EFFECTS OF VITAMIN E SUPPLEMENTATION ON RENAL TISSUE VITAMIN E CONCENTRATION IN MALE RATS

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To the Dean of the Graduate School:

I am submitting herewith a thesis written by Brianna Coulson entitled "Effects of Vitamin E Supplementation on Renal Tissue Vitamin E Concentration in Male Rats." I have examined this thesis for form and content and recommended that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major of Nutrition.

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DEDICATION

To my parents, Chris and Bobbie Woods,
Thank you for you love, dedication, guidance and encouragement.

To my husband,
Thank you for your continuous support, patience, love and encouragement.

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ABSTRACT

BRIANNA COULSON

EFFECTS OF VITAMIN E SUPPLEMENTATION ON RENAL TISSUE VITAMIN E CONCENTRATION IN MALE RATS

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Alpha-tocopherol is a well known antioxidant present in foods. This vitamin has been studied in the past in the prevention and treatment of neoplasms in human and in animals, however, the effects of supplementation on the lipids of the tissues has yet to be discovered. The purpose of this study was to investigate the relationship between DL-alpha tocopherol supplementation and the tissue content of renal and hepatic triglyceride and total cholesterol concentrations. In a two week study, twenty-four male Fisher 344 rats were randomly assigned into three groups: 75 IU D-alpha-tocopherol, 355 IU D-alpha-tocopherol, and 750 IU D-alpha-tocopherol. Renal total cholesterol and triglyceride concentrations were not significantly different among treatment groups. Hepatic total cholesterol concentration were not significantly different among treatment groups. However, there were significant differences in hepatic triglycerides concentrations between the 75 IU D-alpha-tocopherol and the 355 IU and 750 IU groups. Results of this study showed that the supplementation of D-alpha-tocopherol increase hepatic triglyceride content but had no effect on hepatic total cholesterol, or renal total cholesterol and triglyceride.

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CHAPTER I

INTRODUCTION

Cancer is the second leading cause of death in the United States, killing approximately one out of every four persons. It is estimated that approximately 17 million new cancer cases have been diagnosed since 1990 and this estimate does not include noninvasive or squamous skill cell cancer. Around 560,000 of these cases will die each year of cancer, and more than 1,500 patients die each day. Along with this statistics are also the fact that most of the forms of cancer in the United States are preventable. Scientific evidence suggests that one-third of the 560,000 new cases of cancer are related to nutrition, physical activity, obesity, and other lifestyle factors that could have otherwise been prevented (Society, 2003).

Renal Cell Carcinoma (RCC) is a deadly form of cancer, affecting the kidneys and renal pelvis. This form of cancer has approximately 31,900 new cases each year, with more than half (19,500) affecting men. This form of cancer also has a very low survival rate, with 12,500 dying each year and men still being more affected, comprising 8,600 of the deaths (Society, 2003). It is also responsible for 80-85% of malignant tumors of the kidney in adults, and for approximately 2% of cancer deaths worldwide (Bosetti, 2006). It is no surprise that this form of cancer has been linked to those with hypertension, those who are obese, non-active, and smoke (Society, 2003; Murai, 2004).

There is an increasing body of literature that suggests that dietary composition with other environmental factors, influence the rate and generation of renal cell carcinoma. Vitamin E, especially alpha tocopherol, has been promoted for its antioxidant and lipid membrane preserving affects, is being investigated as a preventative agent in cancer initiation and progression by being incorporated into foods as supplements. Studies have been conducted in vivo and in vitro (Wolk, 1996). Preliminary studies involving Wistar rats examined vitamin E would preserve and prevent iron-induced oxidative nephrotoxicity and renal cancer. The study concluded that vitamin E inhibited lipid peroxidation, cancer induced apoptosis and cancer formation (Zhang, 1997). Other studies have also focused on other forms of vitamin E, such as gamma and delta tocopherol, in the diet as a prevention of cancer with beneficial results (Jiang, 2004).

Some studies of the effects of vitamin E in the prevention of cancer suggestion that further research is needed before the vitamin can be used for the prevention of cancers such as RCC. Also, the potential for high levels of vitamin E to have undesirable side effects should be investigated. These side effects could include changes in the function and/or composition of the kidney as well as other organs and tissues, such as the liver. Changes could include alterations in the lipid (triglyceride and cholesterol) concentration of kidney and liver.

Significance Statement

This project is significant as there is a need to have information as the effect of high dietary levels of vitamin E as the lipid composition of kidney and liver.

Null Hypotheses

Liver Tissue— There will be no effect of high dietary concentration of vitamin E on either the cholesterol or triglyceride concentration of the liver.

Kidney Tissue— There will be no effect of high dietary concentration of vitamin E on either the cholesterol or triglyceride concentration of the kidney.

CHAPTER II

REVIEW OF LITERATURE

Cancer

Cancer is defined as a group of cells within the body reproducing at an abnormally accelerated rated. These cells without regard or regulation continue to growth without purpose, often resulting in loss of function to surrounding cells, organs and tissue. Cancer can also spread or metastasize, to other organs and parts of the body causing uncontrolled growth in those areas of the body as well. This condition often results in death. In the United States alone approximately 2.5 million cases of cancer are diagnosed every year, and a cost totaling over 206 billion dollars in 2006. It is also estimated that 1,500 people day per day in 2007 in the United States from cancer, which totals approximately 559,650 deaths per year (American Cancer Society, 2007).

Cancer is caused by a variety of factors, both internal and external. Internal factors include hormones, inherited mutations, immune conditions and mutations from metabolism. External factors, such as smoking, diet, pollutants, chemicals, radiation, and infectious organisms, are currently our focus in prevention. Researchers state that most cancers do not involve genetic components, and it is estimated that only about five percent of cancer types involve a strongly inherited gene mutation. This gene mutation, in turn, results in the cancer itself. It is more common to have genetic harm, or mutation of

the DNA strand as a result of damage that occurred during one's lifetime (American Cancer Society, 2007).

The associated risk of developing cancer can be defined into two terms, lifetime and relative risk. Lifetime risk is the chance of a person developing cancer within his or her lifetime; currently one in three women in the United States will develop cancer within her lifetime, and one in two men in his lifetime. Relative risk is a measure of the strength of the relationship between a risk factors and a particular cancer (American Cancer Society, 2007)

Ten year cancer survival rates for the United States were 66% between 1996 and 2002. Some have speculated that this number is up from previous studies due to early detection and improvements in treatment methods. Others speculate that early prevention techniques are continuing to play a role in decreased incidence of preventable cancers; however, the survival rate fluctuates greatly on the type and stage of the cancer itself (American Cancer Society, 2007).

Prevention is thought to be the key to controlling this disease, along with early detection and treatment, and is a method of delaying or preventing the condition from occurring through an action or avoidance of a particular activity. It is estimated that about one-third of the cancer deaths projected in 2007, approximately 184,684 cases, could have been prevented by changing associated habits, such as smoking, nutrition, obesity and physical inactivity. Risk of developing cancer still has age relationship;

approximately seventy-seven percent of all cancers diagnosed in 2007 were in people aged fifty-five or older (American Cancer Society, 2007).

Renal cell carcinoma (RCC) is a form of kidney cancer in which the cancerous cells are located in the lining of the kidney tubules. The majority of the malignant renal tumors in the United States (approximately 90%), are classified as RCC and originate from the proximal tubal epithelium (Robert Amato, 2006; American Cancer Society, 2007). This form of cancer usually grows in one single mass; however, it has been documented to be found in more than one part of the kidney or in both kidneys at once. RCC is typically asymptomatic in its early stages, creating difficulty in detection (Masaru Marai, 2004). If RCC is detected at an early stage, a common procedure, a radical nephrectomy, can sometimes successfully be used to treat this condition; however, as many as 20-30% of cases develop metastatic disease following this surgical procedure (Amato, 2006). Chemotherapy and radiation are also recommended, with low survival rates and outcomes for these patients. In one-third of newly diagnosed patients, the cancer has already metastasized to the lungs and other organs by the time that it is diagnosed (MedlinePlus, 2008). The five year survival rate for these patients is less than two percent (Amato, 2006).

