SELENIUM SUPPLEMENTATION AS A TREATMENT FOR MILD-MODERATE DEPRESSION IN THE ELDERLY

by

LELA RUTH TATUM, B.S., M.S.

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ABSTRACT

Prior to the elucidation of selenium's role in the action of glutathione peroxidase in 1973, the majority of the research of the mineral involved the debilitative effects in its action as a pro-oxidant in ruminants. Since this time, most research has focused on the antioxidative capabilities of this mineral/enzyme. Minimal contributions of the role of selenium in the brain and nervous system have been presented in the scientific literature. Those studies which do attempt to establish a connection a benefit of selenium to healthy brain and nervous system functioning do so by examining the effects of nutrition as a whole in conditions and populations severely affected by neurological disease such as a Alzheimer's disease in the elderly.

This dissertation examines the role of selenium on depression in the elderly. The subjects were randomly separated into two treatment groups examining two selenium compounds and a control group. The treatment period lasted a total of seven weeks and psychological measures in the form of the Beck Depression Inventory II (BDI-II) were given at the beginning and terminus of the study. Biological measures were made in the form of total plasma selenium concentration. With positive correlations, selenium compounds may be a cost-effective and safer treatment options for elderly people with mild to moderate depression.

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CHAPTER I

INTRODUCTION

Selenium was first understood to be biologically essential for the prevention of liver necrosis in vitamin E deficient rats in research conducted by Klaus and Schwarz in 1957. When present in the diet, selenium induces the synthesis of antioxidant enzymes such as cytosolic glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase as well as the 5'-deiodinases. Selenium is found in most all human tissues and is reported to have immune enhancing effects (Burk, 1989; Combs & Combs, 1986; Rayman, 2000).

Dietary Selenium Sources and Absorption

Selenium exists in soils as basic ferric selenide, calcium selenate, and elemental selenium. Organic compounds such as selenomethionine are derived from plants. Concentrations of selenium compounds in the soil, and foods produced from these soils, can vary greatly by region (Combs & Combs, 1986). Depending on the locale from which the animal acquires its diet, dietary intake of selenium also will vary.

In the human diet, whole grains and meats are the best sources of selenium to be consumed. Particularly high in the mineral are fish and organ meats, such as liver and kidney, as well as muscle tissue which serves as the major reservoir for selenium in the animal (Burk, 1989; Combs & Combs, 1986; Schwarz & Pathak, 1975). Selenium exists in several different forms depending on the food consumed. Selenomethionine is the major form in grains, soy yeast, and animal/plant tissue. In onions, garlic, and leeks the

major compound is Se-methylselenocysteine (SeMC) (Whanger, 2002). Inorganic forms such as selenite and selenate are converted into selenocysteine *in vivo* for incorporation into glutathione peroxidase (GSHPx). For humans, a DRI of 55μg/day has been established (Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids, 2000).

The absorption and retention of selenium from the gastrointestinal tract is dependent on the chemical form and the amount ingested. Studies on physiological levels of radioselenium indicate that the duodenum is the main site of absorption in humans, with no absorption from the rumen in ruminants (Koenig, Rode, Cohen & Buckley, 1997; Rannen, Hylander, Ladefoged, Staun, Tjelleson & Jarnum, 1996). Selenomethionine is virtually 100% absorbed and then incorporated into human protein. It is later converted to selenocysteine whereas inorganic compounds are readily excreted. Absorbed selenium is at first carried mostly in the plasma in association with plasma proteins such as selenoprotein-P and is then deposited in all tissues. Most of the tissue selenium is highly conserved and after transition from selenium adequate or selenium toxic diets to a lower dietary selenium intake, the loss of selenium is rapid at first then tapers off (Schwartz & Pathak, 1975).

Biochemical Function of Selenium

Selenium's first biochemical role was described in 1973 as a component of the antioxidant enzyme glutathione peroxidase (GSHPx) (Rotruck, Pope, Ganther, Swanson, Hafeman & Hoekstra, 1973). The prominent role of GSHPx is to act as a catalyst for the reduction of peroxides to form water in an effort to prevent attack of cell membranes and

nucleic acids by reactive oxygen species such as hydrogen peroxide. GSHPx's action is similar to that of catalase except that it is more efficient and plays a limited role in bacterial metabolism (Schwarz & Pathak, 1975).

The reaction scheme is as follows:

$$H_2O_2 + 2\,GSH \xrightarrow{GSHPx} GSSG + 2\,H_2O$$

$$ROOH + 2\,GSH \xrightarrow{GSHPx} GSSG + ROH.$$

When neither catalase nor GSHPx is present working alongside superoxide dismutase, superoxide would ultimately become the much more destructive hydroxyl radical (·OH).

Glutathione peroxidase works in conjunction with glutathione to convert the potentially damaging hydrogen peroxide and hydroperoxides into the benign species of water and alcohol, respectively. A second selenium enzyme, phospholipid hydroperoxide glutathione peroxidase (PHGPx) was isolated in 1985 (Ursini, Maiorino & Gregolin, 1985). This enzyme solved the mystery as to the method by which phospholipid hydroperoxides are detoxified. This information indicated that both components of the cell, aqueous and lipid, are under the protective control of two distinct selenium containing enzymes.

Selenium exists as the amino acid selenocysteine in both of these enzymes. Selenocysteine is a derivative of serine from which the oxygen of the hydroxyl group is replaced by selenium at the active site. Selenocysteine is incorporated into selenoproteins at UGA codons which usually exists as a stop codon. Selenium is essential in the diet due to the fact that both enzymes are dependent upon it for their actions as antioxidants. Once an animal has reached a selenium deficiency from the lack

of dietary selenium, oxidative damage is rapid. This results in liver necrosis in rodents, myopathies in several species and exudative diathesis in chickens (Schwarz & Pathak, 1975). The most common manifestation of a long-term selenium deficiency in humans is a cardiomyopathy known as Keshan disease.

Many studies have focused on examining the effect of selenium on various cancer cell lines (Schrauzer, 1992; Spallholz, 1994). Many epidemiological studies have shown that selenium may play a role in the prevention of skin, colorectal, and prostate cancers. Selenium is thought to have a function in the induction of cellular apoptosis. A hypothesis exists that thiols possibly initiate apoptosis in HL60 cells (which are prone to undergo apoptosis in response to a number of agents) by introducing oxidative stress to the system. Apoptosis is an adaptive mechanism of the cell that leads to death rather than permitting free radical induced mutations in DNA that may lead to development of cancerous cell lines.

Selenoproteins also play a role in the development of thyroid hormones, prevention of cardiovascular disease, and regulation of immune function. In addition to residing in the functional site of the glutathione peroxidases, selenocysteine is crucial in the development of the iodothyronine deiodinases (thyroid hormone activity), thioredoxin reductases (regulation of gene expression, cell proliferation), and methionine sulfoxide reductases for protein repair (Stadtman, 1996). Through genomic mapping it has been found that more than 30 genes code for the incorporation of selenocysteine. Of these resultant enzymes, the list of those with unknown functions continues to grow (Castellano, Morozova, Morey, Berry, Serras, Corominas, & Guigo, 2001)

Biological Basis for Depression

Estimates are that 5 to 12% of men and 10 to 20% of women in the United States will suffer from a major depressive episode at some time in their life. The part of the brain which is of greatest interest to those who study the physical causes of depression is the limbic system. The structures within this system include the hypothalamus, hippocampus, and amygdale. Many cases of depression apparently stem, at least partially, from disturbances in brain circuits that convey signals through certain neurotransmitters of the monoamine class. These biochemicals, all derivatives of amino acids, include serotonin, norepinephrine and dopamine; of these, only evidence relating to norepinephrine and serotonin is abundant in the literature (Nemeroff, 1998).

While the incidence of depression in modern society is striking, there was been limited research on how neurotransmitters affect mood. What is hypothesized, however, is that antidepressant medications function by regulating the amount of these neurotransmitters in the brain. However, it is not clear how or why there is varied response to these medications. Antidepressant pharmaceuticals are not effective in all cases and depending on brain chemistry, what effectively treats one person may not have any effect on another.

What limits the study of neurotransmitters and depression is the difficulty by which they are studied. Neurotransmitters are present in very small quantities, are present in only certain regions of the brain, and have a very short half-life. Because of these factors, neurotransmitters cannot be measured directly and only their metabolites are available to be studied in blood, urine, and cerebrospinal fluid.

As evidenced by the variability in response to treatment strategies, it is unknown whether changes in neurotransmitter levels are a cause of depression and/or the disease state itself causes changes in concentrations of neurotransmitters in the brain. It has been hypothesized that behavior can affect brain chemistry as well. Stress or trauma may cause changes in brain chemistry which manifest as depressive symptoms. Alternatively, behavior modification and utilization of coping strategies may alleviate depression.

In addition to the nervous system, the endocrine system has garnered attention as to its role in the development of depression. There have been reports of abnormal hormonal levels in people experiencing clinical depression although they have otherwise healthy endocrine glands. Hormonal disorders may be intertwined with the changes in brain chemistry that are seen in depression. Conditions such as thyroid disorders, Cushing's disease and Addison's disease all include depression as a symptom of their etiology. The endocrine system's connection to the brain is at the hypothalamus which in turn regulates the function of the pituitary gland. The neurotransmitters of the hypothalamus, serotonin, dopamine, and norepinephrine all play a role in the management of hormone function.

CHAPTER II

REVIEW OF LITERATURE

Within the past thirty years there has been a wealth of information published recognizing a link between nutrition and mental health. Many nutrients and herbals have been studied to determine their effects on mental functioning in humans. Most frequently studied are those that are purported to halt memory loss (van Dongen, van Rossum, Kessels, Sielhorst & Knipschild, 2000) or treatment of depression (Bottiglieri, Laundry, Crellin, Toone, Carney & Reynolds, 2000; Benton, Griffiths & Haller, 1997; Baldewicz et al, 1998; Penninx, Guralnik, Ferucci, Fried, Allen & Stabler, 2000; Cervantes, Ghadirian & Vida, 1999). The mechanisms through which these nutrients act upon depression are not completely understood but appear to be varied and correlate with serum levels of nutrients and herbal compounds.

Depression and Oxidative Stress

Through enzymatic action of the glutathione peroxidases and the action of selenoprotein P, selenium plays a role in the reduction of hydrogen peroxide and hydroperoxides to reduce oxidative stress in the system. This holds true for neurological tissue as well as other tissue types in the human body. Selenium also acts as a "binding agent" and thus eliminates heavy metals from a system. Both of these functions of selenoproteins are important in the maintenance of good mental health as oxidative damage in the brain has been linked to neurological diseases such as Alzheimer's Disease and Parkinson's Disease.

