COMPARISON OF BLOOD PARAMETERS ASSOCIATED WITH EXERCISE AND THE TOTAL ANTIOXIDANT POWER IN SLED DOGS SUPPLEMENTED WITH BLUEBERRIES

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

Kriya L. Dunlap, B.S.

Fairbanks, Alaska

December 2003

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Abstract

Oxidative damage from free radicals plays an important role in several diseases such as cancer, Alzheimer's disease, and heart disease. Research indicates that exercise may contribute to oxidative stress. Fruits, such as blueberries, are good antioxidants because they contain phenolics that preferentially react with free radicals. Maintaining antioxidant levels by supplementing the diet with blueberries may prevent exercise-induced oxidative damage. Additionally, oxidative damage from exercise can temporarily suppress the immune system. The goal of our study was to compare antioxidant levels in sled dogs supplemented with blueberries.

Total antioxidant power (TAP), haptoglobin, isoprostane and other blood parameters were measured in plasma samples from racing sled dogs before exercise, post-exercise, 24 hours post-exercise, and 48 hours post-exercise. Though isoprostane levels did not change throughout the study, creatine kinase levels increased post-exercise for all exercise dogs regardless of blueberry supplementation. Conflicting data makes it unclear as to whether blueberry supplementation reduces muscle damage, adding confusion to the lack of sound antioxidant data available for dogs. Regardless, dogs fed blueberries had a significantly higher TAP than control post-exercise. This suggests that dogs fed blueberries while exercising as compared to dogs fed a control diet while exercising, may be better protected against oxidative damage.

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List of Abbreviations

ROS	Reactive Oxygen Species
FR	Free Radicals
ADP	Adenosine Diphosphate
APR	Acute Phase Response
ATP	Adenosine Triphophate
BLU	Blueberry
BUN/CK	Blood Urea Nitrogen/ Creatine Kinase ratio
CK	Creatine Kinase
CON	Control
Hb	Hemoglobin
Hp	Haptoglobin
RUN	Runners
T1	Pre-exercise
T2	Post-exercise
T3	24 hours post-exercise
T4	48 hours post-exercise

Acknowledgements

Like every project, this was a collaborative effort and I would be remiss if due credit was not given. Foremost, I am grateful for the guidance and encouragement of Dr. Lawrence Duffy. And though I could not possibly extend the full length of my gratitude, I am blessed to have acquired such a great advisor, educator, and friend.

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Chapter 1

INTRODUCTION

While exercise has many benefits (Clyman, 2001; Kell et al., 2001; Elphick et al., 2003) strenuous exercise produces free radicals, which increases Reactive Oxygen Species (ROS) in athletes (Clarkson and Thompson, 2000; Mastaloudis et al., 2001). Free radicals, including those containing oxygen atoms, have an unpaired electron and are very reactive with other molecules (Stadtman ER, 1996; German JB, 1998; Halliwell, 2002). The production of free radicals and consequent oxidative damage is associated with an increased risk of hyperlipidaemia, hyperalbunminaemia, ischemic heart disease (Dogra et al., 2001; Drew et al., 2001; Block et al., 2002), cancer and other cardiovascular diseases related to aging (Joseph et al., 1999; Bickford et al., 2000). Mammals have both endogenous and diet related protective molecules, called antioxidants, which scavenge excess free radicals. Common antioxidants are ascorbic acid, melatonin, glutathione, biliverdin, and vitamin E. If there is a depletion of the antioxidant defense system or an increase in radical production, lipid peroxidation and subsequent cell damage occur (Balakrishnan and Anuradha, 1998; Block et al., 2002; Lesgards et al., 2002). Several studies have shown that supplementing the diet with antioxidant rich foods can significantly reduce oxidative stress (Baskin et al., 2000; Clarkson and Thompson, 2000; Block et al., 2002; Morrow, 2002).

Exercise induced oxidative damage has been reported in a number of species, including horses (Hargreaves et al., 2002; Kirschvink et al., 2002), dogs (Baskin et al., 2000; Piercy et al., 2000), and humans (Balakrishnan and Anuradha, 1998; Mastaloudis et al., 2001). In human athletes preparing for a marathon, a 10 to 20-fold increase in whole body oxygen consumption and a 100 to 200-fold increase in oxygen uptake by the active skeletal muscle was observed. Normal oxygen metabolism in aerobic organisms results in an increase in the production of free radicals because of an inherent inefficiency in electron transport and cytochrome oxidase in the mitochondria. (Mastaloudis et al., 2001).

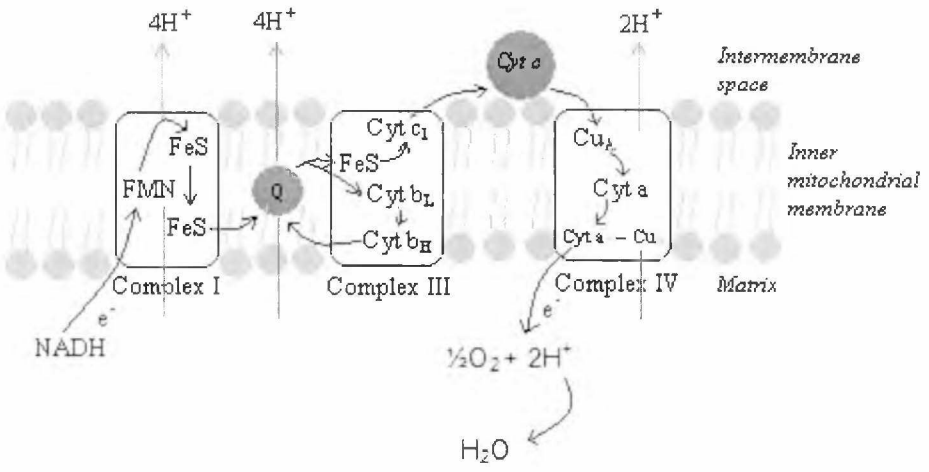

Figure 1.1. The movement of electrons through the Electron transport chain from NADH to O_2. Electrons are transferred between Complexes I and III by coenzyme Q (Q) and between Complexes III and IV by cytochrome c. Complex II is not shown, but it transfers electrons from succinate to coenzyme Q.

The ROS generated by exercise is derived from the electron transport chain (Figure 1.1) in the mitochondria of activated phagocytes at the sites of muscle damage (Baskin et al., 2000). The leakage of electrons from the mitochondrial electron transport chain modifies DNA, membrane lipids, and proteins, especially the iron containing proteins. ROS also disrupt calcium homeostasis (Hoffman et al., 1997; German, 1998; Mastaloudis et al., 2001).

Fruits and vegetables are good antioxidant supplements because, not only do they contain Vitamin C and E, but also phenols and polyphenols (Figure 1.2). These types of molecules preferentially react with free radicals and are responsible for most of the fruit's antioxidant capacity (Swanson, 1998; Javanovic, 2000; Prior and Cao, 2000; Fuhrman, 2002; Kay and Holub, 2002; Lesgards et al., 2002). Variations exist in the ability of certain phenolic antioxidants to scavenge free radicals (Javanovic, 2000; Fuhrman, 2002).

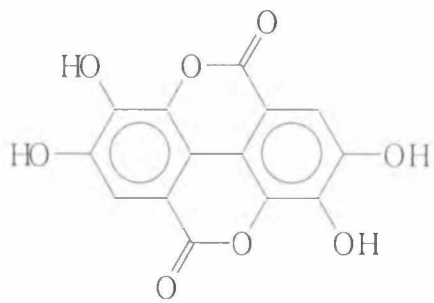

Figure 1.2. Structure of the phenolic acid, Ellagic acid found in blueberries.

In a study looking at several neuronal and behavioral parameters, Joseph et al. (1999) compared rats with age related deficits fed aqueous extracts of blueberries, strawberries and spinach. The antioxidant benefits were exhibited for all the supplemented groups, but rodents supplemented with blueberries revealed the most dramatic benefits (Joseph et al., 1999). They suggested that the interactions between flavanoids (Figure 1.3) and other phytochemicals in blueberries contribute to their augmented capacity as an antioxidant. The polyphenols and flavanoids, thought to be the most important antioxidant components in blueberries (Fuhrman, 2002; Kay and Holub, 2002), like Vitamin E and C, are not restricted to preserving or repairing membrane integrity, but have numerous antioxidant roles. Functioning as reducing agents, as hydrogen atom-donating antioxidants, as singlet oxygen quenchers, and even as metal ion chelaters (Fuhrman, 2002), flavanoids and polyphenols are capable of helping repair any damaged DNA base or protein amino acid (Javanovic, 2000).

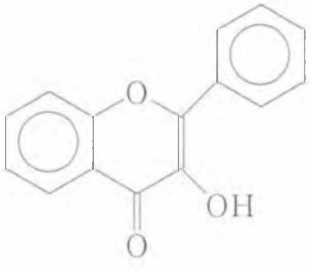

Figure 1.3. Structure of the flavanoid, Flavanol found in the family *Ericaceae*.

Racing sled dogs are excellent models for studying health effects related to exercise. Much of their biochemical and endocrine mechanisms are similar to humans

(Kararli, 1995; Felsburg, 2002), yet their basal metabolic rate and energy expenditure is 3-8 times greater (Hinchcliff et al., 1997). This increased volume of oxygen entering the mitochondrial electron transport chain, compounded with the added stress of exercise, makes racing sled dogs extremely prone to oxidative stress and ideal models for studying exercise induced oxidative damage.

Canine athletes rely more heavily on lipid metabolism because of their elevated energy requirements, rather than carbohydrate metabolism as in human athletes (Kronfeld et al., 1977; Downey, 1980; Reynolds et al., 1994). Kronfeld et al (1977) found that dogs do not share the same deleterious health risks associated with a high fat diet as humans do. Nonetheless, a high fat diet increases the propensity of fatty acid oxidation. Because of the implications of a high fat diet, Kay et al (2002) tested the serum antioxidant levels in humans fed a high fat diet with a blueberry supplement and found that, indeed, test subjects with the blueberry supplement had higher serum antioxidant status compared to a control group.

In racing sled dogs fed a diet high in vitamin E, β-carotene, and lutein, Piercy et al (2000) reported no protection by these antioxidants against muscle damage, as judged by creatine kinase levels in the blood. In a previous study they did observe an increase in resistance of lipoproteins to oxidative damage and a reduction in exercise-induced oxidative damage of DNA in dogs supplemented with Vitamin E (Baskin et al., 2000). Vitamin E (alpha-tocopherol) is known to scavenge peroxyl radicals in human athletes and inhibit the production of superoxide molecules (Mastaloudis et al., 2001).

1.1 Isoprostanes

Repetitive exercise is associated with an increase in plasma isoprostanes (Hinchcliff et al., 2000; Mastaloudis et al., 2001), as well as an increase in plasma creatine kinase (CK) levels (an indicator of muscle damage) (Roberts, 1979; Hinchcliff et al., 2000; Hargreaves et al., 2002). A sled dog race is a multiple day event at a very high intensity and it is expected that these dogs are at an increased risk of oxidative damage (Hinchcliff et al., 2000).

