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## Oxidative Stress for an Acute Bout of Bikram Yoga in Healthy, Trained Adults

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OXIDATIVE STRESS FOR AN ACUTE BOUT OF BIKRAM YOGA IN  
HEALTHY, TRAINED ADULTS

by

Kimberly Anne Trocio

Bachelor of Science in Kinesiology  
Indiana University  
2005

A thesis submitted in partial fulfillment  
of the requirements for the

**Masters of Science in Exercise Physiology**

**Department of Kinesiology and Nutrition Sciences  
School of Allied Health Sciences  
Division of Health Sciences  
The Graduate College**

**University of Nevada, Las Vegas  
May 2013**

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## THE GRADUATE COLLEGE

We recommend the thesis prepared under our supervision by

Kimberly Trocio

entitled

Oxidative Stress for an Acute Bout of Bikram Yoga in Healthy, Trained Adults

be accepted in partial fulfillment of the requirements for the degree of

**Master of Science in Exercise Physiology**

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**May 2013**

## ABSTRACT

### **Oxidative Stress for an Acute Bout of Bikram Yoga in Healthy, Trained Adults**

by

Kimberly Trocio

Dr. Antonio Santo, Thesis Committee Chair  
Professor of Kinesiology and Nutrition Sciences  
University of Nevada, Las Vegas

**BACKGROUND:** Generation of free radicals resulting in oxidative damage has been linked to cellular damage, aging, and human disease. Many studies have reported that physical exercise can contribute to oxidative stress. Further, exercise in a hyperthermic environment can promote additional oxidative stress. It is important to consider that practices in yoga may be beneficial in reducing oxidative stress according to some studies. The compound effects of exercise and hyperthermic exposure are experienced in the practice of Bikram yoga, the original “hot” yoga. Because Bikram yoga and other hot exercise classes are an increasingly growing fitness trend, it is necessary to investigate whether there are benefits and inherent risks associated with participation. Specifically, markers of oxidative stress may provide insight into the safety and benefit for practical application of Bikram yoga. The proposed research will acknowledge two products of oxidative stress by means of lipid peroxidation and the antioxidant defense mechanism.

**PURPOSE:** The purpose of this study is to examine the effect of one Bikram Yoga session on oxidative stress markers in healthy, trained adults. **METHODS:** Fourteen healthy, Bikram yoga trained adults (7 females & 7 males) with a mean age, height, weight, BMI, and % body fat of  $35.86 \pm 9.09$  y,  $171.51 \pm 9.37$  cm,  $76.23 \pm 15.03$  kg,  $25.90 \pm 4.83$ , and  $20.96 \pm 5.17$  respectively, with  $2.13 \pm 1.82$  years of experience,

completed a Bikram yoga session in its standard hot environment (approximately 40.6°Celsius, 40% humidity)(HOT) and a Bikram yoga session in a thermoneutral environment (22.2°Celsius and 50% humidity)(CON) on two separate laboratory visits. Oxidative stress variables, plasma thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) were investigated. Hematocrit (HMT), heart rate (HR), and oral body temperature (TEMP) were also observed. PASW Statistics 20 software was used to analyze the data. **RESULTS:** Oxidative stress in participation of one session of Bikram yoga performed in its standard hot environment is not significantly different when performed in a thermoneutral environment by healthy, trained adults. There was no significant interaction or main effects for TBARS (time:  $p=0.886$ , condition:  $p=0.480$ , interaction:  $p=0.507$ ), GSH(time:  $p=0.161$ , condition:  $p=0.414$ , interaction:  $p=0.525$ ), or hematocrit (time:  $p=0.581$ , condition:  $p=0.148$ , interaction:  $p=0.106$ ). The practice of Bikram yoga in the HOT revealed elevated HR (60 min:  $p=0.005$ , 75 min:  $p=0.034$ , 105 min:  $p=0.033$ ) and TEMP (15-90min:  $p<0.001$  throughout testing in comparison to CON. HR was significantly different dependent upon the specific Bikram yoga pose. Oral temperatures reached significance throughout class participation. **CONCLUSION:** The lack of significant change observed in TBARS, GSH, and hematocrit indicates that the trained participants did not undergo significant oxidative stress regardless of increased oral body temperature or heart rates from the practice of Bikram yoga in the heat. Explanation of comparable levels of oxidative stress may be due to the experience and acclimatization to the practice. These data suggests that while the hyperthermic environment provides cardiovascular benefits, it does not significantly contribute to more oxidative stress in an acute bout of Bikram yoga for the Bikram trained.

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## CHAPTER 1

### INTRODUCTION

It is important to investigate free radicals and oxidative stress, because it is associated with cellular damage, the aging process, and human disease (Golden, Hinerfeld, & Melov, 2002). Free radicals are unstable, highly reactive molecules that are generated as a result of normal metabolism of all living organisms and have biological advantage if maintained. However, free radicals can potentially be dangerous products of cellular metabolism in that they can directly influence cell growth, development, and survival. In an effort to achieve stability, free radicals desire and attempt to "steal" an electron from healthy molecules to fulfill their unpaired electron in their valence electron shell. The end result is damage to healthy cells and commences a chain reaction of free radical production potentially causing significant cellular damage. The cellular damage by free radicals is referred to as oxidative stress, and it occurs when the generation of free radicals exceeds the body's ability to neutralize and repair the resulting damage via the antioxidant defense system. Accumulation of these free radicals can result in failure of normal cell function, consequently causing an inflammatory response, cell death, and oxidation of lipids, proteins, and DNA (Fisher-Wellman & Bloomer, 2009; Mattson, 2006; Powers & Jackson, 2008). More importantly, excessive exposure to oxidative stress can contribute to the acceleration of aging and implication in the cause of diseases including cancer, cardiovascular disease, arthritis, Parkinson's disease, Alzheimer's

disease, and other biological diseases. For those predisposed to these types of devastating conditions, it is important to be aware of what can happen at the basic cellular level in order to explore preventative measures. Furthermore, external factors such as environmental and physical stress has the ability to increase radical production causing a disturbance in normal redox state (Vollaard, Shearman, & Cooper, 2005; Zhao, Fujiwara, & Kondo, 2006). The compound effects of prolonged aerobic exercise and a hyperthermic environment in hot exercise classes such as Bikram yoga have the potential to exacerbate cellular damage.

Because Bikram yoga is considered to be an aerobic activity and is performed in a heated environment, participants are susceptible to free radical production and oxidative damage as opposed to a practice in a neutral thermal environment. Specifically, Bikram yoga classes are performed for exactly 90 minutes, consist of a sequence of 26 postures ( Appendix II) and two breathing techniques, and are required to be practiced in a room heated to the extreme temperature of 105°F (approximately 40.6°C) with 40% humidity. It is purported that performing the exercise in the heated room is essential to receiving maximum benefits and not to harm the body ("About Bikram Yoga," 2010). Additionally, it is claimed that this practice offers advantages on a cellular and biochemical level, but there is a dearth of scientific evidence to support the benefits.

Because of Bikram yoga's increasing recognition, clinical trials are necessary to draw adequate conclusions regarding its effectiveness and safety for its participants. In addition to the obvious concerns of heat stress, it is important to examine the oxidative influence of Bikram yoga so participants can apply these findings to their own practice

and take precautions if necessary. The proposed study will allow comparison and identification of any disparity between Bikram sessions performed in its standard hot condition versus a thermal neutral condition. The proposed study will investigate two different products of oxidative stress in regard to lipid peroxidation and antioxidant defense. Examining each product of oxidative stress will reveal any significant oxidative stresses caused by a Bikram session and the capability of the antioxidant defense system of the participants. Furthermore, elements of heat stress including participants' heart rates, oral body temperature, and dehydration statuses will be investigated for both sessions. In any event, the results will reveal awareness in the safety and impact of Bikram yoga at a cellular level. The findings of the proposed research will assist in understanding the relationship of heat stress and prolonged exercise on oxidative status in one session of Bikram yoga.

### Purpose of the Study

The purpose of this study is to examine the effect of one Bikram Yoga session on oxidative stress markers in healthy, trained adults.

### Research Questions

#### *Research Hypothesis*

There will be significantly greater levels of oxidative stress in healthy, trained adults for one session of Bikram yoga in comparison to a thermal neutral environment.

### *Null Hypothesis*

There will be no change in oxidative stress in healthy, trained adults for one session of Bikram yoga between the hyperthermic and thermal neutral environments.

### *Alternate Hypothesis*

There will be significantly lower levels of oxidative stress in healthy, trained adults for one session of Bikram yoga in comparison to a thermal neutral environment.

### Significance of the Study

There has been very limited research regarding the safety and efficacy of Bikram yoga. Because Bikram yoga and other hot exercise classes are an increasingly growing fitness trend, it is necessary to elucidate whether there are benefits and inherent risks associated with participation. Specifically, markers of oxidative stress may provide insight into the safety and benefit for practical application of Bikram yoga. The proposed research will acknowledge two products of oxidative stress by means of lipid peroxidation and the antioxidant defense mechanism. Further, the study will be able to disclose information regarding the competence of antioxidant defenses in Bikram trained participants. Because there has been limited research, performing this experiment may open the door of opportunity for other students and researchers to make further investigations on the subject of this study. Research application and testing are vital in the advancement of scientific knowledge of answering why oxidative stress occurs and how to prevent and treat the cellular damage that are inflicted on humans and other living things.

## CHAPTER 2

### REVIEW OF LITERATURE

#### Free Radicals

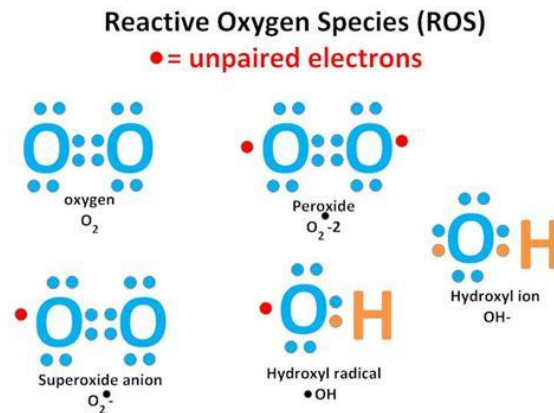
A free radical is any species capable of independent existence, containing one or more unpaired electron in its outermost shell (Halliwell, 1991). Free radicals are produced by an electron transfer that requires a high input of energy (Cheeseman & Slater, 1993). An electron is a negatively charged particle that revolves around the nucleus of an atom. Nearly all electrons in the human body exist in pairs, and these atoms or molecules with paired electrons are considered to be stable. Conversely, when there is only one electron in the outer orbital or when an electron becomes unpaired, that atom or molecule is considered unstable and converts to a free radical. A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom (Karlsson, 1997). A covalent bond is a chemical bond that involves sharing a pair of electrons between atoms in a molecule. When reacting with other radicals or molecules, a free radical has the ability to generate additional radicals (Sen, 2001). Reduction is the process in which free radicals attempt to become stable by "stealing" electrons from nucleic acids, lipids, or any nearby molecule. The molecule that acquires an electron is considered reduced, or stabilized, and the molecule that loses an



electron is considered oxidized. The loss of an electron through oxidation is what drives the generation of free radicals. If this acquiring of electrons is left rampant by the body's natural defense systems, the generation of free radicals promotes a chain reaction potentially resulting in cellular damage and disease.

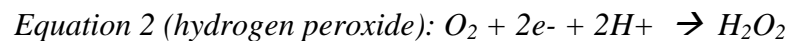
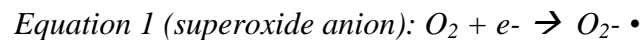
The most imperative and critical of free radicals in the biological system are derived from oxygen. Due to its atomic structure comprising of two unpaired electrons in its outermost shells, oxygen is highly reactive and extremely susceptible to radical formation or oxidation (Sen, 2001). These free radicals and non-radical derivatives of oxygen are collectively termed as reactive oxygen species (ROS). ROS are formed during the reduction of oxygen to water. About 98% of inspired oxygen is reduced to water ( $\text{H}_2\text{O}$ ) during lipolysis, processes of inflammation, the production of chemical energy, along with several other biologic processes (Kerr, Bender, & Monti, 1996; Wilson, Pierce, & Clancy, 2001). However, the incomplete reduction of the remaining 2% leads to the formation of three major ROS: superoxide ( $\text{O}_2^{\bullet-}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and hydroxyl ( $\bullet\text{OH}$ ). Another radical derived from oxygen is singlet oxygen,  $^1\text{O}_2$ . This is an excited form of oxygen in which one of the electrons transfers to a superior orbital following absorption of energy. Figure 1 displays the atomic structure of the ROS, superoxide anion, peroxide, and hydroxyl radical, in comparison to the stable oxygen and hydroxyl ion.

**Figure 1 Reactive Oxygen Species**  
(Wall, 2009)



### *Superoxide ion ( $O_2^{\bullet-}$ )*

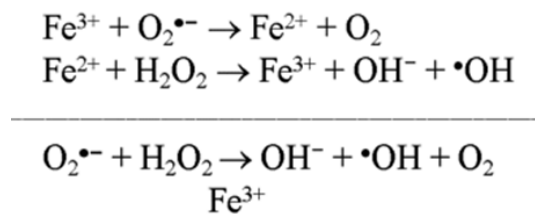
Superoxide anions ( $O_2^{\bullet-}$ ) are generated when oxygen ( $O_2$ ) acquires an additional electron, leaving the molecule with only one unpaired electron. Reduction of oxygen results in the production of superoxide anions [Equation 1]. Superoxide anion has an oxidizing function, where it is reduced to hydrogen peroxide ( $H_2O_2$ ), or a reducing function, in which it is oxidized back to oxygen [Equation 2]. Hydrogen peroxide is a strong oxidizing agent and can easily be converted to form a number of oxidants including the most reactive hydroxyl radical ( $\bullet OH$ ).  $O_2^{\bullet-}$  is continuously being formed within the mitochondria, and the rate of generation is dependent upon the amount of oxygen in circulation at a given time.



### *Hydroxyl Radical ( $\bullet\text{OH}$ )*

Though hydroxyl is short-lived, it is an extremely reactive radical that can damage various cellular components, unless further reduced to water (Dillard, Litov, Savin, Dumelin, & Tappel, 1978). Detrimental effects of ROS can include DNA damage, cell membrane destruction, and protein mutation. Hydroxyl radical can be formed from  $\text{O}_2\bullet^-$  and  $\text{H}_2\text{O}_2$  via the Harber-Weiss reaction (Kehrer, 2000) in Figure 2.

