ABSTRACT

Title of Dissertation: EVALUATING THE EFFECTS OF WEIGHT LOSS ON EXERCISE-INDUCED OXIDATIVE STRESS IN OBESE/OVERWEIGHT SOLDIERS

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Exercise is known to increase reactive oxygen species, a condition recognized as oxidative stress. Obese individuals may experience even greater amounts of oxidative stress after exercise compared to normal weight people. It is not clear how weight loss affects exercise-induced oxidative stress in overweight subjects. The objectives of this study were to 1) evaluate the effect of the Army Physical Fitness Test (APFT) on biomarkers of oxidative stress in overweight/obese soldiers 2) determine the effects of dietary antioxidants, fitness level, body composition on exercise-induced oxidative stress, and 3) determine the effect of weight loss on changes in biomarkers of oxidative stress as a result of the APFT. A total of 60 subjects (35 M, 25 F) were recruited. After completing the 1st APFT (n=47), subjects followed a 3-month weight loss program and then completed the 2nd APFT (n=29). Blood measurements of the oxidative stress biomarkers creatine kinase (CK), C-reactive protein (CRP), glutathione peroxidase (GPX), and superoxide dismutase (SOD) were taken pre, immediately after and 24hrs after each exercise test. Dietary antioxidant intake, fitness level and body

composition were also assessed at each APFT. After completing the 1st APFT, subjects showed a significant increase in CK and CRP levels immediately post-exercise and in CK at 24hrs post-exercise. There was a significant decrease in GPX immediately post-exercise but no significant change in SOD following exercise. Each of the oxidative stress biomarkers were found to be influenced by the antioxidant vitamins A, C and E, fitness level, total fat mass and total fat percentage. There were also significant interactions between fitness level and vitamins A, C, and E, and between fitness level and total fat mass and total fat percentage. There was no significant effect of attempted weight loss on the exercise-induced changes in the biomarkers, but there were significant changes in BMI, fat mass and fat percentage after the weight loss period. In conclusion, the APFT produced oxidative stress in overweight subjects which was not affected by attempted weight loss. Changes in oxidative stress biomarkers at the different time points were significantly affected by dietary antioxidants, fitness level, and body composition.

EVALUATING THE EFFECTS OF WEIGHT LOSS ON EXERCISE-INDUCED OXIDATIVE STRESS IN OVERWEIGHT/OBESE SOLDIERS

by

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Dedication

This research study is dedicated to
all members of the Armed Forces
with whom I am proud to serve.
They are the inspiration behind this research.

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and each of my committee members
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individual contributions to this research study.

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List of Abbreviations

8-EPG	8-isoprostane F2α
8-OHdG	8-hydroxy-2'-deoxyguanosine
ADP	adenosine diphosphate
ANCOVA	analysis of covariance
APFT	Army Physical Fitness Test
BMI	body mass index
BPM	beats per minute
CAT	catalase
CK	creatine kinase
CRP	c-reactive protein
CV%	coefficient of variation
DASH	Dietary Approaches to Stop Hypertension
DHQ	dietary history questionnaire
dl	deciliter
DRI	Dietary Reference Intakes
DXA	dual energy x-ray absorptiometry
F	female
GPX	glutathione peroxidase
HIPAA	Health Information Portability & Accountability Act
HRP	horse radish protein
ID	identification
IL-1	interleukin-1
IL-6	interleukin-6
IRB	Institutional Review Board
IU	international unit
kg	kilogram
LA	lactic acid
LDL	low density lipoprotein
LPO	lipid peroxides
M	male
m	meter
MDA	malondialdehyde
ml	mililiter
nc	not calculated
ng	nanogram
nm	nanometer
ORAC	oxygen radical-absorbing capacity
PEROX	lipid peroxidation
ROS	reactive oxygen species
SOD	superoxide dismutase
TBARS	thiobarbituric reactive acid substances

TNF-α	tumor necrosis factor - alpha
ToFM	total fat mass
ToLM	total lean mass
ToPer	total fat percentage
TrFM	trunk fat mass
TrPer	trunk fat percentage
U	Unit
Vit	vitamin
VLCD	very low-calorie diet
VO2max	maximal aerobic capacity

Chapter 1: Introduction

Oxidative stress exposes the body to free radicals and reactive oxygen species (ROS), which may be linked to a variety of disease states, such as cardiovascular disease and cancer. Oxidative stress occurs when the production of free radicals and ROS, produced in an environment of excess oxygen, exceeds the scavenging capacity of cell and tissue antioxidants (1).

Both aerobic and resistance types of exercise have been shown to produce oxidative stress (2-4). Greater amounts of oxidative stress are associated with exercise at high intensities and/or long durations. It also has been shown that exercise training may decrease an individual's oxidative response to exercise, hence, trained individuals typically experience less oxidative stress following exercise than untrained individuals (1, 5).

Recently, it has been suggested that obese individuals may be susceptible to greater levels of oxidative stress than normal weight people. Furthermore, obese individuals may experience even greater amounts of oxidative stress following exercise (6, 7). Vincent et al. showed that obese subjects had a significantly higher level of oxidative stress biomarkers following an acute bout of aerobic and resistance exercise compared to normal weight subjects (6); however, the effect of weight loss on exercise-induced oxidative stress in obese individuals has not been adequately investigated.

The military has a keen interest in overweight and obesity. These are important matters to the military since excess body weight may adversely impact a soldier's overall health and readiness for combat or deployment. Since all

soldiers are required to perform the Army Physical Fitness Test (APFT) twice annually (in addition to other types of exercise) overweight soldiers may be at increased risk for exercise-induced oxidative stress compared to normal weight soldiers.

It is not known how exercise-induced oxidative stress produced by military specific activities, such as the APFT, affects service members, especially overweight or obese soldiers. The effects of dietary antioxidant intake, fitness level and body composition on exercise-induced oxidative stress have not been investigated in this population. Findings from this study may be useful in future changes to the Military Dietary Reference Intakes (DRI's), supplementation to military rations, and health promotion guidelines to military service members.

The overall objective of this study was to examine the relationship between weight loss and exercise-induced oxidative stress following the APFT in overweight/obese soldiers. Our hypotheses were as follows:

Hypothesis 1) The APFT produces oxidative stress in overweight soldiers

Hypothesis 2) Soldiers who consume greater amounts of antioxidants will experience less oxidative stress following the APFT.

Hypothesis 3) Soldiers who are more fit will experience less oxidative stress following the APFT.

Hypothesis 4) Soldiers who have less body fat will experience less oxidative stress following the APFT.

Hypothesis 5) Soldiers who have changes in their weight will experience changes in biomarkers of oxidative stress following the APFT.

Chapter 2: Literature Review

Oxidative stress is a condition whereby free radicals or reactive oxygen species (ROS) form in cells and tissues beyond the ability of the body's antioxidative processes to neutralize them (1). The production rate of ROS depends primarily on an individual's metabolic rate (8). During exercise, total oxygen consumption can increase by as much as 10 to 20-fold compared to the resting state (9). The metabolic rate also increases, which leads to a significant increase in the production of ROS and free radicals, thereby damaging a variety of body tissues.

Oxidative stress can hinder normal biochemical pathways and is associated with decreased contractile function of muscles, muscle fatigue and heart arrhythmias. Oxidative stress also has been associated with heart disease, hypertension, obesity, diabetes, neoplasia, glomerular diseases, and age-related brain disorders (6, 10).

Exercise and oxidative stress

There has been a considerable amount of research regarding the effects of exercise on oxidative stress; however, the extent of oxidative stress and the degree of free radical production that occur during and after exercise remains controversial. Among the unresolved issues are the effects that exercise intensity, frequency, and duration have on exercise-induced oxidative stress, and whether differences in exercise-induced oxidative stress exist between trained and untrained individuals.

There are several proposed mechanisms for explaining why free radicals form as a result of exercise. The oxygen consumption and metabolic rate increase during exercise which may result in increased oxygen conversion to ROS (8, 11). A second possibility is that damage to muscles during exercise produces inflammation, which releases superoxide (12). A third possibility is that during intense aerobic or resistance exercise, an environment of hypoxia may exist as muscles remain active without obtaining adequate amounts of oxygen. As exercise intensity decreases and reoxygenation of muscles occurs, increases in ROS can be seen (12).

The APFT is a rigorous exercise test that is used to test the fitness status of soldiers. Most individuals perform each of the tests to exhaustion. Many soldiers perform the 2-mile run phase of the APFT at very high aerobic levels and are possibly in an anaerobic state during the event. An increase in exercise intensity would be expected to produce tissue damage or inflammation, resulting in elevated levels of biomarkers of oxidative stress (13). Also, soldiers attempt to perform as many pushups and sit-ups as they can in the allotted time, potentially leading to a hypoxic condition and exposure to damaging free radicals. Studies examining the extent of oxidative stress produced by the APFT have yet to be completed.

Duration of exercise

Wozniak et al. studied the effects of altitude on oxidative stress in trained sportsmen who participated in high altitude training for approximately 2 hours/day for 18 days. Blood samples taken at specified times during the study period were

used for assessing biomarkers of oxidative stress. Levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and lactic acid (LA) showed increases at every measurement compared to control subjects at both low altitude and at high altitude (14). Although the main goal of the study was to investigate the effect of altitude on oxidative stress, the authors showed that increases in oxidative stress were associated with exercising for longer durations and with repeated bouts of prolonged exercise performed over consecutive days.

Mastaloudis et al. observed an increase in vitamin E turnover after an ultra-endurance event (50km trail race). The investigators hypothesized that the increase in vitamin E turnover may be an indicator of lipid peroxidation. Compared to their sedentary counterparts, the runners in this study had higher levels of F2-isoprostanes at all time points. F2-isoprostanes are believed to be a sensitive indicator of lipid peroxidation (15). Soldiers in the Army perform many activities that would be similar to this ultra-endurance race, such as road marching and field training. Although the APFT typically takes a soldier less than 30 minutes to complete, the results of this study may provide the groundwork for studying military activities of longer duration.

Intensity of Exercise

Many researchers agree that oxidative stress may be related to exercise intensity. Exercising at a high intensity can produce muscle damage and lead to an increase in metabolic demand, both of which increase the likelihood of oxidative stress (13). Goto et al. compared forearm blood flow before and after a 12-week intervention in which subjects exercised at 25%, 50%, and 75%

VO2max. The indices of oxidative stress measured were malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG). They observed that the high intensity (75% VO2max) exercise produced the highest levels of MDA and 8-OHdG. Interestingly, the moderate exercise group (50% VO2max) experienced decreased levels of these markers after 12 weeks of exercise training (3). These results provide support for the suggestion that regular, moderate exercise may be ideal for combating exercise-induced oxidative stress.

Chung et al. also studied subjects exercising at 75-80% VO2max to evaluate the effect of menstrual phase on oxidative stress. Their results showed that exercise at 75-80% VO2max produced moderate amounts of oxidative stress and that menstrual phase had no effect on the levels of oxidative stress (2).

Significant amounts of ROS may be produced during short-term intense exercise. Using the Bruce treadmill test, Lawson et al. reported a significant increase in oxidative stress biomarkers in healthy subjects following short-term physical activity at a duration and moderately-high intensity similar to the running portion of the APFT. Levels of MDA and SOD were increased 10 minutes after initiating exercise and at 10 minutes post-exercise by 256% and 60%, respectively. It was not known if the subjects were trained or untrained in this study (4).

It has also been suggested that isometric exercises (e.g. push-ups and sit-ups) may generate ROS. The mechanism appears to be related to hypoxia and ischemia mediated through the xanthine oxidase pathway, or as a result of acidosis and metabolite accumulation. Metabolites include adenosine (which may

generate ROS) or aldehydes and ketones, which are products of lipid peroxidation (13, 16).

Rodriguez et al. evaluated the effect of a high-intensity isometric, ischemic forearm exercise on concentrations of lactate, aldehyde and ketones. The subjects performed a handgrip exercise after having the blood flow in the exercising arm occluded. Blood samples were taken before the test, immediately after and at 1, 3, and 10 minutes post-exercise. The investigators found significant increases in both MDA and lactate immediately post-exercise. The investigators suggested that lactic acid may also contribute to the increased oxidative stress. Lactic acid accumulation may contribute to acidosis which may foster lipid peroxidation and MDA formation (16). These findings provide support that additional oxidative stress resulting from push-ups and sit-ups may be produced during the APFT.

Training status

Many studies have shown an attenuation of oxidative stress as a result of training. Some researchers have shown a positive correlation between time spent doing endurance exercise and levels of antioxidant enzymes (13, 17). In cardiac rehabilitation patients, Leaf et al. observed that after a 12-week training program MDA decreased and elevated MDA was attenuated following exercise. The subjects were able to perform at greater intensities and for a longer duration with less oxidative stress after the training program. They also found that physical deconditioning was correlated with an increase in free radical production following exercise. These researchers further reported that trained subjects had a

higher lactic acid threshold after training. Lactic acid threshold is the level of intensity of exercise when lactate levels begin to build and affect performance. These researchers found that lactic acid levels were negatively correlated with resting MDA levels. (1).

Elousa et al. measured antioxidant enzyme activity response to physical activity and found varying results. After a 16-week training period, SOD, glutathione peroxidase (GPX) and glutathione reductase were increased both at rest and after exercise compared to pre-training levels. In contrast, a single bout of exercise in both trained and untrained subjects did not result in a significant increase in SOD. Although training allows the body to adapt to exercise by increasing levels of antioxidant enzymes, a single, acute bout of exercise did not result in elevated SOD levels. This study demonstrated that 16 weeks of training may provide beneficial upregulation of antioxidant enzymes. The researchers also found that LDL was more resistant to oxidation after training (5).

In an animal experiment, Ji et al. measured the acute effects of exercise on antioxidant enzymes in untrained rats. After an acute bout of exercise, all antioxidant enzymes (SOD, GPX, CAT, and glutathione reductase) were increased in skeletal muscle of untrained rats; however, after an 8-week training period, only resting GPX levels were significantly elevated compared to the sedentary controls. The researchers believe that the increase in GPX is the most important adaptive response in defending against oxidative stress (17).

In summary, these experiments demonstrate that exercise intensity and training can affect the amount of oxidative stress produced after a bout of

exercise. Subjects who were more trained generally had lower levels of several oxidative stress biomarkers following exercise. They also tended to have higher antioxidant enzyme levels at rest which would indicate an adaptation to regular exercise.

Obesity and oxidative stress

Epidemiological studies provide strong evidence that obesity predisposes individuals to many other chronic diseases, including heart disease, diabetes mellitus, hypertension, and certain types of cancer. An underlying mechanism may be that obesity exacerbates oxidative stress. Compared to normal weight individuals, obese individuals have decreased plasma levels of antioxidants such as vitamin E, vitamin C and β-carotene (6), suggesting that their antioxidant defenses may be impaired or they may be battling enormous oxidative stress levels. They also have greater pools of lipids that are susceptible to oxidation (6). Moreover, obese or overweight individuals typically have higher leptin levels than normal weight people, and research has suggested that leptin, IL-1, IL-6, TNF-α, and other cytokines may contribute to greater levels of oxidative stress by stimulating intracellular production of free radicals (11, 18).

Acute exercise in any individual can increase oxidative stress, but the oxidative stress resulting from exercise is likely to be greater in obese individuals, which may lead to harmful effects. For example, acute exercise may exacerbate complications of obesity such as diabetes mellitus and hypertension. The acute exercise may disrupt blood sugar control and increase blood pressure.

Vincent et al. investigated the potential for obese individuals to have greater

amounts of oxidative stress after acute exercise. Oxidative stress levels were measured after aerobic and resistance exercise in both obese and normal weight subjects. They found that the obese group had average increases in the lipid peroxidation marker thiobarbituric reactive acid substances (TBARS) of 42% and 41% after resistance and aerobic exercise, respectively compared to increases of 7.1% and 26.9% in the non-obese group. Similarly, lipid peroxidation (PEROX) increased more significantly in obese individuals than in non-obese subjects after both resistance exercise (100% vs. 85%) and aerobic exercise (70% vs. 62%). These researchers also found a significant negative correlation between dietary antioxidant intake and levels of lipid hydroperoxides (6).

Roberts et al. investigated the combined effects of diet and exercise in obese individuals to determine whether a low-fat, high-fiber diet plus daily exercise could improve oxidative stress and nitric oxide availability (7). They found that after only 21 days, subjects on this diet/exercise regimen experienced reduced oxidative stress markers (8-isoprostaglandin F2α), increased nitric oxide levels, decreased insulin levels, and decreased blood pressure. This study showed that a low-fat, high-fiber diet coupled with daily exercise can attenuate oxidative stress in obese individuals. Subjects lost an average of 3.7 kg during the study. An interesting aspect of this study was that caloric intake was not restricted -- subjects could eat as much as they wanted of the available foods, which were provided buffet style. Allowing subjects to choose foods within certain dietary guidelines can produce desired results. Although the separate effects of diet and exercise were not considered in this study, other researchers have found that

diet alone may decrease oxidative stress. Antioxidants derived from a dietary supplement or from fruits and vegetables resulted in improvements in biomarkers of oxidative stress (19-21).

Antioxidant role in oxidative stress

Cells have naturally occurring antioxidants that neutralize free radicals. Superoxide dismutase (SOD) and glutathione peroxidase (GPX) are important antioxidant enzymes produced by cells that fight against oxidative damage (22). SOD activity is dependent upon zinc and copper and in the absence of these minerals, the enzyme is completely inhibited (23). GPX requires selenium and is inhibited in the mineral's absence or deficiency (24).

Changes in SOD and GPX activities following exercise have been reported by several authors (4, 10, 14, 22). Training or regular exercise results in increased activity levels in SOD and GPX, which are associated with a reduction in free-radical induced injury. Conversely, low levels of both enzymes are associated with greater oxidative stress in a variety of diseases including cardiovascular disease and diabetes (10).

Certain antioxidant nutrients, such as vitamin E and vitamin C, have key metabolic roles in ameliorating oxidative stress. Vitamin E (α-tocopherol) acts as a chain-breaking antioxidant which intercepts lipid peroxide radicals, thereby halting lipid peroxidation (22, 25). Vitamin C neutralizes free radicals through its chemical reduction capabilities. It also spares vitamin E by donating an electron, allowing vitamin E to be regenerated and to continue neutralizing free radicals (26, 27).

An increase in the dietary intake of antioxidants results in decreased levels of oxidative stress. Thompson et al. hypothesized that increasing the consumption of fruits and vegetables would result in a decrease in urine and blood levels of biomarkers of oxidative stress. Over a 14-day intervention period, subjects (n=28) increased their consumption of fruits and vegetables from about 6 to 12 servings a day. The biomarkers assayed included 8-OHdG, MDA and 8-isoprostane F2α (8-EPG). The authors found that the increased fruit and vegetable consumption significantly lowered levels of 8-OHdG and 8-EPG but only had a minimal effect on lowering MDA levels (19).

