EFFECTS OF A DIET RICH IN VITAMIN E ON SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR IN HEALTHY ADULTS

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To the Dean of the Graduate School:

I am submitting herewith a thesis written by Brenda Malone Cluff entitled "Effects of a Diet Rich in Vitamin E on Serum Vascular Endothelial Growth Factor in Healthy Adults." I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Nutrition.

Dr. Victorine Imrhan, Major Professor

We have read this thesis and recommend its acceptance:

Department Chair

Accepted:

Dean of the Graduate School

ABSTRACT

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EFFECTS OF A DIET RICH IN VITAMIN E ON SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR IN HEALTHY ADULTS

AUGUST 2009

Studies indicate that Vitamin E may downregulate vascular endothelial growth factor (VEGF), one of several factors involved in angiogenesis. Regulation and expression of VEGF may have implications in both exacerbation of atherosclerotic heart disease and therapy in ischemic heart disease. In the current study, plasma VEGF levels were measured at baseline and after four weeks in healthy individuals who consumed a diet providing 15mg of vitamin E. Simple Effects Analysis was used to compare baseline and intervention VEGF levels against controls. A significant difference ($p<0.001$) was found in VEGF levels after four weeks of intervention compared to controls, with a 17% (on average) decrease in VEGF concentration in the experimental group. This study shows that dietary vitamin E decreases serum VEGF concentration in healthy adults, independent of serum lipid levels.

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CHAPTER I

INTRODUCTION

Vascular Endothelial Growth Factor (VEGF) was first discovered in 1983 by Senger and his colleagues, who were able to partially isolate the factor, which they called vascular permeability factor (VPF). The amino acid sequence and structure of the protein were identified in the latter part of the decade. Meanwhile, another group of researchers, Ferrara and Henzel, were simultaneously working with VEGF and independently found its partial amino acid sequence in 1989. They called it *vascular endothelial growth factor*, the name by which we refer to this factor today (1,2). cDNA cloning of VPF and VEGF led to the discovery that the two were the same molecule: a molecule which, early on, was thought to play a role in physiological and pathological angiogenesis (1). Still today, an increase in VEGF expression is considered to be one of the hallmark features of pathological angiogenesis (1,2). In light of this fact, numerous studies have been conducted to determine exactly what role VEGF and its receptors play in pathological angiogenesis, including the possibility of VEGF therapy in ischemic heart disease (3-10).

Vitamin E may play a role in VEGF regulation. Several studies targeting vitamin E and VEGF regulation have been conducted to date (11-14). The tocotrienol form of vitamin E in particular has been shown to have anti-angiogenic potential, both *in vitro* and *in vivo* (12,15,16). Additionally, a study by Rodriguez et al. suggests that inhibition

of VEGF overexpression in ischemic heart disease may be one benefit of vitamin E consumption (14).

While there are studies which indicate that VEGF may have a therapeutic role in angiogenesis, VEGF must be carefully regulated to potentiate a positive rather than pathological angiogenic response. In atherosclerotic conditions, VEGF is upregulated, in part, by macrophages and oxidized LDL cholesterol (7,17). Studies indicate that upregulation of VEGF may exacerbate symptoms of atherosclerotic heart disease, worsening atherosclerotic conditions (6,7,18-20). Vitamin E has already been established as having a beneficial role in heart disease (21-22), possibly in part through prevention of VEGF overexpression in the vascular endothelium. However, there are no studies to date, known to the author, which investigate the relationship between intake of vitamin E-rich foods and VEGF levels in human blood. Knowing the effect of a diet rich in vitamin E on serum VEGF would give further insight into the protective mechanisms of vitamin E in atherosclerotic heart disease.

The purpose of this study is to evaluate the effect of a diet composed of foods high in vitamin E on serum VEGF levels in healthy adults. We hypothesize that healthy adults consuming a diet containing approximately 15mg vitamin E per day will have a lower concentration of serum vascular endothelial growth factor than those consuming their usual diets, independent of serum lipid levels.

CHAPTER II

REVIEW OF THE LITERATURE

Properties of VEGF

The term VEGF typically refers to VEGF-A, one of a family of VEGF molecules. In fact, the VEGF-A itself has four different primary isoforms, depending on how exon splicing on the VEGF-A gene occurs. The isoforms have 121, 165, 189, and 206 amino acids, depending on where the sequence is cleaved. $VEGF_{165}$ is the predominant isoform of the four, which may be due to its excellent biological characteristics as it has intermediate properties compared to its counterparts. For example, $VEGF_{121}$ is a freely diffusible, highly acidic protein that cannot bind to heparin. The 189 and 206 isoforms, on the other hand, are very basic and strongly bind to heparin, and they are found solely in the extracellular matrix. $VEGF_{165}$ shares properties of $VEGF_{121}$ and the 189 and 206 isoforms in that it is bound to the extracellular matrix, while some is also diffused, giving it a higher mitogenic potential than its sister isoforms that lack the heparin-binding domain. $VEGF_{165}$ also has greater mitogenic potential than $VEGF_{121}$. These qualities make $VEGF_{165}$ the most bioavailable of the VEGF-A isoforms (1).

Other members of the VEGF family include VEGF-B, VEGF-C, VEGF-D, VEGF-E, and Placental Growth Factor (PlGF), each having its own set of isoforms (2).