**Figure 2 Haber-Weiss Reaction**  
(Tedesco, Martínez, & González, 1997)



The presence of a transition metal, such as ferrous iron ( $\text{Fe}^{3+}$ ), is necessary for hydroxyl radical formation. The interaction of copper or iron and  $\text{H}_2\text{O}_2$  also produce  $\text{OH}\bullet$  as first observed by Fenton. These reactions are significant as the substrates are found within the body and could readily create interactions. The iron-catalyzed Haber–Weiss reaction, involving Fenton chemistry, is considered to be the major mechanism by which the highly reactive hydroxyl radical is generated in biological systems (Liochev, 1999). In the net equation, superoxide reacts with hydrogen peroxide to produce the hydroxyl radical. The Haber-Weiss reaction provide a means to generate more toxic radicals from

the less reactive superoxide and hydrogen peroxide that could be generated enzymatically (Kehrer, 2000).

### *Hydrogen Peroxide ( $H_2O_2$ )*

Hydrogen peroxide formation is derived from numerous reactions including dismutation of superoxide by SOD (superoxide dismutase) and a number of enzyme systems (Powers & Jackson, 2008).  $H_2O_2$  is stable and has a relatively long half-life (Powers & Jackson, 2008). While  $H_2O_2$  is unable to oxidize DNA or lipids directly, it can inactivate some enzymes, usually by oxidation of essential thiol groups (Halliwell & Gutteridge, 2007; Powers & Jackson, 2008). Exposure of cells to large doses of  $H_2O_2$  can cause inhibition of glycolysis resulting in ATP depletion (Halliwell & Gutteridge, 2007). Hydrogen peroxide is distinctive in its ability to be converted to the highly damaging hydroxyl radical or be catalyzed and excreted harmlessly as water. Glutathione peroxidase is essential for the conversion of glutathione to oxidized glutathione, during which  $H_2O_2$  is converted to water (Hunt et al., 2000). If  $H_2O_2$  is not converted into water singlet oxygen ( $^1O_2$ ), another free radical, is formed.

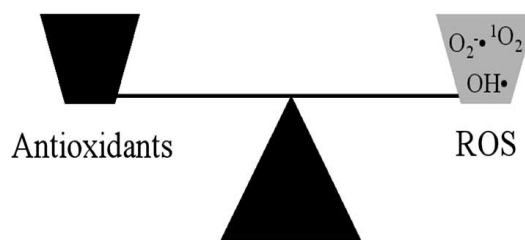
### *Singlet Oxygen ( $^1O_2$ )*

Singlet oxygen is technically not a free radical but can be formed concurrently with radical reactions and may consequently cause further detrimental reactions. Singlet oxygen violates Hund's rule of electron filling in that it has eight outer electrons existing in pairs leaving one orbital of the same energy level empty. When oxygen is energetically excited one of the electrons can jump to empty orbital creating unpaired electrons

(Karlsson, 1997). Singlet oxygen can then transfer the energy to a new molecule and act as a catalyst for free radical formation. The molecule can also interact with other molecules leading to the formation of new free radicals.

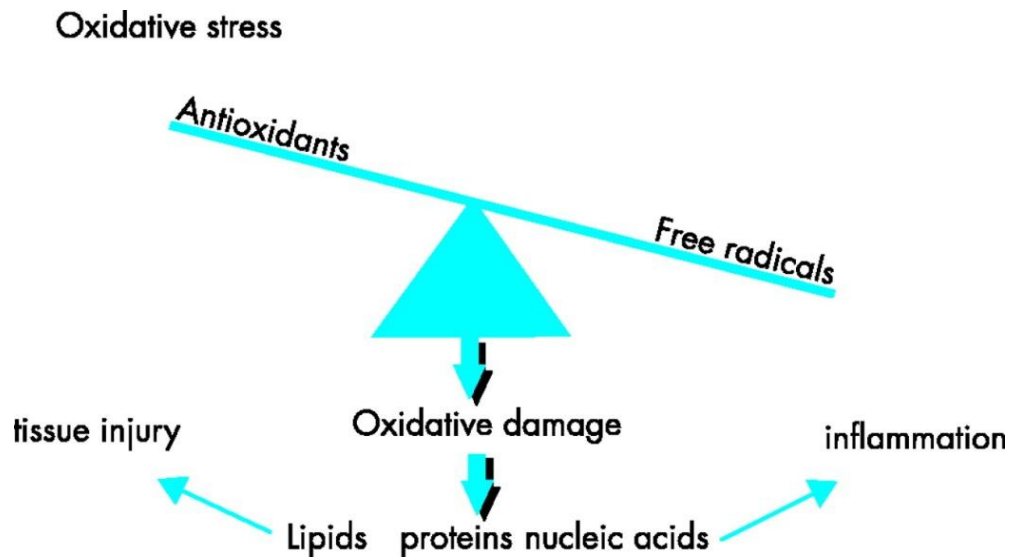
### Oxidative Stress

Generation of ROS is a natural process within the aerobic organism during normal metabolism. ROS production is present under normal conditions at rest in which healthy individuals possess an antioxidant defense system that provides a balance between production, removal, and no net increase in ROS, and oxidized antioxidants are regenerated or reduced to keep pace with ROS production (See Figure 3). Inherently, as metabolic processes progress, it can create an immense amount of ROS. When ROS formation exceeds the body's ability to neutralize and eliminate them, it results in an oxidative imbalance. Further, this imbalance of oxidant and antioxidant levels is referred to as oxidative stress (See Figure 4).



**Figure 3 Balance**

(Deaton & Marlin, 2003) There is a balance between ROS production and antioxidant defenses under normal conditions for healthy individuals.



**Figure 4 Imbalance**

(Deaton & Marlin, 2003) Oxidative stress results from formation of oxidants exceeding the body's ability of the antioxidant systems to remove ROS.

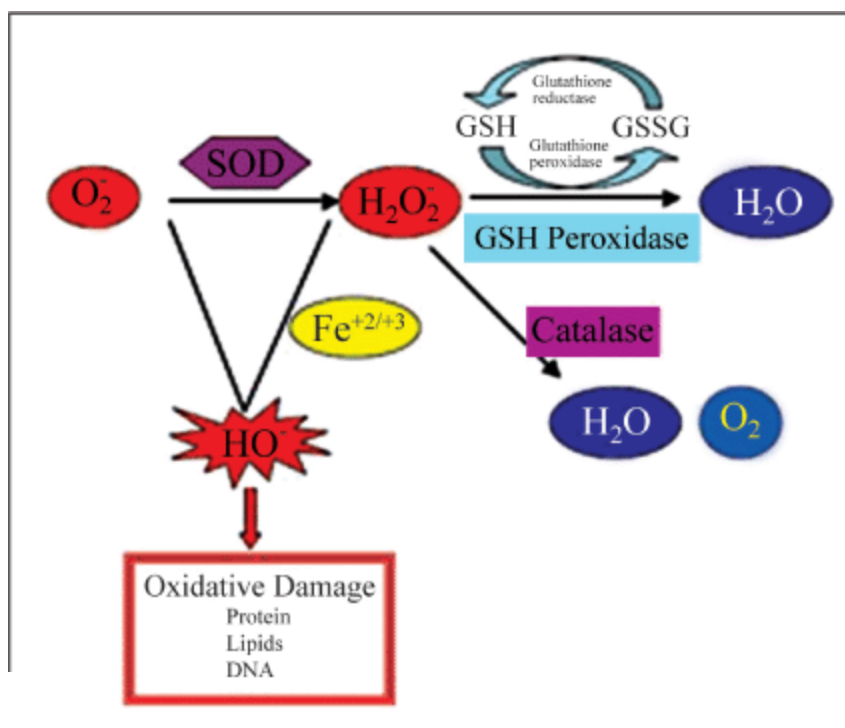
The body's antioxidant defense system serves to protect the cells from excess ROS production and is comprised of endogenous and exogenous compounds (Urso & Clarkson, 2003). Oxidative stress that occurs in the cells, as a consequence of an inequity between the pro-oxidant and antioxidant systems, causes harm to biomolecules such as nucleic acids, proteins, structural carbohydrates, and lipids causing tissue damage and inflammation (Sies & Cadenas, 1985).

*Biological Effects of ROS*

ROS are vital components in the immunity response, specifically by combating against antigens during phagocytosis. This role is especially high in demand in

developments of inflammation. Inflammation can be caused by physical exercise, in particularly intense exercise. Although many studies emphasize the harmful effects of free radicals, ROS also play an important role in cellular signals or in biogenesis of cells. ROS has the ability to serve as cell messengers and to modify oxidation-reduction (redox) status. Further, ROS are recognized of involvement for enzyme activation, drug detoxification or in facilitation of glycogen repletion. In addition, ROS play an essential role in muscular contraction in which inhibition of ROS production leads to a loss of muscular fibers' contractile force. Conversely, increasing ROS leads to increased contractile force. However, a significant amount of ROS in muscular tissue is implicated by muscular fatigue and represent one of the negative effects of ROS.

In addition to those favorable effects, ROS also has potential adverse effects. ROS are not inherently harmful; however, in response to chronic exposure to excessive and/or ectopic production, the system can become unbalanced (free radicals > defenses), potentially resulting in a shift in the intracellular redox balance towards a more oxidizing environment, in turn promoting oxidative damage, inflammation, ill-health, and disease (Fisher-Wellman & Bloomer, 2009). More specifically, damaging effects comprise of oxidative damage to nucleic acids, lipids, and proteins, as well as by changes in gene expression that promote apoptosis within healthy cells and systemic inflammation, and lastly, the functional decline with aging and pathophysiology (Chung et al., 2008; Droge, 2002) .



**Figure 5**  
**Oxidative Stress**

- An unbalance between the production of prooxidants and antioxidants in cells might lead to a strengthened production of free radicals like activated oxygen ( $O_2^-$ ) and reactive oxygen species which could lead to serious cellular damage

(Wakamatsu, Dogru, & Tsubota, 2008)

### *Lipid Peroxidation*

Highly reactive intermediates of oxidative stress include the superoxide anion radical ( $O_2^{\bullet-}$ ), hydrogen peroxide ( $H_2O_2$ ), and the hydroxyl radical ( $\bullet OH$ ). These agents have been shown to be responsible for oxidative damage by initiating lipid peroxidation. Lipid peroxidation refers to the oxidative degradation of lipids and their formation of their products that contribute to advancement of free radical reactions. Lipid peroxidation is the mechanism of radical chain reactions consisting of three major stages: initiation, propagation, and termination (Catala, 2006).

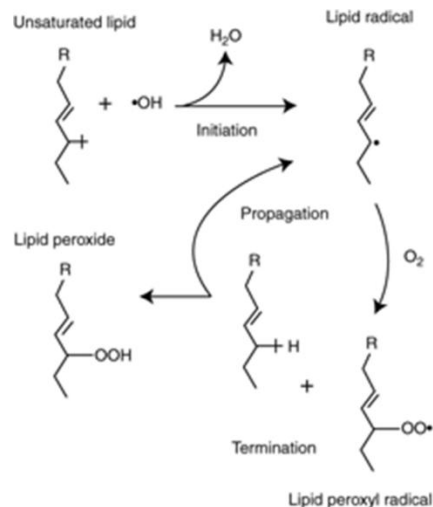


Lipid peroxidation is the initiation of free radical attack on the cell when a free radical steals an electron from the lipid membrane. Initiation of lipid peroxidation occurs when a radical species with significant oxidizing effects, such as the hydroxyl radical ( $\bullet\text{OH}$ ), removes an allylic hydrogen from polyunsaturated fatty acids (PUFAs) forming a lipid radical. PUFAs are abundant in cellular membranes and in low-density lipoproteins (LDL) (Dekkers, Van Doornen, & Kemper, 1996). The PUFAs allow for fluidity of cellular membranes, maintenance of an equilibrated gradient of concentration, membrane permeability, and inflammation. In addition, initiation can be stimulated by ions or chelates of transition metals, like iron, which provoke the toxicity of  $\text{H}_2\text{O}_2$  by formation of hydroxyl radicals (Hall, 1997).

The carbon-carbon double bonds in PUFAs are targeted by ROS, because the site weakens the carbon-hydrogen bond granting accessible abstraction of hydrogen for transformation of additional ROS. An immediate rearrangement occurs in effort to stabilize the carbon-centered radical, forming a more stable lipid radical, whose dienes are conjugated (Gutteridge, 1995). This conjugated diene (a hydrocarbon chain that has two double bonds separated by a single bond) reacts with oxygen to form a proxy radical, the lipid peroxy radical ( $\text{LOO}\bullet$ ). Propagation reactions can continue by the lipid peroxy radical abstracting an allylic hydrogen atom from another adjacent PUFA, resulting in a lipid hydroperoxide ( $\text{LOOH}$ ) and a second lipid radical. This second lipid radical can progress in the corresponding reactions as the first for generation of additional lipid hydroperoxides. The propagation step can undergo numerous rounds of peroxide generation before a termination event occurs. The termination event can be the result of

any reaction with another radical, protein, or compound that acts as a free radical trap, forming a stable end product (Davies, 1996).

The biophysical consequences of peroxidation on membrane phospholipids can be both extensive and highly destructive (Hall, 1997). Lipids altered by peroxidation are subjected to disruption of permeability of cell membranes, reduction to facilitate an equilibrated concentration gradient, decrease in electrical resistance, and inactivation of cross-linked proteins (Radák et al., 1999; Richter, 1987). Lipid peroxidation inflicts the chemical modification of fatty acids resulting in oxidative stress. Since lipid peroxidation is a self-propagating chain-reaction, the initial oxidation of only a few lipid molecules can result in significant tissue damage. An accumulation of lipid peroxides in human cells over time has been known to be associated with numerous pathological conditions.



**Figure 6 Lipid Peroxidation**

(Wikipedia, 2013)

### *Antioxidant Defense*

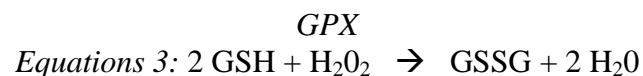
An antioxidant can be defined as a substance that has the ability to reduce the severity of oxidative stress by either forming a less active radical or suppressing the damaging free radical chain reaction on substrates such as proteins, lipids, carbohydrates or DNA (Dekkers et al., 1996). The role of the antioxidative defense mechanism is to combat threats posed by the overproduction of ROS and to stabilize the imbalance of the prooxidant-antioxidant system. Scavenging or detoxification of excess ROS is achieved by an efficient antioxidative system comprising of active non-enzymatic (primarily from food sources) and enzymatic (endogenous) antioxidants (Powers & Lennon, 2000). The efficiency of the antioxidant mechanism is dependent on nutritional consumption and endogenous antioxidant enzyme production. Certain factors including exercise, the external environment, training, nutrition, and aging may promote the inclination and modulation of overproduction of ROS. Because exercise and heat stress increases free radical production, the capabilities of the antioxidant defense system is of critical importance.

An acute bout of exercise is known to increase the activities of antioxidant enzymes, including, superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX) in skeletal muscle, heart, and liver (Jenkins, 1988; Ji, 1993). Skeletal muscle has one of the lowest antioxidant enzyme levels in the body, however, oxygen flux can increase up to several dozen-fold during strenuous exercise. The resultant large increase in ROS is the likely trigger of antioxidant enzyme synthesis.

