The Effect of Wild Blueberry Consumption on the Inflammatory Response, Oxidative Stress and DNA Damage Associated with Exercise

Taylor K. Bloedon

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THE EFFECT OF WILD BLUEBERRY CONSUMPTION ON THE
INFLAMMATORY RESPONSE, OXIDATIVE STRESS AND
DNA DAMAGE ASSOCIATED WITH EXERCISE

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A DISSERTATION
Submitted in Partial Fulfillment of the
Requirements of the Degree of
Doctor of Philosophy
(in Food and Nutrition Sciences)

The Graduate School
The University of Maine
December 2013

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On behalf of the Graduate Committee for Taylor Bloedon I affirm this manuscript is the final and accepted dissertation. Signatures of all committee members are on file with the Graduate School at the University of Maine, 42 Stodder Hall, Orono, Maine.

_________________________________________________________

Dorothy J. Klimis-Zacas, Ph.D. December 2, 2013
ACKNOWLEDGEMENTS

I would first like to thank my advisor, Dr. Dorothy Klimis-Zacas and my co-advisor, Dr. Robert Lehnhard. With their direct guidance and support I was able to obtain funding for my research, as well as funding through the Kinesiology and Physical Education Department to support me as a graduate student. I cannot begin to express how much I value the knowledge and expertise both advisors have shared with me throughout this process. Perhaps most of all, I am thankful for their willingness to accept me for who I am and to appreciate, or at least to pretend to, my unique sense of humor and learning style. Their generosity, kindness and friendship were at times the only thing that made this process manageable. I would also like to thank my advisory committee, Dr. Patrizia Riso, Dr. Adrianne White and Dr. Rodney Bushway for their support and advise.

I would like to thank the University of Maine’s Faculty Research Fund, the Wild Blueberry Association of North America (WBANA), and the Graduate Student Group for funding my research and support from Dr. Jason Bolton, Stefano Vendrame, Chelsea Wagner and Tom Merrow to aid in processing the blueberries and for biochemical analyses, was greatly appreciated.

Last and certainly not least, I would like to thank my family. Their encouragement and ability to always put a smile on my face was invaluable. Most importantly, I thank my daughter Elliot and my husband Charlie. Without their love and support I would not have been able to achieve this goal. Charlie, for the many breakfasts, lunches, dinners, stacked wood and warm fires, clean clothes, hugs when needed, and healthy and happy daughter, I thank you.
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LIST OF ABBREVIATIONS

ROS, Reactive oxygen species
TNF-α, Tumor necrosis factor-alpha
IL-6, Interleukin-6
NSAIDS, Non-steroidal anti-inflammatory drugs
OH, Hydroxyl radical
WB, Wild blueberries
NO, Nitric oxide
O$_{2}^{•-}$, Superoxide

•OH, Hydroxyl radical
ROO•, Peroxyl radical
H$_{2}$O$_{2}$, Hydrogen peroxide
cGMP, Cyclic guanosine monophosphate
Mn-SOD, Manganese-superoxide dismutase
LPS, Lipopolysaccharide
8-OHdG, 8-Hydroxy-2’-deoxyguanosine
ETC, Electron transport chain
FADH$_{2}$, Flavin adenine dinucleotide
SR, Sarcoplasmic reticulum
ER, Endoplasmic reticulum
EPR, Electron paramagnetic resonance
TBARS, Thiobarbituric acid method
KE, Knee extensor
WR_{max}, Maximal work rate
WBC, White blood cells
PBN, Alpha-phenyl-tert-butyl-nitrone
CK, Creatine kinase
GPx, Glutathione peroxidase
ELISA, Enzyme-linked immunosorbent assay
N, Newtons
LDH, Lactate dehydrogenase
CRP, C-reactive protein
TAS, Total antioxidant status
DOMS, Delayed onset muscle soreness
MVIC, Maximum voluntary isometric contraction
1RM, One rep max
CA, Montmorency cherry juice concentrate
FC, Isoenergetic fruit concentrate placebo
MVC, Maximum voluntary contractions
hsCRP, High-sensitivity C-reactive protein
Carboxy-H_2DCFDA, Carboxy-dihydro-2',5'-dichlorohydrofluorescein diacetate
FRAP, Ferric reducing antioxidant power
BB, Blueberry
CON, Control
KN, Natural killer cells
5-OHMU, 5-hydroxymethyl-2'-deoxyuridine
BMI, Body mass index

$\text{VO}_2^{\text{max}}$, Maximal exercise capacity

IV, Intravenous

Lbs, Pounds

% fat, Percent body fat

PBS, Phosphate buffered saline

SD, Standard deviation

U.S., United States

DRI, Dietary Reference Intake

RDA, Recommended Dietary Allowances

EERs, Estimated energy requirements

Mn, Manganese

AI, Adequate Intake

Se, Selenium

Zn, Zinc

Kcals, Calories

SOD, Superoxide dismutase

RPE, Rate of perceived excursion

ADL, Activity of daily living

CVD, Cardiovascular disease
At all levels of intensity and duration, exercise is known to cause an increase in the generation of reactive oxygen species (ROS). When derivatives of oxygen occur and exist independently with one or more unpaired electrons they are known as “free radicals” (Halliwell and Gutteridge 2007). Since atoms possess electrons that are usually associated in pairs, free radicals have the potential to act negatively in the body. Reactive oxygen species refer to oxygen-centered radicals as well as nonradical but reactive derivatives of oxygen (Halliwell and Gutteridge 2007). When ROS are created in excess, resulting in the disruption of the pro-oxidant/antioxidant balance, the physiological system is said to be in a state of “oxidative stress”. The harmful effects of oxidative stress include inflammation, damage to DNA, decreased ability to replenish muscle glycogen and an increased resistance to insulin (Dokken, Saengsirisuwan et al. 2008).

In well-conditioned individuals, the increase in ROS is proportionally met by an increase in antioxidants, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), as a result of training adaptations. However, when trained or untrained individuals perform unaccustomed, high intensity exercise, ROS and other free radicals are generated at a rate that can exceed the body’s antioxidant capacity (Urso and Clarkson 2003, Proske and Allen 2005). Thus the body is placed in a state of oxidative stress, which, in turn leads to bio-molecular
damage beyond the body’s natural ability to repair (Nikolaidis, Jamurtas et al. 2008).


Cytokines can also modulate the inflammatory response by exerting inhibitory effects on immune cells. This inhibition results in the restriction of growth, differentiation and function of these cells (Weinstock, Konig et al. 1997, Ostrowski, Rohde et al. 1999, Pedersen and Hoffman-Goetz 2000, Kimura, Suzui et al. 2001, Moldoveanu, Shephard et al. 2001, Suzuki, Nakaji et al. 2002). In addition to exercise, prior studies have reported that a variety of perturbations,
such as obesity and ageing can cause an increase in the expression of mRNA for cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 among others (Colahan, Kollias-Bakert et al. 2002, Ainsworth, Appleton et al. 2003, Barton, Williamson et al. 2003, Liburt, McKeever et al. 2006, Streltsova, McKeever et al. 2006, Donovan, Jackson et al. 2007, Adams, Katepalli et al. 2009).

A common medical prescription for the treatment of inflammation caused by cytokines, as well as by oxidative stress, is non-steroidal anti-inflammatory drugs (NSAIDS). Aspirin, ibuprofen and naproxen all fall into this class of drugs. Active individuals at all levels, regularly use NSAIDS to treat the pain associated with over-stressed (inflamed) muscles. The annual dollar amount for worldwide sales in NSAIDS is billions of dollars. Although this class of drugs is an effective treatment for inflammation, they can also cause serious secondary medical conditions. Side effects of NSAIDS use include nausea, diarrhea, ulcers, decrease blood clotting ability, reduced blood flow to kidneys and increased edema. The possible harm associated with chronic NSAIDS use has urged research for a more viable and safer alternative. The pharmacology being investigated includes compounds produced in the laboratory as well as those that occur naturally in foods. Naturally occurring sources with an anti-inflammatory capacity include omega-3 fatty acids, black tea, orange peel, cranberries, tart cherries, and many other darker pigmented fruits and vegetables. The specific anti-inflammatory/antioxidant compounds contained in many of these foods are named anthocyanins.
Anthocyanins are natural pigments in food responsible for the colors blue, purple, red and orange. Thus, they are found in a variety of berries, purple carrots, eggplant and purple potatoes. Anthocyanins are a type of polyphenol or flavonoid that possess many antioxidant properties. They have the ability to scavenge ROS, inhibit lipid peroxidation and to chelate metal ions (Basu, Rhone et al. 2010, Del Bo, Ciappellano et al. 2010, Hurst, Wells et al. 2010). This particular type of polyphenol found in deep-colored food can be detected in the blood plasma, making it an excellent bio-marker for the bioavailability of the antioxidant properties contained in anthocyanins (de Pascual-Teresa, Moreno et al. 2010).

The antioxidant activity of flavonoids, in general, is contingent upon their molecular structure such as the number and position of the hydroxyl group (OH). The resulting evidence in previous work suggests that these flavonoids have the ability to scavenge superoxide anions and hydroxyl radicals by donating a hydrogen atom, in turn stopping the reproduction stage of a radical chain reaction (Cook and Samman 1996). One type of flavonoid in particular known to scavenge free radicals is anthocyanins. Low-bush wild blueberries (WB) (vaccinium angustifolium) contain anthocyanins and have been found to have one of the highest recorded antioxidant capacities of any fruit or vegetable (Kalt, Ryan et al. 2001, Kalea, Lamari et al. 2006, Basu, Rhone et al. 2010). Previous studies in animal models have shown the antioxidant properties of WB to decrease ROS and restore the balance between ROS and nitric oxide (NO) (Youdim, McDonald et al. 2002, Basu, Rhone et al. 2010, Vendrame, Daugherty
et al. 2013). Human studies, with the consumption of foods lower in anthocyanins than WB, have demonstrated similar protection from oxidative stress and inflammation (Lyall, Hurst et al. 2009, Hurst, Wells et al. 2010). To date, researchers have not utilized exercise to determine the effect of a diet that includes whole WB on oxidative stress, inflammation and DNA damage in one comprehensive model. Therefore, the purpose of this study is to determine if a diet enriched with whole WB is an effective inhibitor of the intracellular damage associated with oxidative stress.

Goal

The goal of this project is to be the first of its kind using human subjects to investigate the effect of WB consumption on exercise-induced oxidative stress, inflammation and subsequent DNA damage. This study will focus on the following Hypotheses:

Hypothesis #1

Following an eight-week WB intervention, will there be a reduction in post-exercise plasma concentrations of TNFα, and IL-6? Rationale: Exercise has been shown to elicit cellular damage, resulting in an increase in inflammatory markers (Cannon, Meydani et al. 1991, Kimura, Suzui et al. 2001, Hurst, Wells et al. 2010, Bernecker, Scherr et al. 2011). Antioxidants such as anthocyanins have been shown to reduce plasma inflammatory markers (Lyall, Hurst et al. 2009, Hurst, Wells et al. 2010). Wild Blueberries have been found to contain some of
the highest levels of such antioxidants compared to other fruits or vegetables (Kalea, Lamari et al. 2006).

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Method of Assessment</th>
</tr>
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<tbody>
<tr>
<td>Decrease in plasma inflammatory markers, TNFα and IL-6</td>
<td>• ELISA Assay, R&amp;D Systems Kit</td>
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**Hypothesis #2**

Following an eight-week WB intervention, will there be a reduction in oxidative stress and subsequent DNA damage? **Rationale:** Strenuous or unfamiliar exercise has been shown to increase oxidative stress and damage DNA strands (Dunlap, Reynolds et al. 2006, Paik, Jin et al. 2009, Hurst, Wells et al. 2010). The damage to DNA strands can also cause further oxidative stress and increase release of ROS. This, in turn, can cause injury and hinder the cellular repair process.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Method of Assessment</th>
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<tbody>
<tr>
<td>Decrease in oxidative stress (Mn-SOD)</td>
<td>• ELISA Assay, R&amp;D Systems Kit</td>
</tr>
<tr>
<td>Decrease in damage to DNA strands</td>
<td>• Comet Assay</td>
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CHAPTER 2
REVIEW OF LITERATURE

Regular, moderate physical activity has been shown to have numerous health benefits, including lowering the relative risk for developing many chronic diseases such as Type 2 diabetes and cardiovascular disease (Thorell, Borjesson et al. 2009). Habitual physical activity can lead to an improved ability by the body to defend against reactive oxygen species (ROS) that cause oxidative stress and inflammation. This positive effect of training can also significantly reduce post-exercise oxidative stress as well as damage to DNA strands (Urso and Clarkson 2003, Proske and Allen 2005). On the other hand, chronic, high intensity exercise can create unmanageably high levels of ROS resulting in significant damage to muscle cells (Davies, Quintanilha et al. 1982). Such damage can occur following a single bout of unfamiliar work in sedentary individuals. Since sedentary individuals have not stressed their antioxidant capacity through regular exercise, their body’s natural antioxidant pathways have not adapted to the large increase in ROS production created with strenuous exercise. The lack of adaptation creates and imbalance between pro-oxidants and anti-oxidants, making them more susceptible to oxidative stress and elevated inflammatory cytokines with potential for systemic damage that reaches beyond the working muscle itself (Tozzi-Ciancarelli, Penco et al. 2002, Petersen and Pedersen 2005).
The following review is presented in three parts. Part one discusses the specific mechanisms underlying the production of ROS, the creation of oxidative stress, the production of inflammatory cytokines and the steps that lead to DNA damage. The second part reviews the role of exercise in these potentially damaging mechanisms. Finally, literature regarding the dietary consumption of exogenous sources of antioxidants will be reviewed, particularly as it occurs in combination with exercise.

**Reactive Oxygen Species and Oxidative Stress**

In living tissue, “free radical” is a label given to an atom containing one or more unpaired electron(s) that is able to exist independently (Halliwell and Gutteridge 2007). The most well known free radical in the human body is the hydrogen ion (H⁺). However, in the catabolic process of converting energy from carbohydrates, fats and protein, oxygen, (a universal electron acceptor) free radicals are generated. These oxygen centered radicals, as well as non-radicals but reactive derivatives of oxygen, are collectively known as reactive oxygen species (ROS) (Halliwell and Gutteridge 2007). These ROS are products of normal metabolism that have both beneficial and damaging effects in a living system.

Superoxide (O₂⁻) is a common ROS that is generally considered relatively unreactive compared to other radical species. In biological systems, most of the superoxide production occurs in the mitochondrial electron transport chain where oxygen is reduced to water. Due to its relatively long half-life, superoxide is able
to go through diffusion within the cell, increasing the number of potential targets (Halliwell and Gutteridge 2007). For this reason superoxide is considered to be a “primary” ROS. Primary ROS may continue to react with other molecules and generate “secondary” ROS such as hydroxyl radical (·OH), peroxyl radicals (ROO’), and other non-radical derivatives of oxygen, such as hydrogen peroxide (H₂O₂) (Stadtman 2004).

Under normal physiological conditions, ROS are neutralized by an elaborate endogenous antioxidant system that helps to maintain homeostasis or a level of ROS that is beneficial to a living system (Urso and Clarkson 2003). In moderate amounts, ROS may assist in keeping cells healthy by controlling cell division and help to preserve homeostasis by acting as signal transducers (Mittal and Murad 1977). In 1977, Mittal and Murad provided evidence that the hydroxyl radical stimulates activation of guanylate cyclase and formation of cyclic guanosine monophosphate (cGMP) (Mittal and Murad, 1977). Cyclic guanosine monophosphate not only is a regulator of glycogenolysis, cellular apoptosis and ion channel conductance, but it also helps relax smooth muscle tissues. In blood vessels, relaxation of vascular smooth muscle leads to vasodilation and increased blood flow. Since Mittal and Murad’s 1977 research, studies have shown that living systems have not only adapted to a coexistence with ROS but have developed various mechanisms for their beneficial use in various physiological functions. On the other hand, the increase of ROS in cells has been shown to damage lipids, proteins, and DNA, inhibiting their normal function. This damage can increase tumor growth and cancer progression, as well as the aging
processes and risk for Alzheimer’s disease (Valko, Rhodes et al. 2006). The harmful effect of free radicals causing potential biological damage is termed oxidative stress (Kovacic and Jacintho 2001, Valko, Rhodes et al. 2006).

Oxidation is a chemical modification process through which cells of all organisms sense ROS by influencing how proteins communicate with each other. Oxidative stress occurs when ROS are created in excess, resulting in the disruption of the pro-oxidant/antioxidant balance. Under these circumstances ROS outnumber antioxidants due to increased ROS levels, decreased antioxidant properties, or a combination of both. The level of imbalance between pro-oxidants and antioxidants determines the extent of oxidative stress (Halliwell and Gutteridge 2007). There are a variety of mechanisms set in place to decrease oxidative stress, one being manganese-superoxide dismutase (Mn-SOD). Manganese-superoxide dismutase is an inducible antioxidant enzyme that is located in the mitochondria and protects against oxidative damage caused by ROS. It catalyzes the dismutation of superoxide radicals to oxygen and hydrogen peroxide and has been shown to play an important role in mediating protection against lipopolysaccharide (LPS) and other oxidative events (McCord and Fridovich 1969).

**Inflammatory Cytokines**

Inflammation is a protective reaction to injury, irritation or infection that can be elicited both locally and systemically. Without inflammation, tissues would not heal which could pose increasing harm to the organism. *Acute* inflammation is
the first defense to harmful stimuli. The acute response includes an increase in movement of plasma and leukocytes to the damaged tissue (Abbas and Litchman 2009). This localization of fluid and white blood cells is followed by a series of biochemical events that involve both the vascular and immune systems within the damaged cell. The result of these responses in the injured tissue is redness, swelling, pain and loss of function for a limited time. The reduction of these maladies, and the return to a normal, healthy state depends on the severity of injury incurred (Abbas and Litchman 2009). On the other hand, the recovery (healing) from inflammation may be interrupted by another perturbation. The result of frequent, cyclical healing and destruction of tissue is chronic inflammation. This prolonged process can lead to health issues ranging from hay fever to cancer (Abbas and Litchman 2009).

In response to injury or distress, cytokines are released intracellularly. These low molecular weight proteins play a key role in the inflammatory process (Weinstock, Konig et al. 1997, Ostrowski, Rohde et al. 1999, Pedersen and Hoffman-Goetz 2000, Kimura, Suzui et al. 2001, Moldoveanu, Shephard et al. 2001, Suzuki, Nakaji et al. 2002). The word 'cytokine' is translated from Greek roots meaning ‘to put cells into motion’ (Thomson 1994). Almost all nucleated cells synthesize cytokines and present cytokine receptors on their plasma membranes. Cytokines act as bases that complete an assembly of independent receptor subunits on the surface of target cells. When these combine, they produce an intracellular signal that alters target cell function (Taniguchi 1995).

Tumor necrosis factor-alpha is often used as a marker for systemic inflammation in chronic diseases such as cardiovascular disease (Devaux, Scholz et al. 1997). A second cytokine, IL-6 has also been documented to function in the cascade of events involved with inflammation. A normal or desirable level of IL-6 is < 1.04 pg mL (Musselman, Miller et al. 2001). Interleukin-6 is commonly used to study the inflammatory effects of exercise. However, results for IL-6 are conflicting, suggesting that this cytokine has both pro-inflammatory and anti-inflammatory roles (Weinstock, Konig et al. 1997, Ostrowski, Rohde et al. 1999, Pedersen and Hoffman-Goetz 2000, Kimura, Suzui et al. 2001, Moldoveanu, Shephard et al. 2001, Suzuki, Nakaji et al. 2002).

**DNA Damage**

DNA undergoes damaging events every day. This damage can come from a variety sources such as the sun (ultraviolet light), radiation, smoking and
nutritional deficiencies. Fortunately, most DNA damage that occurs is a result of small errors in DNA code. These small DNA errors are referred to as mutations and are often harmless. Other types of DNA damage, such as a DNA strand break, can have more serious ramifications. Fixing a break in a DNA strand is a complex operation, increasing the possibility of inaccuracy during repair. DNA may be modified in a variety of ways, which can ultimately lead to mutations and instability. These mutations could result in the development of cancers and speed up the aging process. A common source of DNA strand breakage comes from oxidative stress (Stadtman 2004).