In order to quantify the oxidative damage caused by exercise in racing sled dogs, the plasma isoprostane levels may be examined. Isoprostanes are prostaglandin like compounds, created by the free radical attack on esterified arachidonic acid (Fig. 1.4.) in the cell membrane (Fig. 1.5) (Pratico et al., 2001; Halliwell, 2002; Morrow, 2002). After damage, isoprostanes are cleaved from the lipid by a phospholipase. Then they enter and circulate in the plasma, eventually being excreted in urine. The biological activity of isoprostanes, both in vitro and in vivo, suggest that they act as mediators of the cellular effects of oxidative stress (Morrow et al., 1995; Hoffman et al., 1997; Stein and Leskiw, 2000; Pratico et al., 2001; Dillon et al., 2002; Morrow, 2002). In fact, Morrow et al (1995) demonstrated that F_2-isoprostane levels were a better indicator of lipid peroxidation than malondialdehyde, a widely used assay.

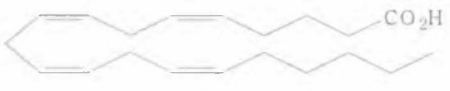

Figure 1.4. Arachidonic acid.

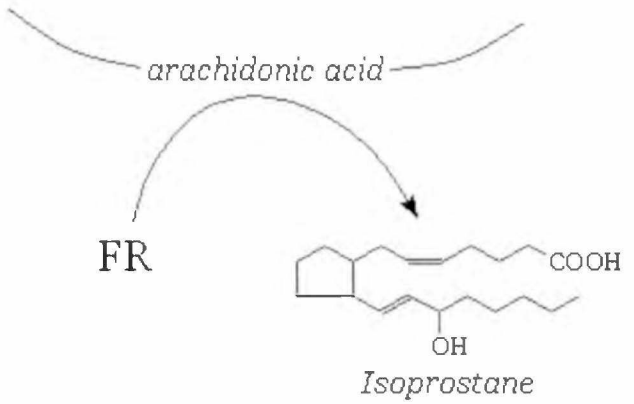

Figure 1.5. Free radical (FR) attack of arachidonic acid in the cell wall results in the release of an isoprostane.

As mentioned previously, sled dogs are maintained on a high fat diet and thus have elevated levels of free fatty acids (Kronfeld et al., 1977; Downey, 1980; Reynolds et al., 1994). Isoprostanes can be formed by the autoxidation of purified polyunsaturated fatty acids (Morrow, 2002). It is, therefore, likely that sled dogs will have elevated plasma isoprostane levels due to the enhanced opportunity for fatty acid oxidation. Antioxidant rich diets have been shown to inhibit the formation of isoprostanes in animal models under oxidative duress (Morrow, 2002). In the current study, plasma F_2–isoprostane levels were measured to determine whether a diet supplemented with blueberries would attenuate lipid peroxidation during and after exercise in racing sled dogs.

1.2 Creatine Kinase

Creatine kinase (CK) is the enzyme responsible for transferring the high-energy phosphate bond from creatine phosphate to adenosine diphosphate (ADP) to yield adenosine triphosphate (ATP) (Roberts, 1979). When an organelle that contains CK, such as the cytosol or inner mitochondrial membrane of myocytes are disrupted, CK leaks from the cell into the circulatory system (Roberts, 1979; Hargreaves et al., 2002). Elevated CK levels are associated with shock, myxedema, pulmonary emboli, pneumonia, radiotherapy, chronic lung disease, surgery, and exercise (Roberts, 1979).

Piercy et al (2000) used plasma CK levels as an index of muscle damage in sled dogs, resulting from lipid peroxidation after a 58 km run. They did not observe protection against muscle damage in sled dogs supplemented with vitamin E as compared with control dogs. However, Hargreaves et al (2002) reported a correlation between antioxidant status and CK levels in endurance horses. Other investigators have also reported elevated CK levels in the blood after exercise (Hinchcliff et al., 1993; Fallon et al., 2001; Evans et al., 2002). In the current study we used plasma CK levels as a biomarker for muscle damage.

1.3 Haptoglobin, Albumin, and Acute Phase Proteins

The advantageous properties of blueberries, as a supplement, are that they are not only limited to oxidative relief. Blueberries may produce other beneficial effects involved in the immune system. The phenolic compounds in blueberries have been shown to promote membrane fluidity, antagonize arachidonic acid transport, and suppress the 5-lipoxygenase pathway, thus reducing inflammatory responses (Joseph et al., 1999).

The stress of exercise can cause temporary suppression of the immune system (Shephard et al., 1998; Fallon et al., 2001). The measurement of certain plasma proteins, associated with the acute-phase response (APR), is often used to evaluate immune function (Duffy, 1996; Zenteno-Savin et al., 1997). Levels of APR proteins such as haptoglobin and albumin can be affected by psychological, environmental and physical stress (Duffy, 1996; Zenteno-Savin et al., 1997; Fallon et al., 2001). A significant stress stimulates the liver to increase the synthesis and secretion of APR proteins (Duffy, 1996).

Typically, in an animal experiencing the APR, Haptoglobin (Hp) levels will be elevated and albumin levels will be depressed (Duffy, 1996; Fallon, et al., 2001; Zenteno-Savin et al., 1997). Albumin also acts as a protein antioxidant (Dogra et al., 2001). Oxidative stress implicated in coronary heart disease may be a result of hypoalbuminaemia (Dogra et al., 2001). Both plasma haptoglobin and albumin levels were determined as a surrogate monitor of the immune system in the present study.

1.4 Total Antioxidant Power and Uric Acid

The overall capacity of blood constituents to quench free radicals is often determined by using biochemical assays, which measure the ability of a sample to reduce a specific oxidant (Piercy et al., 2000; Guohua, 2002; Lesgards et al., 2002). These assays do not measure specific antioxidants, but can give insight into the animal's overall antioxidant defense system. Lesgards et al (2002) used such an assay in determining various lifestyle factors on overall antioxidant levels in the blood. They found that people who ate a diet high in fruits and vegetables had greater antioxidant protection that people who had a diet deficient in fruits and vegetables.

Uric acid is a common biological antioxidant that is frequently measured as an indicator of antioxidant status (Piercy et al., 2000; Mastaloudis et al., 2001; Kirschvink et al., 2002). In many of the antioxidant capacity assays, including ours, it is used as a standard in which the samples are compared. Blood levels of uric acid generally increase as a result of exercise (Piercy et al., 2000; Mastaloudis et al., 2001). Mastaloudis et al 2001 postulates that this phenomenon is likely due to the increased rate of ATP catabolism.

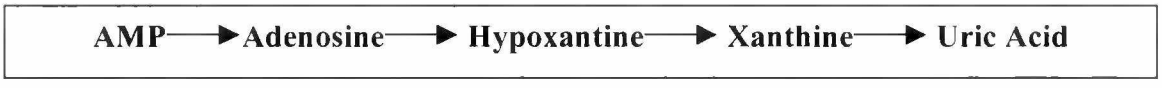

Figure 1.6. Purine catabolism.

The overall purpose of this study was to observe if supplementing the diet of racing sled dogs with blueberries would attenuate exercise-induced muscle damage. Our results reveal conflicting evidence. Many parameters associated with oxidative stress were not affected by blueberry supplementation, yet over-all antioxidant status was elevated in blueberry supplemented dogs.

Chapter 2

MATERIAL AND METHODS

2.1 Sled Dogs

The Institute of Animal Use and Care Committee at the University of Alaska Fairbanks approved this study. The dogs that were used in this study were typical racing sled dogs owned by Dave Monson and Susan Butcher of Trail Breaker Kennel. Thirty-six dogs, designated as the study dogs, were separated by the kennel owners into 3 equal groups, balanced for age, sex and ability (Table 2.1.). The 3 groups were, control (CON), runners (RUN), and blueberry (BLU). Ages for the dogs ranged from 1 to 12 years. A total of Twenty-one females and 15 males entered this study, 3 of which were not sexually intact (2 females and 1 male). One dog was eliminated from the RUN group prior to the termination of the study due to a foot injury. Housing arrangements consisted of 2-m chains on which the dogs were tethered for the duration of the study (8 weeks). Each dog had access to his or her own house. For the 2 months prior to the onset of this 4-day study, all 36 dogs were housed at a kennel on the property of Trail Breaker Kennel in Fairbanks, Alaska. Ambient temperatures during the 2 months of the study ranged from 10°C to 25°C. The temperature was 10°C on both days that the dogs exercised (August 9th and 10th).

Table 2.1. Study dogs listed alphabetically with their corresponding group, age, sex and ability. Ability was rated on a scale of 1-3: 1 is above average, 2 is average, and 3 is below average

DOG	GROUP	AGE	SEX	ABILITY
Blue	CON	1	M	3
Casey	CON	4	M	1
Cowlick	CON	5	F	1
Duter	CON	1	M	1
Harrison	CON	1	M	3
Hepsaba	CON	1	F	1
Lacey	CON	4	F	2
Mas	CON	12	F*	1
Muskrat	CON	7	M	2
Ra	CON	1	F	2
Rocky	CON	4	M	3
Sophia	CON	1	F	3
AVERAGE		3.5	50%M	1.9
Ajax	RUN	1	M	1
Alta	RUN	4	M	2
Bella	RUN	1	F	3
Belock	RUN	1	M	2
Birch	RUN	4	M	1
Flag	RUN	5	M	2
Nellie	RUN	1	F	2
Nenana	RUN	5	F*	2
Pheobe	RUN	3	F	1
Rosier	RUN	1	F	3
Tisbury	RUN	1	F	2
		2.5	45.5%M	1.9
Brook	BLU	1	F	2
Canyon	BLU	3	F	3
Diomede	BLU	1	F	1
Indi	BLU	1	M	1
Kantishna	BLU	2	F	2
Leeda	BLU	2	F	2

Mohogany	BLU	2	M*	1
Orca	BLU	1	F	2
Simon	BLU	1	M	1
Spark	BLU	2	M	2
Whaler	BLU	1	M	2
Yutana	BLU	5	F	2
		1.8	41.6%M	1.8

*Indicates neutered animals

2.2. Diet

The three groups were fed Purina Pro Plan Performance; with BLU group having a blueberry supplement. To insure that the dogs were acclimated to the diets, they were maintained on the diets for 2 months preceding the study. As stated, CON and RUN were fed a standard high energy, high protein commercial diet (Table 2.2.). BLU were fed the same diet, but 2% (about 20g) of their diet by weight was supplemented with wild organic blueberries from Oregon (Table 2.3.). A measured amount of food (approximately 1 lb/day) was fed to each dog. The amount varied slightly throughout the study for each dog in order to maintain ideal body condition. Ideal body condition is defined as easily palpable ribs and vertebral spinal processes, with a slight depression between the wings of the ileum (Laflamme, 1997; Reynolds et al., 1999). The dogs were fed once a day in the morning. During the actual experiment the dogs were fed 12 hours prior to exercise to insure that the dogs were in a post-absorptive state.

Table 2.2. Nutrition facts and ingredients for Purina Pro Plan Performance (control diet).

Crude Protein	30.0%
Crude Fat	20.0%
Crude Fiber	3.0%
Moisture	12.0%
Linoleic Acid	1.8%
Calcium	0.9%
Phosphorus	0.7%

Ingredients: Chicken, corn gluten meal, brewers rice, beef tallow preserved with mixed-tocopherols (source of Vitamin E), ground yellow corn poultry by-product meal, corn bran, animal digest, egg product, dicalcium phosphate, potassium chloride, calcium carbonate, salt, choline chloride, L-Lysine monohydrochloride, zinc oxide, ferrous sulfate, vitamin supplements (E,A, B-12, D-3), riboflavin supplement, niacin, calcium pantothenate, manganese sulfate, biotin, thiamine mononitrate, folic acid, copper sulfate, pyridoxine hydrochloride, garlic oil, menadione sodium bisulfite complex (source of vitamin K activity), calcium iodate, sodium selenite.

