the 0 h and 24 h, and all nine participants’ data was analyzed at both 30 min and 60 min, proving concentration to be detectible. Results for IL-6 varied with a range of 1.70-37.0 pg/mL among participants. However, many participants’ data was undetectable and could not be used due to a % CV greater than 15%, thus several samples were lost. As seen in Table 2, only 5 participant’s data could be used for 0 h, 7 for 30 min, 6 for 60 min, and 6
for 24 h. A range of 4.63-37.00 pg/mL was found at 0 h, 1.89-23.77 pg/mL at 30 min, 1.70-27.09 pg/mL at 60 min, and 2.68-10.49 pg/mL at 24 h.

Previous studies showed that several cytokines could be detected during and after strenuous exercise (Pederson & Hoffman-Goetz, 2000; Pederson et al. 2001). Local response to an infection or tissue injury involves the production of cytokines, which are released at the site of inflammation. These cytokines facilitate the influx of lymphocytes, neutrophils, monocytes and other cells, which participate in the healing. This is accompanied by the acute phase response, which includes the production of cytokines such as IL-6 and IL-1β. These cytokines are usually referred to as inflammatory or pro-inflammatory cytokines. A previous study measuring both IL-6 and IL-1β, among several other markers, showed that plasma concentrations of the markers were elevated in untrained men in response to exercise. The trained runners tended to have higher activity of inflammatory markers prior to exercise but did not increase further in response to exercise (Pederson, 2000). In a study involving marathon runners, levels of TNF-α and IL-1β increased twofold and IL-6 levels increased up to 100-fold (Pederson, 2000). An increase in TNF-α and IL-1β is accompanied by a dramatic increase in IL-6. A previous study was performed to test the hypothesis that inflammatory cytokines are produced in skeletal muscle in response to intense long-duration exercise (Ostrowski et al, 1998). Muscle biopsies and blood samples taken before and after a marathon race showed a marked increase in IL-6. Thus, Ostrowski’s study showed that duration of high-intensity exercise plays a role in inflammation.
There are several factors that explain the variable results on pro-inflammatory and inflammation-responsive cytokines in relation to exercise. The type of physical activity, as well as the intensity and duration of the exercise, can affect the cytokine profile. Increased levels have mostly been described after exercise involving an eccentric component. Downhill running and eccentric cycling at high intensities, greater than 75% VO$_{2\text{max}}$, stimulate a greater increase in the plasma concentrations of IL-6 and IL-1 than other forms of moderate intensity exercise (Peake, Nosaka & Suzuki, 2012). Thus, the release of inflammatory markers is more likely related to the intensity and duration of exercise as well as increased core temperature rather than muscle damage.

The one hour run at 60% of VO$_{2\text{max}}$ intensity did not provide significant results in the concentration of inflammatory markers, showing that this duration and intensity does not produce a pro-inflammatory effect. The fact that the runners were well-conditioned athletes, meaning their calculated VO$_{2\text{max}}$ was greater than 45%, places them as above average and excellent according to ACSM guidelines. If the participants were untrained, inflammatory concentration may have increased. In addition to inflammatory markers, this high intensity exercise did not have a significant effect on pH, meaning no athlete had a lactic acid build-up. Build-ups of lactic acid occur when the body does not have enough oxygen to supply to working muscles during strenuous activity. When oxygen is limited, the body converts pyruvate to lactate, allowing energy production to continue so that exercise can continue (Nicholson, 2001). High lactate levels lead to an increase in acid in the muscle cells, slowing the capacity for more work (Marcinik, Potts, Schlabach, & Dawson, 1991). Because there was no increase in acidity during the run, the well-
conditioned athletes had not met their lactate threshold at the 60% intensity. If participants had not met their lactate threshold, then pH would not drop. When pH drops, inflammation increases. However, pH never decreased and this inflammation never spiked. As above-average runners, they were able to run aerobically for the entire hour.

Results of the study showed that no relationship existed between pH and the inflammatory biomarkers as seen in figures 3 and 4. The hypothesis had proposed an inverted relationship, in that as the concentration of inflammatory markers increased, pH would decrease. During intense exercise, Robergs and colleagues propose that the increase in blood and muscle lactate and the coincident decrease in pH in both tissues has been traditionally explained by the production of lactic acid (2004). A previous study in arthritic patients found that inflammation gives rise to local acidosis, increasing hydrogen concentration and decreasing systemic pH to 6.5 (Goldie & Nachemson, 1970). A study performed on the effect of a single soccer game on muscle damage and inflammation showed both a post-game increase of leukocytes, cytokines, and cortisol, as well as a 72-hour increase in uric acid. The 60-minute run in my research study did not produce a significant change in the inflammatory markers, and thus systemic pH was not affected.