VEGF binds to tyrosine kinase receptors (VEGFR) located on the surface of vascular endothelial cells and monocytes. There are two such known receptors: VEGFR-

1 and VEGFR-2 (1,2). There is also a receptor called VEGFR-3 that binds VEGF-C and VEGF-D but not VEGF-A. Neuropilins are considered to be coreceptors of VEGF, as well (1). Please refer to Figure 1 for a schematic depicting the breakdown of which VEGFR receptors bind the respective members of the VEGF family.

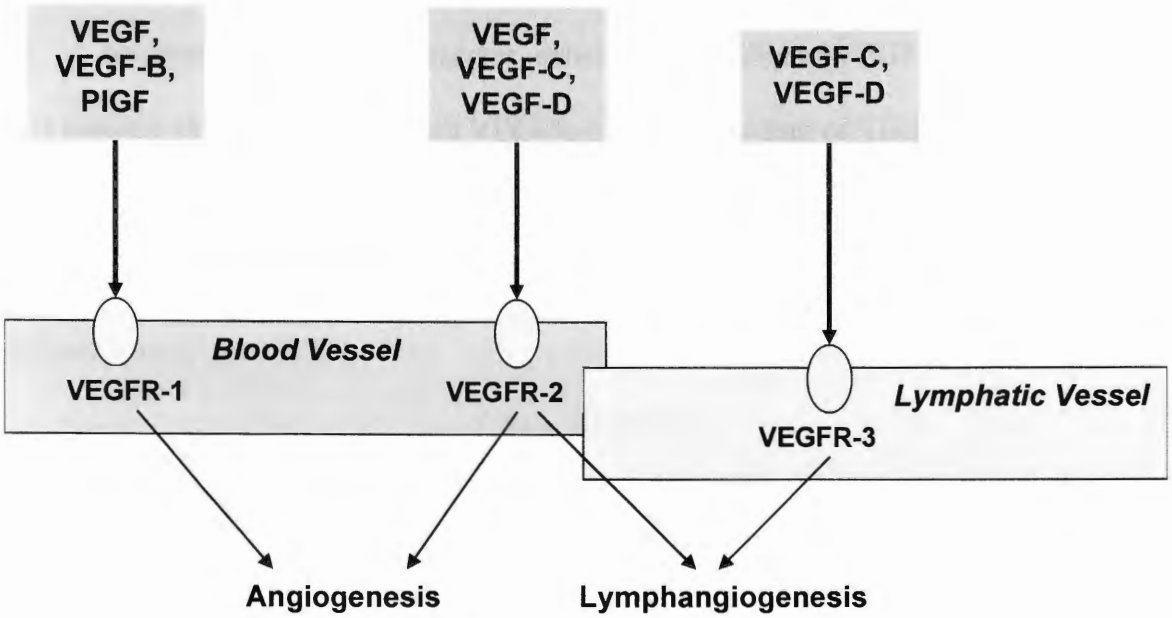

Figure 1. VEGF Isoforms and their membrane-bound respective receptors. VEGFR-1 and VEGFR-2 are predominantly located on the surface of vascular endothelial cells; VEGFR-3 is located on lymphatic endothelial cells, as shown. (Adapted from Ferrara N. Vascular endothelial growth factor: Basic science and clinical progress. Endocrine Reviews. 2004;24:581-611)

Notably, VEGF-A binds to both VEGFR-1 and VEGFR-2. VEGFR-1 has a tendency to sequester VEGF-A as it has poor ability to initiate a mitogenic response. It inhibits the interaction of VEGF-A with its other receptor, VEGFR-2, which has the opposite effect of VEGFR-1 in that it does mediate a mitogenic response. In the hypoxic state, VEGFR-1 expression is upregulated (a mechanism regulated by Hypoxia Inducible

Factor-1, or HIF-1) (1). Therefore, VEGF tends to be sequestered in hypoxic conditions, leading to increased angiogenesis. VEGFR-2 plays a key role in developmental angiogenesis, mediating the mitogenic and angiogenic properties of VEGF in growth and development. VEGFR-2 also promotes cell survival in development, contributing to the antiapoptotic abilities of VEGF in human umbilical vein endothelial cells (1).

Neuropilin, a VEGF co-receptor, enhances the ability of VEGFR-2 to bind VEGF. It is possible that one way in which VEGFR-1 inhibits binding of VEGF to VEGFR-2 is through competitive binding to neuropilin. The neuropilin co-receptor may be partly responsible for the increased mitogenic potential of $VEGF_{165}$ compared to its highly-diffusible isoform, $VEGF_{121}$ (1).

Effects of VEGF

Angiogenesis

VEGF is best known for its direct angiogenic potential. Angiogenesis involves both the formation of new vessels using previously differentiated cells (sprouting from existing vasculature) and the branching and extension of existing vessels. The latter occurs when endothelial cells from the existing vessels actually regroup to form new vasculature. This process is similar but not identical to arteriogenesis, which involves the growth and transformation of existing vessels into smaller capillaries (23-25).

The mechanism by which VEGF stimulates angiogenesis involves a complex signaling pathway. Figure 2 illustrates a simple schematic of this process. In a hypoxic state, binding of VEGF to its receptor leads to an increase in phospholipase C (PLC), which upregulates diacylglycerol (DAG). Protein Kinase C (PKC) is then upregulated by

DAG, leading to phosphorylation and activation of nitric oxide synthase (eNOS). A subsequent increase in nitric oxide (NO) activity is responsible for angiogenesis, supporting the fact that NO levels are increased in ischemia (26).