## Glutathione

Glutathione is the major non-enzymatic endogenous antioxidant that has been the focus of extensive research of exercise induced oxidative stress. GSH plays a multi-functional role in protecting tissues from oxidative damage during exercise. GSH reduces hydrogen peroxides and organic peroxides via reactions catalyzed by glutathione peroxidase (GPX) and serves as a scavenger of singlet oxygen and  $\bullet\text{OH}$  (Ji, 1995). The primary preventative antioxidant pathway for hydroperoxide (free radical) removal involves the action of GPX that oxidizes glutathione (GSH) into glutathione disulfide (GSSG) in the cytosol and mitochondria (Jones, 2008).

GPX, present in cell cytosol and mitochondria, catalyzes the oxidation of glutathione (GSSG) and has the ability to transform  $\text{H}_2\text{O}_2$  into water (Equation 3). Oxidized glutathione (GSSG) is subsequently reduced by glutathione reductase (GR) with the expense of NADPH (Equation 4). The ratio of GSSG to total glutathione (GSH + GSSG) also known as the glutathione redox ratio (GRR) is suspected as a sensitive indicator of oxidative stress.



Changes in blood glutathione as decreases in GSH and increases in GSSG, with no change in total glutathione (TGSH) have been reported following a variety of non-eccentric aerobic exercise protocols (Fisher-Wellman & Bloomer, 2009). In addition,

increases in TGS<sub>H</sub> rarely been reported post-exercise. The authors concluded that increases in TGS<sub>H</sub> may be due to the distribution of GSSG from other tissues in addition to the blood. Increases in TGS<sub>H</sub> could also be due to training status, as individuals with higher training status may be better conditioned to maintain adequate GSH levels during exercise (Leeuwenburgh & Heinecke, 2001). According to a few studies, glutathione status typically returns to basal levels within 15-30 minutes of recovery.

Endurance exercise training has been shown to improve glutathione protection against oxidative stress in rats and humans (Douris et al., 2009; Elokda & Nielsen, 2007; Kihlstrom, 1990; Leeuwenburgh & Heinecke, 2001). A previous study showed endurance swim training in rats provided enhanced protection of the heart by way of elevated GSH levels and a more efficient NADPH supply system to the heart (Kihlstrom, 1990). Likewise, rats were trained by running for two hours per day, five days per week, for 10 weeks (Leeuwenburgh & Heinecke, 2001). The authors found a substantial (62%) increase in GPx activity and increased GSH in the skeletal muscle, with no changes in blood GSH levels. In humans, the data on the effects of training on the glutathione system in humans is very limited. Two studies have shown enhanced glutathione protection as a result of superior aerobic fitness (Douris et al., 2009, Ortenblad, Madsen, & Djurhuus, 1997) while others have shown directly the effects of training on the glutathione system (Elokda & Nielsen, 2007; Miyazak, Oh-ishi, Ookawara, Toshinai, & Ha, 2001). One study simulated cardiac rehabilitation exercise (40 min/day three days/week for six weeks) consisting of aerobic training, circuit weight training, or a combination of both (Elokda & Nielsen, 2007). The authors found that training increased

resting levels of GSH and decreased resting levels of GSSG and the effects were greater in the combination of aerobic and circuit weight training compared to the exercises alone. Similarly, 12 weeks of running for 60 min at 80%  $\text{VO}_2$  max five days per week increased GPx activity by 12% (Miyazak et al., 2001). Assessing individuals of varying fitness levels, Douris et al. (2009) found individuals who practiced martial arts for one hour per day twice a week for at least four years had increased resting GSH and decreased resting GSSG while being better able to respond to an acute bout of oxidative stress compared to their sedentary counterparts. Finally, Ortenblad et al. (1997) found increased GPx activity in the resting muscle of jump trained athletes compared to untrained subjects. These results seem to suggest that exercise training of varying intensities and modes can increase the efficiency and function of the glutathione system, likely leading to a decrease in exercise-induced oxidative stress and potential for disease development.

### Exercise-Induced Oxidative Stress

Many studies have reported that physical exercise can promote oxidative stress. Aerobic exercise is identified as the fundamental cause of the abnormal elevated levels of ROS due to the increased demands of oxygen consumption ( $\text{VO}_2$ ). Under normal conditions, excess ROS produced by the mitochondrial electron transport system are scavenged by the endogenous antioxidants (Rock, Jacob, & Bowen, 1996). However, if radical formation overwhelms the capacity of antioxidant defenses, the free radicals evades from the mitochondria and oxidizes in the lipids, proteins, and other cell

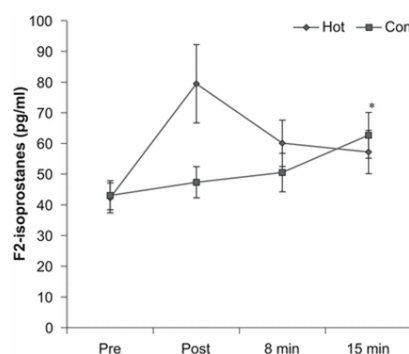
components resulting in oxidative stress. Consequently, these events can prevent normal cellular functions and promote degradation of cellular biochemical processes.

It has been investigated that acute aerobic exercise has the potential to result in increased ROS production (Ashton et al., 1998; Davies, Quintanilha, Brooks, & Packer, 1982). Bloomer et al. (2005) compared oxidative modification of blood proteins, lipids, DNA, and glutathione during the 24 hour period following both aerobic and anaerobic exercise bout in cross-trained men. The results suggest that thirty minutes of aerobic and anaerobic exercise in cross-trained men can increase and differentially affect certain oxidative biomarkers, and can result in a different magnitude of oxidation based on the macromolecule. Specifically, the researchers found significant increases in PC post-anaerobic exercise compared to pre-exercise, an increasing trend but insignificant MDA levels for both conditions, and significant increases in GSH immediately post-aerobic. Different exercise protocols may result in varying levels of ROS production dependent upon intensity and duration. Typically, as intensity and/or duration of exercise increases, the development of oxidative stress is more favorable. Factors that may influence the antioxidant defense mechanism include age, training status, and nutritional intake.

### Exercise in Hyperthermic Environments

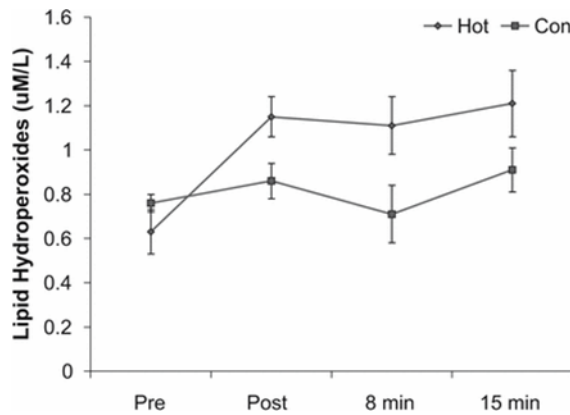
A hyperthermic environment can influence oxidative stress levels both at the resting state and exercising state. It seems that heat stress not only produces oxidants but impairs the enzyme system that is necessary for detoxification of  $H_2O_2$  and organic hydroperoxides (Ohtsuka, Yabunaka, Fujisawa, Watanabe, & Agishi, 1994). The

imposition of increased metabolic rate may be accounted from the compromised antioxidant defense mechanism owing to the overproduction of ROS. It is well established that heat stress reduces stroke volume (SV) and subsequently increases heart rate (HR) in effort to maintain cardiac output during moderately intense exercise (Gonzalez-Alonso, Mora-Rodriguez, & Coyle, 2000; Rowell, Bruce, Conn, & Kusumi, 1966). Even in normal environments prolonged exercise may produce mild hyperthermia along with increased heart rate (HR), and increased core and skin temperatures (Nassis & Geladas, 2002). These conditions are exacerbated in hyperthermic environments when thermoregulation is impaired (Ely, Chevront, Kenefick, & Sawka, 2010). A McAnulty et al. study in 2004 examined lipid oxidative markers (F2 isoprostanes and lipid hydroperoxides) and lactate after low intensity (50% of  $\text{VO}_2\text{max}$ ) running in a hyperthermic environment versus a neutral environment for 6 males subjects. They concluded that hyperthermia increases oxidative stress and selectively affects specific lipid markers, independent of oxygen consumption (results displayed in Figure 8 and 9).



**Figure 7 F2-Isoprostanes** F2-isoprostanes were significantly elevated in the HOT protocol versus CON, as well as elevated over time in both groups (McAnulty et al., 2004).





**Figure 8 Lipid Hydroperoxides** Lipid hydroperoxides were significantly elevated over time, but the pattern of change was not different between protocols (McAnulty et al., 2004).

Further, exercise in the heat has the inclination to increase the risk for state of dehydration which is indicative of elevated oxidative stress (Paik et al., 2009). It is well established that increased HR during hyperthermic exercise results from increased sweat rates and blood volume redistribution which in turn decreases plasma and blood volume (Montain & Coyle, 1992). The independent and combined effects of environmental heat stress and dehydration can challenge the limits of human temperature regulation and aerobic performance (Chevront, Kenefick, Montain, & Sawka, 2010).

### Yoga and Oxidative Stress

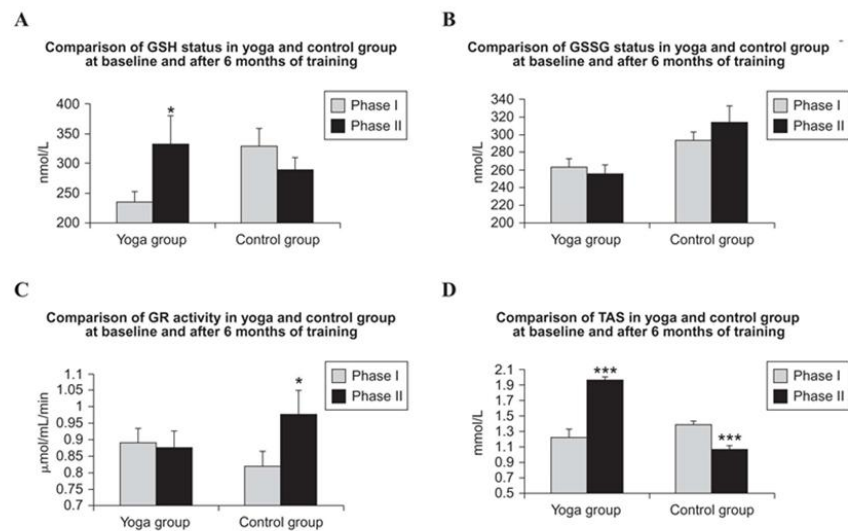
Yoga is an ancient discipline designed to bring balance and health to the physical, mental, emotional, and spiritual dimensions of the individual. Yoga performs *asanas* (postures), *pranayama* (breathing techniques), and meditation to integrate the mind,

body, and spirit. Not until the 20<sup>th</sup> century did the ancient practice become increasingly prevalent in Western society if effort to pursue a healthy lifestyle.

An increasing number of scientific research supports the belief that certain yoga practices can improve physiological and psychological functions. Yoga has been found to have beneficial effects on blood glucose levels in diabetics and individuals with chronic health conditions (Bijlani et al., 2005) (Gokal & Shillito, 2007). Additionally, yoga has been shown to be effective in relieving symptoms of mental illness including depression and anxiety (Shapiro & Cline, 2004; Woolery, Myers, Sternlieb, & Zeltzer, 2004). In a Ross & Thomas (2010) review, their findings concluded that studies comparing the effects of yoga and exercise portrays the indication that in both healthy and ill populations, yoga may be as effective or better than exercise at improving a variety of health-related outcome measures including blood glucose, blood lipids, and oxidative stress (Hagins, Moore, & Rundle, 2007; Sinha, Singh, Monga, & Ray, 2007; Yurtkuran, Alp, & Dilek, 2007). Only few studies found yoga practice as less beneficial from measures of energy expenditure, metabolic equivalents, VO<sub>2</sub>max, and mood (Hagins et al., 2007).

Few studies explored the effect of yoga on oxidative stress. The Sinha et al. (2007) study of 51 healthy males, the researchers found yoga to be more effective than running with flexibility training in improving measures of antioxidant status. Reduced glutathione (GSH) increased in the yoga group and decreased in the exercise group. Similarly, glutathione reductase (GR) increased significantly only in the exercise group, while total antioxidant status increased significantly in the yoga group and decreased

significantly in the exercise group. Figure 10 displays the results of the Sinha et al. (2007) study. A study of 77 type 2 diabetic patients found that yoga resulted in similar measurements as exercise regarding levels of MDA , superoxide dismutase (SOD) and catalase activity (Gordon et al., 2008). Though these studies indicate yoga may be beneficial in reducing oxidative stress, additional research is warranted to provide stronger evidence.



**FIG. 1.** Comparison of the levels of reduced glutathione (A), oxidized glutathione (B), and glutathione reductase (C), as well as total antioxidant status (D) in the yoga and control groups. Phase I is before training. Phase II is after 6 months of training. \* $p < 0.05$ ; \*\*\* $p < 0.001$ .

## Figure 9 Antioxidant Variables (Sinha et al., 2007)

Importantly, with the emerging popularity of Bikram yoga, a form of yoga practiced in high heat, there is a need for clinical trials to substantiate conclusions regarding the indication of oxidative stress in the hyperthermic environment. Scientific

literature suggests that exercise and hyperthermia both independently and adversely affect the balance of the oxidant and antioxidant systems. However, the practice of yoga has been investigated and has demonstrated favorable oxidative effects. This thesis pursues the investigation of the hyperthermic effect in Bikram yoga on oxidative stress biomarkers.

## CHAPTER 3

### METHODOLOGY

#### Limitations and Delimitations

##### *Limitations*

Because the sample consisted of Bikram yoga trained, healthy adults that represented only a small percentage of the general population, it can pose a threat to external validity. Some poses demonstrated in Bikram yoga are of high difficulty, and it is necessary for the participants to be able to complete the class properly and with no assistance. The main reasons for selecting these individuals were because of their acclimated condition to the hot temperature and the insurance of full class participation and completion. To protect internal validity, “Bikram’s Beginning Yoga CD”, a recording of the creator’s script instructing the class, was chosen for the study’s class instruction. This eliminated the variety in teaching influences of certified Bikram Yoga instructors. In addition, validity may be influenced due to the large variation of acute effects of exercise amongst individuals. Selecting participants of similar physical condition including body composition and training level limited these variations across the individuals.

The pre-test and post-test blood draws was determined for before and after effects. Post-30 time frame was selected to monitor change in levels over a short period

of time for comparison of the two conditions. A longer period of time may present implications of subject discomfort following 90 minutes of exercise and an overnight fast and further complications associated with research design.