In 2005, more than 35,000 people were diagnosed with this form of cancer, and approximately 12,000 died. RCC presently ranks at the tenth leading cause of death due to cancer, and constitutes three percent of all solid neoplasms. The incidence of renal cell

carcinoma has been increasing not only in the United States and other Western countries, but worldwide. Incidence rates have increased 10-fold around the world, with higher rates being more prevalent in Western countries (American Cancer Society, 2007).

In those patients who are diagnosed with renal cell carcinoma, 62% are male and only 38% were female. The majority of those who were diagnosed was between the ages 50-70 years old and was typically African American. Risk factors for this type of cancer include smoking, obesity, high blood pressure, exposure to harsh chemicals, family history, low intake of fruit and vegetables and intake of certain medications (Masaru Marai, 2004; American Cancer Society, 2007). Some disease conditions, like von Hippel-Lindau Disease, also showed a strong correlation with the development of RCC. Physical activity and increased the consumption of increased amounts of fruits and vegetables are encouraged with this condition, along with cessation of smoking and exposure to harsh chemicals, to prevent the development of this cancer (American Cancer Society, 2007).

Inflammation, or the inflammatory process by which the body combats injury, damage, disease, and infection, is currently being research as a cause of RCC and other forms of cancer. It is hypothesized that chronic inflammation in the kidneys, which is due to a multitude of factors, can be caused damage to the cells of the kidney resulting in renal cell carcinoma. It is also been hypothesized that lipid peroxidation, or the oxidative degeneration of lipids by which free radicals that take electrons from the cell membrane, could lead to the development of RCC. Due to the nature of free radical oxidation and

inflammation, Vitamin E, a natural antioxidant is being researched as a possible method of prevention of RCC (American Cancer Society, 2007; Marai, 2004).

Vitamin E

Vitamin E or alpha-tocopherol, was first discovered at the University of California at Berkley in 1922. Since that time at least eight vitamers of vitamin E have been discovered with differing levels of biological activity. These vitamers are classified according to their structure and categorized into either tocopherols or tocotrienols. Tocopherols have a saturated phytyl tail attached to their chromanol ring, whereas tocotrienols have an unsaturated aliphatic tail. These classes are further divided into the eight isoforms α- (alpha), β- (beta), γ- (gamma), or δ- (delta), which designated their methyl placement on the chromanol ring. The alpha forms contain three methyl groups, whereas the beta and gamma forms have two and delta has one. Each class is further classified according to their stereochemistry, R and S are used to designate the asymmetrical form of vitamin E. Synthetic forms of alpha tocopherol, which include all racemic (all-rac) alpha-tocopheral acetate and all-rac alpha tocopherol succinate, are used in vitamin supplements and fortified foods. When supplemented, synthetic forms of the often contain a mixture of eight stereoisomers and are not as active as the naturally occurring form, RRR- alpha-tocopherol. Synthetic forms of alpha-tocopherol also increase plasma level of alpha-tocopherol to have of the concentration as the naturally occurring form (Pham, 2005). Naturally occurring alpha -tocopherol is found in lipid

rich plant products and vegetable oils, while rice bran and palm oil have high concentrations of tocotrienols (Packer, Weber, & Rimbach, 2001).

Each of these isoforms have a different bioavailability. Tocopherols are found freely in foods and require no further action, whereas tocotrienols are esterified and need to be hydrolyzed before absorption. Synthetic ester forms of tocopherols also require digestion prior to absorption. Pancreatic esterase and duodenal mucosal esterase in the lumen or brush border of the intestines are thought to hydrolyze tocotrienols and synthetic forms of alpha tocopherol. The half-life for RRR-alpha-tocopherol is also longer, lasting around 48 hours, while SRR-alpha tocopherol lasts only thirteen (Gropper, 2005; Packer, Weber, & Rimbach, 2001).

In the humans, all forms of vitamin E are absorbed by passive diffusion in the jejunum via the formation of micelles from bile salts to allow the lipid soluble vitamin to be absorbed. The presence of dietary lipids in the gut aids in the digestion and absorption of vitamin E; however, the optimum level of lipids required is yet to be discovered. The efficiency of the absorption of vitamin E ranges from 20-80%. Once in the enterocyte, the absorbed tocopherol is incorporated, along with other lipid components, into chylomicrons which enter the lymphatic system and then the general circulation. Chylomicrons, which enter the lymphatic system and then the general circulation at the thoracic duct, are broken down into chylomicron remnants by the action of lipoprotein lipase. Chylomicron remnants are taken up by the liver and tocopherols in these particles

are removed. In humans, the liver also contains a transfer protein, alpha-tocopherol transfer protein (α-TTP)[1], that preferentially enriches VLDLs[2] and consequently other lipoproteins with RRR-alpha-tocoperhol (Gropper, 2005). The affinity of α-TTP for other forms of vitamin E varies, β- and γ-tocopherol have a thirty-eight and nine percent affinity with the α-TTP protein respectively, while SRR-α-tocopherol has eleven percent affinity and α-tocotrienol has twelve. The specificity of the transfer protein helps to regulate the level of plasma vitamin e and the other forms of vitamin e are not resecreted into circulation due to poor recognition. α -TTP has been found in low levels in other tissues within the body including the brain, spleen, lung and kidney (Gropper, 2005).

It has been shown in past studies that the uptake and distribution after oral ingestion of tocotrienols is much less than tocopherols. In one particular study, hamsters were fed a mixture of vitamin E isoforms, and tocopherol was preferentially absorbed. In humans however, tocotrienols can still be detected in the postprandial plasma and found in all classes of lipoproteins (Hayes, 1993; Packer, Weber, & Rimbach, 2001). Tocotrienols have been shown to have a greater antioxidant/free radical savaging capability than tocopherols and an interaction with HMG[3] CoA reductase that produces a cholesterol reducing effect, however, since they are not biologically available studies have not focused on their role in cancer prevention (Packer, Weber, & Rimbach, 2001).

[1] A liver protein responsible for transferring alpha-tocopherol from liver tissue into systemic circulation
[2] Very Low Density Lipoprotein: a protein that transports fats to tissues in the body
[3] 3-hydroxy-3-methyl-glutaryl-CoA reductase

Uptake into the cells of alpha-tocopherol occurs simultaneously as lipoproteins are taken up by tissues. Thus, the uptake of alpha-tocopherol can occur through several pathways: as receptor-mediated uptake of LDLs[4] occurs; through lipoprotein lipase-mediated hydrolysis of chylomicrons and VLDLs; through HDL[5]-mediated nutrient delivery, or some other unknown mechanism. For entrance into the membrane, a phospholipid transfer protein is thought to be used. Once within the cell alpha-tocopherol is thought to participate primarily in antioxidant reactions and cell signaling (Gropper, 2005).

The main function of alpha-tocopherol is that of an antioxidant and the maintenance of the structural integrity of cell membranes. The structure of alpha-tocopherol, specifically the methyl groups in the phenolic ring, allows for hydrogen ions to be released and donated to free radicals (Gropper, 2005). The pathway by which alpha-tocopherol protects cell membranes is by preventing oxidation (peroxidation) of unsaturated fatty acids that are located within the cell membrane in the form of phospholipids and triglycerides. Phospholipids are also part of the mitochondrial and endoplasmic reticulum membrane and are also at risk for oxidative damage through the production of free radicals (Gropper, 2005). Free radicals are single unpaired electrons that are formed during normal bodily functions, such as metabolism and upon exposure to environmental risk factors like smoking, chemicals or pollutants. Alpha-tocopherol has

[4] Low Density Lipoprotein
[5] High Density Lipoprotein

also shown to protect low density lipoproteins oxidation (Gropper, 2005; Packer, Weber, & Rimbach, 2001).