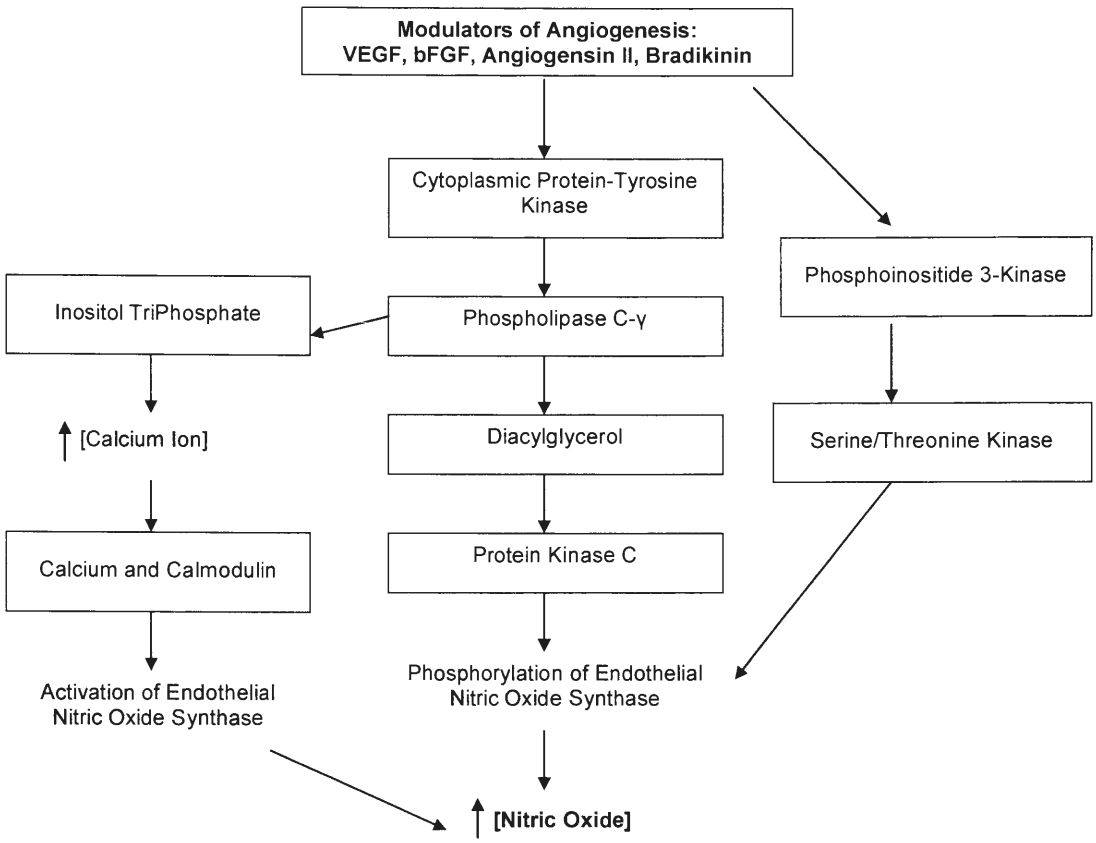

Figure 2. Proposed mechanism for angiogenic potential of VEGF. (Adapted from Contreras DL, Robles HV, Romo E, Rios A, Escalante B. The role of nitric oxide in the post-ischemic revascularization process. *Pharmacol Ther.* 2006;112:553-563.)

Rajesh et al. conducted an *in vitro* study in which human umbilical vein endothelial cells were treated with VEGF, after which expression of platelet endothelial cell adhesion moledule-1 (PECAM-1) was observed. Researchers suggest that the angiogenic activity of VEGF is dependent on lactoscylceramide (LacCer), a

glycosphingolipid that acts as a precursor for more complex glycosphingolipids. LacCer stimulates PECAM-1 gene and protein expression via recruitment of PKC and phospholipase A (PLA). PECAM-1 is necessary for VEGF and LacCer-induced angiogenesis, though the exact mechanism of PECAM-1 involvement is not established (27).

Vascular Permeability and Vasodilation

VEGF is also known to increase vascular permeability and cause vasodilation, common features seen in pathological angiogenesis. In this condition, blood vessels have higher permeability to water and macromolecules, and they are leaky with poorly controlled exchange. It is believed that the role of VEGF in increasing vascular permeability is independent of its angiogenic potential. One proposed mechanism for increased permeability by VEGF involves its receptor, VEGFR-2. The latter stimulates PLC, which in turn produces DAG, as found in the angiogenic signaling pathway of VEGF. This increase in DAG, stimulated by VEGF, leads to an influx of calcium and subsequent production of nitric oxide by nitric oxide synthase. Nitric oxide then upregulates cGMP, which causes increased vascular permeability by an unknown mechanism (2).

Heightened vascular permeability is not typically seen under normal physiological conditions of vasculature growth. The only form of "normal" angiogenesis where there is increased vascular permeability is in wound healing, which may be due to tissue inflammation rather than angiogenesis per se (2).

Regulation of VEGF

Numerous compounds and factors are involved in the regulation of VEGF. The following is a descriptive overview of compounds and conditions involved in the regulation of VEGF.

Macrophages

Macrophages express VEGF receptors and produce VEGF. In turn, VEGF also increases the concentration of macrophages in a given area. This may be one reason that VEGF has pathological consequences; macrophages lead to intimal hyperplasia, plaque instability (with increased risk for plaque rupture), and increased risk of thrombotic occlusion from rupture. A study by Celletti et al. involved the administration of VEGF or albumin (control) to apolipoproteinE (ApoE) deficient, cholesterol-fed mice. The mice treated with a 2μg/kg dose of intraperitoneal VEGF had significant increases in plaque area and thickness compared to controls. The experiment was repeated in rabbits with similar results. The researchers believe that macrophage infiltration as a result of VEGF administration was partly responsible for the observed increases in plaque area and instability (7). Moulton et al. support this idea, stating that inhibition of plaque angiogenesis, such as through VEGF inhibition, leads to a decrease in macrophage infiltration, thus improving stability of atherosclerotic plaque (28).