The hydroxyl radical (·OH) is known to react with all components of the DNA molecule, damaging the deoxyribose backbone and both the purine and pyrimidine bases (Stadtman 2004). The formation of 8-Hydroxy-2’-deoxyguanosine (8-OHdG) is one of the most extensively studied DNA lesions (Radak, Kumagai et al. 2007). The interaction of ·OH with the nucleobases of the DNA strand, leads to the relatively easy formation of 8-OHdG in the nucleoside (Valavanidis, Vlachogianni et al. 2009). The presence of 8-OHdG on the replicating strand of DNA leads to loss of base-pairing specificity, misreading of adjacent pyrimidines, or insertion of adenine opposite to the lesion (van Loon and Hubscher 2009). Mutations may arise due to misincorporation of adenine opposite the 8-OHdG lesion resulting in guanine:cytosine to adenine:thymine transversion mutations; common mutations in cancer cells (van Loon and Hubscher 2009). Once cancer cells form and mutate they begin to multiply and can metastasize into surrounding tissue. If the body is under enough oxidative
stress it is unable to repair the damage and the spread of cancer may continue (van Loon and Hubscher 2009). Research has documented the ability of exogenous antioxidants, such as the anthocyanins in WB and broccoli to assist in decreasing excess breaks in DNA strands (Riso, Martini et al. 2009, Del Bo, Riso et al. 2013).

**Exercise and Reactive Oxygen Species**

As previously discussed, the mitochondria is where 90% of oxygen consumed by the body is reduced to water, making the mitochondrial electron transport chain (ETC) the location where the majority of ROS are produced under normal physiological conditions (Ames, Shigenaga et al. 1995). During exercise, oxygen consumption may increase 10 – 20-fold in the whole body and 100 – 200-fold in skeletal muscle. This increase results in a concomitant increase in energy transduction through the ETC (Pittaluga, Parisi et al. 2006). During this increased rate of transduction, a small number of electrons escape prematurely and attach themselves to molecular oxygen, this produces superoxide anion radicals at a rate that can exceed the body’s natural antioxidant defense capacity, resulting in oxidative stress (Urso and Clarkson 2003, Stadtman 2004, Proske and Allen 2005). This excess ROS can cause an increase in inflammation and damage cellular lipids, proteins and DNA (Nikolaidis, Jamurtas et al. 2008).
Exercise and Oxidative Stress

The transition from the two-electron, nicotinamide dinucleotide (NADH) and flavin adenine dinucleotide (FADH$_2$), to one-electron transfer in the ubiquinone-cytochrome-c reductase (Complex 3) system of the ETC are known sources of increased oxidative stress during exercise (Chance, Sies et al. 1979, Paik, Jin et al. 2009). It is at this segment of the ETC that $O_2^·−$ is produced. Although, $O_2^·−$ is reduced to $H_2O_2$ by mitochondrial superoxide dismutase (SOD), creating a secondary free radical, $·OH$ (Halliwell and Gutteridge 2007). The hydroxyl radical is probably the most indiscriminately powerful oxidant produced in biological systems. Early studies seeking proof of such damage from free radicals include work by Dillard et al. (1978) and Davies et al. (1982).

In one of the first studies to determine that exercise leads to oxidative stress, Dillard et al. (1978) revealed a 1.8-fold increase in lipid peroxidation. Ten participants engaged in 60 minutes of cycling at ~50% of maximum oxygen consumption ($VO_{2\text{max}}$) with and without exposure to 0.3 ppm ozone. While participants were exposed to the 0.3 ppm of ozone they displayed increased lung residual volume and decreased vital capacity, maximal midexpiratory flow rate and forced expiratory volume in one second. Participants then had their breath collected into a spirometer filled with hydrocarbon-scrubbed air. This revealed an increase in exhaled pentane from the stress of the exercise but no effect of ozone. By measuring exhaled pentane levels, Dillard et al. (1978) was able to show an increase in lipid peroxidation due to exercise. They also supplemented participants with 1,200 IU of vitamin E following exercise and found that after two
weeks the mean production of pentane was significantly lowered both at rest and
during exercise (Dillard, Litov et al. 1978).

Following this study, Davies et al. (1982) compared rats exposed to
exhaustive exercise to those exposed to vitamin E deficiency and examined the
effects of free radicals on lipid peroxidation and mitochondrial, sarcoplasmic
reticulum (SR) and endoplasmic reticulum (ER) integrity (Davies, Quintanilha et
al. 1982). After being subjected to a submaximal work intensity endurance test
on a motorized rodent treadmill, the rats were immediately euthanized and
muscles (gastrocnemius, soleus and plantaris) and liver homogenates were
extracted and tested for free radicals by electron paramagnetic resonance (EPR)
spectroscopy. Since measuring free radicals directly in humans is limited due to
their short life span and human subject related issues, EPR appears to be a
favorable alternative method (Ashton, Rowlands et al. 1998). Lipid peroxidation
was measured by thiobarbituric acid method (TBARS) and SR and ER
membrane activity were assessed by latency measurements of alkaline
phosphatase activity (Davies, Quintanilha et al. 1982). Free radicals were
observed by EPR in all homogenates of muscle and liver and were increased two
to three-fold following exhaustive exercise. It was determined that the increase in
exercise could lead to faster turnover rates of ubisemiquinone and hemoglobin
autooxidation, thus resulting in higher levels of superoxide radical generation
(Davies, Quintanilha et al. 1982). Latency calculations for SR and ER revealed a
decrease in membrane integrity following exhaustive exercise, as well as with
vitamin E deficiency. Lipid peroxidation was also significantly increased by both
exhaustive exercise and vitamin E deficiency. More recently, Bailey et al. (2003), have continued to use EPR to detect free radical production during exercise (Bailey, Davies et al. 2003).

Using the single-leg knee extensor (KE) model, Bailey et al. (2003) was able to test seven healthy men at incremental exercise, at 25 and 70% of their established maximal work rate (WR\text{max}), for three minutes at each interval to achieve steady-state pulmonary VO\textsubscript{2}. Upon completion of the exercise session, a sample of the femoral arterial and venous blood was taken for the direct measurement of free radicals in blood gases. In addition, a duplicate assessment of femoral vein blood flow was collected, along with continuous measure of arterial and venous blood pressures (Bailey, Davies et al. 2003). Following the exercise sessions EPR was able to detect free radicals in the circulation of the participants, particularly higher levels found in venous compared to arterial circulation, showing evidence of free radical outflow from a working muscle bed (Bailey, Davies et al. 2003). The increase in oxygen consumption and subsequent ROS production that occurs during strenuous exercise is of concern due to the potential damage inflicted on cell structures, nucleic acids, lipids and proteins, as well as increase systemic inflammation (Nikolaidis, Jamurtas et al. 2008).

**Exercise and Inflammatory Cytokines**

During regular bouts of exercise, the immune system plays an important role in the breakdown and repair process of muscle and connective tissue. When the exercise is intense enough to cause muscle damage the inflammatory
response can be great (Peake, Nosaka et al. 2005). The main purpose of this response is to remove cellular debris from the damaged tissue area, promoting repair of damaged myofibers, blood vessels, nerve fibers and extracellular matrix after the damaging exercise (Walker and Fantone 1994). This process of attraction and migration of blood cells to the affected area and production of various inflammatory cytokines can occur within minutes to hours post-injury (Walker and Fantone 1994).

The first group of white blood cells (WBCs) involved in the inflammatory process are neutrophils, which arrive immediately after injury, as early as 45 minutes and may stay present for up to three days (Hikida, Staron et al. 1983). The increase in neutrophils after exercise-induced muscle damage corresponds with elevated cytokines, including interleukin (IL)-1 and tumor necrosis factor-alpha (TNFα) concentrations, indicating a pro-inflammatory response (Dubravec, Spriggs et al. 1990). Depending on the muscle and amount of muscle mass recruited during the exercise, the level of systemic inflammation varies. The greater the recruitment and burden on the working muscles, the greater the local and systemic response (Cannon, Meydani et al. 1991).

Unlike in the damaged muscle itself, pro-inflammatory cytokines seem to be inhibited systemically. Though the process is unclear at this time, IL-6 may be involved (Pedersen, Steensberg et al. 2003, Petersen and Pedersen 2005). The expression both TNFα and IL-1 lead to an increase in IL-6, which is known to act as both a pro-inflammatory and anti-inflammatory cytokine. It is seen first in circulation immediately after damaging exercise (Pedersen, Steensberg et al.
Following the initial role as a pro-inflammatory cytokine, IL-6 will suppress further production of TNFα and IL-1 by assisting in the upregulation of TNFα and IL-1 receptors, while also stimulating the release of anti-inflammatory cytokines such as IL-10 (Tilg, Dinarello et al. 1997, Petersen and Pedersen 2005, Bernecker, Scherr et al. 2011). While the exact mechanism for IL-6’s dual role as both a pro-inflammatory and anti-inflammatory cytokine is unclear, the significant increase following strenuous exercise has been well documented, making it an excellent marker to assess the level of stress following an exercise session.

Research by Mendham et al. (2012) investigated the acute effects of either cycle ergometry or modified rugby on inflammation and glucose regulation within the Indigenous Australian population (Mendham, Coutts et al. 2012). Ten sedentary men completed a graded exercise test to establish their VO_{2\text{max}} before being randomly placed in either the rugby group or the cycle ergometry group. Participants exercised for 40 minutes then rested for seven days before completing the other exercise session for the crossover design. Fasting venous blood was collected pre-, post-, 30, 60, and 240 minutes post-exercise and analyzed for glucose, insulin, cortisol and inflammatory markers TNFα, IL-1β, IL-6, IL-1 receptor agonist (ra) and C-reactive protein (CRP) (Mendham, Coutts et al. 2012). Following exercise, IL-6 and IL-1ra were significantly increased within the 240-minute post-exercise period, despite the exercise protocol. No significant changes were observed within or between exercise protocols for TNFα, IL-1 β, or CRP. The cycle ergometer exercise protocol consisted of four,
10 minute bouts at 80 – 85% VO$_{2\text{max}}$ with two minutes of passive recoveries. The rugby sessions followed the same time protocol but intensity was measured using the rate of perceived exertion. This study showed that even acute bouts of exercise can increase inflammation.

Similarly, Bernecker et al. (2011) examined marathon runners to evaluate the effect of acute vigorous exercise and its impact on TNFα and IL-6 (Bernecker, Scherr et al. 2011). Thirteen male marathon runners who had successfully completed at least one other marathon, and who were already planning on running the test marathon were selected to partake in the study. All participants maintained a three-day food log and were asked to limit consumption of NSAIDS, fatty foods and large doses of vitamins and minerals prior to the race. During the race each participant wore hear rate monitors to determine exercise intensity. Blood samples were collected immediately pre- and within 1 hour post- race (Bernecker, Scherr et al. 2011). Following the race there was a significant increase in all markers of inflammation (IL-6 and TNFα). In addition, the mRNA gene expression for TNFα and IL-6 were expressed in blood mononuclear cells in all runners both pre- and post- race with no significant differences found (Bernecker, Scherr et al. 2011). These finding suggest that during acute intense exercise, the significant increase in TNFα and IL-6 in the plasma but not blood mononuclear cells post- marathon, may be due to a local production of TNFα and IL-6 and release from the stressed muscle tissues (Bernecker, Scherr et al. 2011). Understanding the role of oxidative stress and
inflammation caused by strenuous exercise is important due to the negative consequences of prolonged inflammation.

Exercise and DNA Damage

Prolonged oxidative stress and inflammation that is not met equally with endogenous mechanisms to lower the damage can cause further damage to muscle, proteins and lead to an increased risk for DNA damage, premature aging and chronic diseases due to their destructive nature (Paik, Jin et al. 2009).

The hydroxyl radical is often formed during exercise-induced oxidative stress and is known to react with all components of the DNA molecule, damaging the deoxyribose backbone and both the purine and pyrimidine bases (Stadtman 2004). The formation of 8-Hydroxy-2′-deoxyguanosine (8-OHdG) is one of the most extensively studied DNA lesions (Radak, Kumagai et al. 2007). 8-Hydroxy-2′-deoxyguanosine accumulates in DNA, causing lesions that disrupt genome integrity (Radak, Kumagai et al. 2007). Such damage to DNA strands can lead to decreased recovery after exercise, injuries and decreased exercise performance (Paik, Jin et al. 2009). While many of these studies examine either trained or untrained individuals, few have examined recreational exercisers, specifically those who engage in regular, strenuous exercise in gymnasiums.

Gandhi and Chopra (2009) examined men and women who exercise to keep fit and matched them to comparable sedentary individuals. They obtained the estimated VO$_{2\text{max}}$ of 40 healthy participants (35 male, 5 female) by the McArdle Step-Test followed by a blood draw 15 minutes post-...
was performed before their daily workout. Participants were asked to maintain their regular fitness routines throughout the study. Fifteen healthy sedentary controls that had never exercised were also assessed during the study. DNA damage was assessed using the comet assay. Cells that are damaged will display migration from the origin and a tail trailing behind the cell, the longer the tail, the greater the damage. Results revealed the length of DNA migration and the % DNA in tails of physically active individuals were significantly increased compared to the sedentary controls (Gandhi and Chopra 2009). Researchers suggest that these results are due to the increased energy demand during strenuous exercise and subsequent increase in oxygen uptake, resulting in an increase in free radicals that damage the DNA strands (Gandhi and Chopra 2009).

A similar study investigating DNA damage compared 12 male marathon runners to 20 sedentary healthy males. Blood was collected from the runners the day before the race, immediately after and then 24, 72 hours, one week and two weeks after the marathon (Tsai, Hsu et al. 2001). DNA damage was assessed using the comet assay and urinary 8-OHdG. The metabolic products of nitric oxide, nitrite and nitrate, were measured along with plasma lipid peroxidation metabolites and creatine kinase. Damage to DNA, as shown by strand breaks in the comet assay, reached its peak 24 hours following the race and did not decrease until two weeks post-race. These consecutive changes in DNA damage were statistically significant (Tsai, Hsu et al. 2001). Urinary 8-OHdG levels increased two-fold immediately post-race and remained elevated through
the one-week follow-up. Creatine kinase increased more than two-fold following
the race and did not normalize until two weeks post-race, indicating a significant
amount of muscle damage (Tsai, Hsu et al. 2001). Plasma nitrite/nitrate
increased 1.6-fold after the race and steadily declined after the race while plasma
lipid peroxidation increased 4.5-fold one day after the race and remained
elevated until the end of the two week follow-up (Tsai, Hsu et al. 2001).
Researchers concluded that long duration activity such as the marathon, leads to
oxidative DNA damage in peripheral blood mononuclear cells (Tsai, Hsu et al.
2001).

To further investigate the mechanism to the perceived cause of DNA
damage observed during exercise, Fogarty et al. (2011) examined the effects of
an acute bout of strenuous exercise on DNA oxidation and free radical
production. They obtained the VO_{2max} of 12 healthy, moderately trained
(recreational activity no more than three times per week) males on a cycle
ergometer, one week prior to the experimental protocol (Fogarty, Hughes et al.
2011). During the experimental protocol, participants completed three
consecutive stages of exercise, each lasting five minutes in duration on a cycle
ergometer. The intensity of these stages was equivalent to 40, 70 or 100% of
VO_{2max} based on preliminary tests. Blood was collected immediately after each
session during the 15-minute rest period (Fogarty, Hughes et al. 2011).
Participants were assessed on serum lipid hydroperoxides to test for lipid
oxidation, serum protein carbonyls to detect muscle damage, lipid soluble
antioxidants (plasma retinol, alpha-tocopherol, gamma-tocopherol, beta-
carotene, lycopene and xanthophyll to test for antioxidant status, and EPR was used to measure free radicals (Fogarty, Hughes et al. 2011).

Results revealed an increase in cell DNA damage at moderate (70% of $\text{VO}_{2\text{max}}$) and high (100% of $\text{VO}_{2\text{max}}$) compared to low intensity exercise, the damage observed increased with each increment in hydrogen peroxide concentration ($p < 0.05$) (Fogarty, Hughes et al. 2011). While lipid peroxidation significantly increased throughout exercise there was no change in protein oxidation. The EPR used to detect lipid-derived free radicals revealed a positive correlation between alpha-phenyl-tert-butyl-nitrone (PBN) and exercise intensity. Greater concentrations of PBN-adduct were seen at 70 and 100% $\text{VO}_{2\text{max}}$ compared to at rest. There was also a positive correlation between PBN-adduct and DNA damage and between PBN-adduct and lipid peroxidation (Fogarty, Hughes et al. 2011). This study concluded that with an increase in exercise intensity, there is a change in lipid peroxidation and nucleic acid oxidation. Despite the significant increase in lipid peroxidation found at 40% $\text{VO}_{2\text{max}}$ compared to at rest, there was not a significant increase in DNA damage at 40% $\text{VO}_{2\text{max}}$, indicating that the increase in lipid-derived free radicals may not be the sole reason for the subsequent DNA damage with increasing intensity. Due to these results, the authors suggest the need for further investigations on a direct cause (Fogarty, Hughes et al. 2011).

A current treatment method used to treat inflammation caused by oxidative stress is non-steroidal anti-inflammatory drugs (NSAIDS). This method of treatment is commonly used to decrease muscle soreness. Many active
individuals, particularly chronic exercisers and weekend warriors who are largely sedentary besides the occasional strenuous exercise session, use NSAIDS to ease the pain caused from the exercise-induced oxidative damage. The problem with this treatment is that habitual use of NSAIDS can lead to health issues such as stomach ulcers or reduced blood flow to the kidneys. Due to the negative side effects of NSAIDS, many researchers have been looking for more natural alternatives to decrease the oxidative stress and inflammation caused by strenuous exercise, allowing individuals to capitalize on the benefits of exercise without the added risk of cellular damage. Treatments being investigated have included a variety of compounds that occur naturally in foods. Naturally occurring sources with an anti-inflammatory capacity include omega-3 fatty acids, black tea, orange peel, cranberries, tart cherries, and many other darker pigmented fruits and vegetables. The specific antioxidant/anti-inflammatory compounds contained in many of these foods are anthocyanins. Thus, investigating the effects of whole foods, containing anthocyanins on exercise-induced oxidative stress, inflammation and DNA damage is essential.

**Exogenous Antioxidants and Exercise**

Anthocyanins are natural pigments found in a variety of plants that are responsible for the colors blue, purple, red and orange. Anthocyanins are a type of polyphenol, which possess many antioxidant properties such as the ability to scavenge ROS, inhibit lipid peroxidation and to chelate metal ions (Basu, Rhone et al. 2010, Del Bo, Ciappellano et al. 2010, Hurst, Wells et al. 2010, Vendrame,
Daugherty et al. 2013). This particular type of polyphenol can be detected in the plasma in its original form found in the plant food itself. This makes it an excellent marker to check bioavailability of the antioxidant properties found within anthocyanins (de Pascual-Teresa, Moreno et al. 2010). To date, the anthocyanin content in low-bush wild blueberries (WB) (*vaccinium angustifolium*) has been found to have one of the highest recorded antioxidant capacities of fruits and vegetables (Kalea, Lamari et al. 2006, Basu, Rhone et al. 2010). The antioxidant properties found in WB have been found to decrease ROS and restore balance between ROS and NO (Youdim, McDonald et al. 2002, Basu, Rhone et al. 2010).

Wild blueberries are one of the most often consumed berries in the United States and are particularly abundant in the State of Maine. Among other berries rich in anthocyanins, blueberries have been found to show more positive effects in reducing oxidative stress (Hurst, Wells et al. 2010).

Research by Hurst and Wells et al. (2010), examined three fruit polyphenolics, blueberries being the noted fruit, and their effect on oxidative stress-induced skeletal muscle damage *in vitro*. With the two other fruits, which were unidentified in the study, the researchers found that the extracts derived from blueberries exhibited the most protection against ROS in myotubes stressed to mimic exercise-induced oxidative stress (Hurst, Wells et al. 2010). The blueberries provided 54% protection (*p* < 0.05) while the two unnamed fruit extracts failed to provide any protection (Hurst, Wells et al. 2010).

