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THE EFFECTS OF EXERCISE UPON INDICATORS OF LIPID PEROXIDATION

A Thesis Presented to the Faculty of
the Division of Health, Physical
Education, and Recreation
Ithaca College

In Partial Fulfillment of the
Requirements for the Degree
Master of Science

---

by
Melissa U. Tompkins

May 1989

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Melissa U. Tompkins

Submitted in partial fulfillment of the requirements for the degree of Master of Science in the Division of Health, Physical Education, and Recreation at Ithaca College has been approved.

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THE EFFECTS OF EXERCISE UPON INDICATORS OF LIPID PEROXIDATION

by

Melissa U. Tompkins

An Abstract

of a thesis submitted in partial fulfillment

of the requirements for the degree of

Master of Science in the Division

of Health, Physical Education,

and Recreation at

Ithaca College

May 1989

Thesis Advisors: Dr. G. A. Sforzo

Dr. Robert Jenkins

ABSTRACT

The purpose of this study was to determine the amount of thiobarbituric reaction products (TBAR), a biochemical marker of malondialdehyde (MDA), in human sweat under exercise and control conditions at two anatomical sites. Three sources of data were used for this purpose. The first included a peak oxygen consumption ($\dot{V}O2$) graded exercise test in which a subject cycled on an ergometer until exhaustion to determine a workload for additional testing. The second source was a sweat sample collected after a submaximal bout of exercise on a cycle ergometer. The third source was a sweat sample taken from the subject while seated in a hot whirlpool bath. The assay detected TBAR in all sweat samples. In an ANOVA test, a significant condition-by-site interaction, $F(1, 12) = 6.38$, $p < .05$, was found. Repeated measures t tests were performed to determine how the conditions differed at each site. The results of those tests revealed a significant difference, $t(12) = 4.77$, $p < .05$, between the bike and whirlpool TBAR values at the arm site only. Based on the results of this study, it was concluded that TBAR can be detected in sweat. Since TBAR react with MDA, a potentially harmful reaction product of lipid peroxidation, these data may indicate that the body removes MDA by secreting it in sweat. Also, since a greater amount of TBAR were found

in the sweat of exercising subjects at the arm site, this may indicate that the body utilizes the sweating mechanism to rid itself of the MDA. Presumably, the MDA was produced from an increased rate of lipid peroxidation in association with the elevated oxygen consumption related to increased physical activity.

ACKNOWLEDGMENTS

The investigator wishes to express her sincere appreciation to everyone involved in the completion of this study:

1. Dr. Gary A. Sforzo, my thesis advisor, for his most valued suggestions and corrections throughout the writing of this thesis.

2. Dr. Robert R. Jenkins, who gave me the opportunity to research this topic, and for all his guidance, advice, and support throughout the investigation.

3. And last but not least, my loved ones, Al and John, for their encouragement, patience, and understanding throughout these past 2 years.

DEDICATION

This thesis is dedicated to my grandparents,

Danny & Boko

Your love, support, and encouragement have made it possible for me to come this far in pursuit of my academic goals.

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Chapter 1

INTRODUCTION

It has recently been determined that oxygen at ambient concentrations can result in oxygen stress and potential cellular damage in aerobic organisms by generating oxygen free radicals (Gutteridge, Rowley, Halliwell, Cooper, & Heeley, 1985). A free radical may be defined as any atom, group of atoms, or molecule in a particular state with at least one unpaired electron occupying an outer orbital (Del Maestro, 1980). The membranes of mammalian cells contain large amounts of polyunsaturated fatty acids which are very susceptible to a process of degradation known as lipid peroxidation (Rouser, Nelson, Fleicher, & Simon, 1968). Many oxygen radicals are extremely reactive and could attack polyunsaturated fatty acids in membranes resulting not only in trauma to the cell but the release of lipid peroxidation products that may diffuse away and be associated with other harmful reactions related to cancer (Halliwell & Gutteridge, 1986; Packer, 1984), reoxygenation injury (Del Maestro, 1980), or degenerative factors related to the aging process (Mead, 1976). Presently, there is not enough evidence to determine how the body actually handles oxygen centered free radicals and lipid peroxidation. The relationship

of exercise to free radical chemistry has become a topic of recent interest. There is evidence that oxygen radical production increases in association with the increased oxygen consumption associated with exercise (Davies, Quintanilha, Brooks, & Packer, 1982; Dillard, Litov, Savin, Dumelin, & Tappel, 1978). It is important to determine the underlying physiological mechanism by which exercise increases oxygen radicals and how the body might protect itself from increased radical production.

One of the chemical by-products resulting from lipid peroxidation, malondialdehyde (MDA), has been widely used as an indication that peroxidation has occurred in reaction systems. Numerous studies that have demonstrated evidence for the presence of MDA in the liver (Brady, Brady, & Ullrey, 1979; Davies et al., 1982; Suzuki, Katamine, & Tatsumi, 1983), skeletal muscle (Brady et al., 1979; Davies et al., 1982; Salminen & Vihko, 1983), brain and heart (Suzuki et al., 1983). However, few studies of lipid peroxidation have been completed on humans. Wilbur, Bernheim, and Shapiro (1949) reported that human sweat yielded a compound that reacted with thiobarbituric reaction products (TBAR) to produce a characteristic reddish-orange color. The presence of TBAR is generally accepted as evidence for

the presence of MDA. The purposes of this study were three-fold: first, to determine whether MDA as determined by TBAR is released through sweat in detectable amounts; second, to determine if a difference exists between the amount of TBAR excreted in sweat induced by exercise or heat; and, third, to determine if a difference exists in TBAR excretion in varying anatomical locations.

Scope of Problem

This study was conducted to investigate if a lipid peroxidation reaction product (i.e., TBAR) could be detected in human sweat induced by exercise or heat and if TBAR release differed by anatomical location. Thirteen undergraduate students from Ithaca College volunteered to serve as subjects and were administered a graded exercise test on a cycle ergometer, to determine their peak oxygen consumption. Each subject underwent further tests to provide sweat samples for TBAR analysis under two conditions: exercise and whirlpool. The sequence of testing for the two conditions was arranged randomly for each subject. The samples collected were analyzed to detect TBAR levels in sweat and differences between the TBAR content of samples collected from the arm and back sites.

Statement of Problem

Can traces of TBAR be found in sweat and, if so, are there differences between the values found in exercise and nonexercise conditions or between back and arm sites?

Hypotheses

The hypotheses of this study were as follows:

Ho: There will be no detectable amounts of TBAR in sweat regardless of condition or location from which it was obtained.

Ha1: TBAR will be detectable, but no difference in the sweat TBAR content during exercise and nonexercise conditions will be detected.

Ha2: TBAR will be detectable, but no difference in the sweat TBAR content between the back or arm sites will be detected.

Assumptions of Study

The following were assumptions of the study:

1. The subjects responded to the directions of the investigator and used maximum effort during the graded exercise testing session.

2. Free radical generation caused lipid peroxidation which resulted in the measured TBAR production.

3. The subjects washed down with water prior to

testing, and this was effective in eliminating any contamination of the sample.