**Limitations**

There are several limitations to this study. Firstly, the subjects were well-trained male runners. Whether the results can be applied to untrained people and/or male subjects remains unclear. Secondly, the study had a small sample size due to the selective inclusion and exclusion criteria, as well as Salimetrics ELISA kit costs. A 96-well plate was used to run the analysis, and thus, in order to sample all participants’ saliva in
duplicate, only nine subjects could be used in the study. Additionally, because the data required having a % CV no greater than 15, several data points were discarded due to inaccuracy, leaving the n for each time point even smaller. More participants would have prevented from a great loss of data in analysis. A third limitation was in regard to the 24-hour salivary collection. Participants were not instructed to limit food intake or physical activity prior to collection at the 24 h mark, and thus concentration of the cytokines may not have been reliable.

**Applications**

No known studies had used saliva as a means of detecting inflammation in athletes, with previous studies using blood samples for analysis. The most commonly used laboratory diagnostic procedures involve the analysis of cellular and chemical constituents of blood. As a diagnostic fluid, saliva offers distinctive advantages over serum because individuals with modest training can collect it non-invasively. This is important for the dietetic profession, which does not receive training with blood collection as opposed to other scientific professions. This can allow dietetic professionals a feasible and accurate way to diagnose clinical diseases. Saliva can also provide a cost-effective approach for screening large populations. It is also a feasible method that can be performed during bouts of exercise. Saliva samples are useful for the diagnosis of hereditary disorders, autoimmune diseases, malignant and infectious diseases, endocrine disorders, as well as drug use (Salimetrics, 2014). The results of this study, although not significant, did prove the accuracy of salivary testing, producing a perfect standard curve. This showed that the procedures for analyses were done correctly. The data also showed
detectible readings and detectible changes among the participants with wide ranges of cytokine concentrations. Thus, saliva can be used as an appropriate medium for the diagnosis of disease in addition to blood and serum for several scientific fields of all skill levels.

Most importantly, this study confirmed the J curve model, which supports the theory that the immune system has a training effect. The curve proposes that trained athletes have improved immune function and fewer upper respiratory infections (URIs) compared to sedentary individuals. Regular exercise can help attenuate the age related decline in immune function. However, if exercise is too frequent and too intense, it can actually lead to an increase in URIs. The stress of strenuous exercise and overtraining can suppress the immune system, leading to frequent illness and injury. The curve shows that moderate intensity activity provides the lowest risk of URIs, while sedentary and overtrained lifestyles can increase URI risk. This is important in regard to this research study, because it supports the J curve. The athletes were performing at moderate intensity and thus there was no increase in inflammatory markers. Studies in which individuals competed in high intensity and longer duration sports such as marathons and sprint-trials did show an increase in inflammatory markers. Intensity, frequency, duration, and type of physical activity do play a big role in immune and inflammatory status in individuals.

Further Studies

Further studies are needed to detect inflammation in athletes using salivary measurements to confirm these results. Studies involving a larger number of participants may offer significant changes in cytokine concentrations, as well as increasing both the
intensity and duration of the exercise. There are also factors such as intensity, duration, frequency and type of physical activity that can affect results. There needs to be more studies conducted to reiterate the J curve. This will support the claim that at both low and high intensities there is a greater decline in immune function. Further studies can help confirm that inflammation increases at high intensities, greater than 75% VO$_{2\text{max}}$ and is unaffected at moderate intensity. This research can help sedentary individuals understand the importance of regular physical activity and can also educate trained individuals on the importance of proper training to avoid overtraining.

**Conclusion**

It is shown in this study that a high-intensity (60% of VO$_{2\text{max}}$) run does not lead to an increase in inflammatory markers and it does not alter pH. Results of this study show that saliva can be used as an accurate medium for detecting inflammation, in addition to blood and serum.
APPENDICES
APPENDIX A

HEALTH QUESTIONNAIRE
Appendix A

Exercise-induced Inflammation Study
Participant Questionnaire

*IRB statement: Individuals who have disease cannot participate in this study

The purpose of this study is to determine, through salivary analysis, the effect of pH on the inflammatory biomarkers TNF-α, IL-6, and IL-1β, in male college athletes during and after high intensity endurance exercise.