Cholesterol and Lipids

There is a correlation between blood lipid levels and serum levels of VEGF. Trapé et al. analyzed serum VEGF from 18 patients (10 male, mean age 48 years) with hypercholesterolemia, using serum samples from 12 subjects with a total cholesterol of

<5.1 mmol/L as controls. Patients with hypercholesterolemia had significantly higher serum VEGF than controls; both total cholesterol and LDL cholesterol had a significant positive correlation with serum VEGF, as did serum triglycerides. After baseline measurements were taken, hypercholesterolemic patients were treated with pravastatin, a cholesterol-lowering statin drug. As total cholesterol and LDL serum levels dropped from the influence of pravastatin, a drop was likewise seen in serum VEGF. Researchers surmise that the statin-induced decrease in serum concentrations of IL-1, IL-6, and TNF may be related to the ability of statins to downregulate VEGF. This may be one reason behind improved plaque stability with statin drug administration (29).

A study by Inoue et al. implies that cholesterol and VEGF levels have a stronger relationship than correlation alone. An *in vitro* model shows the direct upregulation of VEGF by oxidized LDL, likely through activation of proliferator-activated receptor-γ (PPARγ). Oxidized LDL was shown to strongly upregulate VEGF in human coronary artery endothelial cells (HCAECs); native LDL, however, had no effect. Researchers hypothesize that the activation of PPARγ by oxidized LDL is responsible for this upregulatory effect; this is one possible reason why VEGF is upregulated in atherosclerosis, a condition where oxidized LDL is available in abundance (17).

Leptin

Aydin et al. conducted a human study targeting leptin's regulation of VEGF in cyanotic congenital heart disease (CHD). Serum VEGF and leptin levels were monitored in two groups of pediatric patients with cyanotic and acyanotic CHD. Ten females and eight males, one to 9.8 years of age, made up the first group, while there were 11 females

and nine males in the second group, 13 months to 10.1 years in age. In cyanotic conditions, the study found that there was a positive correlation between serum leptin levels and serum VEGF. While VEGF levels negatively correlated with the severity of hypoxia in cyanotic patients, the levels of serum VEGF were significantly higher in cyanotic patients than in acyanotic patients. The same was also true of leptin levels after correcting for differences in BMI. The researchers reported that hypoxia can lead to an increase in leptin levels even in children with a low BMI. The study does not suggest any mechanisms or exact relationships involving leptin and VEGF but simply notes a positive correlation between VEGF and leptin in an oxygen-deprived environment (30).

Hemoglobin A1c

A study by Lim et al. indicates that hemoglobin A1C (HgbA1c) levels can predict the presence of VEGF. Fifty-six patients with diabetes (31 males) and 38 patients with both diabetes and CVD (24 males) were monitored over the course of one year. Thirty-four control subjects (19 males) were also monitored. Individuals with diabetes (DM) tended to have higher VEGF levels than individuals without DM. A positive correlation was found between HgbA1c and VEGF in diabetic patients (with or without CVD). The data in this study suggest that elevated blood glucose is a key factor in regulating circulating serum VEGF. Perhaps this is due in part to the ability of hyperglycemia to mimic a hypoxic state. According to Tilton et al., hyperglycemia upregulates the sorbitol pathway, increasing NADH in the cytosol—the same effect seen in hypoxia. With increased NADH, superoxide levels rise, stimulating an increase in VEGF, suggesting that VEGF may play a role in diabetic vascular dysfunction (31).

Prostaglandin E1

An *in vitro* study conducted by Weiss et al. on adult cardiac myocytes shows that prostaglandin E1 (PGE-1) can initiate angiogenesis in a VEGF-mediated manner. PGE-1 causes an increase in cAMP levels within the myocytes, leading to increased PKA action. In other words, according to this study, PGE-1 upregulates VEGF expression through a cAMP- and PKA-dependent mechanism (30).

Mehrabi et al. found similar results in a study conducted on hearts from patients with ischemic cardiomyopathy. Diseased hearts were obtained from a total of 28 patients with ischemic cardiomyopathy after they received heart transplants. Before undergoing the heart transplant, 14 of these patients were on PGE-1 treatment, while there were 14 patients who had not been on this treatment. When compared to hearts that had not undergone PGE-1 treatment, PGE-1-treated hearts had significantly higher VEGF-positive cells, indicating that VEGF was upregulated by PGE-1. Researchers reported that PGE-1 may provide a cardio-protective benefit in ischemic heart disease by stimulating neoangiogenesis in areas of myocardial infarct via increased VEGF expression (10). A very similar study conducted by Mehrabi and colleagues yielded similar results, with PGE-1 treatment correlating with increased VEGF-positive cells in cardiac scar tissue compared to controls (9).