4. The technique of sweat collection was an appropriate method of sampling.

Definition of Terms

The following terms were operationally defined for the purpose of this study:

1. Aerobic power: the greatest volume of oxygen used by the cells of the body per unit of time (Lamb, 1978). Peak oxygen consumption ($\dot{V}O2$) was the measure of aerobic power determined in the present study during maximum cycle ergometry. Leg fatigue may have caused test termination before true maximal $\dot{V}O2$ could be obtained.

2. Malondialdehyde (MDA): a product of prostaglandin endoperoxide metabolism and nonspecific lipid peroxidation resulted from free radical oxidation of lipids (Marnett, Buck, Tuttle, Basu, & Bull, 1985).

3. Lipid peroxidation (LIPOX): a series of oxygen free radical reactions resulting in a peroxide or hydroperoxide ($H2O2$) that will tend to spontaneously degenerate. The degeneration of peroxides and hydroperoxides produces additional radical centers and leads to structurally devastating consequences (Holman, 1954).

4. Superoxide Dismutase (SOD): a scavenging enzyme which functions to catalyze the dismutation of the superoxide anion radical (O2-) rendering less harmful products H2O2 and O2 (Fridovich, 1976).

5. Thiobarbituric Acid (TBA): a reagent solution used for a colorimetric assay of MDA (Marnett et al., 1985).

6. Oxygen Radical: molecular oxygen with unpaired electrons occupying the outer orbitals (Del Maestro, 1980).

7. Aerobically Conditioned: a subject who had exercised on a regular basis to improve cardiovascular fitness.

8. Graded Exercise Test: a test to determine maximum oxygen consumption ability on a cycle ergometer. Maximal effort is required as the workload gradually increases until exhaustion is achieved.

Delimitations of Study

The delimitations of the study were as follows:

1. Thirteen undergraduate male students from Ithaca College were recruited as subjects.

2. Only subjects who were not aerobically conditioned in the previous 6 months before testing began participated in the study.

3. Following a 10 min warm-up, 65% peak $\dot{V}O_2$ was

the only exercise condition studied.

4. Only back and upper arm sites were sampled during testing.

5. Samples were only taken from the exercise condition between 20 and 30 min after cycling began.

6. Samples were only taken from the whirlpool condition between 10 and 20 min into testing.

7. Only gauze pad absorption was used to collect samples.

Limitations of Study

The limitations of the study were as follows:

1. The subjects were volunteers and might not be entirely representative of the total population of college-aged male undergraduates.

2. The relatively small sample size limits the power of the statistical analysis.

3. Results may only apply when a warm-up period of 10 min, followed by exercise at 65% peak $\dot{V}O_2$ for 20-30 min, is performed in the exercise condition.

4. Results may only apply to samples taken from the whirlpool condition between 10-20 min into testing.

5. Results may only apply to samples from the back and upper arm sites.

6. A different method of sweat collection might produce different results.

Chapter 2

REVIEW OF RELATED LITERATURE

This chapter reviews literature related to free radical chemistry, the relationship of exercise and oxygen radical production, eccrine glands, and the appearance of products of oxygen radical chemistry in sweat.

Free Radical Chemistry

In order for the human species to survive, the ability to consume and use oxygen is essential. Scientists have long studied atomic oxygen and how the human body utilizes it. However, it has recently been found that ambient oxygen consumption does have some potentially harmful side-effects.

Atomic oxygen contains an unpaired electron in the outer valence shell, thus making it a radical. Fridovich (1978) explained that oxygen radicals are extremely reactive, having a strong tendency to interact with other atoms and form a chemical bond. As oxygen radicals establish bonds, they combine with atoms producing intermediates, such as hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH\cdot$). Oxygen radical products are dangerous to the living system due to their reactivity. Oxygen radicals and radical intermediates can cause cell membranes to become leaky. Although

oxygen free radicals are constantly produced when oxygen is reduced, the human body has an elaborate chemical defense system to scavenge and neutralize radicals.

One line of defense against free radical attack is through enzymes which break down the radicals into harmless products. Examples of these enzymes include superoxide dismutase (SOD), which converts O2- to H2O2 and O2, and peroxidases, which reduce H2O2 to H2O. However, the hydroxyl radical has no catalyzing enzyme and tends to form when free metals such as iron are present (Del Maestro, 1980). When it does form, the hydroxyl radical can attack anything in a cell, particularly the cell membrane. Cell membranes consist of phospholipids, a compound containing one saturated and two unsaturated fatty acids. The molecular structure of an unsaturated fatty acid provides less hydrogen atoms on a carbon chain, which increases the number of active carbons and, thus, increases the susceptibility of unsaturated fatty acids to radical attack. Demopoulos (1973) suggested that hydrogen bonding may indirectly protect some molecular sites against hydrogen abstraction by free radicals because of the tight packing and the restrictive mobility it induces. Demopoulos also pointed out that the content and degree of unsaturation are of major importance in

determining the likelihood of free radical damage to membranes of a cell population. When a hydroxyl radical attacks a cell, the unsaturated fatty acid of a membrane phospholipid becomes separated, and a chain reaction of attack to cell membranes can occur from the decreased energy coupling efficiency.

Compounds such as reduced glutathione (GSH), oxidized glutathione (GSSH), and vitamins play an important role as antioxidants. Examples of such antioxidants include Vitamin C and Vitamin E. Vitamin E is found in cell membranes and is known to react with hydroxyl radicals.

Free radical attacks on cell membranes may result in lipid peroxidation, which occurs as a series of chemical reactions including a rearrangement of the polyunsaturated fatty acids into a form called conjugated dienes and finally into a chemical compound malondialdehyde (MDA).

The danger of oxygen radical generation and lipid peroxidation has been explained in a paper by Del Maestro (1980). In that paper, Del Maestro reviewed the possible role of free radicals in pathological states including ischemic injury, cancer, degenerative disorders related to aging, oxygen toxicity, Vitamin E deficiency, and ultraviolet radiation injury. The lipid

peroxidation product, MDA, has also been shown to be toxic, carcinogenic, and mutagenic (Nair, Cooper, Vietti, & Turner, 1986). Research related to free radical chemistry in vivo has been slow since it is not easy to detect the radicals or hydrogen peroxide in the body, although it has been shown that thiobarbituric reaction products (TBAR) are formed in the body (Dillard et al., 1978). The latter finding is generally accepted as an indicator of the presence of lipid peroxidation. In a study by Marnett et al. (1985) using mice, TBAR were found to be metabolized uniformly in the living system.

Relationship of Oxygen Radical Production and Exercise

In the last decade, aerobic exercise such as running, swimming, biking, and aerobic dance have become very popular. The popularity of aerobic exercise has been accompanied by the idea that increasing oxygen consumption capacity results in an unquestionable fitness and health benefit. However, aerobic exercise disturbs cardiovascular, muscular, and metabolic homeostasis. Most exercise physiologists as well as the general population consider cardiovascular fitness important as a preventative measure against heart disease. Aerobic exercise is a method often used to

increase cardiovascular fitness and to rehabilitate individuals after acute myocardial infarction. However, enhanced mitochondrial respiration during exercise generates oxygen radicals as a by-product of oxidative metabolism (Chance, Sies, & Boveris, 1979; Davies et al., 1982), and the possible risk associated with this has received little consideration.