Free radicals damage the phospholipid membrane by starting a series of reactions that can be terminated by alpha-tocopherol. The series of reactions occurs as follows: initiation, propagation, and termination, the last of which involves alpha tocopherol. Initiation typically begins with an initiator, a naturally produced free radical, taking an electron from its surroundings, like the cell membrane. This action damages the structure of the membrane forming peroxyl or a lipid center radical. From this point, peroxyl membranes can continue to damage other surrounding membranes, looking to replace their lost electrons and repair their structural integrity, this step is called propagation. Termination is the final step where alpha-tocopherol is involved. During termination, vitamin e that is located near the peroxyl radicals (or damaged membrane) can react and donate an electron to the peroxyl radical before it can interact with other phospholipids within the cell. Thus, alpha-tocopherol terminates the propagation cycle (Gropper, 2005).

However, alpha-tocopherol does not work in isolation from other antioxidants; rather it is part of an interlinking set of redox antioxidant cycles, which perform reduction/oxidation cycles repeatedly .It is hypothesized that vitamin E acts catalytically, that is it is efficiently reduced from its free radical (chromanoxyl) form to return back to its reduced native state. This catalysis occurs through the interactions between water- and lipid-soluble micronutrients, such as vitamin C, glutathione, and carotenoids, by both

enzymatic and nonenzymatic pathways that regenerate vitamin E from its tocopheroxyl radical back to tocopherol form. Vitamin C can regenerate alpha tocopherol directly, and thiol antioxidants, such as glutathione and lipoic acid, can regenerate alpha tocopherol indirectly via vitamin C. Under conditions in which these mechanisms act synergistically to keep the steady-state concentration of alpha tocopherol radicals low, the loss of alpha tocopherol is prevented. These recycling effects have been seen in human oxidized LDL and in membranes enriched with tocopherols or tocotrienols (Gropper, 2005; Packer, Weber, & Rimbach, 2001).

Vitamin E is also currently being researched for its role in cell signaling. Recent studies have shown that alpha tocopherol is capable of inhibiting the activity of protein kinase C (an important cell signaling molecule) and phosphatidylinositol 3-kinase. Changes in cell proliferation (especially smooth muscle cell), platelet aggregation and NADPH-oxidase activation are associated with alterations in the activity of these kinases (Azzi, 2004). Some reports also show that vitamin E has had an effect on the expression and activity of immune and inflammatory cells, and to inhibit platelet aggregation and enhance vasodilation (Pham, 2005).

Alpha-Tocopherol and Cancer Prevention

Decades of research on vitamin E suggest that supplementing the diet with vitamin E is a safe way to increase intake of the vitamin (Medicine, 2000). Some epidemiological studies have just shown a decrease incidence of mortality. The Alpha-

Tocopherol and Beta-Carotene Prevention Study (ATBC) studied whether certain vitamin supplements could prevent lung cancer and other cancers in a group of 29,133 Finnish make smokers. The 50-69 year old participants took a daily supplement for five to eight years and were assigned to groups, receiving on a daily basis: 50 mg of alpha-tocopherol, 20 mg beta-carotene, both or a placebo. While the results for beta-carotene showed an 18% increase in mortality (per 100,000), the results showed that vitamin E had no effect on lung cancer, but showed a 32% decrease (per 100,000), in the number of new prostate cancer cases and a 41% decrease (per 100,000), in mortality due to prostate cancer. Within the six-year post-intervention period, the low prostate cancer incidence rates trended toward normal in the alpha-tocopherol supplementation group but remained below the placebo group (Institute et al., 2003). Wright et al, reported data on the Alpha-Tocopherol and Beta-Carotene Prevention Study (ATBC), and when they analyzed the data reported higher circulating concentrations of alpha-tocopherol, within the normal range, were associated with significantly lower total and cause specific mortality (Wright, 2006).

Malmberg et al. (2002), studied twelve patients with advanced colorectal cancer to determine if short term vitamin E supplementation would help with immune function. The researchers supplemented the diet for two weeks with 750 mg vitamin E[6] prior to chemotherapy or radiation treatment. The supplementation resulted in significantly

[6] Form not specified

increased CD4:CD8[7] ratios and enhanced capacity of their T cells to produce T helper 1 cytokines interleukins and IFN-γ (Interferon- gamma, an important immunologic compound associated with the body's immunological response and tumor suppression). These authors also concluded that vitamin E may be used to improve and support immune function in patients with advanced cancer (Malmberg et al., 2002). Another study using with melanoma Bl6-F10 cells and free radical levels in monkey kidney cells, showed that alpha-tocopherol acid succinate decreased free radical levels in the monkey cells at concentrations of 1μg/ml, and a significant decrease of BL6-F10 growth was observed at a concentration of 5 μg/ml alpha-tocoperhol acid succinate. They also concluded that the inhibitory effect seen in on the BL6-F10 cell growth was not due to the antioxidant properties of alpha-tocopherol but may be due to its potential role within the cells, such as regulation of cellular enzymes involved in growth (Ottino, 1997).

Within the realm of renal cell carcinoma, research has shown a positive correlation between the supplementation of vitamin E in the diet and decreased incidence of chemically induced RCC. In a study by Zhang et al., male Wistar rats were fed an alpha-tocopherol sufficient diet (2 international units (I.U.) alpha-tocopherol /100 g) and a alpha-tocopherol supplemented (50 units/100g) diet and were treated with Fe-NTA[8] to induce renal cancer. The results revealed that only five percent of the vitamin E supplemented rats developed cancer, while forty-four percent of the vitamin E sufficient

[7] A subgroup of lymphocytes important in utilizing and activating other immune cells
[8] ferric nitrilotriacetate: a pro-oxidant used to create oxidative damage and renal cancer

rats did. The authors concluded that vitamin E, as an antioxidant, is an effective chemopreventative measure when free radicals play an important role in carcinogenesis (Zhang, 1997). Another study by Iqbal et al., confirmed these findings and also concluded that pretreatment with vitamin E reduced lipid peroxidation in rat kidney cells and that there was a dose-dependent relationship with the amount of vitamin E used and the amount of lipid peroxidation seen. With the higher doses of 2 mg vitamin e/animal/day, the lipid peroxidation levels almost reduced to the control values (Iqbal, 1998).

Certain risk factors for developing RCC, including lipid peroxidation, have also been shown decreased incidence when the diet was supplemented with alpha-tocopherol. Lipid peroxidation, especially during the induction period, showed a marked increase in the development of RCC and malignant mesenchylmal tumors when compared to a normal renal cortex in human kidneys (Nikiforova, 2001). In another study designed to explore the effects of vitamin E supplementation on nitric oxide[9] inhibition and its effects on renal injury, male Sprague Dawley rats were fed a standard diet containing a high dose of alpha-tocopherol acetate (0.7 g/kg body weight per day). These high doses of alpha-tocopherol acetate, prevented oxidation and maintained renal vascular and glomerular function and structural integrity. Alpha-tocopherol acetate doses also showed a decrease in the development of proteinuria but not in hypertension (Attia, 2001). Another mechanism by which All-RAC-alpha-tocopherol acetate has been shown to prevent

[9] Nitric Oxide deficiency increases superoxide dismutase (0_2^-) activity, an enzyme which creates a large amount of free radicals.

oxidative damage, is by upregulating a glycoprotein-specific chaperone gene, calnexin, and a sugar transferring enzyme, glucosaminyltransferase-3, in rat tubular cells after they were fed an 500 mg/kg DL-alpha-tocopherol acetate supplemented diet prior to oxidative damage. Calnexin and glucosaminyltransferase-3 have been associated with reduced acute oxidative tubular damage, which is a subsequent indicator of renal carcinogenesis (Lee et al., 2006).