Similar research examining exercise-induced oxidative stress *in vivo* by Lyall, et al. (2009), also found that anthocyanins, from blackcurrant extract,
provided protection from oxidative stress. They examined 10 healthy individuals (five males, five females), who participated in regular physical activity, though at varying levels of ability and fitness. Participants underwent VO\textsubscript{2max} testing and two familiarization sessions before completing a three-week intervention trial of a 30-minute row exercise maintained at 80% of their individual VO\textsubscript{2max}. Before the 30-minute row test participants were given two capsules of either the placebo or the black currant extract. Immediately upon completion of the exercise trial, the participants were given another two capsules. In this double-blind crossover study each participant participated in both the control and the experimental groups with a week washout period between the row trials. Plasma protein carbonyls, TNF\textalpha, ROS generating capability and creatine kinase (CK) activity were analyzed to assess the effects of the extract on exercise-induced oxidative stress.

Results showed a significant 1.4-fold increase in plasma protein carbonyls immediately after exercise ($p < 0.05$) in the placebo group ($0.6 \pm 0.1$ to $0.9 \pm 0.1$ mmol/mg protein pre- compared to post- exercise). In contrast, an increase in plasma protein carbonyls post-exercise was prevented in the black currant group ($0.3 \pm 0.1$ to $0.9 \pm 0.1$ mmol/mg protein pre-compared to post-exercise) ($p < 0.05$). A comparative difference was also seen in ROS generation post- exercise ($p < 0.05$) with the placebo group experiencing a four-fold increase in ROS production while the black currant group experienced attenuation in ROS production. The placebo group was also found to have a 1.3-fold increase in CK activity ($p < 0.05$), which remained elevated 24 hours post-exercise, while the
black currant group had significantly lower CK activity ($p < 0.05$). Plasma TNFα levels did not differ between groups before the 30-minute row trial, although the black currant group experienced a significantly lower level post-exercise ($2,087 \pm 317$ pg/ml to $1,457 \pm 180$ pg/ml) ($p < 0.05$) (Lyall, Hurst et al. 2009). As evidenced by previous studies, anthocyanins are absorbed and present in the plasma within about 30 minutes of ingestion (Mazza, Kay et al. 2002), which may explain why the black current group experienced the ROS protection following the 30-minute row trial. Results from this study, examining healthy, moderately active individuals showed that ingested anthocyanins are capable of protecting exercise-induced oxidative stress.

Similar results were determined examining another anthocyanin-rich berry and its effect on exercise-induced oxidative stress (Pilaczynska-Szczesniak, Skarpanska-Steinborn et al. 2005). Pilaczynska-Szczesniak et al. (2005) examined 19 male members of the Polish rowing team during their one-month training camp before regular training and competition. Rowers were randomly assigned to either the treatment group or the control group. They were instructed to consume 150 mL of a chokeberry juice, containing 23 mg/100mL of anthocyanins or matched placebo juice three times a day for four weeks. All participants completed a 2,000-meter rowing exercise test both before and after supplementation. This exercise was intended to act as the individuals’ control to compare the graded rowing test for maximal power. Markers of damage, including CK, SOD, glutathione peroxidase (GPx) and thiobarbituric acid reactive substances (TBARS) were measured during the control rowing trial and maximal
power rowing trial. While no changes were found between the two groups of rowers before supplementation of chokeberry juice, TBARS concentrations taken one-minute after the maximal power trial was significantly lower in the supplemented group than the control group ($p < 0.05$). Glutathione peroxidase was also significantly lower in the treatment group ($p < 0.05$) and while SOD displayed lower values in the supplemented group, the findings were not significant. Creatine kinase levels were reported to be significantly higher ($p < 0.05$) in the control group post- maximal power rowing trial than that of the chokeberry treatment group (Pilaczynska-Szczesniak, Skarpanska-Steinborn et al. 2005).

Fruits other than those from dark colored berries contain flavonoids such as lycopene. Tomatoes are an example of one of those fruits. In 2012 researchers investigated the effects of tomato juice on serum concentrations of 8-oxodG after extensive physical activity (Harms-Ringdahl, Jenssen et al. 2012). Harms-Ringhahl et al. (2012) examined 15 healthy, untrained individuals (seven female, eight male, ages 24 – 43). Participants exercised at 80% of their predicted max heart rate (220 – age = maximum heart rate) for 20 minutes on an ergometer bicycle. Blood was collected on day 0, day 35, day 70 and day 105 post-exercise testing. Two samples were collected at each session, one before and one 60 seconds after the 20 minute exercise session. Each participant acted as his or her own control. Participants were then asked to consume 150 ml of tomato juice containing 0.1 mg lycopene per ml, per day for five weeks, followed by a five-week washout without tomato juice before the final five weeks with
tomato juice (Harms-Ringdahl, Jenssen et al. 2012). Serum 8-hydroxy-2’dehoxyguanosine (8-oxodG) was analyzed as a marker of oxidative stress. Levels of 8-oxodG were not significantly different from the start of the intervention before the exercise sessions. The 20 minutes of exercise increased levels of 8-oxodG by an average of 42% compared to the background levels. Following the five-week intake of tomato juice, 8-oxodG remained basically unchanged when compared before and after 20 minutes of exercise (Harms-Ringdahl, Jenssen et al. 2012). Following the five-week washout period the level of 8-oxodG increased 84% after exercise and after the last five weeks involving tomato juice consumption, 8-oxodG levels were near the same as before exercise. These results revealed that 8-oxodG significantly increased following 20 minutes of cycling at 80% of their estimated maximum heart rate suggesting a correlation between 8-oxodG and ROS production. Results also showed that consumption of 150 ml of tomato juice (containing 15 mg lycopene) daily for five weeks protects nucleotides from ROS produced by exercise. Researchers concluded that long term consumption of tomato juice may reduce oxidative stress levels caused by exercise, diabetes, cardiovascular disease or inflammatory conditions (Harms-Ringdahl, Jenssen et al. 2012). Other fruits known to be high in antioxidants and flavonoids have been studied to better understand their effects on exercise induced oxidative stress. One fruit that has been gaining recent popularity are tart cherries. Researchers Connolly and colleagues (2006) designed a randomized, placebo controlled, crossover design aimed at testing the efficacy of a tart cherry juice blend in preventing symptoms of exercise-induced muscle
damage. Sixteen healthy males volunteered to undergo baseline testing for pain, muscle tenderness, relaxed elbow angle and isomeric elbow flexion strength. At this time that participants received either the placebo beverage or cherry juice blend. The tart cherry juice was composed of Montmorencier cherries and a commercially available apple juice and was mixed in a proprietary ratio. One, 12-ounce bottle of juice contained 50 – 60 cherries, 600 mg of phenolic compounds and 40 mg anthocyanins. The placebo was a mix of unsweetened black cherry Kool-aid soft drink mix and water with enough sugar added to match that of the cherry juice.

Participants were provided with 12-ounce bottles and instructed to consume one bottle in the morning and another in the evening for the succeeding eight days. Participants were asked to refrain from exercising their upper extremities during the study, along with avoiding anti-inflammatory medications (Connolly, McHugh et al. 2006). On the forth day of the study, participants returned to the lab and performed a bout of eccentric elbow flexion contractions. The exercise regimen consisted of 40 (2 x 20) maximal eccentric contractions of the elbow flexors using a modified preacher curl apparatus. During these sessions participants were asked to use maximal force during contractions while researchers assisted in forcing the subjects elbow into full extension. This allowed the starting position of the elbow to be fully extended for maximal resistance and movement with the elbow at about 130° flexion. Each flexion lasted about three seconds with about 12 seconds rest in between repetitions. Participants were allowed a three-minute rest between sets (Connolly, McHugh
et al. 2006). On each of the remaining four days participants were examined for pain, muscle tenderness and relaxed elbow angle and strength.

Pain was measured by asking participants to verbally rate their overall discomfort during the elbow flexion and extension while doing activities of daily living (Connolly, McHugh et al. 2006). Muscle tenderness was assessed in force using a standard manual muscle myometer. Muscle strength was measured by a force transducer during isometric elbow flexion exercises at 130°, 90°, and 30° angles, with two contractions, each lasting 30 seconds with a 60 second rest in between (Connolly, McHugh et al. 2006). Two weeks after baseline testing, participants returned to the lab and repeated the testing procedures with the opposite arm, again with either the placebo or cherry juice. Participants then followed the same procedure as they did during the first trial (Connolly, McHugh et al. 2006).

Reported outcomes for this study demonstrated that isometric elbow flexion strength loss was significantly greater in the placebo trial than the cherry juice trial ($p < 0.0001$), although no difference was found between test angles for isometric elbow flexion exercises (Connolly, McHugh et al. 2006). The loss in strength was averaged over all three test angles and found to be significantly greater during the placebo trial than during the cherry juice trial (22% and 4%, respectively) ($p < 0.0001$). There was also a significant difference seen in pain development between trials with pain levels being reported as higher during the placebo trial than during the cherry juice trial ($p = 0.051$). The pain in the cherry juice trial peaked at 24 hours and then declined, while during the placebo trial the
pain continued to increase to a peak at 48 hours (Connolly, McHugh et al. 2006). No differences were found for muscle tenderness or range of motion between trials (Connolly, McHugh et al. 2006). This randomized crossover design was one of the first studies to investigate the effects of tart cherry juice and its effect on muscle damage. The reported findings suggest that ingestion of a tart cherry juice blend may decrease some of the symptoms of exercise induced muscle damage, particularly reducing loss of strength. The proposed benefit of the tart cherries was likely their ability to reduce inflammation and oxidative stress due in part to the anthocyanins in the berries.

While these investigators did not directly measure inflammation or oxidative stress, Howatson and colleagues did examine these measures to provide more insight into the potential mechanisms of supplementing with cherries before and after marathon (Howatson, McHugh et al. 2010). Twenty recreational marathon runners (13 male and 7 female) were administered either tart cherry juice or a fruit flavored placebo beverage based on their predicted finish time in a pseudo-randomized fashion. Participants were asked to consume the beverage twice daily for the five days before the marathon and for 48 hours after (Howatson, McHugh et al. 2010). The tart cherry juice blend used the same formula as Connely et al. (2006) with an additional 560 mg of other flavonols (quercetin, kaempferol and isoramnetin and their glucosides), flavanols (catechin, epicatechin and procyanidins and their glucosides) and phenolic acids (neochlorogenic acid, 3-coumaroylquinic acid, chlorogenic acid and ellagic acid). Blood was drawn six days before the marathon, the day before, immediately after
and at 24 and 48 hours after the marathon. Blood samples were analyzed for muscle damage with CK and lactate dehydrogenase (LDH), inflammation with C-reactive protein (CRP), IL-6 and uric acid and antioxidant status with total antioxidant status (TAS) and TBARS and protein carbonyls. Muscle soreness (DOMS) and maximum voluntary isometric contraction (MVIC) were also measured after the marathon (Howatson, McHugh et al. 2010).

Measurement of MVIC and DOMS were not assessed pre-race, but were assessed immediately following the marathon, 24 and 48 hours after. Results indicated similar levels of MVIC between the two groups following the race, although the cherry juice group had a significantly faster recovery in strength over the following 48 hours than the placebo group ($p < 0.024$). There were no significant differences found for DOMS between the two groups. Levels of inflammation (IL-6) showed a significant group effect and group by time interaction ($p = 0.009$ and $p < 0.001$, respectively). Immediately following the race, the cherry juice group had a significantly smaller increase in IL-6 ($p < 0.001$) and CRP ($p \leq 0.025$) than did the placebo group. Serum uric acid was elevated post-race and at 24 hours post-race in the placebo group but not in the cherry juice group ($p \leq 0.006$). Expressed as a percentage of baseline, TAS was significantly higher in the cherry juice group compared to the placebo group ($p < 0.001$) following the five-day supplementation. Following the marathon both groups demonstrated a significant increase in TAS ($p < 0.01$, for both groups), although only the cherry juice group remained elevated 24 hours after ($p < 0.01$) (Howatson, McHugh et al. 2010). This study demonstrated that
consuming cherry juice for five days before, the day of and for the two days after running a marathon may have a positive impact on recovery of muscle strength following the race. As well, the juice demonstrated a decrease in inflammation and uric acid following strenuous activity, which can aid in decreasing recovery time. These results suggest that a more natural alternative to pharmaceuticals such as NSAIDS is available to treat inflammation caused by exercise.

The positive results supporting the potential anti-inflammatory effect of cherry juice following strenuous exercise encouraged Bowtell et al. (2011) to further the investigation of cherry juice using single leg exercises to induce oxidative stress and assessing the impact of Montmorency cherry juice (Bowtell, Sumners et al. 2011). Ten healthy, well-trained men were asked to perform a single leg knee extension one-rep max (1RM), one week before the first trial to measure initial leg strength for the study. The participants completed a muscle damaging exercise trial followed by a two-week washout period before repeating the exercise trial using the other leg. Participants consumed 30 mL of either Montmorency cherry juice concentrate (CA) or an isoenergetic fruit concentrate placebo (FC) twice a day for 10 days. On day eight of 10 for each trial period, participants completed the damaging exercise trial (Bowtell, Sumners et al. 2011).

The damaging exercise trial included a warm up of three sets of five repetitions of single-leg knee extension exercises at 50% 1RM separated by a two minute rest then three more reps of maximum voluntary contractions (MVC) at 70% knee flexion angle, each separated by a two minute rest period. After a
five-minute rest, participants completed 10 sets of 10 single-leg knee extensions at 80% 1RM with elongated eccentric phase lasting three seconds, again with two-minute rest between sets. Once the 10 sets at 80% 1RM were completed the participants were again asked to complete the three MVC. Blood samples were taken ten minutes after the last MVC. Following a 24 and 48-hour recovery, participants returned to the lab at the same time each day having fasted overnight for another blood draw. They then repeated the warm-up and three single-leg knee extensions MVC, with two minutes of rest between (Bowtell, Sumners et al. 2011). Creatine kinase, high-sensitivity C-reactive protein (hsCRP), total nitrotyrosine, protein carbonyls and total antioxidant capacity were analyzed using serum samples from each time point. An in-line force transducer was used to measure knee extension force throughout the exercise trials. In addition, pressure-pain threshold was measured using a handheld algometer before, immediately after, 24 and 48 hours after completing the exercise protocol. This assessment involved application of a steadily increasing pressure to the belly of the rectus femoris, vastus lateralis and vastus medialis muscles until the participant decided a point of discomfort had been reached (Bowtell, Sumners et al. 2011).

The relative work completed by the participants did not differ between trials although the knee extension MVC force decreased 64% on average after completing the intensive exercise trial ($p < 0.001$) (Bowtell, Sumners et al. 2011). The force recovery was significantly faster during the CA trial compared to the FC trial with levels returning to 90.9% and 84.9% respectively after 24 hours of
recovery and 92.9% and 88.5% after 48 hours of recovery. In addition, both absolute change and absolute MVC force was significantly higher in the CA trial than the FC trial. While there were significant decreases in pressure point threshold in all three muscles at each recovery time, indicating muscle soreness, there were no differences seen between trials. The significant increase in CK seen after the exercise session was similar in both groups, indicating no significant differences between the trials. Similarly, the changes in protein carbonyls correlated with the changes in muscle recovery, although there were no differences seen between the FC and the CA trials (Bowtell, Sumners et al. 2011). The main outcome in this study was the enhanced recovery of knee extensor maximum isometric strength after the intensive knee extensor resistance training session seen by consuming the Montmorency cherry juice.

In 2012, McLeay and colleagues examined the effects of another berry high in anthocyanins, blueberries, using a similar design. McLeay and colleagues (2012) investigated the effects of blueberry consumption on markers of exercise induced muscle damage and inflammation after strenuous eccentric exercise. Ten healthy females who had been participating in recreational level activity at least twice per week for the past year were recruited to participate in the study. This randomized crossover design investigated the effects of consuming blueberries on performance of one leg on a Biodex isokinetic dynamometer. The same performance was evaluated after the control condition on the contralateral leg at least one month later, depending on the participants’ menstrual cycle (McLeay, Barnes et al. 2012). Participants were tested during the luteal phase of
their menstrual cycle for both exercise trials so that hormone levels and body temperature would be similar during both sessions. All participants were asked to refrain from any exercise other than the necessary walking for the 48 hours prior to the trial and until 60 hours post-trial.

Blood was drawn on the day of the trial prior to a five-minute warm up on a Monark cycle ergometer (McLeay, Barnes et al. 2012). Participants then performed the pre-damage performance testing which included five maximal efforts of each isometric, concentric and eccentric contractions of the quadriceps muscle while seated on the isokinetic dynamometer. Each test was separated by five minutes of passive recovery. Once participants completed the exercise protocol they consumed either the blueberry or control beverage. The blueberry beverage contained 200 g of frozen New Zealand blueberries, a banana, and 200 mL apple juice. The control beverage contained the banana and apple juice but instead of blueberries contained 25 g of dextrose needed to make the drinks isocaloric. Participants returned to the lab two more times, once for a standardized lunch, which included muesli bars (whole grain granola bars) and another beverage, then later that evening for another blood sample, warm-up and the eccentric bout of exercise. This session was followed by a standardized meal. They were also asked to keep a food record for the remainder of the study so that the diet patterns could be replicated on the next round of testing. The eccentric exercise was 300 maximal eccentric repetitions using the quadriceps to induce muscle damage. The following three mornings (12, 36 and 60 hours post-exercise) participants were asked to return to the lab for blood samples,
performance tests, ratings of muscle soreness and a standardized breakfast. The breakfast included the allocated control or blueberry beverage (McLeay, Barnes et al. 2012).

Muscle performance was evaluated by measuring the torque generated during a series of isometric, eccentric and concentric exercises over a 60 hour recovery period. In order to assess perceived muscle soreness participants were asked to step up onto a 40 cm box then step down before rating the perceived level of muscle soreness on a scale from 0 to 10 (0 = no soreness, 10 = very, very painful). Creatine kinase and protein carbonyls were used as biochemical markers of muscle damage for blood draws pre-damage, and 12, 36 and 60 hours post-damaging exercise. Plasma ROS-generating potential was measured with hydrolysed carboxy-dihydro-2',5'-dichloro-hydrofluorescein diacetate (carboxy-H$_2$DCFDA). Inflammation was assessed by plasma IL-6 and the ferric reducing antioxidant power (FRAP) assay was used to detect antioxidant capacity (McLeay, Barnes et al. 2012).

Results documented significant differences in percent change from pre-damage evaluation in peak and average isometric, concentric and eccentric torque to the different time points ($p < 0.001$). The blueberry treatment did not seem to have an effect on torque compared to the control group, although the rate of recovery following isometric tension was significantly faster with the blueberry drinkers in the first 36 hours ($p < 0.047$) (McLeay, Barnes et al. 2012). No overall differences were found between groups for perceived muscle soreness. Between time points (12 and 36 hours post-exercise) the
improvement of the blueberry group was highly significant ($p = 0.0002$) compared to the control group ($p = 0.031$) as was the 60 hour recovery time ($p = 0.008$ and $p = 0.049$) for the blueberry group compared to the control group respectively (McLeay, Barnes et al. 2012).

Following the 300 eccentric contractions, there was a significant increase in oxidative stress and inflammation in both the control and blueberry group. After 12 hours, the oxidative stress markers, ROS-generating potential and protein carbonyls significantly increased in both groups ($p < 0.01$). After 36 hours there was a gradual decrease in ROS-generating potential in the blueberry group, while the control group remained elevated ($p < 0.01$). Plasma protein carbonyls also showed a significant increase at 12 hours for both groups with the blueberry group showing an accelerated decrease in carbonyls compared to control group though not significant ($p = 0.06$). Serum CK showed a gradual and significant increase in both groups ($p < 0.05$) and no differences found for IL-6 between groups. Prior to the onset of exercise the blueberries showed no effect on plasma antioxidant capacity, although between the pre-treatment and the 60 hour recovery time point there was a significant treatment x time interaction ($p = 0.038$) (McLeay, Barnes et al. 2012). This study revealed that blueberry consumption pre- and post- eccentric exercise has the potential to decrease recovery time and reduce exercise-induced oxidative stress.