The electron transport system in mitochondria is a major source of free radical production. Although aerobic cells contain enzymatic and nonenzymatic scavenger systems against oxygen free radical production, it has not been determined whether such scavenger systems can totally protect cells. For instance, periods of high oxygen consumption, as occurs in the endurance trained athlete during strenuous exercise, may provide an oxidative stress sufficient to overwhelm the protective mechanisms.

Lovelin, Cottle, Pyke, Kavanagh, and Belcastro (1987) suggested that, during exercise, the redistribution of blood flow to working skeletal muscles may leave some tissues hypoxic and possibly more susceptible to lipid peroxidation. Lovelin et al. also conjectured that mitochondrial swelling, edema formation, proteinuria, and hemolysis seen following exercise might be due to exercise-induced free radical

generation. They pointed out that certain factors, such as glucose depletion and disturbances in the reduction/oxidation capability of a cell, may affect free radical generation and lipid peroxidation. A number of free radical scavenging enzymes require NADH and/or NADPH as co-factors for activity. Thus, the stress of exercise, by causing substrate depletion and modification of the NADH:NADPH ratio may affect free radical production and lipid peroxidation by altering the co-factor availability for free radical scavenging enzyme function.

The information regarding the relationship of exercise and free radical production is somewhat limited at the moment. There are far more animal than human studies due to the difficulty in detection of free radicals in living systems. Animal studies have provided data relative to the influence of endurance training on radical production in various tissues and organs, such as the liver (Davies et al., 1982; Suzuki et al., 1983), skeletal muscle (Davies et al., 1982; Salminen & Vihko, 1983), brain (Suzuki et al., 1983), heart (Suzuki et al., 1983), lungs (Quintanilha, 1984), and urine (Marnett et al., 1985). Human studies have been limited to the analysis of breath (Balke, Snider, & Bull, 1984; Dillard et al., 1978) and blood (Kanter,

Kaminsky, La Ham-Saeger, Lesmes, & Nequin, 1986a, 1986b; Lovelin et al., 1987; Ohno, Sata, Yamashita, Doi, Arai, Kondo, & Taniguchi, 1986).

Perhaps the first study to test for the oxidation of unsaturated fatty acids and the formation of peroxides was by Wilbur et al. (1949). They found a TBAR test sensitive to changes produced in certain unsaturated fatty acids and, when TBAR was combined with human skin and sweat samples, a color value was detected. No further studies in relation to human samples were reported until 1978 when Dillard et al. looked at the amount of pentane in expired air as an index of lipid peroxidation and found an increase during 1 hr of submaximal oxygen consumption (50% $\dot{V}O_2max$) exercise. However, pentane increase was significantly reduced by Vitamin E. It was concluded that exercise induced lipid peroxidation in humans, but Vitamin E administration reduced lipid peroxidation.

In 1979, Brady et al. did an exercise study using rats and reported an increase in lipid peroxidation in the liver and muscle subsequent to swimming exercise. The lipid peroxidation increase was reduced in liver by Vitamin E supplementation in the deficient group. Brady et al. explained that Vitamin E is imbedded in cell membranes and scavenges free radicals, thus helping to

protect the membrane against lipid peroxidation. Similarly, Davies et al. (1982), using an electron spin resonance method, found a proportional two- to three-fold increase in free radical concentration of the muscle and liver of rats following exhaustive exercise on the treadmill. Exercising the rats to exhaustion also showed increased lipid peroxidation and free radical production. A study by Suzuki et al. (1983), investigated voluntary wheel exercise in rats fed either diets high or low in rancid oil. They found that exercise reduced MDA accumulation in most tissues, but an increased accumulation of lipid peroxidation was noted in the excised brains of rats. It was speculated that lipid peroxidation may transfer from one tissue to another. It was interesting to note that more than 80% of the total lipid peroxidation in the rat's whole body was distributed in carcass portions including subcutaneous adipose tissue, skeletal muscle, and skin. The remaining lipid peroxidation was found in blood plasma and other major organs. It was shown that MDA was not localized in any one specific area.

Salminen & Vihko (1983) studied peroxidation in skeletal muscle of endurance trained mice and found a significant difference in the rate of peroxidation between red and white skeletal muscle. The red skeletal

muscle contained more peroxidizable lipids than white muscle, suggesting that greater lipid peroxidation might be due to the higher content of mitochondria, which contains phospholipids and unsaturated fatty acids. It was also reported that endurance training decreased the lipid peroxidation rate in both muscle types. The lower lipid peroxidation rate might have been related to higher concentrations of Vitamin E in microsomal membranes, which regulate the susceptibility of cell membranes to lipid peroxidation.

As research continued, Marnett et al. (1985) investigated the distribution and oxidation of MDA in mice that were injected with MDA. It was reported that a majority of the metabolic pool of MDA was converted to carbon dioxide pathways, and MDA was also detected in urine. After the animals were sacrificed, TBAR were found in the tissues and organs. The authors suggested that MDA is rapidly and completely incorporated into the pathways of intermediary metabolism.

There have been only a few human studies related to the area of exercise and free radical chemistry. For example, a study by Balke et al. (1984) analyzed expired respiratory gas collected 60 and 100 min following 20 min of submaximal (50% $\dot{V}O_2max$) leg cycling. Significant increases in ethane, a hydrocarbon gas related to lipid

peroxidation, were found.

Gutteridge et al. (1985) determined the levels of copper and iron complexes in the sweat of athletes immediately after exercise. Iron and copper are known to increase both hydroxyl radical formation and lipid peroxidation. Gutteridge et al. demonstrated that copper and iron complexes found in human sweat were capable of stimulating lipid peroxidation but speculated that excretion of such metals in sweat may be a defense mechanism to reduce lipid peroxidation. In a subsequent study by Halliwell & Gutteridge (1986), they determined that iron and copper complexes mediate damage by oxygen radicals.

Kanter et al. (1986a, 1986b) studied TBAR levels in blood of ultramarathon runners and found a significant correlation ($p < .05$) between TBAR and elevated serum enzymes. It was suggested that lipid peroxidation, from which TBAR are derived, might be linked to muscle tissue damage occurring after long distance running.

More recently, Lovelin et al. (1987) examined the plasma TBAR levels in humans after various exercise intensities. A 26% increase in plasma lipid peroxides was found following bicycle exercise to voluntary exhaustion, while plasma MDA levels had decreased below resting levels after 5 min incremental exercise bouts of

40% and 70% $\dot{V}O_2max$ with a 5 min rest between each workload. The results of Lovelin et al. suggest that exhaustive maximal exercise induced free radical generation, while short periods of submaximal exercise might have inhibited free radical generation and lipid peroxidation.