Cyclooxygenase-2 (COX-2)

Because COX-2 is an early respondent to hypoxia, there is some evidence of a link between COX-2 and VEGF according to a study conducted by Wu et al. Researchers observed the relationship between VEGF and COX-2 expression in rat

cardiac myocytes, and a positive correlation was discovered between VEGF expression and COX-2 upregulation. Therefore, researchers suggest that the rise of COX-2 in hypoxic conditions is secondary to a rise in VEGF expression. The increase in COX-2 can actually lead to a cardioprotective effect; while COX-2 induces the inflammatory process, it later plays a key role in its resolution (33). In fact, there seems to be a cardioprotective effect of VEGF upregulation by COX-2: VEGF leads to angiogenesis and COX-2 production via endothelial cells, resulting in an increase in proarachidonic acid and subsequent cardioprotection. A study by Dowd et al. showed that inhibition of COX-2 leads to cardiac injury, also suggesting a cardioprotective effect of COX-2 (34). One proposed pathway for this protective effect is illustrated in a schematic found in Figure 3.

C-Reactive Protein

C-reactive protein (CRP) is known to predict cardiovascular events and is cited as an inhibitor of angiogenesis. Perhaps a part of its ability to inhibit angiogenesis is related to its effect on VEGF expression. Yang et al. conducted a study involving the addition of CRP to human coronary artery endothelial cells (*in vitro*) to observe the regulation of VEGF by CRP. Researchers found that while CRP did not slow endothelial cell proliferation, it did decrease the prevalence of VEGF receptors. VEGF-mediated (specifically, $VEGF_{165}$) capillary cell proliferation was inhibited as a result (35).

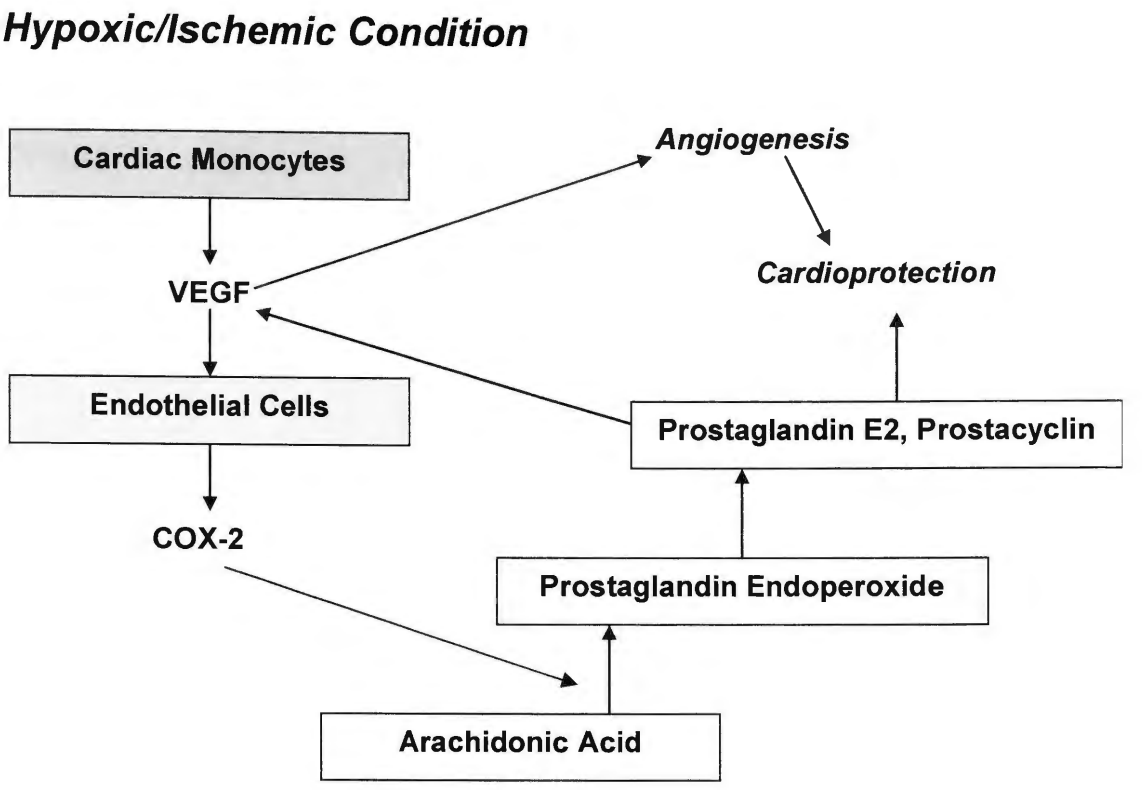

Figure 3. Proposed pathway for COX-2 gene regulation by myocytes in hypoxic conditions. (Adapted from Wu G, Mannam AP, Wu J, Kirbis S, Shie JL, Chen C, Laham RJ, Sellke FW, Li J. Hypoxia induces myocyte-dependent COX-2 regulation in endothelial cells: role of VEGF. *Am J Physiol Heart Circ Physiol.* 2003;285:2420-2429.

Chondromodulin

Chondromodulin is described by some researchers as the antithesis of VEGF in that it inhibits pathological angiogenesis. While chondromodulin is found in normal cardiac valves, it is greatly reduced in humans suffering from valvular heart disease. There are at least two proposed mechanisms for why this may happen. One relates to a transcription factor called Cbfal, which is upregulated in atherosclerotic aortic valves. It is thought that this protein represses chondromodulin while stimulating VEGF (34).

Another possibility is loss of valvular interstitial cells in valvular heart disease; loss of these cells likely leads to a decrease in chondromodulin and subsequently upregulates VEGF-A (36).