Lopes et al. investigated the effect of the Dietary Approaches to Stop Hypertension (DASH) diet on lowering blood pressure and oxidative stress in obese and normal weight individuals. The subjects in this study followed either the DASH diet or a diet low in antioxidants to determine the separate effects of dietary antioxidants. Subjects who followed the DASH diet averaged about 6.1 servings of fruits and vegetables per day. The researchers found that the DASH diet raised the antioxidant capacity and reduced levels of oxidative stress biomarkers in the obese subjects. Obese individuals also experienced a reduction in blood pressure. The DASH diet had a similar effect on the lean subjects. The increase in oxidative stress was greater in the other two diet groups as compared to the DASH diet group. Lean subjects were normotensive prior to the study and did not experience any significant change as a result of the DASH diet (21).

To study the effect of dietary patterns versus dietary supplements on lipid

peroxidation, Miller et al. divided 123 subjects into 3 test diet groups: a control group, a fruit and vegetable diet group (9 servings of fruits and vegetables per day), and a combination diet group (approximately 10 servings of fruits and vegetables per day plus low-fat foods). Subjects followed the test diet for 8 weeks. The outcome variables measured were breath ethane, MDA and oxygen radical-absorbing capacity (ORAC). The authors found no significant differences in MDA among the different groups. Compared to the control group, breath ethane was lower in subjects consuming both the fruit and vegetable diet and the combination diet, with the combination diet group having the lowest ethane levels. The fruit and vegetable diet resulted in the highest ORAC levels, followed closely by the combination diet (20). The results suggest that a combination of increased antioxidant intake from fruits and vegetables and possibly a low-fat diet can improve certain indicators of oxidative stress.

Overweight and Obesity in the Military

The issue of overweight and obesity is a particularly sensitive subject to the United States military. Soldiers who are overweight by military standards are required to enroll in a weight management program to enable them to lose a sufficient amount of weight to meet weight and body fat standards. They are provided with nutrition education and dietary advice aimed at helping them choose foods that are appropriate for losing weight. They also are provided with education about exercise and are usually required to participate in exercise programs within their units that will facilitate weight loss.

In 2004, the United States Army Institute of Environmental Medicine released

evidence to support changes to the current Army Weight Control Program. The study included 1521 active duty males and 1257 active duty females stationed in North Carolina, South Carolina and Missouri. They found that 11% of male Soldiers and 23% of female Soldiers were overweight and exceeded Army standards for body fat (28). The study had several key findings. First, the authors showed that if only body mass index (BMI) was used as an indicator of overweight and obesity, the prevalence of overweight in soldiers closely matched the rates of the civilian population. By BMI alone, 38% of male Soldiers and 35% of female Soldiers were overweight.

Second, the study demonstrated how using BMI does not accurately reflect overweight and obesity rates in the military population. Using BMI may lead to overestimating body fat in active and muscular individuals, such as soldiers and athletes. BMI is more suited for assessing one's likelihood of developing diseases related to weight, not necessarily related to percent body fat.

Following Army standards used for calculating percent body fat, this study measured waist circumference at the smallest circumference midway between the xiphoid process of the sternum and the umbilicus anteriorly, and between the lowest lateral portion of the rib cage and the iliac crest laterally. This site is different than the waist circumference site used for assessing the risk of chronic diseases related to weight.

Body fat standards based on age for soldiers range from 20-26% and 30-36% for males and females, respectively. After determining body fat percentage using the proposed Army standard, the overweight rates listed above (11% for male

and 23% for female soldiers) accurately represent the rate of overweight in the military population (28).

The costs associated with overweight and obesity in the military can be measured in several ways. Direct expenses may result from increased medical care while on active duty or during retirement. There are also indirect expenses arising from lost productivity or time away from work. In a study evaluating the costs of excess body weight among Air Force personnel, Robbins et al. found that excess body weight resulted in medical expenditures of approximately $19.3 million dollars, or 5.6% of all medical care costs in 1997. They also reported 11,234 days of lost productivity amounting to a monetary cost of $3.5 million. Thus, total costs related to excess body fat were approximately $22.8 million (29).

It has been established that obesity may predispose an individual to a variety of comorbidities such as heart disease, diabetes, and arthritis. Furthermore, as an individual ages, the costs associated with treating these conditions may increase. Individuals who retire from the military may receive medical care until death. In a recent study to examine the burden of obesity among a sample of retired military members and their family members, Kress et al. found that 80% of the male and 60% of the female retirees were overweight or obese. There was a positive relationship between body weight and the number of comorbidities (30). The investigators did not report calculated dollar cost of this burden, but the study illustrated the magnitude of the problem and the need to identify strategies to alleviate it.

Preventing or managing obesity as early as possible in the military, as well as in the general population, may help to alleviate the risk of chronic diseases related to obesity. The Army Physical Fitness Test (APFT) is typically taken twice a year. Over a twenty year career, a soldier could accumulate an immeasurable amount of damage as a result of exercise-induced oxidative stress. Unfortunately, it is not possible to measure the adverse effects that accumulate as a result of oxidative stress. However, it seems reasonable to assume that certain individuals, because of their diet, fitness or body composition, may be exposed to greater amounts of oxidative stress which may increase their risk of developing chronic diseases.

Summary

A number of factors, including exercise, obesity and diet contribute to oxidative stress. An individual who is sedentary, obese, and who has a low dietary intake of antioxidants would presumably be exposed to greater than normal amounts of ROS after engaging in an acute bout of vigorous exercise. By making positive changes such as increasing fitness, losing weight, and increasing dietary antioxidants, oxidative stress may be reduced, thereby reducing the likelihood of developing diseases. The results of previously reported studies suggest that positive changes in modifiable risk factors for disease are plausible. These changes could potentially have a great impact on preventing chronic diseases among soldiers in the military as well as in the general population.

Chapter 3: The Army Physical Fitness Test Causes Oxidative Stress in Overweight Soldiers; Relationship of Diet, Fitness Status, and Body Composition to Exercise-Induced Oxidative Stress

Abstract

Exercise is known to increase reactive oxygen species, a condition recognized as oxidative stress. It's been observed that obese subject may experience even greater amounts of oxidative stress after exercise. The objectives of this study were to determine whether the Army Physical Fitness Test (APFT) produces changes in biomarkers of oxidative stress and to evaluate the impact of dietary antioxidant intake, fitness level and body composition on changes in APFT-induced biomarkers of oxidative stress. Forty-seven subjects were asked to perform an APFT. Outcome parameters, including biomarkers of oxidative stress and inflammation, were measured before and after the APFT. Outcome parameters included creatine kinase (CK), c-reactive protein (CRP), glutathione peroxidase (GPX), and superoxide dismutase (SOD). Dietary antioxidant intake, fitness level and body composition were also measured. Results showed a significant increase in CK levels immediately post-exercise and at 24hrs post-exercise. There was also a significant increase in CRP immediately post-exercise and at 24hrs post-exercise. There was a significant decrease in GPX immediately post-exercise. There was no significant change in SOD following exercise. CK levels immediately after exercise were found to be influenced by vitamin A, C, and E, and the interactions between fitness level and vitamins A and E. CRP levels immediately post-exercise were found to be influenced by vitamins A, C and E, fitness level, total fat mass and total fat

percentage. There were also significant interactions between fitness level and vitamin E, total fat mass and total fat percentage. GPX levels immediately post-exercise were found to be influenced by vitamins A and C, fitness level, total fat mass and the interactions between fitness level and vitamin A and fitness level and total fat mass. SOD levels immediately post-exercise were found to be influenced by fitness level, body fat percent and the interaction between them. In conclusion, the APFT causes oxidative stress in overweight subjects. The associations between dietary antioxidants, fitness level and body composition seen with each of the biomarkers provide support for future research in this area and possibly in changes to military standards.

Introduction

Oxidative stress is a condition whereby the formation of free radicals or reactive oxygen species (ROS) in cells exceeds the capacity of tissue oxidative processes to neutralize them (1). The production rate of ROS depends primarily on the metabolic rate of the cell (8). During exercise, total oxygen consumption can increase by as much as 10 to 20-fold (9). The metabolic rate also increases, leading to a significant increase in the production of ROS and free radicals, which can damage a variety of body tissues.

There are several mechanisms to explain how free radicals form during exercise. One possibility is that exercise-induced damage to muscles results in inflammation and the release of the free radical, superoxide (12). Another possible mechanism is that intense aerobic or resistance exercise produces an hypoxic environment leading to ischemia, as muscles are deprived of adequate

oxygen. As exercise intensity decreases, reperfusion and reoxygenation of muscles occurs resulting in an increase in ROS production (12).

Fitness level is a determinant of the level of oxidative stress arising from exercise. Studies comparing trained and untrained subjects indicated that trained individuals had higher blood levels of antioxidant enzymes both at rest and immediately post-exercise, suggesting that training results in an adaptation to exercise-induced oxidative stress (10).After undergoing training, levels of free radicals and other oxidative stress biomarkers were lower than before training. Similarly, it has been shown that untrained individuals experience a greater rise in oxidative stress biomarkers after exercising than trained subjects.

Body composition also may be a determining factor in the response to exercise-induced oxidative stress. Vincent et. al. (6) reported that obese individuals experienced more lipid peroxidation after resistance and aerobic exercise sessions compared to normal weight subjects.

Several theories have been suggested to explain why obese individuals may be more susceptible to exercise-induced oxidative stress than normal weight individuals. Oxidative stress may be associated with increased respiration, chronic inflammation, hyperglycemia, decreased antioxidant defenses, increased lipid levels susceptible to oxidation, increased endothelial sources of ROS and hyperleptinemia, conditions more likely to be seen with obesity (11).

It is not known is how the Army Physical Fitness Test (APFT) affects oxidative stress in overweight soldiers. It is also not known how dietary antioxidant intake, fitness level or body composition affects the APFT-induced oxidative stress

experienced in overweight/obese soldiers. Therefore, the objectives of this study were to: 1) Determine whether the APFT produces oxidative stress in overweight/obese soldiers 2) Evaluate the impact of dietary antioxidant intake on biomarkers of oxidative stress following the APFT in overweight/obese soldiers 3) Evaluate the impact of fitness level on biomarkers of oxidative stress following the APFT in overweight/obese 4) Evaluate the impact of body composition on biomarkers of oxidative stress following the APFT in overweight/obese soldiers.

Methodology

Subjects

A total of 60 subjects (35 male, 25 female) were recruited from among military personnel who were beneficiaries of the Walter Reed Army Healthcare System. Subjects who volunteered to participate underwent an initial screening evaluation to determine if they met the inclusion criteria: 1) $\geq$18 years old 2) overweight by Army standards (BMI $\geq$ 28) 3) Not actively losing weight 4) Eligible to participate in the Army Weight Control Program and 5) Able to perform the APFT. Anyone with a history of heart disease, uncontrolled diabetes, or medical conditions that may impact oxidative stress markers were not eligible to participate and were excluded from the study.

Study Design

Informed consent was obtained after subjects were given a brief description of the study. Once subjects provided written and verbal consent, baseline anthropometry, body composition, fitness assessment and dietary data were collected. Subjects were scheduled to take the APFT as soon as possible. A

total of 47 subjects (27 male, 20 female) completed this baseline APFT (22% dropout), and this was the sample for the remainder of the study.

The protocol for this study was approved by the Human Use Committee at Walter Reed Army Medical Center and by the Institutional Review Board at the University of Maryland College Park.

Biochemical assays

Blood samples were drawn from each individual to measure oxidative stress biomarkers and antioxidant enzyme activity before and after each APFT. Blood samples were taken immediately before (but no more than 1 hour prior to) the APFT, immediately following the APFT, and at 24 hours after the APFT. There was an average lag time of approximately 5-7 minutes between the completion of the APFT and the blood collection immediately after exercise.

The biomarkers for oxidative stress that were assayed included creatine kinase (CK), a marker of muscle cell damage; C-reactive protein (CRP), a marker for inflammation; and lipid peroxides (LPO).

The biomarkers of antioxidant enzymes that were measured were superoxide dismutase (SOD) and glutathione peroxidase (GPX).

Blood (1 10-ml and 4 7-ml tubes) was collected from each subject in the Pathology Laboratory at Walter Reed Army Medical Center, Washington, DC. The blood samples for the CK and CRP assays were collected in a serum separator tube with no anticoagulant or preservative and were processed immediately. Serum was separated immediately from the clot. CK was analyzed using Vitros CK clinical chemistry slides. CK catalyzes the conversion of creatine

phosphate and ADP to creatine and ATP. Glycerol kinase then catalyzes the conversion of ATP and glycerol to L-a-glycerophosphate and ADP. L-a-glycerophosphate and oxygen are then converted to dihydroxyacetone phosphate and hydrogen peroxide by L-a-glycerophosphate oxidase. Finally, a leuco dye precursor is oxidized by hydrogen peroxide in the presence of peroxidase to form a dye. The slide was read using a Vitros 950 Chemistry System (Johnson and Johnson Clinical Diagnostics, Rochester, NY).

CRP was analyzed using a CRP latex assay which uses an immunoturbidimetric reaction. Human CRP from the sample combines with latex particles coated with monoclonal anti-CRP antibodies. The precipitate was determined turbidimetrically at 552 nm on a Roche Cobas Integra system (Roche Diagnostic Systems, Somerville, NJ).

Blood samples for analyzing GPX and SOD were drawn into heparinized tubes, and samples for the LPO assay were collected in sodium citrate tubes. Samples were transported on ice to the Department of Clinical Investigation research laboratory at Walter Reed. Samples were centrifuged at 4°C and 2,000 rpm for 10 minutes to separate the plasma from the whole blood. Plasma samples were aliquoted, frozen and stored at -70°C until analysis.

SOD was analyzed with a colorimetric ELISA assay kit (Oncogene, San Diego, CA, kit no. QIA97). In brief, samples were incubated on a 96-well plate coated with monoclonal antibody (mouse) to human Cu/Zn SOD and HRP-conjugated anti-Cu/Zn SOD monoclonal antibody. Tetramethyl-benzidine was used to produce a fluorescent signal. The assay was read on a Molecular

Devices spectrophotometer at 455nm. Samples were expressed in ng/ml. All samples were performed in duplicate. The CV% for this assay was 16%.

GPX was analyzed using a colorimetric ELISA assay kit (Calbiochem, Darmstadt, Germany, kit no. 353918). Samples were incubated in wells of a divided 96-well microplate that were coated with a polyclonal antibody specific for human plasma GPX. GPX was detected using a biotinylated polyclonal antibody to plasma GPX. GPX was measured after adding p-nitrophenylphosphate to produce a fluorescent signal. The assay was read on a Molecular Devices spectrophotometer at 405 nm. Samples were expressed in ng/ml. All samples were performed in duplicate. The CV% for this assay was 7%.

LPO was analyzed with a colorimetric assay kit (Calbiochem, Darmstadt, Germany, kit no. 437634). The agent, 1,1,3,3-tetraethoxypropane was used as a standard for determining MDA. Samples were incubated with N-methyl-2-phenylindole to produce a chromophore. The assay was read on a Molecular Devices spectrophotometer at 500nm. The assay failed to produce readable results.

Anthropometry and body composition

Height was measured to the nearest 0.01 centimeter using a digital stadiometer. Weight was measured to the nearest 0.1 kg using a digital scale. Body mass index (BMI) values were calculated as body mass (kg)/height $(m)^2$. Body composition (lean body mass and body fat) of the whole body was measured using a fan beam dual energy X-ray absorptiometry (DXA) (Hologic Discovery Wi, Bedford, MA, USA). Subjects were asked to remove all metal

objects and wear light clothing with no metal. They were positioned in a supine position for the scan. The DXA machine was calibrated daily according to manufacturer recommendations. Results were analyzed using QDR software for Windows XP.

Training status analysis

Training status was assessed by using a submaximal cycle ergometer exercise test. Testing was completed at baseline data collection and at least 48 hours before the APFT in order to avoid carryover effects for the biomarker measurements. The submaximal cycle ergometer test provided an estimate of the volume of oxygen or oxygen consumption (VO2max) of each subject. The YMCA Submaximal Cycle Ergometer Test protocol is routinely used to test fitness and has been validated as a predictor of VO2max and ultimately cardiorespiratory fitness level. The YMCA cycle ergometer test has a significant correlation with a Bruce treadmill VO2max test. For all subjects the correlation was r=0.77 (p<0.05) which was r=0.63 (p<0.05) for males and r=0.90 (p<0.05) for females. (31). The cycle ergometer used in the test was the Monark 818 E cycle ergometer. This protocol uses two to four 3-minute stages of continuous exercise that are designed to raise the steady heart rate between 110 beats per minute and 85% of age-predicted maximal heart rate for at least two consecutive stages. Each stage is performed for 3 minutes and the subject's heart rate is measured during the last 15-30 seconds of the 2nd and 3rd minutes. If the two heart rates weren't between 5 beats per minute (BPM) of each other, subjects continued that stage for 1 additional minute. Blood pressure and rate of

perceived exertion also were measured at the end of each stage. The resistance setting on the cycle ergometer is increased from 0.5 kg during the first stage to 3.5 kg in the final stage, depending on the heart rate of the subject. Participants were instructed to refrain from ingesting food, alcohol, caffeine, and using tobacco products within 3 hours of testing. Heart rate was assessed with a Polar heart rate T31 monitor (Polar Electro Inc. Lake Success, NY). A fan was used to minimize effect of heat on thermoregulation during the test. Work rate produced during each stage was used to estimate VO2max. Predicted VO2max was calculated using the TriFit software (Polar/Healthfirst, Albuquerque, NM).

Dietary analysis

Dietary antioxidant intake was determined by using a 3-day food diary and by using the National Cancer Institute Diet History Questionnaire-web version (DHQ). Subjects were instructed to log into their diaries every food, beverage and dietary supplement that they consumed. Food diaries were reviewed for accuracy by registered dietitians. The same dietitian entered the food diaries into the nutrient analysis program, Nutribase v6.08 (Cybersoft, 2005). In regards to the DHQ, subjects were provided secure log-in ID's and passwords. The DHQ is validated for antioxidant intake (32). Due to poor completion rate, these data were not used for analysis and are not reported here.

Army Physical Fitness Test

The Army Physical Fitness Test (APFT) is a standardized fitness test that is given to all soldiers at least twice per year. It consists of performing as many pushups and sit-ups during two-minute periods and then running two miles as

quickly as possible. Subjects were instructed to perform this APFT at the same effort level as they would an official APFT. Also, subjects were instructed to avoid strenuous exercise for 24 hours prior to the test, to include the cycle ergometer fitness test. Push-ups and sit-ups were performed in a gymnasium using exercise mats. The 2-mile run was performed on a protected, outdoor track. Subjects were given a 5-minute rest period between the sit-ups, push-ups and 2-mile run events.

Power Analysis

Power was based on expected increases in MDA (n=12) and SOD (n=13), and on expected weight loss (n=16). We used the greatest number of subjects and added 5 subjects for each of the covariates we investigated (fitness level, vitamins A, C, E, body fat). We calculated that 35 subjects would be needed to reach the desired significance level of 0.05 and 80% power. Sixty subjects were recruited to account for an estimated 25-50% drop-out rate based on clinic statistics and experience with a similar research study. Forty-seven subjects completed the baseline APFT (22% dropout).