In general, there is substantial literature derived from animal studies that imply oxygen radical generation is increased by exercise, although human studies have been limited. However, both animal and human research have consistently demonstrated that exercise may alter oxygen radical production and result in lipid peroxidation.

Eccrine Glands

The human eccrine sweat gland is a dermal exocrine gland involved in thermoregulation. It is a single tubule consisting of two parts: a secretory component, which produces the primary fluid secretion, and a ductal component, which modifies the secretion, reabsorbs sodium chloride, and conducts the fluid to the skin surface (Schultz, 1969). In the skin, the epidermis must be supplied with water originating from skin blood vessels to allow evaporation from the dry skin surface to occur (Kuno, 1956). Therefore, heat is lost through sweating only when sweat evaporates. According to Kuno,

there is an estimated 2.38 million sweat glands contained in the exposed areas of human skin. However, there are considerable individual differences in the patterns of sweat distribution. Regionally, the forehead, neck, some larger areas of the anterior and posterior surfaces of the trunk, and back of the hand sweat the most. The sides of the chest and the extremities produce relatively less sweat. Nielsen (1969) found the fully developed, steady state sweat response is not seen until 30 to 40 min after the start of exercise, while Ayling (1986) reported that the initial sweating response to exercise occurs within 1.5 s in a warm environment (37.5 °C). Sweating during exercise is thought to be controlled by a combination of thermal and sympathetic nervous system activity.

Verde, Shephard, Corey, and Moore (1982) have pointed out that there is some disagreement about the composition of human sweat. Factors influencing sweat composition include sweat inducing mechanisms (i.e., exercise or heat exposure), duration of sweat, the rate of sweat secretion, and the method of sampling. It is known, however, that sweat is about 99% water. The solid component only makes up approximately 0.3 to 0.8% of the total sweat. Various studies (Kuno, 1956; Pilardeau, Vaysse, Garnier, Joublin, and Valeri, 1979;

Robinson & Robinson, 1954) have analyzed sweat composition and found that the solid component contains sodium chloride, magnesium, potassium, vitamins, lactic acid, iron, and copper.

Appearance of Products of Oxygen Radical Chemistry in Sweat

In an attempt to gather information about free radical generation in humans, research still remains in the developmental stage primarily due to the difficulty in detecting lipid peroxidation in humans (animal studies have used excised organs for research). However, it has been well established that MDA, a product of lipid peroxidation, is uniformly metabolized throughout the living system when exogenously administered (Marnett et al., 1985), and in human research TBAR have been detected in blood plasma of exercised humans (Lovelin et al., 1987).

Currently, there is no research directly linking TBAR with exercise sweat, although metals that stimulate lipid peroxidation have been found in sweat. For instance, iron and copper, which are capable of stimulating lipid peroxidation, have been found (Gutteridge, 1984). In a recent study by Campbell and Anderson (1987), the effects of aerobic exercise and training on specific trace minerals including copper was

investigated. Although the study was not directly related to sweat, the fact that serum copper levels increased with exercise could lead to the speculation that copper levels in sweat may correlate with blood production and possibly be available to stimulate lipid peroxidation. Human sweat contains elements originating from blood plasma, some of which include iron and copper complexes, and both metals may increase lipid peroxidation (Gutteridge et al., 1985). However, the only human study relative to sweat composition and lipid peroxidation was by Gutteridge et al. Although TBAR were not measured directly in sweat collected from athletes in that study, they did report that sweat stimulated peroxidation of lipid vessicles incubated with such sweat.

Summary

With the increasing popularity of aerobic exercise in today's society, a concern about the long-term effects of increased oxygen radicals in exercising humans has begun to develop. Although the human body possesses numerous biochemical mechanisms to enable it to survive in the oxygenated atmosphere in which we live, there is a possibility that under certain circumstances oxygen may be harmful to the body. Free radicals generated in the body have been shown to be

involved in such harmful reactions as post ischemic damage, cancer, degenerative aging factors, oxygen toxicity, and ultraviolet radiation injury (Del Maestro, 1980). It is necessary for the body to protect itself from these harmful reactions. Research has provided evidence of lipid peroxidation in blood plasma (Kanter et al., 1986a, 1986b; Lovelin et al., 1987), tissues (Gutteridge et al., 1985; Wilbur et al., 1949), and expired air (Balke et al., 1984; Dillard et al., 1978) subsequent to aerobic exercise. The more advanced work involving endurance training and diet control of oxygen radical chemistry on animals has enabled researchers to study similar and additional tissues of the whole body. The thermoregulatory system may play a role in the protection against oxidant stress. Sweating may be a natural excretion mechanism to rid the body of the by-products of lipid peroxidation that otherwise might diffuse through the blood and result in tissue damage away from the original site of production.

Chapter 3

METHODS AND PROCEDURES

This chapter outlines the methods and procedures used in this study. Specifically, this chapter deals with (a) selection of subjects, (b) testing procedures and instrumentation, and (c) treatment of data.

Selection of Subjects

This study was conducted in the spring of 1987. Subjects were recruited using an announcement to classes in the School of Health, Physical Education, and Recreation (Appendix A) at Ithaca College and 13 males, ranging in age from 18 to 22 yr volunteered to participate. Each subject filled out a medical history questionnaire (Appendix B) and also read and signed an informed consent form describing the test procedures (Appendix C). If a candidate could be classified as apparently healthy and had no contraindications for graded exercise testing, as outlined by the American College of Sports Medicine (1986), he was allowed to participate.

Testing Procedures and Instrumentation

All subjects first completed and followed the same testing format for the graded exercise test. However, the order for performance of the two remaining data collection sessions was randomly assigned (i.e.,

submaximal exercise and whirlpool bath). Standard directions were given for each test with an opportunity for further explanation if requested. Each of the procedures used during this study are described in more detail in the following sections.

Graded Exercise Test

Aerobic capacity (peak $\dot{V}O_2$) was assessed by cycling to exhaustion on a Jaeger cycle ergometer (Model ER 40). Each subject was weighed and familiarized with the cycling procedure prior to the actual testing session. Subjects were seated on the cycle while the seat height adjustment was made enabling the subject to engage nearly full leg extension during pedaling. The subject was fitted with a Daniels breathing valve, supporting headgear, and a noseclip to prevent air leakage. Respiratory gas data were derived from an Applied Electrochemistry SA-3 oxygen analyzer, a Beckman LB-2 carbon dioxide analyzer, and a Rayfield gas meter providing input to an Apple IIe computer which determined the following printed data: ventilatory volume (L/min), corrected $\dot{V}O_2$ (mL/kg/min), VCO_2 (L/min), and respiratory quotient. Subjects sat for 3 min breathing into the breathing valve while the computer recorded initial resting values. The exercise protocol began at a workload of 50 W, and increased 50 W every

2 min until 200 W was reached. Thereafter, workloads increased 25 W for each 2 min stage. The pedal speed was set at 50 revolutions per min and a metronome was utilized to assist subjects in maintaining a steady pace. Following the graded exercise test, subjects rested until heart rate returned to near resting levels before being permitted to leave the laboratory.