Another study investigating the effects of endurance exercise and the impact of consuming blueberries to reduce the subsequent oxidative stress was by McAnulty and Colleagues (2011). Twenty-five trained participants (unidentified
as male or female) were randomly placed into either the blueberry (N = 13) or control (N = 12) group for this study. Participants completed a graded exercise test adapted for runners using the MedGraphics CPX metabolic system to determine VO$_{2\text{max}}$ one week prior to the start of the intervention. Participants were given individual servings of 250 g of blueberries. They were advised to incorporate the 250 g of blueberries into their daily diet for 6 weeks. The berries were only to be eaten within the 24-hour period, no specific time frame of when to consume them was given. In addition, participants were asked to avoid certain foods high in vitamin C and E as well as to refrain from consuming additional nutritional supplements and anti-inflammatory medications during the study. Food logs were recorded to ensure homogeneity among participants’ diet (McAnulty, Nieman et al. 2011). The control group followed their usual diet during the six weeks. After the six weeks, participants returned to the lab and completed a 2.5-hour treadmill run (McAnulty, Nieman et al. 2011).

During the follow-up visit, participants had their first blood draw and muscle biopsy taken. Within the one-hour prior to the exercise test, participants in the blueberry group consumed an additional 375 g serving of blueberries before completing the 2.5-hour run at about 72% of their initial VO$_{2\text{max}}$. During the run, participants had metabolic measures assessed every 30 minutes to verify workload. Participants were only allowed water. Blood samples and muscle biopsies were taken immediately after exercise and again one hour later. (McAnulty, Nieman et al. 2011). Blood was analyzed for F$_2$-isoprostanes for oxidative stress, cortisol, cytokines IL-1ra, IL-6, IL-8 and IL-10, homocysteine,
leukocytes, T-cell function, natural killer (KN) and lymphocyte cell counts for inflammation and immune system expression. Antioxidant status was determined using the FRAP assay. Urine was also collected and tested for modifications of DNA damage through 8-hydroxy-2-deoxy guanosine (8-OHDG) and RNA damage through 5-hydroxymethyl-2'-deoxyuridine (5-OHMD) (McAnulty, Nieman et al. 2011).

Results indicated that consuming blueberries had a significant impact on F2-isoprostanes by reducing the increase in levels following exercise in the blueberry group compared to the control group (55% and 129% respectively, \( p = 0.016 \)) (McAnulty, Nieman et al. 2011). No differences were found between groups for muscle NFkB activity or cortisol following exercise. Modified urinary 5-OHMD declined significantly following exercise in the blueberry group compared to the control group (\( p = 0.028 \)) though the marker for DNA damage was not significantly changed. The blueberry group exhibited greater antioxidant capacity compared to the control group (23% and 17% respectively, \( p \leq 0.001 \)) (McAnulty, Nieman et al. 2011). Following the 2.5 hour run, IL-10, IL-1ra, IL-6, IL-8 and homocysteine were elevated significantly for both groups although only IL-10 showed a significant difference with the blueberry group having greater protection (\( p = 0.045 \)). Changes in NK cells were higher in the blueberry group compared to the control group at all three time points showing significant group (\( p = 0.003 \)) and time (\( p \leq 0.001 \)) differences. No significant differences were found in the pattern of change for lymphocytes between groups (McAnulty, Nieman et al. 2011). These results show that consuming 250 g of blueberries
daily for six weeks, along with an additional 375 g of berries consumed one hour prior to running 2.5 hours at ~ 72% VO$_{2\text{max}}$ may reduce F$_2$-isoprostanes and urinary markers for RNA damage. Perhaps a finding that is unique to this study is the changes in NK cells, with the blueberry group showing almost double the amount before exercise compared to the control group. Also of interest was that the levels of NK cells remained elevated throughout exercise in the blueberry group (McAnulty, Nieman et al. 2011). This is of particular interest because NK cells in the blood typically decrease after prolonged exercise. Having significantly higher amounts before exercise appeared to help to keep levels high following exercise, providing more protection. The significant increased in levels of IL-10 seen in the blueberry group also provide additional anti-inflammatory protection needed following exhaustive endurance exercise (McAnulty, Nieman et al. 2011).

Findings from this research are in support with previously discussed studies, showing that anthocyanin supplementation can reduce some oxidative damage and muscle membrane damage caused by strenuous exercise.

While this study by McAnulty et al. (2011), was the first of its kind, it was not without flaws. Without having a baseline of data before the intervention it may be hard to determine the true affects of the blueberry intervention. As well, having the additional acute ingestion of berries prior to exercise effects the interpretation as to whether the chronic blueberry ingestion or the acute dose was responsible for the outcomes. Another issue with this study was the freedom at which the participants were given to consume the berries on their own. This may have led to participants missing several doses of berries or perhaps
consuming them along with dairy, which has been shown to effect bioavailability.

Further research taking these issues into account would benefit the compilation of studies investigating antioxidants, particularly whole fruits such as blueberries and their effects on exercise induced oxidative stress. While few studies have examined both oxidative stress and DNA damage resulting from a session of strenuous exercise in sedentary individuals in one model, evidence suggests a need to expand on previous findings and provide a better understanding of oxidative stress, DNA damage and inflammation and repair with wild blueberry consumption. Thus, the purpose of this study is to determine if a diet of whole WB is an effective inhibitor of the intracellular damage associated with exercise in sedentary humans.
CHAPTER 3
METHODS AND MATERIALS

Study Population

Eleven untrained males between the ages of 21 and 30 were recruited for this study. Males were chosen for this study to avoid changes in inflammation status based on monthly menstruation (Finn 1986, Kawano, Motoyama et al. 1996). Participants were recruited through flyers posted throughout the University of Maine campus, as well as through General Announcements on the FirstClass email server (see appendix A and B). Males meeting the initial requirements (male, aged 21 – 30 years, untrained, not allergic to blueberries, physically able to exercise, particularly walking and or running on a treadmill and were not opposed to having blood draws) were instructed to call for additional information. A phone interview was conducted with potential participants discussing their ability to report to the lab daily for eight weeks to receive the wild blueberry (WB) purée (see appendix C) and to confirm their fitness status. Meeting the “untrained” criteria was defined as the performance of purposeful exercise no more than twice a week for the previous year. Participants meeting initial eligibility requirements completed a follow-up screening. Participants were first provided with a description of the study and were informed of its risks and benefits both verbally and in writing (see appendix D). If they agreed to participate, they were asked to sign a written informed consent at that time (appendix D). Next, they were asked to complete questionnaires assessing their
history of physical activity using the Global Physical Activity Questionnaire (GPAQ) (see appendix E) (World Health Organization 2001), food intake and eating habits (see appendix F) to assess dietary patterns and consumption of foods high in anthocyanins such as berries and red wine, and a medical history questionnaire to rule out any major health conditions (see appendix G) (Del Bo, Riso et al. 2013). This screening process took about one hour to complete. Participants were notified of their eligibility within a week. Individuals with health conditions such as type II diabetes, hypertension, cancer, uncontrolled asthma, any heart condition, a body mass index (BMI) > 30 or a waist circumference > 42 inches were not eligible for the study.

**Experimental Design**

All subjects performed a preliminary treadmill graded exercise test to determine their maximal exercise capacity ($\text{VO}_{2\text{max}}$) using the Bruce Protocol, outlined in Table 3.1 (Med Graffics - Optima II system, Minneapolis, MN). Two weeks following this test, participants were asked to return to the Human Performance Lab in Lengyel Gym at The University of Maine and perform one hour of treadmill exercise at 70% of their maximal capacity. Participants were instructed to consume two pieces of whole-wheat toast with light butter and jam and as much water as desired, two hours before arriving at the lab. Blood was first taken within five minutes before exercise, immediately after, and 30 minutes, one hour, three hours and six hours after the exercise session. Refer to Figure 3.1 for the experimental design.
Table 3.1. The Bruce Protocol for determining VO$_{2\text{max}}$ Testing

<table>
<thead>
<tr>
<th>Stage</th>
<th>Speed MPH</th>
<th>Grade (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.7</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>3.4</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>4.2</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>18</td>
</tr>
<tr>
<td>6</td>
<td>5.5</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>6.0</td>
<td>22</td>
</tr>
<tr>
<td>8</td>
<td>6.5</td>
<td>24</td>
</tr>
<tr>
<td>9</td>
<td>7.0</td>
<td>26</td>
</tr>
</tbody>
</table>

The Bruce Protocol for VO$_{2\text{max}}$ testing increases in speed and grade every three minutes until complete exhaustion.

Following exercise participants were asked to remain in the lab and were allowed water only until the final blood draw was taken. Participants were able to use this time to read, work or rest. Two weeks following this exercise session, the subjects began an eight-week course of WB purée consumption. During the eight-week feeding period, participants came to the conference room (room 157, Hitchner Hall) daily to receive and consume their WB purée. The purée contained 300g of berries (equivalent to two cups). Wyman’s Company donated the WB in frozen form. Participants reported to the lab between 7am and 10am Monday through Friday to consume the WB purée. On Fridays, participants were provided cooler bags with frozen WB purée for Saturday and Sunday’s consumption. They were provided with defrosting instructions as well as a list of foods to avoid, particularly dairy for 30 minutes before and after consuming the WB purée to prevent interference with bioavailability. Participants returned the cooler bags the next Monday morning.
Figure 3.1. Experimental Design

- Baseline
- Pre-Exercise Session 1 Blood Draw
- Post-Exercise Session 1 Blood Draw at Time 0 min, 30 min, 1 h, 3 h, 6h
- 2-week rest
- Graded Exercise Test VO$_2$max
- Exercise Session 1 at 70% VO$_2$max for 60 min
- Wild Blueberry Treatment 8 Weeks
- Pre-Exercise Session 2 Blood Draw
- Post-Exercise Session 2 Blood Draw at Time 0 min, 30 min, 1 h, 3 h, 6h
- Exercise Session 2 at 70% VO$_2$max for 60 min
Throughout the experiment, participants were required to avoid foods rich in anthocyanins such as berries and red wine, and to limit the intake of foods rich in other types of antioxidants such as oranges, and tomatoes (see appendix H). In addition, participants requested to avoid the use of all herbs and medications, such as non-steroidal anti-inflammatory drugs (NSAIDS), that are known to affect inflammation and immune function and limit any multivitamin use to no more than 100% of the United States Daily Value. Participants were also instructed to remain at the current level of activity (≤ two days/week), to report any changes to diet, exercise or health that may impact the study, and to limit alcohol consumption (≤ two drinks/day).

At the end of this eight-week period, participants performed a second bout of one-hour exercise at 70% of their maximal capacity (VO$_{2\text{max}}$). The second round of blood draws were taken at this time, within five minutes before exercise, immediately after, and 30 minutes, one hour, three hours and six hours after the exercise session (Figure 3.1).

A total of six, 10mL samples were taken at each exercise session for a total of 120 mL during the study. To allow for these multiple draws, a trained phlebotomist inserted a catheter (intravenous (IV) line) upon arrival to the Human Performance Lab. The catheter was periodically flushed with sodium citrate (Ricca Chemical, Arlington, TX) either by the phlebotomist or the participant to prevent clotting. Using a catheter was more practical and comfortable for the participant rather than a multitude of individual sticks with a needle. Blood samples were collected in tubes (Fisher Scientific, Pittsburg, PA), and
immediately placed on ice. Within one hour of collection, the samples were taken to Dr. Dorothy Klimis-Zacas’s Clinical Laboratory in Hitchner Hall room 214, at The University of Maine where blood was centrifuged at 2500 RPM for 15 minutes (Sorvall RT1 centrifuge, Thermo SCIENTIFIC Asheville, NC). Plasma was separated and stored at -80° C for later analysis in Hitchner Hall.

**Blueberry Processing**

Two thousand grams of frozen WB were placed on a wire tray and evenly distributed. The berries were placed on a belt, which fed through a steam convection cooker (Laitram Coolsteam Convection Cooker, model - pilot cooker Harahan, LA) set at 77° C for four minutes and 15 seconds. The blueberries were steam-blanch to an internal temperature of 70°C, then immediately chilled to 30° C using a spray gun and hose with tap water. The processing protocol outlined by Rossi et al. (2003) was used (Rossi, Giussani et al. 2003). The berries were then puréed for 10 seconds at 3000 RPM in an RSI 6V Robot Coupe commercial food processor (Robot Coupe Scientific, Industrial Division, Robot Coupe, USA Inc. Ridgewood, CA) until puréed but not liquid. Three hundred grams of berries were then placed into vacuum seal bags and laid flat on a tray that was transferred to a freezer set at -35°C for 120 minutes until partially frozen. Bags were then vacuum-sealed and frozen at -20°C until use. The bags of WB purée were consumed within four weeks. Prior to consumption the berries were defrosted overnight at 37° C.
Anthocyanin Concentration in Wild Blueberries

Wild blueberries were provided as a composite from Wyman's (Cherryfield, ME, USA). Ten, 300 g vacuum-sealed bags of the previously steam blanched and frozen blueberries were defrosted overnight at 37°C. The total concentration of anthocyanins in the WB purée was determined spectrophotometrically. Briefly, 50 μL aliquots of the extract were diluted 1:10 with 450 μL of distilled water in a tube while being protected from light. Two hundred μL of the diluted sample was transferred in each of two clean tubes before the addition of 200 μL buffer (0.4 M CH₃COONa) at pH 4.5 to one tube then vortexed. Two hundred μL of buffer (0.025 M KCl) pH 1 was added to the other tube and vortexed. The absorbance was measured with a spectrophotometer twice for each sample at the following wavelengths: 510 and 700 nm (Perkin-Elmer Lambda 20, Waltham, MA, USA). The blanched WB purée contained 51 mg/L of anthocyanins and 331 mg/L of phenolics.

Anthropometrics

Anthropometric measurements taken during the initial screening included height, weight, waist circumference (just above the iliac crest) and body composition using the 7-site skin-fold caliper method (chest, midaxillary, triceps, subscapular, abdomen, suprailiac, thigh) (Lange Skinfold Caliper, Beta Technology, Gays Mills, WI). The post-intervention body composition measurements were recorded at the beginning of the post-exercise session (Appendix G). Weight (lbs), body mass index (BMI) and percent fat (%fat) were
measured to analyze the effect of the eight-week WB diet intervention on body composition. All measurements were taken by a single, trained individual to ensure validity. Body mass index was calculated [(weight (lbs)/ height (inches) 2) x 704.5] and percent fat was calculated [((4.95/body density)-4.5) x 100] (McArdle, Katch et al. 2006).

Diet Analysis

Participants were given a three-day food intake log (appendix I) to record their daily food and beverage intake during the follow-up screening before baseline testing. Participants were instructed to specify the exact amount of each food item, the initial state of the food (fresh, frozen, or canned), the preparation type (fried, baked, boiled), the brand names (or any comparable brand names), provide food labels when available and any complete recipes including the number of servings. During exercise session one, a Registered Dietitian reviewed the food logs with the participants to ensure accuracy. Following the intervention, participants completed a second three-day food log. This log was returned during exercise session two and reviewed with the participant. A Registered Dietitian analyzed both sets of food logs using The Food Processor, Nutrition and Fitness Software (esha Research, Salem, OR). Nine of the 10 participants completed both pre- and post-intervention three-day food intake logs and included in the analysis.

Table 3.2 displays the macronutrient nutrition profile analyzed with The Food Processor Software. Vitamins and minerals that work as antioxidants were
measured using both *The Food Processor* software and the *USDA Database for the Flavonoid Content of Selected Foods, Release 3 (2011)* USDA. *The Food Processor* program provides a limited amount of non-flavonoid antioxidants. The antioxidants analyzed from this program are included in Table 3.2. The *USDA Database for the Flavonoid Content of Selected Foods, Release 3 (2011)* was used to analyze the flavonoid content of the participant’s diets, pre- and post-intervention. The selected flavonoids measured are included in Table 3.2. Each flavonoid was converted to milligrams per 100 gram, edible portion (mg/100 g, edible portion) corresponding to the food items. The amount of each flavonoid was then calculated for the given weight (g) of the food items consumed. The average amount of each flavonoid (mg) consumed by each participant before and after WB intervention, as well as the average of each flavonoid consumed as a group before and after the WB intervention were calculated. Post-intervention dietary analysis did not include the provided WB.
Table 3.2. Nutrient Profile Analysis of the Study Population

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>Vitamin/Mineral</th>
<th>Plant Bioactives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Calories (Kcal)</td>
<td>Beta-carotene (mcg)</td>
<td>Anthocyanidins</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>Vitamin C (mg)</td>
<td>Cyanidin</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>Vitamin E (mg)</td>
<td>Delphinidin</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>Manganese (mg)</td>
<td>Malvidin</td>
</tr>
<tr>
<td></td>
<td>Selenium (µg)</td>
<td>Pelargonidin</td>
</tr>
<tr>
<td></td>
<td>Zinc (mg)</td>
<td>Peonidin</td>
</tr>
<tr>
<td></td>
<td>Lycopene (mg)</td>
<td>Petunidin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flavonols</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kaempferol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myricetin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quercetin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flavonones</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hesperetin</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flavon-3-ols</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Catechins</td>
</tr>
</tbody>
</table>

Nutrients and plant bioactives were analyzed by The USDA Database for the Flavonoid Content of Selected Foods, Release 3 (2011) and The Food Processor, Nutrition and Fitness Software (esha Research, Salem, OR).

Biochemical Assays

Manganese Superoxide Dismutase (Mn-SOD), Tumor necrosis factor-alpha (TNFα) and interleukin-6 (IL-6) were measured in plasma samples with the R&D Quantikine ELISA assay kit (DYC3419, DTA00C, HS600B, R&D Systems, Minneapolis, MN) according to manufacturer instructions. Analyses were completed on eight of the 10 participants due to individual physiological outliers. Blood was collected via venous puncture and placed on ice no longer than one hour. Blood samples were centrifuged at 2300 RPM for 15 minutes (Sorvall RT1 centrifuge, Thermo SCIENTIFIC Asheville, NC). Plasma was separated, aliquoted into 500 µL tubes and stored at -80°C until the assays were performed. Colorimetric detection of Mn-SOD, TNFα and IL-6 as an azo dye product of the Griess reaction was performed at 450 nm with correction lengths of 570, 450 nm.
and at 540 nm and 490 nm with a correction of 650 nm for Mn-SOD, TNFα and IL-6 respectively, using a Spectra Max Plus 384 plate reader (Molecular Devices Inc., Sunnyvale, CA).

An alkaline version of the single cell electrophoresis or “comet assay” was performed to detect DNA damage. For lymphocyte extraction, one mL of blood was removed from the fresh blood sample. Next, 250 μL of blood was mixed with 250 μL culture medium RPMI (Roswell Part Memorial Institution). The blood and RPMI mixture was layered on 500 μL hystopaque (Select Science) and centrifuged at 5000 RPM for five minutes. The lymphocyte layer was then collected and washed with about one mL phosphate buffered saline (PBS) and centrifuged at max speed for 10 seconds to pellet lymphocytes. Phosphate buffered saline was then removed and the pellet was resuspended in one mL of 50% RPMI (40% fetal bovine serum and 10% dimethyl sulfoxide (DMSO)). Samples were then immediately stored at -80° C until they were placed on dry ice and sent overnight to the Linus Pauling Institute of Science at Oregon State University where they were again stored at -80° C until the analysis was performed two weeks later. Before use, the lymphocytes were defrosted and transferred to eppendorf 1.5 mL tubes and immediately centrifuged at max speed for about four minutes. The supernatant was removed and the samples were then resuspended in about one mL PBS and mixed. Single-cell electrophoresis was conducted according to protocol outlined by Anderson and Laubenthal (2013) (Anderson and Laubenthal 2013). A fluorescent microscope (Nikon E400) and Comet Assay software (Comet Assay IV, Perspective Instruments, Cleaver
Scientific, Rugby, Warwickshire, England) was used to score the comet-like nuclei, randomly scoring a minimum of 25 cells per slide. DNA damage was recognized as a fluorescent core followed by fragments or “tail” and the damaged DNA fragments migrated during electrophoresis. Damage was calculated as %DNA in tail.