Statistical Analysis

Paired t-tests were used to determine the significance of any observed difference in descriptive statistics and in oxidative stress before and after the APFT. Partial Pearson's correlations and Analysis of Covariance (ANCOVA) were performed to determine the extent to which a relationship exists between fitness level, dietary antioxidant intake, body composition and exercise-related changes in CK, CRP, GPX and SOD. Analyses were controlled for gender, age,

BMI and baseline values of dependent variables. Mean values were used for continuous variables. Class variable (gender) results were reported for an idealized experiment with 50% males and 50% females. In the event there were extreme outliers, the criterion of 2 standard deviations (SD) was used to eliminate a subject from an analysis. For CK analysis, 2 outliers were eliminated (5 and 7 SD's). For CRP analysis, 5 outliers were eliminated (2, 3, 3, 3, 4 SD's). For GPX analysis, 2 outliers were eliminated (5 and 5 SD's). For SOD analysis, 1 outlier was eliminated (5 and 7 SD's). The statistical analyses were performed using the SAS System for Windows V9.1 (SAS Institute, Cary, NC, 2002). All values were considered significant at $P < 0.05$.

Results

Table 1 reports baseline subject characteristics for males, females, and all subjects. Average age for all subjects was 33.7 (7.9) years. Males were taller, weighed more, and had more lean mass than females. Females had greater trunk fat percentage and total fat percentage than males. Males had higher intakes of calories, protein, fat and selenium. Males had slightly higher, although not significant, predicted maximal aerobic capacity (VO2max) measurements than females.

Oxidative stress biomarkers

Changes in oxidative stress biomarkers from baseline to each of the 2 time points after exercise are reported in Table 2. Changes in oxidative stress biomarkers are reported only for CK, CRP, GPX and SOD. The LPO assay failed to produce readable data; therefore those data are not reported.

Compared to the pre-exercise values, there was a significant increase in CK immediately post-exercise and at 24hrs post-exercise for both males and females. For all subjects, CK increased by 30% at post-exercise compared to pre-exercise levels (223.1 vs. 171.5 U/L), and by 80% at the 24hr post-exercise time point compared to pre-exercise (308.1 vs. 171.5 U/L). Relative to baseline, CK values in male subjects increased by 31% immediately post-exercise (270.1 vs. 206.2 U/L), and by 68% at 24hrs post-exercise (346.7 vs. 206.2 U/L). Similar changes were observed in female subjects. Compared to pre-exercise values there was a 28% increase immediately post-exercise (153.9 vs. 120.4 U/L), and a 55% increase at the 24hr post-exercise time point (186.0 vs. 120.4 U/L) in females.

Compared to the pre-exercise values, there was a significant increase in CRP immediately post-exercise for both males and females. There was a slight, but not significant increase for males and a slight decrease for females at 24hrs post-exercise. For all subjects, CRP increased by 6% at post-exercise compared to pre-exercise levels (0.221 vs. 0.209 mg/dL), and decreased by 6% at the 24hr post-exercise time point compared to pre-exercise (0.196 vs. 0.209 mg/dL). Relative to baseline, CRP values in males increased by 6% immediately post-exercise (0.179 vs. 0.169 mg/dL), and by 11% at 24hr post-exercise compared to pre-exercise (0.187 vs. 0.169 mg/dL). A different trend was observed in female subjects. Compared to pre-exercise values, there was a 5% increase in CRP immediately post-exercise (0.289 vs. 0.276 mg/dL), but a 22% decrease at 24hrs post-exercise compared to pre-exercise (0.215 vs. 0.276 mg/dL) in females.

GPX and SOD values were measured at only 2 time points: pre-exercise and immediately post-exercise. GPX tended to decrease after exercise but was not significant compared to the pre-exercise time point (79.0 ± 5.6 vs. 85.5 ± 6.7 ng/ml, p=0.06).

SOD tended to increase after exercise, but there was no significant difference in mean SOD values post-exercise compared to the pre-exercise values (0.95 ± 0.12 vs. 0.91 ± 0.10 ng/ml, p= 0.69).

Pearson's Correlations

Partial Pearson's correlations were used to determine the strength of the relationship between dietary antioxidant intake, fitness level (predicted VO2max) and body composition and the observed changes in biomarkers of oxidative stress after removing the effects of age, gender and BMI (Table 3). Several noteworthy correlations between dietary antioxidant intake and markers of oxidative stress were observed. Dietary intake of vitamin A was negatively correlated with CK values at 24hrs post-exercise (r= -0.68, p=0.03) and positively correlated with baseline CRP measurements (r=0.68, p=0.03). Vitamin A was positively correlated with SOD levels immediately post-exercise (r=0.64, p=0.04). Dietary intake of vitamin E was correlated with CRP immediately post-exercise (r=0.84, p=0.003). Predicted VO2max was positively correlated with baseline GPX levels (r=0.59, p=0.01), but negatively correlated with GPX immediately post-exercise(r=-0.60, p=0.01). Body composition was found to be related to CRP levels. Total fat percentage (r=0.51, p=0.03), trunk fat mass (r=0.53, p=0.02) and trunk fat percentage (r=0.56, p=0.01) were positively correlated with

baseline CRP levels. Trunk fat percentage was found to be positively correlated with baseline SOD levels (r=0.46, p=0.05). Significant relationships were found between total lean mass and CK values immediately (r=-0.49, p=0.04) and at 24hr post-exercise (r=0.54, p=0.02). Total lean mass was negatively correlated with baseline CRP levels (r=-0.47, p=0.05).

ANCOVA

ANCOVA was used to determine the effect that fitness level (Predicted VO2max), dietary antioxidant intake and body composition had on post-exercise and 24hr post-exercise values for CK, CRP, GPX and SOD. Interactions between fitness level and dietary antioxidant intake and between fitness level and body composition were also investigated for each of the biomarkers. Analyses were controlled for gender, pre-exercise values of CK, CRP, GPX, and SOD, age, and BMI. Appendix A contains the regression equations that were generated during the ANCOVA process. These include variables that were included in the final models.

The increase in CK immediately post-exercise was associated with low dietary intake of vitamin A (p=0.04), and higher intakes of vitamin C (p=0.01) and vitamin E (p=0.004). We also found that the interaction between fitness level and intake of vitamin A (p=0.04) was positively associated with CK levels immediately post-exercise. The interaction between fitness level and intake of vitamin E (p=0.003) was negatively associated with CK levels immediately post-exercise. However, at the 24hr time point, there were no significant associations of any

diet, fitness or body composition factors, or of any of their interactions, on the increase in CK levels at 24hr post-exercise.

Any individual associations between fitness level (Predicted VO2max), dietary antioxidant intake, body composition and between the difference in the increase in CRP levels immediately post-exercise or between the difference in the increase in CRP levels at the 24hr post-exercise time point could not be determined.

Changes in GPX were associated with higher fitness levels (p=0.05), higher intakes of vitamin C (p=0.02), lower intakes of vitamin A (p=0.05) and also with greater total fat mass (p=0.03). Changes were also positively associated with the interaction between fitness level and vitamin A (p=0.05) and negatively associated with the interaction between fitness level and total fat mass (p=0.03).

Changes in SOD were found to be associated with higher fitness levels (p=0.04) and higher total fat percentage (p=0.006); however, the interaction between these two variables (p=0.03) was negatively associated with SOD levels immediately post-exercise.

Discussion

The APFT resulted in increases in CK and CRP and resulted in slight decreases in GPX. There was no change in SOD as a result of the APFT.

We found an increase in CK immediately following exercise and a further increase at 24 hrs after exercise. These findings are consistent with the results of other studies (33-39), which showed that peak CK values occurred 24hrs or more after exercise. Several of these studies found peak CK values at 6 hrs (40), 24

hrs (38, 39), 72 hrs (33, 34, 37), and 96 hrs (35). Since we only collected post-exercise samples immediately after exercise and at 24 hours after exercise, it is not clear whether our subjects would have experienced greater CK levels if samples had been taken at additional time points beyond 24hrs after exercise. The increase in CK seen immediately after the APFT and after 24hrs may reflect the intensity of exercise that's necessary to generate exercise-induced oxidative stress.

With respect to fitness and antioxidant intakes, we found that dietary intakes of vitamins C, E and A were associated with the increase in CK immediately after exercise. Interestingly, it was noted that while fitness level, as measured by predicted VO2max, did not affect CK values, there was an association between fitness level and vitamins A and E and the CK values immediately after exercise. At 24hrs after exercise, vitamin A was negatively correlated with the increase in CK levels at 24hrs post-exercise.

Bloomer and colleagues examined whether an antioxidant supplement of 400 IU vitamin E, 1 gm vitamin C and 90 µg selenium would attenuate an increase in CK following eccentric exercise (35). Eccentric exercise involves developing tension on a muscle as it lengthens, for example lowering a dumbbell using the biceps muscle. They found that the subjects taking the antioxidant supplement did not experience an increase in CK until 72 hours post-exercise. After 72 hours post-exercise, CK values in subjects taking the antioxidant supplement were significantly greater than the values seen before exercise, but still significantly lower than a placebo group (35). This suggests that the suppression of CK by

antioxidant supplementation is greatest immediately after exercise, but this attenuation may persist for up to 3 days after exercise. The subjects in our study had much lower intakes of dietary antioxidants, but it's possible that the subjects with the highest intakes may have experienced a similar CK response to exercise. Our correlation analysis suggests that higher intakes of vitamin A were associated with a small increase in CK 24hrs after exercise.

Sacheck et al. tested vitamin E supplementation on the exercise-induced effects on CK levels in young (mean age = 26.4 years) and old men (mean age = 71.1 years). Following exercise, both young and older males experienced increases in CK levels, but the increase after exercise appeared to be blunted in the group of young males taking the vitamin E supplement (38).

In a study by Mastaloudis, plasma antioxidants (vitamin C and vitamin E) did not attenuate the increase in muscle damage as measured by CK following a 50-km ultramarathon (39). This exercise event was substantially longer than the APFT in our study and makes it difficult to directly compare the two studies. Creatine kinase peaked at 24hr post exercise, which is similar to the pattern of increase we observed in this study. However, subjects in their study were trained runners (average VO2max = 58 ml/kg/min) in contrast to the average for subjects in our study who were not trained (predicted VO2max = 34.5 ml/kg/min). It's possible that trained subjects do not have as much muscle damage as untrained subjects when performing similar exercise. We didn't observe an association between fitness level and CK increases immediately post-exercise or at 24hrs post-exercise. It remains unclear how antioxidant intake may affect the increase

in CK values after exercise. Further investigation may elucidate which individuals may benefit from proper doses of supplementation. It may be that trained individuals do not benefit as much as untrained individuals from antioxidant supplementation since trained individuals may have less muscle damage following exercise.

We found a significant increase in CRP immediately following exercise, but not at 24 hrs. The average baseline value of CRP before exercise was 0.209 mg/dL, which is above desired levels. CRP levels are commonly used as a measure of inflammation. It is believed that obesity may be characterized by a state of chronic inflammation. Our subjects were obese on average (mean BMI 31.1 kg/m^2) and may have been in a state of chronic inflammation prior to the exercise test. According to the American Heart Association, levels below 1 mg/L (or 0.1 mg/dL) are considered desirable and indicate a low risk for cardiovascular disease. Levels between 1 and 3 mg/L put one at moderate risk for cardiovascular disease, and above 3 mg/L is considered high risk (41). Most (68%) of our subjects were in the moderate and high risk categories (mean CRP = 0.209 ± 0.033 mg/dL). As part of the protocol, we requested individuals to not participate in the APFT on a day when they were ill or had an acute injury to avoid the possibility that CRP may be elevated prior to the APFT. Nevertheless, it is possible that subjects may have participated in the APFT despite being in a state of acute inflammation resulting from a fever or an injury and failed to notify the research team. However, our results are similar to those of other studies that evaluated the levels of CRP in overweight and obese individuals. Mora et al.

observed a slight correlation between BMI and CRP in females (r=0.47, p=0.15). They also observed median CRP levels greater than 0.26 mg/dL for females with BMI > 25.8 kg/m^2 and CRP levels greater than 0.45 mg/dL for females with BMI > 29.3 kg/m^2 (42). In a study with overweight/obese subjects, Roberts et al. found mean CRP levels to be 0.24 mg/dL (43).

We found significant correlations between body composition and baseline CRP levels. Total body fat percentage was positively correlated with CRP levels (r=0.51, p=0.03). In addition, trunk fat mass and trunk fat percentage were positively correlated with CRP levels at baseline (r=0.53, p=0.02 and r=0.56, p=0.01, respectively). Taken together, these results are similar to the results previously discussed (7, 42) and support the theory that greater amounts of body fat are associated with higher CRP levels.

Additionally, we observed a positive correlation between vitamin A and baseline CRP levels (r=0.68, p=0.03) and a positive correlation between vitamin E and the increase in CRP levels immediately post-exercise (r=0.84, p=0.003). This is contrary to the findings of Phillips et al. who showed that an antioxidant supplement containing 300 mg mixed tocopherols, 800 mg docosahexaenoate, and 300 mg flavonoids reduced the elevation in CRP after exercise (33). The difference in the results between Philips' study and ours may be that their combination of antioxidants was sufficiently great enough to produce a significantly different response when compared to the low antioxidant intake seen in our subjects. It is also possible that any effect of dietary antioxidants on CRP

levels was negated by the high caloric intake and obese status of our subjects, since obesity is associated with elevated CRP levels.

Mora et al. noted an inverse relationship between CRP levels and fitness level (42) whereas we did not find a significant relationship between baseline CRP and fitness level (r=-0.09, p=0.74). Their results suggest that individuals who are trained have less inflammation and the potential for greater levels of oxidative stress than individuals who are untrained. One reason our results did not show a significant relationship may be that our subjects were clustered together around a lower predicted VO2max which did not allow an adequate comparison. Subjects in our study had low fitness levels and had greater amounts of body fat. It's possible that they would experience greater levels of oxidative stress than subjects better trained and having less body fat.

GPX was only measured immediately after exercise and not at 24hrs post-exercise because previous studies have indicated that GPX levels peak immediately after exercise (5). Increased levels are often associated with decreased oxidative stress. This study found a decrease in GPX (p=0.06) following exercise. It's been suggested that a decrease in antioxidant enzymes after exercise indicates that the antioxidant defenses were overcome and not able to combat the exercise-induced oxidative stress (11). We found that there were significant associations between fitness level, vitamins A and C, total fat mass and changes in GPX levels. Higher fitness level and higher antioxidant intake were associated with greater GPX levels. In contrast, the interaction between fitness level and body fat mass was associated with decreased GPX

levels. The subjects in this study generally had lower fitness levels and higher body fat mass, which would correspond with the observed decrease in GPX levels after exercise.

Aguilo found similar results in a study with trained cyclists (mean VO2max = 80.2 ml/kg/min) participating in an exhaustive mountain stage climb (171 km) that took an average of 270 minutes to complete. These researchers found a decrease in GPX immediately following the bicycle race, which returned to baseline after 3 hours of recovery (44). The decrease seen after exercise in their study may indicate that the antioxidant defenses were overcome and not able to scavenge free radicals generated during exercise. The investigators speculated that once exercise and the oxidant stress were stopped, the antioxidant defenses were restored. The subjects in our study were not nearly as trained (mean predicted VO2max = 34.5 ml/kg/min), however it may be suggested that exercise was sufficient to overcome the antioxidant defenses of the subjects in each study.

Other authors found smaller decreases in GPX after exercise compared to pre-exercise levels. Groussard et al. reported a slight nonsignificant decrease in GPX following a Wingate cycle ergometer exercise test (45). These subjects were young men (mean age = 22.2 years) who were lean (14% body fat) and physically active but not undergoing a training regimen. It is possible that these subjects would have had greater decreases in GPX levels after exercise if they had higher body fat percentages.

The subjects in our study were obese (mean body fat % = 31%). We observed a decrease in GPX levels after exercise. We also found a slight negative correlation between body fat mass and changes in GPX levels after exercise ($r=0.42$, $p=0.08$). This suggests that body fat may inhibit GPX levels. When we used ANCOVA to take into account the effects of dietary antioxidant intake, fitness level and body composition, we found that the interaction between fitness level and body fat mass was associated with lower levels of GPX immediately after exercise.

In contrast, Elousa et al. found that immediately after exercise, GPX increased significantly; however by 30 minutes GPX returned to baseline levels (5). Subjects in this study were younger (mean age = 20 yrs) than our subjects (mean age = 34 yrs), but they had similar fitness levels to our subjects (mean Predicted VO2max = 37.43 ml/kg/min). It is possible that the exercise intensity in the Elousa study was not sufficient to overwhelm the antioxidant enzyme defenses, which may have resulted in a decrease in GPX levels as we observed in our study. Elousa et al. did not evaluate dietary antioxidant intakes, so it is not possible to speculate on the role of diet on GPX.

We found that vitamin A intake was associated with lower levels of GPX after exercise, whereas the interaction between fitness level and vitamin A intake was associated with increased levels. Vitamin C intake was associated with higher levels of GPX immediately post-exercise. In summary, our results and data from other studies suggest that given sufficient exercise intensity or duration, antioxidant defenses could be overwhelmed, resulting in decreased GPX levels

following exercise. Furthermore, body fat may be negatively correlated with GPX levels and may inhibit the ability of GPX to combat oxidative stress. It's possible that dietary antioxidant intake and fitness level together may enhance GPX levels during exercise.

Similar to our findings, Elousa et al. reported that there was no significant difference in SOD values before and after exercise in untrained subjects. Nevertheless, they did observe a positive relationship between fitness level and SOD levels (p=0.002) (5). We found that predicted VO2max was associated with higher levels of SOD after exercise and also that body fat percentage was associated with SOD levels after exercise. In addition, we found that an interaction effect between fitness and with total fat percentage resulted in lower levels of SOD after exercise. These results suggest that, similar to GPX, lower fitness levels and a greater percentage of body fat may cause a decrease in SOD, potentially limiting its ability to scavenge free radicals produced during exercise.

Limitations

This study had several limitations. The subjects were free-living soldiers stationed in the Washington, DC metropolitan area. Diets and physical activity were not controlled before the baseline measurements or before the APFT. The accuracy of food records is always a potential limitation. Subjects may not document everything that they eat or drink and often underestimate portion sizes. (To minimize inaccurate reporting, food diaries were thoroughly reviewed by registered dietitians). We also did not look at other dietary antioxidants as part of

this study since adding more covariates to the ANCOVA would have increased the sample size required. It's possible that other nutrients such as selenium or specific phytochemicals may have affected the biomarkers evaluated.

Subjects were instructed to treat the test APFT as they would a regular APFT: avoiding exercise the day before and putting at least as much effort into the test as they normally would. Since this exercise test did not count toward their military record, it is possible that some subjects did not exert as much effort as they otherwise would. The goal of the APFT is to perform as many pushups and sit-ups and to run as fast as possible in the time allowed, which leads to exhaustion for most individuals. However, intensity of exercise and workload were not directly measured. Also, since these soldiers lived in the Washington, DC area, they may not be representative of soldiers stationed elsewhere in the Army. Assignments in Washington, DC are generally more administrative than in other areas; therefore, soldiers tend to be older and more sedentary.

There was also no control group for comparison. A stronger design would have included a control group of normal weight soldiers. We were also not able to evaluate the effects of the APFT on lipid peroxidation due to lack of readable results from the LPO assay.