In other disease conditions where the kidney is damaged, vitamin E supplementation has also shown to ameliorate renal injury. Trachtman et al. (1996), investigated the potential of vitamin E supplementation (100 IU/kg[10]) restored renal function in male Lewis rats with Immunoglobin A (IgA) nephropathy. The authors concluded that a diet moderately enriched, only a 3-fold increase above basal, with vitamin E ameliorates renal function and prevents structural changes that are associated with IgA nephropathy. The authors further concluded that this effect was due to a reduction in renal cortical malondiadehyde content and a decrease of TGF-beta 1[11] mRNA in the kidney (Trachtman et al.). Some studies have shown that DL-alpha-tocopherol acetate supplementation to the diet improved GFR, blood pressure, and levels of angiotensin converting enzyme all improved in Sprague Dawley rats that had chronic cadmium poisoning (Choi et al., 2003). In pigs with iron-irritant coil induced renal artery

[10] Form not specified

[11] Transforming growth factor beta- a polypeptide growth factor that is commonly association with preeclampsia and tumor progression

stenosis, vitamin E (100 IU/kg[12]) and vitamin C (1000 mg) supplementation improved renal hemodynamics, decreased oxidative stress, inflammation and fibrosis if the ischemic kidney (Chade et al., 2003). Another study involving acute renal failure, demonstrated that the antioxidant savaging ability of the kidney decreases with age and that supplementation of vitamin E is essential for protecting the kidney against acute renal failure (Shimizu, 2004). Calcium oxalate crystallization within the kidney is associated with oxidative stress and often results in tubular damage. In a study by Huang et al (2007), it was found that male Wistar rats who received 200 mg/kg body weight vitamin E intraperitoneally had a decreased tubular cell death and in fact, cell proliferation was seen at all time points and calcium oxalate deposition was decreased. There was even enhancement of defensive roles of osteopontinin and Tamm-Horsfall protein (Huang et al.). In another study it was demonstrated that vitamin E and selenium in combination inhibited oxalate synthesis, reduced renal tissue lipid peroxidation, and enhanced enzymatic and non enzymatic antioxidant status in the kidney (Kumar, 2001). Naziroglu et al. (2004), concluded that high doses of alpha-tocopherol (1000 mg/kg body weight) in the diet with selenium might play a role in preventing cisplatin[13]-induced nephropathy, a condition associated with increased rate of lipid peroxidation (indicated by increased production of malondialdehyde) and decreased renal activity of scavenging enzymes (glutathione peroxidase) in female Wistar rats (Mustafa Nazırolu).

[12] Form not specified
[13] Cisplatin, a cytotoxic agent used in the treatment of cancer

Gamma-Tocopherol

Gamma tocopherol, which occurs naturally in the RRR, is form of vitamin E most commonly consumed in the American diet, has been long been overlooked for beneficial properties relevant to human health. This is due to the fact that gamma tocopherol has a lesser bioavailability and bioactivity than alpha tocopherol and that when alpha-tocopherol is supplemented or high in the diet, gamma-tocopherol levels decreased. This is due to the fact that gamma tocopherol is absorbed well by the body but not retained within the tissues. However, new research suggests that previous ideas about the role of gamma tocopherol may have not reflected its importance relative to human health. It was previously thought that the tissue and plasma concentrations of gamma tocopherol were low and storage limited to adipose tissue, that its potential health benefits were minimal. However, Burton et al. (1998) reported that gamma tocopherol can make up approximately 30-50% of the total vitamin E concentrations in human skin, muscle, vein and adipose tissues. More importantly, it was reported that gamma tocopherol concentrations in the tissue appear to be 20-40 times greater than those of plasma (Burton et al., 1998). It is speculated that human gamma tocopherol concentrations in skin and msucle are almost 20-50 times higher than those measured in rodents. Another well reported finding was that with alpha tocopherol supplementation, tissue and plasma gamma tocopherol levels are suppressed, whereas with gamma tocopherol supplementation both tocopherols increase in concentration (Jiang, Christen, Shigenaga, & Ames, 2001).

The biological activity of gamma tocopherol has also been questioned when compared to alpha tocopherol; however, this may be due to an error in the determination of the biological activity itself. Biological activity of vitamin E has typically been determined by using the rat fetal assay, where biological activity is defined as the ability of supplemented tocopherols (or tocotrienols) to prevent embryo death in mothers who are vitamin E depleted. In this assay, alpha tocopherol shows the greatest bioactivity, whereas gamma tocopherol only exhibits 10-30% of the activity of alpha tocopherol. However, this difference in biological activity may be caused by the discrepancy in the retention of alpha and gamma tocopherol in rodents, which is reflected by the lower plasma and tissue concentrations of gamma tocopherol than alpha tocopherol. This discrepancy can also be explained by their differences in the metabolism of the two compounds (Jiang, Christen, Shigenaga, & Ames, 2001).

Both alpha and gamma tocopherol, along with dietary fat, are taken absorbed within the intestine without discrimination, and then secreted in chylomicrons into the lymphatic system with triacylglceryols (triglycerides) and cholesterol. This nonpreferrential incorporation of alpha and gamma tocopherol in chylomicrons was shown after supplementation with equal amounts of the two tocopherols showed equal amounts of both tocopherols in the lipoproteins produced. After chylomicrons transport lipids and vitamin E to the muscle, adipose, and brain, the resultant chylomicron remnants are taken up in the liver. As alpha tocopherol is incorporated into VLDL, gamma tocopherol appears to be catabolized to hydrophilic gamma CEHC (carboxyethyl

hydroxychromans) by cytocrome P450-dependent process and is then primarily excreted into the urine. (Cytrochrome P450 is a group of enzymes used to breakdown toxins and drugs within the liver. This enzymatic process is known to produce a large number of free radicals and had been implicated in cellular oxidative damage. Gamma CEHC has shown to possess a natiuretic activity, which may have some physiological purpose.)

It has also been shown that both gamma CEHC and gamma tocopherol contain anti-inflammatory properties (Jiang, Christen, Shigenaga, & Ames, 2001). Gamma tocopherol and CEHC inhibit prostaglandin E2 synthesis in lipopolysaccharide-stimulated macrophages and in interleukin 1-activated epithelial cells by fifty prevent at a concentration of 4-10 μmol gamma-tocopherol/L. It was also revealed that gamma-tocopherol and CEHC directly inhibit cyclooxygenase-2 (COX-2) activity in intact cells, however, have no effect on the COX-2 protein expression (Jiang et al., 2001). Cooney et al. found that gamma-tocopherol has greater potentency than alpha tocopherol in inhibiting neoplastic transformation of C3H/10T1/2 cells (Cooney, Franke, Harwood, Hatch-Pigott, Custer, & Mordan, 1993). The anti-inflammatory activity of γ-tocopherol could partially explain this difference in potency.