**Statistics**

A two-way ANOVA for repeated measures using intervention and time as dependent variables was used to determine any statistically significant ($p < 0.05$) differences in the biochemical markers: in one group (pre-intervention vs. post-intervention) at six time points (pre-exercise, immediately following exercise, 30 minutes, one hour, three hour, and six hours post-exercise). A paired, two-tailed, t-test was used to determine statistical significant ($p < 0.05$) difference in nutrients, antioxidants, and body composition measurements before and after the WB intervention. The IBM SPSS Statistics Program, Version 21 (Armonk, NY) was used to perform statistical analysis. Results are presented in mean ± standard deviation.
CHAPTER 4
RESULTS

Population Characteristics

Of the 11 participants that were recruited, 10 completed the intervention in its entirety (a 90% participation rate). Demographics of the 10 participants are presented in Table 4.1. The mean ± standard deviation (SD) of participants’ age was 22.8 ± 1.8, ranging from 21 to 26 years. The United States (U.S.) national average for weight in males over the age of 20, (85.6 ± 2.33 kg) is higher than the average weight of participants (76.9 ± 8.9 kg), although not statistically significant (p > 0.05) (Fryar, Gu et al. 2012). Participants were comparable to the national average for height (70.25 ± 3.1 inches compared to 69.3 ± 0.13 inches) although they were significantly below the national average in waist circumference (34.09 ± 3.3 inches compared to 36.9 ± 0.80 inches (p ≤ 0.03). Body mass index (BMI) was also significantly lower than the national average (24.25 ± 3.4 kg/m² compared to 27.0 ± 0.31 kg/m², p ≤ 0.02) (Fryar, Gu et al. 2012). Despite being sedentary, (exercising 1.34 ± 0.7 days/week) the participants in this study had significantly lower levels of body fat compared to the average male between the ages of 19 and 30 years (14.2 ± 5.5 compared to 22%, p ≤ 0.002) (Fryar, Gu et al. 2012). Their average VO₂max (45.18 ± 8.2 ml/kg/min) was considered slightly above average (34 – 43.9 ml/kg/min) for males ages 20 – 29, though not significant (McArdle, Katch et al. 2006). Refer to Figure 4.1 for national averages comparisons.
### Table 4.1. Study Populations’ Characteristics\(^a\)

<table>
<thead>
<tr>
<th>Variables</th>
<th>National Average for US Males ages 20-29</th>
<th>Pre-Intervention (n = 10)</th>
<th>Post-Intervention (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>20-29</td>
<td>22.8 ± 1.9</td>
<td>22.9 ± 1.8</td>
</tr>
<tr>
<td>Height (in)</td>
<td>69.9 ± 0.13</td>
<td>70.25 ± 3.1</td>
<td>70.25 ± 3.1</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>85.6 ± 2.33</td>
<td>76.90 ± 8.9</td>
<td>76.85 ± 10.0</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>27.0 ± 0.31</td>
<td>24.25 ± 3.4(^*)</td>
<td>24.22 ± 3.6(^*)</td>
</tr>
<tr>
<td>Waist circumference (in)</td>
<td>36.9 ± 0.80</td>
<td>34.09 ± 3.3(^*)</td>
<td>34.09 ± 3.6(^*)</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>22(%)</td>
<td>14.20 ± 5.5(^*)</td>
<td>14.15 ± 4.9(^*)</td>
</tr>
<tr>
<td>Physical Activity (d/wk)</td>
<td>--</td>
<td>1.34 ± 0.7</td>
<td>0.31 ± 0.3(^**)</td>
</tr>
<tr>
<td>(\text{VO}_2\text{max} (ml/kg/min))</td>
<td>34 – 43.9</td>
<td>45.18 ± 8.2</td>
<td>NA</td>
</tr>
</tbody>
</table>

\(^a\)Data are expressed as mean ± SD
(BMI) body mass index, physical activity (days/week purposeful exercise)
\(^*\)Statistically significant (p ≤ 0.05) from National Average
\(^**\)Statistically significant (p ≤ 0.02) pre-post

### Figure 4.1. Study Populations’ Characteristics Compared to National Averages\(^a\)

There were no significant differences for any anthropometric measurements taken pre- and post-intervention (percent body fat, weight, height, BMI, waist circumference) (p > 0.05 for all measures) among participants. Days
engaged in purposeful physical activity significantly decreased throughout the study, from \(1.34 \pm 0.7\) days/week pre-intervention, to \(0.31 \pm 0.3\) days per week post-intervention \((p < 0.001)\) (Figure 4.2.).

**Figure 4.2.** Study Populations’ Purposeful Physical Activity Levels Pre-Post-Intervention\(^a\)

![Graph showing purposeful activity levels pre- and post-intervention.](image)

\(^a\)Data are expressed as mean ± SD

\(^*\)Statistically significant \((p \leq 0.05)\)

**Diets**

Participants completed a three-day food log pre- and post-intervention. Of the 10 participants to complete the study, nine participants completed both the pre- and post- diet logs. Table 4.2 presents the nutrient intake of nine participants’ pre- and post- intervention, compared with the Dietary Reference Intakes (DRIs) and Recommended Dietary Allowances (RDAs) for males ages 19 – 30 years old.
<table>
<thead>
<tr>
<th>Nutrients</th>
<th>DRI (RDA)</th>
<th>Pre-Intervention (n = 9)</th>
<th>Post-Intervention (n = 9)</th>
<th>p value pre-post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kcals</td>
<td>2209.26</td>
<td>2649.7 ± 506.2*</td>
<td>2432.5 ± 634.6</td>
<td>p ≤ 0.422</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.8g/kg (61.52)</td>
<td>91.5 ± 27.2*</td>
<td>73.9 ± 12.8*</td>
<td>p ≤ 0.085</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>45-65% of energy</td>
<td>350.8 ± 77.3</td>
<td>295.4 ± 88.8</td>
<td>p ≤ 0.150</td>
</tr>
<tr>
<td></td>
<td>303.7 = 55% of EER</td>
<td>298.1 – 430.6</td>
<td>273.7 – 395.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>130g/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat (g)</td>
<td>20-35% of energy</td>
<td>36% of energy</td>
<td>40% of energy</td>
<td>p ≤ 0.841</td>
</tr>
<tr>
<td></td>
<td>73.6g = 30% of EER</td>
<td>96.7 ± 23.5</td>
<td>99.1 ± 31.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30g/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-carotene (mcg)</td>
<td>NA</td>
<td>1650.1 ± 1294.1</td>
<td>2266.0 ± 2695.7</td>
<td>p ≤ 0.393</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>90mg/day</td>
<td>114.0 ± 122.4</td>
<td>60.1 ± 45.0</td>
<td>p ≤ 0.244</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>15mg/day</td>
<td>12.0 ± 9.4</td>
<td>6.1 ± 3.8*</td>
<td>p ≤ 0.070</td>
</tr>
<tr>
<td>Manganese (mg)</td>
<td>2.3mg/day</td>
<td>4.1 ± 1.9*</td>
<td>2.6 ± 1.9</td>
<td>p ≤ 0.008</td>
</tr>
<tr>
<td>Selenium (μg)</td>
<td>55μg/day</td>
<td>120.8 ± 38.0*</td>
<td>79.3 ± 18.0*</td>
<td>p ≤ 0.010</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>11mg/day</td>
<td>12.5 ± 5.0</td>
<td>7.5 ± 2.7*</td>
<td>p ≤ 0.033</td>
</tr>
<tr>
<td>Lycopene (mg)</td>
<td>NA</td>
<td>5886.3 ± 7941.8</td>
<td>2701.3 ± 5159.3</td>
<td>p ≤ 0.234</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD
Dietary Reference Intakes (DRI), Recommended Dietary Allowances (RDAs) are set to meet the needs of almost all (97 to 98 percent) individuals in a group.
Estimated energy requirement (EER) based on men ages 19 years and older: EER = [662 – (9.53 X age)] + PA* X [(15.91 X wt) + (539.6 X ht)], using the average age (23), wt (77kg) and ht (70in) for all participants and a physical activity factor (PA) for sedentary men (1.0)
Based on the average weight of participants in study (0.8g/kg/body weight)
Adequate Intake (AI)
Minimum requirement
Recommended ranged based on 45-65% of total energy
Grams of carbohydrate to meet 55% of EER (2209.26) Grams of fat to meet 30% of EER (2209.26)
* Statistically significant (p ≤ 0.05) from DRI (RDA) recommendations

*a Data are expressed as mean ± SD
  Dietary Reference Intakes (DRI), Recommended Dietary Allowances (RDAs) are set to meet the needs of almost all (97 to 98 percent) individuals in a group.
b Estimated energy requirement (EER) based on men ages 19 years and older: EER = [662 – (9.53 X age)] + PA* X [(15.91 X wt) + (539.6 X ht)], using the average age (23), wt (77kg) and ht (70in) for all participants and a physical activity factor (PA) for sedentary men (1.0)
c Based on the average weight of participants in study (0.8g/kg/body weight)
d Adequate Intake (AI)
e Minimum requirement
f Recommended ranged based on 45-65% of total energy
g Grams of carbohydrate to meet 55% of EER (2209.26) Grams of fat to meet 30% of EER (2209.26)
* Statistically significant (p ≤ 0.05) from DRI (RDA) recommendations
On average, participants’ daily consumption of kcals was 2649.7 ± 506.2 prior to the intervention. This amount is significantly higher than the recommended kcal intake (2209.26 kcals) based on the estimated energy requirements (EERs) for sedentary males ages 19 and older ($p \leq 0.031$). Protein recommendations were based on the RDA of 0.8 g/kg body weight. Based on the average weight of the participants (77 kg), the protein recommendation is 61.52 g/day or an Adequate Intake (AI) of 56 g/day. On average, participants consumed, 91.5g ± 27.2 of protein pre-intervention, an amount significantly higher than the recommendation ($p \leq 0.011$). Compared to the RDAs (90 mg/day and 15 mg/day), participants consumed comparable levels of vitamin C and vitamin E (114 ± 122.4 mg and 12.0 ± 9.4 mg, respectively) ($p > 0.05$). Manganese (Mn) consumption was significantly higher than the set AI prior to the intervention (4.1 ± 1.9 mg/day compared to 2.3 mg/day, $p \leq 0.025$). Selenium (Se) was found to be significantly higher than the RDA (55 μg/day) pre-intervention (120.8 ± 38.0, $p \leq 0.001$). Pre-intervention, participants consumed adequate levels on zinc (Zn) compared to the RDA (12.5 ± 5.0 mg 11 mg/day, $p > 0.05$). Refer to Figure 4.3 for DRI, RDA comparisons. In data not shown, participants’ three-day food intake logs indicated lower levels of foods containing high levels of Zn, Se and Mn, such as fresh seafood and ground beef, as well as lower levels of fruits and vegetables listed for participants to limit or eliminate during the intervention (see Appendix I).
**Figure 4.3.** Study Populations’ Dietary Intakes compared to the Dietary Reference Intakes and Recommended Dietary Allowances for Sedentary Males Ages 19 – 30 years  

A  

No significant changes were observed pre- or post-intervention for macronutrients; Calories (kcal/day), protein (g/day), carbohydrates (g/day) or total fat (g/day) pre-post-intervention (see Table 4.2). Antioxidant levels did not significantly change for beta-carotene, vitamin C, vitamin E, or lycopene ($p >$...
A significant decrease was detected in Mn ($p \leq 0.008$), Se ($p \leq 0.010$) and Zn ($p \leq 0.033$) pre-post-intervention. Table 4.3 shows the level of flavonoids (anthocyanins, flavonols, flavones, flavanones, and flavon-3-ols) pre-post-intervention. No significant changes ($p > 0.05$) were detected. The table does not include the 51 mg/L of anthocyanins or the 331 mg/L of phenolics consumed in the steam blanched WB purée.

**Table 4.3. Study Population Plant Bioactive Consumption Pre-Post-Intervention**

<table>
<thead>
<tr>
<th>Plant Bioactives</th>
<th>Pre-Intervention (n = 9)</th>
<th>Post-Intervention (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthocyanidins (mg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyanidin</td>
<td>0.21 ± 0.29</td>
<td>0.09 ± 0.13</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>1.56 ± 1.90</td>
<td>0.49 ± 0.63</td>
</tr>
<tr>
<td>Malvidin</td>
<td>2.67 ± 3.45</td>
<td>0.42 ± 1.13</td>
</tr>
<tr>
<td>Pelargonidin</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Peonidin</td>
<td>0.11 ± 0.18</td>
<td>0.06 ± 0.14</td>
</tr>
<tr>
<td>Petunidin</td>
<td>0.98 ± 1.61</td>
<td>0.16 ± 0.33</td>
</tr>
<tr>
<td><strong>Flavonols (mg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isorhamnetin</td>
<td>0.02 ± 0.03</td>
<td>0.06 ± 0.10</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>0.13 ± 0.18</td>
<td>0.11 ± 0.17</td>
</tr>
<tr>
<td>Myricetin</td>
<td>0.08 ± 0.13</td>
<td>0.07 ± 0.10</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1.14 ± 0.71</td>
<td>1.12 ± 0.98</td>
</tr>
<tr>
<td><strong>Flavones (mg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apigenin</td>
<td>0.00 ± 0.00</td>
<td>0.03 ± 0.09</td>
</tr>
<tr>
<td>Luteolin</td>
<td>0.18 ± 0.17</td>
<td>0.12 ± 0.90</td>
</tr>
<tr>
<td><strong>Flavanones (mg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eriodictyol</td>
<td>0.00 ± 0.00</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Hesperetin</td>
<td>0.41 ± 1.12</td>
<td>1.22 ± 3.45</td>
</tr>
<tr>
<td>Naringenin</td>
<td>0.22 ± 0.56</td>
<td>0.33 ± 0.56</td>
</tr>
<tr>
<td><strong>Flavan-3-ols (mg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catechins</td>
<td>0.49 ± 0.64</td>
<td>0.68 ± 0.69</td>
</tr>
<tr>
<td>Gallic acid esters</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Epicatechins</td>
<td>0.40 ± 0.60</td>
<td>0.94 ± 1.32</td>
</tr>
<tr>
<td>Epicatechin 3-gallate</td>
<td>0.12 ± 0.02</td>
<td>0.18 ± 0.51</td>
</tr>
<tr>
<td>Epigallocatechin</td>
<td>0.03 ± 0.03</td>
<td>0.29 ± 0.69</td>
</tr>
<tr>
<td>Epigallocatechin 3-gallate</td>
<td>0.02 ± 0.03</td>
<td>0.30 ± 0.81</td>
</tr>
<tr>
<td>Gallo catechin</td>
<td>0.00 ± 0.00</td>
<td>0.04 ± 0.11</td>
</tr>
<tr>
<td>Theaflavins</td>
<td>0.00 ± 0.00</td>
<td>0.05 ± 0.14</td>
</tr>
<tr>
<td>Theaflavin-3, 3’-digallate</td>
<td>0.00 ± 0.00</td>
<td>0.05 ± 0.15</td>
</tr>
<tr>
<td>Theaflavin-3’-gallate</td>
<td>0.00 ± 0.00</td>
<td>0.04 ± 0.13</td>
</tr>
<tr>
<td>Theaflavin-3-gallate</td>
<td>0.00 ± 0.00</td>
<td>0.04 ± 0.11</td>
</tr>
<tr>
<td>Thearubigins</td>
<td>0.04 ± 0.12</td>
<td>2.38 ± 7.13</td>
</tr>
</tbody>
</table>

aData are expressed as mean ± SD
Plasma Manganese Superoxide Dismutase (Mn-SOD)

Figure 4.4 displays the plasma concentrations of Mn-SOD pre-post-intervention. A significant interaction between pre- and post- intervention over time was detected ($p \leq 0.014$). There were no significant post-hoc differences between any time points pre- or post-intervention. Before treatment, plasma Mn-SOD concentrations tended to increase 30 minutes after exercise, then steadily decreased. Following the intervention, the concentration of Mn-SOD tended to decrease 30 minutes after exercise, then increased back to baseline levels six hours post-exercise (Table 4.4).

Figure 4.4. Effect of Wild Blueberry Intervention on Plasma Manganese Superoxide Dismutase (Mn-SOD) Concentrations Following Strenuous Exercise$^a$

$^a$Data expressed as mean ± SD
Statistically significant interaction pre/post intervention across time ($p \leq 0.014$)
Table 4.4. Effect of Wild Blueberry Intervention on Plasma Mangangese Superoxide Dismutase (Mn-SOD) Concentrations Following Strenuous Exercise

<table>
<thead>
<tr>
<th>Time</th>
<th>Pre-Intervention (N = 6) pg/mL</th>
<th>Post-Intervention (N = 6) pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-exercise</td>
<td>5310.8 ± 3326.9</td>
<td>5961.6 ± 3693.7</td>
</tr>
<tr>
<td>0 min</td>
<td>5751.1 ± 2395.5</td>
<td>5826.4 ± 2310.8</td>
</tr>
<tr>
<td>30 min</td>
<td>7084.6 ± 3073.6</td>
<td>4795.6 ± 2404.2</td>
</tr>
<tr>
<td>1 hr</td>
<td>6925.2 ± 3758.2</td>
<td>5593.2 ± 4125.0</td>
</tr>
<tr>
<td>3 hr</td>
<td>6527.2 ± 3564.1</td>
<td>6906.3 ± 2573.7</td>
</tr>
<tr>
<td>6 hr</td>
<td>5693.3 ± 3775.8</td>
<td>5309.9 ± 3665.2</td>
</tr>
</tbody>
</table>

aData expressed mean ± SD
Statistically significant interaction pre/post intervention across time (p ≤ 0.014)

DNA Single Strand Breaks

DNA single strand breaks were evaluated by the percent of DNA in the tail after single cell gel electrophoresis "comet assay". Figure 4.5 displays an undamaged comet (A) and a damaged comet (B) as it appears under the fluorescent microscope. Figure 4.6 and Table 4.5 presents the results of DNA strand breaks registered at each time point (pre-exercise, 0 minutes, 30 minutes, one hour, three hours, and six hours post-exercise) pre- and post-intervention. No significant changes in DNA damage were detected at any time-points before or after exercise (Figure 4.6 and Table 4.5). The WB intervention did not have a significant effect on DNA damage. Additionally, no differences were found for time or the interaction between time and pre-post-intervention (Figure 4.6 and Table 4.5).
Figure 4.5. DNA Strand Before and After Single Strand Breaks As Seen Under A Fluorescent Microscope

A. 
B.

Damaged and undamaged DNA strand “Comet” as seen under a fluorescent microscope (Nikon E400) and Comet Assay software (Comet Assay IV, Perspective Instruments, Cleaver Scientific, Rugby, Warwickshire, England), before (A) and after (B) damage

Figure 4.6. Effect of Wild Blueberry Intervention on DNA Damage Following Strenuous Exercise

%DNA in Tail

Pre 0 min 30 min 1 hr 3 hr 6 hr
Pre-Intervention Post-Intervention

aData expressed as mean ± SD
No significant differences were found for time or pre/post intervention
Table 4.5. Effect of Wild Blueberry Intervention on DNA Damage Following Strenuous Exercise$^a$

<table>
<thead>
<tr>
<th>Time</th>
<th>Pre-Intervention (N = 8)</th>
<th>Post-Intervention (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% DNA in Tail</td>
<td>% DNA in Tail</td>
</tr>
<tr>
<td>Pre-Exercise</td>
<td>66.8 ± 13.8</td>
<td>66.6 ± 16.8</td>
</tr>
<tr>
<td>0 min</td>
<td>62.4 ± 7.8</td>
<td>70.2 ± 15.8</td>
</tr>
<tr>
<td>30 min</td>
<td>66.2 ± 13.2</td>
<td>67.3 ± 18.9</td>
</tr>
<tr>
<td>1 hr</td>
<td>62.2 ± 12.5</td>
<td>74.0 ± 16.2</td>
</tr>
<tr>
<td>3 hr</td>
<td>53.8 ± 9.5</td>
<td>61.7 ± 13.0</td>
</tr>
<tr>
<td>6 hr</td>
<td>57.4 ± 13.2</td>
<td>65.7 ± 14.1</td>
</tr>
</tbody>
</table>

$^a$Data are expressed as mean ± SD

Inflammatory Markers

Interleukin-6 (IL-6). Treadmill walking at 70% of VO$_{2\text{max}}$ for one hour caused a significant increase in inflammation (IL-6) ($p \leq 0.003$) (Figure 4.7).