Conclusions

We can conclude from this study that the APFT does cause oxidative stress in overweight soldiers. There was a significant increase in CK immediately post-exercise and at 24hrs post-exercise. There was a significant increase in CRP immediately after exercise, but not at 24hrs after exercise. We found a slight

decrease in GPX after exercise, whereas we found no significant change in SOD following exercise. Fitness level and dietary antioxidant intake were positively associated with higher levels of GPX and SOD. Body fat was positively associated with greater levels of CK immediately after exercise, with higher baseline CRP levels, and with higher levels of SOD after exercise. We would expect that increasing fitness level, increasing antioxidant intakes and decreasing body fat would attenuate the potentially damaging effects of intense exercise. Future studies may help to elucidate ideal levels of each of these factors in controlling oxidative stress, and perhaps in lowering the risk of chronic diseases.

Table 1. Descriptive Statistics for Subjects Completing the Baseline APFT

	Males	Females	All Subjects
	n=28	n=19	n=47
Age (yr)	33.1 (8.3)	34.4 (7.1)	33.7 (7.9)
Height (cm)	176.8 (6.6)	165.1 (8.4)*	172.0 (6.8)
Weight (kg)	99.8 (9.9)	82.3 (11.0)*	92.1 (9.5)
BMI (kg/m^2)	31.9 (2.8)	29.9 (2.3)*	31.1 (2.6)
Body composition	n=28	n=19	n=47
Trunk Fat Mass (kg)	14.0 (2.6)	13.1 (2.8)	13.6 (2.5)
Trunk Fat %	30.2 (4.0)	35.4 (5.2)*	32.3 (4.5)
Total Fat Mass (kg)	26.8 (5.0)	28.7 (4.0)	27.6 (4.6)
Total Fat %	27.2 (3.8)	36.0 (3.7)*	30.8 (3.7)
Total Lean Mass (kg)	68.2 (6.9)	48.4 (5.9)*	60.2 (6.5)
Fitness Level	n=23	n=15	n=43
Predicted VO2max (ml/kg/min)	35.9 (8.7)	32.5 (5.1)	34.5 (7.4)
Dietary Intake	n=23	n=15	n=38
Total Calories	2102 (785)	1574 (441)*	1894 (672
Total CHO (gm)	268 (113)	208 (70)	245 (98)
% Total Calories	51 (9)	53 (9)	52 (9)
Total Protein (gm)	92 (35)	71 (18)*	83 (30)
% Total Calories	18 (5)	18 (4)	18 (5)
Total Fat (gm)	74 (31)	53 (21)*	66 (28)
% Total Calories	32 (6)	30 (7)	31 (6)
Vitamin A (mcg RE)	321 (279)	228 (221)	285 (258)
Vitamin E (mg ATE)	2.0 (1.4)	1.6 (2.3)	1.8 (1.8)
Vitamin C (mg)	347 (808)	233 (544)	302 (717)
Selenium (mcg)	67 (46)	37 (27)*	55 (40)
Copper (mg)	0.67 (0.50)	0.41 (0.24)	0.56 (0.42)
Zinc (mg)	9.0 (7.7)	5.7 (4.5)	7.7 (6.7)

Body composition determined by DEXA. Trunk Fat % = trunk fat mass/total trunk fat mass.
Fitness level determined by submaximal cycle ergometer.
Dietary intake determined by 3-day food diary.
Student's t-test was used to determine significance between values. Values are means (SD).
* Significant difference compared to males, $p < 0.05$.

Table 2. Mean Changes From Baseline Levels of Exercise-Induced Oxidative Stress Biomarkers Immediately After Exercise (Post) and at 24 hours After Exercise (24hr)

	CK (U/L)		CRP (mg/dL)		GPX (ng/ml)	SOD (ng/ml)
Subjects	Post	24hr	Post	24hr	Post	Post
Male	63.9 ± 6.0†	143.7 ± 32.0†	0.010 ± 0.004*	0.043 ± 0.028	-8.7 ± 5.0	-0.04 ± 0.15
Female	33.5 ± 2.9†	90.2 ± 18.5*	0.014± 0.005*	-0.009 ± 0.031	-3.6 ± 4.4	0.15 ± 0.09
All	51.6 ± 4.4†	130.9 ± 24.9†	0.012 ± 0.003†	0.028 ± 0.022	-6.5 ± 3.4	0.04 ± 0.01

All outcome measures reported without significant outliers. Values are means ± SE. Paired t-tests were used to determine significance between values. Differences from pre-exercise were significant at * $p < 0.05$, † < 0.001

Table 3. Partial Pearson's Correlations for Baseline and Changes from Baseline in Exercise-Induced Oxidative Stress Biomarkers

		CK			CRP			GPX		SOD	
	n	Pre	Post	24hr	Pre	Post	24hr	Pre	Post	Pre	Post
VO2max	20	0.00	0.02	-0.07	-0.09	0.05	0.24	0.59*	-0.60*	-0.44	0.12
Vit C	13	-0.11	-0.10	-0.02	-0.05	-0.09	-0.02	0.33	-0.40	-0.52	0.51
Vit A	13	0.44	0.56	-0.68*	0.68*	0.61	0.13	0.06	-0.11	-0.17	0.64*
Vit E	13	-0.09	0.06	-0.16	0.50	0.84*	0.21	0.39	-0.29	-0.41	0.43
ToFM	21	0.00	0.03	-0.14	0.35	-0.06	0.06	-0.22	0.42	0.32	0.10
ToPer	21	0.18	0.25	-0.35	0.51*	-0.13	0.13	-0.18	0.32	0.37	0.17
TrFM	21	0.11	0.15	-0.20	0.53*	0.05	-0.19	-0.06	0.30	0.43	0.13
TrPer	21	0.27	0.31	-0.33	0.56*	-0.19	-0.12	-0.16	0.32	0.46*	0.21
ToLM	21	-0.42	-0.49*	0.54*	-0.47*	-0.12	-0.19	-0.03	0.11	-0.26	-0.21

Correlations controlled for age, gender, and BMI. ToFM, total fat mass; ToPer, total fat percent; TrFM, trunk fat mass; TrPer, trunk fat percent; ToLM, total lean mass. Correlations completed for pre-exercise values (Pre) and on the differences after exercise (Post and 24hr).
* Correlations were significant at $p < 0.05$

Chapter 4: Weight Loss Effects on Exercise-Induced Oxidative Stress Biomarkers in Overweight/Obese Soldiers

Abstract

Obesity is known to increase levels of inflammation. Obese individuals may be in a chronic state of increased oxidative stress and exercise may exacerbate this condition. The purpose of this study was to examine the effect of weight loss on exercise-induced oxidative stress following the Army Physical Fitness Test (APFT). Sixty subjects were recruited for this study. Subjects were asked to complete an APFT where blood markers of oxidative stress (CK, CRP, SOD, GPX) were measured before and after the APFT. Dietary antioxidant intake, fitness level and body composition were also assessed. Subjects attempted to lose 10 pounds as part of a weight control program. After a 10 pound weight loss or after 3 months, whichever occurred first, subjects completed a 2nd APFT. Blood markers of oxidative stress were collected before and after the APFT. Dietary antioxidant intake, fitness level and body composition were also measured again. Twenty-nine subjects completed the entire study protocol. There was no significant effect of weight loss on the exercise-induced changes in the biomarkers after the weight loss period. Changes in BMI were found to be associated with changes in CRP levels immediately post-exercise. Changes in total body fat mass and total body fat % were found to be associated with changes in CRP levels at 24hrs post-exercise. Total body fat mass was found to be associated with changes in GPX levels and SOD levels immediately post-exercise. We also found a significant increase in baseline SOD levels (1.28 vs.

0.74 ng/ml, p=0.04) and SOD levels immediately post-exercise (1.68 vs. 0.93 ng/ml, p=0.006) after the 2nd APFT compared to the 1st APFT. There was also a significant increase in SOD following the 2nd APFT that was not observed after the 1st APFT. In conclusion, weight loss (fat mass and lean mass) did not affect changes in exercise-induced oxidative stress biomarkers, whereas body fat decreases did appear to have an effect on the change in biomarkers.

Introduction

There are many factors that may play a role in the levels of oxidative stress that are experienced when an individual exercises. Dietary intake of antioxidants and level of fitness have been established as possible links (1, 5, 19, 21); however it has also been shown that body composition may have a part in exercise-induced oxidative stress as well. Vincent et al. compared obese subjects to normal weight subjects, and found that the obese individuals had greater amounts of lipid peroxidation after resistance and aerobic exercise (6).

Exercise can increase the oxygen consumption of skeletal muscle by 10 to 20-fold, thereby increasing the change for developing reactive oxygen species (ROS). A variety of theories may explain why these individuals have more exercise-induced oxidative stress than normal weight individuals. In addition to the increased respiration previously mentioned, oxidative stress may be related to chronic inflammation, hyperglycemia, decreased antioxidant defenses, increased lipid levels susceptible to oxidation, increased endothelial sources of ROS and hyperleptinemia (11).

As the focus on treating obesity continues to intensify, so does the research related to the metabolic effects of weight loss. It has been hypothesized that weight loss may help to decrease oxidative stress, one of the negative consequences of obesity. Several studies have demonstrated this. In studying weight loss and oxidative stress, Roberts et al. found that after only 21 days, the subjects experienced a reduction in oxidative stress markers (8-isoprostaglandin F2α), increased levels of nitric oxide, decreased insulin, and decreased blood pressure. This was an early study showing that a low-fat, high-fiber diet coupled with daily exercise can attenuate oxidative stress in obese individuals. Subjects lost an average of 3.7 kg during the study (7). More recently, Roberts et al. found that after a 3.6 kg weight loss, obese subjects had decreases in oxidative stress markers 8-isoprostanes by 35% and in the enzymatic oxidant myeloperoxidase by 20%. C-reactive protein (CRP) decreased by 39% (43).

Clement et al. studied inflammation-regulated genes in white adipose tissue of obese and nonobese women. The obese women followed a very low calorie diet (VLCD) for 28 days. They found an increase in anti-inflammatory molecules and a decrease in inflammation-related markers (46).

To our knowledge, the effect of weight loss on exercise-induced oxidative stress in overweight or obese individuals has not been investigated. Therefore, the purpose of this study was to evaluate the effect that weight loss has on exercise-induced oxidative stress in overweight or obese soldiers as a result of the Army Physical Fitness Test (APFT).

Methodology

Subjects

Subjects were recruited from among military personnel who were beneficiaries of the Walter Reed Army Healthcare System. Sixty (35 male, 25 female) subjects volunteered for this study. Forty-seven subjects (27 male, 20 female) completed the baseline Army Physical Fitness Test (APFT) (22% dropout). Thirty-three subjects returned for follow-up measurements and 29 completed the APFT (52% dropout). Inclusion criteria included: 1) subjects were at least 18 years old 2) subjects had a BMI $\geq$ 28 3) subjects were not actively losing weight 4) subjects were eligible to participate in the Army Weight Control Program and 5) subjects were able to perform the APFT. Subjects were screened for participation and anyone with a history of heart disease, hypertension, uncontrolled diabetes, or medical conditions that may impact oxidative stress markers were not eligible to participate and were excluded from the study.

Study Design

Informed consent was obtained after a brief description of the study. Once subjects provided written and verbal consent, baseline anthropometry and body composition measurements were taken. Subjects were provided instructions on completing a diet history questionnaire as well as a 3-day food diary. For the diary, subjects were asked to log everything that they ate or drank and any dietary supplements that they may have consumed. Subjects also completed a fitness assessment. Subjects were scheduled to take the APFT within two weeks

of baseline anthropometry and body composition measurements. They were provided instructions relating to the APFT which included avoiding strenuous exercise for at least 24hrs prior to the test. Any subject reporting an illness or injury was rescheduled to a later time when the illness (to include presence of a fever) or injury had been resolved. If there were more than two weeks between baseline measurements and APFT, the subject's weight was checked to determine the need for remeasuring body composition. After baseline data were collected, subjects attended a weight loss class or an individual appointment with a dietitian from the wellness clinic (not associated with this study) and received a weight loss plan. After subjects lost 10 pounds or after a period of 3 months, whichever occurred first, subjects were scheduled for the 2nd APFT measurements. The second set of measurements included dietary intake, fitness assessment, and body composition as previously described. Subjects also completed a 2nd APFT.

The protocol for this study was approved by the Human Use Committee at Walter Reed Army Medical Center and by the Institutional Review Board at the University of Maryland College Park.

Biochemical assays

Biochemical assays measured included CK, CRP, GPX, SOD and LPO. Blood samples were drawn in the Pathology Lab at Walter Reed Army Medical Center. CK and CRP were collected in serum separator tubes and analyzed immediately. GPX and SOD were collected in heparinized tubes and LPO samples were collected in sodium citrate tubes. Plasma was separated and

stored as previously described. Analyses for all assays were completed as previously described in Chapter 3.

Anthropometry and body composition

Height was measured to the nearest 0.01 centimeter using a digital stadiometer. Weight was measured to the nearest 0.01 kg using a digital scale. BMI was calculated as body mass (kg)/height $(m)^2$. Body composition (lean body mass and fat mass) of the whole body was measured by DXA as previously described in Chapter 3.

Training status analysis

Training status was assessed using a submaximal cycle ergometer exercise test to predict VO2max as previously described in Chapter 3.

Dietary analysis

Dietary antioxidant intake was determined by using a 3-day food diary and by using a diet history questionnaire as previously described in Chapter 3. For the second diet history questionnaire, subjects were instructed to answer the questions with reference to the length of time they participated in the study. Data are not reported due to very poor completion rate (3 DHQ's completed).

Army Physical Fitness Test

Subjects completed each APFT as previously described in Chapter 3. Additionally, for the 2^{nd} APFT subjects were instructed to perform this APFT at the same intensity or level of work as the 1^{st} APFT to enable comparison of the two tests.

Power Analysis

Power was based on expected changes in MDA (n=12), SOD (n=13), and weight loss (n=16). We used the greatest number of subjects and added 5 subjects for each of the covariates we investigated (fitness level, vitamins A, C, E, body fat). We calculated that 35 subjects would be needed to reach the desired significance level of 0.05 and 80% power. Sixty subjects were recruited to account for an estimated 25-50% drop-out rate based on clinic statistics and experience with a similar research study. Twenty-nine subjects completed the 2nd APFT (52% dropout).

Statistical Analysis

Paired t-tests were used to determine if there were any differences in any of the descriptive statistics and in any oxidative stress biomarkers measured before and after both APFTs. Pearson's correlations and Analysis of Covariance (ANCOVA) were performed to determine the extent to which a relationship exists between fitness level, dietary antioxidant intake, body composition and exercise-related changes in CK, CRP, GPX and SOD. Changes in dietary antioxidant intake, fitness level and body composition were used as covariates for data analyses before and after the weight loss period. In the event there were extreme outliers, the criterion of 2 standard deviations was used to eliminate a subject from an analysis. There was 1 observation eliminated for the CK analysis and 3 observations were eliminated for the CRP analysis. The statistical analyses were performed using the SAS System for Windows V9.1 (SAS Institute, Cary, NC, 2002). All values were considered significant at $P < 0.05$.

Results

A total of 29 subjects (18 males, 11 females) finished the entire protocol (both APFTs). Table 1 reports subjects completing both APFTs. Subjects had significant decreases in weight, BMI, trunk fat mass, trunk fat percentage, total body fat mass, total body fat percentage, total lean mass and total dietary fat intake. There was a significant increase in fitness level. There were slight, although non-significant, decreases in vitamin A, vitamin E, selenium, copper, and zinc. Changes in dietary intake were assessed using the 3-day food diary. There was extremely low compliance in completing the diet history questionnaire (3 of 29 subjects). This questionnaire took most subjects at least 90 minutes to complete and many subjects would not complete it more than once. Therefore, these data are not reported here.

Oxidative stress biomarkers

Mean levels of oxidative stress biomarkers for both the 1st and the 2nd APFTs in addition to mean changes for each biomarker are reported in Table 2. In all subjects, the 2nd APFT CK levels increased 27% immediately post-exercise compared to the pre-exercise value (247.0 vs. 194.9 U/L), and by 59% at the 24hr post-exercise time point (308.5 vs. 194.9 U/L). Relative to the 1st APFT, there were no significant differences in CK levels at each of the time points. We also found there was no attenuation in the exercise-induced change in CK levels immediately post-exercise and at 24hrs post-exercise between the 1st APFT and the 2nd APFT (Table 2).

Following the second APFT, CRP levels increased 6% immediately post-exercise compared to pre-exercise (0.155 vs. 0.146 mg/dL), and by 88% at the 24hr post-exercise time point (0.274 vs. 0.146 mg/dL) in all subjects. In comparison to the 1st APFT, there were no significant differences in CRP levels at each of the time points. Once again, we found there was no reduction in the exercise-induced change in CRP levels immediately post-exercise and at 24hrs post-exercise between the 1st APFT and the 2nd APFT (Table 2).

GPX values were measured at only 2 time points: pre-exercise and immediately post-exercise. For the 2nd APFT, GPX levels increased by 4% immediately post-exercise compared to the pre-exercise time point (74.8 vs. 71.8 ng/ml). There were no significant differences in GPX levels at the pre-exercise or immediately post-exercise time points in comparison to the 1st APFT. We found there was no alteration in the exercise-induced change in GPX levels immediately post-exercise between the 1st APFT and the 2nd APFT (Table 2).

SOD was also measured at two time points only: pre-exercise and immediately post-exercise. There was a 31% increase in SOD levels immediately post-exercise compared to pre-exercise levels after the 2nd APFT (1.68 vs.1.28 ng/ml). At the 2nd APFT, there was a 73% increase in pre-exercise SOD levels compared to the pre-exercise SOD levels for the 1st APFT (1.28 vs. 0.74 ng/ml). There was also an 81% increase in SOD levels immediately post-exercise at the 2nd APFT compared to the 1st APFT (1.68 vs. 0.93 ng/ml). We found there was no difference in the exercise-induced change in SOD levels immediately post-exercise between the 1st APFT and the 2nd APFT (Table 2).

Pearson's correlations

Pearson's correlations were used to determine the strength of the relationship between dietary antioxidant intake, fitness level (predicted VO2max) and body composition and the observed changes in biomarkers of oxidative stress. The correlation coefficients for these relationships are seen in Table 3. After the 3-month weight loss period, there was a positive correlation found between the change in vitamin A intake and in the change in baseline GPX levels (r=0.68, p=0.04). In addition, there was a significant positive correlation found between the change in total body fat percentage and the change in GPX levels after exercise (r=0.39, p=0.04). There was a positive correlation between the change in trunk fat percentage and the change in GPX levels immediately post-exercise (r=0.40, p=0.03). We found a positive correlation between the change in total lean mass and the change in baseline CRP levels after the 3-month weight loss period (r=0.44, p=0.02) and also between total lean mass and the change in CRP levels immediately post-exercise (r=0.38, p=0.05).

ANCOVA

ANCOVA was used to determine the effect that changes in weight, BMI, fitness level, total fat mass and total fat percentage had on changes in each of the biomarkers of oxidative stress. The compliance rate of subjects returning food diaries (11 of 29, 38%) was small enough so that we were not able to include antioxidant intake information in the ANCOVA. Even though our analysis showed no significant differences in the exercise-induced changes for each of the markers after the 3-month weight loss period, we continued the analysis of

covariance since the differences were based on some subjects experiencing increases and others experienced decreases.