Submaximal Exercise Trial

Sweat was collected during exercise while a subject cycled at 65% peak $\dot{V}O_2$ as determined from the graded exercise test. The ambient room condition during testing remained at 22 °C with the humidity of 48%. To collect sweat, a clean towel was placed around the shoulders of each subject and a wire frame was placed over the towel. Next, an alcohol pad was used to wipe off the site areas from which sweat was to be collected. A clean plastic poncho was then draped over the subject's shoulders. The exercise protocol consisted of a 10 min warm-up period cycling at 50 W, followed by 15 to 20 min at 65% peak $\dot{V}O_2$, and a 5 min active recovery period at 50 W. Pedal speed was set at 50 revolutions per min. Subjects cycled for 20 min before sweat was sampled to ensure that any pre-exercise sweat was flushed from the body. Between 20 and 30 min into exercise, a 0.5 to 1.0 mL sweat sample was taken from

the upper arm and a 0.5 to 1.0 mL sample from the back of each subject, whereby each sample was placed into separate test tubes. Sweat was collected by absorbing samples into sterile gauze pads and squeezing the sample into the collection tube. Surgical gloves were worn to prevent contamination of the sample. The sweat samples were frozen up to three weeks at -80 °C until subsequent analysis.

Whirlpool (Control) Trial

Sweat was collected under a nonexercise, sedentary condition while subjects sat in a warm bath (40.5 °C). Each subject was familiarized with the test. A clean towel was placed around the subject's shoulders. An alcohol pad was used to wipe off the site areas for sweat collection. A clean plastic poncho was draped over each subject's shoulders, and then around the tank as the subject was seated in the tank. Subjects sat in the bath with the water level waist high for 15 to 20 min. Following sample collection, subjects were helped out of the bath and seated for a cool down period before being permitted to leave the room. The sweat samples were collected between 10 and 20 min of testing. Sweat samples equal to 0.5 to 1.0 mL from the arm and the back of each subject were placed into separate test tubes. Collection and storage of samples was done as described

in the exercise condition.

TBAR Assay

When malondialdehyde (MDA) is combined with thiobarbituric acid (TBA), a reddish-orange color is produced. MDA was determined by the procedure of OhKana, Ohishi, and Yagi (1986). A 1% solution of TBA was made by mixing 100 mg of TBA to 10 mL of distilled water in a beaker. This solution was warmed and stirred until the TBA dissolved. A 0.2 mL aliquot of each sweat sample was placed into separate 15 mL centrifuge tubes. Two hundred μL of sodium dodecyl sulfate (SDS), a detergent which frees MDA from bound substances was added. Next, 1.5 mL of a 20% acetic acid solution (pH 3.5) was added and mixed. This solution was heated for 1 hr, followed by cooling to room temperature. Five mL of n-butanol were then added and the solution was vortexed thoroughly. The TBA-MDA complex was extracted into the butanol layer by shaking. The tubes were then centrifuged for 10 min. The amount of thiobarbituric reaction products (TBAR) in the samples was quantified by placing aliquots in fluorometric cuvettes and reading fluorescence in an LS-5 Spectroluminometer set at 515 nm excitation and 553 nm emission. Three aliquots were fluorometrically read from each sample with the average value used in data analysis. These readings were then

converted into nmol/L by reference to a standard curve which was established using tetraethoxypropane as the source of TBAR (Kwon & Watts, 1963).

Treatment of Data

A univariate 2 x 2 (Condition x Site) ANOVA was used to identify differences that may have existed in TBAR values between bike and whirlpool conditions and between arm and back sites. Additionally, multiple regression analysis was used to compare peak $\dot{V}O_2$ with exercise TBAR at the back and arm sites. The reliability of the three measurements for each sample was determined by a treatment by subject two-way ANOVA on the three measurements from each sample, and statistical significance was tested at the .05 level.

Chapter 4

ANALYSIS OF DATA

This study was conducted to investigate first, if malondialdehyde (MDA) was detectable in human sweat, as evidenced by thiobarbituric reaction products (TBAR) and second, if TBAR were present, to examine any significant differences between the selected variables of conditions and sites. Data collection and the statistical analysis of these data are described in this chapter. Sections in this chapter include the following: (a) description of subjects, (b) reliability of TBAR assay, (c) multiple regression analysis, and (d) analysis of variance (ANOVA).

Description of Subjects

Subjects' physical characteristics are reported in Table 1. The subjects' aged from 18 to 22 yr and their weights were 62 to 130 kg. Peak $\dot{V}O_2$, as determined by the graded exercise test, were between 28.2 mL/kg/min and 60.0 mL/kg/min. Subjects' workload (65% peak $\dot{V}O_2$) during the exercise session ranged from 150 W to 225 W. These values indicated a wide range of weights and fitness levels within subjects.

Reliability of TBAR Assay

A treatment by subject two-way ANOVA was used to investigate the reliability of three TBAR values

Table 1

Physical Characteristics of Subjects

Subject	Age (yr)	Weight (kg)	Peak $\dot{V}O_2$ (mL/kg/min)	GXT Peak Workload (W)	65% Peak Workload (W)
1	22	107	31.4	225	150
2	19	62	53.1	225	175
3	19	130	28.2	225	150
4	18	63	60.0	250	200
5	19	111	40.9	275	200
6	22	84	44.0	275	200
7	19	107	29.8	225	150
8	18	100	33.4	225	175
9	20	73	52.9	275	200
10	19	80	54.6	275	225
11	21	83	47.3	250	200
12	19	85	40.4	225	150
13	18	113	33.1	275	200
M	19.5	92.2	42.3	248.0	182.7
SD	1.4	20.8	10.7	23.9	25.8

obtained from aliquots of the same sample. These were conducted for each separate condition (i.e., whirlpool arm TBAR, whirlpool back TBAR, bike arm TBAR, and bike back TBAR), then an intraclass correlation (R) was calculated by the formula:
R = MSSubjects - MSInteraction/MSSubjects. The values from this intraclass correlation were combined to assess the reliability of the assay technique. The mean R was equal to .980, with Rs ranging from .968 to .996. These results indicated an acceptable reliability of the assay technique to determine TBAR values.

Multiple Regression Analysis

A multiple regression analysis was conducted to identify any significant relationship of peak $\dot{V}O2$ with arm and back exercise TBAR. However, results showed $R2$ was equal to .12, and no significance was found ($p < .05$). Therefore, it does not appear there was any significant relationship between peak oxygen consumption values and TBAR values.