Oxidative variables selected were designated by the categories of lipid peroxidation and anti-oxidant status. Selection was influenced by availability of lab materials and cost. The oral body temperature determination, using not the most accurate measurement tool, was limited by availability of equipment and limited funds. The length of the class was pre-determined by the Bikram class requirements and thusly, enables the flexibility of changing the exercise time regardless of oral body temperature levels.

### *Delimitations*

Though interaction and the surroundings for the Bikram Yoga class were controlled, the personal motivation or effort of the participant could not. The participants were advised to maintain a normal diet and stay well-hydrated the day prior and during testing. For future studies, it may be worthy to note for the participants to keep a food/drink log to ensure they consume the same amount of food and liquids for each particular condition in order to reduce variability in baseline measures between conditions. Also, it may be advantageous to better control participant's water consumption during and after performing the Bikram class as hydration status may be a compounding factor for results.

## Subject Characteristics

A sample of 15 healthy, trained individuals was recruited to voluntarily participate in this study. Subject characteristics are presented in Table 1. The participants included in the study are adults aged 18-45 years, free of medical conditions that limit physical activity, and have participated in Bikram yoga on average at least three times a week in the last three months. The Physical Activity Readiness Questionnaire (PAR-Q) was used to screen recruits at high-risk for a cardiovascular complication. Individuals who answered no to all the questions were able to participate in the study. Individuals taking antioxidant supplements within 3 months prior to testing were excluded from participation. These antioxidant supplements including vitamin A, vitamin C, vitamin E, or selenium owe potential effects on oxidative stress markers. In addition, individuals that are considered well below average for body composition according to the American College of Sports Medicine Guidelines were not able to participate. Moreover, obese individuals based on their Body Mass Index were excluded from the study.

Participants were asked to maintain their usual diet and be well hydrated for the duration of the study. Strenuous physical activity within 24 hours of testing and caffeine consumption on the days of testing was prohibited. To minimize diurnal rhythm effects, participants were instructed to complete an overnight fast of 8-12 hours on the mornings of testing. Participants were instructed to follow the BIA guidelines prior to testing (Table 1).

**Table 1 Subject Characteristics**

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<i>Age (years)</i>	$35.86 \pm 9.09$
<i>Height (centimeters)</i>	$171.51 \pm 9.37$
<i>Weight (kilograms)</i>	$76.23 \pm 15.03$
<i>Body Mass Index (kg/m<sup>2</sup>)</i>	$25.90 \pm 4.83$
<i>Body Fat (%)</i>	$20.96 \pm 5.17$
<i>Bikram Experience (years)</i>	$2.13 \pm 1.82$

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**Table 2 BIA Guidelines**

- 
1. No eating or drinking within 4 hours of test.
  2. No moderate or vigorous exercise within 12 hours of the test.
  3. Void (urinate) completely within 30 minutes of the test.
  4. Abstain from alcohol consumption within 48 hours of the test.
  5. Do not ingest diuretics, including caffeine, the day of assessment.
- 

*From Advanced Fitness Assessment and Exercise Prescription, Fifth Edition, by Vivian H. Heyward, 2006, Champaign, IL: Human Kinetics.*

### Instrumentation

Height was obtained using an anthropometer, Body mass was measured using a weighing scale at pre-test and post-test to determine the net loss of body water during the Bikram yoga sessions. Body Mass Index (BMI) was determined by the equation  $\text{mass/height}^2$  (kg/m<sup>2</sup>). An ELG III Tetrapolar Bioelectrical Impedance Analysis (BIA) System was used to determine the participant's body composition. A Taylor Sybron Corporation Sling Psychrometer measured ambient temperature and humidity for both conditions. A Polar Heart Rate Monitor and belt were used to monitor heart rate for the duration of testing. A digital oral thermometer was administered for obtainment of oral



temperature readings of the participant. Baseline measurements of heart rate and oral body temperature were taken followed by measurements every 15 minutes until test termination.

The experimental condition was performed in an environmental chamber. See Appendix VII for specifications. The environmental chamber was constructed in a 10 ft. x 10 ft. x 11ft. room in MPE 312 at UNLV. A single layer of visqueen clear plastic sheet lined the walls and ceiling which provided further insulation inside the room. A door on one side of the chamber provided entry to the chamber. A sheet of visqueen plastic was placed at the doorway and was cut to allow the sheet of plastic to open upwards. During class participation, the plastic “door” was at the closed position but not sealed. The chamber was designed to hold the maximum capacity of 3 participants.

Heaters and humidifier/dehumidifier units provided for temperature and humidity regulation inside the chamber. Temperature and humidity readings were monitored every 15 minutes in preparation and for the duration of testing. If the temperature was too low, the heater was turned on at a higher setting. If the temperature was above the desired temperature, the heater was reduced or turned off. If the humidity was too high, the dehumidifier setting was increased. If the humidity was too low, the humidifier was turned on. Based on the room temperature and humidity, the settings were adjusted to maintain the proper levels.

Five hundred milliliters of water was provided to participants to drink ad libitum during each of the conditions and remaining fluid was measured with a graduated cylinder at the end of the trials for determination of water consumption.

Blood samples were drawn via finger prick by use of Capiject Lancets (Fisher Scientific) and Multivettes (Fisher Scientific) for capillary blood collection. After blood collections were complete, the samples were centrifuged, prepared and stored in multiple aliquots in 1.4 mL Posi-Click Tubes (Denville Scientific) at -80°C until analysis. GSH and TBARS were analyzed using an enzyme linked immunosorbent assay (ELISA) following the procedures as outlined by the manufacturer (MBL and Cayman Chemical). The centrifugation of blood samples and additional measurements using a metric ruler were performed for hematocrit analysis.

### Research Design

In this randomized control study, participants who satisfied the study criteria participated in two different conditions: the experimental and control. At each visit, the participant performed the specific yoga protocol in one of the two conditions. A random integer generator (accessed at [random.org](http://random.org)) was used to determine the order of placement in each condition.

The subject assigned in the experimental condition (HOT) participated in a session of Bikram Yoga in a controlled environment with a temperature of 40.6°Celsius (105°Fahrenheit) and 40% humidity. In contrast, the control condition (CON) required the subject to participate in a bout Bikram Yoga exercises in a neutral temperature with a room temperature of about 22.2°Celsius (72°Fahrenheit) and 50% humidity. Participants performed each condition separated by least three days. All testing was conducted in a controlled environmental chamber where room temperature and humidity was manually

regulated. The test location was located at the Human Performance Lab (MPE 312) at the University of Nevada, Las Vegas. Testing on both days was conducted at the same location and at the same time of day in the morning. A pre-recorded audio recording, "Bikram's Beginning Yoga Class" instructed by Bikram Choudhury, was used for both HOT and CON conditions. All subjects listened and participated along with the recording of the Bikram yoga class for each day of testing. To consider participation of the study to be complete, participants were recommended to participate for the entire 90 minutes of instruction while inside the environmental chamber. However, as stated in the informed consent, the participant may have chosen to withdraw from testing at any time they felt uncomfortable and/or wished to discontinue testing.

At least one CPR and AED Certified Member of the research team closely monitored the participant(s) of any signs/symptoms of heat stress at all times while testing. The research team member was located directly outside of the chamber for most of the duration of the class. Because the visqueen plastic lining the chamber is clear, the research team member was able to visually monitor the subject at all times. At every 15 minutes during the class session, a member of the research team entered the chamber in order to monitor and record the subjects' body temperature and heart rate, check ambient temperature and humidity inside the chamber (about 2-3 minutes at a time), and to visually and verbally check on the subject. The research team member observed for any visual symptoms of heat stress from outside and inside of the chamber. Signs/symptoms of heat stress include light-headedness, dizziness, nausea, faintness, fatigue, headache, weakness, and profuse sweating. If symptoms did occur, the research team member

would proceed into the chamber and verbally ask the subject how they are feeling and if they are able to continue testing. If the subject was not feeling well and unable to continue, the participant was to be removed from the hot environment immediately, cooled off, and provided water for consumption. The member(s) of the research team may assist in cooling off the participant by wetting the participant's skin with water and fanning them with air. In addition, if the subject's heart rate exceeded their predicted maximum heart rate ( $220 - \text{age}$ ) or body temperature exceeded  $40^{\circ}\text{C}$  (or  $104^{\circ}\text{F}$ ) at any time during testing, they were forced to withdraw from the study for their safety.

Five hundred milliliters of water was provided to participants to drink ad libitum during each of the conditions and remaining fluid was measured at the end of the class participation for consumption. Body mass was also measured at pre-test and post class participation for each of the conditions to determine the net loss of body water. At post-test participants were provided with water in the amount of net body water lost during the Bikram yoga class [Net body water lost = pretest body weight – posttest body weight – amount of water consumed]. The water was required to be consumed by participant before the last blood draw at post-30 minutes. Snacks and refreshments were provided to the participants each day at the termination of testing.

The PI and Co-PI withdrew all blood samples for duration of the study. Blood samples were drawn via finger prick by means of capillary blood collection. Samples were drawn at the index finger, middle finger, or ring finger all at the distal phalanx. Samples of six hundred microliters of blood were drawn from automated single-use lancets into multivettes containing anticoagulant and immediately placed on ice until

participation was complete. The blood samples was used to determine hematocrit, GSH, and plasma TBARS. The samples were centrifuged at 1,000g for 20 minutes at 4°C. The plasma was stored in multiple aliquots at -80°C until analysis.

Blood samples were prepared in BHS 122 and stored in BHS 119 until all blood collections were complete. Time allowed for storage from blood collection to analysis was no more than three months. Once all blood samples were collected, the blood samples were analyzed at BHS 122 for the specified oxidative stress markers outlined in the protocol. The blood samples collected for this was not used for any other further research or study. Excess samples were destroyed and disposed of after the study was complete.

**Table 3 Research Design**

Condition	Pre-Test	Participation	Post-Test	Post-30
<b>Control Trial</b> (72°F/22.2°C, 50%H) [CON]	<i>Blood Draw</i>	<i>Bikram yoga session</i>	<i>Blood Draw</i>	<i>Blood Draw</i>
<b>Experimental Trial</b> (105°F/40.6°C, 40%H) [HOT]	<i>Blood Draw</i>	<i>Bikram yoga session</i>	<i>Blood Draw</i>	<i>Blood Draw</i>
<b>Both Trials</b>	<i>Heart Rate and Oral Body Temperature</i>			

## Collection of the Data

### *Subject Characteristics*

Prior to class participation, body weight, height, and body composition of participants were measured and recorded at the testing site on D1.

### *Temperature and Humidity*

Ambient temperature and humidity were evaluated by the members of the research team prior and during each day of testing to ensure proper measurements at 40.6° Celsius (105° Fahrenheit) and 40% humidity.

### *Hydration*

Hematocrit measurements for hydration status at each blood draw was determined from blood analysis. Measurements of water consumption during testing and body mass differences at pre- and post-test were also examined. Net body water loss determined the amount of water the participant to consume from post to post-30.

### *Oral Body Temperature and Heart Rate*

Oral body temperature and heart rate was recorded every 15 minutes starting at the beginning of the Bikram Yoga Class till termination of testing.

### *Blood Sample Collection*

Six hundred microliter blood samples was collected from the participant's index, middle, and ring fingertips at pre-test, post-test, and 30 minutes post-test. At pre-test and post-test, blood samples was drawn before, within five minutes following exercise and 30 minutes post-exercise. Before any blood collection, the fingertip was sterilized with 70%

isopropyl alcohol. Using gloves for protection, the PI or Co-PI punctured the site using a single-use automated lancet. The first drop of blood was wiped away using gauze and then collected the blood into multivettes treated with anticoagulant to prevent clotting. Gauze was applied on the site from which the lancet is withdrawn, and an adhesive bandage was applied to protect the puncture site after collection. The single-use lancet was disposed in the proper biohazard waste container. After each blood collection, the blood sample multivettes were immediately placed on ice in a cooler until all collections are complete.

The samples of blood will be used for analysis of hematocrit, glutathione (GSH), and thiobarbituric acid reactive substances (TBARS). After blood collection was complete, the samples were centrifuged at 1,000 x gravity for 20 minutes at 4°C and separated to plasma. Hematocrit was measured immediately after centrifugation. The sample was observed, and a metric ruler was used to measure the height of the components of whole blood for each individual sample. The value of hematocrit was found by dividing the height of red blood cells by the total height and then multiplying the value by 100. After hematocrit analysis, the plasma of the samples were prepared and stored in multiple aliquots at -80°C until analysis of the oxidative stress variables. Blood samples was prepared in BHS 122 and stored at BHS 119. GSH and plasma TBARS was measured and analyzed in BHS 122 using commercially available assay kits following procedures outlined by the manufacturer (MBL and Cayman Chemical).

All materials used for blood collection and analysis, including gloves, disposable lancets, multivettes, gauze, bandages, and pipettes, was disposed in the proper biohazard

waste or sharps biohazard waste containers. The areas of blood collection were completely sanitized with a 1:10 dilution of bleach solution after each blood collection. If a spill occurs, the spill was cleaned immediately by placing paper towel over the spill, flood with a 1:10 dilution of bleach, let it sit for 15-20 minutes, and then finished by cleaning the area. Further, once the study was fully completed the entire testing area was thoroughly decontaminated using the same protocol for a spill using the bleach dilution.

Hydration status was determined via hematocrit determination. GSH and plasma TBARS were measured and analyzed using commercially available assay kits following procedures outlined by the manufacturer (MBL and Cayman Chemical).

### Data Analysis Methods

The hematocrit and selected oxidative stress markers were analyzed using a two condition (experimental and control) by three (Pre-test , Post-test, and Post-30minutes) analysis using a repeated measure analysis of variance (ANOVA) for a crossover design to assess condition and time. In addition, a repeated measures factorial ANOVA between the two conditions by nine times (0, 15, 30, 45, 60, 75, 90, 105, and 120 minutes) was used to compare for differences in oral body temperature and heart rate. Dependent t tests were used to analyze differences in water consumption during class participation, water consumption between Post-test and Post-30, and total body weight lost in the two conditions. All descriptive data was presented as means  $\pm$  standar error of the means (SEM). The level of significance was set at 0.05. Statistical Package for Social Sciences Software (SPSS 20) was used for all statistical analyses.