While the role selenium may play in the propagation of brain diseases has been broached, the limitations of enzymatic research in living nervous tissue restricts the inferences which can been made. From cadaver studies it has been shown that regions of the brain containing more gray matter have higher selenium levels and selenium appears to concentrate in glandular parts in the brain. Additionally, it has been shown in animal studies that selenium is highly conserved in brain when the element is deficient from the diet. This implies that selenium may play a critical role in reducing oxidative stress in this essentially closed system. It is also known that glutathione peroxidase activity in animal brain is age-related showing a continuous decrease with age.

As with other brain diseases, it has been suggested that some forms of depression may be consistent with the presence of oxidative stress in the brain. A 2003 study (Khanzode, Dakhale, Khanzode, Saoji & Palasodkar, 2003) examined the effect of the selective serotonin reuptake inhibitors (SSRIs), fluoxetine and citalopram on antioxidant (superoxide dismutase) activity, ascorbic acid concentrations, and lipid peroxidation. In major depression there is a significant increase in superoxide dismutase and malondialdehyde (an indicator of lipid peroxidation) and decreased ascorbic acid in the plasma when compared to age/sex matched controls. After treatment with the standard SSRIs, the trend was reversed.

Selenium and Mental Health

Much more research has been conducted with selenium to determine its safety and efficacy of the mineral as a supplement than for use as a mood altering treatment (Kelly & MacEvilly, 2001). According to published research, what is known is that those

patients who are chronically depressed have statistically significant lower plasma selenium concentrations than those who do not report experiencing depression. The current average daily intake of selenium in the United States for adults is approximately 76μg, (Longnecker et al, 1991). In the United Kingdom the average intake of selenium is 43 μg of selenium/day (Benton & Cook, 1991) which is below the recommendation of 55μg/day (Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids, 2000). The 1991 study by Benton and Cook was the first time selenium's effect on depression was examined. The subjects monitored themselves and reported their feelings and mood over a period of five weeks while their diet was supplemented with 100μg of selenium per day of yeast-based selenium tablets. The Benton and Cook study (1991) reported that the lower the selenium content of the diet, the greater likelihood the subjects reported experiencing anxiety and depression. When selenium was added to the diet, reported anxiety and depression subsided.

Hawkes and Hornbostel (1995) examined the effect of selenium on the mood of a small group of healthy men. Little effect was observed in this group purportedly due to the fact that these men were cognitively normal and had higher dietary intake levels of selenium than subjects in previous studies. This result suggests that selenium supplementation will not elevate the mood of normal subjects but may have an effect on those who experience mental depression.

Diagnosis of Depression in the Elderly

The diagnosis of mental illness presents some unique challenges in the aged population. There are many contributing factors that must be taken into consideration

when attempting to determine a diagnosis in this population. Importance is placed upon evaluating elderly clients for depression in that with proper treatment it may extend the lifespan and increase the quality of life of these people (Heidrich, 1994).

There is the common belief that depression is part of the normal aging process. This misconception exists among young and old alike. Often, the elderly are reluctant to admit that they are suffering from depression for fear of being stigmatized by family members and society as being "lazy" or "self-absorbed" (Marwaha & Livingston, 2002). Because of this fear, the elderly may present with markedly different symptoms than younger clients. In the clinical setting, these symptoms could be attributed to co-morbid disease processes and the depression remains undiagnosed all together (Stewart, 2003).

Events encountered in old age also play a part in cognitive functioning and depressive illness. Onsets of pure major depression and generalized anxiety disorders are predicted by higher ratings of loss and humiliation (Kendler, Hettema, Butera, Gardner & Prescott, 2003). Losses may include physical losses, such as the death of loved ones, or social losses such as loss of one's job due to retirement or disease. Humiliation may be the result of decreased physical stamina and strength and feelings of physical inadequacy. One of the greatest predictors of depressive symptoms is the degree of social integration of the person (Ramos & Wilmoth, 2003). More intensive exchanges with relatives, being married, and satisfaction with family relationships decrease depressive symptoms. In the same vein, the patients' view of aloneness tends to change depending on the stage of treatment. In a study conducted with older women being treated for depression, it was found that these women identified aloneness with being vulnerable, fearful, helpless, and

as having a loss of control of self. After treatment these women viewed aloneness as being self-reliant, hopeful, and resourceful and as having self-determination and self-reflection (Pierce, Wilkinson & Anderson, 2003).

Treatment for all sufferers of depression is important and could be vital to the elderly. In the August 2003 edition of the *Journal of Aging and Health* evidence is presented that cognitive impairment and depression exacerbate the impact of stroke and diabetes (Fultz, Ofstedal, Herzog & Wallace, 2003). It has also been shown that treatment with antidepressants may have a neuroprotective effect. The longer the period of time a person experiences a depressive episode, the greater the loss in hippocampal volume (Sheline, Gado & Kraemer, 2003). The risk of suicide is also greater in the elderly. According to recent statistics, suicide rates amongst the elderly are about twice that of the population as a whole. The largest group at risk are caucasian men over the age of 80. The suicide rate for this group is six times the current overall rate and three times the rate of African-American males over 80 years old. This high rate among white males over 80 is important because the very elderly age group (85 years and older) is the fastest growing sub-population of elderly adults in the United States. Studies show that individuals suffering from a major affective disorder have a greater than 50% higher suicide rate than the general population. Lifetime risk for suicide in the general population is 1%, compared with 15% for persons suffering from depression and 15% for alcoholics. Studies of alcoholics revealed that between 30% and 60% suffer from depression, and a significant proportion of alcoholics have other persons in their family suffering from depression (Institute on Aging, 2003).

Treatment of depressive disorders in the elderly poses a new array of problems. Side effects of certain drug therapies can be injurious to those persons who are frail or under a physician's care for other conditions. Elderly clients tend to be prescribed other medications for conditions unrelated to their depression. The potential for drug-drug and drug-disease interactions may affect the relative safety and efficacy of medications to treat depression (Oslin, TenHave, Streim, Datto, Weintraub, DiFilippo, & Katz, 2003). Since 1985, however, rates of prescribing antidepressants more than doubled. (Harman et al., 2003). Despite this, it cannot be taken for granted that a significant proportion of the population is not compliant with the prescribed treatment regimen. Typically, selective serotonin reuptake inhibitors (SSRIs) are prescribed. These drugs may not be tolerated as well in the elderly as in the non-elderly population. Adverse effects are more common in these patients due to diminished cardiovascular function. Evidence of increased number of falls due to administration of SSRIs and tri-cyclic antidepressants are thought to be directly related to these drugs' cardiovascular effects. The mechanism whereby antidepressants increase this risk is complex and may include orthostatic hypotension, arrhythmias, sedation and confusion (Kurzthaler, Hotter, Miller, Kemmler, Halder, Rhomberg & Fleischhacker, 2001).

The morbidity of depression is very high in elderly and they are at risk for depression (Zung, Broadhead & Roth, 1993). In addition to pharmacotherapy, those receiving the greatest amount of social support from others leads to a greater decrease in depression. Other factors such as functional ability to perform daily activities,

satisfaction with economic situations, and regular food habits contribute significantly to a decrease in depression in the older-elderly (Demura & Sato, 2003).

Purpose of Present Study

This study attempted to examine the effect of selenium supplementation on mild to moderate depression in the elderly. The attempt was made to ascertain if differing forms of selenium, Se methylselenocysteine (SeMC) and selenomethionine, would be retained and utilized by elderly subjects to alleviate mild to moderate depression. Supplements of 200 micrograms of selenium in the form of a seleno-amino acid were taken by the subjects over a period of 7 weeks. It was hypothesized that this level, as well as these forms of seleno-amino acids, would have an effect on the self-reported symptoms and associated BDI-II scores.

CHAPTER III

MATERIALS AND METHODS

Human Subjects

A proposal for the approval to use human subjects was prepared and presented to the Texas Tech University Institutional Review Board Human Subjects Committee (see Appendix A). Permission was granted on October 10, 2003 to conduct the study. Volunteer recruitment commenced in November 2003 by way of informational posting via a senior group newsletter, and by an informal presentation to senior center groups from two communities in West Texas..

Targeted persons included those volunteers who were between the ages of 60 and 80. Those subjects who had uncontrolled medical or psychiatric illness other than mild to moderate depression were excluded from participation in the study. Additionally, if subjects expressed any form of suicidal behavior they were excluded and referred to a professional mental health provider. At the 0.8 power, 54 subjects were required for the study. Recruitment, enrollment and data collection took place in two communities in West Texas. Subjects were given a personal interview to determine eligibility in private meeting rooms with the researcher and nurse practitioner present.

Preliminary Data Collection

Subjects were notified of the voluntary nature of the study and asked to provide basic demographic information and report their daily intake of prescriptions, vitamin, minerals and herbal preparations. Subjects were then asked to read and understand

consent forms for administration of the Beck Depression Inventory (BDI-II) and for procedures involved in the research study (See Appendix A). If the subject agreed with the terms of the study, the BDI-II was self-administered. If a subject was unable to self-administer the BDI-II due to sensory or educational impairment then it was orally administered by the researcher. Those that answered in the affirmative to question nine of the BDI-II were asked to complete a No Self-Harm Contract which referred the individual for immediate psychiatric assessment (See Appendix B). Only subjects falling into the mild or moderate levels of depression were included in the study. The Beck Intrepretrack computer software program generates scores for each completed BDI-II diagnostic assessment form. Scores with accompanying levels of depression, i.e., minimal, mild, moderate and severe were provided by the Beck software.

Collection of blood samples was obtained by a nurse practitioner from the subjects agreeing to the terms outlined in the Study Participation Consent Form (Appendix A). Prior to collection of blood samples, subjects were also required to read and grant consent for the venipuncture procedure. Approximately 10mL of whole blood was collected into Vacutainer collection tubes with citrate as the added anticoagulant. Samples were given code numbers and the researcher did not know to which study group the subjects were assigned during the selenium analysis procedure. Whole blood was then separated by centrifugation. Plasma and erythrocytes were stored at -4°C for the planned determination of baseline plasma selenium by the method of Spallholz et al., (1978) and erythrocyte glutathione peroxidase activity by the method of Paglia and Valentine, 1967.