Fibroblast Growth Factor 2

The angiogenic effect of VEGF may be dependent on fibroblast growth factor-2 (FGF-2) according to a study conducted by Nillesen et al. (37). The researchers prepared five different collagen scaffolds with varied combinations of FGF2, VEGF, and heparin, then implanted these scaffolds into the backs of three-month-old male Wistar rats. Scaffolds were removed with surrounding tissue at three, seven, and 21 days after implantation. The explants were analyzed for histology and immunohistochemistry. VEGF and FGF2 together promoted blood vessel formation in scaffolds but seemed incapable of doing so independently. When VEGF and FGF-2 were injected together, there was a decrease in the time needed for initiation of angiogenic benefit compared to VEGF injection alone. Thus, VEGF combined with FGF2 may promote good vasculature formation in ischemic heart disease, preventing ischemic damage and supplying a means for more rapid angiogenesis. Results in this study suggest that combination therapy rather than VEGF alone may be most effective in treating ischemic heart disease (37).

Role of VEGF in Ischemic Heart Disease

Seeking to understand the role of VEGF in ischemic heart disease is difficult because of seemingly conflicting studies. Some studies report a cardioprotective effect

from VEGF upregulation in ischemic heart disease (8-10,37-40), while other studies indicate that VEGF expression in heart disease may be harmful (6-7,41-42).

VEGF and Atherosclerotic Plaque Instability

There are at least two mechanisms by which VEGF may augment the size of atherosclerotic plaques. The first method is a direct one, where VEGF acts on the plaque by entering the plaque via the *vasa vasorum* and then stimulating angiogenesis in the same. Another mechanism involves the release of endothelial precursor cells from the bone marrow, stimulated by VEGF. After their release, such cells may enter atherosclerotic plaques and there cause neovascularization (7,23).

Celletti et al. (6) fed 16 New Zealand white male rabbits a high (0.25%) cholesterol diet for 21 days, followed by intramuscular administration of rhVEGF or albumin (control). After seven to 21 days, rabbit thoracic aortas were harvested, and measurements of plaque area, circumference, and thickness were taken. Prevalence of macrophages was also determined. Seven days after its administration, researchers found that intramuscular rhVEGF correlated with an increase in plaque thickness, area, and circumference in rabbits on a high-cholesterol diet. Twenty-one days after rhVEGF administration, a significant increase in macrophages was observed (6). Another study by Celletti and colleagues found similar results in mice, showing that VEGF siginificantly increased macrophage levels in bone marrow, with subsequent increase in plaque area (7). Moulton et al. have shown in an animal study that atherosclerotic plaques release angiogenic promoters, resulting in macrophage infiltration and exacerbation of plaque growth. Conversely, inhibition of angiogenesis in atherosclerotic plaque led to fewer

macrophages and increased plaque stability. These findings are consistent with *in vitro* studies where researchers found that inhibition of angiogenesis and neovascularization led to subsequent inhibition of atherosclerotic progression, indicating that angiogenesis is needed for plaque growth, while its inhibition improves plaque stability (41-42).

Therapeutic Roles of VEGF in Ischemic Heart Disease

There is more published research that supports a therapeutic role of VEGF in heart disease than a harmful one. Henry et al. conducted as study called the VIVA Trial (vascular endothelial growth factor in ischemia for vascular angiogenesis). One hundred seventy-eight men with stable angina, 40 to 75 years of age, were assigned to a placebo group (63 subjects), a low dosage of rhVEGF (56 subjects), or a high dosage of VEGF (59 subjects); treatments were administered every three days for nine total days. At baseline, 60 days, and 120 days, subjects were required to exercise on a treadmill as long as they could comfortably do so. Researchers found that, at 120 days, subjects who had received a high-dosage of rhVEGF had significant improvement in their angina as well as a small (24 second) but significant increase in exercise duration compared to the two other groups (8).

Hamada et al. conducted another human study involving VEGF in heart disease. HUVECS were incubated with serum from twenty-one patients with cyanotic heart disease and 17 control subjects. Samples were taken before and after total cardiac repair in five of these patients. Because hypoxia stimulates VEGF-induced angiogenesis, serum from patients with cyanotic heart disease stimulated an increased rate of HUVEC tube formation compared to controls. The five post-cardiac repair samples stimulated less

tube formation than the corresponding samples taken before cardiac repair was complete. Researchers additionally added VEGF blockers to the serum of six patients, verifying that VEGF was the stimulus behind HUVEC tube formation. Blocking VEGF led to a decrease in tube formation, in contrast to the lack of a significant decrease in tube formation when other proteins (bFGF, erythropoietin, angiopoietin 1, MCP-1, and haptoglobin) were blocked. With supplementation of VEGF after blockage, HUVEC endothelial tube formation again increased (38).

Further support for a therapeutic role of VEGF is explored next.

VEGF Gene Therapy

Too much VEGF in the wrong place can be harmful rather than helpful in heart disease. Ozawa et al. conducted a study on ears from adult male rats, where VEGF was locally delivered to nonishcemic tissue via $VEGF_{164}$-expressing myoblasts. Researchers found that when more VEGF was expressed by the myoblasts, abnormal, leaky, and bulbous vasculature resulted, indicating that uncontrolled release of VEGF leads to extensive and abnormal vasculature. However, when myoblasts were cloned and made to produce smaller percentages of VEGF at a given location in the rat ears, homogenous and normal angiogenesis resulted. Researchers concluded that controlled release of VEGF leads to normal angiogenesis and vasculature, preventing immature and bulbous vasculature for which VEGF can be the cause if unregulated (43). Lee et al. found that administration of a large amount of VEGF directly to an atherosclerotic plaque, for example, leads to plaque growth, instability, and rupture (44). The theory behind gene

therapy, then, is to regulate the expression of VEGF rather than administering it in large quantities directly to the bloodstream (44).