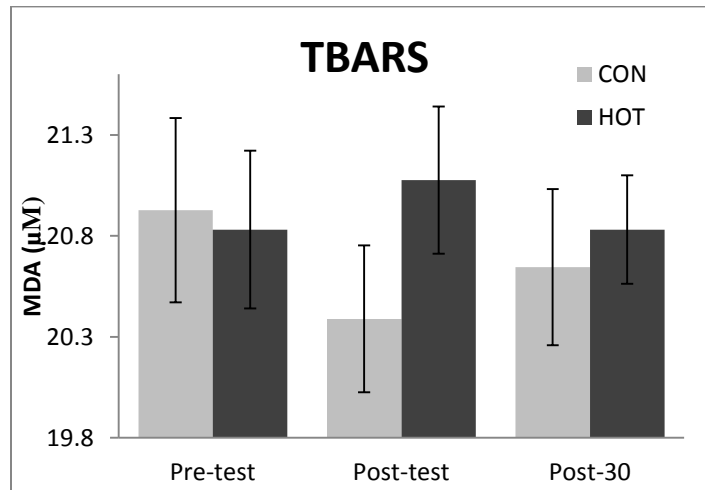


## CHAPTER 4

### RESULTS

#### TBARS

No significant main effects or interactions were observed for plasma TBARS (time:  $F_{2, 52} = 0.121$ ,  $p=0.886$ , condition:  $F_{1, 26} = 0.514$ ,  $p = 0.480$ , interaction  $F_{2, 52} = 0.688$ ,  $p=0.507$ ). The means  $\pm$  SEM for MDA levels are presented in Figure 10.



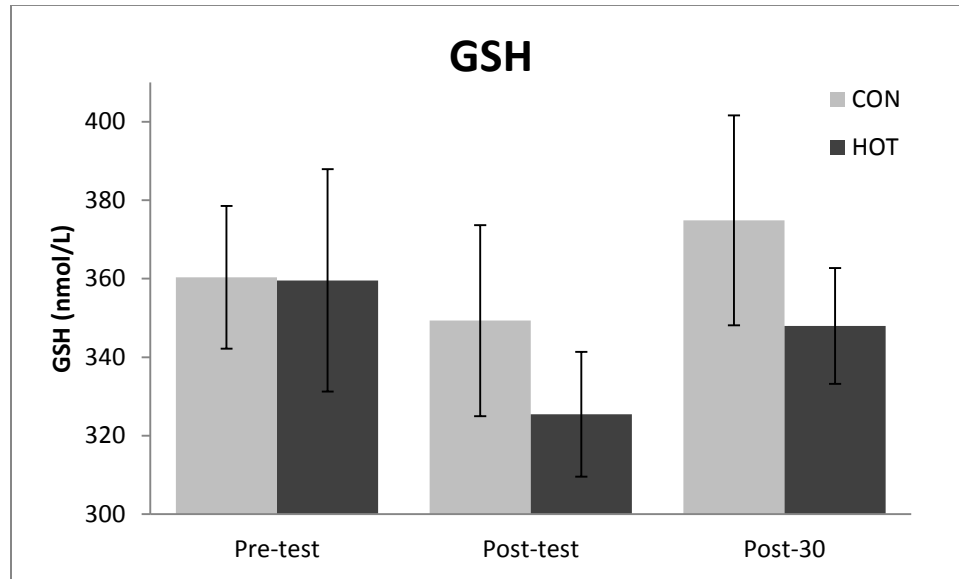
**Figure 10 TBARS** Presented are means  $\pm$  SEM

TBARS response to 90 minutes of Bikram yoga performed in a thermal environment (HOT) and thermoneutral environment (CON) by healthy, trained adults ( $n=14$ ) before the test (Pre-test), immediately after (Post-test) and 30 minutes following the bout (Post-30). ( $p>0.05$ )

## GSH

No significant main effects or interactions were observed for GSH (time:  $F_{2, 52} = 1.890$ ,  $p=0.161$ , condition:  $F_{1,26} = 0.414$ ,  $p = 0.525$ , interaction:  $F_{2, 52} = 0.531$ ,  $p=0.591$ ).

The means + SEM for GSH are presented in Figure 11.

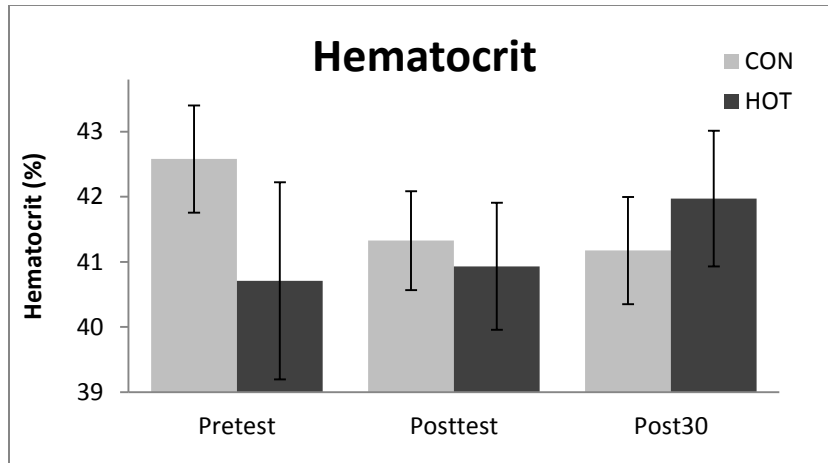


**Figure 11 GSH** Presented is means  $\pm$  SEM

GSH response to 90 minutes of Bikram yoga performed in a thermal environment (HOT) and thermoneutral environment (CON) by healthy, trained adults ( $n=14$ ) before the test (Pre-test), immediately after (Post-test) and 30 minutes following the bout (Post-30). ( $p>0.05$ )

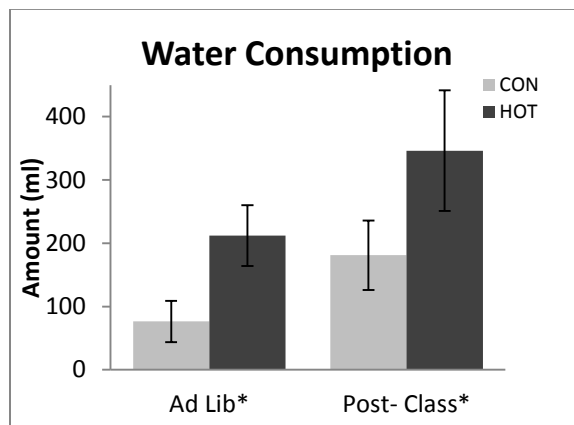
## Hematocrit and Water Consumption

No significant main effects or interactions were observed for hematocrit (time:  $F_{1,450, 37.706} = 0.445$ ,  $p=0.581$ , condition:  $F_{1, 26} = 0.148$ ,  $p = 0.704$ , interaction:  $F_{1,450, 37.706} = 2.552$ ,  $p=0.106$ ). The means + SEM for hematocrit are presented in Figure 12. Water consumption during participation of the class (ad libitum) and water given post-class are displayed in Figure 15a, and body weight lost is presented in Figure 15b (means+ SEM).



**Figure 12 Hematocrit** Presented are means  $\pm$  SEM

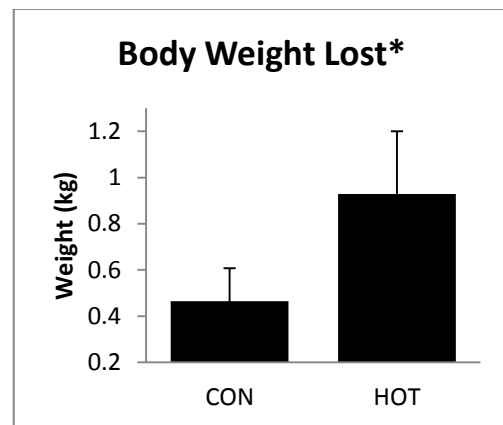
Hematocrit measurements in response to 90 minutes of Bikram yoga performed in a thermal environment (HOT) and thermoneutral environment (CON) by healthy, trained adults ( $n=14$ ) before the test (Pre-test), immediately after (Post-test) and 30 minutes following the bout (Post-30). ( $p>0.05$ )



**Figure 13a Water Consumption**

Presented are means  $\pm$  SEM ( $n = 14$ )

Water consumption ad libitum during the 90 water Bikram session minutes (Ad Lib) and consumption from immediately after minutes following conditions till 30 the class. \*Denotes significance (Ad Lib:  $p=0.009$ , Post-Class:  $p=0.069$ )



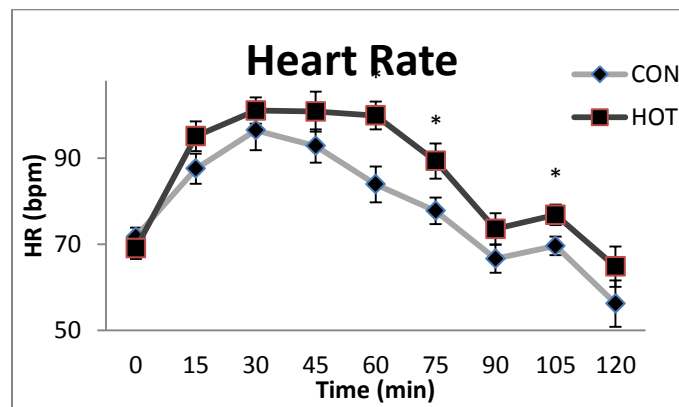
**Figure 13b Body Weight Lost**

Presented are means  $\pm$  SEM ( $n = 14$ )

Body weight lost during the 90 minute Bikram session are presented at thermal (HOT) and thermoneutral(CON) conditions. \*Denotes significance ( $p=0.026$ )

## Heart Rate

Though the main effect for condition ( $F_{1,26}=3.851$ ,  $p = .061$ ) failed to reach significance, the main effect for time ( $F_{8,208} = 49.949$ ,  $p < .001$ ) and interaction between the protocols ( $F_{8,208} = 2.288$ ,  $p=0.026$ ) were significant. This result indicates that there is a difference in heart rates among both conditions over time. Independent t-tests were used to examine condition differences at each of the 15 minute time intervals. The HOT condition presented elevated HR at all time intervals and were significantly different at 60, 75, and 105 minutes ( $p= 0.005$ ,  $0.034$ , and  $0.033$ , respectively) during class participation in comparison to the control condition. Pairwise comparisons revealed heart rates to be variable dependent on the specific Bikram yoga pose (60 and 75 min) and activity during post-test (105 min). The bow pose and rabbit pose (refer to Appendix II) were the particular Bikram postures that significantly elevated heart rates. The means  $\pm$  SEM for HR are presented in Figure 14.

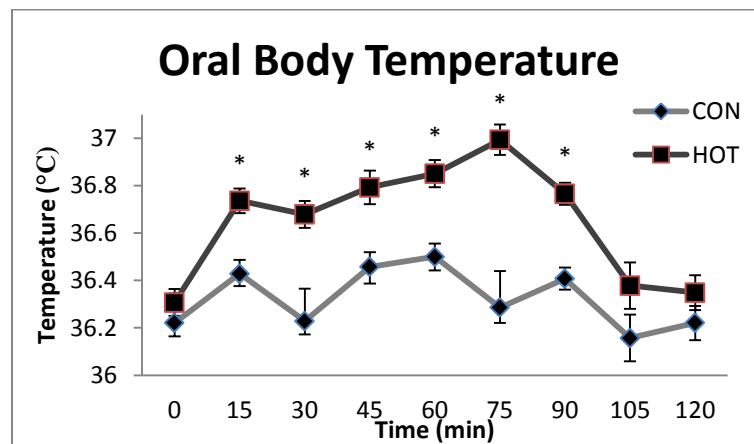


**Figure 14 Heart Rate** Presented are means  $\pm$  SEM

HR response to 90 minutes of Bikram yoga performed in a thermal environment (HOT) and thermoneutral environment (CON) by healthy, trained adults ( $n=14$ ) before the test (Pre-test), immediately after (Post-test) and 30 minutes following the bout (Post-30). \*Denotes significance at 60, 75, and 105 minutes ( $p= 0.005$ ,  $0.034$ , and  $0.033$ , respectively)

## Oral Body Temperature

The main effects for time ( $F_{6,113, 158.94} = 11.64, p < .001$ ).and condition ( $F_{1, 26} = 22.69, p < .001$ ) were both significant. Additionally, the interaction between protocols also reached significance ( $F_{8, 208} = 3.34, p = .001$ ). Simple main effects analysis was conducted to determine the nature of the interaction. Independent t-tests were used to examine condition differences at each of the 15 minute time intervals. The HOT condition presented elevated oral body temperatures at all time intervals and were significantly different at 15, 30, 45, 60, 75, and 90 minutes ( $p < .001, 0.005, < .001, < .001, < .001, \text{ and } < .001$ , respectively) during class participation in comparison to the control condition. Oral body temperature measurements taken at room temperature at the post session time intervals (105 and 120 minutes) were consistent with the baseline measurement ( $p = 1.00$ ). Figure 14 presents the means  $\pm$  SEM for oral body temperature.



**Figure 15 Oral Body Temperature** Presented are means  $\pm$  SEM

Oral body temperature response to 90 minutes of Bikram yoga performed in a thermal environment (HOT) and thermoneutral environment (CON) by healthy, trained adults ( $n = 14$ ) before the test (Pre-test), immediately after (Post-test) and 30 minutes following the bout (Post-30). \*Denotes significance from 15 - 90 minutes ( $p < 0.001$ ).

## **CHAPTER 5**

### **DISCUSSION AND CONCLUSIONS**

#### **Discussion**

The present study investigated the oxidative modification of plasma TBARS and glutathione for one session of Bikram yoga in its standard hot environment versus a thermoneutral environment performed by healthy, trained adults. To our knowledge, this is the first investigation to evaluate oxidative stress associated with the practice of Bikram yoga. In addition, we are the first to report heart rate, oral body temperature, and hematocrit measures during a session of Bikram yoga. The major findings of this study suggest that the oxidative stress in participation of one session of Bikram yoga performed in its standard hot environment is not significantly different when performed in a thermoneutral environment based upon products of lipid peroxidation and glutathione oxidation. Changes in hematocrit were not significant. The practice of Bikram yoga in the hot condition revealed elevated heart rates and oral body temperatures throughout testing.

Results from the present study indicate that there were no significant main effects or interaction for the lipid peroxidation variable, TBARS. Though these findings were not statistically significant, oxidative stress levels based on lipid peroxidative plasma

TBARS returned back to baseline in a comparable time between both conditions. It is well established that acute aerobic exercise has the potential to result in increased free radical production (Ashton et al., 1998; Davies et al., 1982). Dufaux et al. (1997) did not observe increased levels of plasma TBARS in distance running for moderately trained participants. However, Bloomer et al. (2005) examined oxidative stress via TBARS and found no significant change in oxidative stress in aerobic (70%  $\text{VO}_{2\text{max}}$  cycling) and anaerobic exercise (squatting) for 30 minutes in moderately trained participants. Hyperthermic influence on the lipid peroxidation variables,  $\text{F}_2$ -isoprostanes (FIP) and lipid hydroperoxides (LPO) were investigated in low intensity exercise (running on a treadmill at 50%  $\text{VO}_{2\text{max}}$  for  $49.8 \pm 4.6$  minutes) (means  $\pm$  SD). (McAnulty et al., 2005). Contrary to our findings, McAnulty et al. (2005) found that a hyperthermic environment can further increased lipid peroxidation significantly. Both intensity and duration are components that can contribute to the production of free radical production resulting in oxidative stress (Bloomer, Davis, Consitt, & Wideman, 2007). Our findings that are conflicted to the literature is most likely due to a few factors including lower stress levels incurred due to mode of exercise (Bikram yoga versus running, cycling, and squatting), longer exposure to the heat, and the lipid peroxidation variables analyzed. Additionally, the present study evaluated 14 Bikram trained individuals, while the other studies evaluated 6 moderately trained males. The higher training status of our participants versus the moderately trained of the other study could have promoted better tolerance of exercise- induced oxidative stress and acclimatization to the heat. The majority of research investigating oxidative stress in acute exercise includes aerobic submaximal and

maximal efforts on the treadmill or cycle ergometer with no influence to environmental factors (Fisher-Wellman & Bloomer, 2009). The present study is the first to evaluate oxidative stress in an acute bout of Bikram yoga performed under hyperthermic exposure. We speculated no significant change in lipid peroxidation levels between CON and HOT for a session of Bikram yoga.