Subjects enrolled in the study were assigned random numbers for confidentiality purposes. The subjects were subsequently randomly assigned to one of three treatment groups. Subjects were unaware of the group to which they were assigned. The groups consisted of a control group, which received an oral placebo, and two experimental groups. All subjects received their treatment or placebo in capsule form and were instructed to self-administer it once daily by mouth. Subjects assigned to experimental group number one received selenium orally as selenium methyl selenocysteine (SeMC) and those assigned to experimental group number two received oral selenomethionine. Both experimental groups received a supplement containing 200μg selenium per day. Group number three received a placebo of inert substances. All groups received their supplement in a capsule form provided by PharmaSe, Inc. (Lubbock, Texas). Subjects were instructed to consume their supplement once daily for a period of seven weeks. The only reported side effect of selenium at this dose is mild stomach upset. Any volunteer who found any symptom bothersome was informed that they could have remove themselves from the study at any time. After the treatment period, participants were contacted again for a follow-up self-administered BDI-II for determination of changes in depression scores and a repeat venipuncture for determination of plasma selenium concentrations.

Beck Depression Inventory – II

The Beck Depression Inventory II (BDI-II) was the chosen assessment instrument in the current study. To reduce any possible systematic error, an identical testing environment was provided to all individuals who were screened. The instrument was self-administered thus the issue of the researcher introducing bias into the study was minimized. The instrument was analyzed via scoring by the Interpretrack software with the data verified for accuracy (i.e., responses are indicated properly and in a consistent manner) by the researcher. Before beginning the study, the software was tested to ensure proper analysis of test subjects' responses.

Predictive validity has been tested using the BDI to discriminate adjustment level in grade school students (Albert & Beck, 1975). Concurrent validity has been tested using clinician ratings of depression with correlations ranging from 0.62 to 0.66 (Foa, Riggs, Dancu, & Rothbaum, 1993) as well as the 1990 study by Groth-Marnat which found moderate correlations between the BDI and other depression measurement scales (Groth –Marnat, 1990). The BDI correlated weakly with the Hamilton score and was shown to differentiate between minor and major depressive illness (Schotte, Maes, Cluydts & Cosyns, 1997). These results support the construct validity of the BDI in depressive subjects.

In reference to the BDI portion of the current study, and in many psychological/behavioral studies, consistency over time is less of a concern than internal consistency. It is hypothesized that the respondents' mood will change and thus their scores on the instrument will vary. Overall, the Beck is considered very reliable with

internal consistencies ranging from 0.73 to 0.92 (mean = 0.86). For the BDI-II the alpha coefficient has been measured at 0.92. Concern exists over the test-retest reliability. Beck, Ward, Mendelson, Mock & Erbaugh (1961) suggested that if the BDI was re-administered in a short period of time the scores could be inflated due to the effects of memory. However, it was shown that there was a 40% decline in BDI scores over a period of 8 weeks which accounted for 10% of the variance (Ahava, Iannone, Grebstein & Schirling, 1998).

The BDI is generally considered to be one of the predominant tools in the diagnosis of depression. The tool has been tested in several different populations (Ahava et al., 1998; Chochinov, Wilson, Enns & Lander, 1997; Lykouras, et al., 1998; Krefetz, Steer, Gulab & Beck, 2002; Aben et al., 2002; Addington, Addington, Maticka-Tyndale & Joyce, 1992; Novy, Nelson, Francis & Turk, 1995; Carter and Dacey, 1996; Kojima, Furukawa, Takahashi, Kawai, Nagaya & Tokudome, 2002). Thus, it can be considered valid and reliable across different ethnic and cultural groups in the discrimination between psychiatric patients and non-psychiatric patients as well as patients with major depressive disorders.

Selenium Analysis

A quantitative analysis of plasma selenium concentration was performed by the method of Spallholz, Collins & Schartz (1978). To prepare the samples for analysis, the plasma was first digested. A solution was prepared in which 75ml of concentration sulfuric acid was added to 5g of sodium molybdate in 75ml of water. After cooling, 100ml of 70-72% perchloric acid was added to give a final volume of 250ml.

Digestion of the samples was accomplished by the following protocol. Into 19x150mm Pyrex test tubes 1ml of plasma was placed. A selenium standard of 100 ng/ml was prepared along with the samples. To each tube, 3ml of the digestion mixture was added. Each tube was heated over a Fisher burner until white fumes were driven off and the volume of each tube was equal to approximately 1ml. This procedure resulted in a digestion mixture that was free of carbon.

Following digestion, 6ml of 0.008 M ethylenediaminetetraacetate (EDTA) was added. The EDTA is necessary to bind cations which may interfere with the selenium analysis. Two milliliters of concentrated ammonium hydroxide (NH_4OH) was added to each tube and eventually brought to a pH of approximately 2.0 by adding either additional NH_4OH or hydrochloric acid (HCl). All samples were brought up to 12.5ml with distilled water.

At this time, a diaminonapthalene (DAN) solution was prepared for selenium analysis by dissolving 50mg DAN in 50ml of diluted sulfuric acid (H_2SO_4) – 14ml concentrated H_2SO_4 diluted to 100ml. Once dissolved, this solution was transferred to a separatory funnel to which 50ml of spectrophotometric grade cyclohexane was added. After being shaken for two minutes, the DAN solution was allowed to separate from the cyclohexane for five minutes. The lower phase was collected and the cyclohexane was discarded. Four washes were required to sufficiently remove any interfering fluorometric material.

Once the DAN solution was prepared, 4ml was transferred to each tube designated for selenium analysis after which they were incubated in a 50°C water bath for

twenty minutes. After incubation, the tubes were cooled, and 4ml of cyclohexane was added. The tubes were capped and vortexed for five minutes and then placed in a centrifuge for five minutes to assure that all the aqueous material was eliminated from the cyclohexane layer.

Finally, the cyclohexane phase was transferred directly into quartz cuvettes. Fluorescence from the resulting 4,5-benzopiazselenol complex in the samples was read by excitation at 363nm and emission at 525nm in a Perkin Elmer 650-40 Fluorescence Spectrophotometer (Norwalk, Connecticut). (See Appendix C for Protocol)

Erythrocyte Glutathione Peroxidase Activity

Erythrocyte glutathione peroxidase activity was determined using the method of Paglia and Valentine (1967). Equipment failure and ultimate loss of erythrocyte fraction of samples caused these data to not be collected. This protocol can be found in Appendix D.

Statistical Analysis

Descriptive statistics, correlation, and variance statistics were completed via SPSS version 12 software. Interpretative data regarding the Beck Depression Inventory-II was provided via the Beck Interpretrack software (The Psychological Corporation, 2000). Paired t-tests were run on the data due to the small number of groups and small number of subjects for both the pre and post-test BDI-II and plasma selenium determinations (pre and post treatment). Significance was found between the variables of pre- and post-BDI-II, pre-treatment plasma selenium and post-treatment plasma selenium, pre-test BDI-II

and pre-treatment plasma selenium as well as post-test BDI-II and post-treatment plasma selenium. Results were determined for the study participants as a whole and that of individual research groups as well. Significance was reported at the $p<0.05$ level and any greater significance level was noted.

CHAPTER IV

RESULTS

Demographic Information

Data were collected from the period of January through April of 2004 in two West Texas communities. There were a combined total of twenty three volunteers who were included in the study. All participants resided in the communities and were members of local Senior Citizen's groups. Of the twenty three subjects, 20 were women and 3 volunteers were men. Ages ranged from 53 years to 83 years with a mean age of 63 years.

Eighty-six percent of the subjects were married and the remaining fourteen percent were divorced or widowed. All volunteers had obtained a minimum of a high school diploma and 39% had college or advanced degrees. The majority of participants reported pharmaceutical drug use with estrogen the most common replacement therapy or osteoarthritis medications. Other medications included those for hypertension and hypercholesteremia. Nearly 80% of the respondents reported daily multivitamin use. The most common supplement was Centrum Silver which contained 20μg Se. Thirty-eight percent of the study participants (Control Group 1, n=3; Experimental Group 2, n=2; Experimental Group 3, n=3) had at some time been diagnosed with depression and of those, 71% had taken antidepressant medications. No respondent was currently taking any prescription antidepressant medication. The most common cause of an identifiable subject stressor was the care of an ill family member. Almost 20% of all subjects

answered in the affirmative to this item. Demographic information is presented in Table 1 and Figures 1 through 3.

Table 1. Summary of Frequency Distribution of Background Variables Control and Experimental Groups

Background Variables	SeMC (n=7)		Selenomethionine (n=8)		Control (n=8)	
	N	%	n	%	n	%
Age (years)						
53-59	1	14.3	4	50.0	1	12.5
60-65	1	14.3	0	0	1	12.5
66-70	4	57.1	0	0	0	0
71-75	1	14.3	2	25.0	0	0
76-80	0	0	1	12.5	2	25.0
81-85	0	0	1	12.5	0	0
Gender						
Male	1	14	1	12	1	12
Female	6	86	7	88	7	88
Education Level						
High School	4	50	3	37	3	37
College/Bachelors	1	14	5	63	3	37
College/Advanced	2	28	0	0	2	25
Hospitalization	6	85	7	87	3	87
Heart Attach/Stroke	1	14	0	0	0	0
Prescription or Non-Prescription Medication	7	100	7	87	6	75
Herbal Preparations	1	14	1	12	0	0
Vitamin/Mineral Supplements	6	85	5	63	7	87
Depression Diagnosis	3	42	2	25	3	37
Prescription Antidepressants	3	42	1	12	2	25