Analysis of Variance (ANOVA)

The dependent variable used for this analysis was TBAR, measured at two sites and under two conditions to determine if a statistically significant difference existed between or within conditions and sites. As reported in Table 2, results of this analysis showed a

Table 2

ANOVA Summary Table

Source of Variation	SS	df	MS	F
Between Subjects	239378.31	12	19948.19	1262.33*
Within Subjects				
Condition	98223.08	1	98223.08	2.78
Subject within Condition	423585.92	12	35298.83	
Site	53632.69	1	53632.69	3.37
Subject within Site	191076.31	12	15923.03	
Condition-by-Site	173538.77	1	173538.77	6.38*
Subject within Condition-by-Site	326520.23	12	27210.02	
Total	1505955.31	51		

* $p < .05$.

significant condition-by-site interaction, $F(1, 12) = 6.38$, $p < .05$ (Figure 1). Because the condition-by-site interaction was significant, the main effects of condition and site cannot be interpreted. Therefore, a simple main effects test using repeated measures t tests to specifically determine how the conditions statistically differed at each site was performed. As shown in Table 3, the results of the simple main effects indicated a significant difference ($t(12) = 4.77$, $p < .05$) was found between bike and whirlpool TBAR values at the arm site. However, no difference was found for these conditions at the back site. Furthermore, no differences were found between the arm and back sites within either the exercise or the nonexercise condition. Because TBAR was detected in all sweat samples, the null hypothesis was rejected, and it was concluded that TBAR could be detected in sweat. Since the main effects of the ANOVA test, which would indicate differences between conditions and sites, were not tested due to an interaction found, the null hypotheses Ha1 and Ha2 were uninterpretable.

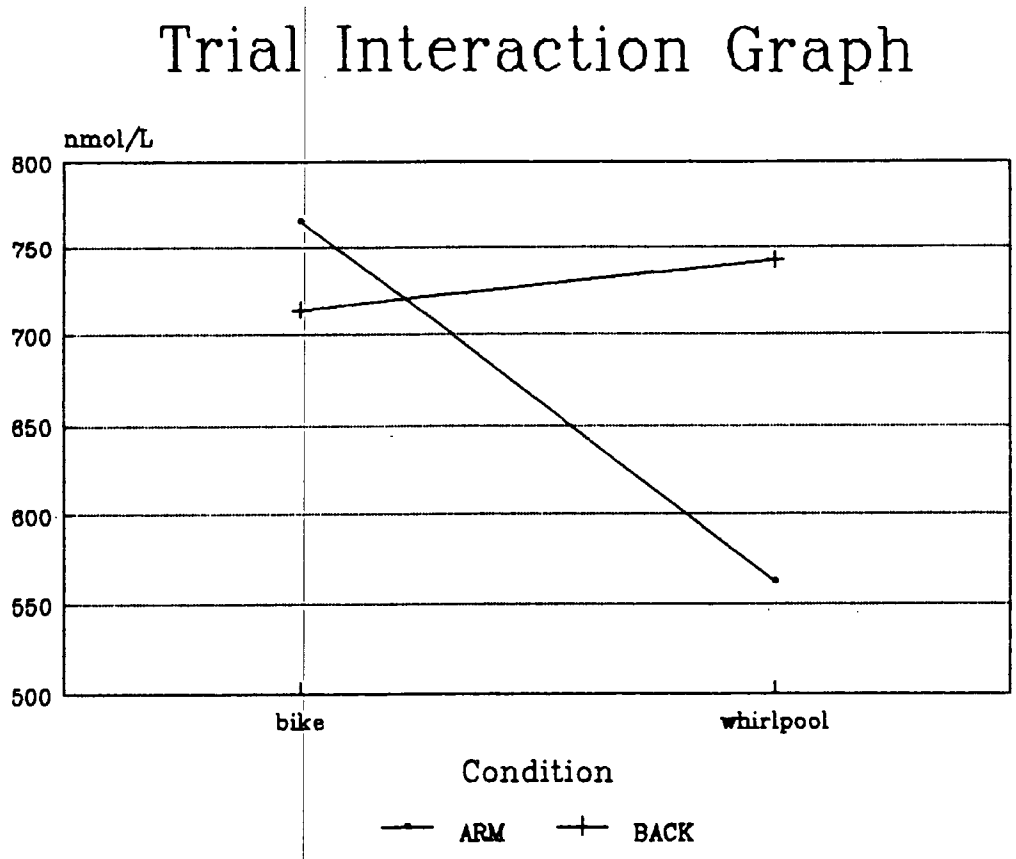

Figure 1. Difference between arm and back TBAR at bike and whirlpool conditions.

Table 3

Simple Main Effects Following Condition-by-Site Interaction

	Site
	Arm
Condition (N = 13)	
Whirlpool	562.5 ± 25.4*
Bike	765.0 ± 34.0*
Whirlpool and Bike	663.8 ± 20.7
	Back
Condition (N = 13)	
Whirlpool	742.3 ± 43.1
Bike	713.7 ± 30.8
Whirlpool and Bike	728.0 ± 30.8
	Arm and Back
Condition (N = 13)	
Whirlpool	652.4 ± 30.4
Bike	739.3 ± 34.6
Whirlpool and Bike	

Note. All variables are M ± SD for TBAR in nmol/L.

* $p < .05$, between whirlpool and bike conditions.

Chapter 5

DISCUSSION OF RESULTS

The results of this study show that thiobarbituric reaction products (TBAR), a biochemical marker for malondialdehyde (MDA), can be detected in sweat. The ability to detect TBAR in sweat allowed differences in TBAR under exercise and nonexercise conditions to be studied. A discussion of the results is contained in this chapter under the following sections: (a) TBAR in various tissues, (b) influence of exercise on TBAR levels, and (c) importance of the sweating mechanism to TBAR clearance.

TBAR in Various Tissues

Prior to this investigation, evidence that lipid peroxidation occurred in exercising humans was presented by Dillard et al. (1978), who found ethane and pentane, products of lipid peroxidation, in expired gas. However, blood was the only human tissue or fluid where evidence of TBAR, specifically, MDA, had been detected in relation to exercise (Kanter et al., 1986a, 1986b; Lovelin et al., 1987). The present study found that TBAR were detectable in all sweat samples, regardless of the condition under which sweat was obtained. All samples were centrifuged to remove any cell debris, which may have been collected from the skin. Therefore,

it is unlikely that TBAR resulted from peroxidation skin contaminants in the sweat. These data demonstrate that it is reasonable to evaluate lipid peroxidation in sweat, as well as the more costly expensive gas chromatographic methods for expired air and more invasive methods for blood.

Influence of Exercise on TBAR Levels

Several investigators have shown that elevated oxygen consumption related to exercise may lead to increased lipid peroxidation (Davies et al., 1982; Dillard et al., 1978; Salminen & Vihko, 1983). In this study, a higher TBAR value was found in exercise sweat than nonexercise sweat at the arm site only. This finding may have resulted from lipid peroxidation during exercise or, exercise may have produced a washout of TBAR substance already present. The latter explanation is unlikely since TBAR values in resting humans are lower than detection limits.

In this study, a multiple regression analysis was conducted to determine the relationship of peak $\dot{V}O_2$ to arm and back TBAR levels in exercise sweat and no substantial correlation was found. First, this indicates there may not be any correlation between chronic exercise and sweat TBAR, however, it may have reflected the fact that peak $\dot{V}O_2$ was not determined

during the sample collection session, but rather on a different day. Perhaps such factors as diet or previous daily activity, which were not controlled for in the present study, could alter the relationship between aerobic capacity and TBAR levels in exercise sweat.