The antioxidant capacity of gamma-tocopherol has been widely disregarded due to the lack of one electron donating methyl group on the chromanol ring, like alpha tocoperhol. However, the unsubstituted C-5 position of gamma tocopherol appears to make it better able to trap lipophilic free radicals, like reactive nitrogen oxide species (RNOS). RNOS which have been implicated in numerous chronic inflammation-related

diseases, such as cancer, cardiovascular disease, and neurodegenerative disorders. RNOS that are formed during inflammation include superoxide dismutase, nitrogen dioxide, peroxynitrie, etc. In recent studies, researchers have found that gamma-tocopherol is superior to alpha-tocopherol in detoxifying nitrogen dioxide. Researchers showed that alpha-tocopherol in a reaction with nitrogen dioxide leads to the formation of a nitrosating intermediate that generates nitroso products, where as gamma tocopherol reduces nitrogen dioxide to a less harmful nitric oxide or traps the nitrogen dioxide within its structure and forms 5-nitro-gamma-tocopherol (Jiang, 2001).

Research has also focused on the possibility of the role of gamma tocopherol in the prevention of cancer. Giuliano et al. (1997) reported that serum concentrations of gamma and alpha tocopherol were significantly lower, twenty-four percent, in women with reoccurring positive papillomavirus infection, which has been linked to cervical cancer (Giuliano et al.). Helzlsouer et al. (2000) conducted a case-control study to determine the relationship between alpha-tocopherol, gamma-tocopherol, and selenium and the incidence of prostate cancer. Men in the highest quintile of plasma gamma-tocopherol concentrations had a 5-fold reduction in the risk of prostate cancer when they were compared with the lowest quintile. These authors also found that the protective effects of elevated selenium and alpha-tocopherol concentrations were only observed if the gamma-tocopherol concentrations were also high (Helzlsouer et al.). In contrast, a case-control study of patients with invasive cervical cancer in Latin America revealed that they had higher serum concentrations of gamma-tocopherol than control subjects

(Potischman, 1991). Zheng et al. reported a positive correlation between serum gamma-tocopherol and selenium concentrations and the risk of developing oral and pharyngeal cancer (Zheng, 1993). A study by Jiang et al (2004), showed that gamma-tocopherol inhibited the proliferation or prostate cancer cells (LNCaP) and lung cancer cells (A549) by inducing apoptosis via interrupting the de novo sphingolipid pathway in the cancer cell line (Jiang, 2004).

Researchers continue to study the effects of vitamin E and its role in cancer prevention. Evidence thus far still remains inconclusive regarding any one component/form responsible for cancer prevention.

CHAPTER III

MATERIALS AND METHODS

Animals and Experimental Design

This study is part of a larger project. Briefly, twenty-four male Fisher 344 rats were obtained for the project. All rats obtained weighed approximately 160 grams. The Institutional Animal Care and Use Committee (IACUC) of Texas Woman's University, Houston Center, approved this research project. The rats were housed in individual stainless steel cages. A temperature of 24± 2°C and a twelve-hour dark-light cycle were maintained. The rats were fed standard lab chow diet during the acclimation period of 2-3 days. After acclimation, rats were randomly assigned into three groups (Table 1), each consisting of eight rats, and fed the corresponding diets described in Table 2 for two weeks.

Table 1

Experimental Dietary Groups

Group	Diet	Number
1	Casein + 75 I.U.[14] per kg of RRR-alpha-tocopherol	8
2	Casein + 355 I.U. per kg of RRR-alpha-tocopherol	8
3	Casein + 750 I.U. per kg of RRR-alpha-tocopherol	8

[14] International units

Table 2

Composition of Experimental Diets

	1	2	3
Casein	235	235	235
Vitamin Mix (AIN[15] 76A)	12.8	12.8	12.8
DL-Methionine	3.5	3.5	3.5
Mineral Mix (AIN-76)	41.2	41.2	41.2
Starch (Cornstarch)	10.1	10.1	10.1
Cholinebitartrate	2.4	2.4	2.4
Fiber	59	59	59
Soybean Oil	200	200	200
Sucrose	34	34	34
RRR-alpha-tocopherol (I.U./kg diet)	75	355	750

Food and water were available to the rats ad libitum, and fresh food was given daily. Rats were anesthetized with Nembutal and sacrificed three hours into the light cycle. Tissues, liver and kidney, were excised and weighed and stored at -80°C.

Extraction of hepatic and renal lipids for determination of triglycerides and total cholesterol (Appendix A) was performed using the modified version of Hara and Radon

[15] American Institute of Nutrition

(1978). Biochemical determination of liver triglycerides was done by the glycerophosphateoxidase method using a Stanbio Kit 2000, and liver cholesterol was analyzed by the cholesterol esterase/cholesterol oxidase method using a Stanbio kit 1010

Statistical Analyses

Statistical analyses were performed using SPSS. Values for the concentrations of both triglyceride and cholesterol for kidney and liver were analyzed using ANOVA, with Tukey's test being used for post hoc intergroup comparisons, where appropriate. The level of significance was $p \leq 0.05$ for all comparisons.

CHAPTER IV

RESULTS

Total Cholesterol

Data for the cholesterol concentration (mg/g) for kidney and liver are given as mean values ± standard deviations in Table 3. No between group differences were noted. Values for ANOVA for these two parameters are given in Tables 4 and 5.

Table 3

Concentration of Triglycerides (TG) and Cholesterol in the Kidney and Liver of Male Fisher 344 Rats Fed Experimental Diets for 14 Days

	Group 1[1]	Group 2[2]	Group 3[3]
Kidney Triglyceride (mg/g)	36.5 ± 8.68	44.3 ± 14.5	36.7 ± 9.26
Kidney Cholesterol (mg/g)	7.14 ± 2.5	7.2 ± 1.37	5.6 ± 1.87
Liver Triglyceride (mg/g)	21.5 ± 5.08	33.68 ± 7.7	34.81 ± 7.62
Liver Cholesterol (mg/g)	6.36 ± 0.96	7.6 ± 2.56	7.64 ± 1.85

[1]Group 1: Casein + 75 I.U. per kg of d-alpha-tocopherol

[2]Group 2: Casein + 355 I.U. per kg of d-alpha-tocopherol

[3]Group 3: Casein + 750 I.U. per kg of d-alpha-tocopherol

Table 4

One-Way ANOVA Summary Table for Renal Cholesterol Concentration for Male Fisher 344 Rats Fed Experimental Diets for 14 Days

Source	SS	Df	MS	F	Sig
Between Groups	1311.37	2	655.68	1.68	.21
Within Groups	8175.48	21	389.3		
Total	9486.86	23			

Table 5

One-Way ANOVA Summary Table for Hepatic Cholesterol Concentration for Male Fisher 344 Rats Fed Experimental Diets for 14 Days

Source	SS	Df	MS	F	Sig
Between Groups	850.52	2	425.26	1.168	0.330
Within Groups	7644.7	21	364		
Total	8495.2	23			

Triglycerides

Data for the triglyceride concentration (mg/g) for kidney and liver are given as mean values ± standard deviations in Table 4. No between group differences were noted for the renal concentration of triglycerides. By contrast, between group differences were noted for liver, specifically, the values for group 1(21.5 mg/g) was significantly lower than the value for group 2 (33.7 mg/g, p=0.006) and the value for group 3 (34.8 mg/g, p=0.003). Values for groups 2 and 3 were not significantly different. Values for the ANOVA for these parameters are summarized in Tables 7 and 8. Post Hoc results are summarized in Table 9.

All data were analyzed using SPSS for Z-scores to evaluate if outliers were present in any of the data. One outlier was found in the kidney triglycerides however, when analyzed further this value did not change the results of the data set.