Table 2.3. Nutrition facts for Safeway Select's frozen wild organic blueberries.

Amount per serving (serving size 1 cup or 140 grams)	
Carbohydrate	16 g
Fat	0 g
Protein	less than 1 g

2.3. Exercise

All dogs were allowed only minimal exercise on their chains for 2 months preceding the study. Activity levels varied depending on the individual dog. During these 2 months, the experimental diet was administered. At the closure of this adaptation period, the RUN and BLU groups were exercised 7 miles at 15 miles per hour for 2 consecutive days. This speed and distance was chosen based on previous studies that

determined that on average, 7 miles at 15 miles per hour is approximately 70% VO_2 max for most sled dogs (Ordway et al., 1984). The exercise groups were run in 2 teams of 8 dogs and 1 team of 7 dogs, in front of an All Terrain Vehicle for consistency, with no relevance to which group they were in. CON dogs did not exercise.

2.4. Blood Sampling

All dogs were bled before exercise (T1), immediately upon their return (T2), 24 hours post exercise (T3), and 48 hours post exercise (T4). Blood was drawn by venipuncture from the jugular into three 5 ml heparinized vacutainer tubes. Plasma was obtained by centrifugation at 2500 X g for 10 min, transferred into freezer vials, flash frozen in liquid nitrogen and stored at –70°C until they were analyzed.

2.5. Biochemical Analyses

The biochemical analyses, including haptoglobin, isoprostane, and total anti-oxidant power, were done at the University of Alaska Fairbanks.

A commercial assay from Oxford Biomedical Research laboratory (#TA 01) was used to determine plasma total antioxidant power. In this assay, the ability of all the antioxidants in the sample to reduce Cu^{++} to Cu^{+} was applied as an index of the sample's antioxidant capacity. The antioxidant concentrations of the samples were then determined by further extrapolation from a standard curve developed from known

concentrations of uric acid. The procedure supplied with the assay was followed (Oxford Biomedical Research, 2001).

Plasma Isoprostane levels were determined by using a competitive inhibition assay, purchased from Oxford Biomedical Research laboratory (#EA 84). The isoprostane, 15-isoprostane F_{2t} in the samples or standards competes with 15-isoprostane F_{2t} conjugated to horseradish peroxidase (provided in the kit) for an antibody, specific for this isoprostane that is coated in the wells. When the substrate is added to the conjugate, a blue color forms in proportion to the amount of conjugate bound, and inversely proportional to the amount of sample or standard bound to the microplate. The directions supplied with the kit were followed, eliminating the solid-phase extraction step (Oxford Biomedical Research, 2002). To compensate for this missing step we ran duplicates of all samples, and duplicates of samples that were spiked with an addition 12.5 ng/ml of the provided standard. The extraction step allows for accurate quantification of isoprostane levels, by reducing any matrix effects due to isoprostanes bound to proteins in the sample. Spiking the sample with a known concentration of standard may not allow for specific values to be compared but allows for overall trends in isoprostane levels to be compared.

A commercial assay, purchased from Tri-Delta Diagnostics, Inc. (Phase Haptoglobin Assay), was used to determine plasma haptoglobin levels. Hemoglobin, like other heme-proteins, can catalyze the reduction of hydrogen peroxide to water. The peroxidase activity of hemoglobin is preserved by combining with haptoglobin. Haptoglobin levels in the samples and standards are directly proportional to the

peroxidase activity of hemoglobin. Chromogen, supplied by the kit, detecting hemoglobin peroxidase activity exhibits a color in proportion to the amount of haptoglobin present. The procedure supplied with the kit was followed (Tri-delta Diagnostics, 2001).

2.6 Clinical Analysis

Complete chemistry screens were performed at Fairbanks Memorial Hospital. The values of interest were albumin, albumin/globulin ratio, bilirubin, blood urea nitrogen/creatine ratios, creatine kinase and uric acid levels. All analyses were done on the samples at the same time, within one month of collection. The raw data is contained in the appendix.

2.7. Statistical Analysis

All data was analyzed using SAS statistical software and found to be normally distributed. Analysis of variance was used to analyze all the data to evaluate the effects of treatment and exercise on blood parameters. Significant differences between treatment groups were analyzed further using the Tukey multiple comparison procedure. Differences were considered significant at $P \leq 0.05$.

Chapter 3

RESULTS

The blueberry group (BLU) and the runners (RUN) were compared to the control (CON) for each day in which blood was collected (T1,T2, T3, T4). In addition to eating only the control diet for the duration of the study, CON animals did not exercise. For the isoprostane, total antioxidant power, and haptoglobin an entire blood collection day was run using one kit. This approach was used to reduce variability and reagent related differences. Therefore, comparisons were made only in relation to the CON group for that day, since CON samples should not vary greatly throughout the duration of the study. Since we were unable to control for interassay variability the means for the CON dogs did change throughout the study.

Groups of dogs were compared using SAS statistical software as described in the methods. Various tests were run on the values to ensure homogeneity of variance, and normality. Analysis of variance was performed on all the data followed by post hoc analysis using Tukey's studentized range. Differences were considered significant at $P \leq 0.05$.

3.1. Isoprostanes

Isoprostane levels for BLU and RUN did not significantly differ from CON throughout the study. Each group showed an increasing trend compared to pre-exercise values. This non-significant trend peaked post-exercise and gradually declined toward control values at 48 hours post-exercise.

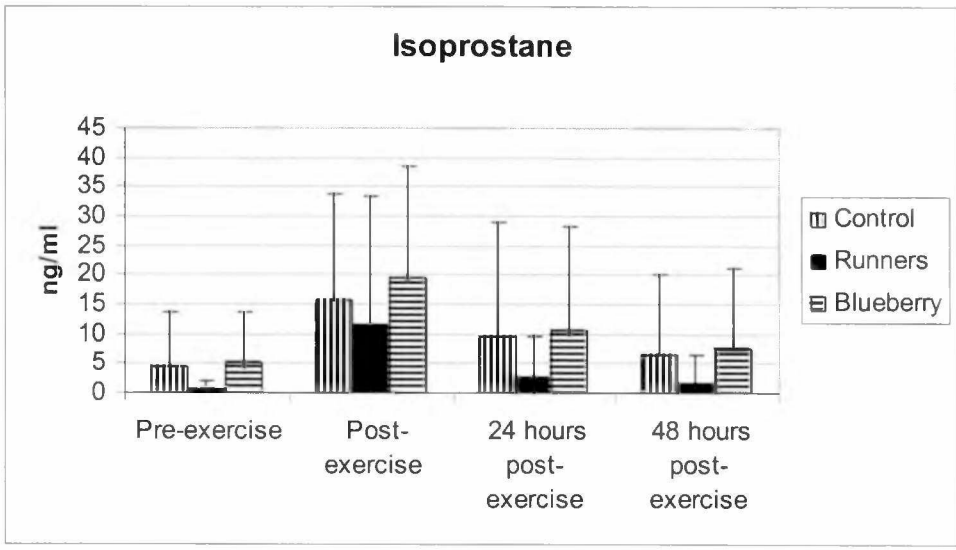

Figure 3.1. Means and standard deviations of isoprostane levels for each group over the 4 collection days.

Interestingly, the mean values for RUN tended to be lower than CON or BLU groups.

Table 3.1. Actual values (ng/ml) of the means and standard deviations of isoprostane levels for each group over the 4 collection days.

	Pre-exercise	Post-exercise	24 hours post-exercise	48 hours post-exercise
CON avg	4.50	15.61	9.57	6.49
CON stdeva	9.07	18.16	19.51	13.45
RUN avg	0.62	11.63	2.62	1.65
RUN stdeva	1.30	21.79	6.97	4.65
BLU avg	4.95	19.49	10.70	7.58
BLU stdeva	8.67	18.92	17.52	13.52

3.2. Creatine Kinase

Creatine kinase levels for BLU and RUN groups were significantly higher than CON group post-exercise. BLU levels remained significantly higher than CON 24 hours post-exercise, returning to baseline by 48 hours post-exercise (Table 3.3).

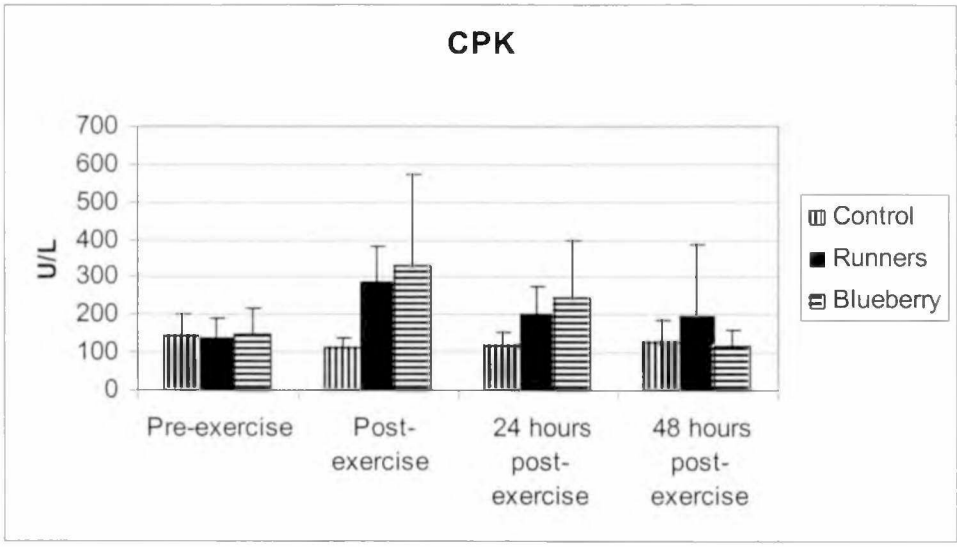

Figure 3.2. Means and standard deviations of creatine kinase levels for each group over the 4 collection days.

The creatine kinase control dog's mean values showed less variation during the 48 hours of the experiment than the isoprostane mean values. But, both BLU and RUN groups showed the biggest change post-exercise, followed by gradual decline to pre-exercise values at 48 hours post-exercise.

Table 3.2. Actual values (U/L) of the means and standard deviations of creatine kinase levels for each group over the 4 collection days. Asterisks indicates significant difference of at least p=0.05 using tukey's studentized range.

	Pre-exercise	Post-exercise	24 hours post-exercise	48 hours post-exercise
CON avg	140.91	111.41* **	115.33*	125.25
CON stdeva	61.42	27.66	36.53	61.36
RUN avg	140.18	286.54**	202.00	194.64
RUN stdeva	51.511	96.17	73.27	190.69
BLU avg	147.75	329.58*	244.08*	117.75
BLU stdeva	67.73	245.66	152.23	43.86

Blood Urea Nitrogen/ creatine kinase ratios (BUN/CK) for BLU and RUN did not significantly differ from CON throughout the study. A decrease following exercise was observed (Fig 3.3) with a return toward pre-exercise levels 48 hours post-exercise. As creatine kinase levels increased and decreased as seen in Fig 3.2, the ratios would be expected to change.