Attempts to determine the best means of gene delivery of VEGF has been explored in a study by Lee et al. (44). Researchers attempted to use a water-soluble lipopolymer (WSLP) as a gene carrier. Male New Zealand white rabbits were injected with a WSLP, either carrying VEGF or plasmid (the placebo). This WSLP system was found to be a safe means of regulated VEGF expression in ischemic rabbit hearts. Interestingly, there was an increase in VEGF in hypoxic but not in normal cells under the WSLP system (44). This is supported by the fact that endothelial cell VEGF receptors have been shown to be upregulated in hypoxic conditions (45). In fact, as noted previously, VEGF can have a harmful effect on non-ischemic tissues when administered without tight control. Springer et al. reported that consistent expression of VEGF in non-ischemic tissues led to embryonic-like vasculature and hemangiomas in mice. This study illustrates the criticality in careful regulation of VEGF expression to avoid adverse effects. Such regulation is possible through gene-mediated expression (46).

Mice given arterial injury had higher endothelial repair when injury was preceded by intravenous administration of an adenovirus expressing VEGF in a study by Hutter et al. Fifteen mice received VEGF-expressing adenovirus, 15 received VEGF-trap-expressing adenovirus, 17 received adenoviruses expressing both VEGF and VEGF-trap, and 20 received a control solution without any VEGF or VEGF-trap expression. These different mediums were also tested on HUVEC's in varying degrees. Two weeks after injection and arterial injury, mice were euthanized and arteries examined. VEGF

treatment alone led to both significantly decreased mouse arterial neointima formation and increased HUVEC cell proliferation compared to controls. Conversely, VEGF-trap administration inhibited cell proliferation and led to increased neointima formation compared to other interventions (39).

A human study involving VEGF gene therapy was conducted by Sarkar et al. Seven adults with angina (two female) were treated with 0.25mg (or 1.0mg in two patients) of plasmid phVEGF-A_{165} into the pericardium of patients via a thoracotomy. Plasma VEGF concentrations significantly increased over the course of six days post gene transfer. The study saw only subjective improvements in exercise ability and increased myocardial perfusion. However, the study did determine the safety of administration of VEGF in human patients with chronic angina (19).

There is, of course, at least one study that found few, if any, benefits from genetically-delivered VEGF. A human study was conducted by Ripa et al., who gave patients in a hospital an intramyocardial injection of VEGF-A_{165} in a plasmid vector. While no adverse effects were noted, there did not seem to be any health benefits to VEGF gene-delivery expression compared to the control groups (47). Gene-mediated expression of VEGF, however, cannot be ruled out as a potential treatment for ischemic heart disease in the future.

Vitamin E in Angiogenesis

Vitamin E is an essential, fat-soluble vitamin in the human diet. While there are eight total isoforms of the vitamin, there are two basic forms: tocopherol and tocotrienol (16) (see Figure 4).

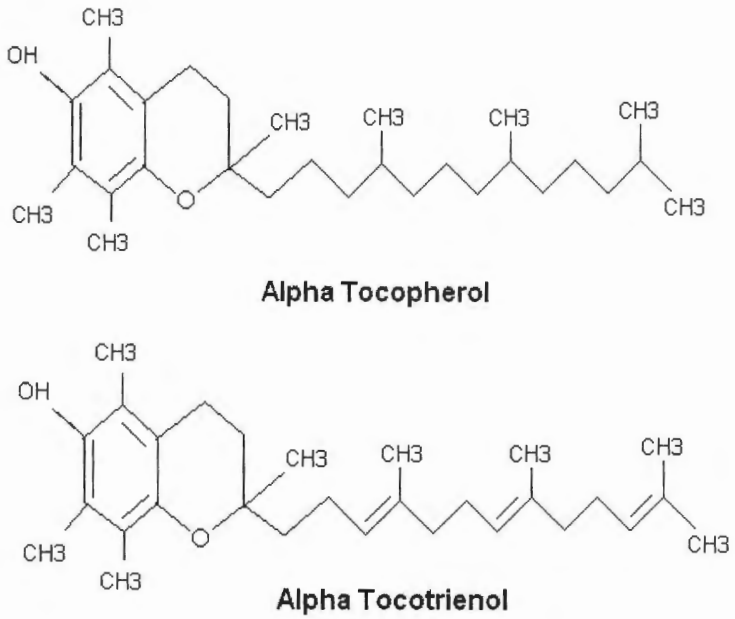

Figure 4. Structure of tocopherol and tocotrienol forms of vitamin E.

Tocopherol and its isoforms are found primarily in nuts and oils, while tocotrienol and its isoforms are predominantly found in palm oil, cereal grains, and rice bran. Out of all of the vitamin E isoforms, alpha tocopherol has the highest biological activity (16). However, some studies suggest that it is tocotrienol, not tocopherol, which has the greatest effect on angiogenesis (12,16,21,48). Several studies have been conducted using bovine aortic endothelial cells incubated with different isomers of vitamin E at various concentrations. All isomers of tocotrienol (and especially the delta isomer) reduced the

size of bovine aortic endothelial cell (BAEC) endothelial tubes. Isomers of tocopherol, however, had no effect on BAEC growth. Perhaps this is related to the ability of tocotrienol to pass easily through cell membranes with its unsaturated side chain (refer to figure 4 for chemical structures) (12,21,48).