Table 6

One-Way ANOVA Summary Table for Renal Triglyceride Concentration for Male Fisher 344 Rats Fed Experimental Diets for 14 Days

Source	SS	Df	MS	F	Sig
Between Groups	31903	2	15951.52	1.288	0.297
Within Groups	260139.8	21	12387.6		
Total	292042	23			

Table 7

One-Way ANOVA Summary Table for Hepatic Triglyceride Concentration for Male Fisher 344 Rats Fed Experimental Diets for 14 Days

Source	SS	Df	MS	F	Sig
Between Groups	86528.68	2	43264.34	8.970	0.002
Within Groups	101283.8	21	4823		
Total	187812.54	23			

Table 8

One-Way ANOVA Tukey Post Hoc Summary Table for Hepatic Triglyceride Concentration for Male Fisher 344 Rats Fed Experimental Diets for 14 Days

Comparisons		Std. Error	Sig.
Group 1	Group 2	34.72	.006
Group 1	Group 3	34.72	.003
Group 2	Group 3	34.72	.944

CHAPTER V

DISCUSSION

Although the composition of the diets varied widely in vitamin E concentration, there was no significant between group differences for the concentration of renal triglycerides, renal cholesterol or hepatic cholesterol. However, there were between group differences for the hepatic concentration of triglycerides. Rats consuming the baseline diet that contained 75 I.U. of alpha tocopherol had significantly lower liver triglyceride concentration than those consuming diets containing 355 I.U or 750 I.U. of RRR-alpha-tocopherol. These results are similar to those found by Husveth et al. (2000) who supplemented the diets of broiler chickens fed broiler fish oil or beef tallow diets with vitamin E[16] (100 IU/kg diet). They found a significant increase in the amount of polyunsaturated triglyceride content in the liver due to dietary alpha-tocopherol supplementation. A study by Yang et al. (1976), who supplemented DL-alpha-tocopherol (all-rac-tocopherol-acetate) into the diets of female weanling Wistar at six concentrations (0, 25, 250, 2,500, 10,000, and 25,000 IU/kg diet) and were supplemented for 8 and 16 months until sacrifice concluded that the liver lipids were higher in rats supplemented with 250 IU/kg diet than 0 and 25 IU/kg diet at eight months of supplementation but not for 16 months. In another study by Sklan, who studied the liver triglyceride and cholesterol concentration of Leghorn male chicks after supplementing the diet with

[16] Form not specified

10 mg/g/kg D-alpha-tocopherol acetate, found enhanced liver total fatty acids when compared with the control. When further analyzed, the total fatty acids present in the liver mostly consisted of triglycerides, which may show that triglycerides compose most of the storage form of alpha-tocopherol. However, unlike our study Sklan also found an increased concentration of cholesterol in the liver and a decreased fatty acid synthesis with supplementation of alpha-tocopherol.

Parola (1992), found that if alpha-tocpherol was supplemented in the diets of rats with carbon tetrachloride induced liver damage, the liver of those rats would increase their alpha-tocopherol storage threefold over the control. The researchers also determined that this increased storage showed a marked decrease in oxidative damage done to the liver. Garcia-Martinez et al. (2007) reported that when alpha-tocopherol was supplemented in the diets of diabetic rats that plasma alpha-tocopherol levels[17] stayed consistent or were normalized, while liver storage of alpha-tocopherol increased.

In another study by Leonard et al., five week old male *Mttp*[18] mice were injected with 500 μg of polyinosinic-polycytidylic ribonucleic acid every other day for eight days, inducing an inactiviation of *Mttp*, the protein responsible for the secretion of VLDL (very low density lipoprotein) in the liver. After the knock out of this protein the rats were separated into two different groups, one fed standard lab chow for 6 weeks and the other fed deuterated alpha and gamma tocopherol acetate in a 1:1 molar ratio added to

[17] Form not specified
[18] Microsomal triglyceride transfer protein

tocopherol stripped corn oil to be supplemented to the diets. Once analyzed, the plasma cholesterol and triglyceride rich VLDL disappeared from the plasma, while there was an accumulation of neutral lipids in the liver. When they analyzed the other organs, like the kidney and muscle, they had slightly reduced levels of alpha-tocopherol. Other reviews on the metabolism of vitamin E, have suggested that alpha-tocopherol is incorporated into hepatocytes through a selective uptake from HDL (high density lipoprotein) through the SR-BI (selective high-density lipoprotein) receptor. Rast fed a vitamin E-free diet showed an 11-fold increase the expression of the SR-BI protein in the hepatic cell membranes, which was then partially reversed by refeeding. In relevance to our study, measuring the HDL lipoprotein might be a better source of analysis for the total amount of triglyceride and alpha-tocopherol within the liver tissue.

In conflicting results with our study, Deyhim (2007), found that diets supplemented for four months with a mixture of vitamin E[19], at concentrations of 62.5 or 656 mg/kg diet, did not (P> 0.1) affect the concentrations of cholesterol or triglyceride concentration in the liver of 1 year-old male Sprague-Dawley rats. Interestingly, these rats were separated into four groups, of which three groups went through an orchiectomy to assess whether the male sex hormone, testosterone, or one of its metabolites regulates the lipid profiles in these rats. In another study involving the vitamin E supplementation with ovariectomized female Sprague Dawley rats at concentrations of 75 IU, 300 IU, 750 IU alpha-tocopherol/kg of diet, by Lucas et al. (2006), the liver triglyceride content

[19] 24.4% delta-tocopherol, 1.8% beta-tocopherol, 61% gamma-tocopherol, 12.8% alpha-tocopherol

increased after orchiectomy and this effect was reduced by 13% by vitamin E supplementation of 525 IU/kg diet suggesting that the male sex hormone might modulate lipid profiles in the liver.

Other researchers, such as Radcliffe et al., have found no significant effect on liver triglyceride content when varying the dietary level of vitamin E by using two vegetable oils (corn and cottonseed oil); in female Sprague-Dawley rats fed diets either containing 100 g/kg of cottonseed oil or corn oil, which both contain both RRR-alpha-tocopherol and RRR-gamma-tocopherol, with the level of alpha tocopherol being higher, but the level of gamma-tocopherol lesser, and more cottonseed oil than corn oil. These results may due to the fact that in this particular study we used male Fisher rats, which may have an effect on the triglyceride storage. Previous studies have shown that the hormone testosterone has an effect on triglyceride storage within the liver. In other studies noted by Radcliffe, there have been difference in the High Density Lipoprotein content of both the serum and the liver between female and male rats, which could affect the triglyceride and alpha-tocopherol content of the liver and peripheral tissues.

The results of this study indicate that rats fed a diet supplemented with 355 I.U. and 750 I.U. of RRR-alpha-tocopherol had a significantly higher liver triglyceride content. Thus, including RRR-alpha-tocopherol in the diet is a possible approach to increasing the hepatic triglyceride and Vitamin E storage in the liver.

CHAPTER VI

SUMMARY AND CONCLUSION

The purpose of this research was to investigate the effects of feeding RRR-alpha-tocopherol in three different quantities, on renal and hepatic concentrations of triglycerides and cholesterol using twenty-four six-week old male Fisher 344 rats, who were assigned into three experimental groups, and fed diets containing 75 I.U. RRR-alpha-tocopherol, 355 I.U. R-alpha-tocopherol, and 750 I.U. RRR-alpha-tocopherol for fourteen days.

No differences were found in liver or kidney total cholesterol concentrations for any of the treatment groups, so the null hypothesis is accepted. Kidney triglyceride concentration was also analyzed and found to have no significant differences among the diet groups, so the null hypothesis is accepted. Rats consuming the 355 I.U. D-alpha-tocopherol and the 750 I.U. D-alpha-tocopherol were found to have a significantly higher concentration of liver triglyceride when compared to the control diet group containing 75 I.U. D-alpha-tocopherol, therefore the null hypotheses were rejected.

The renal and hepatic triglyceride and total cholesterol concentration levels were analyzed to determine whether positive, negative or no correlation existed between the various values. There was no correlation found between the triglyceride and total cholesterol values found in the liver or the kidney.