While Kanter et al. (1986a) reported exercise related increases in blood lipid peroxidation, their subjects had engaged in marathon events. Different work and fitness levels were involved and, thus, no direct comparison to this study is possible. Lovelin et al. (1987) investigated the levels of MDA in the plasma of humans during exercise at both submaximal and maximal levels of oxygen consumption. They detected MDA in the plasma after exhaustive exercise but not after submaximal exercise. The data of the present study appear to be in conflict with those of Lovelin et al. since evidence of lipid peroxidation (TBAR) was detected in sweat under submaximal exercise. The Lovelin et al. finding of no rise in TBAR at submaximal exercise may be explained by adequate removal of TBAR into sweat. Perhaps a plasma rise is not observed until the total production exceeds the system's ability to remove TBAR. For a better comparison with the work of Lovelin et al., it would have been interesting to have measured blood TBAR in the present study, however this was not done.

Importance of the Sweating Mechanism to TBAR Clearance

It has already been shown that products of lipid peroxidation are released from the body in the expired air of exercising individuals (Dillard et al., 1978). It has also been established that TBAR, end products of lipid peroxidation, are present in human blood plasma (Marnett et al., 1985). It is important to know what happens to plasma TBAR once they are generated. Is there a way for the body to clear TBAR in order to prevent further harmful reactions from occurring? Unfortunately, the difficulty in answering that question in humans is due, in part, to the difficulty in studying internal organs and tissues as in animal studies. Therefore, research is primarily limited to the study of lipid peroxidation products in samples of respiratory gas, blood, and sweat. The latter is the only substance that is easily obtained by non-invasive means and requires no elaborate equipment for analysis. When an individual sweats, blood is shunted to the skin surface and sweat, as a filtrate of blood, is released through the pores to cool down the body. Substances present in the blood, that can cross membranes may appear in sweat. The loss of TBAR in sweat may serve as a protective mechanism to remove the undesired lipid peroxidation end products. Thus, the thermoregulatory system may assist

in the protection of the body from any potential harm from lipid peroxidation products produced as a result of an exercise induced increase in oxygen consumption.

This study found detectable amounts of TBAR in sweat, but perhaps most interesting was the significant difference within subjects between the exercise and nonexercise conditions at the arm site only. Since it has been found that loosely bound iron can activate oxygen to the radical species and stimulate lipid peroxidation (Halliwell & Gutteridge, 1986), it may be that the higher level of TBAR found at the arm site was due to a greater iron content. However, in an unpublished investigation done to determine the presence of iron in the same sweat samples from this study, greater bleomysin-detectable iron was found at the back than arm site (Jenkins, 1987). This finding agrees with Gutteridge et al. (1985) who also found greater values of loosely bound iron at the back than the arm site. Therefore, iron content does not explain the difference of TBAR at the arm and back sites. If a higher level of iron had been present at the arm site, than it would be possible that the TBAR originated on the skin surface itself. However, since the iron content was not higher at the arm site, TBAR was most likely formed elsewhere and excreted via sweating.

Other possible explanations for the differences between arm and back sweat include the possibility that a variation in the rate of sweat secretion occurred at the different sites. No attempt to determine sweating rate by site was made. There was also no attempt to determine sweat rate by condition and it is possible though unlikely, that condensation of whirlpool water on the plastic poncho resulted in a dilution of some samples. A final explanation for the present results may involve an unknown eccrine site specific mechanism for TBAR elimination. Similar to the suggestions of Halliwell and Gutteridge (1986), that OH radical formation in vivo is site specific to areas where free metal stimulation is available, other substances (i.e., Na+, K+, Cl-, glucose, lactate) are secreted by eccrine glands in a site specific manner (Pilardeau et al., 1979). The results of the present study may indicate that the body releases more TBAR in arm than back sweat during exercise.

Summary

In summary, evidence of increased lipid peroxidation has been found in the expired air of exercising humans (Dillard et al., 1978). Blood was the only human tissue or fluid where TBAR was detected with regard to exercise (Kanter et al., 1986a, 1986b; Lovelin

et al., 1987). The present study found TBAR in sweat under both exercise and nonexercise conditions.

Various exercise levels have been studied to determine the occurrence of lipid peroxidation in humans. Research indicates consistently that exhaustive exercise increases lipid peroxidation; however, little research has been done on the effect of training on lipid peroxidation, and the effects of submaximal exercise on TBAR levels remains unclear. In this study, a comparison of the submaximal exercise and nonexercise conditions could not be interpreted due to an interaction found. A higher level of TBAR was found between the two conditions at the arm site only. Possible explanations for this interaction include the body releasing more TBAR at the arm site, the rate of sweat production, or secretion elements in the sweat glands.

The results of this study do show the possibility that, as lipid peroxidation occurs in individuals consuming oxygen at high levels during exercise, the body removes potentially harmful TBAR through sweat.

Chapter 6

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

This study was undertaken to determine if thiobarbituric reaction products (TBAR), a biochemical marker for malondialdehyde (MDA) were released through exercise or heat induced sweat. The subjects were 13 undergraduate male students from Ithaca College.

The study involved three testing sessions for each subject. First, a pretest on a cycle ergometer was executed to obtain a peak $\dot{V}O2$ in order to determine a relative workload for the exercise condition. The second and third tests consisted of a bike exercise and a whirlpool bath session, respectively, during which sweat samples were collected. The samples were then analyzed for TBAR and an ANOVA was used to analyze the reliability of the three TBAR values for each sample from the subject. The results of these tests indicated an acceptable reliability for the TBAR analysis (R = .980). A second ANOVA was computed to determine whether a significant difference in TBAR concentration existed between the two conditions or at the two sites. Further post hoc testing was conducted using a simple main effects test. Results revealed a significant difference within subjects in the bike and whirlpool

conditions at the arm site ($p < .05$). A multiple regression analysis was performed to identify any significant relationship of peak $\dot{V}O2$ with arm and back exercise TBAR. However, no significant relationship was demonstrated.

Conclusions

The results of this study yielded the following conclusions regarding TBAR in sweat and the effect of exercise and nonexercise conditions upon TBAR concentration in sweat:

1. TBAR can be measured in sweat and may serve as an indicator of lipid peroxidation occurring in the body.

2. Significantly higher values of TBAR were found in the exercise condition than in the nonexercise condition at the arm site only.

Recommendations

The following recommendations for further study were made after completion of this investigation:

1. Further study should be conducted in the area of free radical chemistry and the exercising human. TBAR can be detected in blood and sweat. The determination of the origin of TBAR production is necessary as is the examination of the relationship of TBAR release and oxygen consumption. It would certainly

be interesting to measure TBAR in the sweat and blood simultaneously.

2. This study used submaximal bike testing with a work rate set at 65% $\dot{V}O_2$ when sweat samples were collected. A repeat of this data collection during a graded exercise test could help determine if TBAR varies with exercise intensity.