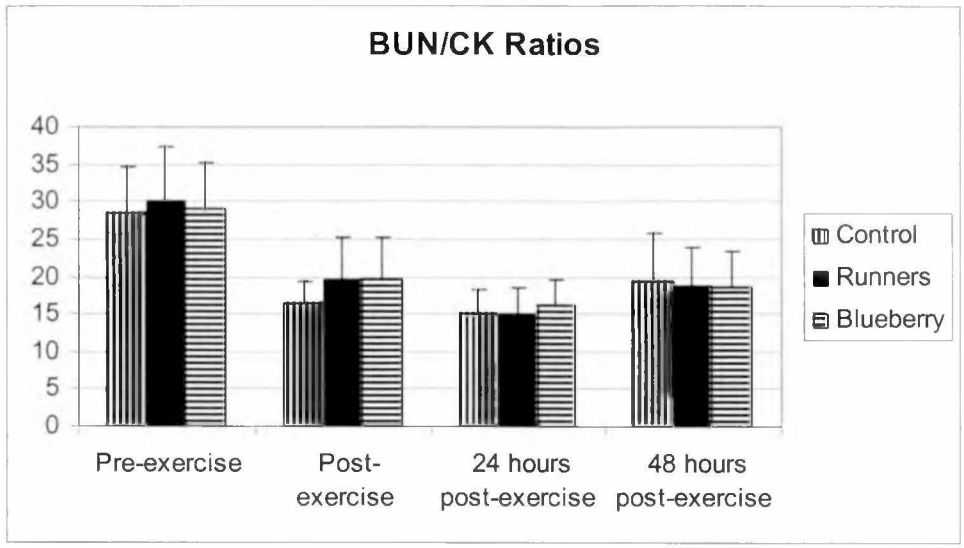

Figure 3.3. Means and standard deviations of BUN/CK ratios for each group over the 4 collection days.

Since blood urea nitrogen can be an indicator of the acute phase response as well as hydration status, it can be concluded that the stress was not extreme in these athletes.

Table 3.3. Actual values of the means and standard deviations of BUN/CR ratios for each group over the 4 collection days.

	Pre-exercise	Post-exercise	24 hours post-exercise	48 hours post-exercise
CON avg	28.47	16.33	15.05	19.23
CON stdeva	6.13	3.07	3.27	6.48
RUN avg	29.95	19.50	15.16	18.88
RUN stdeva	7.26	5.67	3.36	4.89
BLU avg	29.03	19.48	16.10	18.53
BLU stdeva	6.22	5.67	3.52	4.70

3.3. Haptoglobin, Albumin and Acute Phase Proteins

Haptoglobin levels for BLU were significantly lower than CON post-exercise. but had returned to levels comparable with CON by 24 hours post exercise (Table 3.6.). No other mean haptoglobin levels deviated from CON for the length of the study. The RUN group's mean values also tended to be lower post-exercise. The BLU group displayed a better recovery by 24 hours post-exercise than the RUN group.

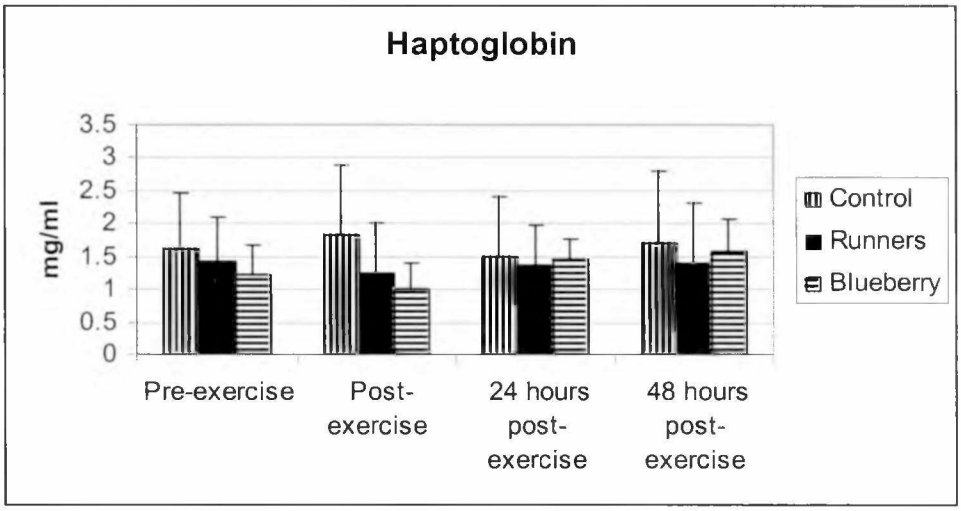

Figure 3.4. Means and standard deviations of haptoglobin levels for each group over the 4 collection days.

In the acute phase response haptoglobin would be expected to increase. The lack of a BUN response and the haptoglobin decrease shows that the exercise used in this experiment did not stress the dogs to the level of an acute phase.

Table 3.4. Actual values (mg/ml) of the means and standard deviations of haptoglobin levels for each group over the 4 collection days. Asterisks indicates significant difference of at least p=0.05 using tukey's studentized range.

	Pre-exercise	Post-exercise	24 hours post-exercise	48 hours post-exercise
CON avg	1.61	1.83*	1.51	1.71
CON stdeva	0.84	1.06	0.89	1.10
RUN avg	1.44	1.24	1.38	1.41
RUN stdeva	0.65	0.77	0.60	0.91
BLU avg	1.22	0.99*	1.46	1.58
BLU stdeva	0.45	0.42	0.31	0.48

Albumin levels for BLU and RUN did not significantly differ from CON throughout the study. This also supports the lack of an acute phase response.

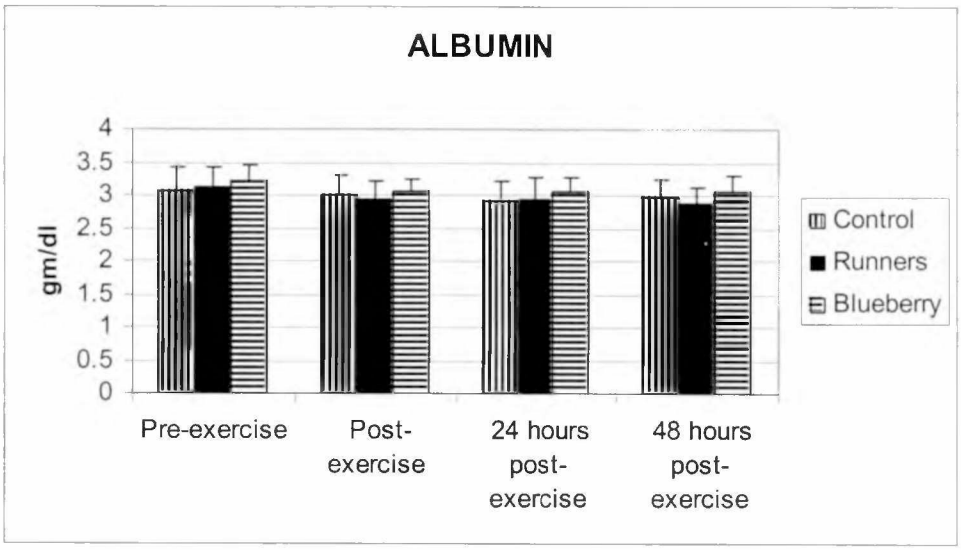

Figure 3.5. Means and standard deviations of albumin levels for each group over the 4 collection days.

Table 3.5. Actual values (gm/dl) of the means and standard deviations of albumin levels for each group over the 4 collection days.

	Pre-exercise	Post-exercise	24 hours post-exercise	48 hours post-exercise
CON avg	3.07	3.00	2.90	2.98
CON stdeva	0.37	0.30	0.30	0.28
RUN avg	3.13	2.96	2.93	2.86
RUN stdeva	0.31	0.29	0.35	0.27
BLU avg	3.20	3.05	3.06	3.06
BLU stdeva	0.25	0.18	0.22	0.24

Albumin/ Globulin ratios for BLU and RUN did not significantly differ from CON throughout the study.

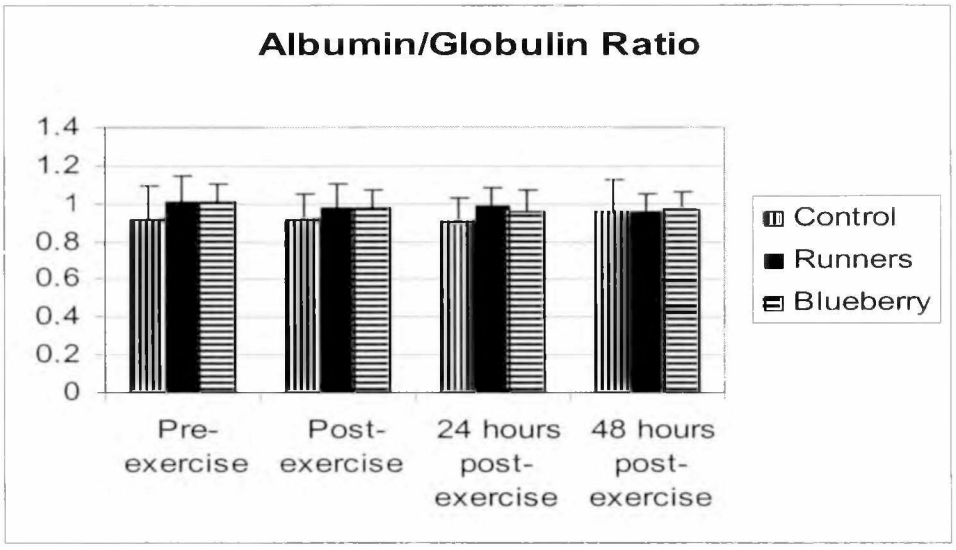

Figure 3.6. Means and standard deviations of albumin/globulin ratios for each group over the 4 collection days.

Table 3.6. Actual values of the means and standard deviations of albumin/globulin ratios for each group over the 4 collection days.

	Pre-exercise	Post-exercise	24 hours post-exercise	48 hours post-exercise
CON avg	0.91	0.91	0.90	0.95
CON stdeva	0.19	0.14	0.13	0.17
RUN avg	1.01	0.97	0.98	0.95
RUN stdeva	0.14	0.13	0.10	0.09
BLU avg	1.01	0.98	0.96	0.97
BLU stdeva	0.09	0.10	0.12	0.10

3.4. Total Antioxidant Power and Uric Acid

Total antioxidant power for BLU post exercise was significantly higher than CON (Table 3.10). No other significant differences were observed between treatment groups at 24 or 48 hours post-exercise. Means 24 hours post-exercise were lower than other days. Each day was run on a separate kit, on a separate day to eliminate inter-assay variability. There is slight differences between kits and even unrecognizable changes that occur between laboratory sessions. Thus, comparisons can not be made between days.