Name: ___________________________________________ Ht: __________ Wt: __________

Gender: ______________ Age: __________

Birthdate: ________________________________

Address: ____________________________________________________________________________________

City: ______________ State: _____ ZIP: ________

Phone: ______________

Emergency contact: ___________________________Phone: ___________________________

Personal physician: ___________________________Phone: ___________________________

E-mail: _____________________________________________________________________________________
Health History

Have you ever had a definite or suspected heart attack or stroke? Yes
No

Do you have any other cardiovascular or pulmonary (lung) disease? Yes
No

Do you have a history of: diabetes, thyroid, kidney, liver disease? Yes
No

(Circle all that apply)

Have you ever been told by a health professional that you have had an abnormal resting or exercise electrocardiogram (EKG)? Yes
No

Have you ever been diagnosed with an inflammatory condition? Yes
No

Do you have an autoimmune disease? Yes
No

Pain or discomfort in the chest or surrounding areas that occurs when you engage in physical activity? Yes
No

Shortness of breath Yes
No

Unexplained dizziness or fainting Yes
No

Difficulty breathing/asthma Yes
No

Swelling of the ankles (recurrant and unrelated to injury) Yes
No
Heart palpitations (irregularity or racing of the heart)  

Yes  
No

Known heart murmur  

Yes  
No

Had surgery or been diagnosed with disease in past 3 months  

Yes  
No

Have you had high blood cholesterol within the past 12 months?  

Yes  
No

Do you currently smoke cigarettes or have quit within past 6 months?  

Yes  
No

Do you have high blood pressure?  

Yes  
No

Exercise and Physical Activity

Describe your regular physical activity or exercise program

Type: ________________________________

Frequency: __________ days per week
Duration: _______________ minutes
Intensity:  low    moderate    high  (circle one)
Running History

How long have you been running competitively?
______________________________________________________________

How many days a week do you run?
______________________________________________________________

What is your typical weekly mileage? High (above 35 miles) □     Moderate □     Low (below 10 miles) □

What is your average distance training run?
______________________________________________________________

What is the distance of your longest run to date? _______ When?
______________________________________________________________

What are your typical times for the following running events?

<table>
<thead>
<tr>
<th>DISTANCE</th>
<th>AVERAGE TIME</th>
<th>BEST TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10K</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Other</td>
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</tr>
</tbody>
</table>

I have answered the previous questions accurately and completely. I understand that my answers will be kept private and will result in either the selection or rejection from the
proposed research study. If you have any further questions, please contact Alyssa Tyler at Apiunno@kent.edu or (440)781-6408.

Signature: ___________________________________________ Date:

_________________________

Printed name: ______________________________________
APPENDIX B

RECRUITMENT EMAIL
Appendix B

Recruitment Email

Dear ________,
You are invited to participate in a research study, designed by nutrition graduate student, Alyssa Tyler, and advisor, Natalie Caine-Bish.

The purpose of this research study is to examine the effect of high intensity exercise on pH and the inflammatory biomarkers tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) in male college athletes. A secondary purpose is to determine a correlation between pH and the inflammatory biomarkers. High intensity exercise can cause the body to create an acidic environment and release inflammatory mediators, which can induce muscle damage and cell death in high concentrations.

Results of the study will hopefully reveal the changes in inflammatory biomarker release as a result of exercise intensity, recruited muscle mass, and duration. This study will help to understand and prevent localized muscle injury as a result of acute inflammation by understanding the shifts in cytokine concentration. Controlling systemic pH and preventing its drop to a dangerous acidic level can prevent the injury of both polymorphonuclear cells and leukocytes prevented, thus protecting the body against disease.

You will be participating in a complimentary VO₂max assessment and a one-hour run, during which saliva samples will be collected to be used for inflammatory analysis.

If you are interested in participating in this study, please email Apiunno@kent.edu for more information.

Thank you,
Alyssa Tyler
APPENDIX C
CONSENT FORM
Appendix C

Informed Consent to Participate in a Research Study

**Study Title:** The effect of high intensity exercise on pH and inflammatory markers

**Principle Investigator:** Dr. Natalie Caine-Bish  
**Co-Investigator:** Alyssa Tyler

You are being invited to participate in a research study. This consent form will provide you with information on the research project, what you will need to do, and the associated risks and benefits of the research. Your participation is voluntary. Please read this form carefully. It is important that you ask questions and fully understand the research in order to make an informed decision. You will receive a copy of this document to take with you.

**Purpose**

The purpose of this research study is to examine the effect of high intensity exercise on pH and the inflammatory biomarkers tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) in male college athletes. A secondary purpose is to determine a relationship between pH and the inflammatory biomarkers. High intensity exercise can cause the body to create an acidic environment and release inflammatory mediators, which can induce muscle damage and cell death in high concentrations.

Results of the study will hopefully reveal the changes in inflammatory biomarker release as a result of exercise intensity, recruited muscle mass, and duration. This study will help to understand and prevent localized muscle injury as a result of acute inflammation by understanding the shifts in cytokine concentration. Controlling systemic pH and preventing its drop to a dangerous acidic level can prevent the injury of both polymorphonuclear cells and leukocytes prevented, thus protecting the body against disease.