3. The effect of variables such as time of day of testing, diet, and monitoring daily activity and sleep patterns could have an effect on TBAR levels found in sweat, and this should be investigated.

4. The measurement of free metal complexes, particularly iron and copper in sweat along with TBAR levels might provide more insight into the body's adaptation to exercise as it relates to lipid peroxidation.

5. A training study should be conducted to determine how TBAR might vary as an individual increases aerobic capacity.

Appendix A

RECRUITING CONVERSATION

OUTLINE EXAMPLE

My name is Melissa Tompkins. I am a graduate student working on my Masters Degree in Physical Education at Ithaca College. I am conducting a research project related to oxygen use and exercise. It is now known that harmful reaction products occur when the body consumes oxygen. These reaction products result in the formation of a compound called malondialdehyde that is potentially harmful. My research project is designed to determine whether exercise increases the production of this compound and whether sweat may be a vehicle by which the body excretes the compound.

I am seeking male volunteers that have not actively engaged in a regular exercise program or a sport program within the last 6 months requiring aerobic endurance to participate in the study. Your time involvement will be 3-5 hr and will require exercise to allow me to record your oxygen consumption ability, sitting in a hot whirlpool, and exercising on a bicycle to stimulate sweating.

If you are interested in participating in this study, or would like any further information, would you please write your name and phone number down and I shall

contact you.

Appendix B

INFORMED CONSENT FORM

1. a) Purpose of the study. To determine whether exercise increases the production of a chemical compound (malondialdehyde) in sweat. Malondialdehyde is a by-product of a series of chemical reactions involving oxygen. If this compound remains in the body it could produce harmful effects. This study will determine whether sweat may be a way of the body excreting the compound and thus decrease the chance of harm to the body.

   b) Benefits. You will gain knowledge about oxygen use as it relates to exercise and the importance of the sweating mechanism.

2. Method. You will be asked to participate either on 3 or 5 nonconsecutive days during the month of March. The testing days will be arranged at mutually convenient times on weekdays from 8:00-5:00. Each of the testing days will require approximately 1 hr of your time. You will be asked to abstain from consuming any caffeine, tobacco, alcohol, or drugs for at least 3 hr prior to your testing times. Testing sites will be on campus at the Exercise Physiology lab in Hill Center and the

Physical Therapy clinic in Smiddy Hall. Testing procedures will be as follows:

Day 1

You will be tested for maximum effort oxygen consumption ability (peak $\dot{V}O2$) which requires strenuous exercise on a cycle ergometer for approximately 20-30 min. You will breathe into an air valve which will record the amount of air inspired and expired to determine peak $\dot{V}O2$. You will begin by simply sitting, then pedal at a steady pace while the workload increases. At the point where you feel fatigued and want to stop, you will be slowed down to a stop while you sit and continue breathing into the air tube. This test will take place in the Exercise Physiology lab. Approximately 1 hr of your time is needed for explanation and testing.

Day 2 and 3

You will be selected to participate in one of these tests on Day 2, and will participate in the other test on Day 3. On each of these testing days you will be asked to shower without soap prior to coming in for the test.

Nonexercise test

You will sit on an elevated seat in a hot whirlpool

bath of 40.5 °C up to the navel for approximately 20 min in the Physical Therapy clinic. A plastic poncho will be draped over your shoulders. A 1 mL sweat sample will be collected from your arm and back using sterile gauze pads to absorb sweat.

Exercise test

You will exercise on a stationary bike in the Exercise Physiology lab for approximately 30 min. The work load will be determined at 65% peak $\dot{V}O_2$. The room temperature will be 22 °C in normal humidity. A plastic poncho will be draped over your shoulders, and a 1 mL sweat sample will be collected as in the nonexercise condition.

Retest

Half of the subjects will be asked to repeat Day 2 and Day 3 testing conditions on two other nonconsecutive days to determine test reliability.

3. Will this hurt? Exercise at moderate and heavy work loads is required. Extreme risk of physical harm does not exist; however, there is evidence which indicates that certain body changes may occur during the cycle testing. These include abnormal blood pressure, fainting, abnormal heart beat, or in extremely rare cases, heart attack. The other risk is the possibility of an adverse reaction from heat

while sitting in a hot whirlpool both. These symptoms include dizziness, nausea or extreme fatigue. Precautions will be taken by closely monitoring how you are feeling during the test. Testing will be stopped should you feel any of these symptoms arise. The most likely negative effects of participation in this study are some minor discomfort or temporary muscle soreness.

4. Need more information? Additional information can be obtained from either Melissa Tompkins (753-9933) or Dr. Robert Jenkins (274-3974) or Dr. Gary Sforzo (274-3359). All questions are welcomed and will be answered.

5. Withdrawal from the Study. Participation is voluntary. You are free to withdraw your consent and discontinue at any time. In addition, if you are unable to tolerate either the exercise or whirlpool conditions, you may withdraw from the study.

6. Will the data be maintained in confidence? You will be identified throughout the testing procedure by subject number, (e.g., Subject 10) to ensure confidentiality of information collected. Subsequent data will only be reported in an anonymous fashion.

7. I have read the above and I understand its contents and I agree to participate in the study. I further agree to accurately complete a medical history questionnaire prior to participation. I am 18 years of age or older and do not know of any physical restrictions that should preclude my participation in this project.

_____ _____

Signature Date

Appendix C

HEALTH QUESTIONNAIRE

A. Have you ever experienced any of the following:

	YES	NO
1. Heart attack, coronary bypass, or other cardiac surgery	_____	_____
2. Chest discomfort--especially with exertion	_____	_____
3. High blood pressure	_____	_____
4. Extra, skipped, or rapid heart beat/palpitations	_____	_____
5. Heart murmur or unusual cardiac findings	_____	_____
6. Rheumatic fever	_____	_____
7. Ankle swelling	_____	_____
8. Peripheral vascular disease	_____	_____
9. Phlebitis, emboli	_____	_____
10. Unusual shortness of breath	_____	_____
11. Lightheadedness or fainting	_____	_____
12. Pulmonary disease including asthma, emphysema and bronchitis	_____	_____
13. Abnormal blood lipids	_____	_____
14. Diabetes	_____	_____
15. Stroke	_____	_____

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16. Emotional disorders _____ _____

17. Drug allergies _____ _____

18. Orthopedic problems, arthritis _____ _____

19. Unusual eating or sleeping behaviors _____ _____

B. Do you have any immediate family history of:

1. Coronary disease _____ _____

2. Sudden death _____ _____

3. Congenital heart disease _____ _____

C. Have you:

1. been on any medications in the last month? _____ _____

2. been ill, hospitalized or had any surgery within the last month? _____ _____

3. Have you ever experienced an adverse reaction (e.g., nausea, fainting, etc.) to high temperatures or humidity while exercising? _____ _____

4. Have you ever experienced an adverse reaction (e.g., nausea, fainting, etc.) from a hot whirlpool bath? _____ _____

D. Do you participate in a sport program or a regular exercise program?

If yes, what type of exercise _____

frequency of exercise _____

duration of exercise _____

intensity of exercise _____

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