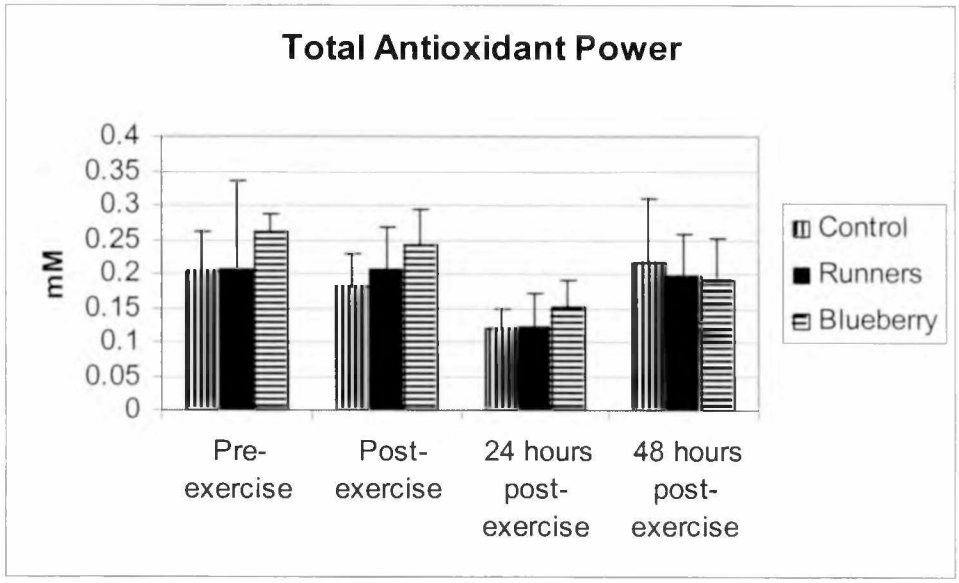

Figure 3.7. Means and standard deviations of total antioxidant power for each group over the 4 collection days.

Table 3.7. Actual values (mM) of the means and standard deviations of total antioxidant power for each group over the 4 collection days. Asterisks indicates significant difference of at least p=0.05 using tukey's studentized range.

	Pre-exercise	Post-exercise	24 hours post-exercise	48 hours post-exercise
CON avg	0.20	0.18*	0.12	0.23
CON stdeva	0.06	0.05	0.03	0.09
RUN avg	0.21	0.21	0.12	0.20
RUN stdeva	0.13	0.06	0.05	0.06
BLU avg	0.26	0.24*	0.15	0.19
BLU stdeva	0.03	0.05	0.04	0.06

Uric acid levels for BLU and RUN were significantly higher than CON post exercise (Table 3.12). The mean uric acid values for the two exercise groups were not different from CON values for any of the other time periods.

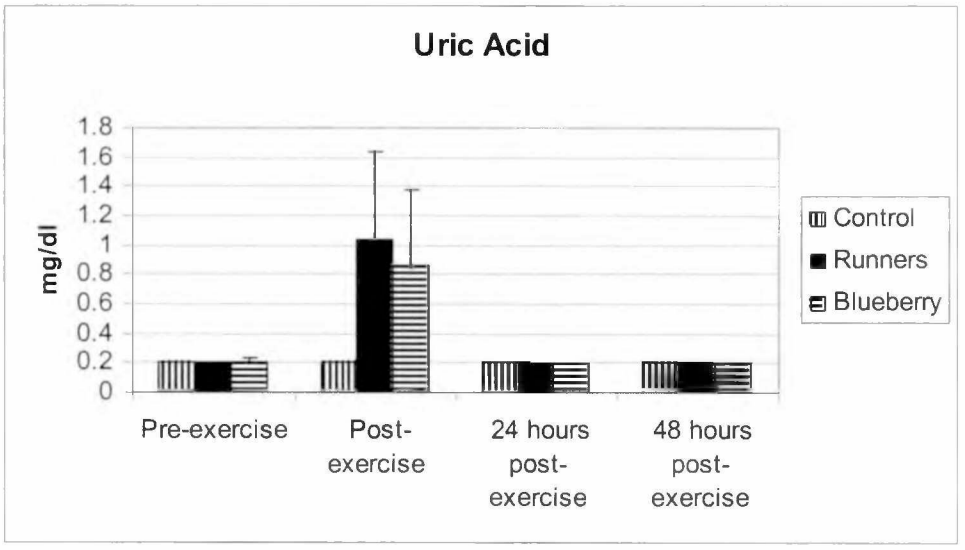

Figure 3.8. Means and standard deviations of uric acid levels for each group over the 4 collection days.

The increase in uric acid post-exercise was dramatic in both the RUN and BLU. The BLU group displayed a smaller trend in uric acid levels as compared to RUN.

Table 3.8. Actual values (mg/dl) of the means and standard deviations of uric acid levels for each group over the 4 collection days. Asterisks indicates significant difference of at least p=0.05 using tukey's studentized range.

	Pre-exercise	Post-exercise	24 hours post-exercise	48 hours post-exercise
CON avg	0.20	0.20* **	0.2	0.2
CON stdeva	>E-08	>E-08	>E-08	>E-08
RUN avg	0.20	1.04**	0.2	0.2
RUN stdeva	>E-08	0.61	>E-08	>E-08
BLU avg	0.21	0.86*	0.2	0.2
BLU stdeva	0.03	0.52	>E-08	>E-08

Bilirubin levels for BLU and RUN did not significantly differ from CON throughout the study.

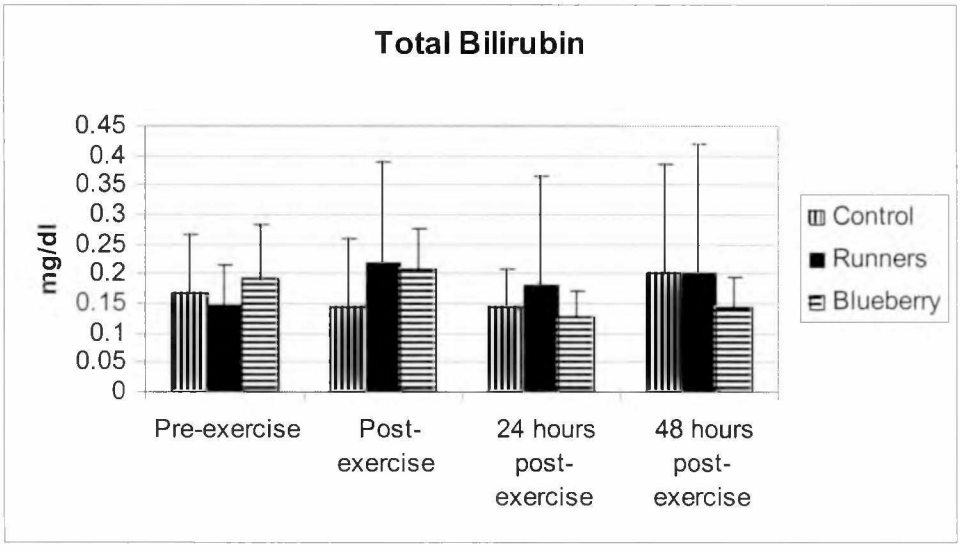

Figure 3.9. Means and standard deviations of bilirubin levels for each group over the 4 collection days.

Table 3.9. Actual values (mg/dl) of the means and standard deviations of bilirubin levels for each group over the 4 collection days.

	Pre-exercise	Post-exercise	24 hours post-exercise	48 hours post-exercise
CON avg	0.17	0.14	0.14	0.20
CON stdeva	0.10	0.12	0.07	0.19
RUN avg	0.15	0.22	0.18	0.20
RUN stdeva	0.07	0.17	0.18	0.22
BLU avg	0.19	0.21	0.13	0.14
BLU stdeva	0.09	0.07	0.05	0.05

Chapter 4

DISCUSSION AND FUTURE DIRECTION

Supplementing young healthy sled dogs with blueberries failed to attenuate muscle damage caused by a period of exercise at 75% VO_2 maximum. Creatine kinase levels, an indicator of muscle damage (Roberts, 1979; Hargreaves et al., 2002), was significantly elevated after exercise in both exercise groups (RUN and BLU) regardless of dietary supplementation with blueberries. In fact, BLU remained significantly higher than control (CON) 24 hours after exercise and though RUN also displayed an increased trend, the difference was insignificant. Interesting, mean creatine kinase levels for all groups remained inside the reference range (25-467 U/L) for dogs regardless of exercise. Elevated creatine kinase levels in this case indicates that the exercise may have been severe enough to elicit some damage, but not enough to push creatine kinase levels outside the normal range. In addition, supplementing the diet with blueberries was not enough to combat this any stress that was accrued.

Another index of muscle damage is elevated plasma and urine 15-isoprostane F_{2t} levels (Morrow et al., 1995; Hoffman et al., 1997; Stein and Leskiw, 2000; Pratico et al., 2001; Dillon et al., 2002; Morrow, 2002). Isoprostanes are released when free radicals attack esterified arachidonic acid on the cell membrane allowing them to enter the circulatory system (Pratico et al., 2001; Halliwell, 2002; Morrow, 2002). Because of the nature of isoprostane formation they are a biomarker of oxidative stress, and more specifically lipid peroxidation (Morrow et al., 1995; Hoffman et al., 1997; Stein and

Leskiw, 2000; Pratico et al., 2001; Dillon et al., 2002; Morrow, 2002). Throughout the duration of the study, no significant variations of mean 15-isoprostane F_{2t} levels were observed, although BLU and CON tended to be higher than RUN at every blood collection time.

There are two possible explanations for the isoprostane results not paralleling the creatine kinase patterns. One technical and one metabolic. The procedure included in the Oxford Biomedical assay called for an extraction process when using plasma samples to liberate any isoprostanes that may have been bound to proteins in the blood (Oxford Biomedical Research, 2002). In our assay we excluded this step and compensated for matrix effects by spiking each sample with a known concentration of 15-isoprostane F_{2t} and compared these with unspiked samples. This modified procedure has been used by others. The means of spiked samples did not deviate significantly between groups. However, the trends observed for each spiked sample did not correlate well with the amount of spike administered to each sample. This indicated that plasma matrix effects were significant and differed between dogs. Hinchcliff et al (2000) applied a similar technique with the same assay. In the past few years, slight changes in the kit prevented a comparable approach to elicit positive results. We used the negative results for the unspiked data, which, due to high variability, yielded the same non-significant conclusion.

The second explanation is based on metabolic differences indicated by these biomarkers. If the isoprostane results are accurate, a plausible explanation for the lack of change in isoprostane levels, at the same time as a remarkable increase in creatine kinase

levels would indicate that the muscle damage produced during exercise was not caused by oxidative stress, but from some other stressor. Significantly higher total antioxidant power (TAP) for the blueberry supplemented group supports this idea. Both groups that exercised, BLU and RUN, experienced significantly higher creatine kinase levels post-exercise, yet only the BLU group had significantly higher TAP than CON post-exercise. If the observed muscle damage was due to oxidative stress than theoretically the boosted circulation of antioxidants in the blood should have partially offset the muscle damage, if blood levels reflect organ availability. However, BLU dogs experienced elevated creatine kinase levels for a longer period of time than the RUN, extending 24 hours post exercise. The TAP of the RUN did appear to exceed CON post exercise. This was likely a result of significantly elevated uric acid levels for both the BLU and the RUN post exercise. Normal range for uric acid values in the dog is less than 0.4 mg/dl. Values for exercising dogs exceeded this range post-exercise. Bilirubin, also an inherent antioxidant, did not change for any group throughout the study, staying within the reference range for dogs (0.1-0.7 mg/dl). Poly phenols in blueberries were most likely responsible for the additional TAP for the BLU.