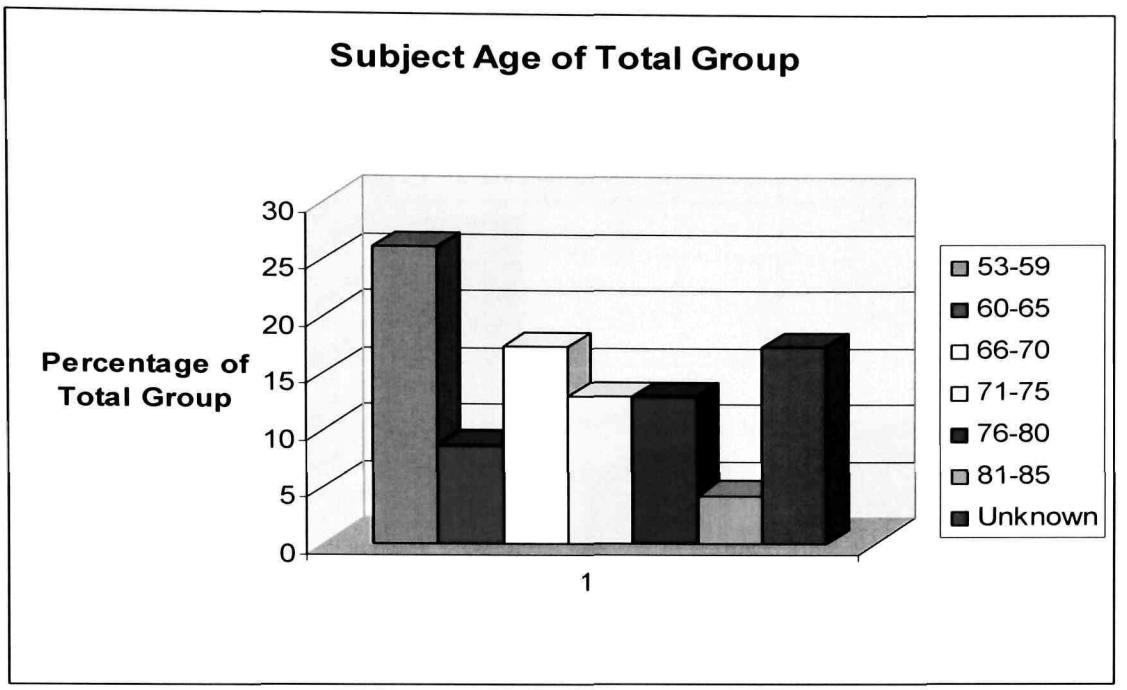

Figure 1. Subject Age

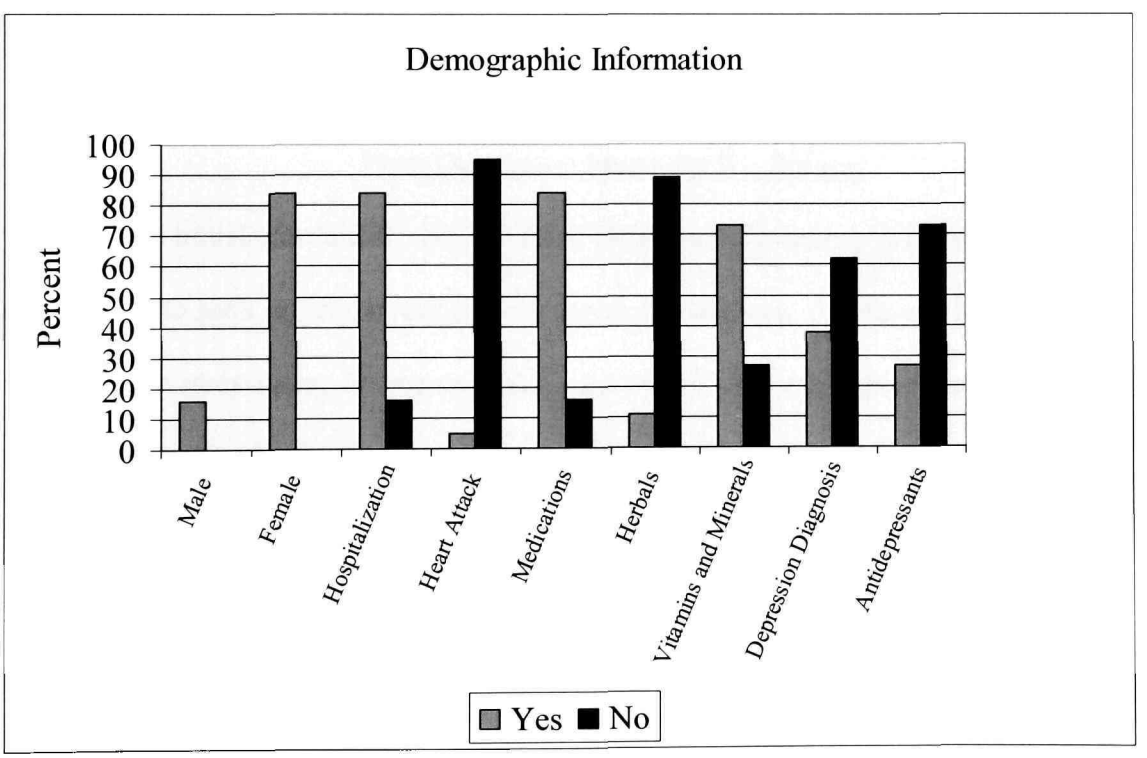

Figure 2. Demographic Characteristics

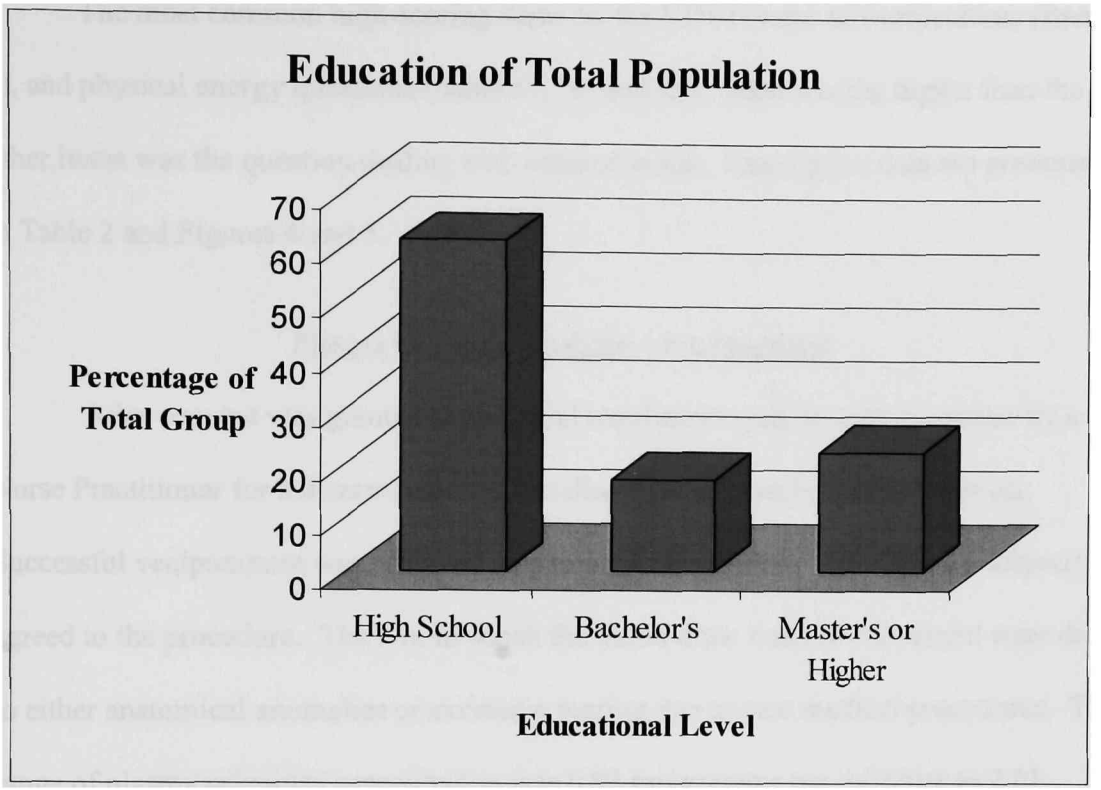

Figure 3. Highest Educational Level Attained

Beck Depression Inventory II – Pre-test

At the initial enrollment visit, the Beck Depression Inventory-II (BDI-II) was administered to each subject agreeing to the terms of the study. A total of 23 participants completed the assessment. Scores ranged from a low of zero to a high score of twenty-four. When analyzed using the Beck Interpretrak Software (2000), this indicated a range of minimal depression to moderate depression for study participants. The mean score on the BDI-II pretest was 11.7 (standard deviation = 7.5), which indicates the average respondent suffered from mild depression.

The most common high-scoring items on the BDI-II were self-criticalness (Item 8), and physical energy questions (Items 15, 16, and 20). Also scoring higher than the other items was the question dealing with interest in sex. Descriptive data are presented in Table 2 and Figures 4 and 5.

Plasma Selenium Analysis – Pre-treatment

After consent was granted at the initial enrollment visit, blood was drawn by a Nurse Practitioner for a determination of baseline plasma selenium concentration. Successful venipuncture was achieved on a total of 18 subjects. All of the 23 subjects agreed to the procedure. The five in which the blood draw was not successful were due to either anatomical anomalies or excessive scaring due to past medical procedures. The range of plasma selenium concentration was 0.83 micrograms per milliliter to 2.01 micrograms per milliliter. Mean concentration of pre-treatment plasma selenium was

Table 2. Beck Depression Inventory – II Pre-treatment Scores

Treatment	**n**	**Minimum**	**Maximum**	**Mean**	**SD**
Pre Treatment BDI-II – Total Group	23	0	24	11.7	7.5
SeMC Group Pre-treatment	8	0	23	11.5	7.6
Selenomethionine Pre-treatment	7	7	24	14.7	5.8
Control Pre-treatment	8	0	21	11	8.1