We could not determine any significant associations between changes in weight, BMI, fitness level, total fat mass, total fat percentage and the difference in the increase in CK levels immediately post-exercise or on the difference in the increase in CK levels at the 24hr post-exercise time point.

The difference in the exercise-related change in CRP levels immediately post-exercise was found to be influenced by the change in BMI after the 3-month weight loss period. The difference in exercise-related changes in CRP levels at the 24hr post-exercise time point was found to be associated with changes in total body fat mass and total body fat percentage after the 3-month weight loss period.

Differences in exercise-induced changes in GPX levels post-exercise were found to be slightly influenced by changes in total body fat mass (p=0.06) after the 3-month weight loss period.

Similarly, differences in exercise-induced changes in SOD levels post-exercise were found to be influenced by changes in total body fat mass after the 3-month weight loss period.

Discussion

Weight loss did not significantly alter the effects of exercise-induced oxidative stress in overweight soldiers. However, this study found that weight loss was associated with an increase in the levels of SOD before and after exercise which were significantly greater than the levels before weight loss. The 2nd APFT

produced an increase in SOD after exercise that was not present before weight loss. Weight loss did not appear to affect change in any of the other markers.

The goal for weight loss in this study was at least 10 pounds, whereas the actual average weight loss was 5.06 ± 6.38 pounds (2.3 ± 3.9 kg). There were several subjects who gained rather than lost weight, which most likely had an impact on the analysis. Despite observing no effect of weight loss on the overall mean difference in exercise-induced changes in each of the oxidative stress biomarkers, we did find associations between changes in fat mass and fat percentage and changes in CRP levels at 24hr post-exercise. We also found associations between changes in fat mass and changes in both GPX and SOD levels immediately post-exercise. The average total fat mass decrease was 2.6 ± 4.6 pounds (1.2 ± 2.1 kg).

In this study, there was no significant difference in the exercise-induced change seen in CK levels immediately post-exercise or at the 24hr post-exercise time point. There were also no significant correlations between dietary antioxidant intake, fitness level or body composition. It's possible that the changes in each of these variables was not enough to illicit a noticeable change in CK. In Chapter 3, we examined the effects of dietary antioxidant intake, fitness level and body composition on CK levels. We found that dietary intakes of vitamins A, C, and E and the interactions between fitness level and vitamin A and fitness level and vitamin E were associated with the increase in CK levels immediately post-exercise. Vitamin A intake was negatively correlated with the increase in CK levels 24hrs post-exercise. Total lean mass was negatively

correlated with baseline CK levels and the increase in CK levels immediately post-exercise. It was positively correlated with the increase in CK levels 24hrs post-exercise. There were no significant changes in dietary antioxidants after the 3-month weight loss period. The negligible change in antioxidant intake may not have been sufficient to elicit a change in the increase in exercise-related CK levels after attempted weight loss. There was a significant increase in predicted VO2max after the 3-month weight loss period (37.2 vs. 32.8 ml/kg/min); however this change was not associated with changes in CK levels at any time point. Greater improvement in fitness level may have produced a noticeable change.

Excess body weight, and by extension body fat, have been found to be associated with increased levels of inflammation and oxidative stress. Marcell found that subjects who had more body fat had greater levels of inflammation measured by CRP (47). In an early study investigating the theory that exercise-induced oxidative stress may be greater in obese subjects, Vincent found that compared to normal weight individuals, obese subjects experienced greater amounts of lipid peroxidation after resistance and aerobic exercise (6). These data coincide with the correlations we observed in Chapter 3. Total body fat percentage, trunk fat mass, and trunk fat percentage were positively correlated with higher baseline levels of CRP. These data support the theory that obese individuals may experience more oxidative stress following exercise.

In response to this theory, weight loss has been targeted as one avenue to reduce oxidative stress in overweight persons. Clement et al. examined the gene expression profiles for inflammation of obese and lean subjects. Gene profiles of

subcutaneous white adipose tissue were analyzed before and after obese subjects followed very low-calorie diets for 28 days. The researchers observed an improvement in the genetic inflammatory/anti-inflammatory profile in the adipose tissue of obese subjects, which resulted in comparable profiles to lean subjects (46).

Heilbronn et al. observed a significant decrease in DNA damage, but not in protein carbonyl concentration after overweight subjects (25 < BMI < 30) lost weight. Biomarkers were measured before and after a 6-month weight loss program. Subjects in this study lost between 1% (control group) and 14% (test groups) of their body weight. All three test groups (calorie restriction alone, calorie restriction with exercise, and very low-calorie diet alone) had significant changes in weight and consequently in the decrease in DNA damage (48).

Roberts et al. used a 21-day diet and exercise intervention to test the effect of weight loss on markers of oxidative stress in obese subjects (mean BMI = 35.4). Subjects followed a low-fat diet and exercised daily. Biomarkers were measured before and after the weight loss intervention. After weight loss, these researchers observed significant decreases in the oxidative stress biomarkers myeloperoxide, 8-isoprostanes, and CRP (43).

Okita et al. found similar results in a group of female subjects (mean BMI = 27.4) who followed a 2-month exercise program to facilitate weight loss. Interestingly, these researchers noted that subjects in the highest quartile for weight loss had nonsignificant changes in CRP, whereas subjects in the other three quartiles who lost less weight had significant decreases in CRP. The

investigators suggested that this unusual finding may have been a result of rapid weight loss and overtraining, which may have lead to increased inflammation (49). Jae found a comparable trend with respect to weight loss quartiles. Subjects in the two lowest quartiles for weight loss had increases in CRP, while subjects in the two highest quartiles for weight loss had proportional decreases in CRP (50).

Collectively, these studies support the hypothesis that weight loss improves inflammation. We saw only modest weight loss in our study. It is possible that if our subjects had lost more weight, exercise-induced CRP levels would have been lower. Since we were not able to evaluate the role of dietary antioxidant intake on exercise-induced changes in CRP levels, we cannot make conclusions about their effectiveness. However, based on our observed correlation between change in vitamin A intake and change in baseline CRP levels, future studies may show significant effects of dietary antioxidants on attenuating inflammation following exercise.

To our knowledge, this is the first study to show a relationship between weight loss and increases in antioxidant enzyme levels (SOD). It is also the first study to show a possible link between changes in total body fat mass and both GPX and SOD. Vincent suggests that antioxidant defenses in obese individuals are diminished, due to increases in ROS which overwhelm and subsequently deplete antioxidant enzyme levels (11). It appears plausible that weight loss may reverse this effect, as suggested by our results which showed an increase in SOD levels following weight loss and immediately post-exercise.

Limitations

Our study had a few limitations that may have restricted the interpretation of the data. The number of subjects returning food diaries was low enough to preclude dietary antioxidant intake from being included in the ANCOVA. These data would have allowed for a more complete ANCOVA. The number of subjects returning for the follow-up study was lower than desired. A larger sample size may have allowed us to observe a difference in our biomarkers of interest. It also may have allowed us to investigate more covariates and/or their interactions with other covariates of interest. Also, the average weight loss was less than anticipated. Not all subjects lost weight and several gained a considerable amount. There was no formal or standard weight loss program used with every subject. Each subject received weight loss counseling by registered dietitians, but was not required to follow a specific intervention. The study may have been improved with more stringent diet and exercise protocols. There was no control group of overweight soldiers who did not attempt weight loss. A study comparing normal weight subjects, overweight/obese not attempting weight loss and overweight/obese subjects attempting weight loss would have been a stronger study design.

Conclusions

There are very few data available on the effects of weight loss on antioxidant enzymes, including GPX and SOD which were examined in this study. We found a significant increase in baseline SOD levels and SOD levels immediately post-exercise after a 3-month weight loss period. Our study showed a possible

relationship between changes in body fat and exercise-induced changes in CRP levels, GPX levels, and SOD levels. This study provides support for future research examining the role of weight loss in alleviating the effects of exercise-induced oxidative stress in overweight and obese individuals.

Table 1. Descriptive Statistics for Subjects Completing Both APFTs

	1st APFT	2nd APFT	Difference
n=29			
Age (yr)	34.4 (7.7)	-	nc
Height (cm)	173.1 (9.6)	-	nc
Weight (kg)	92.2 (13.5)	89.9 (12.7)	-2.3 (2.9)†
BMI (kg/m^2)	30.8 (2.9)	30.1 (2.7)	-0.8 (1.0)†
Body Composition (n=29)			
Trunk Fat Mass (kg)	13.6 (3.0)	12.8 (2.8)	-0.8 (1.4)†
Trunk Fat %	32.1 (5.3)	31.0 (6.1)	-1.1 (2.1)†*
Total Fat Mass (kg)	27.4 (4.8)	26.1 (4.9)	-1.2 (2.1)†
Total Fat %	30.4 (5.5)	30.0 (6.3)	-0.6 (1.5)*
Total Lean Mass (kg)	60.1 (11.5)	59.7 (11.7)	-0.8 (1.7)†
Fitness Level (n=20)			
Predicted VO2max (ml/kg/min)	32.8 (7.6)	37.2 (7.8)	4.4 (8.8)*
Dietary Intake (n=11)			
Total Calories	1417 (269)	1267 (406)	-150 (344)
Total CHO (gm)	164 (41)	165 (55)	0.5 (39)
% Total Calories	46 (11)	52 (13)	5 (9)
Total Protein (gm)	71 (18)	68 (16)	-3 (19)
% Total Calories	20 (5)	22 (4)	2 (5)
Total Fat (gm)	55 (20)	37 (21)	-17 (20)*
% Total Calories	34 (7)	26 (10)	-8 (8)*
Vitamin A (mcg RE)	250 (187)	201 (192)	-49 (302)
Vitamin E (mg ATE)	2.3 (2.7)	1.0 (0.6)	-1.4 (2.8)
Vitamin C (mg)	73 (44)	70 (73)	-3 (49)
Selenium (mcg)	41 (34)	29 (26)	-13 (34)
Copper (mg)	0.47 (0.28)	0.40 (0.31)	-0.6 (0.50)
Zinc (mg)	5.8 (4.3)	4.1 (2.3)	-1.7 (5.1)

Body composition determined by DXA.
Trunk Fat % = trunk fat mass/total trunk fat mass.
Fitness level determined by submaximal cycle ergometer.
Dietary intake determined by 3-day food diary.
Student's t-tests were used to determine significance between values. Values reported are means ± SD.
nc=not calculated.
Differences were significant at p * < 0.05, † < 0.01

Table 2. Mean Levels of Exercise-Induced Oxidative Stress Biomarkers for Both APFTs and Mean Changes for All Subjects

Biomarker	n	Time Point	1st APFT	2nd APFT	Change (2nd APFT – 1st APFT)
CK (U/L)	27	Pre	168.5 ± 30.2	194.9 ± 35.8	26.3 ± 32.1
	27	Post	224.6 ± 36.9	247.0 ± 41.4	22.4 ± 38.2
	11	24hr	334.4 ± 85.3	308.5 ± 69.2	-25.9 ± 47.2
	27	Post-Pre	56.1 ± 8.5*	52.1 ± 6.2*	-4.0 ± 7.7
	11	24hr-pre	167.6 ± 48.1*	85.5 ± 40.7	-82.2 ± 53.5
CRP (mg/dL)	24	Pre	0.203 ± 0.037	0.146 ± 0.033	-0.054 ± 0.029
	24	Post	0.214 ± 0.039	0.155 ± 0.035	-0.057 ± 0.033
	10	24hr	0.265 ± 0.071	0.274 ± 0.113	0.009 ± 0.050
	24	Post-Pre	0.011 ± 0.005*	0.009 ± 0.003*	-0.003 ± 0.006
	10	24hr-Pre	0.071 ± 0.041	0.083 ± 0.058	0.011 ± 0.025
GPX (ng/ml)	28	Pre	75.5 ± 7.6	71.8 ± 10.0	-3.6 ± 9.6
	28	Post	78.0 ± 6.6	74.8 ± 9.9	-3.2 ± 9.5
	28	Post-Pre	2.5 ± 6.4	3.0 ± 5.2	0.4 ± 8.3
SOD (ng/ml)	19	Pre	0.74 ± 0.13	1.28 ± 0.17	0.54 ± 0.24**
	19	Post	0.93 ± 0.16	1.68 ± 0.20	0.74 ± 0.24**
	19	Post-Pre	0.19 ± 0.13	0.40 ± 0.14*	0.20 ± 0.19

Time Point: Pre = value for blood sample taken immediately before exercise; Post = value for blood sample taken immediately after exercise; 24hr = value for blood sample taken 24hrs after exercise; Post-Pre = difference between blood samples taken post-exercise and pre-exercise; 24hr-Pre = difference between blood samples taken 24hrs post-exercise and pre-exercise.
1st APFT = blood samples taken at 1st APFT;
2nd APFT = blood samples taken at 2nd APFT;
Change = difference between samples from 1st APFT and 2nd APFT
All outcome measures reported without significant outliers. Values are means ± SE.
Paired t-tests were used to determine significance between values.
* Differences from pre-exercise levels were significant at $p < 0.05$.
** Differences between 1st and 2nd APFT were significant at $p < 0.05$.

Table 3. Pearson's Correlations for Changes in Covariates and Changes in Exercise-Induced Oxidative Stress Biomarkers After the 2nd APFT.

	CK			CRP			GPX		SOD	
	Pre	Post	24hr	Pre	Post	24hr	Pre	Post	Pre	Post
VO2max	0.00	-0.26	-0.61	0.23	0.02	-0.41	0.01	-0.07	0.07	0.02
Vit C	0.28	0.59	0.35	-0.18	0.42	0.61	0.29	-0.13	-0.12	0.22
Vit A	0.16	0.04	0.05	0.09	0.49	0.05	0.60*	0.30	-0.29	0.26
Vit E	0.10	-0.07	-0.36	0.38	0.37	-0.39	0.18	0.01	-0.49	-0.09
ToFM	-0.14	-0.12	-0.02	-0.01	0.27	-0.16	-0.22	0.31	0.10	-0.34
ToPer	-0.15	-0.13	0.12	-0.14	0.14	-0.15	-0.23	0.39*	0.10	-0.31
TrFM	0.00	0.00	-0.24	-0.08	0.14	-0.22	-0.19	0.31	0.18	-0.32
TrPer	-0.06	-0.05	-0.03	-0.12	0.12	-0.19	-0.27	0.40*	0.25	-0.29
ToLM	0.04	0.06	-0.32	0.44*	0.38*	-0.20	0.14	-0.31	-0.13	-0.15

ToFM, total fat mass; ToPer, total fat percent; TrFM, trunk fat mass; TrPer, trunk fat percent; ToLM, total lean mass.
Correlations completed with the changes of each covariate (2nd APFT – 1st APFT)
Pre = change in baseline value of each biomarker
Post = change in the increase in each biomarker immediately post-exercise (Post – Pre)
24hr = change in the increase in each biomarker 24hrs post-exercise (24hr – Pre)
* Correlations were significant at $p < 0.05$

Chapter 5: Conclusions

Major findings

In relation to our study objectives:

Hypothesis 1) The APFT produces oxidative stress in overweight soldiers.

- There were significant increases in CK levels immediately post-exercise and at 24hrs post-exercise. Normal levels for CK are 55-170 U/L. Baseline levels for CK were 171 U/L and increased to as high as 347 U/L. Subjects did experience some muscle damage as a result of exercise that was consistent with other studies examining the effect of exercise on oxidative stress and muscle damage.
- There were significant increases in CRP levels immediately post-exercise. CRP < 0.01 mg/dL indicates low inflammation. The mean baseline value for CRP was 0.209 mg/dL, which is greater than desirable (<0.01 mg/dL) according to the American Heart Association. This indicates that subjects were in a state of chronic inflammation that generally worsened after exercise.
- There was a slight decrease in GPX levels immediately post-exercise. There are no established normal/desirable levels for GPX so we cannot make comparisons between this group of subjects and the rest of the population.
- There was no significant change in SOD levels after the APFT. There are no established normal/desirable levels for SOD so we cannot make comparisons between this group of subjects and the rest of the population.
- In summary, the APFT does cause oxidative stress in overweight soldiers.

Hypothesis 2) Soldiers who consume greater amounts of antioxidants will experience less oxidative stress following the APFT.

- Dietary antioxidant intake was associated with changes in biomarkers of oxidative stress.
- Vitamin A was negatively correlated with CK levels at 24hrs post-exercise. It was also positively correlated with baseline CRP levels and with SOD levels after exercise.
- Vitamin E was positively correlated with CRP levels immediately post-exercise.
- There were significant associations between dietary antioxidants and increases in CK and CRP levels immediately post-exercise and also with GPX immediately post-exercise.
- In summary, dietary antioxidant intake appears to have a potentially protective role against oxidative stress in overweight soldiers.

Hypothesis 3) Soldiers who are more fit will experience less oxidative stress following the APFT.

- Predicted VO2max was significantly correlated with GPX levels before and after exercise.
- Predicted VO2max was associated with increases in CRP levels, GPX levels and SOD levels immediately post-exercise.
- There also were associations with the interactions between fitness level and dietary antioxidants with increases in CK levels, CRP levels and GPX levels immediately post-exercise. There were associations with

interactions between fitness level and body fat with increases in all of the biomarkers of oxidative stress immediately after exercise.

- In summary, fitness level appears to be associated with less oxidative stress and may have a protective role.

Hypothesis 4) Soldiers who have less body fat will experience less oxidative stress following the APFT.

- Body fat was associated with changes in CRP levels, GPX levels and SOD levels immediately post-exercise.
- The interactions between body fat and fitness level are discussed above.
- In summary, body fat appears to increase oxidative stress in overweight soldiers and may possibly inhibit antioxidant enzyme activity.

Hypothesis 5) Soldiers who lose weight will experience less oxidative stress following the APFT.

- Weight loss did not result in significant decreases in oxidative stress following the APFT.
- Changes in body fat mass, BMI and body fat percentage were found to be influential in the changes seen in CRP levels immediately post-exercise and at 24hrs post-exercise, and in GPX and SOD levels immediately post-exercise.
- We found a significant increase in SOD that was not present before weight loss. There were also increased levels of SOD before and after exercise compared to before weight loss.
- In summary, it appears that decreased levels of body fat may protect

against oxidative stress. It also appears that fat loss results in improved levels of antioxidant enzymes (SOD).

Strengths and Limitations

There were several strengths and limitations of this study. Strengths include:

- This is the first study to examine the effect of weight loss on exercise-induced oxidative stress in overweight/obese soldiers. This is the first study to show a relationship between weight loss and increases in antioxidant enzymes.
- Subjects served as their own control for the weight loss analyses.
- All variables (i.e., body composition, fitness level, and diet) were measured before and after weight loss.

Limitations:

- Diet and exercise were not controlled. Dietary analysis is limited due to the poor return rate. There are also limitations in analysis of food records that relate to accuracy of reported portion sizes and actual foods consumed.
- Weight loss varied and did not reach the goal of 10 pounds per person. Some subjects gained a significant amount of weight.
- Subjects may not have performed the APFT to exhaustion.
- Subjects may not have performed the APFT at the same intensity level before and after weight loss.
- There was a poor follow-up rate. A larger follow-up sample size may have facilitated investigating more covariates and interactions. We were able to

investigate our main objective, though, which was weight loss and decreases in body fat.