With its deep rich color, it is not surprising that blueberries are now heralded as one of the leading antioxidants, both natural and synthetic. Just supplementing 2% of the diet with blueberries is reported to prevent age related maladies associated with oxidative stress (Joseph et al., 1999). Joseph et al (1999) reported that the phenolic compounds in blueberries might inhibit inflammatory responses through various mechanisms. These benefits are probably due to a cocktail of nutrients in a synergistic web that once

unwound, may not provide the same integrity in individuals, if components are fed separately. Evidence exists that compounds in blueberries may play a role in boosting the immune system. Markers associated with immune function for sled dogs supplemented with blueberries failed to significantly differ from control throughout the study. Albumin, albumin/globulin ratios, bilirubin, and blood urea nitrogen/ creatine kinase ratios remained stable and all within the reference range for dogs. Haptoglobin levels for the BLU group were significantly lower than CON post-exercise, though still within the reference range for dogs (0.3-3.5). Animals who experience an acute phase response are expected to display elevated levels of haptoglobin (Duffy, 1996). The observed drop in haptoglobin may be acting as a marker of red blood cell stress. Hemoglobin is released from lysed red blood cells and binds to haptoglobin, lowing haptoglobin levels.

The immune profiles for these dogs suggest that the exercise used in this study was not strenuous enough to elicit an acute phase response. Though it is tempting to speculate that the reduced haptoglobin for BLU was due to enhanced immune function, it is more likely due to hemolysis in the samples. Haptoglobin (Hp) is influential at blocking the exchange of heme between methemoglobin and albumin by binding free hemoglobin (Hb) (Zenteno-Savin et al., 1997; Ben-David et al., 2001). Damage to red blood cells causes heme to be released, thus negatively effecting haptoglobin levels (Montgomery et al., 1980; Marks et al., 1996). Five plasma samples that were collected post-exercise appeared to be hemolyzed. Three of the 5 were collected from blueberry dogs; the other groups each having one hemolized sample apiece.

In our study, supplementing the diet with blueberries appears to increase the amount of antioxidants available to the animal. The elevated level of antioxidants, however, did not combat muscle damage associated with exercise as suggested by enhanced creatine kinase levels. In the future, a different method for assessing muscle damage caused by oxidative stress should be used in order to mechanistically evaluate the potential of blueberries in reducing exercise stress to cells. Further, it is necessary to have an exercise regime that will challenge the immune system in order to evaluate any benefits to the immune system obtained from blueberries supplementation.

Because of the many advantages of using sled dogs as research animals, including large homogenous sample sizes, there is many directions that can be explored in the future. Sled dogs are often exposed to the same environmental hazards as humans in the extreme climate of the circumpolar. This makes sled dogs ideal models for studying the effects of environmental and nutritional toxins/influences on the immune system and subsequently the effects of dietary intervention to compensate for any impairments. In many villages, natives still follow a subsistence lifestyle, exposing themselves to both climate and to many contaminants that have accumulated in the fish and game that they consume. The sled dogs in these villages are also maintained on indigenous food and therefore can be used as models for researching the effects that a subsistence diet might have on the immune system. One such study was performed in northern New York at Cornell University. It was suspected that the inhabitants of a small town on the St. Lawrence River were exposed to dangerously high levels of PCBs. The study involved feeding research dogs varying levels of PCBs and testing different parameter to find

reliable biomarkers for PCB exposure. A kennel of sled dogs living on a reservation was maintained on fish from the river and were used as the first research subjects in a series of work, studying the level and effects of PCBs on the local people and animals (Korytko et al., 1999) .

In the current study, blueberries were used as an antioxidant in combating muscle damage, but Joseph et al (1999) found that supplementing the diet of rats with blueberries helped with deleterious cognitive functions associated with age. For eons people have anthropomorphized a dogs age with the familiar 7-year analogy for every 1-year of a dog to equal that of a human. Though the relationship is not entirely linear, age comparisons can be made between the two species (Felsburg, 2002). Therefore extrapolations can be made from immune profiles of dogs corresponding to different age brackets. Cognitive tests and subsequent dietary interventions could provide valuable nutritional information. The ongoing search to find the fountain of youth so that we cannot only live longer but healthier is forever propelling future research.

Chapter 5

LITERATURE CITED

Balakrishnan SD, Anuradha CV (1998) Exercise, depletion of antioxidants and antioxidant manipulation. Cell Biochem Funct 16:269-275.

Baskin CR, Hinchcliff KW, DiSilvestro RA, Reinhart GA, Hayek MG, Chew BP, Burr JR, Swenson RA (2000) Effects of dietary antioxidant supplementation on oxidative damage and resistance to oxidative damage during prolonged exercise in sled dogs. Am J Vet Res 61:886-891.

Ben-David M, Duffy LK, Bowyer RT (2001) Biomarker responses in river otters experimentally exposed to oil contamination. J Wildl Dis 37:489-508.

Bickford PC, Gould T, Briederick L, Chadman K, Pollock A, Young D, Shukitt-Hale B, Joseph J (2000) Antioxidant-rich diets improve cerebellar physiology and motor learning in aged rats. Brain Res 866:211-217.

Block G, Dietrich M, Norkus EP, Morrow JD, Hudes M, Caan B, Packer L (2002) Factors associated with oxidative stress in human populations. Am J Epidemiol 156:274-285.

Clarkson PM, Thompson HS (2000) Antioxidants: what role do they play in physical activity and health? Am J Clin Nutr 72:637S-646S.

Clyman B (2001) Exercise in the treatment of osteoarthritis. Curr Rheumatol Rep 3:520-523.

Dillon SA, Lowe GM, Billington D, Rahman K (2002) Dietary supplementation with aged garlic extract reduces plasma and urine concentrations of 8-iso-prostaglandin F(2 alpha) in smoking and nonsmoking men and women. J Nutr 132:168-171.

Dogra G, Ward N, Croft KD, Mori TA, Barrett PH, Herrmann SE, Irish AB, Watts GF (2001) Oxidant stress in nephrotic syndrome: comparison of F(2)-isoprostanes and plasma antioxidant potential. Nephrol Dial Transplant 16:1626-1630.

Downey RK, DS; Banta, CA (1980) Diet of Beagles affects stamina. J Am Anim Hosp Assoc 16:273-277.

Drew KL, Rice ME, Kuhn TB, Smith MA (2001) Neuroprotective adaptations in hibernation: therapeutic implications for ischemia-reperfusion, traumatic brain injury and neurodegenerative diseases. Free Radic Biol Med 31:563-573.

Duffy LB, RT (1996) Acute Phase Proteins and Cytokines in Alaskan Mammals as Markers of Chronic Exposure to Environmental Pollutants. American Fisheries Society Symposium 18:809-813.

Elphick GF, Wieseler-Frank J, Greenwood BN, Campisi J, Fleshner M (2003) B-1 cell (CD5+/CD11b+) numbers and nIgM levels are elevated in physically active versus sedentary rats. J Appl Physiol.

Evans RK, Knight KL, Draper DO, Parcell AC (2002) Effects of warm-up before eccentric exercise on indirect markers of muscle damage. Med Sci Sports Exerc 34:1892-1899.

Fallon KE, Fallon SK, Boston T (2001) The acute phase response and exercise: court and field sports. Br J Sports Med 35:170-173.

Felsburg PJ (2002) Overview of immune system development in the dog: comparison with humans. Hum Exp Toxicol 21:487-492.

Fuhrman B AM (2002) Polyphenols and Flavonoids Protect LDL Against Atherogenic Modifications. In: Handbook of Antioxidants (Cadenas E PL, ed), pp 303-336. New York: Marcel Dekker Inc.

Fuhrman BaA, M (2002) Polyphenols and Flavonoids Protect LDL Against Atherogenic Modifications. In: Handbook of Antioxidants (Cadenas E PL, ed), pp 303-336. New York: Marcel Dekker Inc.

German JB DC (1998) Phytochemicals and Targets of Chronic Disease. In: Phytochemicals a New Paradigm (Bidlack WR OS, Meskin MS, Jahner D, ed), pp 13-32. Lancaster: Technomic Publishing Company, Inc.

Guohua CR, RL (2002) Measurement of Total Antioxidant Capacity in Nutritional and Clinical Studies. In: Handbook of Antioxidants, 2nd Edition (Cadenas E PL, ed). New York: Marcel Dekker Inc.

Halliwell B (2002) Food-derived Antioxidants: How to evaluate their importance in food and *In vivo*. In: Handbook of antioxidants, 2nd Edition (L CEaP, ed), pp 1-34. New York: Marcel Dekker Inc.

Hargreaves BJ, Kronfeld DS, Waldron JN, Lopes MA, Gay LS, Saker KE, Cooper WL, Sklan DJ, Harris PA (2002) Antioxidant status and muscle cell leakage during endurance exercise. Equine Vet J Suppl:116-121.

Hinchcliff KW, Reinhart GA, Burr JR, Schreier CJ, Swenson RA (1997) Metabolizable energy intake and sustained energy expenditure of Alaskan sled dogs during heavy exertion in the cold. Am J Vet Res 58:1457-1462.

Hinchcliff KW, Reinhart GA, DiSilvestro R, Reynolds A, Blostein-Fujii A, Swenson RA (2000) Oxidant stress in sled dogs subjected to repetitive endurance exercise. Am J Vet Res 61:512-517.

Hinchcliff KW, Olson J, Crusberg C, Kenyon J, Long R, Royle W, Weber W, Burr J (1993) Serum biochemical changes in dogs competing in a long-distance sled race. J Am Vet Med Assoc 202:401-405.

Hoffman SW, Moore S, Ellis EF (1997) Isoprostanes: free radical-generated prostaglandins with constrictor effects on cerebral arterioles. Stroke 28:844-849.

Javanovic SS, MG (2000) Antioxidants in Nutrition. In: Reactive Oxygen Species (CC C, ed), pp 327-334. New York: Annals of the New York Academy of Sciences.

Javanovic SV SM (2000) Antioxidants in Nutrition. In: Reactive Oxygen Species (CC C, ed), pp 327-334. New York: Annals of the New York Academy of Sciences.

Joseph JA, Shukitt-Hale B, Denisova NA, Bielinski D, Martin A, McEwen JJ, Bickford PC (1999) Reversals of age-related declines in neuronal signal transduction, cognitive, and motor behavioral deficits with blueberry, spinach, or strawberry dietary supplementation. J Neurosci 19:8114-8121.

Kararli TT (1995) Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm Drug Dispos 16:351-380.

Kay CD, Holub BJ (2002) The effect of wild blueberry (*Vaccinium angustifolium*) consumption on postprandial serum antioxidant status in human subjects. Br J Nutr 88:389-398.

Kell RT, Bell G, Quinney A (2001) Musculoskeletal fitness, health outcomes and quality of life. Sports Med 31:863-873.

Kirschvink N, Art T, de Moffarts B, Smith N, Marlin D, Roberts C, Lekeux P (2002) Relationship between markers of blood oxidant status and physiological variables in healthy and heaves-affected horses after exercise. Equine Vet J Suppl:159-164.

Korytko PJ, Casey AC, Bush B, Quimby FW (1999) Induction of hepatic cytochromes P450 in dogs exposed to a chronic low dose of polychlorinated biphenyls. Toxicol Sci 47:52-61.

Kronfeld DS, Hammel EP, Ramberg CF, Jr., Dunlap HL, Jr. (1977) Hematological and metabolic responses to training in racing sled dogs fed diets containing medium, low, or zero carbohydrate. Am J Clin Nutr 30:419-430.

Laflamme D (1997) Development and Validation of a body Condition Score System for Dogs. Canine Practice 22:10-15.

Lesgards JF, Durand P, Lassarre M, Stocker P, Lesgards G, Lanteaume A, Prost M, Lehucher-Michel MP (2002) Assessment of lifestyle effects on the overall antioxidant capacity of healthy subjects. Environ Health Perspect 110:479-486.