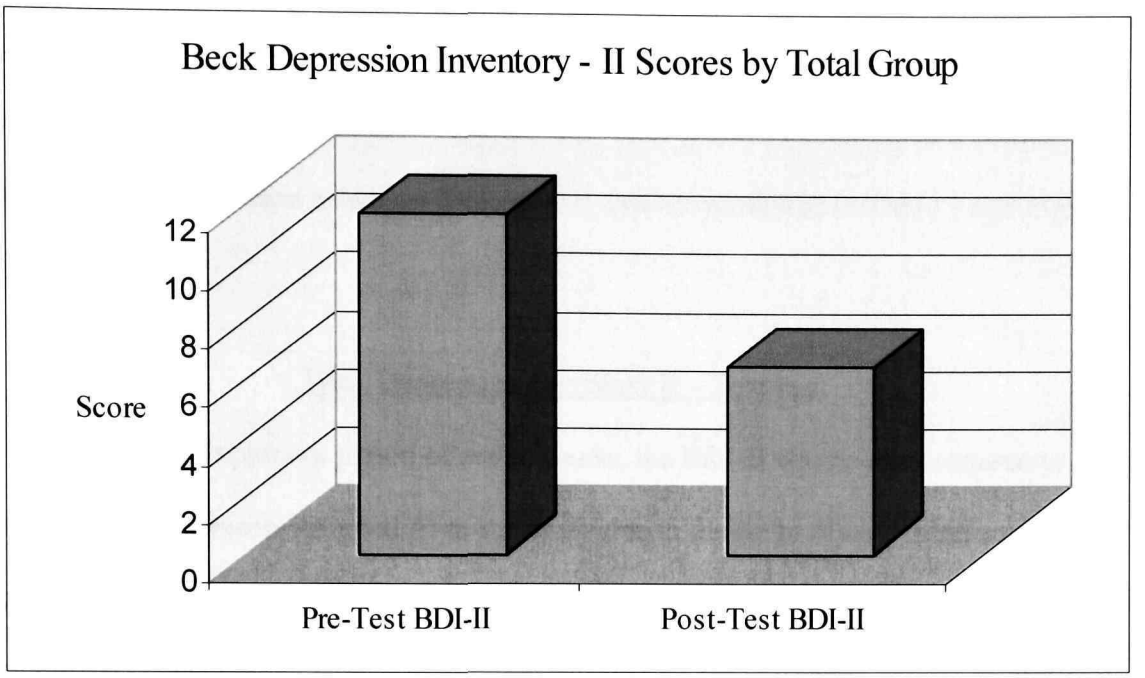

Figure 4. Mean BDI-II Scores for All Participants

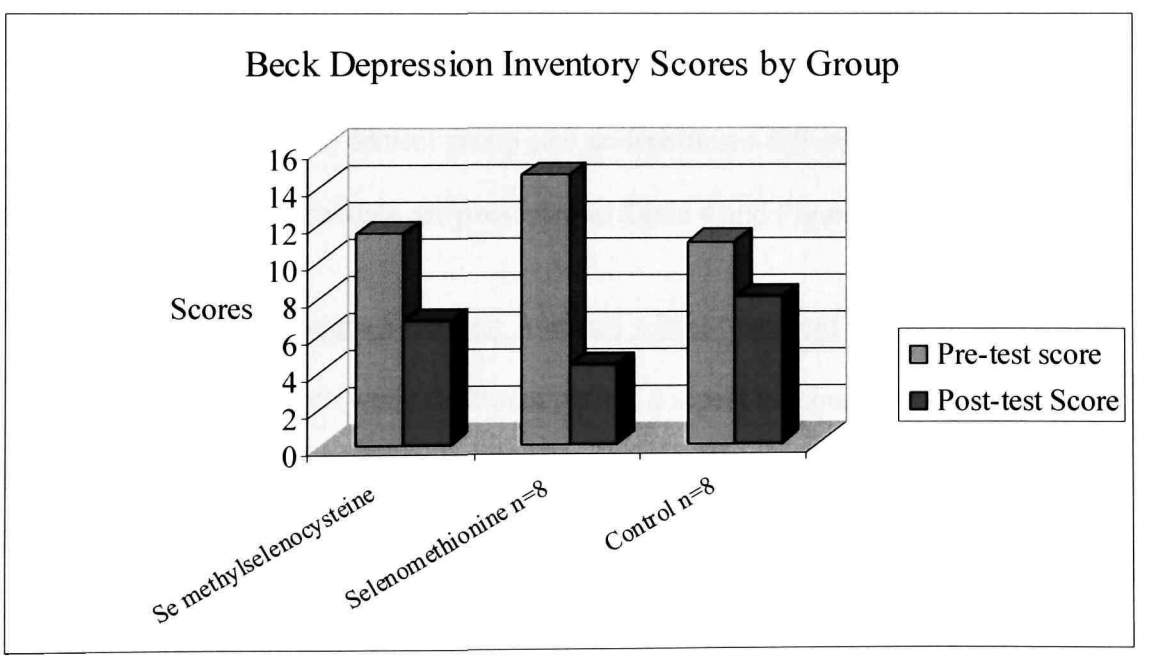

Figure 5. Pre- and Post-BDI-II Scores as Reported by Control and Se Treatment Groups

1.27 micrograms per milliliter (standard deviation = 0.36). Average plasma selenium concentration from the literature is approximately 1.00 microgram per milliliter (Hawkes et al., 2003). The subjects in this study fell into what would be considered a normal range for this for plasma selenium. Descriptive data are presented in Table 3 and Figure 6 and 7.

Beck Depression Inventory II – Post-test

After the treatment period of seven weeks, the BDI-II was re-administered to all subjects. Two subjects resigned from the study due to illness or other commitments. From the remaining 21 subjects, all successfully completed the follow-up assessment. Follow-up scores ranged from 0 to 16. Mean score on the assessment was 6.6 (standard deviation = 5.5). This indicates an approximately five-point reduction in mean BDI-II scores bringing the mean categorical determination to minimal-mild depression via the Beck Interpretrack software. The experimental groups both saw significant decreases ($p < 0.05$) in total scores. The control group saw no significant difference in pre-test and post-test scores. Statistical data are presented in Table 4 and Figures 4 and 5.

Plasma Selenium Analysis – Post-treatment

Following the seven week treatment period, a repeat venipuncture was performed on the 18 participants from which blood samples were taken at the beginning of the study. The 2 participants who resigned from the study were excluded from the data bringing the follow-up sample size to 16 participants. Repeat plasma selenium concentrations ranged from 1.07 to 2.06 micrograms per milliliter of plasma. The mean

plasma selenium concentration was 1.67 micrograms per milliliter (standard deviation = 0.33).

Table 3. Plasma Selenium – Pre-treatment

Treatment	**n**	**Minimum µg/ml**	**Maximum µg/ml**	**Mean µg/ml**	**SD**
Pre-treatment Plasma Selenium – Total Group	18	0.83	2.01	1.27	0.36
SeMC Group Pre-treatment	6	0.83	1.87	1.23	0.37
Selenomethionine Pre-treatment	6	0.88	2.01	1.34	0.42
Control Pre-treatment	6	1.08	1.31	1.17	0.13

* Control and treatment group means were not significantly different.

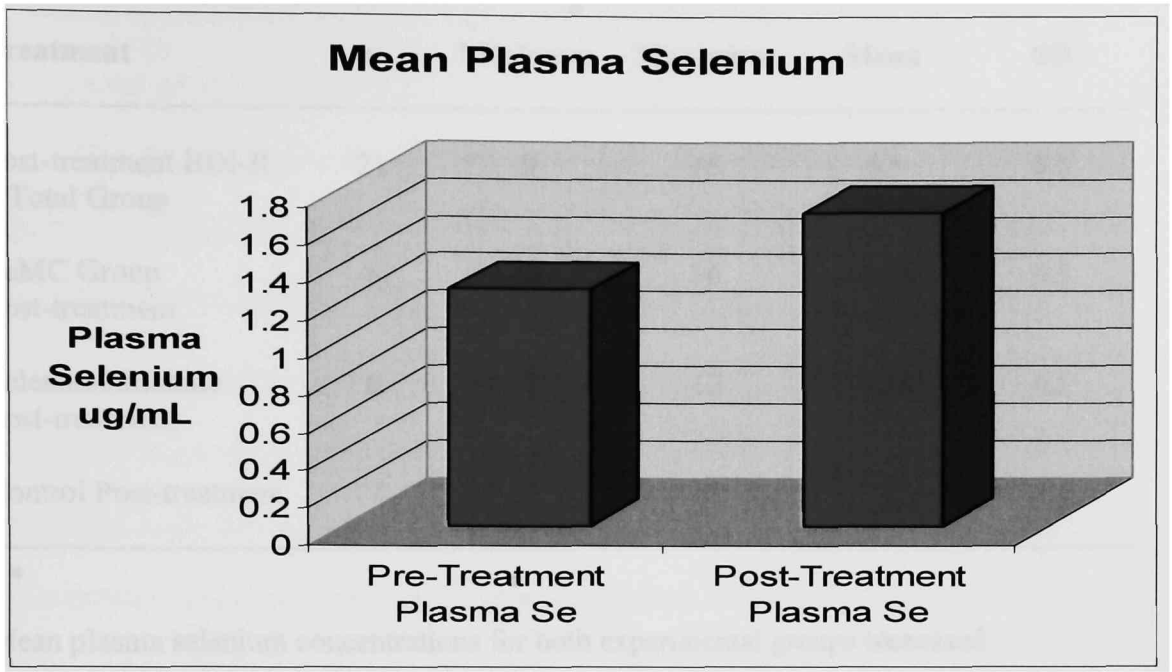

Figure 6. Plasma Selenium for All Participants

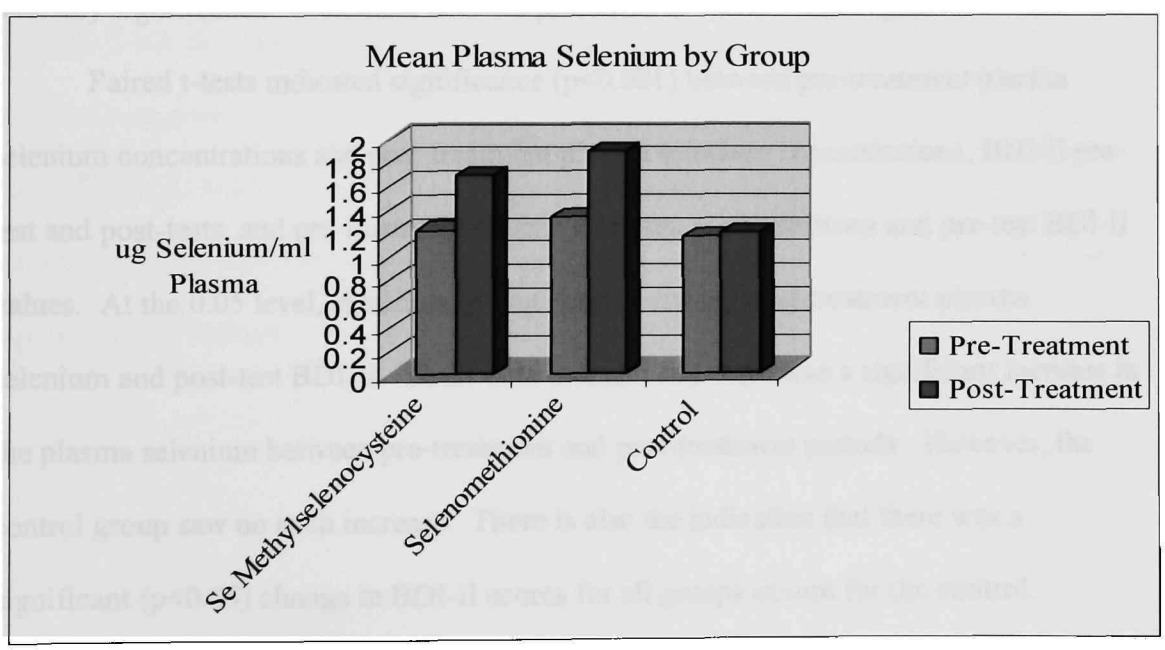

Figure 7. Mean Plasma Selenium by Research Group

Table 4. BDI-II Scores – Post-treatment

Treatment	n	Minimum	Maximum	Mean	SD
Post-treatment BDI-II – Total Group	21	0	16	6.5	5.5
SeMC Group Post-treatment	8	0	16	6.75	6.3
Selenomethionine Post-treatment	6	7	13	4.28	6.5
Control Post-treatment	7	0	15	8	7.1

Mean plasma selenium concentrations for both experimental groups increased significantly ($p < .001$). The control group's mean value also increased slightly but not with any significance. Statistical data are presented in Table 5 and Figures 6 and 7.