Future directions

This study provided evidence to support further research into the effect of weight loss on exercise-induced oxidative stress. There was promising evidence that decreasing body fat may lead to desirable changes in oxidative stress biomarkers. Future studies may also examine the relationship between weight loss, fitness level, and dietary antioxidant intake on cytokine decreases and their role in oxidative stress. This study also showed that dietary antioxidants may play an important role in decreasing the negative effects of intense exercise of short duration. Future studies into other military specific physical activities of longer duration such as road marching, field training or patrol may show a need for increased intakes of these vitamins. In addition, if future research shows a stronger relationship between dietary antioxidants and decreases in exercise-induced oxidative stress, there may be evidence to support an increase in the military specific Dietary Reference Intakes for antioxidants and/or to increase supplementation of military rations.

Appendix A: Regression equations

Baseline analysis (Chapter 3)

Changes in CK: Immediately post-exercise

$CK_{females}$ = 19.78 + 0.11(ckpre) – 0.74(BMI) + 0.47(VO2max) – 0.40(vitamin A) + 0.001(vitamin C) + 76.77(vitamin E) + 0.01(VO2max*vitamin A) – 2.17(VO2max*vitamin E)

CK_{males} = 332.63 + 0.11(ckpre) – 9.14(BMI) + 0.47(VO2max) – 0.40(vitamin A) + 0.001(vitamin C) + 76.77(vitamin E) + 0.01(VO2max*vitamin A) – 2.17(VO2max*vitamin E)

24hrs post-exercise

There were no significant effects found in the ANCOVA

Changes in CRP: Immediately post-exercise and 24hr post-exercise

There were no significant effects found in the ANCOVA

Changes in GPX: Immediately post-exercise

$GPX_{females}$ = -301.14 – 0.34(gpxpre) + 1.33(age) – 4.02(BMI) + 9.46(VO2max) – 0.29(vitamin A) + 0.01(vitamin C) + 0.02(total fat mass) + 0.009(VO2max*vitamin A) – 0.0004(VO2max*total fat mass)

GPX_{males} = -208.55 – 1.52(gpxpre) + 1.33(age) – 4.02(BMI) + 9.46(VO2max) – 0.29(vitamin A) + 0.01(vitamin C) + 0.02(total fat mass) + 0.009(VO2max*vitamin A) – 0.0004(VO2max*total fat mass)

Changes in SOD: immediately post-exercise

$SOD_{females}$ = -8.05 – 0.32(sodpre) + 0.06(age) – 0.06(BMI) + 0.22(VO2max) + 0.0001(vitamin C) + 0.37(total fat %) – 0.008(VO2max*tot fat%)

SOD_{males} = -13.01 – 0.32(sodpre) + 0.06(age) + 0.22(BMI) + 0.22(VO2max) + 0.0001(vitamin C) + 0.37(total fat %)– 0.008(VO2max*tot fat%)

Changes in Biomarkers after 3-month weight loss period analysis (Chapter 4)

Changes in CK: Immediately post-exercise and 24hrs post-exercise

There were no significant effects found in the ANCOVA

Changes in CRP: Immediately Post-exercise

CRP = 0.01 + 0.01(change in BMI)

24hr post-exercise

CRP = -0.03 – 0.0001(change in total fat mass) + 0.13(change in total fat %)

Changes in GPX: Immediately Post-Exercise

GPX = 10.21 + 0.008(change in total fat mass)

Changes in SOD: Immediately post-exercise

SOD = 0.10 – 0.0001(change in total fat mass)

Appendix B: Data Collection Form

SUBJECT ID: _____

Data Collection Form

Measurement	Baseline			After Weight Loss		
Date						
Anthropometry:						
Height						
Weight						
BMI						
Body composition:						
Trunk fat mass						
Trunk % fat mass						
Total fat mass						
% fat mass						
Total lean mass						
Physical activity assessment:						
VO2max						
Minutes exercise per week						
Dietary assessment:	**DHQ**	**3-day**		**DHQ**	**3-day**	
* attached						
Blood measurements:	**PRE**	**POST**	**24HR**	**PRE**	**POST**	**24HR**
CK						
CRP						
LPO						
SOD						
GPX						
APFT						
Total score						
Run time						
Run points						
Pushups						
Pushups points						
Sit-ups						
Sit-ups points						

Appendix C: Screening Form

SUBJECT ID: _____

General Data and Medical History Questionnaire

Please fill out all information. This information will be used to assess your eligibility for participation in this study and for maintaining a record of your participation. All information will be kept confidential.

Gender: Male Female (circle one) Date of Birth: _____

Have you lost weight in the last month? YES NO (circle one)

If so, how much? _____ lbs

Have you been on profile during the last 6 months? YES NO (circle one)

If yes, what were the restrictions?:

_____

Are you able to take the Army Physical Fitness Test? YES NO (circle one)

Are you pregnant, think you may be pregnant or planning to get pregnant?

YES NO (circle one)

Do you currently smoke? YES NO (circle one)

Please list any medical conditions for which you are being seen by a medical professional (for example: high blood pressure, diabetes, weight, etc.)

Please list all medications that you currently take:

Please list any vitamin or mineral supplements that you take. Also please list any other supplements that you take:

Appendix D: Consent Form

VOLUNTEER AGREEMENT AFFIDAVIT

For use of this form, see AR 70-25 or AR 40-38; the proponent agency is OTSG

PRIVACY ACT OF 1974

Authority: 10 USC 3013, 44 USC 3101, and 10 USC 1071-1087.

Principle Purpose: To document voluntary participation in the Clinical Investigation and Research Program. SSN and home address will be used for identification and locating purposes.

Routine Uses: The SSN and home address will be used for identification and locating purposes. Information derived from the study will be used to document the study; implementation of medical programs; adjudication of claims; and for the mandatory reporting of medical conditions as required by law. Information may be furnished to Federal, State and local agencies.

Disclosure: The furnishing of your SSN and home address is mandatory and necessary to provide identification and to contact you. If future information indicates that your health may be adversely affected. Failure to provide the information may preclude your voluntary participation in this investigational study.

Approved by the WRAMC HUC/IRB on 25 Oct 2005 for WU # 05-93003 (2).
This form expires on 12 July 2006 Initials

PART A(1) – VOLUNTEER AFFIDAVIT

Volunteer Subjects in Approved Department of the Army Research Studies

Volunteers under the provisions of AR 40-38 and AR 70-25 are authorized all necessary medical care for injury or disease which is the proximate result of their participation in such studies.

I, _____, SSN _____

having full capacity to consent and having attained my _____ birthday, do hereby volunteer/give consent as legal representative for _____ to participate in _____

EVALUATING THE EFFECTS OF EXERCISE-INDUCED OXIDATIVE STRESS IN OVERWEIGHT SOLDIERS BEFORE AND AFTER WEIGHT LOSS

under the direction of **LTC Veronica Thurmond, AN, PhD, Nursing Research Service (202) 782-9887**

conducted at **WALTER REED ARMY MEDICAL CENTER, WASHINGTON, DC 20307-5001**

(Name of Institution)

The implications of my voluntary participation/consent as legal representative; duration and purpose of the research study; the methods and means by which it is to be conducted; and the inconveniences and hazards that may reasonably be expected have been explained to me by

MAJ Anne Andrews, MSIS, RD, LD, Nutrition Care Directorate, (202) 782-5331

I have been given an opportunity to ask questions concerning this investigational study. Any such questions were answered to my full and complete satisfaction. Should any further questions arise concerning my rights/the rights of the person I represent on study-related injury, I may contact

CENTER JUDGE ADVOCATE OFFICE – (202) 782-1550 OR DSN 662-1550

at **WALTER REED ARMY MEDICAL CENTER, WASHINGTON, DC 20307-5001**

[Name, Address and Phone Number of Hospital (Include Area Code)]

I understand that I may at any time during the course of this study revoke my consent and withdraw/have the person I represent withdrawn from the study without further penalty or loss of benefits; however, I /the person I represent may be required (military volunteer) or requested (civilian volunteer) to undergo certain examination if, in the opinion of the attending physician, such examinations are necessary for my/the person I represent's health and well-being. My/the person I represent's refusal to participate will involve no penalty or loss of benefits to which I/the person I represent is otherwise entitled.

LIMITATIONS TO MEDICAL CARE ARE DESCRIBED IN PART B

PART A (2) – ASSENT VOLUNTEER AFFIDAVIT *(MINOR CHILD)*

I, _____ SSN _____ having full capacity to assent and having attained my _____ birthday, do hereby volunteer for _____ to participate in _____

under the direction of _____

Conducted at _____

(Name of Institution

NOT APPLICABLE

(Continue on Reverse)

PART A(2) – ASSENT VOLUNTEER AFFIDAVIT (MINOR CHILD) (Cont'd)

The implications of my voluntary participation; the nature, duration, and purpose of the research study; the methods and means by which it is to be conducted; and the inconveniences and hazards that may reasonably be expected have been explained to me by

I have been given an opportunity to ask questions concerning this investigational study. Any such questions were answered to my full and complete satisfaction. Should any further questions arise concerning my rights I may contact

CENTER JUDGE ADVOCATE OFFICE – (202) 782-1550 OR DSN 662-1550

at **WALTER REED ARMY MEDICAL CENTER, WASHINGTON, DC 20307-5001**
[Name, Address, and Phone Number of Hospital (Include Area Code)]

I understand that I may at any time during the course of this study revoke my assent and withdraw from the study without further penalty or loss of benefits; however, I may be requested to undergo certain examinations if, in the opinion of the attending physician, such examinations are necessary for my health and well-being. My refusal to participate will involve no penalty or loss of benefits to which I am otherwise entitled.

LIMITATIONS TO MEDICAL CARE ARE DESCRIBED IN PART B

PART B - TO BE COMPLETED BY INVESTIGATOR

INSTRUCTIONS FOR ELEMENTS OF INFORMED CONSENT: (Provide a detailed explanation in accordance with Appendix C, AR 40-38 or AR 70-25

DESCRIPTION OF THIS STUDY

You are being asked to be in this research study because you are currently in the Army Weight Control Program (AWCP) or your body mass index is greater than or equal to 28. Your participation is entirely voluntary. Refusal to participate will not result in any penalty or loss of benefits to which you are otherwise entitled. If you choose not to participate in this research study, it will in no way impact your involvement in the AWCP.

The purpose of this study is to determine how the Army Physical Fitness Test affects the production of potentially harmful substances in the body called free radicals in overweight/obese soldiers. It will also determine if weight loss may be beneficial in lowering the production of these substances.

Other studies have shown that more free radicals are produced after exercise in overweight people compared to normal weight people.

If you agree to be in this study, you will also be asked to perform an Army Physical Fitness Test (APFT) once at the beginning of the study and once after you lose 10 pounds. These APFT's are separate and in addition to what the AWCP may have scheduled. A total of about 4-5 tablespoons of blood will be taken before and after each of the APFT's. Your body fat and lean body mass will be measured using a machine called DEXA (Dual Energy X-Ray Absorptiometry) at WRAMC. The DEXA scan uses X-rays to determine your lean body mass and body fat and is considered superior to other measurements of body fat. This is routinely used to test level of physical activity fitness. In this test, you will be asked to bicycle for two 3-

Approved by the WRAMC HUC/IRB on 25 Oct 2005 for WU # 05-93003 (2).
This form expires on 12 July 2006 Initials

I do ☐ do not ☐ (check one & initial) consent to the inclusion of this form in my outpatient medical treatment record.

SIGNATURE OF VOLUNTEER	DATE	SIGNATURE OF LEGAL GUARDIAN (If volunteer is a minor)
PERMANENT ADDRESS OF VOLUNTEER	TYPED NAME OF WITNESS	
	SIGNATURE OF WITNESS NOT APPLICABLE	DATE

REVERSE OF DA FORM 5303-R, MAY 89

PART B – TO BE COMPLETED BY INVESTIGATOR (Cont'd)

minute stages of continuous exercise to increase your heart rate. You will also be asked to complete several forms to collect general information about your dietary intake and physical activity level.

After a 10 pound weight reduction or after 3 months, you will be asked to perform a second APFT and we will perform the same measurements as the first time.

AMOUNT OF TIME FOR YOU TO COMPLETE THIS STUDY

You will be part of this study for approximately 3 months or until you have lost at least 10 pounds. During this time, you will be asked to visit the Nutrition Research Laboratory at WRAMC for a total of 6 times. Each visit will range from 15-90 minutes.

On visits 1 and 4, you will be asked to complete the questionnaires and have the DEXA scan and cycle ergometer tests done. These visits will take approximately 90 minutes each.

On visits 2 and 5, you will be asked to take the APFT and have your blood drawn before and after the test. These visits will take approximately 60 minutes each.

On visits 3 and 6, you will be asked to provide the final blood sample. This visit will take approximately 15 minutes.

APPROXIMATE NUMBER OF PEOPLE TAKING PART IN THIS STUDY

Up to 65 overweight soldiers will be recruited to participate in this study.

POSSIBLE RISKS OR DISCOMFORTS FROM BEING IN THIS STUDY

There will be some discomfort from drawing blood, and you will have swelling and a bruise at the site of the needle stick. Some people feel dizzy or light-headed for a few minutes after blood is drawn.

Your DEXA scans involve exposure to radiation. Although it can vary from person to person, your total whole-body radiation exposure of approximately 0.004 rem from these examinations will be well below the levels for adverse health effects to occur. The Health Physics Society in a Policy Statement dated 1996 indicated that for exposures below 10 rem, the risks of health effects are either too small to be observed or are non-existent.

POSSIBLE BENEFITS OF BEING IN THIS STUDY

You will not benefit from being in this study, however, the information that we learn from your participation may help us determine if there need to be changes in the military rations, physical fitness program or possibly health promotion guidelines.

Approved by the WRAMC HUC/IRB on 25 Oct 2005 for WU # 05-93003 (2).
This form expires on 12 July 2006 Initials

SIGNATURE OF VOLUNTEER	DATE	SIGNATURE OF LEGAL GUARDIAN (If volunteer is a minor)
PERMANENT ADDRESS OF VOLUNTEER	TYPED NAME OF WITNESS NOT APPLICABLE	
	SIGNATURE OF WITNESS	DATE

REVERSE OF DA FORM 5303-R, MAY 89

PART B – TO BE COMPLETED BY INVESTIGATOR (Cont'd)

CONFIDENTIALITY (PRIVACY) OF YOUR IDENTITY AND YOUR RESEARCH RECORDS

The principal investigator will keep records of your being in this study. These records may be looked at by people for the Walter Reed Department of Clinical Investigation, the Walter Reed Human Use Committee, the Army Clinical Investigation Regulatory Office (CIRO) and other government agencies as part of their duties. These duties include making sure that research subjects are protected. Confidentiality of your records will be protected to the extent possible under existing regulations and laws. Complete confidentiality cannot be promised, particularly for military personnel, because information bearing on your health may be required to be reported to appropriate medical or command authorities. Your name will not appear in any published paper or presentation related to this study.

This research study meets the confidentiality requirements of the Health Insurance Portability and Accountability Act (HIPAA). A HIPAA Authorization form for this study will be provided to you separately and you will be asked to sign that form.

If you decide to also participate in a similar study entitled, "Evaluation of Behavioral Theory and Integrated Internet/Telephone Technologies to Support Military Obesity and Weight Management Programs", certain information may be shared between the investigators. This information includes your DEXA scan and your 3-day food diary.

CONDITIONS UNDER WHICH YOU ARE TAKING PART IN THIS STUDY MAY BE STOPPED WITHOUT YOUR CONSENT

Your taking part in this study may be stopped without your consent if remaining in the study might be dangerous or harmful to you. Your taking part in this study may also be stopped without your consent if the military mission requires it, or if you become ineligible for medical care at military hospitals.

ELIGIBILITY AND PAYMENT FOR BEING IN THIS STUDY

You will not receive any payment for being in this study.

COMPENSATION TO YOU IF INJURED AND LIMITS TO YOUR MEDICAL CARE

Should you be injured as a direct result of being in this study, you will be provided medical care for that injury at no cost to you. You will not receive any compensation (payment) for injury. You should also understand that this is not a waiver or release of your legal rights. You should discuss this issue thoroughly with the principal investigator before you enroll in this study.

Medical care is limited to the care normally allowed for Department of Defense health care beneficiaries (patients eligible for care at military hospitals and clinics). Necessary medical care does not include in-home or nursing home care.

Approved by the WRAMC HUC/IRB on 25 Oct 2005 for WU # 05-93003 (2).
This form expires on 12 July 2006 Initials

SIGNATURE OF VOLUNTEER	DATE	SIGNATURE OF LEGAL GUARDIAN (If volunteer is a minor)
PERMANENT ADDRESS OF VOLUNTEER	TYPED NAME OF WITNESS NOT APPLICABLE	
	SIGNATURE OF WITNESS	DATE

REVERSE OF DA FORM 5303-R, MAY 89

PART B – TO BE COMPLETED BY INVESTIGATOR (Cont'd)

WHAT WILL HAPPEN IF YOU DECIDE TO STOP TAKING PART IN THIS STUDY AND INSTRUCTIONS FOR STOPPING EARLY

You have the right to withdraw from this study at any time. If you decide to stop taking part in this study, you should tell the principal investigator as soon as possible. By leaving this study at any time, you will in no way risk losing your right to medical care.

Please feel free to ask any questions that will allow you to clearly understand this study.

A copy of this consent will be provided to you.

SIGNATURE OF VOLUNTEER	DATE	SIGNATURE OF LEGAL GUARDIAN (If volunteer is a minor)
PERMANENT ADDRESS OF VOLUNTEER	TYPED NAME OF WITNESS NOT APPLICABLE	
	SIGNATURE OF WITNESS	DATE

REVERSE OF DA FORM 5303-R, MAY 89

Appendix E: HIPAA Consent Form

Authorization for Research Use of Protected Health Information

Protocol Title: Evaluating the Effects of Exercise-Induced Oxidative Stress in Overweight Soldiers Before and After Weight Loss

Principal Investigator: LTC Veronica Thurmond, AN, PhD **Work Unit #: 05-93003**

The Federal Health Insurance Portability and Accountability Act (HIPAA) includes a Privacy Rule that gives special safeguards to Protected Health Information (PHI) that is identifiable, in other words, can be directly linked to you (for example, by your name, Social Security Number, birth date, etc.). We are required to advise you how your PHI will be used.

1. What information will be collected?

For this research study, we will be collecting information about your diet, physical activity, personal and medical background, height, weight, waist size, body fat and lean body mass as well laboratory measurements related to oxidative stress and weight. Your name, e-mail address, telephone number, and date of birth will also be collected.

2. Who may use my PHI within the Military Healthcare System?

The members of the WRAMC research team will have access to your health information in order to find out if you qualify to participate in this study, to administer research treatments, to monitor your progress, and to analyze the research data. Additionally, your PHI may be made available to health oversight groups such as the WRAMC Department of Clinical Investigation and Human Use Committee.

3. What persons outside of the Military Healthcare System who are under the HIPAA requirements will receive my PHI?

The Principal Investigator or designee will send your research data that is collected to the University of Maryland for analysis by a statistician; however, all personal identifiers will be removed and the identity of the data will be unknown

4. What is the purpose for using or disclosing my Protected Health Information (PHI)?

The purpose of this study is to determine how the Army Physical Fitness Test affects the production of potentially harmful substances in the body called free radicals in overweight/obese soldiers. It will also determine if weight loss may be beneficial in lowering the production of these substances.