Marks DB, Marks AS, Smith CM (1996) Basic Medical Biochemistry a clinical approach. Baltimore: Williams and Wilkins a waverly company.

Mastaloudis A, Leonard SW, Traber MG (2001) Oxidative stress in athletes during extreme endurance exercise. Free Radic Biol Med 31:911-922.

Montgomery R, Dryer RL, Conway TW, Spector AA (1980) Biochemistry a case-oriented approach, 3rd Edition. St. Louis: The C.V. Mosby Company.

Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ, 2nd (1995) Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N Engl J Med 332:1198-1203.

Morrow JD ZW, Van der Ende DS, Reich EE, Terry ES, Cos B, Sanchez SC, Montine TJ, Roberts LJ (2002) Quantification of Isoprostanes as Indicators of Oxidant Stress In Vivo. In: Handbook of Antioxidants, 2nd Edition (L CEaP, ed), pp 57-71. New York: Marcel Dekker Inc.

Morrow JZ, WE; Van der Ende, DS; Reich, EE; Terry, ES; Cos, B; Sanchez, SC; Montine, TJ; Roberts, LJ (2002) Quantification of Isoprostanes as Indicators of Oxidant Stress In Vivo. In: Handbook of Antioxidants, 2nd Edition (L CEaP, ed), pp 57-71. New York: Marcel Dekker Inc.

Ordway GA, Floyd DL, Longhurst JC, Mitchell JH (1984) Oxygen consumption and hemodynamic responses during graded treadmill exercise in the dog. J Appl Physiol 57:601-607.

Oxford Biomedical Research I (2001) Colorimetric Microplate Assay for Total Antioxidant Power Product No. TA 01. In, pp 1-5.

Oxford Biomedical Research I (2002) Enzyme Immunoassay for Isoprostane Product No. EA 84. In, pp 1-8.

Piercy RJ, Hinchcliff KW, DiSilvestro RA, Reinhart GA, Baskin CR, Hayek MG, Burr JR, Swenson RA (2000) Effect of dietary supplements containing antioxidants on attenuation of muscle damage in exercising sled dogs. Am J Vet Res 61:1438-1445.

Pratico D, Lawson JA, Rokach J, FitzGerald GA (2001) The isoprostanes in biology and medicine. Trends Endocrinol Metab 12:243-247.

Prior RL, Cao G (2000) Analysis of botanicals and dietary supplements for antioxidant capacity: a review. J AOAC Int 83:950-956.

Reynolds AJ, Fuhrer L, Dunlap HL, Finke MD, Kallfelz FA (1994) Lipid metabolite responses to diet and training in sled dogs. J Nutr 124:2754S-2759S.

Reynolds AJ, Reinhart GA, Carey DP, Simmerman DA, Frank DA, Kallfelz FA (1999) Effect of protein intake during training on biochemical and performance variables in sled dogs. Am J Vet Res 60:789-795.

Roberts R (1979) Creatine Kinase Isozymes as Diagnostic and Prognostic Indices of Myocardial Infarction. In: Isozymes: Current Topics in Biological and Medical Research (Rattazzi MS, JG, ed), pp 115-154. New York: Alan R. Liss, Inc.

Shephard RJ, Castellani JW, Shek PN (1998) Immune deficits induced by strenuous exertion under adverse environmental conditions: manifestations and countermeasures. Crit Rev Immunol 18:545-568.

Stadtman ER BB (1996) Reactive oxygen-mediated protein oxidation in aging and disease. Drug Metab Rev 30:225-243.

Stein TP, Leskiw MJ (2000) Oxidant damage during and after spaceflight. Am J Physiol Endocrinol Metab 278:E375-382.

Swanson C (1998) Vegetables, Fruits, and Cancer Risk: The Role of Phytochemicals. In: Phytochemicals a New Paradigm (Bidlack WR OS, Meskin MS, Jahner D, ed), pp 1-12. Lancaster: Technomic Publishing Company, Inc.

Tri-delta Diagnostics I (2001) Instruction Manual: Phase Range Haptoglobin Assay. In, pp 1-6.

Zenteno-Savin T, Castellini MA, Rea LD, Fadely BS (1997) Plasma haptoglobin levels in threatened Alaskan pinniped populations. J Wildl Dis 33:64-71.

Appendix. Plasma Analysis Data.

Table 1. Albumin values (gm/dl).

Dog	Group	T1	T2	T3	T4
Blue	CON	3.1	3	3.1	3.2
Casey	CON	3	3	2.8	2.8
Cowlick	CON	3.1	3.4	3.1	3.5
Duter	CON	2.5	2.4	2.4	2.7
Harrison	CON	2.4	2.4	2.3	2.8
Hepsaba	CON	3.2	3.2	3.1	3
Lacey	CON	3	3	3	2.8
Mas	CON	2.9	3	2.8	2.8
Muskrat	CON	3.2	3.1	3.1	2.8
Ra	CON	3.2	3.1	2.8	2.9
Rocky	CON	3.4	3.2	3	2.9
Sophia	CON	3.8	3.2	3.3	3.5
Ajax	RUN	2.9	2.6	2.9	3.1
Alta	RUN	2.7	2.7	2.2	2.4
Bella	RUN	2.9	2.7	3.1	2.8
Belock	RUN	2.8	2.6	2.5	2.6
Birch	RUN	3.6	3.4	3.4	3.2
Flag	RUN	3.4	2.9	2.8	3.1
Nellie	RUN	3.5	3.2	3.2	3.1
Nenana	RUN	3.1	3.3	3	2.8
Pheobe	RUN	3.3	3.1	3.3	3.1
Rosier	RUN	3.3	2.7	2.8	2.6
Tisbury	RUN	2.9	3	3	2.7
Brook	BLU	3.2	2.9	3.1	3.1
Canyon	BLU	2.7	3.1	3	3.2
Diomede	BLU	3.2	3.1	2.6	2.8
Indi	BLU	3.2	3.2	3.1	2.8
Kantishna	BLU	3.2	3	3.1	3.2
Leeda	BLU	3.6	3.4	3.3	3.3
Mohogany	BLU	3	2.9	3	2.8
Orca	BLU	3.5	3.2	3.3	3.5
Simon	BLU	3.5	3.1	3.2	3.2
Spark	BLU	3.1	3.1	3.1	3.1
Whaler	BLU	3	2.7	2.7	2.8
Yutana	BLU	3.2	2.9	3.2	2.9

Table 2. Albumin/Globulin ratios.

Dog	Group	T1	T2	T3	T4
Blue	CON	0.9	0.9	0.9	1
Casey	CON	0.8	0.9	0.8	0.9
Cowlick	CON	1.1	1.2	1.1	1.3
Duter	CON	0.8	0.8	0.8	0.9
Harrison	CON	0.7	0.7	0.7	0.8
Hepsaba	CON	1	1	1	1
Lacey	CON	0.7	0.8	0.8	0.8
Mas	CON	0.7	0.8	0.8	0.8
Muskrat	CON	0.9	0.8	0.9	0.8
Ra	CON	1.1	1	1	1.1
Rocky	CON	0.9	0.9	0.9	0.8
Sophia	CON	1.3	1.1	1.1	1.2
Ajax	RUN	0.9	0.9	1	1
Alta	RUN	0.8	0.8	0.8	0.8
Bella	RUN	1	0.9	1	0.9
Belock	RUN	0.8	0.8	0.8	0.8
Birch	RUN	1.2	1.1	1.1	1.1
Flag	RUN	1.1	1	1	1
Nellie	RUN	1.2	1.1	1.1	1
Nenana	RUN	1	1.2	1	1
Pheobe	RUN	1	1	1	1
Rosier	RUN	1.1	0.9	1	0.9
Tisbury	RUN	1	1	1	1
Brook	BLU	0.9	0.9	1	1
Canyon	BLU	1	1	1	1
Diomede	BLU	1	1	0.8	0.9
Indi	BLU	1.1	1	1	1
Kantishna	BLU	1	0.9	0.9	0.9
Leeda	BLU	1	0.9	0.9	0.9
Mohogany	BLU	0.9	0.9	0.8	0.8
Orca	BLU	1.2	1.1	1.2	1.2
Simon	BLU	1.1	1.2	1.1	1
Spark	BLU	1	1	1	1
Whaler	BLU	1	0.9	0.9	1
Yutana	BLU	0.9	0.9	0.9	0.9

Table 3. Total bilirubin (mg/dl).

Dog	Group	T1	T2	T3	T4
Blue	CON	0.2	0.1	0.1	0.6
Casey	CON	0.1	0.1	0.1	0.1
Cowlick	CON	0.1	0.5	0.2	0.5
Duter	CON	0.1	0.1	0.1	0.1
Harrison	CON	0.1	0.1	0.1	0.1
Hepsaba	CON	0.1	0.1	0.1	0.1
Lacey	CON	0.2	0.1	0.3	0.1
Mas	CON	0.1	0.1	0.1	0.1
Muskrat	CON	0.2	0.1	0.2	0.1
Ra	CON	0.1	0.1	0.1	0.1
Rocky	CON	0.3	0.2	0.2	0.1
Sophia	CON	0.4	0.1	0.1	0.4
Ajax	RUN	0.1	0.1	0.1	0.4
Alta	RUN	0.2	0.1	0.1	0.1
Bella	RUN	0.1	0.2	0.7	0.1
Belock	RUN	0.3	0.2	0.1	0.1
Birch	RUN	0.2	0.3	0.3	0.1
Flag	RUN	0.1	0.2	0.1	0.8
Nellie	RUN	0.1	0.1	0.1	0.1
Nenana	RUN	0.1	0.7	0.1	0.2
Pheobe	RUN	0.2	0.2	0.2	0.1
Rosier	RUN	0.1	0.1	0.1	0.1
Tisbury	RUN	0.1	0.2	0.1	0.1
Brook	BLU	0.4	0.3	0.2	0.2
Canyon	BLU	0.2	0.1	0.1	0.1
Diomede	BLU	0.2	0.3	0.1	0.1
Indi	BLU	0.2	0.2	0.1	0.2
Kantishna	BLU	0.1	0.2	0.1	0.2
Leeda	BLU	0.2	0.3	0.2	0.2
Mohogany	BLU	0.1	0.2	0.2	0.1
Orca	BLU	0.3	0.2	0.1	0.2
Simon	BLU	0.2	0.2	0.1	0.1
Spark	BLU	0.2	0.2	0.1	0.1
Whaler	BLU	0.1	0.1	0.1	0.1
Yutana	BLU	0.1	0.2	0.1	0.1

Table 4. Blood urea nitrogen/ creatine kinase ratios.