Paired t-tests indicated significance ($p<0.001$) between pre-treatment plasma selenium concentrations and post-treatment plasma selenium concentrations, BDI-II pre-test and post-tests, and pre-treatment plasma selenium concentrations and pre-test BDI-II values. At the 0.05 level, significance was found between post-treatment plasma selenium and post-test BDI-II. These data indicate that there was a significant increase in the plasma selenium between pre-treatment and post-treatment periods. However, the control group saw no such increase. There is also the indication that there was a significant ($p<0.05$) change in BDI-II scores for all groups except for the control. Additionally, there was a strong correlation between pre-test BDI-II and post-test BDI-II scores meaning that responses to pre-test questions may indicate responses to post-test

questions. Likewise, the data reflect a significant ($p<0.001$) rise in plasma selenium correlating to a reduction in BDI-II score at the end of the treatment period for the experimental groups (See Table 6).

The strongest correlation among all the data was between pre-BDI-II and post-BDI-II. This data generated a Pearson's R of 0.720 which is significant at the 0.01 level (2-tailed test). This indicates that the higher scoring participants on the pre-test were likely to have higher scores on the post-test. A correlation was also found between medication usage and post-treatment selenium analysis (R=0.532) (persons who reported medication usage had higher plasma selenium concentration). Negative correlations were found between prior depression diagnosis and post-treatment plasma selenium (R=-0.518), and depression diagnosis/pre-BDI-II data (R=-0.592). This means prior depression diagnosis predicted a lower plasma selenium concentration than average and pre-BDI-II scores. Prior antidepressant medication usage and pre-BDI-II score also reflected a negative correlation (R=-0.566), as well as antidepressant medication usage and post-BDI-II scores (R=-0.602). As a result, this indicates prior antidepressant medication usage predicted decreases in both pre- and post-BDI-II scores. Statistical data is presented in Table 6.

Table 5. Plasma Selenium – Post-treatment

Treatment	n	Minimum	Maximum	Mean	SD
Post-treatment Plasma Selenium	16	1.07	2.06	1.68	0.34
SeMC Group Post-treatment	5	1.44	2.06	1.70	0.25
Selenomethionine Post-treatment	6	1.37	2.03	1.90	0.23
Control Post-treatment	5	1.07	1.20	1.20	0.07

Table 6. Correlational Data/Paired t-test

Factor	Pearson's R
Pre test BDI-II / Post test BDI-II	0.720**
Medication usage/Post-treatment Plasma Selenium	0.532
Depression Diagnosis/Post-test BDI-II	-0.518
Depression Diagnosis/Pre-test BDI-II	-0.592
Antidepressant Usage/Pre-test BDI-II	-0.566
Antidepressant Usage/Post-test BDI-II	-0.602

** Significant at the 0.01 level.
All other relationships are significant at the 0.05 level.

Table 6. Continued

Paired Samples t-test (Total Population)	Significance
Pre-treatment Plasma Selenium Post-treatment Plasma Selenium	<0.001
Pre-test BDI-II Post-test BDI-II	0.001
Pre-treatment Plasma Selenium Pre-test BDI-II	<0.001
Post-treatment Plasma Selenium Post-test BDI-II	0.002

Paired Sample t-test by Group	SeMC	Selenomethionine	Control
Pre-test BDI-II Post Test BDI-II	0.002*	0.010*	0.605
Pre-treatment Plasma Selenium Pre-test BDI-II	0.010*	0.001**	0.399
Pre-test BDI-II Post-test BDI-II	0.048*	0.002*	0.225
Post-treatment Plasma Selenium Post-test BDI-II	0.063	0.039*	0.305

* $p<0.05$; ** $p<0.001$

CHAPTER V

DISCUSSION AND CONCLUSIONS

While the sample number was too low to draw any concrete conclusions, the data clearly indicate that selenium supplementation significantly affects plasma concentrations of the mineral in subjects in this study group. During the 7 week treatment period there were also significant decreases in depression scores. One can infer that the selenium supplementation may have, in part, affected the participants' mood in the experimental groups as the control group subjects did not see significant decreases in depression scores or plasma selenium concentrations. Other mitigating factors must be considered. It is possible that study participants changed their diet over the 7-week treatment period and thus had feelings of more energy. It is also possible that participants anticipated positive results and as a result may have responded more favorably in the post-test BDI-II. Ideally, the treatment period should have been longer than 7 weeks to account for memory of pre-test questions. Memory of pre-test questions may have led to artificial improvement in depression scores. It was noted during the post-test deliverance that a few subjects mentioned they were attempting to recall what their responses had been during the pre-test.

Nevertheless, one must recognize the differences in the data collected in this study between the experimental (selenium-treated) groups and control group. There were significant decreases in depression scores coupled with increased plasma selenium levels in subjects in the experimental groups. While the depression score data can be somewhat subjective, the plasma selenium concentrations are purely objective and preliminary

conclusions can be drawn to the effect that increases in plasma selenium can lead to the amelioration of depressive symptoms in this population. Particularly striking are the improvements in the energy-related items from the BDI-II.

From post-interviews with the subjects several participants in the experimental groups reported that their ability to "handle stress" appeared to increase. These responses were unsolicited and were not followed by any leading questions. No such self-observations were reported by the members of the control group. These feelings of well-being may have led to the improvement in the mental-clarity and energy related items listed on the BDI-II.

The fact that this was a volunteer study and that the participants were all fairly well educated may have had an effect on the outcomes. It was noted at the conclusion of the study that compliance seemed to be high in that the final pill count was appropriate for the time period. Additionally, this population appeared to be well-versed in the disease process of depression and eager to offer their altruistic assistance. Had the participant group been larger and randomly selected from a non-volunteer, less-educated group suffering from depression, I feel the results may have been more variable.

More thorough research would be beneficial examining the link between selenium and depression. The sample size should be much larger, at least eighteen subjects per research group. This is adequate at the 0.8 power level. Lengthening the treatment period should also be considered. The average life-span of an erythrocyte is approximately 120 days. Increasing the treatment period to this length may be beneficial in observing changes in glutathione peroxidase activity more precisely and more

assuredly due to the experimental treatment. In future studies a diet history may also be beneficial to the researcher. Despite the majority of the participants reporting use of multivitamins, the range of plasma selenium was fairly broad. Determining what proportion is obtained through the diet and what proportion is retained due to consumption of multivitamin supplements or genetic predispositions may introduce some interesting research questions. The final modification which should be considered is the documentation of subjective comments by research participants. The use of a survey should be adequate in this case. The participants could be asked about their motivation for participating in this type of research study, how much knowledge they have of depression, selenium, and the connections between nutrition and mental health, as well as any physical or psychological changes they experience over treatment period.

In future studies it would be interesting to explore how the endocrine system may be involved with selenium and the glutathione peroxidases in the alleviation of mood and depressive disorders. Particularly interesting, especially in this population of predominantly young-elderly females, would be the role the thyroid gland plays. This study enrolled primarily women (86%) yet none reported having any history of hypothyroidism. This statistic is fairly surprising as the incidence in this population as a whole is much higher. In one study, nearly 6% of women over 60 had hypothyroidism, and some experts estimate that as many as 20% of women in this age group have a subclinical condition (Hershman & Hassani, 2004). In fact, the symptoms of hypothyroidism and menopause are very similar and hypothyroidism may easily be missed. It is known that selenium plays a role in the development of thyroid hormones.

Recent research has noted a link between selenium and hypothyroidism (Kvicala & Zamrazil, 2003; Sher, 2000; Olivieri, Girelli, Stanzial, Rossi, Bassi & Corrocher, 1996). What is the incidence of hypothyroidism in these clients from West Texas? Is there any correlation between hypothyroidism and low plasma selenium/reduced enzyme activity? Does selenium supplementation in hypothyroid patients lead to improved endocrine function? These are just a few of the research questions which I hope some day will be explored.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL

Tatum, Willie

From: Lela Tatum [Lela.Tatum@ttu.edu]
Sent: Monday, June 14, 2004 1:51 PM
To: Tatum, Willie
Subject: Fw: changes to the protocol

----- Original Message -----
From: Mcglynn, Richard
To: Johnston, Elizabeth
Cc: Tatum, Lela
Sent: Friday, October 10, 2003 12:15 PM
Subject: RE: changes to the protocol

Elizabeth-
I approve this proposal as amended. Data collection can begin at once.

Richard P. McGlynn
Professor of Psychology
Director, Experimental Psychology Division
Chair, Human Subjects Protection Committee (IRB)
Department of Psychology
Box 2051
Texas Tech University
Lubbock, TX 79409
Voice: 806-742-3711 ext 255
Fax: 806-742-0818
r.mcglynn@ttu.edu

-----Original Message-----
From: Tatum, Lela
Sent: Thursday, October 09, 2003 9:50 AM
To: Johnston, Elizabeth; Boylan, Mallory
Cc: Mcglynn, Richard
Subject: Re: changes to the protocol

Attached you'll find the revisions required to the proposal.
Thanks,
Lela

----- Original Message -----
From: Johnston, Elizabeth
To: Tatum, Lela ; Boylan, Mallory
Cc: Mcglynn, Richard
Sent: Thursday, October 09, 2003 9:22 AM
Subject: changes to the protocol

Attached is a copy of the letter I am sending today about the required chances needed to your protocol. It is exactly what was talked about while you were at the full board - no surprises.

APPENDIX B

INSTITUTIONAL REVIEW BOARD PROPOSAL

Proposal for Selenium Supplementation as a Treatment for Depression in the Elderly

I. Rationale:

Selenium was first understood to be biologically essential for the prevention of liver necrosis in vitamin E deficient rats in work done by Klaus Schwarz in 1957. When present in the diet, selenium induces the synthesis of antioxidant enzymes such as cytosolic glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase as well as the 5'-deiodinases. Selenium is found in most all human tissues and is reported to have immune enhancing effects.

The absorption and retention of selenium from the gastrointestinal tract is dependent on the chemical form and the amount ingested. Studies on physiological levels of radioselenium indicated that the duodenum is the main site of absorption. Selenomethionine is virtually 100% absorbed and well retained whereas inorganic compounds are readily excreted. Absorbed selenium is at first carried most in plasma in association with plasma proteins and is then deposited in all tissues. Most of the tissue selenium is highly labile and after a transfer from selenium adequate or selenium toxic diets to lower levels, the loss of selenium is rapid at first then tapers off.

Estimates exist that 5-12 percent of men and 10-20 percent of women in the United States will suffer from a major depressive episode at some time in their life. Many cases of depression apparently stem, at least in part, from disturbances in brain circuits that convey signals through certain neurotransmitters of the monoamine class. These biochemicals, all derivatives of amino acids, include serotonin, norepinephrine and dopamine; of these, only evidence relating to norepinephrine and serotonin is abundant in the literature.