The present study shows that D-alpha-tocopherol, when supplemented at or above 355 I.U. can significantly increase hepatic triglyceride level, when compared to the control diet of 75 I.U. D-alpha-tocopherol. A study by Husveth et al. (2000) reported that animals fed diets supplemented with alpha-tocopherol showed an increase in the amount of polyunsaturated triglyceride content in the liver and that this increase may increase the hepatic antioxidant capacity.

The increased storage of triglycerides in the liver associated with supplementation of RRR-alpha-tocopherol may as act as a chemopreventive agent by increasing the antioxidant capacity of the liver tissue. Findings from this study and other research suggest that there is no significant accumulation of total cholesterol in the kidney or liver, or any significant accumulation of triglycerides in the kidney due to D-alpha-tocopherol supplementation. The significant differences found in the liver triglyceride values suggest further research is needed to determine the mechanisms whereby D-alpha-tocopherol increases the antioxidative capacity of the liver.

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APPENDIX A

Lipid Extraction Procedure

Lipid Extraction Procedure

Note: All of this work is done under Yellow Light.

Day 1

1. Weigh sample, write down weight.
2. Homogenize it with the machine.
3. Calculate the proportion amount of Hexane Isopropanol (3:2) to add. For a 500 mg weight, use 9 ml of Hexane IP.
4. Vigorously shake solution, then vortex.
5. Store in freezer overnight without light.

Day 2

1. After been in the freezer- vortex until no solute remains on the bottom.
2. Rinse the filter paper with Hexane IP before filter into a waste container.
3. Fold filter paper, and filter the solution into a labeled test tube with gravity. Cover the solution to avoid evaporation.
4. Add 0.5 ml additional Hexane IP into the original test tube, vortex and filter.
5. Discard the old test tube.
6. Refill the new test tube with the filtrate up to its original volume of Hexane IP.
7. Allocate into 4 test tubes.

Cover with foil and store into the refrigerator

APPENDIX B

Schematic Diagram for Determination of Triglycerides

Schematic Diagram for Determination of Triglycerides

Extraction of hepatic lipids for the determination of triglycerides or cholesterol (Hara A., Radin N. Lipid Extraction of tissues with a tow-toxicity solvent. *Analytical Biochemistry.* 1978:90:420-426.)

500 mg tissue

↓

Add 9 ml hexane:isopropanol (3:2)

↓

Homogenize and let stand for 1 hr and re-homogenize

↓

Filter (use #2 filter paper) into a calibrated test tube

<u>For Triglyceride Determination (TG)</u>

Pipet 20 μL triglyceride standard [200 mg/dL]

↓

Take 200 μL filtrate of sample and combine 400 μL hexane (dilution ratio 3:1)

↓

Pipet 50 μL of dilute filtrate into three test tubes

↓

Evaporate under N_2 to dryness all four test tubes

↓

Prepare a blank test tube (empty)

↓

Add 1 mL triglyceride enzymatic reagent to each test tube.
(The blank, the standard, and the three samples tubes that have been dried)

↓

Cover each test tube and gently invert

↓

Let test tubes standard at room temperature for minimum of 10 minutes

↓

Read at 500 nm within 60 minutes

↓

Calculate triglycerides in tissue, expressed as mg/g of tissue

APPENDIX C

Schematic Diagram for Determination of Cholesterol

Schematic Diagram for Determination of Cholesterol

500 mg tissue

↓

Add 9 ml hexane:isopropanol (3:2)

↓

Homogenize and let stand for 1 hr and re-homogenize

↓

Filter (use #2 filter paper) into a calibrated test tube

<u>For Cholesterol Determination (TC)</u>

Pipet 20 µL cholesterol standard [200 mg/dL]

↓

Take 200 µL filtrate of sample (10x usual volume) (3 samples)

↓

Evaporate under N_2 to dryness all four test tubes

↓

Prepare a blank test tube (empty)

↓

Add 1 mL cholesterol enzymatic reagent to each test tube.
(The blank, the standard, and the three samples tubes that have been dried)