Dog	Group	T1	T2	T3	T4
Blue	CON	27	16.3	15	18.8
Casey	CON	25.6	16.3	15	17.5
Cowlick	CON	27	21.3	16.7	21.1
Duter	CON	21.3	16.3	20	36.7
Harrison	CON	25	12.9	12.5	22.5
Hepsaba	CON	24.5	11.1	11.1	12.2
Lacey	CON	38.8	20	17.1	17.1
Mas	CON	23.3	16.3	12.2	18.8
Muskrat	CON	25	14.3	11.4	12.9
Ra	CON	35	17.5	17.8	21.3
Rocky	CON	29.1	13.6	11.8	13
Sophia	CON	40	20	20	18.8
Ajax	RUN	42.2	23.8	16.3	25
Alta	RUN	20	12.7	15.6	16.7
Bella	RUN	36.7	24.3	18.6	20
Belock	RUN	37.5	27.8	15.7	21.4
Birch	RUN	22	16	13.8	14.4
Flag	RUN	20	13.3	12.9	17.1
Nellie	RUN	28.9	16.7	11.4	15.7
Nenana	RUN	31.1	19	15	21.3
Pheobe	RUN	32.2	15.6	13.3	15
Rosier	RUN	31.1	28.6	22.9	28.6
Tisbury	RUN	27.8	16.7	11.3	12.5
Brook	BLU	40	23.8	18.6	21.4
Canyon	BLU	13.8	15	18.8	28.9
Diomede	BLU	29	17.8	11	14.4
Indi	BLU	32	24.5	17.8	17.8
Kantishna	BLU	29	13.3	12.5	23.8
Leeda	BLU	31.1	20	17.1	21.4
Mohogany	BLU	33	18.8	14.4	18.8
Orca	BLU	27.8	18.8	12.9	14.3
Simon	BLU	30	24	17.5	18.8
Spark	BLU	26.4	15.5	14.4	15
Whaler	BLU	24	19	14.4	14
Yutana	BLU	32.2	23.3	23.8	13.8

Table 5. Creatine kinase values (U/L).

Dog	Group	T1	T2	T3	T4
Blue	CON	128	108	157	109
Casey	CON	90	48	77	82
Cowlick	CON	244	115	114	95
Duter	CON	154	140	105	125
Harrison	CON	282	125	82	148
Hepsaba	CON	136	119	108	59
Lacey	CON	84	83	105	248
Mas	CON	124	109	91	78
Muskrat	CON	90	101	98	89
Ra	CON	115	135	129	244
Rocky	CON	138	101	210	98
Sophia	CON	106	153	108	128
Ajax	RUN	185	283	298	250
Alta	RUN	123	241	94	160
Bella	RUN	139	305	229	185
Belock	RUN	132	504	263	108
Birch	RUN	183	375	324	750
Flag	RUN	85	187	191	117
Nellie	RUN	95	312	190	122
Nenana	RUN	66	133	157	97
Pheobe	RUN	240	270	98	73
Rosier	RUN	176	276	193	168
Tisbury	RUN	118	266	185	111
Brook	BLU	135	182	140	83
Canyon	BLU	243	589	419	196
Diomede	BLU	251	306	133	71
Indi	BLU	92	283	143	87
Kantishna	BLU	136	158	125	89
Leeda	BLU	94	154	317	97
Mohogany	BLU	173	715	354	185
Orca	BLU	112	224	178	148
Simon	BLU	137	823	604	144
Spark	BLU	52	136	91	109
Whaler	BLU	135	182	140	83
Yutana	BLU	243	589	419	196

Table 6. Haptoglobin values (mg/ml).

Dog	Group	T1	T2	T3	T4
Blue	CON	2.2	1.18	0.76	0.68
Casey	CON	1.48	2.76	2.18	2.8
Cowlick	CON	0.45	0.42	0.19	0.36
Duter	CON	2.74	2.69	2.28	2.8
Harrison	CON	2.4	2.89	2.28	2.9
Hepsaba	CON	1.62	1.69	1.4	1.3
Lacey	CON	0.52	2.87	2.47	2.82
Mas	CON	1.27	2.49	2.27	1.9
Muskrat	CON	0.56	1.67	1.52	1.6
Ra	CON	1.1	0.21	0.19	0.34
Rocky	CON	2.24	2.71	2.08	2.76
Sophia	CON	2.69	0.33	0.44	0.25
Ajax	RUN	0.89	0.45	0.99	0.6
Alta	RUN	1.71	1.45	1.58	1.83
Bella	RUN	1.67	0.47	1.04	0.64
Belock	RUN	1.66	2.62	2.16	2.7
Birch	RUN	1.74	1.29	1.31	1.57
Flag	RUN	1.38	0.77	1.09	0.79
Nellie	RUN	0.6	1.58	1.88	2.26
Nenana	RUN	1.51	0.62	0.81	0.41
Pheobe	RUN	0.49	2.1	1.85	1.88
Rosier	RUN	1.28	1.95	2.14	2.62
Tisbury	RUN	2.87	0.31	0.28	0.25
Brook	BLU	0.67	0.92	1.43	1.09
Canyon	BLU	1.01	1.02	1.41	1.41
Diomede	BLU	1.64	0.75	1.46	1.57
Indi	BLU	1.29	1.93	1.61	1.68
Kantishna	BLU	1.59	0.93	2.08	2.53
Leeda	BLU	0.77	1.15	1.3	1.08
Mohogany	BLU	0.65	1.37	2.02	2.36
Orca	BLU	1.69	0.45	1.02	1.37
Simon	BLU	0.9	0.8	1.35	1.89
Spark	BLU	1.56	0.9	1.36	1.51
Whaler	BLU	0.98	1.32	1.31	1.51
Yutana	BLU	1.94	0.38	1.21	0.98

Table 7. Isoprostane values (ng/ml).

Dog	Group	T1	T2	T3	T4
Blue	CON	0.005	0.068	0.003	>E-05
Casey	CON	0.004	0.124	0.001	0.003
Cowlick	CON	9.369	24.07	13.35	20.51
Duter	CON	0.003	0.340	0.001	0.002
Harrison	CON	0.014	0.227	0.001	0.566
Hepsaba	CON	2.242	38.34	2.069	0.933
Lacey	CON	0.021	23.05	0.053	0.003
Mas	CON	0.179	27.39	0.342	0.228
Muskrat	CON	31.80	55.55	69.12	44.91
Ra	CON	0.955	2.134	13.90	0.398
Rocky	CON	4.856	7.013	6.060	3.817
Sophia	CON	4.584	9.006	9.958	6.585
Ajax	RUN	>E-08	8.121	0.001	0.001
Alta	RUN	4.327	75.77	23.41	15.61
Bella	RUN	0.001	10.34	0.002	0.001
Belock	RUN	0.002	14.35	>E-04	>E-04
Birch	RUN	0.003	0.188	0.001	0.001
Flag	RUN	1.260	7.913	3.484	1.610
Nellie	RUN	0.004	0.380	0.010	0.027
Nenana	RUN	0.001	0.344	0.000	>E-05
Pheobe	RUN	0.251	4.039	0.667	0.403
Rosier	RUN	0.002	0.112	0.002	0.001
Tisbury	RUN	0.989	6.434	1.312	0.599
Brook	BLU	5.907	49.23	11.34	5.745
Canyon	BLU	0.078	15.12	0.125	0.862
Diomede	BLU	0.004	16.61	0.001	0.001
Indi	BLU	0.250	45.56	0.245	0.128
Kantishna	BLU	12.21	28.11	26.02	3.041
Leeda	BLU	0.004	0.103	0.001	0.001
Mohogany	BLU	0.204	4.758	1.773	1.198
Orca	BLU	0.025	0.377	0.001	0.001
Simon	BLU	8.253	19.07	31.64	11.23
Spark	BLU	0.001	0.066	0.006	0.001
Whaler	BLU	29.34	47.57	54.14	43.40
Yutana	BLU	3.133	7.322	3.109	25.45

Table 8. Total antioxidant power (mM).

Dog	Group	T1	T2	T3	T4
Blue	CON	0.169	0.185	0.119	0.300
Casey	CON	0.145	0.177	0.100	0.184
Cowlick	CON	0.180	0.173	0.096	0.310
Duter	CON	0.267	0.139	0.089	0.170
Harrison	CON	0.323	0.154	0.104	0.2051
Hepsaba	CON	0.183	0.177	0.116	0.181
Lacey	CON	0.145	0.269	0.173	0.437
Mas	CON	0.256	0.188	0.146	0.216
Muskrat	CON	0.222	0.116	0.112	0.121
Ra	CON	0.232	0.146	0.093	0.139
Rocky	CON	0.180	0.273	0.158	0.226
Sophia	CON	0.138	0.177	0.142	0.118
Ajax	RUN	0.243	0.238	0.119	0.300
Alta	RUN	0.250	0.246	0.058	0.233
Bella	RUN	0.187	0.100	0.109	0.128
Belock	RUN	-0.022	0.188	0.150	0.153
Birch	RUN	0.187	0.281	0.242	0.265
Flag	RUN	0.389	0.185	0.081	0.226
Nellie	RUN	0.162	0.208	0.104	0.258
Nenana	RUN	0.445	0.319	0.108	0.149
Pheobe	RUN	0.096	0.177	0.150	0.146
Rosier	RUN	0.169	0.188	0.131	0.174
Tisbury	RUN	0.166	0.146	0.104	0.125
Brook	BLU	0.263	0.307	0.181	0.163
Canyon	BLU	0.263	0.185	0.119	0.160
Diomede	BLU	0.291	0.277	0.093	0.146
Indi	BLU	0.222	0.261	0.150	0.170
Kantishna	BLU	0.256	0.192	0.196	0.272
Leeda	BLU	0.253	0.292	0.173	0.205
Mohogany	BLU	0.277	0.181	0.158	0.174
Orca	BLU	0.236	0.284	0.154	0.279
Simon	BLU	0.291	0.315	0.204	0.233
Spark	BLU	0.305	0.188	0.188	0.268
Whaler	BLU	0.243	0.212	0.089	0.079
Yutana	BLU	0.236	0.208	0.116	0.128

Table 9. Uric acid values (mg/dl).

Dog	Group	T1	T2	T3	T4
Blue	CON	0.2	0.2	0.2	0.2
Casey	CON	0.2	0.2	0.2	0.2
Cowlick	CON	0.2	0.2	0.2	0.2
Duter	CON	0.2	0.2	0.2	0.2
Harrison	CON	0.2	0.2	0.2	0.2
Hepsaba	CON	0.2	0.2	0.2	0.2
Lacey	CON	0.2	0.2	0.2	0.2
Mas	CON	0.2	0.2	0.2	0.2
Muskrat	CON	0.2	0.2	0.2	0.2
Ra	CON	0.2	0.2	0.2	0.2
Rocky	CON	0.2	0.2	0.2	0.2
Sophia	CON	0.2	0.2	0.2	0.2
Ajax	RUN	0.2	1.2	0.2	0.2
Alta	RUN	0.2	0.5	0.2	0.2
Bella	RUN	0.2	0.2	0.2	0.2
Belock	RUN	0.2	1	0.2	0.2
Birch	RUN	0.2	1.5	0.2	0.2
Flag	RUN	0.2	1	0.2	0.2
Nellie	RUN	0.2	1.1	0.2	0.2
Nenana	RUN	0.2	2.5	0.2	0.2
Pheobe	RUN	0.2	0.7	0.2	0.2
Rosier	RUN	0.2	1	0.2	0.2
Tisbury	RUN	0.2	0.7	0.2	0.2
Brook	BLU	0.2	1.2	0.2	0.2
Canyon	BLU	0.3	0.2	0.2	0.2
Diomede	BLU	0.2	0.5	0.2	0.2
Indi	BLU	0.2	1	0.2	0.2
Kantishna	BLU	0.2	0.5	0.2	0.2
Leeda	BLU	0.2	1.1	0.2	0.2
Mohogany	BLU	0.2	0.5	0.2	0.2
Orca	BLU	0.2	0.7	0.2	0.2
Simon	BLU	0.2	2.1	0.2	0.2
Spark	BLU	0.2	0.5	0.2	0.2
Whaler	BLU	0.2	0.7	0.2	0.2
Yutana	BLU	0.2	1.3	0.2	0.2