**Procedures**

1. **Questionnaire:** upon recruitment, participants will be asked to complete a ten-minute questionnaire to assess health and running history. Participants will first be asked basic anthropometric information including height, weight, and BMI. They will also be asked medical history questions to rule out inflammatory, immune, infectious, and cardiac conditions that would prevent appropriate inflammatory marker concentrations. Participants will be asked to include running history information: number of years running, types of races completed, average completion times and personal bests.

2. **Informed consent:** one on one meeting between selected participants and co-investigator to explain the purpose of the study, benefits and risks in participating, voluntary withdrawal, and participant protection.

3. **VO_{2}\text{max}** Assessment: Participants will undergo a metabolic cart maximal exercise treadmill test using a client-selected pace with 3% progressive grade every three minutes until
VO$_{2\text{max}}$ or volitional exhaustion is reached. Participants must have between a 50-65% VO$_2$ to participate. Facemasks will be worn for the metabolic cart assessment.

4. Test day criteria: No food or beverages (other than water) can be consumed 2 hours prior to the 1 hour run. You cannot be ill on test day.

5. You will be asked to run for a period of 1 hour. Upon the first 5 minutes, you will run at a speed needed to reach 60% of your VO$_{2\text{max}}$. We will then determine the speed needed to sustain that 60% intensity. The VO$_{2\text{max}}$ mask will only be worn initially during the 1 hour run to ensure that the appropriate intensity is being met.

6. Test day salivary collection: through passive drool technique, pipettes will be used to collect saliva at four different times: pre-run, halfway mark (30 minute mark), upon completion (1 hour mark), and 24 hours post-completion. Saliva will be initially frozen upon collection. After collection from all participants, it will then used for assaying of 3 inflammatory markers, TNF-α, IL-6, and IL-1β, and pH. Saliva will be discarded after analysis.

**Benefits**

The potential benefits of participating in this study include a complimentary VO$_{2\text{max}}$ testing, a very expensive assessment for individuals. VO$_{2\text{max}}$ testing is a very important test for athletes of all skill level who hope to improve their performance. Determination of maximum oxygen capacity reflects the maximum amount of oxygen in milliliters that one can use in one minute per kilogram of body weight. Those who are fit have higher max values and can exercise more intensely than those who are not as well conditioned. The value tells the potential for endurance athletes. Participation in this study will help to benefit all athletes by understanding the effect of exercise on inflammation in effort to minimize muscle and localized injury.

**Risks and Discomforts**

Because this is a high-intensity endurance exercise lasting two hours, there are potential risks beyond those encountered in everyday life. Possible risks include falling off the treadmill, risk of cardiac arrest during high intensity exercise, muscle cramping, and muscle soreness upon completion. This risk is low due to the fact that selected participants are highly conditioned athletes who have a measured VO$_{2\text{max}}$ of 50-65%, as determined upon initial assessment, and are capable of running for one hour without injury. Monitoring of heart rate throughout the exercise as well as keeping the athletes well hydrated will prevent both cardiac risk and dehydration.

Individuals who have any inflammatory, immune, infectious, or cardiac diseases will be excluded from participation in this study as determined by the initial health questionnaire.

Individuals who arrive ill on test day will be excluded from participating in the study.

**Privacy and Confidentiality**

Anonymity will be protected upon completion of the initial health questionnaire and written consent. Participants will be assigned a reference number for the continuation of the study.
Your study related information will be kept confidential within the limits of the law. Any identifying information will be kept in a secure location and only the researchers will have access to the data. Research participants will not be identified in any publication or presentation of research results; only aggregate data will be used.

Data will be stored for five years in both a locked file and computer database, after which it will be permanently removed and destroyed.

**Voluntary Participation**

Taking part in this research study is entirely up to you. You may choose not to participate or may discontinue your participation at any time without penalty or loss of benefits to which you are otherwise entitled. You will be informed of any new, relevant information that may affect your health, welfare, or willingness to continue your study participation.

**Contact Information**

If you have any questions or concerns about this research, you may contact Alyssa Tyler at 440. 781.6408. This project has been approved by the Kent State University Institutional Review Board. If you have any questions about your rights as a research participant or complaints about the research, you may call the IRB at 330.672.2704.

**Consent Statement and Signature**

THE EFFECT OF HIGH INTENSITY EXERCISE ON PH AND INFLAMMATORY MARKERS

I have read this consent form and have had the opportunity to have my questions answered to my satisfaction. I voluntarily agree to participate in this study. I understand that a copy of this consent will be provided to me for future reference.

_____________________________________________               _________________________
Participant Signature                                                                 Date
APPENDIX D

BASELINE CHARACTERISTICS FOR ALL PARTICIPANTS
Appendix D

Baseline Characteristics for All Participants

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<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>VO2max (mL/kg/min)</th>
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