5. How long will the researchers keep my Protected Health Information?

The WRAMC research team in the Nutrition Care Directorate will keep the research data for up to three years after the end of the study. Then all the information will be destroyed. The master code will be destroyed as soon as all data collection is completed.

A PHOTOCOPY OF THIS FORM MUST BE SIGNED BY ALL VOLUNTEERS.
Approved by the WRAMC Privacy Board on 6 Sep 05 for WU # 05-93003
Edward M. Lund

6. Can I review my own research information?

You may look at your personal research information at any time.

7. Can I cancel this Authorization?
Yes. If you cancel this Authorization, you will no longer be included in the research study. However, the information that has already been collected will be kept by the research team to assure patient safety.

If you want to cancel your Authorization, please contact the Principal Investigator at 202-782-9887.

8. What will happen if I decide not to sign this Authorization?

If you decide not to sign this Authorization, you will not be able to participate in this research study. Refusal to sign this Authorization will not result in any loss of medical benefits to which you are otherwise entitled.

9. Can my Protected Health Information be disclosed to parties not included in this Authorization who are not under the HIPAA requirements?

There is a potential that your research information will be shared with another party not listed in this Authorization in order to meet legal or regulatory requirements. Examples of persons who may access your PHI include representatives of the Army Clinical Investigation Regulatory Office, the Food and Drug Administration, the DHHS Office for Human Research Protections, and the DHHS Office for Civil Rights. This disclosure is unlikely to occur, but in that case, your health information would no longer be protected by the HIPAA Privacy Rule.

10. Who should I contact if I have any complaints?

If you believe your privacy rights have been violated, you may file a written complaint with the WRAMC Privacy Officer, 6900 Georgia Ave., NW, Washington, DC 20307. Telephone: 202-782-3501.

The signature below acknowledges receipt of this Authorization:

Signature: _____ Date: _____

If you are a parent, court-appointed representative, or acting as power of attorney, indicate your authority to act for the participant: _____

Print Name: _____

A copy of this signed Authorization will be provided to you. 7/21/03

A PHOTOCOPY OF THIS FORM MUST BE SIGNED BY ALL VOLUNTEERS.
Approved by the WRAMC Privacy Board on 6 Sep 05 for WU # 05-9303
Edward Milano

Appendix F: IRB Approval Letters

DEPARTMENT OF THE ARMY
WALTER REED ARMY MEDICAL CENTER
WASHINGTON, DC 20307-5001

REPLY TO
ATTENTION OF:

MCHL-CI

12 August 2005

MEMORANDUM FOR 1LT Stacey Mobley, MC, Nutrition Care Directorate, Walter Reed Army Medical Center, Washington, DC 20307-5001

SUBJECT: Approval to Begin Protocol **Work Unit# 05-93003**: Evaluating the Effects of Exercise-Induced Oxidative Stress in Overweight Soldiers Before and After Weight Loss

1. Congratulations! Your protocol was approved with revisions by the Clinical Investigation Committee (CIC) on 21 June 2005 and by the Human Use Committee (HUC) on 12 July 2005 as a "greater than minimal risk" human use protocol. Please use the assigned seven (7) digits **Work Unit# 05-93003** for all correspondence with Department of Clinical Investigation (DCI) regarding this study as noted on item 5 below.

2. The last of the required revisions were received on 8 August 2005. A copy of the minutes from the applicable committee (s) and a final copy of the approved research protocol are attached for your administrative files. **Also, enclosed are the approved stamped consent form that must be duplicated and used for enrolling subjects, and the "STEP-BY-STEP GUIDE..." to be used when consenting subjects**. You may begin work on the project upon receipt of this letter. Your research protocol was approved for an enrollment of up to 65 patients at Walter Reed. This approval is only for **one year**. As part of your continuing review and re-approval and in order to keep your research ongoing, you are required to submit an annual progress report (APR) in the first week of **June** each year.

3. Funding in the total amount of $8,947.55 ($3,973.77 in FY05, $3,973.77 in FY06 for consumable supplies and $1,000 for travel) was approved. All DCI funding is contingent on the availability of funds in the DCI budget and the adherence to applicable spending guidelines and policies. All consumable supplies need to be ordered through DCI prior to 15 August of FY05 as funding after that date may be unavailable. Please coordinate this with Ms. Daisy Word, Chief, Research Administration Service, DCI, Building #6, Room 4009, (202) 782-7859.

4. Significant or unexpected side effects must be reported to the Medical Monitor of this study, LTC Patrick O'Malley, Staff, Department of Medicine, WRAMC.

5. As the principal investigator, you are required by Federal, DoD, and WRAMC regulations to submit the following in a timely fashion to the Department of Clinical Investigation if applicable: (a) addenda delineating any changes in the protocol, (b) PI change, (c) notification of serious or unexpected adverse effects within 24 hours, and (d) publication clearance, travel orders and funding requests.

6. **Also enclosed, is a copy of the NARMC DoD Multiple Project Assurance (MPA) and the WRAMC Federal -Wide Assurance (FWA) that all investigators agree to adhere to in conducting research, as attested to by your submission of a signed Principal Investigator Responsibilities Statement.** If you have any questions, the POC is Kendra Hill at (202) 782-7841.

4 Encls
as

CF: Research Administration Service

SUSAN D. FRASCISCO
LTC, MC
Chief, Research Review Service
Asst. Chief, Department of Clinical Investigation

Version Date: 1/6/2005

Institutional Review Board (IRB)/Independent Ethics Committee (IEC) Authorization Agreement

Name of Institution or Organization Providing IRB Review (Institution/Organization A):
Walter Reed Army Med Center

IRB Registration #: 00000662 Federalwide Assurance (FWA) #, if any: 00000477
Name of IRB (as listed by OHRP):
Walter Reed Army Medical Center #1

Name of Institution Relying on the Designated IRB (Institution B):
University of Maryland, College Park FWA #: FWA00005856

The Officials signing below agree that University of Maryland, College Park may rely on the designated IRB for review and continuing oversight of its human subjects research described below:

(___) This agreement applies to all human subjects research covered by Institution B's FWA.

(_X_) This agreement is limited to the following specific protocol(s):

Name of Research Project: Evaluating the Effects of Exercise-Induced Oxidative Stress in Overweight Soldiers Before and After Weight Loss
Name of Principal Investigator: Mark Kantor, PhD
Name of Co-Investigator: Veronica Thurmond, PhD, RN
Name of Student Investigator: Anne Andrews

Sponsor or Funding Agency: Walter Reed Army Medical Center Department of Clinical Investigation

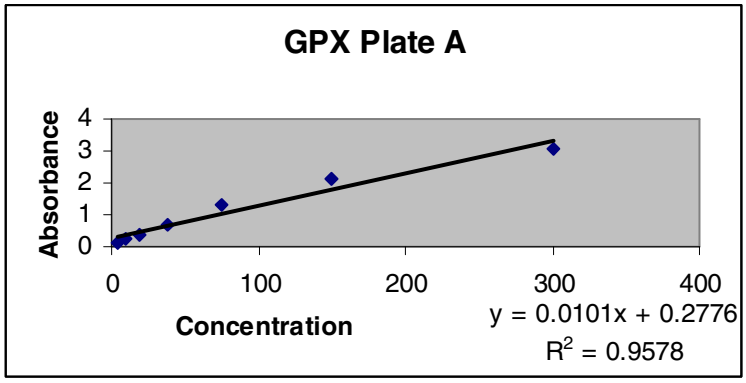
The review performed by the designated IRB will meet the human subject protection requirements of Institution B's OHRP-approved FWA. The IRB at Institution/Organization A will follow written procedures for reporting its findings and actions to appropriate officials at Institution B. Relevant minutes of IRB meetings will be made available to Institution B upon request. Institution B remains responsible for ensuring compliance with the IRB's determinations and with the Terms of its OHRP-approved FWA. This document must be kept on file by both parties and provided to OHRP upon request.

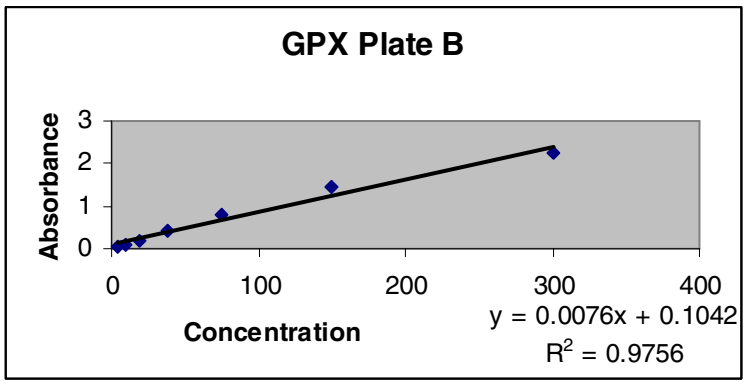
Signature of Signatory Official (Institution/Organization A):
Susan D. Fracisco MD, LTC, MC for Date: 11/18/05
Print Full Name: Maria Sjogren, COL MC Institutional Title: Chief, Department of Clinical Investigation

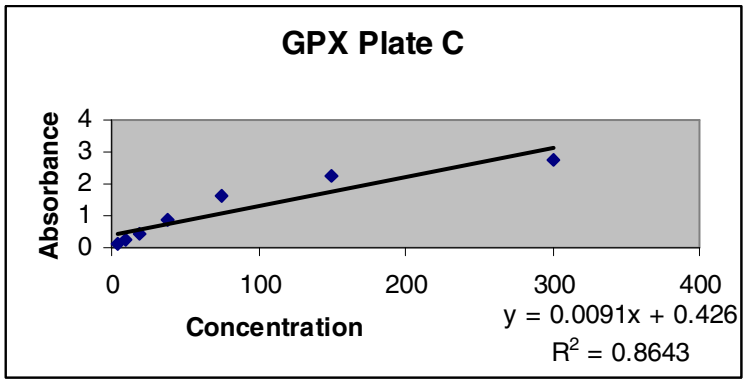
Signature of Signatory Official (Institution B):
Jacques Gansler Date: 10/14/05
Print Full Name: Dr. Jacques Gansler Institutional Title: Vice President for Research

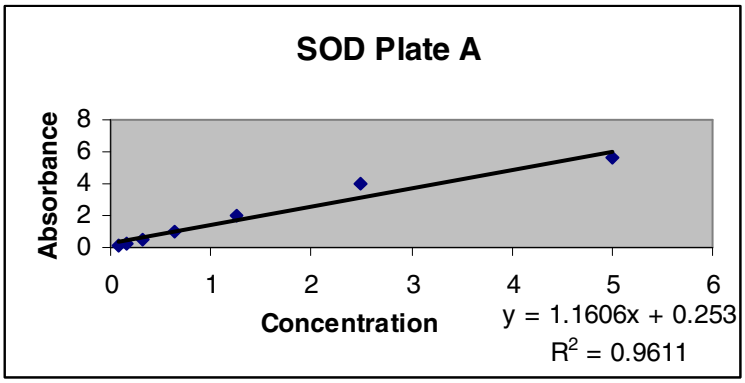
Appendix G: Standard Curves for GPX and SOD

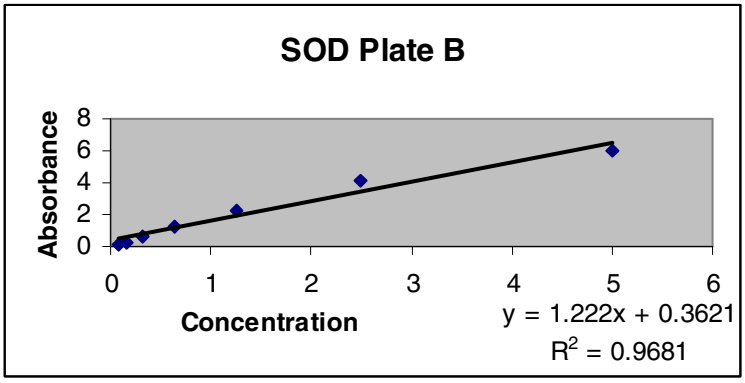
Standard curves for both assays were created by serial dilution.

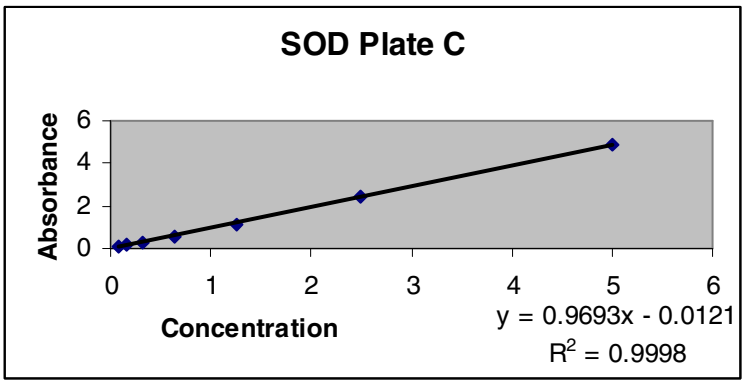







SOD Plate A
Absorbance
8
6
4
2
0
0
1
2
3
4
5
6
Concentration
y = 1.1606x + 0.253
R2 = 0.9611


SOD Plate B
Absorbance
8
6
4
2
0
0
1
2
3
4
5
6
Concentration
y = 1.222x + 0.3621
R2 = 0.9681


SOD Plate C
Absorbance
6
4
2
0
0
1
2
3
4
5
6
Concentration
y = 0.9693x - 0.0121
R2 = 0.9998

Appendix H: Raw data

ID	Age	Gender (1=M 2=F)	HT	WT	2-WT	BMI	2-bmi	VO2	2-vo2
1	25	1	69.1	214.1	.	31.6	.	43.3	.
4	43	1	69.1	221.1	218.5	32.6	32.2	36.7	.
5	29	2	66.5	182.8	176.3	29.2	28.1	34.8	.
7	46	1	66.0	189.8	187.1	30.7	30.3	34.8	.
8	53	2	67.0	176.0	.	27.6	.	37.4	.
11	29	1	66.4	203.8	.	32.5	.	.	.
12	39	1	74.0	226.0	215.0	29.0	27.6	29.7	33.6
13	25	1	73.7	244.3	.	31.7	.	49.8	41.2
14	40	1	68.1	185.3	174.9	28.1	26.6	42.3	42.9
16	35	2	60.4	162.0	160.6	31.3	31.0	33.5	.
17	20	2	61.0	143.0	153.5	27.1	29.1	31.0	40.1
18	27	1	68.0	224.4	217.0	34.2	33.0	35.4	.
19	41	2	68.6	208.0	202.4	31.2	30.3	36.5	36.3
20	37	2	65.6	201.4	.	32.9	.	34.5	.
21	36	1	68.0	216.3	206.1	33.0	31.4	39.8	.
23	38	1	68.0	239.7	227.1	36.5	34.6	29.6	34.1
26	23	1	68.2	218.1	.	33.1	.	.	.
27	38	2	66.8	186.7	183.7	29.4	29.0	34.4	.
28	39	2	64.9	170.6	.	28.5	.	.	.
29	39	1	71.9	266.0	260.4	36.3	35.5	24.6	33.5
30	36	1	64.8	219.0	.	36.8	.	29.4	.
31	27	2	66.9	177.4	174.3	28.0	27.5	27.0	.
32	37	1	72.7	216.7	213.2	28.9	28.4	50.2	45.0
33	27	2	66.8	172.3	173.7	27.2	27.4	29.3	.
34	43	1	70.5	204.3	193.2	29.0	27.4	34.0	31.5
35	36	2	66.2	193.0	199.4	31.0	32.1	24.4	25.8
36	33	2	63.9	187.0	.	32.2	.	29.3	.
37	34	2	64.6	156.0	151.5	26.3	25.6	34.6	34.8
38	24	1	66.6	229.5	227.7	36.4	36.2	44.2	46.1
39	30	1	72.9	252.3	.	33.4	.	31.8	.
40	39	2	66.8	209.3	.	33.1	.	24.2	.
41	29	2	67.6	197.4	193.2	30.4	29.8	34.8	.
43	28	2	62.0	154.1	169.2	28.2	31.0	30.7	.
44	31	1	71.7	231.4	227.3	31.7	31.2	32.6	41.2
45	51	1	69.3	192.6	189.7	28.2	27.8	31.3	52.5
46	29	1	68.5	212.4	197.5	31.9	29.6	29.7	29.8
47	22	1	68.2	194.4	192.0	29.5	29.1	28.7	34.9
50	24	1	70.4	246.3	241.8	35.0	34.3	41.2	.
51	23	1	65.6	177.5	.	29.1	.	55.5	.
52	23	1	72.0	203.8	206.2	27.7	28.0	28.5	52.5
53	37	1	73.6	245.8	245.8	32.0	32.0	24.4	41.8
55	39	2	63.0	161.9	152.6	28.7	27.1	46.0	32.9
56	39	1	72.0	245.6	225.3	33.4	30.7	23.8	25.3
57	26	1	71.9	204.3	.	27.8	.	.	.
58	43	1	68.8	222.7	.	33.2	.	46.6	.
59	38	2	60.1	178.1	166.9	34.8	32.6	29.8	30.1
60	32	2	59.5	155.7	.	30.9	.	32.3	.