Levels of IL-6 significantly increased from rest to immediately following exercise (pre: 0.80 ± 0.17 pg/mL to 30 minutes: 3.0 ± 0.49 pg/mL, $p < 0.001$). They then significantly decreased one hour after exercise (one hour: 2.20 ± 0.59 pg/mL to three hour: 1.46 ± 0.31 pg/mL, $p \leq 0.009$). No differences in IL-6 concentration were seen between pre- and post-intervention at all time points ($p \leq 0.84$).
Figure 4.7. Effect of Wild Blueberry Intervention on Plasma Interleukin-6 (IL-6) Levels.

![Graph showing the effect of Wild Blueberry Intervention on Plasma Interleukin-6 (IL-6) Levels.](image)

Figure 4.7 presents the effect of WB on the pro-inflammatory cytokine TNFα. Participants remained within normal ranges (0 – 22 pg/mL) at all time points. Table 4.6 outlines the results of both IL-6 and TNFα at each time point pre- and post-intervention. No significant changes were seen in TNFα for time or pre-post-intervention, following strenuous exercise, although a marginal trend for decreasing levels was seen following the WB intervention (p ≤ 0.67) (Figure 4.8).

**Tumor Necrosis Factor-alpha (TNFα).** Figure 4.8 presents the effect of WB on the pro-inflammatory cytokine TNFα. Participants remained within normal ranges (0 – 22 pg/mL) at all time points. Table 4.6 outlines the results of both IL-6 and TNFα at each time point pre- and post-intervention. No significant changes were seen in TNFα for time or pre-post-intervention, following strenuous exercise, although a marginal trend for decreasing levels was seen following the WB intervention (p ≤ 0.67) (Figure 4.8).
Figure 4.8. Effect of Wild Blueberry Intervention on Plasma Tumor Necrosis Factor-alpha (TNFα) Levels Following Strenuous Exercise

Table 4.6. Effect of Wild Blueberry Intervention on Plasma Inflammatory Markers Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNFα) Following Strenuous Exercise

<table>
<thead>
<tr>
<th>Time</th>
<th>Pre-Intervention</th>
<th>Post-Intervention</th>
<th>Pre-Intervention</th>
<th>Post-Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Pre-Exercise</td>
<td>7.3 ± 3.4</td>
<td>4.4 ± 2.6</td>
<td>0.8 ± 0.5</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>0 min</td>
<td>7.4 ± 3.7</td>
<td>5.6 ± 3.6</td>
<td>3.1 ± 1.4*</td>
<td>2.7 ± 1.4*</td>
</tr>
<tr>
<td>30 min</td>
<td>9.3 ± 5.8</td>
<td>6.6 ± 3.9</td>
<td>2.2 ± 1.7</td>
<td>2.4 ± 1.6</td>
</tr>
<tr>
<td>1 hr</td>
<td>8.1 ± 3.7</td>
<td>4.1 ± 2.0</td>
<td>1.5 ± 0.9**</td>
<td>1.5 ± 0.5**</td>
</tr>
<tr>
<td>3 hr</td>
<td>8.3 ± 5.0</td>
<td>4.1 ± 1.1</td>
<td>1.5 ± 1.6</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>6 hr</td>
<td>7.2 ± 3.1</td>
<td>4.1 ± 2.2</td>
<td>1.2 ± 1.0</td>
<td>1.9 ± 1.1</td>
</tr>
</tbody>
</table>

aData are expressed as mean ± SD
*Statistically significant (p < 0.05) for time 0 minutes to 30 minutes post-exercise, for pre- and post-Intervention
** Statistically significant (p < 0.05) for time 30 minutes to 1 hr post-exercise, for pre- and post-Intervention
CHAPTER 5
DISCUSSION

Summary

The current study documented the effects of an eight week wild blueberry (WB) intervention aimed at inducing oxidative stress, inflammation and DNA damage on 10 sedentary males exposed to exercise. In order to investigate the effectiveness of WB consumption in reducing the effects of exercise-induced oxidative stress, inflammation and DNA damage only healthy, sedentary males were used. In addition, diet was controlled to eliminate anthocyanin and flavonoid consumption outside of the WB purée provided. Participants also reported daily to consume the WB purée, ensuring intake compliance. Physical activity was also monitored throughout the study, to ensure that participants were maintaining their sedentary lifestyle. Since the population remained unchanged in both anthropometrics and dietary intake during the time of the intervention, potential confounding variables were absent. Findings suggest that the exercise imposed upon participants was sufficient to induce a significant increase in inflammation both pre- and post-intervention, demonstrated by increases in interleukin-6 (IL-6) immediately post-exercise. Though not statistically significant, the WB purée consumption appeared to assist participants’ ability to decrease oxidative stress, demonstrated by a decreasing trend in plasma concentration of manganese superoxide dismutase (Mn-SOD) following exercise. No significant changes were detected in DNA damage or tumor necrosis factor-alpha (TNFα) following exercise or post-intervention.
Population Characteristics

The participants in the current study were significantly thinner than the national average for 20-29 year old males. More specifically, participants in the study were leaner than the average male, having only 14% body fat compared to 22% (Fryar, Gu et al. 2012). In addition, the distribution of body fat was different than the national average, having a significantly smaller waist circumference (Fryar, Gu et al. 2012). These characteristics may have played a role in their response to stress. Studies have shown that adipose tissue around the abdomen can cause an increase in inflammatory adipokines such as C-reactive protein, TNFα and IL-6 (Wajchenberg 2000, Kershaw and Flier 2004). In general, all participants were within “normal” ranges for all parameters, aside from fitness level.

Despite fitting the definition for being sedentary (≤ two days/week purposeful activity for the past year), the participants’ VO2max was above the national average for males ages 19 – 30 (45.18 ± 8.2 compared to 34 – 43.9 ml/kg/min). The fitness level of the participants may explain the low oxidative response to the exercise workload. A sedentary population with a lower VO2max may have elicited a greater oxidative stress response to the same workload. Despite the relatively high VO2max pre-intervention, their communication regarding their decrease in activity during the intervention, and the perceived increase in difficulty during the second exercise session, may explain the increased trend in DNA damage observed post-intervention. Assessing VO2max before completing the second exercise session may have provided a better
representation of current fitness levels. If \( \text{VO}_{2\text{max}} \) dropped during the intervention, the second \( \text{VO}_{2\text{max}} \) assessment may have provided more information regarding the increase in DNA damage seen following the intervention.

**Diet**

The macronutrient distribution in the participants’ diet did not change significantly from pre- to post- intervention. Significant differences were detected when compared to the Dietary Reference Intakes (DRI) and Recommended Dietary Allowances (RDA). The study participants consumed significantly more total Calories (kcals) than the recommendation for sedentary males, ages 19 – 30, and significantly more grams of protein. The additional kcals beyond the recommended levels were adequate for the study population considering the body composition and maintenance of body weight throughout the study. Of the vitamins and minerals evaluated, only manganese (Mn), selenium (Se) and zinc (Zn) decreased following the intervention. A possible explanation for the decrease in these three minerals may have been the observed decrease in fresh seafood and meat in the participants’ diets. In particular, two participants consumed two to three servings of fresh seafood at the beginning of the study (summer living in Maine) and decreased to one or no servings by the end of the study (winter living in Maine). Seafood is a good source of both Zn and Se. On average participants consumed more ground beef (hamburgers) pre-intervention, which is another excellent source of both Zn and Se, compared to post-intervention, lending another possible explanation for the decrease in Zn and Se
intakes post-intervention. Participants were asked to eliminate berries, such as raspberries and strawberries during the study that contain high levels of Mn (Castetbon, Kadio et al. 1997). Finally, tomato products, which are good sources of Mn, were to be consumed no more than three times per week during the study. Participants were asked to consume their regular diets, aside from the foods listed in appendix I. Had we compensated for the vitamin and mineral loss from restricting such foods by providing or suggesting alternative options to ensure a similar level of intake from pre- to post-intervention, these decreases may have been avoided. Although the dietary assessments did not include the 300 g of WB provided during the study, which contains 8.04 mg Mn and 1.88 mg Zn. If we account for the WB intake there would not longer be statistically significant difference pre- and post-intervention for both Mn and Zn. Selenium would not have been affected by the addition of WBs.

**Plasma Manganese Superoxide Dismutase (Mn-SOD)**

A significant interaction across time and pre-post- WB purée feeding was detected in plasma Mn-SOD concentrations in the current study ($p \leq 0.014$). No post-hoc differences were found, indicating that the response pattern for plasma Mn-SOD concentration after strenuous exercise was different pre-post-intervention, but at no specific time points. Normal values of Mn-SOD for healthy males have been found at 99,800 ± 24,800 pg/mL (Kawaguchi, Suzuki et al. 1990). The participants in this study remained within the normal range at all time points both pre- and post-intervention. Although not statistically significant, before
receiving the WB purée for eight weeks, participants had a slight increase in Mn-SOD immediately post-exercise with an increasing trend 30 minutes post-exercise. Following this peak, Mn-SOD concentrations tended to decrease back to baseline. Post-intervention, plasma Mn-SOD concentrations tended to decrease 30 minutes post-exercise, before steadily increasing to a peak three hours post-exercise, though not significant (Figure 4.4). The increase of exogenous antioxidants in the WB purée may have caused the decrease in Mn-SOD following exercise (Cook and Samman 1996). Anthocyanins have the ability to donate a hydrogen atom and scavenge superoxide anions and hydroxyl radicals, reducing oxidative damage (Cook and Samman 1996). Post-intervention, the level of available antioxidants could have delayed the need for Mn-SOD production (Cook and Samman 1996). Few researchers have examined the effects of intense exercise on changes in plasma Mn-SOD concentration. Many researchers have measured SOD expression of either copper/zinc SOD or SOD unspecified.

Su et al. (2008) did not specify the specific type of SOD analyzed, they observed serum SOD concentration with allicin in exercise-induced muscle damage. A significant interaction between treatment and time was detected (Su, Tian et al. 2008). After downhill running for 45 minutes or until the participant reached a heart rate of 180 beats/minute or an rate of perceived excursion (RPE) of ≥ 19, serum SOD concentrations in the allicin group significantly decreased immediately post-exercise. These levels increased back to normal 24 hours post-exercise before decreasing again 48 hours post-exercise. This pattern was
similar to the pattern seen in our study, although in a longer timeline. The control group showed a significant increase in serum SOD concentration immediately after exercise then returned to pre-exercise levels at both 24 and 48 hours post-exercise, much like the participants in our study before the WB intervention (Su, Tian et al. 2008). The differences found between the two groups immediately post-exercise were significantly different ($p < 0.001$), unlike our study. Su et al. (2008) concluded that it was the antioxidant properties of the allicin group that increased the plasma antioxidant capacity, resulting in a decreased need for SOD to scavenge free radicals (Su, Tian et al. 2008). Based on these results it is likely that the participants in the current study were able to utilize the plasma antioxidants available from the WB purée to reduce free radicals post-exercise. A larger sample size in our study may have produced significant differences between pre-post-intervention.

**DNA Damage**

No significant changes were detected in DNA damage following the intervention, nor where there any statistically significant changes at any time points following exercise pre- or post-intervention. Previous researchers have documented DNA strand breaks in normal healthy males not exposed to oxidative stress to range from $2.4 \pm 1.3$ to $11.3 \pm 2.6$ % DNA in tail (Guarnieri, Riso et al. 2007, Riso, Martini et al. 2009, Del Bo, Riso et al. 2013). In the current study, participants’ % DNA in tail was, on average $66.83 \pm 13.80$ pre- and $66.58 \pm 16.80$ post-intervention before the onset of exercise. This level of DNA damage
is significantly higher than would be expected based on the population (healthy sedentary males without any chronic health condition). One possible explanation for the elevated level of DNA damage before the exercise session could stem from the handling of the lymphocytes. The lymphocytes used to detect DNA damage were isolated and then frozen at -80° C until they were defrosted to be used for the comet assay. The process of freezing then defrosting cells may have caused some additional damage both pre- and post-intervention. Although the process of using previously frozen tissues and cells for analysis of DNA strand breaks using the comet assay has been validated, it continues to be debated (Jackson, Pedersen et al. 2013). Previous studies examining exercise-induced DNA damage using the single cell electrophoresis (comet assay) have found a smaller percentage of broken DNA strands in the tail of the “comet” (% DNA in tail), closer to 35 – 55% DNA in tail (Niess, Hartmann et al. 1996, Niess, Baumann et al. 1998).

In 2013 Del Bo’ et al. (2013) investigated the effects of a one time feeding of 300 g of blueberries on 10 healthy males (20 ± 1.6 years of age), fitness levels unspecified. Participants displayed an increase in plasma anthocyanins one hour post- blueberry consumption ($p < 0.001$). A significant decrease in $H_2O_2$ induced DNA damage ($p < 0.01$), was also seen one hour post- blueberry consumption, although no correlations between anthocyanins levels and DNA damage were detected (Del Bo, Riso et al. 2013). A similar reduction in DNA damage was seen when Riso et al. (2009) used $H_2O_2$ to induce oxidative stress in 20 males (10 smokers and 10 nonsmokers). DNA damage was measured both before and
after consuming 200 g of broccoli daily for 10 days. The %DNA in tail for smokers and non-smokers exposed to stress before broccoli intervention was 64.4 ± 11.2 and 59.9 ± 14.2, respectively (Riso, Martini et al. 2009). This level of damage following treatment with H₂O₂ was similar to the extent of damage seen in our study, both pre- and post-intervention. We expected to find a significant decrease in DNA strand breaks following the WB intervention, similar to what was seen following the broccoli diet for both smokers and non smokers (64.4 ± 11.2 and 59.9 ± 14.2 down to 49.5 ± 12.5 and 47.2 ± 7.5, respectively (p < 0.05)) (Riso, Martini et al. 2009). This however, was not the case in our study.

In our study the trend was an increase in DNA damage post- WB intervention. This may be due in part, to the change in physical activity exhibited by the subjects throughout the study. Participants in our study were required to be sedentary (≤ two days/week of purposeful physical activity). The study started in September and finished in December. While participants were considered to be sedentary prior to the start of the study, during the course of the investigation they significantly lowered their level of purposeful physical activity. All participants decreased their physical activity during the course of the study. Purposeful activity (activity performed with the intent to increase cardiovascular or muscular fitness/strength) was the only classification of activity collected upon the completion of the study. However, through general conversation, the participants described themselves as being less active in their daily lives (ADL) as well. They pointed to changes in weather (Fall into Winter) and increase in scholastic demands as being the primary causes for this change in ADL. This
decrease in physical activity may have influenced the relative level of stress imposed for the post-treatment exercise session and the increasing trend observed in DNA damage post-intervention.

The exercise intensity both pre- and post-intervention was set based on the VO$_{2\text{max}}$ attained prior to treatment. If the participants did indeed significantly reduce their overall physical activity over the course of the intervention, they may very well have incurred a significant decrease in VO$_{2\text{max}}$. Which, in turn, would mean that the post-intervention exercise session was performed at a higher relative intensity than prior to the intervention. Therefore, though not statistically significant, the increase in DNA damage following exercise, post-intervention in our study might be expected.

**Inflammatory Markers**

**Plasma Interleukin-6 (IL-6).** The significant increase in interleukin 6 (IL-6) following exercise was as expected based on the type and intensity of exercise (briskly walking at an incline for one hour at 70% of VO$_{2\text{max}}$) for our sedentary participants. Participants' IL-6 levels were within the desirable range (< 1.04 pg mL) pre- exercise then significantly increased immediately post- exercise before significantly decreasing one-hour post- exercise (Musselman, Miller et al. 2001). Levels continued to decrease up to six hours post- exercise, but remained above the desirable range at this time-point (Musselman, Miller et al. 2001, Mendham, Coutts et al. 2012). Our results suggest that the intensity of exercise (70% of VO$_{2\text{max}}$) was at a sufficient level of stress to cause a significant increase in
inflammation. These results are in agreement with other studies (Nieman, Davis et al. 2003, Steinberg, Ba et al. 2007, Su, Tian et al. 2008, Konrad, Nieman et al. 2011, McAnulty, Nieman et al. 2011).

Su et al. (2008) administered either a placebo or an allicin supplement to male (n = 8) and female (n = 8) athletes for two weeks before exposing them to downhill running. Blood was drawn immediately before and after exercise, as well as 24 and 48 hours post-exercise. Both groups demonstrated a significant increase in IL-6 following exercise, although the allicin group had a significantly lower response (1.9-fold increase) in IL-6 compared to the control group (8.9-fold increase) (p < 0.001) (Su, Tian et al. 2008). As well, the allicin group’s IL-6 returned to baseline after 24 hours while the control group remained elevated at 48 hours post-exercise (p < 0.001).

While Su et al. (2008) documented a significant increase in IL-6 for both groups post-exercise and a significant difference in IL-6 between groups, McAnulty et al. (2011) detected changes in IL-6 closer to those found in the current study (McAnulty, Nieman et al. 2011). McAnulty and colleagues (2011) examined the daily blueberry consumption (250 g) for six weeks plus an acute dose of 375 g of blueberries one hour before exercise. Participants (treatment group, n = 13 and control group, n = 12) ran 2.5 hours at ~72% VO$_{2\text{max}}$. Both groups had significant increases in IL-6 post-exercise but no differences in IL-6 were detected between treatment and control groups (McAnulty, Nieman et al. 2011). They did, however detect significant reductions in oxidative stress markers: F$_2$-isoprostanes and urinary 5-OHMU (DNA marker) and significant
increases in IL-10 levels, indicating an increase in anti-inflammatory action (McAnulty, Nieman et al. 2011). These results may suggest that IL-6 is a sensitive marker that corresponds at predictable levels in relation to the level of stress in working muscle, regardless of exogenous or endogenous antioxidant availability. The level of IL-6 activation was much higher immediately after exercise with runners in the McAnulty et al. (2011) study than in our study (15.5 ± 1.9 vs. 3.1 ± 2.7 pg/mL). This may be explained by the impact increased exercise duration has on glycogen stores. The longer the exercise duration, the need for glucose increases and over time glycogen stores become depleted, driving working muscles to rely more heavily on beta-oxidation rather than glycolysis for the production of energy (McArdle, Katch et al. 2006). This increased reliance (stress) on electron transport, and thus the "consumption" of oxygen may be a significant cause of increased oxidative stress. When compared to results of our study, McAnulty et al. (2011) suggested that the duration of exercise (stress) has a greater impact on inflammation than the intensity of the work being performed (one hour vs. 2.5 hours).

Helge and colleagues (2003) suggested that the release of IL-6 from working muscle is positively correlated to work intensity, glucose uptake and plasma adrenaline concentration. This indicates that IL-6 may act differently during an exercise-induced inflammatory response compared with chronic inflammation (Helge, Stallknecht et al. 2003). It was suggested by Gleeson (2000) that the IL-6 response acts as a signal, indicating critically low levels of muscle glycogen and that as working muscles need for blood glucose is rising
In our study, the bout of exercise may have significantly depleted participant’s glycogen stores, causing IL-6 levels to significantly rise immediately after. This may explain why the WB intervention did not decrease IL-6 levels post-exercise. The minimal exercise training our sedentary participants were exposed to in the previous year does not allow for the level of muscle glycogen stores needed to sustain exercise at 70% VO$_{2\text{max}}$ for one hour based on the rate of muscle glycogen adaptations with training (McArdle, Katch et al. 2006). Had participants in our study increased their level of exercise training throughout the study they may have adapted to the glucose demands and increased their glycogen storage capacity, decreasing the rise in IL-6 post-intervention. If participants had not had the WB purée, the significant decrease in physical activity during our study may have lead to an even greater depletion of glycogen levels, and a greater rise in IL-6.