↓

Mix each tube by vortex

↓

Let test tubes standard at room temperature for minimum of 10 minutes

↓

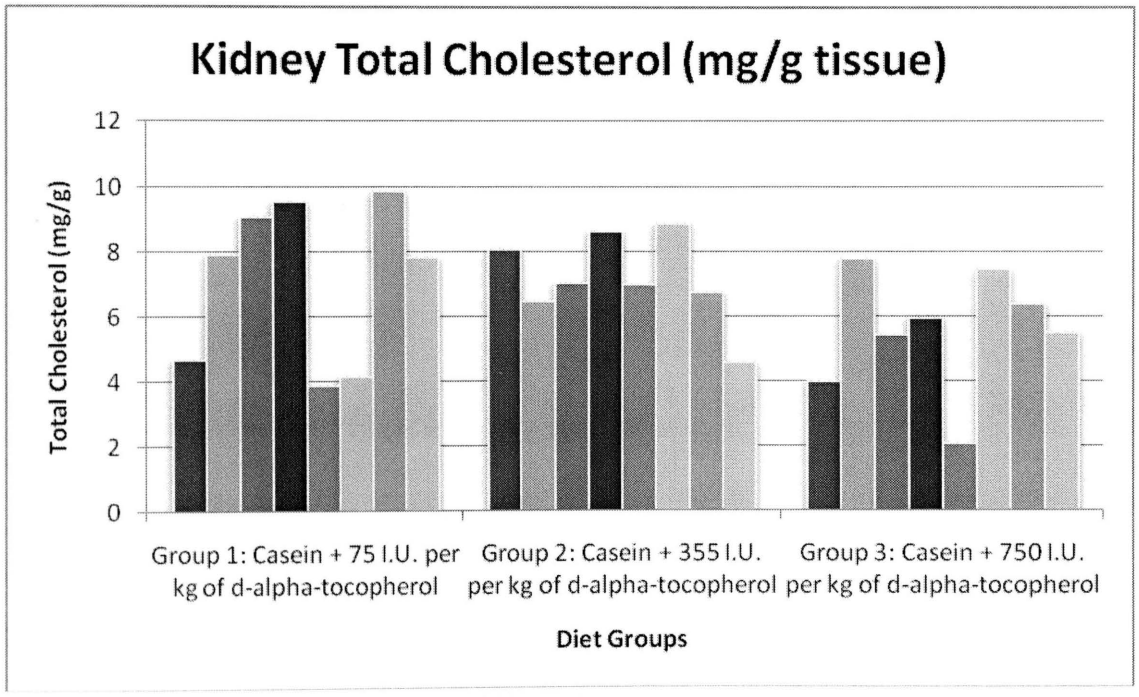
Read at 500 nm within 60 minutes

↓

Calculate triglycerides in tissue, expressed as mg/g of tissue

APPENDIX D

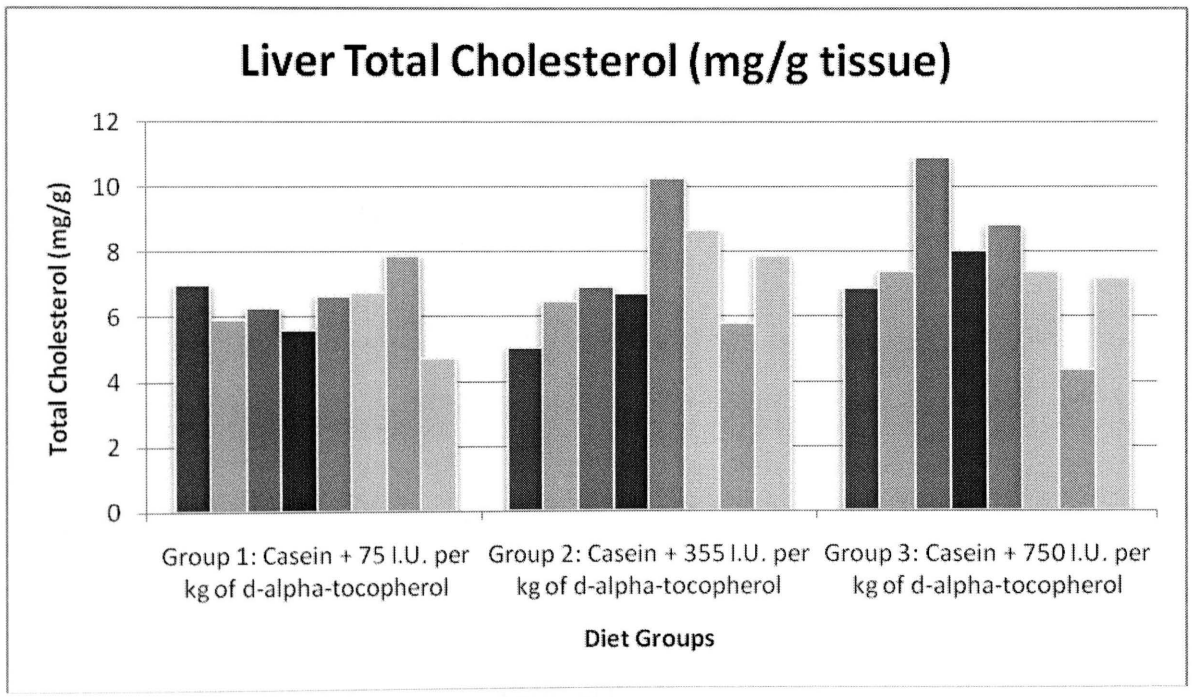
Total Kidney Cholesterol (mg/g Tissue) for Male Fisher 344 Rats Fed Experimental Diets



Total Kidney Cholesterol (mg/g tissue) for Male Fisher Rats fed Experimental Diets

APPENDIX E

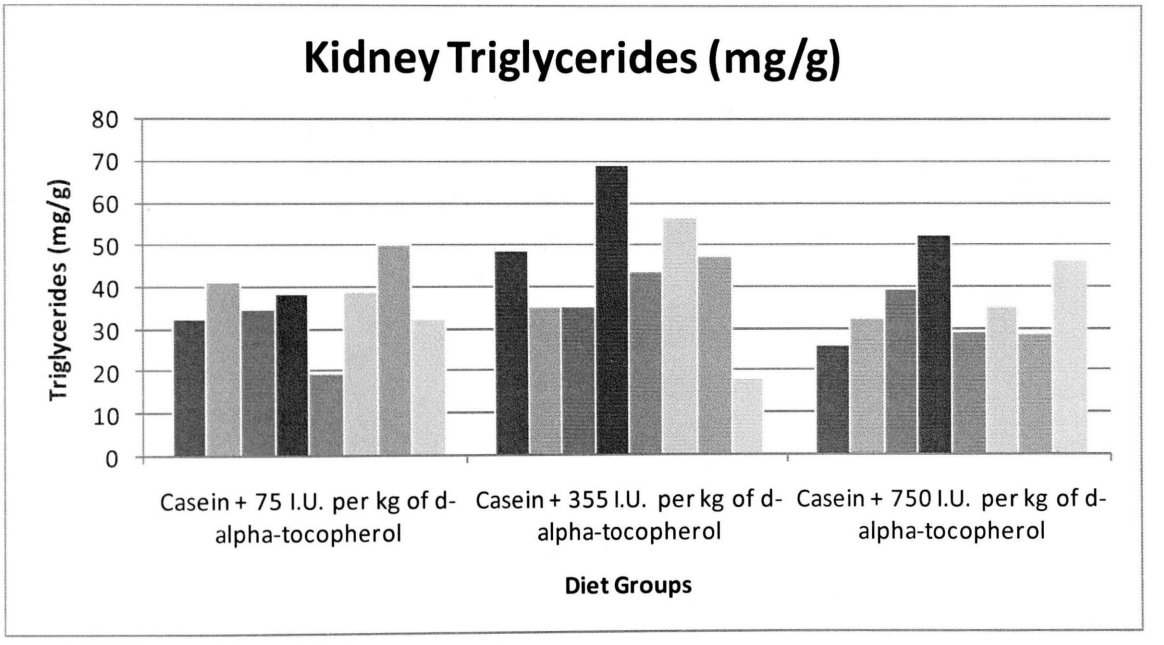
Total Liver Cholesterol (mg/g Tissue) for Male Fisher 344 Rats Fed Experimental Diets



Total Liver Cholesterol (mg/g tissue) for Male Fisher Rats fed Experimental Diets

APPENDIX F

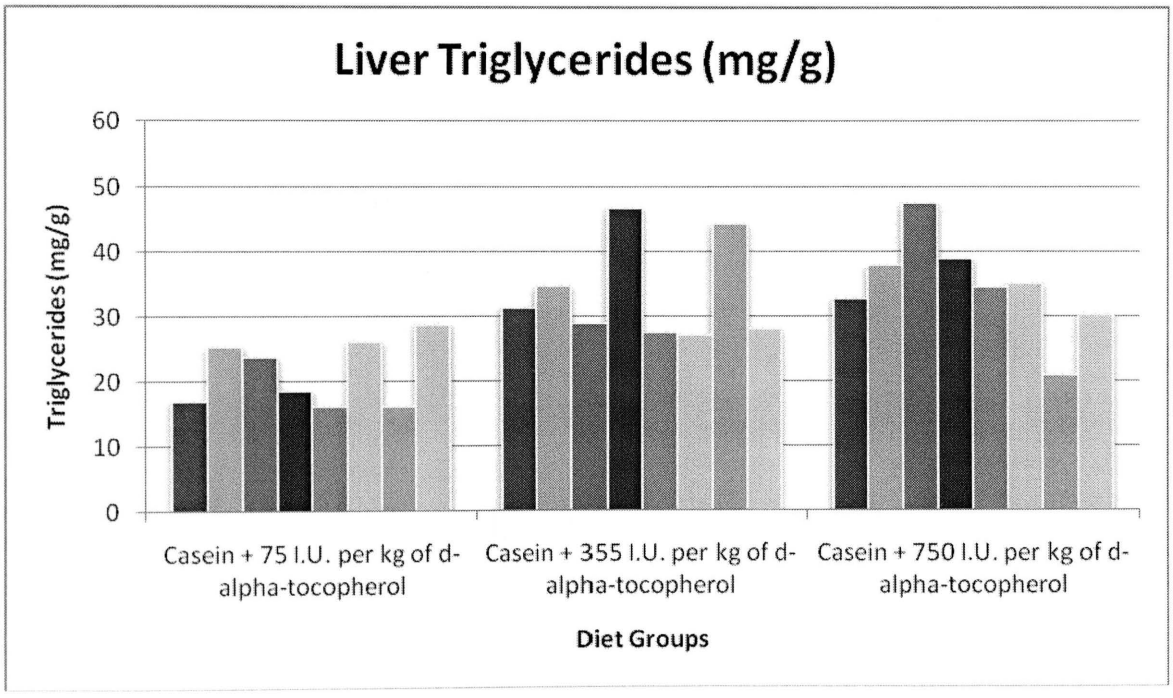
Kidney Triglycerides (mg/g Tissue) for Male Fisher 344 Rats Fed Experimental Diets



Kidney Triglycerides (mg/g tissue) for Male Fisher Rats fed Experimental Diets

APPENDIX G

Liver Triglycerides (mg/g Tissue) for Male Fisher 344 Rats Fed Experimental Diets



Liver Triglycerides (mg/g tissue) for Male Fisher Rats fed Experimental Diets