Likewise, the current study's findings reveal that GSH levels failed to reach significance between conditions, over time, or by means of interaction. A yoga training study (Sinha et al., 2007) found that GSH levels to increase after practicing for 6 months, therefore, increasing the participants ability to combat oxidative stressors. The Sinha et al. (2007) study included 60 members of the Indian navy that were divided into two groups, a yoga group and a control group that participated in the routine physical training exercises (30 minutes of jogging, 10 minutes of stretching, 5 minutes of pull-ups, and 15 minutes of games). Their findings reflect significant differences in GSH levels from pre-training and post-training. The novice yoga status of the participants may influence the levels of improvement. Because the participants in the current study are Bikram trained, they may already have an up regulated antioxidant capacity to counterbalance oxidative stress. These optimal GSH levels may explain why the current findings are not significant. While future research is necessary, we can speculate that the antioxidant defenses appear to be a sufficient balance between free radical production for the Bikram trained participants during the sessions of Bikram yoga. Though not significant, findings suggest that performing Bikram yoga in the heat could produce a better capacity to combat oxidative stress based upon GSH levels.



Hematocrit measurements were not significantly different over time or between conditions. The current protocol allowed participants to drink water ad libitum during both sessions of Bikram and were given water according to the amount of net body water lost post-session as recommended by ACSM (Sawka et al., 2007). This may convey why significant dehydration did not occur in the current study. Our findings were consistent with a study performed by Laitano et al. (2010) who investigated the effect of oxidative stress and other variables on rest and low-intensity exercise in a thermoneutral condition compared to a hyperthermic condition. They found that hematocrit measurements were not significantly different in the euhydrated participants. Based on hematocrit measurements and water consumption, our data suggests that the participants in the present study were euhydrated.

Additionally, Bikram yoga practice in the hot condition revealed elevated oral body temperatures throughout testing in comparison to the control condition. Oral body temperatures were significantly different between conditions over time at all measurements taken during class participation (0, 15, 30, 45, 60, 75, and 90 minutes). Oral body temperatures are responsive to changes in core body temperature, and were administered at the sublingual site for easy accessibility (Lim, Byrne, & Lee, 2008; Moran & Mendal, 2002).

Exercise produced mild hyperthermia in a thermoneutral environment regardless of mode of exercise, in regards to the Nassis & Gelanda study (2002) at 60%  $\text{VO}_{2\text{max}}$  either cycling or running for 90 minutes. Further, Ely et al. (2010) findings observed that

exercise in a modest hyperthermic environment can degrade aerobic performance due to the added heat stress.

Furthermore, any type of exercise, including yoga, typically leads to increased heart rate. Our findings revealed elevated heart rates throughout testing compared with thermoneutral when Bikram yoga was performed in the hot condition. Significance was affirmed at 45, 60, and 105 minutes dependent upon on the specific yoga posture and activity after the class ended. (Miles et al., 2013) examined the acute effects of one session of hatha yoga practice on blood pressure and other cardiovascular responses. Similarly, their findings revealed significant increases in heart rate and cardiac output during yoga practice for both novice and advanced participants, but without the added hyperthermic exposure. This confirms that hyperthermic demands require an additional increase in physiological adjustments when performing Bikram yoga. Moreover, (Miles et al., 2013) examined cardiovascular responses at each yoga posture. They found that the most significant differences in standing postures. Contrary to their findings, our significant changes in heart rate were evident for two postures that were performed on the floor (bow pose and rabbit pose, refer to Appendix II). These data suggest that heart rates can be variable specific to certain yoga postures. This may be due to the shift in blood flow from the core to the periphery of the body due to the compound effects of exercise and the hyperthermic environment. McAnulty et al. (2004) findings indicate that core temperatures and heart rates were significantly elevated in a hyperthermic environment versus a thermoneutral environment for low intensity exercise. Moreover, they also suggest that hyperthermia does increase oxidative stress and selectively affect lipid

oxidative markers. Further investigation is needed to make necessary conclusions regarding cardiovascular benefits associated with Bikram yoga. In addition, Bikram yoga is a very low impact exercise that may be considered an aerobic alternative to possibly acquire cardiorespiratory improvements for individuals that are unable to participate in higher impact activities.

### Conclusions

It is well established that hyperthermic exposure is capable of causing increased free radical production (Flanagan, Moseley, & Buettner, 1998). However, in the present study, the lack of patterns of change observed in TBARS, GSH, and hematocrit indicates that the trained participants did not undergo significant oxidative stress regardless of increased oral body temperature or heart rates from the practice of Bikram yoga in the heat. Explanation of no significant increases in oxidative stress and no threats of dehydration and safety occurring during this study perhaps may be due to the experience and acclimatization to the practice. Performing Bikram yoga increases the likelihood of heat stress, therefore, more precaution may be necessary for untrained individuals. Future research is necessary for better clinical understanding and appreciation for the benefits Bikram yoga.



Department of Kinesiology and Nutrition Sciences

**INFORMED CONSENT FORM**

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**TITLE OF STUDY:** Oxidative stress for an acute bout of Bikram Yoga in healthy, trained adults

**INVESTIGATOR(S):** Dr. Tony Santo, Dr. James Navalta, Kimberly Trocio, Paul Hafen, Michael Jarrett, Krystina Moschella

**CONTACT INFORMATION:** If you have any questions or concerns about the study, please contact Dr. Santo at (702) 895-5329 or Kimberly Trocio at (702)895-2069.

For questions regarding the rights of research subjects, any complaints or comments regarding the manner in which the study is being conducted you may contact the **UNLV Office of Research Integrity – Human Subjects Research** at (702) 895-2794 or toll free at 877-895-2794 or via email at [IRB@unlv.edu](mailto:IRB@unlv.edu).

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**Purpose of the Study**

The purpose of this study is to examine the effect of one Bikram Yoga session on oxidative stress in standard conditions (105°F or 40.6°C, 40% humidity) in comparison with a thermally neutral environment (72°F or 22.2°C, 50% humidity) in healthy, trained adults.

**Participants**

You are being asked to participate in the study because you fit these criteria: you are a healthy adult, age 18-45 years and free of medical conditions that limit physical activity. You must be practicing Bikram Yoga at least 3 times per week and have been doing so for the past 3 months. The Physical Activity Readiness Questionnaire will be used to screen potential study participants who are considered at risk for medical complications. If you are considered well below average for percent body fat, according to the American College of Sports Medicine Guidelines, you will be excluded from the study. If you take antioxidant supplements, including Vitamins A, C, E, or selenium or have been taking these supplements within the last 3 months you are prohibited from participation. Supplementation of these antioxidants will affect the measurements of oxidative stress. Women who are pregnant or those that think they may be pregnant may not participate. Participation is contingent upon the research team's review of your criteria to participate.

**Procedures**

If you volunteer to participate in this study, you will be asked to do the following: you will report to the UNLV Human Performance Laboratory, building MPE room # 312 on two separate days, allowing a minimum of three days between visits. Please allow 2.5 hours for each day of testing. For each day of testing, you will be asked to: (1) fast 8-12 hours prior to testing, (2) not exercise within 12 hours of the test, (3) not consume alcohol within 48 hours of the test, and (4) not ingest beverages containing

Participant Initials \_\_\_\_\_

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*Approved by the UNLV IRB. Protocol 1203-4087*

*Received: 10-09-12 Approved: 05-22-12 Expiration: 05-21-13*

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caffeine on the day of assessment. It is recommended that you bring your own yoga mat and towel for testing.

All testing will be conducted in a controlled environmental chamber where room temperature and humidity are regulated. Heating and humidifier/dehumidifier units will provide for regulation inside the chamber. The chamber capacity is three participants.

During your two separate visits you will participate in one of two different conditions, the experimental and control, in which you will be randomly placed. For the experimental condition, you will participate in a one bout Bikram Yoga Class in the environmental chamber at the standard Bikram Yoga temperature of 40.6°Celsius (105°Fahrenheit) and 40% humidity. In contrast, the control condition will require for you to participate in a one bout Bikram Yoga Class in the environmental chamber at a neutral, room temperature of 22.2°Celsius (72°Fahrenheit) and 50% humidity.

On the first day, the test will begin with height and weight measurements followed by an assessment of body fatness using bioelectrical impedance analysis.

*Bioelectrical Impedance Analysis Procedure:* You will lie on your back on a padded training table with the right hand and right foot exposed. Two electrodes will be attached to your hand and foot and a very low grade electrical current (from a 9 volt dry cell battery) will pass between the electrodes. This current cannot be felt nor is it harmful unless you have a *pacemaker*. The current is resisted by body water and is reported as ohms of resistance. This is introduced into an equation to give percent body fat. The procedure takes approximately 5 minutes.

On both days, you will wear a Polar Heart Rate Monitor for duration of the Bikram Yoga Class. Starting at Pre-test and at every 15 minute interval for the class duration, your heart rate and core body temperature will be recorded. To obtain core body temperature measurements, we will administer an oral thermometer. The oral thermometer will be placed in your mouth under your tongue. After closing your mouth, it will take approximately 30-60 seconds to obtain a measurement. While waiting for the measurement, you will continue your class participation. A bottle of water will be distributed to you before the class begins to drink ad libitum (at your pleasure) during class participation.

"Bikram's Beginning Yoga Class" CD will be provided for class instruction for both conditions for each day of testing. You will listen and participate along with the recording of the Bikram Yoga Class for each day of testing. To complete the study, you are recommended to participate for the entire 90

Participant Initials \_\_\_\_\_  
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Oxidative stress for an acute bout of Bikram Yoga in healthy, trained adults  
Department of Kinesiology and Nutrition Sciences  
University of Nevada, Las Vegas  
Dr. Santo: (702) 895-5329

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minutes of instruction while inside the environmental chamber. However, you may choose to withdraw from testing at any time you feel uncomfortable.

Blood samples of 600 $\mu$ L (about 1/2 teaspoon per blood draw) will be drawn from your fingertips. Three blood draws will be taken before the class begins, immediately after the class ends, and 30 minutes after the class ends during both visits. A member of the research team will draw all blood samples. The member(s) of the research team will monitor you for the duration of testing. After the class ends and the second blood draw complete, you may rest (sitting or lying down) in room temperature outside of the environmental chamber. From that time until the final blood draw, you will be required to consume a specified amount of water (equivalent to your total body water lost). Total body water lost is determined by measuring your body weight again after the class ends and measuring the amount of water you consume during the class.

You will be provided with snacks and refreshments after each visitation. After 3 days, you may participate in the other condition following the same protocol. Please wear the same clothing as you did on the first day.

Your blood samples will be stored until all blood collection for this research study is complete or for no longer than 3 months. Your blood sample will only be used for analysis that is described in this protocol and for no other study. Any remaining samples will be destroyed and disposed.

#### **Benefits of Participation**

There may be little direct benefits to you as a participant in this study. However, you will come away knowing your height, weight, and percent body fat. You will have the opportunity to participate in 2 Bikram Yoga classes and will be able to compare the differences in conditions. Blood analysis will provide you with your red blood cell count, which can indicate how well hydrated you are.

There has been very limited research regarding the safety and efficacy of Bikram Yoga. Because Bikram Yoga and other hot exercise classes are a growing fitness trend, it is necessary to provide valuable scientific knowledge to the participants. This study will provide the opportunity to explore Bikram Yoga at a molecular level by examining oxidative stress biomarkers. Without supportive scientific evidence, we may never know the benefit, if any, practiced in Bikram Yoga. This study may provide a path to future studies regarding Bikram Yoga.

Participant Initials \_\_\_\_\_  
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Oxidative stress for an acute bout of Bikram Yoga in healthy, trained adults  
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**Risks of Participation**

Because you are Bikram trained, this study involves minimal risk to you. Activity in a hot environment for a long period of time may elicit symptoms of heat stress including light-headedness, dizziness, nausea, faintness, fatigue, headache, cool, dry skin, and inability to sweat. The CPR and AED Certified Members of the research team will closely monitor you of any symptoms of heat stress at all times while testing. If symptoms do occur, you will be removed from the hot environment immediately, provided water, cooled off by applying cool, wet cloths on your head, back, and neck, and an electric fan will be placed directly facing you. During blood draws, you may feel pain or discomfort from the puncture of the needle and from collection. Though not common, bruising and soreness of the fingertips at the location of needle insertion may be experienced hours to days after blood collection. Bruises are usually harmless and will disappear with time. If symptoms persist or swelling occurs, it is highly suggested to seek advice from a medical professional.

Prior to testing, you should have been notified to stay well hydrated and maintain a normal diet the days leading up to the test. Testing is scheduled in the mornings to ensure minimal amount of time fasted. Water will be provided during testing, and snacks and refreshments will be provided after each visit to ensure proper recovery. In addition, you are advised to drink plenty of fluids, maintain a normal diet, and to avoid physical, strenuous activity for the 24 hours after participation at each visit.

**Cost /Compensation**

There will not be any financial cost to you to participate in this study. The study will take 5 hours of your time (2.5 hours for 2 days); however there is no compensation for your time. The University of Nevada, Las Vegas may not provide compensation or free medical care for an unanticipated injury sustained as a result of participating in this research study.

**Contact Information**

If you have any questions or concerns about the study, you may contact Dr. Tony Santo at antonio.santo@unlv.edu. For questions regarding the rights of research subjects, any complaints or comments regarding the manner in which the study is being conducted you may contact the UNLV Office of Research Integrity – Human Subjects at 702-895-2794 or toll free at 877-895-2794 or via email at IRB@unlv.edu.

Participant Initials \_\_\_\_\_  
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**Voluntary Participation**

Your participation in this study is completely voluntary. You may refuse to participate in this study or in any part of this study and you may withdraw at any time without prejudice to your relations with the University. You are encouraged to ask questions about this study prior to the beginning or at any time during the study. You will be given a copy of this form.