**Plasma Tumor Necrosis Factor-alpha (TNFα).** In the present study, increases in TNFα were seen immediately after exercise, reaching peak levels 30 minutes post-exercise. However, these increases were not significant to pre-exercise and our participants’ TNFα did not increase beyond a normal or desirable range (0 – 22 pg/mL). No significant differences were determined pre-post intervention. Previous studies have examined the effects of strenuous exercise on TNFα with varying results. Using a randomized crossover design, Mendham et al. (2012) compared the effects of two exercise protocols on inflammation and glucose regulation in ten sedentary males. Participants either participated in cycle ergometry at 80-85% max heart rate for four x 10 minute
bouts with two minutes of passive recovery between each session or modified rugby sessions consisting of 40 minutes (four x 10 minute bouts with two minutes recovery) of a six-a-side, touch football regulation play. They found no significant differences in TNFα within or between protocols. Similar to the current study, TNFα levels stayed well within the normal range (0 – 22 pg/mL) for both the rugby and cycle exercise sessions (McAnulty, Nieman et al. 2011, Mendham, Coutts et al. 2012).

In contrast to the current study, Bernecker et al. (2011) found significant increases in both IL-6 and TNFα in trained (currently engaged in a training regimen for a marathon) males following a marathon, although similar to other studies, levels for both TNFα and IL-6 remained within the normal range (Bernecker, Scherr et al. 2011). On the other hand, Konrad et al. (2011) noted participants’ TNFα levels significantly increase after runners (running 55 km/week or more) performed two hours of treadmill running at 70 – 75% VO2max (Konrad, Nieman et al. 2011). Men and women were randomized into a quercetin or placebo group. Both groups ingested a chewable supplement 15 minutes prior to the two-hour run. Despite a significant increase in plasma quercetin levels in the treatment group, a decrease in TNFα was not seen post- exercise (Konrad, Nieman et al. 2011). These results are similar to the current study, which showed no significant changes in TNFα following the WB intervention. One explanation for the response in TNFα in the current study, particularly in response to exercise, is the inhibitory role that IL-6 plays on TNFα (Moldoveanu, Shephard et al. 2001). Interleukin-6 levels initially increase following the production of TNFα.
This pro-inflammatory cytokine (IL-6) has also been found to have an anti-inflammatory effect, increasing the immune response by stimulating the increase of IL-10, an anti-inflammatory cytokine (Weinstock, Konig et al. 1997, Ostrowski, Rohde et al. 1999, Pedersen and Hoffman-Goetz 2000, Kimura, Suzui et al. 2001, Moldoveanu, Shephard et al. 2001, Suzuki, Nakaji et al. 2002). As well, participants in the Konrad et al. (2011) study ran much longer (two hours) than the participants in the current study (one hour), potentially causing a greater amount of oxidative stress and inflammation resulting in the significant increase in TNFα. The changes in TNFα in our study were not significant. However, the post-intervention decreasing trend in TNFα suggests that significance may have been obtained with less variability between our subjects. A larger experimental number of subjects would have aided in reducing variability.

**Limitations and Future Recommendations**

Additional reasons our study did not identify changes post-intervention, aside from the number of participants and their variability could be explained by the normality of participants. Given the participants’ lower than average BMI, waist circumference, percentage of body fat, and higher than average level of fitness, their response to the rise in exercise-induced inflammation may have been lower compared to a population with above average levels of body composition and lower levels of fitness. Having higher levels of body fat, particularly around the abdomen can cause an increase in inflammatory
adipokines such as C-reactive protein, TNFα and IL-6 (Wajchenberg 2000, Kershaw and Flier 2004).

Future recommendations include adding urinary 5-OHMU or protein carbonyls as companion DNA markers, to provide a more comprehensive assessment of DNA damage. Also, participants should be asked to maintain the same level of physical activity throughout the study, as well as ADL. Doing so will ensure that the second exercise session is performed at the same level of intensity, decreasing risk for greater damage post-intervention compared to pre-intervention. A second round of metabolic testing may be administered to allow for comparison of fitness levels pre- and post-intervention to further guarantee that both exercise sessions are performed at the same relative intensity.

Future studies involving nutraceuticals and exercise should employ an expanded array of biochemical analyses in order to provide a more comprehensive picture of their effects on oxidative stress, inflammation and DNA damage.

Conclusions

In the current study we examined the effects of an eight-week WB intervention on 10 sedentary males exposed to exercise designed to induce oxidative stress, inflammation and DNA damage. Consuming 300 g of WB purée daily for eight weeks did not show significant changes in inflammation, oxidative stress or DNA damage for sedentary males. It was found that exercise at 70% of VO₂max in sedentary males invoked an inflammatory response. As well, the
increase in exogenous anthocyanins from the WB purée available to scavenge free radicals may have postponed the need for an increase in Mn-SOD following exercise. While previous studies have shown the positive effects of WB consumption on inflammation and oxidative stress, the current study warrants further investigation of this nutraceutical's use as an anti-inflammatory agent as it applies to the exercise challenge.

**Significance**

This research examined the effects of consuming WB purée for eight weeks on exercise-induced inflammation, oxidative stress and DNA damage in sedentary males. The majority of the results were in agreement with other studies examining the effects of an exogenous antioxidant source, particularly with the use of anthocyanin containing fruits. The population examined (healthy, sedentary males) in this study can be used as a reference when comparing other sedentary individuals who are not otherwise healthy, making the results useful when examining other causes of oxidative stress and inflammation.

Exercise, in this case, served as a vehicle to induce oxidative stress and inflammation in a sedentary but otherwise healthy population. Oxidative stress in and of itself is known to increase the risk factors associated with cardiovascular disease (CVD) (Strobel, Fassett et al. 2011). Cardiovascular disease remains the number one killer worldwide, despite the preventable nature of many of the chief risk factors. Prevention strategies for CVD are a major concern for public health policies, most including lifestyle changes with a focus on diet and exercise.
Including whole WBs into the diet may prove to not only directly reduce the occurrence of ROS and oxidative stress but also to increase the overall fiber content of the diet, which is one step towards decreasing overall risk factors.

Results from this study are promising and point to the need for further investigation with a larger number of participants to decrease variability. If further studies with a larger number of subjects support the consumption of wild blueberries, the health care profession, commodity groups, and the Wild Blueberry industry can use wild blueberries as anti-inflammatory foods that can be consumed daily without the detrimental effects of medications.


APPENDICES
APPENDIX A

CAMPUS RECRUITMENT FLYER
Research Participants Needed

Wild blueberry consumption, does it reduce inflammation, oxidative stress and muscle damage?

Be a part of an important nutrition and exercise research study

- Are you a male between 21 and 30 years of age?
- Do you currently exercise less than 2 time/week?
- Are you physically able to exercise (run/walk)?
- Do you like wild Maine blueberries?
- Will you be in town from September to December?
- Are you ok with receiving blood draws?

If you answered YES to these questions you may be eligible to participate in a nutrition and exercise research study

The purpose of this study is to determine if a diet with whole wild blueberries is an effective inhibitor of muscle damage associated with exercise in humans.

Benefits include $100, a comprehensive nutrition program and exercise plan upon completion of the study as well as a blueberry hat and t-shirt.

The study is being conducted at both Hitchner Hall and Lengyel Gym at the University of Maine.

Please call or email Taylor Bloedon for more information.

207-581-2464
taylor.bloedon@umit.maine.edu
I am Taylor Bloedon a graduate student in the Department of Food Science and Human Nutrition. I would like to invite you to participate in my research study to determine if a diet of whole wild blueberries can decrease inflammation and muscle damage associated with exercise in humans. You may participate if you are a male between the ages of 21 and 30, currently exercise less than 2 days per week and have done so for at least one year and have no blueberry allergies.

As a participant, you will be asked to perform 3 exercise tests and consume wild blueberries daily for 8 weeks. The total time commitment of the study is 12 weeks. You will be asked to remain sedentary during the study (exercising < 2 days per week), give blood and follow a diet (minimal changes to your regular diet).

Risks for participating are minimal, possibly some minor muscle soreness after each exercise test and minor pain or bruising from blood draws. Benefits include a comprehensive nutrition and exercise program upon completion of the study.

If you would like to participate in this research study, or you have questions, please contact me at 581-2464 or email at Taylor.bloedon@umit.maine.edu.

Thank you for your consideration,

Taylor Bloedon, MS., RD
APPENDIX C

INITIAL CONTACT PHONE SCREENING
Phone Screening (first contact)

Thank you for your interest in our Wild Blueberry and Exercise research study. If you have a few minutes I would like to ask you a couple questions to ensure that you are eligible for the study. All questions and answers will remain confidential and will be destroyed immediately if you do not advance in the study. If you do continue in the study, a code number will be put on the sheet on which I have recorded your responses. This code number is used to protect your identity. A key linking names and code numbers will be kept separate from the data and will be destroyed in 10 years. Data from the study will be stored in a safe and locked file in my office until August when the study ends. After that time data will be stored in Dr. Dorothy Klimis-Zacas’s office for 10 years.

Before we begin, do you have any questions for me?

1. Are you a male between the ages of 21 and 30?  Y  N

2. Do you have any allergies to blueberries?  Y  N

3. How often do you engage in purposeful physical activity? _______________
   -Purposeful PA is activity that you decide to do to exert energy or to accomplish a physical goal.
   -Depending on answer, further questions may be asked to better understand the nature of the activity such as, intensity of activity, goal of activity etc.

4. If no exercise, are you physically able to walk/run?  Y  N

5. Do you have any major health conditions such as Type 2 diabetes, HTN, Heart disease, cancer, severe asthma or breathing issues? Y  N
   -If yes, what condition:__________________________

6. Do you mind having a small needle inserted and kept in place for up to 7 hours for multiple blood draws?  Y  N

7. Will you be able to commit two 7-8 hour days, one at the beginning of the study during the week of May 28th to July 3rd and one at the end of the study during the week of July 30th to August 5th for testing?  Y  N

8. Will you be able to report to Hitchner Hall at the University of Maine campus Monday through Friday,
June 24\textsuperscript{th} to July 29\textsuperscript{th} to receive and consume about 2 cups of Wild Blueberries? \(\text{Y} \quad \text{N}\)

9. The total time commitment for this study is about 24 hours of your time. Is this acceptable for you to commit to participating? \(\text{Y} \quad \text{N}\)

Here is the breakdown of your estimated time commitment:

- Phone screening: 15 minutes
- Health screening: 1 hour
- VO2max test: 1.5 hours
- Exercise Session #1: 8 hours
- Daily Blueberry Feedings: 13 hours (20 mins, 5 days a week for 8 weeks)
- Exercise Session #2: 8 hours
- **Total**: 24 hours

Do you wish to continue with this study? \(\text{Y} \quad \text{N}\)

The next session will include filling out some questionnaires about your physical activity, health status, eating history, as well as collecting your height, weight, waist circumference and body composition. This should take about an hour to 1.5 hours of your time.

If yes, schedule the next in person session: Date: ____________Time: ____________
Informed Consent

You are invited to participate in a research project being conducted by Taylor Bloedon, a graduate student in the Department of Food Science and Human Nutrition, Dr. Dorothy Klimis-Zacas, professor of Clinical Nutrition, and Dr. Robert Lehnhard, professor of Exercise Physiology at the University of Maine. The purpose of the research is to determine if a diet of whole wild blueberries will decrease muscle damage associated with exercise in humans.

What will you be asked to do?

Second Screening In Person (Visit 1)

- Complete a Physical Activity Questionnaire. This will ask you questions about your activity of daily living as well as purposeful or planned physical activity.
- Complete a Food Frequency Questionnaire. This will ask you questions about your regular eating and habits and behaviors, particularly with foods that are high in anthocyanins (what gives foods and beverages the blue, red and purple color).
- Complete a Medical History Questionnaire. This will ask you questions about your current and previous state of health. Questions are focused on major health issues that may interfere with the study or put your safety at risk.
- You may wish to skip questions on any of your questionnaires, although doing so may make it difficult to determine eligibility.
- Have your height, weight, waist circumference and body composition tested (skinfold measurement).
- This visit should take about 1 hour
Visit 2, VO$_2$max Test:
- Arrive to Lengyel Gym 3 hours after first morning meal (time and meal options will be assigned during Visit 1).
- Dress in clothing comfortable to walk/run in. Including running shoes. (No jeans, sandals or slip on shoes)
- You will have your VO$_2$max tested. This involves breathing into a tube while walking/running on a treadmill that will gradually get faster and increase incline until you reach fatigue. This will measure maximum oxygen consumption.
- Once the study is complete you will be scheduled to return to the lab two weeks later.
- This visit should take about 1.5 hours

Visit 3 and 4, Exercise Sessions 1 and 2:
- Visit 3 will take place 2 weeks after Visit 2 (VO$_2$max)
- Visit 4 will take place 8 weeks after Visit 3
- You will be asked to perform (walk/jog) at 70% of your VO$_2$max for 60 minutes on a treadmill.
- You will arrive at Lengyel Gym 1 hour before the exercise session will begin (scheduled time will be given at Visit 2 and 3).
- You will have an intravenous (IV) line inserted into a vein in your hand by licensed phlebotomist prior to the running session. This IV will stay in place during the running, and for 6 hrs after the running is over. The IV is for the purpose of collecting blood both before and after your running session. A total of 6, 10ml (2 teaspoons) blood samples will be collected by a registered phlebotomist. One will be collected before the running begins. The 5 remaining samples will be collected over the 6 hr period immediately following your running session at time 0, 30 minutes, 1 hour, 3 hours, 6 hours. After the last blood draw you will be provided with a meal. These blood samples will be kept frozen for later analysis.
- You will be asked to stay in the lab for the duration of the study (about 7-8 hours). You may bring personal items for entertainment such as work, reading material, lap tops etc. We will also have games and movies available for your entertainment.
- You will be given water to drink as needed and will be asked not to eat anything for the 6 hours after the exercise session. Once the 6 hours are up and you have completed your last blood draw a meal will be provided.
- These visits will take about 8 hours
Throughout the study:
- For the 8 weeks following Visit 3 you will be asked to consume a blueberry puree daily. You will come into Hitchner Hall daily to consume your blueberry puree and on Friday you will pick up 2 additional purees for the weekend. Times to arrive will be scheduled during Visit 3.
- You will be asked to maintain your sedentary lifestyle throughout the study (≤ 2 days per week of planned physical activity)
- Once a week you will be asked questions regarding your diet and exercise behaviors and reminded of foods and beverages to avoid during the study. You will be provided with a list of foods and beverages to avoid.
- Each visit should take about 20 minutes to consume your berries. One day a week may take an additional 10 minutes to review your diet and activity level.

Risks

The potential risks of this study include:

- Increased heart rate, blood pressure, breathing rate and sweating. These are normal responses to exercise and they stop when the exercise is completed.
- You may experience muscle soreness the day following the exercise session. That too is a normal response to exercise training.
- Having an IV inserted into your vein may result in a slight bruise. There is a chance of infection, but it is highly unlikely.
- Continuing to refrain from exercise for the total time of the study (12-14 weeks) may postpone any plans to start an exercise program.
- The increase in dietary fiber may cause some gastrointestinal discomfort (abdominal cramping and or bloating) if you are not used to a moderate to high fiber diet, though any discomfort will be resolved within a few days.

Every effort will be made by the investigators to minimize the risks and discomforts you may experience during your participation in this project.
Benefits and Compensation

Possible benefits include an increase in daily fiber and antioxidant content, which have been shown to help with bowel regularity and improve colon health. Previous research has shown that daily wild blueberry intake can improve the function of the lining of your arteries as well as decrease oxidative stress. Upon full completion of the study you will be eligible to receive $50 as well as the opportunity to receive three free sessions with a Personal Trainer and a Registered Dietitian, in addition to a blueberry t-shirt, hat and the free wild blueberries daily. If you do not complete the study you will not receive the $50 or the free sessions with the Registered Dietitian or the Personal Trainer.

Confidentiality

Your name will not be on any data. A code number will be used to protect your identity. Only Taylor Bloedon, Dr. Dorothy Klimis-Zacas and Dr. Robert Lehnhard will have access to code numbers. Blood samples will be analyzed at The University of Maine in Hitchner Hall. Only an ID number will be on the blood samples and all other data collected during the study. During blood analysis all of the blood sample will be used. Data will be kept in both Dr. Klimis-Zacas’s and Dr. Lehnhard’s locked offices in paper format only. Your name or other identifying information will not be reported in any publications. The key linking your name to the data, as well as all data, will be destroyed after 10 years. All information obtained from you will be treated as privileged and confidential. It will not be released or revealed in any way that identifies you. The information obtained from you will be used for statistical and scientific analysis with all rights of privacy retained. If you do not advance past the second screening your information will be destroyed immediately.
Contact Information

If you have any questions about the research at any time please feel free to contact Taylor Bloedon at:
Phone: (207) 581-2464 or 541-515-5553 (cell phone for immediate assistance)
Email taylor.bloedon@umit.maine.edu

You may also contact either faculty sponsor/co-investigators:
Dr. Dorothy Klimis-Zacas: Dr. Robert Lehnhard
Phone: (207) 581-3124 Phone: (207) 581-2480
Email: dorothy.klimis.zacas@umit.maine.edu Email: Robert.lehnhard@umit.maine.edu

If you have any questions about your rights as a research participant, please contact Gayle Jones, Assistant to the University of Maine’s Protection of Humans Subjects Review Board at:
Phone: (207) 581-1498
Email: gayle.jones@umit.maine.edu

Your signature below indicates that you have read and understood the above information. You will receive a copy of this form.

Name (print): __________________________________________

Name (signature): ___________________________ Date: ________________
This questionnaire is designed to find out about your physical activity in your everyday life.

Please try to answer every question, except when there is a specific request to skip a section.

Your answers will be treated as strictly confidential and will be used only for medical research.
THE QUESTIONNAIRE IS DIVIDED INTO 3 SECTIONS

- **Section A** asks about your physical activity patterns in and around the house.
- **Section B** is about travel to work and your activity at work.
  It may be skipped by people who have not worked at any stage during the last 12 months.
- **Section C** asks about recreations that you may have engaged in during the last 12 months.

What is your date of birth?

What is today's date?

Your sex (Please tick (√) appropriate box)?

---

**Section A**  
**HOME ACTIVITIES**

**GETTING UP AND GOING TO BED**
*Please put a time in each box*

<table>
<thead>
<tr>
<th>Average over the past year</th>
<th>At what time do you normally get up?</th>
<th>At what time do you normally go to bed?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On a weekday</td>
<td></td>
</tr>
<tr>
<td></td>
<td>On a weekend day</td>
<td></td>
</tr>
</tbody>
</table>

**GETTING ABOUT — Apart from going to work**

Which form of transport do you use **most often** apart from your journey to and from work?