Many nutrients and herbals have been studied to determine their effects on mental functioning in humans. Most frequently studied are those that are purported to halt memory loss or treat depression. The mechanisms through which these nutrients act upon depression are not completely understood but appear to be varied and correlate with serum levels of nutrients and herbals.

Much more research has been conducted with selenium to determine its safety and efficacy of the mineral as a supplement than for use as a mood altering treatment. What is known, according to research, is that those patients who are chronically depressed have statistically significant lower plasma selenium concentrations than those who do not report experiencing depression. The current average daily intake of selenium for adults is approximately 43 μg, which is below the recommendation of 55 μg/day. The 1991 study by Benton and Cook was the first time selenium's effects on mood were examined. The subjects monitored themselves and reported their feelings and mood over a period of five weeks while their diet was supplemented with 100 μg/day of yeast-based selenium tablets. The Benton and Cook study reported that the lower the selenium content of the diet, the greater likelihood the subjects reported

experiencing anxiety and depression. When selenium was added to the diet, reported anxiety and depression subsided.

Hawkes and Hornbostel examined the effect of selenium on the mood of a small group of healthy men in 1995. Little effect was observed on this group purportedly due to the fact that these men were cognitively normal and had higher intake levels of selenium than subjects in previous studies. This suggested that selenium supplementation will not elevate mood of 'normal' subjects but may have an effect on those who are depressed.

II. Subjects:

A. Subjects shall be volunteers of generally good health between the ages of 65-80. They will exhibit no sign of mental illness other than mild-moderate depression and shall have no uncontrolled physical illness. Subjects will be excluded from participation if they are currently medicated with any pharmaceutical or herbal anti-depressant treatment. Subjects will also be excluded if they have severe depression and/or have expressed any suicidal behavior.

B. Potential subjects will be residents of assisted living facilities and members of senior social groups (Senior Citizen's Center, etc.) in Lubbock, Texas.

C. Recruitment will take place by means of participant flyer. Contact information is included in the proposed flyer. If the posting does not generate enough interest, an oral presentation will be given at the aforementioned social organizations detailing the research criteria.

III. Protocol:

A. A power analysis conducted indicated that a minimum of 54 subjects spread among three treatment groups (18 subjects per group) should be sufficient for a power at the 0.8 level. Subjects shall be generally healthy adults between the ages of 65-80 who exhibit no sign of mental illness other than depression, or uncontrolled physical illness. Recruitment shall take place from assisted living facilities and senior social groups in Lubbock, Texas by way of participant flyer posting. Potential subjects will be informed of the voluntary nature of the study and asked to report their daily prescription, vitamin, mineral and herbal preparation intake. Subjects taking any psycho-pharmaceutical drug will be excluded. Prior to the research, the Beck Depression Inventory (BDI-II) will be self-administered. If a subject is unable to self-administer the BDI-II due to sensory or educational impairment then it shall be administered orally. Subjects who answer affirmative to the suicidal statements in section nine of the BDI-II Depression Inventory will be asked to complete a No Self-Harm Contract which refers the individual for immediate psychiatric assessment. Anyone experiencing suicidal thoughts or intention will be excluded from the study (see attached Contract.). Only subjects falling

into the mild or moderate levels of depression/low mood will be included in the study. The Beck Intrepretrack computer software program generates scores for each completed BDI-II diagnostic assessment form. Scores with accompanying levels of depression/low mood, i.e., minimal, mild, moderate and severe are provided by the Beck software program.

The subject will also be asked to respond to basic demographic data and 24-hour food frequency/diet history requests. Collection of blood samples will be conducted by a nurse practitioner/phlebotomist for those subjects who have a BDI-II score indicating mild or moderate depression. Approximately 15mL of whole blood will be collected to ascertain baseline levels of plasma selenium and placed immediately into ice then frozen for later analysis. Collection of samples will also be retained at the conclusion of the eight week treatment period for analysis of plasma selenium by the method of Spallholz et. al, 1978 (protocol attached) and erythrocyte glutathione peroxidase activity (protocol attached). A list of referral sites for depression/low mood will be given to each subject prior to entry into the study and again at the conclusion of the study.

There will be three treatment groups. The groups will consist of a control group which will receive an oral placebo and two experimental groups, one of which will receive selenium orally as $Se\text{-}CH_3$ and the second which will receive oral selenomethionine for a period of eight weeks at a level of 200μg daily in one dose. The current maximum level of daily selenium intake that is likely to pose no risk of adverse effects has been established at 400μg for adult human beings. Placebo and selenium products shall be obtained from PharmaSe in capsule form.

B. Participants will be informed of the voluntary nature of this study in both the initial presentation as well as in the consent form. All participants will be identified in published materials only by coded number so as to assure privacy. The supplement of selenium that subjects will receive is a moderate dose. The only reported side effect in some people at this dose has been mild stomach upset. If any side effect becomes bothersome the participant is entitled to remove him or herself from the study. Participants who agree to participate in the initial screening will receive, prior to administration, a list of referrals to mental-health providers. Participants who answer in the affirmative to the BDI-II Question 9 (Suicidal Ideation) will be asked to sign a no-harm contract. If a subject indicates they are suicidal immediate evaluation will be done by Dr. Weiss, who will be on call whenever subjects are participating in the study. If the subject refuses to sign the no-harm contract emergency personnel will be called to intervene. See attached for list of referrals and no-harm contract.

IV. Consent Form

See attached copy.

V. Liability

Participants will be required to have access to a physician or nurse in case of unexpected injury in the course of the study period. Neither Texas Tech nor the researchers shall be held financially liable for any injury as a result of the research protocols. Those participants screened and found to suffer from severe depression shall be referred to a treatment facility. Additionally, all participants screened will be given a list of referrals for treatment. This will be included in the descriptive of the research proposal along with the no-harm statement.

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

A Study Examining the Effect of Selenium (Se) Supplementation on Mood
Mood Questionnaire Consent

- INTRODUCTION

  You are being asked to take part in a research study conducted by Lela Tatum, M.S., and L. Mallory Boylan, PhD., L.D., R.D at Texas Tech University's College of Human Sciences, Department of Food and Nutrition. You have been asked to take part in this study because you are in the age group that we are studying. You will be asked to fill out a short questionnaire to see if you have a lower mood than normal. Based on your answers to the questions you might be asked to take part in the second part of the study.

- PROCEDURES

  If you volunteer to take part in this study, we would ask you to do the following thing:

  I. Answer the questions on the Beck Depression Inventory (BDI-II). This form asks a series of questions to find out about your mood and whether you suffer from lowered mood. If chosen to continue in the second part of the study you will answer these questions again two months later.

- POTENTIAL RISKS AND DISCOMFORTS

  We know of no risks involved in filling out this questionnaire.

- FINANCIAL OBLIGATION

  This research is supported by Texas Tech University. People who take part in the study are not responsible for any costs. All mood questionnaires are free. Neither you nor your insurance company will be billed for your taking part in this research.

- PRIVACY AND CONFIDENTIALITY

  The research team and your doctor and nurses will know that you are taking part in a study. Your information will be kept private, but you and your doctor may have a copy if you want one. No information about you will be given out without your permission. When the results of the research are published or discussed in conferences, no information about you will be included.

- PARTICIPATION AND WITHDRAWAL

Whether or not your take part in this research is your choice. If you agree to take part but later decide not to, you may drop out. You will be taken out of the study if any problems come up which need to be treated by your doctor. If you get sadder you will be taken out of the study and your doctor will be told.

- NEW FINDINGS
  You will be told about any changes that have to be made in the study and you may decide to drop out.

- RIGHTS OF RESEARCH SUBJECTS

  Dr. Boylan has agreed to answer any inquiries I may have concerning the procedures and has informed me that I may contact the Texas Tech University Institutional Review Board for the Protection of Human Subjects by writing to them in care of the Office of Research Services, Texas Tech University, Lubbock, Texas 79409, or by calling 742-3884.

  If this research project causes any physical injury to participants in this project, treatment is not necessarily available at Texas Tech University or the Student Health Center, nor is there necessarily any insurance carried by the University or its personnel applicable to cover any such injury. Financial compensation for any such injury much be provided through the participant's own insurance program. Further information about these matters may be obtained from Dr. Robert M. Sweazy, Senior Associate Vice President for Research, 742-3884, Room 203 Holden Hall, Texas Tech University, Lubbock, Texas 79409-1035.

  I understand that I may not derive therapeutic treatment from participation in this study. I understand that I may discontinue this study at any time I choose without penalty.

SIGNATURE OF RESEARCH SUBJECT
Mood Questionnaire

My signature below shows:

- I have read this form and understand what it means
- I have had a chance to ask questions and have had these questions answered to help me understand the study
- **I agree to take the mood questionnaire to see if I can take part in the study**; and
- I will be given a copy of the signed permission form.

_____
Name of subject

_____ _____
Signature of subject Date

SIGNATURE OF INVESTIGATOR

I have explained the research to the person and answered all of his/her questions. I believe that he/she understands the information and freely decides to participate.

_____
Name of investigator

_____ _____
Signature of investigator Date

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

A Study Examining the Effect of Selenium (Se) Supplementation on Mood

- INTRODUCTION
  You are being asked to take part in a research study conducted by Lela Tatum, M.S., and L. Mallory Boylan, PhD., L.D., R.D. at Texas Tech University's College of Human Sciences, Department of Food and Nutrition. You have been asked to take part in this study because you are the right age and have a score on the mood questionnaire that shows you may have a lower mood than normal. About 75 people will take part in this study. Taking part in this study is your choice. Please read the information below, and ask questions about anything you do not understand, before deciding whether or not to take part.

- PURPOSE OF THE STUDY
  Recent studies show that adding selenium, a mineral found in grains, meats and other foods, to your diet may raise mood in some people. Our study will see if this mineral will help the mood in retired people. We will measure the change in mood scores and find out whether there is any connection with changes in the amount of selenium in your blood after you take the selenium tablets.

- PROCEDURES
  If you volunteer to take part in this study, we would ask you to do the following things:
  I. Give us information about you (ex: age, sex, medications you are taking). Your identity will be private because your name will not be used.
  II. Tell us what you have eaten over the past 24 hours.
  III. Agree to have about a tablespoon's worth of blood taken from your arm at the beginning and at the end of the study to see how much selenium is in your blood. Blood will be taken by a nurse practitioner in a private price such as the clinic of where you live.
  IV. Agree to take a tablet of selenium or a sugar pill for eight weeks. These tablets will be given to you for free.