**Confidentiality**

All information gathered in this study will be kept completely confidential. Only those persons who are directly related to this study (i.e.: researchers, data analysts) will have access to your data. No reference will be made in written or oral materials, which could link you to this study. All records will be stored in the laboratory for a period of 3 years. After 3 years, any documentation with identifiable information (e.g., name) will be destroyed. Unidentifiable data will be stored in locked storage indefinitely.

**Freedom of Consent:**

I have read the above information carefully and I am aware of the tests/procedures to be performed. Knowing these risks and having the opportunity to ask questions, I agree (consent) to participate in this study. I understand that I have a right to withdraw from this study at any time without prejudice. I am at least 18 years old and a copy of the informed consent has been given to me.

\_\_\_\_\_  
Signature of Participant

\_\_\_\_\_  
Date

\_\_\_\_\_  
Participant Name (Please Print)

\_\_\_\_\_  
Signature of Witness

\_\_\_\_\_  
Date

Participant Initials \_\_\_\_\_  
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Oxidative stress for an acute bout of Bikram Yoga in healthy, trained adults  
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## Appendix II BIKRAM YOGA POSES: “ASANAS”



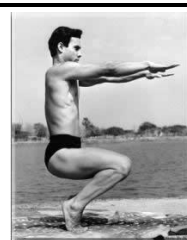
Standing Deep Breathing



Half Moon Pose



Hands to Feet



Awkward Pose



Eagle Pose



Standing Head to Knee



Standing Bow Pose



Balancing Stick



Standing Separate



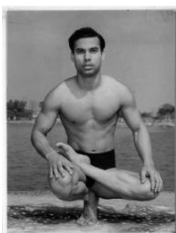
Triangle Pose



Standing Separate Leg



Tree Pose



Toe Stand



Dead Body Pose



Wind-Removing Pose



Cobra Pose



Locust Pose



Full Locust Pose



Bow Pose Fixed



Firm Pose



Half Tortoise Pose



Camel Pose



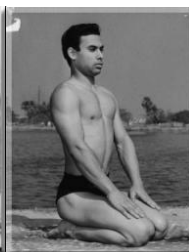
Rabbit Pose



Head to Knee Pose  
and Stretching Pose



Spine-Twisting  
Pose



Blowing in  
Firm Pose

Retrieved from: <http://www.bikramyoga.com/BikramYoga/TwentySixPostures.php>

Physical Activity Readiness  
Questionnaire - PAR-Q  
(revised 2002)

# PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
<input type="checkbox"/>	<input type="checkbox"/>	1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?
<input type="checkbox"/>	<input type="checkbox"/>	2. Do you feel pain in your chest when you do physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	3. In the past month, have you had chest pain when you were not doing physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
<input type="checkbox"/>	<input type="checkbox"/>	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
<input type="checkbox"/>	<input type="checkbox"/>	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
<input type="checkbox"/>	<input type="checkbox"/>	7. Do you know of any other reason why you should not do physical activity?

If  
you  
answered

## YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

## NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active — begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal — this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

### DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever — wait until you feel better; or
- if you are or may be pregnant — talk to your doctor before you start becoming more active.

**PLEASE NOTE:** If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

**Informed Use of the PAR-Q:** The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

**No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.**

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME \_\_\_\_\_

SIGNATURE \_\_\_\_\_

DATE \_\_\_\_\_

SIGNATURE OF PARENT \_\_\_\_\_

WITNESS \_\_\_\_\_

or GUARDIAN (for participants under the age of majority)

**Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.**



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continued on other side...



...continued from other side

# PAR-Q & YOU

Physical Activity Readiness  
Questionnaire - PAR-Q  
(revised 2002)

**CANADA'S**  
**Physical Activity Guide**  
to Healthy Active Living

**Physical activity improves health.**

Every little bit counts, but more is even better – everyone can do it!

Get active your way – build physical activity into your daily life...

- at home
- at school
- at work
- at play
- on the way ...that's active living!

**Endurance**  
4-7 days a week  
Continuous activities for your heart, lungs and circulatory system.

**Flexibility**  
4-7 days a week  
Gentle reaching, bending and stretching activities to keep your muscles relaxed and joints mobile.

**Strength**  
2-4 days a week  
Activities against resistance to strengthen muscles and bones and improve posture.

Starting slowly is very safe for most people. Not sure? Consult your health professional.

For a copy of the *Guide Handbook* and more information:  
1-888-334-9769, or  
[www.paguide.com](http://www.paguide.com)

Eating well is also important. Follow *Canada's Food Guide to Healthy Eating* to make wise food choices.

## Get Active Your Way, Every Day – For Life!

Scientists say accumulate 60 minutes of physical activity every day to stay healthy or improve your health. As you progress to moderate activities you can cut down to 30 minutes, 4 days a week. Add-up your activities in periods of at least 10 minutes each. Start slowly... and build up.

**Time needed depends on effort**

Very Light Effort	Light Effort	Moderate Effort	Vigorous Effort	Maximum Effort
60 minutes	30-60 minutes	20-30 minutes		
• Strolling • Dusting	• Light walking • Volleyball • Easy gardening • Stretching	• Brisk walking • Biking • Raking leaves • Swimming • Dancing • Water aerobics	• Aerobics • Jogging • Hockey • Basketball • Fast swimming • Fast dancing	• Sprinting • Racing

**Range needed to stay healthy**

## You Can Do It – Getting started is easier than you think

Physical activity doesn't have to be very hard. Build physical activities into your daily routine.

- Walk whenever you can – get off the bus early, use the stairs instead of the elevator.
- Reduce inactivity for long periods, like watching TV.
- Get up from the couch and stretch and bend for a few minutes every hour.
- Play actively with your kids.
- Choose to walk, wheel or cycle for short trips.
- Start with a 10 minute walk – gradually increase the time.
- Find out about walking and cycling paths nearby and use them.
- Observe a physical activity class to see if you want to try it.
- Try one class to start – you don't have to make a long-term commitment.
- Do the activities you are doing now, more often.

## Benefits of regular activity: Health risks of inactivity:

Benefits of regular activity:	Health risks of inactivity:
<ul style="list-style-type: none"> <li>• better health</li> <li>• improved fitness</li> <li>• better posture and balance</li> <li>• better self-esteem</li> <li>• weight control</li> <li>• stronger muscles and bones</li> <li>• feeling more energetic</li> <li>• relaxation and reduced stress</li> <li>• continued independent living in later life</li> </ul>	<ul style="list-style-type: none"> <li>• premature death</li> <li>• heart disease</li> <li>• obesity</li> <li>• high blood pressure</li> <li>• adult-onset diabetes</li> <li>• osteoporosis</li> <li>• stroke</li> <li>• depression</li> <li>• colon cancer</li> </ul>

Health Canada Santé Canada

Canadian Society for Exercise Physiology



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Source: Canada's Physical Activity Guide to Healthy Active Living, Health Canada, 1998 <http://www.hc-sc.gc.ca/hppb/paguide/pdf/guideEng.pdf>

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## FITNESS AND HEALTH PROFESSIONALS MAY BE INTERESTED IN THE INFORMATION BELOW:

The following companion forms are available for doctors' use by contacting the Canadian Society for Exercise Physiology (address below):

The **Physical Activity Readiness Medical Examination (PARmed-X)** – to be used by doctors with people who answer YES to one or more questions on the PAR-Q.

The **Physical Activity Readiness Medical Examination for Pregnancy (PARmed-X for Pregnancy)** – to be used by doctors with pregnant patients who wish to become more active.

## References:

- Arraiz, G.A., Wigle, D.T., Mao, Y. (1992). Risk Assessment of Physical Activity and Physical Fitness in the Canada Health Survey Follow-Up Study. *J. Clin. Epidemiol.* 45:4 419-428.
- Mottola, M., Wolfe, L.A. (1994). Active Living and Pregnancy. In: A. Quinney, L. Gauvin, T. Wall (eds.), **Toward Active Living: Proceedings of the International Conference on Physical Activity, Fitness and Health**. Champaign, IL: Human Kinetics.
- PAR-Q Validation Report, British Columbia Ministry of Health, 1978.
- Thomas, S., Reading, J., Shephard, R.J. (1992). Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can. J. Sport Sci.* 17:4 338-345.

For more information, please contact the:

Canadian Society for Exercise Physiology  
202-185 Somerset Street West  
Ottawa, ON K2P 0J2  
Tel. 1-877-651-3755 • FAX (613) 234-3565  
Online: [www.csep.ca](http://www.csep.ca)

The original PAR-Q was developed by the British Columbia Ministry of Health. It has been revised by an Expert Advisory Committee of the Canadian Society for Exercise Physiology chaired by Dr. N. Gledhill (2002).

Disponible en français sous le titre «Questionnaire sur l'aptitude à l'activité physique - Q-AAP (révisé 2002)».



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## ***Appendix IV BODY COMPOSITION***

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### **ACSM Body Composition (% Body Fat) For Males and Females**

#### **Male**

<b>Percentile</b>	<b>Fitness Category</b>	<b>AGE</b>				
		<b>20-29</b>	<b>30-39</b>	<b>40-49</b>	<b>50-59</b>	<b>60+</b>
90	Well Above Average	7.1-11.7	11.3-15.8	13.6-18	15.3-19.7	15.3-20.7
70	Above Average	11.8-15.8	15.9-18.9	18.1-21	19.8-22.6	20.8-23.4
50	Average	15.9-19.4	19-22.2	21.1-24	22.7-25.6	23.5-26.6
30	Below Average	19.5-25.8	22.3-27.2	24.1-28.8	25.7-30.2	26.7-31.1
10	Well Below Average	25.9	27.3	28.9	30.3	31.2

#### **Female**

<b>Percentile</b>	<b>Fitness Category</b>	<b>20-29</b>	<b>30-39</b>	<b>40-49</b>	<b>50-59</b>	<b>60+</b>
90	Well Above Average	14.5-18.9	15.5-19.9	18.5-23.4	21.6-26.5	21.1-27.4
70	Above Average	19-22	20-23	23.5-26.3	26.6-30	27.5-30.8
50	Average	22.1-25.3	23.1-26.9	26.4-30	30.1-33.4	30.9-34.2
30	Below Average	25.4-32	27-32.7	30.1-34.9	33.5-37.8	34.3-39.2
10	Well Below Average	32.1	32.8	35	37.9	39.3

\*Data provided by the Institute for Aerobics Research, Dallas, TX (1994). Study population for the data set was predominately White and college educated.

*Taken from ACSM'S Health-Related Physical Fitness Assessment Manual, 2ndEd. 2008. pg. 59.*

## APPENDIX V TBARS ASSAY

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### *Cayman Chemical*

#### Pre-Assay Preparation

##### *Reagent Preparation*

1. Thiobarbituric Acid (TBA)  
The vial contains 2 g of TBA. It is ready to use to prepare the Color Reagent
2. TBA Acetic Acid  
Each vial contains 20 ml of concentrated acetic acid. Slowly add both vials (40ml) of TBA Acetic Acid to 160 ml of HPLC-grade water. This diluted Acetic Acid Solution is used in preparing the Color Reagent. The diluted Acetic Acid Solution is stable for at least three months at room temperature.
3. TBA Sodium Hydroxide (10X)  
The vial contains a solution of sodium hydroxide (NaOH). Dilute 20 ml of TBA NaOH with 180 ml of HPLC-grade water. This diluted NaOH Solution is used in preparing the Color Reagent. The diluted NaOH Solution is stable for at least three months at room temperature. Store the diluted NaOH Solution in a plastic container suitable for corrosive materials.
4. TBA Malondialdehyde Standard  
The vial contains 500  $\mu$ M Malondialdehyde (MDA) in water. It is ready to use to prepare the standard curve.
5. TBA SDS Solution  
The vial contains a solution of sodium dodecyl sulfate (SDS). The solution is ready to use as supplied.
6. To prepare the Color Reagent:  
The following amount of Color Reagent is sufficient to evaluate 24 samples. Adjust the volumes accordingly if more or less samples are going to be assayed. Weigh 530 mg of TBA and add to  $\geq$  150 ml of diluted TBA Acetic Acid Solution. Add 50 ml of diluted TBA Sodium Hydroxide and mix until the TBA is completely dissolved. The solution is stable for 24 hours.

##### *Sample Preparation*

###### Plasma

1. Collect blood using an anticoagulant such as heparin, EDTA, or citrate.
2. Centrifuge the blood at 700-1,000 x g for 10 minutes at 4°C. Pipette off the top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice. If not assaying the same day, freeze at -80°C. The plasma sample will be stable for one month while stored at -80°C.
3. Plasma does not need to be diluted before assaying.

### Performing the Assay

1. Label vial caps with standard number or sample identification number
2. Add 100  $\mu$ l of sample or standard to appropriately labeled 5 ml vial.
3. Add 100  $\mu$ l of SDS Solution to vial and swirl to mix.
4. Add 4 ml of the Color Reagent forcefully down side of each vial.
5. Cap vials and place vials in foam or some other holder to keep the tubes upright during boiling.
6. Add vials to vigorously boiling water. Boil vials for one hour.
7. After one hour, immediately remove the vials and place in ice bath to stop reaction. Incubate on ice for 10 minutes
8. After 10 minutes, centrifuge the vials for 10 minutes at 1,600 x g at 4°C. Vials may appear clear or cloudy. Cloudiness will clear upon warming to room temperature.
9. Vials are stable at room temperature for 30 minutes.
10. Load 150  $\mu$ l (in duplicate) from each vial to either the clear plate (calorimetric version) or to the black plate (fluorometric version).
11. Read the absorbance at 530-540nm or read fluorescence at an excitation wavelength of 530nm and an emission wavelength of 550nm.

## **APPENDIX VI GSH ASSAY**

### ***MBL International Corporation***

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#### Pre-Assay Preparation

##### *Reagent Preparation and Storage*

1. OPA Probe, Reducing Agent Mix, GSH Quencher  
Dissolve in 1.05 ml of dH<sub>2</sub>O. Pipette up and down to completely dissolve. Store at -20°C. Use within two months.
2. GSH Standard  
Accurately dissolve in 45 µl H<sub>2</sub>O, then add 5 µl of PCA to stabilize the standard GSH stock solution (20 µg/µl). Store at -20°C. Use within two months.

##### *Sample Collection and Storage*

##### Serum or Other Liquid Samples

Add 20 µl 6N PCA to 60 µl samples, vortex to mix, keep on ice for 5 minutes, and then spin for 5 minutes at top speed at 4°C. Collect supernatant. The sample can then be stored at -80°C, stable for a month.