*Please tick (√) one box ONLY per line*

<table>
<thead>
<tr>
<th>Distance of journeys</th>
<th>Car</th>
<th>Walk</th>
<th>Public transport</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>less than one mile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–5 mile(s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 5 miles</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TV or Video Viewing
*Please put a tick (✓) on every line*

<table>
<thead>
<tr>
<th>Hours of TV or Video watched per day</th>
<th>Average over the last 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>On a weekday before 6 pm</td>
<td></td>
</tr>
<tr>
<td>On a weekday after 6 pm</td>
<td></td>
</tr>
<tr>
<td>On a weekend day before 6 pm</td>
<td></td>
</tr>
<tr>
<td>On a weekend day after 6 pm</td>
<td></td>
</tr>
</tbody>
</table>

### Stair Climbing at Home
*Please put a tick (✓) on every line*

<table>
<thead>
<tr>
<th>Number of times you climbed up a flight of stairs (approx 10 steps) each day at home</th>
<th>Average over the last 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>On a weekday</td>
<td></td>
</tr>
<tr>
<td>On a weekend day</td>
<td></td>
</tr>
</tbody>
</table>

### Activities in and Around the Home
*Please put a tick (✓) on every line*

<table>
<thead>
<tr>
<th>Approximate number of hours each week</th>
<th>Average over the last 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Preparing food, cooking and washing up</td>
<td></td>
</tr>
<tr>
<td>Shopping for food and groceries</td>
<td></td>
</tr>
<tr>
<td>Shopping and browsing in shops for other items (e.g. clothes, toys)</td>
<td></td>
</tr>
<tr>
<td>Cleaning the house</td>
<td></td>
</tr>
<tr>
<td>Doing the laundry and ironing</td>
<td></td>
</tr>
<tr>
<td>Caring for pre-school children or babies at home (not as paid employment)</td>
<td></td>
</tr>
<tr>
<td>Caring for handicapped, elderly or disabled people at home (not as paid employment)</td>
<td></td>
</tr>
</tbody>
</table>
Section B  

ACTIVITY AT WORK

Please answer this section only if you have been in paid employment at any time during the last 12 months or you have done regular, organised voluntary work.

If not please go to page 9

TYPES OF WORK DURING THE LAST TWELVE MONTHS

- We would like to know what full or part-time jobs you have done in the last 12 months.
- You may have held a single job or have held two jobs at once.
- If you have changed jobs with the same employer, you should enter it as a change of job only if it entailed a substantial change in physical effort.

EXAMPLE

Someone who worked full-time for 6 months, then retired, rested for 3 months and then started a voluntary job for 6 hours a week, would complete the questions as follows.

<table>
<thead>
<tr>
<th>Name of occupation</th>
<th>Job 1</th>
<th>Job 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>How many hours per week did you usually work?</td>
<td>38</td>
<td>6</td>
</tr>
<tr>
<td>For how many months in the last 12 months did you do this work?</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

ACTIVITY LEVELS AT YOUR WORK

Now we would like you to take the total number of hours you worked per week in each job and divide them up according to your activity level.

Please complete EACH line

<table>
<thead>
<tr>
<th>Activity</th>
<th>Job 1</th>
<th>Job 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting — light work e.g. desk work, or driving a car or truck</td>
<td>✓ 6</td>
<td>✓</td>
</tr>
<tr>
<td>Sitting — moderate work e.g. working heavy levers or riding a mower or forklift truck</td>
<td>✓ 30</td>
<td>✓ 4</td>
</tr>
<tr>
<td>Standing — light work e.g. job technician work or working at a shop counter</td>
<td>✓ 2</td>
<td>✓</td>
</tr>
<tr>
<td>Standing — light/moderate work e.g. light welding or stocking shelves</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

The number of hours in each activity should add up to the number of hours that you worked in each job e.g. 6+30+2=38 (nurse)
What jobs have you held in the last 12 months, and how many months in the year did you do them?

Please complete EACH line

<table>
<thead>
<tr>
<th>Name of occupation</th>
<th>Job 1</th>
<th>Job 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>How many hours per week did you usually work?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For how many months in the last 12 months did you do this work?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ACTIVITY LEVELS AT YOUR WORK

Now we would like you to take the total number of hours you worked per week in each job and divide them up according to your activity level.

Please complete EACH line

<table>
<thead>
<tr>
<th>Sitting — light work (e.g. desk work, or driving a car or truck)</th>
<th>Job 1</th>
<th>Job 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting — moderate work (e.g. working heavy levers or riding a mower or forklift truck)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing — light work (e.g. lab technician work or working at a shop counter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing — light/moderate work (e.g. light welding or stocking shelves)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing — moderate work (e.g. fast rate assembly line work or lifting up to 50 lbs every 5 minutes for a few seconds at a time)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing — moderate/heavy work (e.g. masonry/painting or lifting more than 50 lbs every 5 minutes for a few seconds at a time)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking at work — carrying nothing heavier than a briefcase (e.g. moving about a shop)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking — carrying something heavy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moving, pushing heavy objects objects weighing over 75lbs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Stair or Step Climbing at Work

*Please put a tick (✓) on EACH line where appropriate*

<table>
<thead>
<tr>
<th>Number of times you climbed up a flight of stairs (10 steps) at work</th>
<th>Average Over the Last 12 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Job 1</td>
<td></td>
</tr>
<tr>
<td>Job 2</td>
<td></td>
</tr>
</tbody>
</table>

### Ladder Climbing at Work

*Please put a tick (✓) on EACH line where appropriate*

<table>
<thead>
<tr>
<th>Number of times you climbed up a ladder at work</th>
<th>Average Over the Last 12 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Job 1</td>
<td></td>
</tr>
<tr>
<td>Job 2</td>
<td></td>
</tr>
</tbody>
</table>

### Kneeling and Squatting at Work in Job 1

In an average working day in Job 1 did you

- Kneel for more than one hour in total? No □ Yes □ Don’t know □
- Squat for more than one hour in total? No □ Yes □ Don’t know □
- Get up from kneeling or squatting more than 30 times? No □ Yes □ Don’t know □

### Kneeling and Squatting at Work in Job 2

In an average working day in Job 2 did you

- Kneel for more than one hour in total? No □ Yes □ Don’t know □
- Squat for more than one hour in total? No □ Yes □ Don’t know □
- Get up from kneeling or squatting more than 30 times? No □ Yes □ Don’t know □
### TRAVEL TO AND FROM WORK

**JOB 1**
Please complete EVERY line

<table>
<thead>
<tr>
<th>Roughly how many miles was it from home to Job 1?</th>
</tr>
</thead>
<tbody>
<tr>
<td>How many times a week did you travel from home to Job 1?</td>
</tr>
</tbody>
</table>

**Please tick (√) one box ONLY per line**

<table>
<thead>
<tr>
<th>How did you normally travel to Job 1?</th>
<th>Always</th>
<th>Usually</th>
<th>Occasionally</th>
<th>Never or rarely</th>
</tr>
</thead>
<tbody>
<tr>
<td>By car</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By works or public transport</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By bicycle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**JOB 2 (if appropriate)**
Please complete EVERY line

<table>
<thead>
<tr>
<th>Roughly how many miles was it from home to Job 2?</th>
</tr>
</thead>
<tbody>
<tr>
<td>How many times a week did you travel from home to Job 2?</td>
</tr>
</tbody>
</table>

**Please tick (√) one box ONLY per line**

<table>
<thead>
<tr>
<th>How did you normally travel to Job 2?</th>
<th>Always</th>
<th>Usually</th>
<th>Occasionally</th>
<th>Never or rarely</th>
</tr>
</thead>
<tbody>
<tr>
<td>By car</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By works or public transport</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By bicycle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Section C  RECREATION

The following questions ask about how you spent your leisure time.

Please indicate how often you did each activity on average over the last 12 months.

For activities that are seasonal, e.g. cricket or mowing the lawn, please put the average frequency during the season when you did the activity.

Please indicate the average length of time that you spent doing the activity on each occasion.

EXAMPLE

If you had mowed the lawn every fortnight in the grass cutting season and took 1 hour and 10 minutes on each occasion.

If you went walking for pleasure for 40 minutes once a week.

You would complete the table below as follows:

Please give an answer for the AVERAGE TIME you spent on each activity and the NUMBER OF TIMES you did that activity in the past year.

<table>
<thead>
<tr>
<th>Number of times you did the activity in the last 12 months</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Less than once a month</td>
<td>Once a month</td>
<td>2 to 3 times a month</td>
<td>Once a week</td>
<td>2 to 3 times a week</td>
</tr>
<tr>
<td>Mowing the lawn</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking for pleasure</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Now please complete the table on pages 10 and 11
Please give an answer for the NUMBER OF TIMES you did the following activities in the last 12 months and the AVERAGE TIME you spent on each activity.

Please complete EACH line

<table>
<thead>
<tr>
<th>Number of times you did the activity in the last 12 months</th>
<th>Average time per episode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Swimming — competitive</td>
<td></td>
</tr>
<tr>
<td>Swimming — leisurely</td>
<td></td>
</tr>
<tr>
<td>Backpacking or mountain climbing</td>
<td></td>
</tr>
<tr>
<td>Walking for pleasure — you should not include walking as</td>
<td></td>
</tr>
<tr>
<td>a means of transportation as this was included in</td>
<td></td>
</tr>
<tr>
<td>Sections A &amp; B</td>
<td></td>
</tr>
<tr>
<td>Racing or rough terrain cycling</td>
<td></td>
</tr>
<tr>
<td>Cycling for pleasure — you should not include cycling</td>
<td></td>
</tr>
<tr>
<td>as a means of transportation</td>
<td></td>
</tr>
<tr>
<td>Mowing the lawn — during the grass cutting season</td>
<td></td>
</tr>
<tr>
<td>Watering the lawn or garden in the summer</td>
<td></td>
</tr>
<tr>
<td>Digging, shovelling or chopping wood</td>
<td></td>
</tr>
<tr>
<td>Weeding or pruning</td>
<td></td>
</tr>
<tr>
<td>DIY e.g. carpentry, home or car maintenance</td>
<td></td>
</tr>
<tr>
<td>High impact aerobics or step aerobics</td>
<td></td>
</tr>
<tr>
<td>Other types of aerobics</td>
<td></td>
</tr>
<tr>
<td>Exercises with weights</td>
<td></td>
</tr>
<tr>
<td>Conditioning exercises e.g. using an exercise bike</td>
<td></td>
</tr>
<tr>
<td>or rowing machine</td>
<td></td>
</tr>
</tbody>
</table>

Please continue on the next page
### Number of times you did the activity in the last 12 months

<table>
<thead>
<tr>
<th>Activity</th>
<th>None</th>
<th>Less than once a month</th>
<th>Once a month</th>
<th>2 to 3 times a month</th>
<th>Once a week</th>
<th>2 to 3 times a week</th>
<th>4 to 5 times a week</th>
<th>6 times a week or more</th>
<th>Average time per episode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Hours</td>
</tr>
<tr>
<td>Floor exercises e.g. stretching, bending, keep fit or yoga</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dancing e.g. ballroom or disco</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competitive running</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jogging</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bowling — indoor, lawn or 10 pin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tennis or badminton</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squash</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Table tennis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Golf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Football, rugby or hockey (during the season)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cricket (during the season)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rowing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Netball, volleyball or basketball</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fishing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horse-riding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snooker, billiards or darts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musical instrument playing or singing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ice-skating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sailing, wind-surfing or boating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Martial arts, boxing or wrestling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

You have finished the questionnaire — Thank you
APPENDIX F

FOOD INTAKES AND EATING HABITS QUESTIONNAIRE
Food Intake and Eating Habits

Breakfast

How often do you eat breakfast?

○ Always
○ Never
○ Sometimes

What do you usually eat for breakfast?

How long does it take to eat breakfast?

○ 0-5 minutes
○ 5-10 minutes
○ > 10 minutes

How do you eat it?

○ Home
○ Restaurant
○ Other _______________________

Lunch

How often do you eat lunch?

○ Always
○ Never
○ Sometimes

What do you usually eat for lunch?

How long does it take to eat lunch?

○ < 30 minutes
○ 30-60 minutes
○ > 60 minutes

Where do you eat it?

○ Home
○ Restaurant
○ Cafeteria
○ Other _______________________

After lunch I am usually…

○ Full/satisfied
○ Unsatisfied
Snacks

How often do you have snacks?
- Always
- Never
- Sometimes

How many times per day?
- 1
- 2
- > 2

Where do you get your snacks?
- Home
- Restaurant
- Other

__________________________

Dinner

How often do you eat dinner?
- Always
- Never
- Sometimes

How long does it take to eat dinner?
- < 30 minutes
- 30-60 minutes
- > 60 minutes

Where do you eat it?
- Home
- Restaurant
- Cafeteria
- Other

__________________________

What snacks do you usually have?

What do you usually eat for dinner?

After dinner I am usually…
- Full/satisfied
- Unsatisfied
How often do you eat the following:

<table>
<thead>
<tr>
<th>Item</th>
<th>Daily</th>
<th>4-5 Times/Week</th>
<th>2-3 Times/Week</th>
<th>Once a Week</th>
<th>Twice a Month</th>
<th>Once a Month</th>
<th>4-5 Times/Year</th>
<th>Twice a Year</th>
<th>Never</th>
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<td>Tomatoes</td>
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</tbody>
</table>

Adapted from Del Bo’ et al. (2013)
PERSONAL DATA

Name

Date of birth

Age

Sex

M

F

Address

City

State

Telephone

Email

ANTHROPOMETRICS

Height: _______

Weight: _______

BMI: _______

Waist Circumference: ___________

Body Composition: ___________
Are you currently taking food supplements (vitamins, minerals, herbs etc….)?

☐ Yes  ☐ No

If yes, please indicate what you are taking, how much and how often and what purpose

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Dosage</th>
<th>How often</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
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</table>

Do you smoke?

☐ I do not smoke
☐ 0 – 9 cigarettes/day
☐ 10-19 cigarettes/day
☐ > 20 cigarettes/day

Please indicate the brand of cigarettes you usually smoke:

__________________________________________

Do you use other tobacco products?

☐ Yes  ☐ No

If yes, please indicate what product and how much you use:

__________________________________________

__________________________________________

Do you drink alcohol?

☐ No, never
☐ ≤ 2 servings/week
☐ 2-10 servings/week
☐ > 10 servings/week
Are you following a specific diet?

☐ Yes  ☐ No

If yes, Please specify which diet and for what reason

_________________________________________________________
_________________________________________________________
_________________________________________________________

Do you have food allergies or you do not like any particular food item?

☐ Yes  ☐ No

If yes, please indicate which:

_________________________________________________________

In particular, have you ever had allergic reactions with…

☐ Tomatoes  ☐ Strawberries  ☐ Raspberries  ☐ Blueberries

☐ Cherries  ☐ Blackberries  ☐ Grapes  ☐ Pomegranate

☐ No, Never

Are you currently taking any drugs/medications?

☐ Yes  ☐ No

If yes, please indicate which:

_________________________________________________________

Did you ever undergo surgery?

☐ Yes  ☐ No

If yes, please specify:

_________________________________________________________
Do you currently or have you had any of the following medical conditions:

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Yes/No</th>
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</thead>
<tbody>
<tr>
<td>Food allergies</td>
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<td>Food intolerances</td>
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<td>Anorexia nervosa</td>
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<td>Bulimia nervosa</td>
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<td>Gastritis</td>
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<td>Crohn's disease</td>
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<td>Diverticulitis</td>
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<td>Irritable bowel syndrome</td>
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<td>Coronary heart disease</td>
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<td>Cardiac arythmia</td>
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<td>Palpitations</td>
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<td>High blood pressure</td>
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<td>Dyslipidemia</td>
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<td>Kidney disease</td>
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<td>Anemia</td>
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<td>Type I diabetes</td>
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<td>Type II diabetes</td>
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<td>Osteoporosis</td>
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<td>Liver disease/hepatitis</td>
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<td>Thyroid problems</td>
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<td>Drug allergies</td>
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<td>Latex allergies</td>
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<td>Chronic infection</td>
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<td>Back pain</td>
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<td>Anxiety</td>
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<td>Asthma</td>
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<td>Panic attacks</td>
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<td>Depression</td>
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<td>Other (please specify)</td>
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</table>

Please elaborate on any condition if applicable:

Adapted from Del Bo’ et al. (2013)
APPENDIX H

INSTRUCTIONS TO PARTICIPANTS
Thank you for participating in the Blueberry and Exercise Study. During the next 8 weeks we will be providing you with 300 grams of blueberries in the form of a sorbet to consume daily.

Throughout the study we ask the following of you:
- Come to Hitchner Hall room 152 daily to consume your blueberries
- Follow instructions and consume your blueberries on Saturday and Sunday
- Remain at your current level of activity (≤ 2 days/week)
- Report any changes to diet or exercise or health that may impact the study
- Limit excessive intake of NSAIDS (aspirin, ibuprofen, and naproxen)
- Limit dietary supplements to a daily multivitamin only
  - No additional vitamin C, Omega 3 fatty acids etc

Avoid the following foods:
- Blueberries (aside from those provided)
- Raspberries
- Blackberries
- Cranberries
- Red Grapes
- Red Cabbage
- Strawberries
- Red Wine
- Red Currants
- Black Currants
- Egg Plant
- Chokeberry
- Cherries
- Elderberries
- Plums
- Radishes
- Any Juice from the fruits above

Limit the following:
- Oranges: limit to 2 oranges a week
- Orange juice: limit to 16 oz per week
- Tomato sauce: limit to 3 times per week
- Tomatoes: limit excessive amounts
- Red onions: limit excessive amounts
APPENDIX I

DIETARY ASSESSMENT THREE-DAY FOOD LOG
INSTRUCTIONS FOR RECORDING 3-DAY DIETARY RECORDS

1. Please record each food and beverage item you consume on a separate line. Be sure to include all snacks.

2. Record each item after weighing in exact amounts:
   - liquids in cups or **fluid** ounces
   - vegetables and fruits in cups, grams, or ounces
   - beans, grains, and pasta in cups **dry** or cups **cooked**
   - bread in slices, indicate what kind of bread (brand name and type)
   - meats, fish, poultry and cheeses in ounces
   - nuts in cups, ounces, or grams
   - chips or other snack type foods in cups, ounces, or grams
   - Spread (butter, cream cheese, margarine, etc.) in tsp or Tbs

3. Please specify if food is consumed raw. Also indicate if it was prepared from fresh, frozen, or canned products.

4. Indicate how the foods were prepared, such as fried, baked, boiled, etc.

5. If a food has a mixture of ingredients (sandwich or casserole), list the major ingredients separately in their proportions or amounts.

6. Use brand names whenever possible, or mention comparable brand.

7. For fruits and vegetables, please indicate if the skin was removed.

8. Indicate if dairy products are whole, 2%, or skim.

9. Be sure to include sauces, gravies, milk/sugar in coffee, etc.

10. Check food labels for weights, etc. Candy bars, cheeses, cookies, juices are all labeled with their weights -----Write this information down!

11. Provide any other information you feel might be helpful, such as food labels and/or recipes.

12. Record EVERYTHING edible that goes in your mouth.

13. MOST IMPORTANTLY, eat as you normally would -- please don’t change your usual eating habits or modify your portion size.
3-DAY FOOD/BEVERAGE INTAKE RECORD

Please measure and weigh all food and beverages you eat throughout the day and write them down as you eat them. Remember to give as many details as possible, keep the food label if you think it will help describe the food better than you are able to. Providing us with recipes for homemade foods is helpful for us, too. Please list any vitamin or mineral supplements or any other supplements taken on the backside of this form and attach these labels if possible. It’s best to be as descriptive as possible!

<table>
<thead>
<tr>
<th>Time</th>
<th>Food or Beverage item</th>
<th>Brand/source (manufacturer)</th>
<th>Type of preparation (bake, boil, fry, etc.)</th>
<th>Amount/weight (cups, ounces, grams, tsp, TBS, fluid ounces)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Food or Beverage item</td>
<td>Brand/source (manufacturer)</td>
<td>Type of preparation (bake, boil, fry, etc.)</td>
<td>Amount/weight (cups, ounces, grams, tsp, TBS, fluid ounces)</td>
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List any vitamin/mineral pills or other supplements here:
BIOGRAPHY OF AUTHOR

Taylor Kathryn Bloedon was born in Newburg, Oregon on October 13, 1981. She graduated from Ontario High School in 2000. She attended The University of Oregon where she obtained her Bachelor's degree and graduated with Honors in 2004. She was also member of the Varsity Cross Country and Track and Field Team at The University of Oregon. She then attended Oregon State University where she was awarded her Masters degree in Nutrition and Food Management with a minor in Exercise Science in 2007. Taylor completed her Dietetic Internship at the Maricopa County Community Dietetic Internship in Phoenix, Arizona before passing her exam to become a Registered Dietitian in 2008. Taylor was hired as a full time Lecturer in the Kinesiology and Physical Education Department at the University of Maine in August of 2013. She is a candidate for the Doctor of Philosophy degree in Food and Nutrition Sciences from the University of Maine in December 2013.