ID	Trunk Fat mass	2-trfm	trunk % fat	2-trper	total fat mass	2-tofm	total % fat	2-toper	total lean mass	2-tolm
1	11215.2	.	25.4	.	22036.1	.	23.2	.	69756.8	.
4	13718.5	14591.8	29.5	31.6	25066.6	25733.0	25.3	26.2	70837.5	68970.6
5	11026.1	10315.3	30.2	30.4	29842.6	29176.1	36.4	37.0	49529.0	47106.8
7	15188.9	14462.5	36.2	35.8	26805.1	26784.7	31.5	31.9	55100.3	53848.0
8	14486.4	.	37.7	.	31869.9	.	40.3	.	44480.3	.
11	12480.0	.	28.8	.	23473.9	.	25.8	.	64611.8	.
12	13813.8	11860.7	27.8	26.5	25232.1	23442.9	24.9	24.3	72912.7	69395.3
13	17305.9	.	31.3	.	29909.6	.	27.2	.	76704.9	.
14	9974.2	8574.0	25.3	22.6	17988.4	15590.9	21.6	19.5	62286.7	61335.3
16	13732.2	12499.7	38.5	39.1	26926.3	26950.1	37.0	37.4	43674.5	43123.3
17	11888.5	14018.7	39.1	42.4	25873.7	29769.1	40.3	43.1	36473.2	37301.8
18	13096.7	13006.1	29.0	28.7	25168.1	23745.4	25.5	24.4	70103.0	70413.7
19	12593.3	11978.5	31.2	30.9	29924.8	28634.8	31.9	31.5	60499.4	58938.4
20	15408.7	.	36.6	.	31196.0	.	34.6	.	55785.2	.
21	11794.0	11309.6	26.5	26.4	27083.6	25544.1	28.0	27.7	66046.2	63436.9
23	16453.9	13585.4	31.3	27.1	30018.1	25682.5	27.9	25.2	74294.0	73110.9
26	14607.1	.	30.4	.	26942.3	.	27.5	.	67899.3	.
27	11709.6	12005.0	30.6	30.4	25326.0	25157.8	30.2	30.4	55247.1	54237.2
28	14186.8	.	41.0	.	29812.1	.	38.8	.	44417.6	.
29	17757.1	14526.7	33.1	27.8	33274.3	28878.1	27.9	24.6	81866.7	84225.3
30	11280.6	.	25.1	.	20876.9	.	21.8	.	71549.6	.
31	13026.1	12697.0	35.7	35.5	30324.0	29643.8	37.9	37.8	46919.0	46010.6
32	10886.9	11118.2	24.6	24.4	23215.5	22328.4	23.9	23.3	70775.5	70338.3
33	13972.7	14140.5	39.5	40.0	30819.3	31554.8	39.7	40.4	43985.9	43622.6
34	15280.6	12453.8	33.8	31.0	28927.4	26449.2	31.4	30.4	60091.8	57573.8
35	13726.9	16319.9	36.3	38.4	30361.6	33631.4	35.3	37.1	52737.1	54094.6
36	16091.6	.	39.9	.	31531.8	.	37.5	.	49985.9	.
37	8034.3	7759.6	24.7	24.1	19848.8	19421.4	28.2	28.5	48150.3	46347.4
38	13761.4	12893.2	28.2	27.2	25298.6	24574.9	24.7	24.3	74226.4	73447.4
39	16352.4	.	30.5	.	31138.9	.	27.4	.	78273.1	.
40	16966.7	.	39.4	.	35740.8	.	38.1	.	55056.8	.
41	18835.5	18690.4	42.6	42.8	34873.7	35296.5	39.4	40.7	51234.5	49082.3
43	8992.9	10654.8	29.4	32.5	22805.6	27048.8	32.5	35.6	44972.6	46557.7
44	18082.9	18186.5	37.2	36.8	35232.8	34025.7	33.9	33.3	65777.9	65142.9
45	8459.2	7783.5	20.8	18.9	15101.7	14000.2	17.5	16.4	67845.1	67835.5
46	14896.4	11781.6	35.5	30.3	29847.6	23971.4	31.4	27.1	62329.9	61450.5
47	12884.4	12836.5	31.4	31.3	26876.8	26227.9	30.8	30.5	57303.3	56658.2
50	15435.4	14316.7	31.8	30.4	35135.3	33698.5	31.7	30.9	71665.3	71364.2
51	11065.4	.	29.6	.	21429.3	.	26.9	.	55501.8	.
52	13770.1	12915.3	32.9	30.5	26920.4	25340.5	29.4	27.4	61753.9	64254.5
53	19898.8	17415.5	37.2	33.1	34743.2	32065.7	31.5	29.1	71785.0	74451.2
55	8097.4	6767.3	25.6	23.4	23248.0	20485.0	31.9	30.0	47169.0	45501.1
56	16045.0	13190.3	30.3	27.0	31629.0	27096.8	28.8	26.8	75183.0	70844.3
57	13060.2	.	32.2	.	27712.3	.	30.3	.	60996.0	.
58	13508.3	.	30.0	.	23829.5	.	23.8	.	72371.9	.
59	13326.0	12531.9	35.5	34.6	27508.9	25536.7	34.4	34.0	50005.2	47142.1
60	11892.4	.	38.7	.	27956.1	.	40.0	.	39864.2	.

ID	Kcal	2-kcal	CHO (gm)	2-cho	Pro (gm)	2-pro	Fat (gm)	2-fat
1	2431.1	.	305.8	.	96.3	.	93.2	.
4	2019.9	.	287.7	.	85.3	.	66.7	.
5	1496.3	.	226.3	.	60.7	.	40.1	.
7	1554.1	.	239.6	.	63.7	.	40.7	.
8	.	.	.	.	.	.	.	.
11	2851.8	.	278.2	.	184.9	.	112.1	.
12	3071.1	.	362.0	.	96.6	.	142.2	.
13	1693.9	.	216.4	.	62.1	.	64.0	.
14	1983.9	.	266.7	.	82.5	.	62.4	.
16	1504.4	.	180.4	.	75.1	.	56.0	.
17	2256.3	.	281.4	.	87.7	.	93.0	.
18	2431.6	.	396.2	.	81.9	.	54.8	.
19	1676.6	1093.2	156.2	159.6	90.1	74.1	79.3	23.0
20	902.0	.	113.4	.	46.6	.	29.6	.
21	1237.0	642.9	150.5	95.7	66.6	36.8	45.1	16.5
23	2055.2	.	279.6	.	94.3	.	63.4	.
26	.	.	.	.	.	.	.	.
27	1337.0	.	186.2	.	62.5	.	39.7	.
28	.	.	.	.	.	.	.	.
29	1928.6	2042.4	166.9	176.4	102.4	99.1	95.2	89.3
30	1364.2	.	135.7	.	88.2	.	53.0	.
31	.	1171.4	.	176.0	.	55.8	.	27.0
32	2843.1	.	297.5	.	112.7	.	101.2	.
33	1611.2	1409.4	142.7	172.9	91.2	72.9	71.5	47.4
34	1119.8	1361.7	145.1	165.5	56.6	73.5	39.1	49.0
35	1105.0	.	143.6	.	82.3	.	21.5	.
36	1547.1	.	239.9	.	56.4	.	43.9	.
37	1525.6	1655.5	212.6	243.2	50.8	73.2	54.5	40.2
38	2193.9	.	326.8	.	97.3	.	62.7	.
39	3891.5	.	554.0	.	119.7	.	134.6	.
40	2603.4	.	374.0	.	103.9	.	83.1	.
41	1517.0	1129.3	202.7	188.3	63.8	62.5	54.4	19.6
43	2019.9	.	287.7	.	85.3	.	66.7	.
44	.	2158.4	.	212.4	.	89.2	.	82.8
45	1935.6	.	256.2	.	54.6	.	80.4	.
46	1382.8	1093.2	164.7	159.6	75.8	74.1	46.0	23.0
47	1410.9	.	210.7	.	43.8	.	45.9	.
50	.	.	.	.	.	.	.	.
51	.	.	.	.	.	.	.	.
52	.	.	.	.	.	.	.	.
53	1716.4	.	191.7	.	102.6	.	58.1	.
55	1111.1	1574.8	138.6	213.2	56.5	59.0	35.2	48.1
56	1072.0	715.8	87.5	41.4	79.5	50.1	47.8	37.9
57	3839.0	.	476.4	.	187.8	.	128.2	.
58	2330.7	.	373.3	.	73.2	.	65.8	.
59	1403.3	1217.2	239.5	196.4	45.7	70.8	33.0	18.1
60	.	.	.	.	.	.	.	.

ID	Vit A (mcg RE)	2-vita	Vit E (mg ATE)	2-vite	Vit C (mg)	2-vitc	Sel (mcg)	2-sel	Copper (mg)	2-cop	Zinc (mg)	2-zinc
1	214.5	.	2.8	.	97.3	.	66.3	.	0.5	.	4.8	.
4	392.9	.	0.9	.	9.7	.	29.2	.	0.6	.	8.3	.
5	260.6	.	0.6	.	183.2	.	36.3	.	0.2	.	3.6	.
7	760.1	.	3.0	.	142.6	.	26.4	.	0.4	.	12.9	.
8	.	.	.	.	.	.	.	.	.	.	.	.
11	547.9	.	3.2	.	15.3	.	157.6	.	1.1	.	30.9	.
12	275.8	.	2.1	.	22.6	.	49.8	.	0.5	.	4.9	.
13	119.9	.	0.2	.	3381.9	.	0.2	.	0.0	.	0.3	.
14	45.3	.	0.8	.	34.0	.	78.7	.	0.5	.	4.2	.
16	124.2	.	0.7	.	45.4	.	93.2	.	0.7	.	13.8	.
17	95.4	.	0.7	.	51.2	.	16.3	.	0.3	.	2.3	.
18	482.2	.	0.9	.	1013.8	.	156.2	.	0.3	.	22.8	.
19	106.9	175.2	1.1	0.7	35.8	64.1	46.9	7.3	0.4	0.5	8.6	3.8
20	131.7	.	0.6	.	17.5	.	44.5	.	0.2	.	1.5	.
21	507.9	162.6	2.9	1.2	57.9	20.9	49.3	39.2	0.8	0.5	7.0	3.7
23	748.3	.	5.7	.	198.5	.	74.0	.	1.0	.	17.0	.
26	.	.	.	.	.	.	.	.	.	.	.	.
27	840.9	.	1.3	.	77.2	.	20.9	.	0.4	.	2.7	.
28	.	.	.	.	.	.	.	.	.	.	.	.
29	177.1	239.0	1.7	1.6	23.5	13.2	88.5	86.9	0.3	0.4	3.2	4.8
30	117.0	.	1.2	.	25.6	.	149.6	.	0.5	.	4.8	.
31	.	113.8	.	0.8	.	13.9	.	52.3	.	0.4	.	2.9
32	163.3	.	3.1	.	2107.6	.	73.4	.	2.2	.	22.2	.
33	494.9	238.4	1.4	1.9	119.2	61.9	82.9	58.0	1.1	0.3	15.3	3.5
34	103.6	88.2	0.4	0.2	55.1	27.7	14.3	1.8	0.4	0.0	3.6	0.9
35	50.8	.	1.0	.	159.1	.	59.5	.	0.4	.	4.7	.
36	76.6	.	0.8	.	166.4	.	24.5	.	0.3	.	2.5	.
37	406.8	211.6	2.1	0.5	92.8	85.0	39.8	26.4	0.3	0.1	3.3	3.5
38	1076.2	.	2.5	.	323.0	.	45.7	.	1.0	.	11.3	.
39	179.6	.	1.9	.	22.5	.	73.5	.	0.6	.	5.5	.
40	17.7	.	0.9	.	100.9	.	44.1	.	0.3	.	5.5	.
41	82.6	733.9	1.1	1.2	54.1	78.4	13.4	37.2	0.4	0.5	2.8	5.8
43	392.9	.	0.9	.	2189.5	.	29.2	.	0.6	.	8.3	.
44	.	234.3	.	0.8	.	22.2	.	42.2	.	0.3	.	2.5
45	239.1	.	2.5	.	70.2	.	35.3	.	0.6	.	5.1	.
46	29.5	175.2	0.2	0.7	73.7	64.1	26.7	7.3	0.3	0.5	2.6	3.8
47	10.3	.	0.8	.	83.0	.	33.2	.	0.3	.	3.0	.
50	.	.	.	.	.	.	.	.	.	.	.	.
51	.	.	.	.	.	.	.	.	.	.	.	.
52	.	.	.	.	.	.	.	.	.	.	.	.
53	79.1	.	0.9	.	37.1	.	92.7	.	0.4	.	6.6	.
55	171.9	124.2	1.2	1.7	30.9	68.2	3.0	20.0	0.2	0.5	0.6	7.6
56	496.7	0.0	4.0	0.0	83.7	10.0	93.1	0.00	0.7	0.0	6.7	0.1
57	525.5	.	2.9	.	74.2	.	127.1	.	1.8	.	14.5	.
58	101.1	.	0.8	.	35.5	.	8.7	.	0.5	.	3.9	.
59	167.3	63.0	9.7	1.1	175.5	274.4	0.2	30.3	0.3	1.1	10.1	7.5
60	.	.	.	.	.	.	.	.	.	.	.	.

	CK (U/L)						CRP (mg/dL)					
ID	Pre	2pre	Post	2post	24hr	224h	Pre	2pre	Post	2post	24hr	224hr
1	292	.	371	.	621	.	0.112	.	0.123	.	0.190	.
4	64	113.00	121	135.00	.	.	0.146	0.138	0.185	0.118	.	.
5	102	.	143	.	.	.	0.045	.	.	.	.	.
7	2221	101.00	3014	161.00	1488	.	0.655	0.410	0.673	0.430	0.604	.
8	59	.	.	.	67	.	0.991	.	.	.	0.876	.
11	190	.	268	.	.	.	0.021	.	0.034	.	.	.
12	195	157.00	303	207.00	245	.	0.202	0.035	0.208	0.036	0.125	.
13	249	.	405	.	.	.	0.112	.	0.104	.	.	.
14	217	178.00	284	230.00	368	.	0.057	0.093	0.065	0.113	0.120	0.584
16	112	105.00	129	130.00	206	178.00	0.371	0.410	0.349	0.450	0.396	0.490
17	88	181.00	117	225.00	.	.	0.878	.	0.916	0.974	.	.
18	409	182.00	502	264.00	607	.	0.132	0.030	0.132	0.030	0.152	.
19	273	856.00	322	994.00	.	.	0.021	0.148	.	0.155	.	.
20	.	.	.	.	.	.	0.451	.	0.357	.	.	.
21	63	58.00	92	85.00	91	.	0.051	0.020	0.062	0.023	0.704	.
23	119	60.00	170	92.00	245	219.00	0.459	0.260	0.544	0.310	0.554	0.470
26	207	.	264	.	253	.	0.108	.	0.119	.	0.102	.
27	91	63.00	119	87.00	.	.	0.069	0.090	0.060	0.100	.	.
28	140	.	188	.	.	.	0.169	.	0.188	.	.	.
29	610	527.00	676	605.00	816	811.00	0.156	0.080	0.150	0.080	0.119	0.080
30	362	.	388	.	515	.	0.049	.	0.047	.	0.049	.
31	108	92.00	139	128.00	154	.	0.133	0.230	0.142	0.220	0.127	.
32	90	87.00	129	123.00	166	.	0.058	0.071	0.058	0.076	0.160	.
33	40	37.00	54	57.00	201	89.00	0.063	0.030	0.061	0.040	0.122	0.040
34	96	84.00	129	110.00	164	139.00	0.328	0.610	0.306	0.630	0.743	1.170
35	107	90.00	145	113.00	178	.	0.300	0.260	0.298	0.280	0.405	.
36	155	.	184	.	277	.	0.394	.	0.416	.	0.286	.
37	53	81.00	84	117.00	100	87.00	0.157	0.020	0.188	0.020	0.143	0.050
38	198	139.00	278	198.00	276	428.00	0.054	0.030	0.068	0.030	0.130	0.070
39	135	.	196	.	.	.	0.471	.	0.511	.	.	.
40	117	.	164	.	.	.	0.484	.	0.513	.	.	.
41	116	159.00	144	190.00	.	152.00	0.340	0.070	0.380	0.070	.	0.125
43	146	.	187	.	.	.	0.503	.	0.559	.	.	.
44	72	249.00	105	326.00	104	155.00	0.210	0.320	0.220	0.350	0.194	0.230
45	622	354.00	859	440.00	16626	.	0.020	0.012	0.022	0.012	0.020	.
46	78	469.00	155	593.00	238	458.00	0.110	0.098	0.110	0.101	0.180	0.106
47	.	112.00	.	176.00	.	.	.	0.155	.	0.155	.	.
50	228	262.00	279	.	280	160.00	0.240	0.930	.	.	0.280	0.487
51	69	.	136	.	154	.	0.020	.	0.020	.	0.040	.
52	106	359.00	167	426.00	382	273.00	0.030	0.054	0.030	0.057	0.070	0.036
53	350	343.00	444	435.00	946	556.00	0.670	1.371	0.718	1.436	1.300	1.958
55	68	58.00	84	71.00	.	.	0.052	0.025	0.060	0.026	.	.
56	95	72.00	150	114.00	116	.	0.630	0.202	0.680	0.221	.	0.215
57	195	.	207	.	.	.	0.147	.	0.138	.	.	.
58	466	.	533	.	.	.	0.060	.	0.120	.	.	.
59	110	108.00	163	173.00	.	255.00	0.150	0.019	0.150	0.022	0.029	.
60	221	.	251	.	.	.	0.300	.	0.330	.	.	.

ID	SODPre	2sodpre	sodPost	2sodpost	GPXPre	2gpxpre	gpxPost	2gpxPost
1	1.00	.	0.32	.	72.84	.	67.79	.
4	0.06	2.58	0.39	2.92	91.16	29.94	68.06	54.28
5	0.79	.	1.36	.	217.49	.	159.17	.
7	1.08	2.06	2.58	3.02	73.12	43.90	67.94	34.00
8	0.01	.	0.56	.	118.34	.	104.63	.
11	0.89	.	0.78	.	30.07	.	43.23	.
12	0.54	1.96	0.14	0.67	73.79	31.85	103.13	51.79
13	0.59	.	0.58	.	67.74	37.33	58.21	38.85
14	0.87	1.25	0.96	2.39	78.40	48.56	62.62	44.10
16	0.21	0.85	0.51	1.25	72.84	46.82	67.79	45.95
17	0.28	1.48	0.82	1.73	73.80	43.22	53.84	46.62
18	0.25	0.98	0.40	1.42	62.58	52.14	63.91	34.31
19	0.55	0.68	0.75	1.89	41.68	21.54	42.82	57.93
20	2.01	.	0.03	.	91.16	.	68.06	.
21	0.18	1.55	0.05	2.34	39.60	16.47	77.46	38.28
23	0.17	0.74	0.88	0.86	78.22	55.30	61.56	39.67
26	2.16	.	3.19	.	217.49	.	159.17	.
27	0.52	.	0.39	.	71.69	174.68	67.93	126.11
28	0.13	.	0.17	.	70.12	.	83.98	.
29	1.05	1.27	1.10	1.78	2.59	42.07	103.39	51.93
30	1.11	.	0.27	.	46.48	.	15.47	.
31	1.08	0.35	0.64	0.25	73.12	36.58	67.94	51.80
32	0.02	2.16	0.75	1.85	91.56	30.96	39.42	40.89
33	1.94	1.33	2.54	1.57	30.07	44.27	43.23	26.53
34	1.72	0.06	0.54	0.87	57.79	53.30	73.66	32.44
35	1.18	0.68	1.76	0.55	73.79	60.22	103.13	25.78
36	1.52	.	1.35	.	67.74	.	58.21	.
37	0.74	0.75	1.12	1.01	78.40	27.01	62.62	40.67
38	1.05	2.70	0.82	3.42	118.34	43.18	104.63	62.74
39	1.97	.	2.28	.	73.80	.	53.84	.
40	0.12	.	0.10	.	62.58	.	63.91	.
41	1.22	.	1.08	.	41.68	139.41	42.82	220.80
43	0.71	.	0.73	.	39.60	.	45.66	.
44	1.02	0.90	0.89	2.22	78.22	42.91	61.56	60.37
45	.	.	.	.	171.78	143.88	134.28	177.77
46	1.32	.	2.03	.	2.59	120.68	103.39	144.21
47	1.02	.	0.38	.	46.48	58.14	15.47	62.22
50	1.38	.	0.20	.	57.79	308.44	73.66	.
51	.	.	.	.	102.23	.	125.80	.
52	.	.	.	.	175.18	219.86	185.71	166.73
53	.	.	.	.	.	.	.	.
55	1.82	.	1.33	.	71.69	135.54	67.93	138.75
56	.	.	.	.	137.00	112.79	130.31	104.18
57	2.40	.	1.41	.	70.12	.	83.98	.
58	.	.	.	.	170.69	.	135.43	.
59	.	.	.	.	105.80	135.83	107.48	113.84
60	.	.	.	.	76.77	.	99.21	.

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