#### Performing Assay

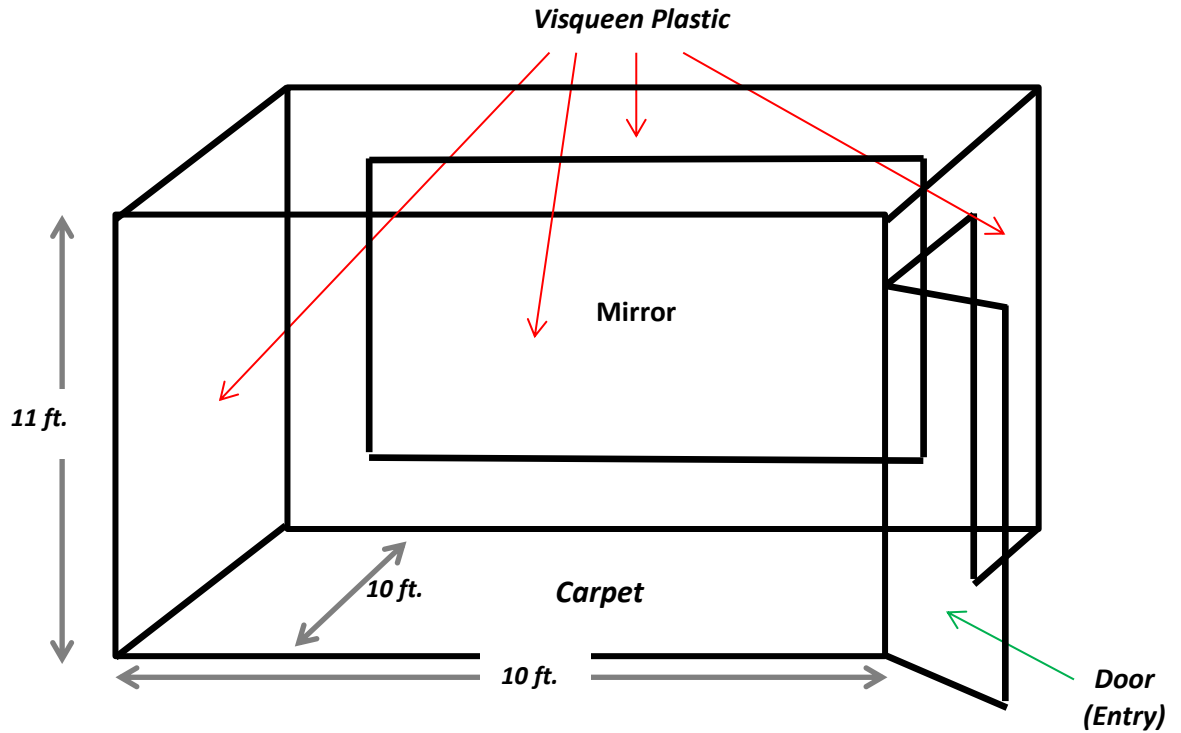
1. Standard Curve: Add 10 µl of the 20 µg/µl standard GSH stock into 990 µl of Assay Buffer to generate 0.2 µg/µl of working standard solution. Add 0, 2, 4, 6, 8, 10 µ into a 96-well plate to generate 0, 0.4, 0.8, 1.2, 1.6, 2.0 µg/µl standard GSH. Bring the volume to 90 µl with Assay Buffer.
2. Preparation of Samples for Assays: Add 20 µl of the 3N KOH to 40 µl of PCA preserved samples to neutralize the samples. Keep on ice for 5 minutes and then spin for 5 minutes at top speed at 4°C. Transfer the supernatant into new tubes. Add 10 µl of the neutralized samples into 96-well plate. Bring the sample volume to 90 µl with Assay Buffer.
3. Assay: Add 10 µl of OPA Probe into the standard and sample wells, mix well, incubate at room temperature for 40 minutes. Read the samples and standards on a fluorescence plate reader equipped with Ex/Em = 340/450 nm.
4. Calculations: Subtract background reading from all standard and sample readings. Plot RFU vs. standard GSH amount. Apply the sample readings to the standard curve to get glutathione amount in each sample.

- a. Glutathione Concentration =  $G_a/S_v$ , where  $G_a$  is the glutathione amount from standard curve and  $S_v$  is the sample volume added to the sample wells.



## **APPENDIX VII ENVIRONMENTAL CHAMBER**

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- Dimensions: 10ft x 10ft x 11ft room located at MPE 318 at UNLV
- Visqueen plastic sheeting completely lined the walls and ceiling of the chamber. Carpet was installed for the flooring of the chamber and a mirror was installed on one wall (requirements for Bikram Yoga).



Tony Santo Photography

## APPENDIX VIII DATA TABLES

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### TBARS

<i>Condition</i>	<i>Time</i>		
	<u>Pre-test</u>	<u>Post-test</u>	<u>Post-30</u>
CON	20.93±0.46	20.39±0.36	20.65±0.39
HOT	20.83±0.39	21.08±0.36	20.83±0.27

---

Values are means  $\pm$  SEM ( $n=14$ )

### GLUTATHIONE

<i>Condition</i>	<i>Time</i>		
	<u>Pre-test</u>	<u>Post-test</u>	<u>Post-30</u>
CON	360.38±18.21	349.33±24.35	374.89±26.77
HOT	359.58±28.34	325.44±15.89	347.50±14.74

---

Values are means  $\pm$  SEM ( $n=14$ )

### HEMATOCRIT

<i>Condition</i>	<i>Time</i>		
	<u>Pre-test</u>	<u>Post-test</u>	<u>Post-30</u>
CON	42.58±0.83	41.33±0.75	41.18±0.82
HOT	40.71±1.52	40.93±0.98	41.97±1.04

---

Values are means  $\pm$  SEM ( $n=14$ )

**HEART RATE** Values are means  $\pm$  SEM (n=14)

<i>Condition</i>	<i>Time</i>								
	<u>0</u>	<u>15</u>	<u>30</u>	<u>45</u>	<u>60*</u>	<u>75*</u>	<u>90</u>	<u>105*</u>	<u>120</u>
<b>CON</b>	72 $\pm$ 2.2	88 $\pm$ 3.5	97 $\pm$ 4.7	93 $\pm$ 3.9	84 $\pm$ 4.2	78 $\pm$ 3.1	67 $\pm$ 3.3	70 $\pm$ 2.2	69 $\pm$ 5.4
<b>HOT</b>	69 $\pm$ 2.5	95 $\pm$ 3.4	101 $\pm$ 3.0	101 $\pm$ 4.7	100 $\pm$ 3.2	89 $\pm$ 4.1	74 $\pm$ 3.7	77 $\pm$ 2.4	72 $\pm$ 4.7

**ORAL BODY TEMPERATURE** Values are means  $\pm$  SEM (n=14)

<i>Condition</i>	<i>Time</i>								
	<u>0</u>	<u>15</u>	<u>30</u>	<u>45</u>	<u>60</u>	<u>75</u>	<u>90</u>	<u>105</u>	<u>120</u>
<b>CON</b>	36.2 $\pm$ 0.12	36.4 $\pm$ 0.06	36.2 $\pm$ 0.14	36.5 $\pm$ 0.06	36.5 $\pm$ 0.06	36.3 $\pm$ 0.15	36.4 $\pm$ 0.05	36.2 $\pm$ 0.10	36.2 $\pm$ 0.07
<b>HOT</b>	36.3 $\pm$ 0.06	36.7 $\pm$ 0.05	36.7 $\pm$ 0.06	36.8 $\pm$ 0.07	36.9 $\pm$ 0.06	37 $\pm$ 0.06	36.8 $\pm$ 0.05	36.4 $\pm$ 0.10	36.3 $\pm$ 0.07

**WATER CONSUMPTION AND LOSS** Values are means  $\pm$  SEM (n=14)

	H2O consumed (ml)	Weight loss (kg)	H2O given (ml)
<b>CON</b>	76.36 $\pm$ 32.77	0.46 $\pm$ 0.14	181.071 $\pm$ 54.76
<b>HOT</b>	212.07 $\pm$ 47.89	0.92 $\pm$ 0.27	346.21 $\pm$ 95.26

## **APPENDIX IX STATISTICAL ANALYSES**

---

Condition: TBARS

Analysis: 2 (cond) by 3 (time) mixed model ANOVA

### **Tests of Within-Subjects Effects**

Source	SS	df	MS	F	Sig.	Effect size	Power
Time	.387	2	.194	.121	.886	.005	.068
Time * Condition	2.205	2	1.103	.688	.507	.026	.160
Error(Time)	83.273	52	1.601				

a. Computed using alpha = .05

### **Tests of Between-Subjects Effects**

Source	SS	df	MS	F	Sig.	Effect size	Power
Condition	1.407	1	1.407	.514	.480	.019	.106
Error	71.133	26	2.736				

a. Computed using alpha = .05

No significant main effects or interactions

Condition: GSH

Analysis: 2 (cond) by 3 (time) mixed model ANOVA

**Tests of Within-Subjects Effects**

Source	SS	df	MS	F	Sig.	Effect size	Power
Time	.010	2	.005	1.890	.161	.068	.375
Time *	.003	2	.001	.531	.591	.020	.133
Condition							
Error(Time)	.140	52	.003				

a. Computed using alpha = .05

**Tests of Between-Subjects Effects**

Source	SS	Df	MS	F	Sig.	Effect size	Power
Condition	.006	1	.006	.414	.525	.016	.095
Error	.390	26	.015				

a. Computed using alpha = .05

No significant main effects or interactions

## HEMATOCRIT

### Tests of Within-Subjects Effects

Measure: MEASURE\_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power <sup>a</sup>
Time	Huynh-Feldt	4.369	1.450	3.013	.445	.581	.017	.108
Time *	Huynh-Feldt	25.047	1.450	17.271	2.552	.106	.089	.409
Condition Error(Time)	Huynh-Feldt	255.209	37.706	6.768				

a. Computed using  
alpha = .05

### Tests of Between-Subjects Effects

Measure: MEASURE\_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power <sup>a</sup>
Condition	5.042	1	5.042	.148	.704	.006	.148	.066

a. Computed using alpha = .05

### Pairwise Comparisons

Measure: MEASURE\_1

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig. <sup>a</sup>	95% Confidence Interval for Difference <sup>a</sup>	
					Lower Bound	Upper Bound
1	2	.515	.673	.835	-1.203	2.233
	3	.070	.701	1.000	-1.717	1.857
2	1	-.515	.673	.835	-2.233	1.203
	3	-.445	.328	.463	-1.283	.393
3	1	-.070	.701	1.000	-1.857	1.717
	2	.445	.328	.463	-.393	1.283

Based on estimated marginal means

a. Adjustment for multiple comparisons: Sidak.

No significant main effects or interactions

## HEART RATE

### Mauchly's Test of Sphericity<sup>a</sup>

Measure: MEASURE\_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon <sup>b</sup>		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
<b>Time</b>	.148	44.259	35	<b>.143</b>	.702	.953	.125

Mauchley's Test result = not significant → sphericity is assumed.

### Tests of Within-Subjects Effects

Measure: MEASURE\_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power <sup>a</sup>
<b>Time</b>	Sphericity Assumed	32691.913	8	4086.489	<b>49.494</b>	<b>.000</b>	.656	395.949	1.000
<b>Time * Condition</b>	Sphericity Assumed	1511.484	8	188.936	<b>2.288</b>	<b>.023</b>	.081	18.306	.871
Error(Time)	Sphericity Assumed	17173.714	208	82.566					

a. Computed using alpha = .05

### Tests of Between-Subjects Effects

Measure: MEASURE\_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power <sup>a</sup>
<b>Condition</b>	3038.194	1	3038.194	<b>3.851</b>	<b>.061</b>	.129	3.851	.472
Error	20513.500	26	788.981					

a. Computed using alpha = .05

The variable "Time" was significant ( $F_{8,208} = 49.494$ ,  $p < .001$ ).

The variable "Condition" was not significant ( $F_{1,26} = 3.851$ ,  $p = .023$ ).

The interaction was significant ( $F_{8,208} = 2.288$ ,  $p = .061$ )

### Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
0	Equal variances assumed	.099	.755	.806	26	.427	2.64286	3.27812
15	Equal variances assumed	.025	.876	-1.536	26	.137	-7.57143	4.93092
30	Equal variances assumed	5.485	.027	-.822	26	.419	-4.57143	5.56304
45	Equal variances assumed	1.058	.313	-1.322	26	.198	-8.00000	6.04990
60	Equal variances assumed	1.621	.214	-3.029	26	.005	-16.00000	5.28207
75	Equal variances assumed	2.096	.160	-2.243	26	.034	-11.57143	5.15985
90	Equal variances assumed	.110	.742	-1.411	26	.170	-6.92857	4.91054
105	Equal variances assumed	.023	.882	-2.248	26	.033	-7.21429	3.20964
120	Equal variances assumed	.882	.356	-.834	26	.412	-3.28571	3.94009

### ORAL BODY TEMPERATURE

#### Mauchly's Test of Sphericity<sup>a</sup>

Measure: MEASURE\_1

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon <sup>b</sup>		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
<b>Time</b>	.062	64.184	35	<b>.002</b>	.590	.764	.125



### Tests of Within-Subjects Effects

Measure: MEASURE\_1

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power <sup>a</sup>
<b>Time</b>	Huynh-Feldt	6.610	6.113	1.081	11.635	<b>.000</b>	.309	1.000
<b>Time *</b>	Sphericity	1.897	8	.237	3.339	<b>.001</b>	.114	.973
<b>Condition</b>	Assumed							
	Sphericity	14.770	208	.071				
Error(Time)	Assumed							
	Huynh-Feldt	14.770	158.940	.093				

a. Computed using alpha = .05

### Tests of Between-Subjects Effects

Measure: MEASURE\_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power <sup>a</sup>
<b>Condition</b>	6.736	1	6.736	22.690	<b>.000</b>	.466	.996
Error	7.718	26	.297				

a. Computed using alpha = .05

The variable “Time” was significant ( $F_{6.113, 158.94} = 11.64, p < .001$ ).  
The variable “Condition” was significant ( $F_{1, 26} = 22.69, p < .001$ ).  
The interaction was significant ( $F_{8, 208} = 3.34, p = .001$ ).

simple main effects analysis

**Independent Samples Test**

	Levene's Test for Equality of Variances		t-test for Equality of Means				
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
0	2.557	.122	-.628	26	.536	-.08571	.13654
<b>15</b>	.238	.630	-3.914	26	<b>.001</b>	-.30714	.07847
<b>30</b>	1.090	.306	-3.046	26	<b>.005</b>	-.45000	.14773
<b>45</b>	.501	.485	-3.577	26	<b>.001</b>	-.33571	.09387
<b>60</b>	.653	.426	-4.394	26	<b>.000</b>	-.35000	.07966
<b>75</b>	.600	.446	-4.255	26	<b>.000</b>	-.70714	.16619
<b>90</b>	.018	.894	-5.411	26	<b>.000</b>	-.35857	.06627
105	.207	.653	-1.597	26	.122	-.22143	.13863
120	.163	.690	-1.238	26	.227	-.12714	.10274

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