- POTENTIAL RISKS AND DISCOMFORTS
  The amount of selenium that you will be getting in the tablet each day is 200 micrograms. The only side effect in some people who take this mineral has been mild stomach upset. You may have slight bruising and some pain from having blood taken but these risks should not be serious.

- FINANCIAL OBLIGATION
  This research is supported by Texas Tech University. People who take part in the study are not responsible for any costs. All tablets and the mood diagnostic questionnaires are free. Neither you nor your insurance company will be billed for your taking part in this research.

- PRIVACY AND CONFIDENTIALITY
  The research team and your doctor and nurses will know that you are taking part in a study. Your information will be kept private, but you and your doctor may have a copy if you want one. No information about you will be given out without your permission. When the results of the research are published or discussed in conferences, no information about you will be included.

- PARTICIPATION AND WITHDRAWAL
  Whether or not your take part in this research is your choice. If you agree to take part but later decide not to, you may drop out. You will be taken out of the study if any problems come up which need to be treated by your doctor. If you get sadder you will be taken out of the study and your doctor will be told.

- NEW FINDINGS
  You will be told about any changes that have to be made in the study and you may decide to drop out.

- RIGHTS OF RESEARCH SUBJECTS

  Dr. Boylan has agreed to answer any inquiries I may have concerning the procedures and has informed me that I may contact the Texas Tech University Institutional Review Board for the Protection of Human Subjects by writing to them in care of the Office of Research Services, Texas Tech University, Lubbock, Texas 79409, or by calling 742-3884.

  If this research project causes any physical injury to participants in this project, treatment is not necessarily available at Texas Tech University or the Student Health Center, nor is there necessarily any insurance carried by the University or its personnel applicable to cover any such injury. Financial compensation for any such injury much be provided through the participant's own insurance program. Further information about these matters may be obtained from Dr. Robert M. Sweazy, Senior Associate Vice President for Research, 742-3884, Room 203 Holden Hall, Texas Tech University, Lubbock, Texas 79409-1035.

I understand that I may not derive therapeutic treatment from participation in this study. I understand that I may discontinue this study at any time I choose without penalty.

SIGNATURE OF RESEARCH SUBJECT
Study Subjects

My signature below shows:

- I have read this form and understand what it means
- I have had a chance to ask questions and have had these questions answered to help me understand the study
- I agree to participate in this research study; and
- I will be given a copy of the signed permission form.

_____
Name of subject

_____ _____
Signature of subject Date

SIGNATURE OF INVESTIGATOR

I have explained the research to the person and answered all of his/her questions. I believe that he/she understands the information and freely decides to participate.

_____
Name of investigator

_____ _____
Signature of investigator Date

ID:_____

Demographic Information

Age:

1) <60 2) 60-65 3) 66-70 4) 71-75 5) 76-80 6) 81-85 7) >86

Education:

1) Elementary School
2) Junior High School
3) Some College
4) Bachelor's Degree
5) Master's Degree or higher

Gender:

1) Male
2) Female

Have you ever been hospitalized? If yes, when and for how long?

Have you ever suffered a stroke or heart attack? If yes, when? How many?

Are you currently taking any prescription or non-prescription medications? If yes, which, and for how long have you taken them?

Do you take any herbal preparations such as St. John's Wort, ginko biloba, ginseng, etc.? If yes, which?

Do you take any vitamin, mineral, or amino acid supplements? If yes, which? How often?

Have you ever been diagnosed with depression? If so, when?

Are you currently taking any medication for depression (such as Prozac, Zoloft, Luvox, Paxil, Celexa, Wellbutrin, Parnate, Nardil, etc.) or any herbals (such as St. John's Wort)? If so, which? How long have you taken these?

Have you experienced any of the following major life changes in the past 6 months?

1) Move to a new home or city
2) Death of a family member
3) Loss of a job or unwillingly retired
4) Major illness of you or a loved one
5) Other stressor (please specify):

APPENDIX C

NO HARM CONTRACT

No-Harm Contract

1. I, _____ agree not to kill myself, or cause any harm to myself during the period from _____ to _____.
2. I agree to get enough sleep and to eat well.
3. I agree to get rid of things I could use to kill myself
4. I agree that if I have a bad time and feel that I might hurt myself, I will call my doctor, nurse, the Lubbock Crisis Intervention Service at 765-8393 or 911 immediately
5. If I refuse to sign this document, emergency personnel will be called to intervene.

Signed: _____ Witnessed: _____

Date: _____

Mental Health / Counseling Providers

Contact Lubbock Crisis Intervention Service	765-8393 – 24 hours a day
Ambulance	911
MHMR Crisis Line	740-1414
UMC Emergency Department	775-8636
Covenant Emergency Department	725-0069

APPENDIX D

FLUOROMETRIC MEASUREMENT OF SELENIUM

FLUOROMETRIC MEASUREMENT OF SELENIUM

Spallholz et al (1978)

Solutions

Digestion mixture

1. Add to 5 g of sodium molybdate 75mL of water
2. Add 75mL of sulfuric acid to the molybdate solution slowly
3. Add 100mL of 70-72% perchloric acid.

Diaminonaphthalene solution

1. Add 35mL of concentrated sulfuric acid to 215mL of water
2. Add 150mL of 3,3'-diaminonaphthalene in 150mL of sulfuric acid solution and transfer to a separatory funnel.
3. Add 150mL of cyclohexane and shake for 10-15 minutes. Use lower sulfuric acid phase for assay.

EDTA (0.006M) solution

1. Make Se standard solution and a water blank for total volume of 1mL in test tubes.
2. Place 1mL of tissue in test tubes. Bring up to a volume of 1mL using water.
3. Add 2mL of the digestion mixture and 2-3 acid washed glass beads
4. Digest each sample until the final volume is about 1mL and light yellow in color.
5. Dilue the samples to 7mL with 0.008M EDTA
6. Add 2.1-2.2mL of concentrated NH4OH to each test tube
7. Adjust pH to 2 using either concentrated NH4OH or H2SO4.
8. Add 4mL of the diaminonaphthalene solution, mix, and incubate in a 50C water bath for 20 minutes
9. After incubation, add 4mL of cyclohexane and shake for 5 minutes
10. Transfer the cyclohexane phase to the cuvette
11. Measure fluorescence from the 4,5-benzopiazselenol complex by excitation at 363nm with the emission measure at 525nm. Autozero against a chloroform blank.
12. Plot data as relative fluorescence intensity against 1ng Se/4mL of cyclohexane.

APPENDIX E

DETERMINATION OF GLUTATHIONE PEROXIDASE ACTIVITY

DETERMINATION OF GLUTATHIONE PEROXIDASE ACTIVITY

modified from Paglia and Valentine (1967)

Solutions

DPB: Dilute buffer (0.2M, pH 7.0)

1. 1.7535g EDTA (6.0mM) + 34.836g K2HPO4/1000mL H2O
2. 1.2275g EDTA (6.0mM) + 19.0526g KH2PO4/700mL H2O
3. 1000mL of 1) + 640mL of 2) = pH 7.0

Before use, dilue the buffer with H2O in a 1:4 ratio

Sodium azide (NaN3) (0.01M)

NaN3(anhydrate) MW=65.01
65mg NaN3/100mL H2O

Potassium chloride (KCl) (0.15M)

KCl MW=74.56
11.18g/1000mL H2O (1.12%)

Sucrose

Sucrose MW=342.3
85.58g/1000mL H2O (8.56%)

Hydrogen peroxide (H2O2) (5.0mM)

H2O2 MW=34.01
20uL of 30.% H2O2 into 35.2mL H2O

Preparation of assay mixture for 10 assays

GSH	2.0mM	12.20mg
NADPH	0.25mM	4.17mg
GR	1.0EU/mL	20.00EU
NaN3	1.0mM	2.00mL
DPB		18.00

Assay mixture should be made daily

Procedure

1. Homogenize tissue in 0.25M sucrose
2. Centrifuge at 26,500g for 20 minutes at 4C
3. Collect the supernatant for analysis
4. Pipette 1.7mL of assay mixture into a cuvette and insert it into a spectrophotometer. The concentration of NADPH should read 0.7 to 1.0 at 340nm
5. Pipette 20 to 200uL of tissue supernatant to the cuvette, use DPB to make 1.9mL/cuvette, and incubate in the spectrophotometer for about 2 minutes
6. Add 0.1mL of 5.0mM H2O2 to start the reaction, and turn on the recorder immediately.
7. Record the starting and final OD for a period of 3-5 minutes.
8. Perform a blank analysis
9. Calculate enzyme activity

To operate:

1. Set spectrophotometer at 340nm in 'Kinetic' mode.
2. Place the cuvette containing 1.7mL assay mixture into the machine. The concentration of NADPH should be between 0.7-1.0 of absorbance
3. Add 200uL of buffer solution or 20uL to 200uL tissue supernatant. Use diluation phosphate buffer to adjust volume to 1.9mL
4. Add 0.1mL H2O2 and start immediately
5. Calculate enzyme activity.

APPENDIX F

BECK DEPRESSION INVENTORY - II

Date:

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness

0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all the time.
3 I am so sad or unhappy that I can't stand it.

2. Pessimism

0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my future is hopeless and will only get worse.

3. Past Failure

0 I do not feel like a failure.
1 I have failed more than I should have.
2 As I look back, I see a lot of failures.
3 I feel I am a total failure as a person.

4. Loss of Pleasure

0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings

0 I don't feel particularly guilty.
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

6. Punishment Feelings

0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. Self-Dislike

0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

8. Self-Criticalness

0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes

0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

10. Crying

0 I don't cry anymore than I used to.
1 I cry more than I used to.
2 I cry over every little thing.
3 I feel like crying, but I can't.

Continued on Back

_____ Subtotal Page 1

Orland 0154018392

APPENDIX G

REQUEST FOR PARTICIPANTS IN STUDY

Research Opportunity!

Wanted: Men and women ages 60-85 to participate in a study about how diet affects mood.

What am I required to do?

You will be required to fill out two brief surveys, take a mineral supplement for 8 weeks (provided free of charge), and have a small amount of blood taken.

Will this inconvenience me?

All services will be provided free of charge. The surveys and blood draws will be completed in the privacy of your home. Additionally, to protect your privacy, you name or any identifying information will not be used.

How can I learn more?

You may contact the researcher at the following telephone number at any time. In about a week you will receive information about when you will be able to visit personally.

Thank you,

Lela Tatum, M.S., Ph.D.(c)
Dr. Mallory Boylan, Ph.D, R.D., L.D. (supervisor)
Texas Tech University
Department of Food and Nutrition
(806) 441-8983
(806)742-3068 (supervisor)