Miyazawa et al. looked at the anti-angiogenic potential of tocotrienol *in vivo* by incubating chick embryo chorioallantoic membrane (CAM) assay with tocotrienol for two days. Additionally, five-week-old male ICR mice were implanted with colon carcinoma cells and then fed high levels of vitamin E orally for five days. A dose-dependent decrease in angiogenesis was observed in the CAM assay with delta-tocotrienol, and the mice had a decrease in tumor cell angiogenesis with high oral intake of tocotrienol. The study does not indicate that the CAM nor the mice were given anything but tocotrienol, so there is no evidence in this study that tocotrienol has a greater *in vivo* anti-angiogenic potential than tocopherol (48). However, Nakagawa et al. conducted a similar study involving incubation of CAM with both tocotrienol and tocopherol; all forms of tocotrienol significantly inhibited angiogenesis whereas tocopherol did not (12).

The study by Nakagawa et al. additionally looked at HUVEC (both treated with delta-tocotrienol and untreated) RNA. DNA chip analysis revealed that the tocotrienol treatment downregulated VEGFR, meaning decreased VEGF signaling and subsequent stunted growth and migration of endothelial cells (12).

Healthy pigs given the alpha-tocopherol form of vitamin E yielded different results. In an *in vivo* study conducted by Daghini et al., 12 female, healthy, domestic pigs

received a normal diet with or without antioxidant supplementation for 12 weeks. Levels of vitamins C and E (alpha-tocopherol form) were evaluated in the plasma via high-performance liquid chromatography at the end of the study. Renal tissues were additionally analyzed for vitamins C and E. After 12 weeks, plasma levels of the vitamins were higher in experimental compared to control groups. Plasma alpha-tocopherol in normal, healthy pigs positively correlated with angiogenesis. Decreased alpha-tocopherol lead to decreased VEGF expression in kidneys and decreased angiogenesis. The study suggests that antioxidant vitamins may actually have a pro-oxidant effect in normal, non-hypoxic conditions (22). Further studies examine in more detail the regulation of VEGF by vitamin E—particularly the alpha-tocopherol isoform of vitamin E (13-14).

Vitamin E and VEGF Regulation

Two-month-old male wild type and apolipoprotein-E-deficient mice were given vitamin C and alpha-tocopherol in their drinking water in a study conducted by Nespereira et al. Another group of mice was given vitamin C and beta-tocopherol. Because VEGF is upregulated by high cholesterol levels (LDL in particular), plasma cholesterol levels were compared. Finally, mice plasma levels of VEGF and VEGFR proteins and VEGF mRNA were determined. Vitamin C and alpha-tocopherol caused a decrease in aortic VEGF and VEGFR-2 in apo-E deficient mice but not in wild type mice, both for the VEGF protein and for VEGF mRNA. Vitamin C and beta-tocopherol were also administered to each group of mice with less reduction of VEGF and VEGFR in the apo-E deficient mice than that which was seen with alpha-tocopherol. The

researchers therefore report a vitamin C- and vitamin E-reduced expression of VEGF and VEGFR in this model of atherosclerosis, particularly with the alpha-tocopherol form (13).

Rodriguez et al. observed anti-oxidant regulation of VEGF in 18 male Yucatan miniature pigs. Pigs were divided into three groups and were fed one of three diets for 12 weeks: standard porcine chow, a high-cholesterol diet (24.5% lard, 4% cholesterol), and a high-cholesterol diet supplemented with antioxidant vitamins C and E (alpha tocopherol); vitamin supplementation for this final diet started on the fourth week of intervention. Pigs given the high-cholesterol diet had a significant upregulation in VEGF and VEGFR-2 expression (both protein and mRNA). When this same diet was administered with vitamins C and E, however, there was no such increase (14).

CHAPTER III

METHODS

This study is part of a larger study entitled "Effects of Dietary Sources of Vitamin E on Serum Lipids and Vitamin E Concentration," conducted in our laboratory. Briefly, blood samples were collected from 30 adult volunteers (see Table 1 for demographic information). Fifteen volunteers were assigned to the experimental group, and 15 were case-matched in the control group. During a one-week acclimatization period, volunteers were given specific study participation instructions, and informed consent was obtained from volunteers before the study was started. All volunteers were given a questionnaire to determine background information, preferred vitamin E-containing foods, and food allergies. Two blood samples were taken from each member of the control group and the experimental group. The initial sample was taken before intervention. After the initial blood draw, the control group participants continued to eat their usual diet, while the experimental group subjects consumed a diet composed of foods rich in vitamin E (hereafter referred to as the vitamin E diet, or VED), adding up to about 15mg per day. Vitamin E-rich foods included nuts and seeds, tomato products, leafy vegetables, other vegetables, fruits, cereals, and beans. VED subjects were given vitamin E-foods based on their completed questionnaire and were instructed to return to retrieve replacements for foods consumed by other individuals in their households. Each participant kept a food diary and remained on the assigned diet for four weeks. At the end of the study (week

five, including the one week acclimatization period), participants' blood was drawn again. Blood plasma was stored at -80 degrees Celsius. Figure 5 displays a simple flow-diagram of the study.

Table 1. Demographics of Study Participants

	Age in Years	**Weight**	**Sex**
Control			
Baseline	32 ± 10 (24-54)	146 ± 21 (120-179)	14 Female 1 Male
Post-Intervention	32 ± 10 (24-54)	146 ± 21 (122-179)	14 Female 1 Male
% Change	0%	0%	0%
Experimental			
Baseline	28 ± 11 (20-50)	135 ± 19 (114-169)	14 Female 1 Male
Post-Intervention	28 ± 11 (20-50)	134 ± 18 (114-167)	14 Female 1 Male
% Change	0%	-0.7%	0%

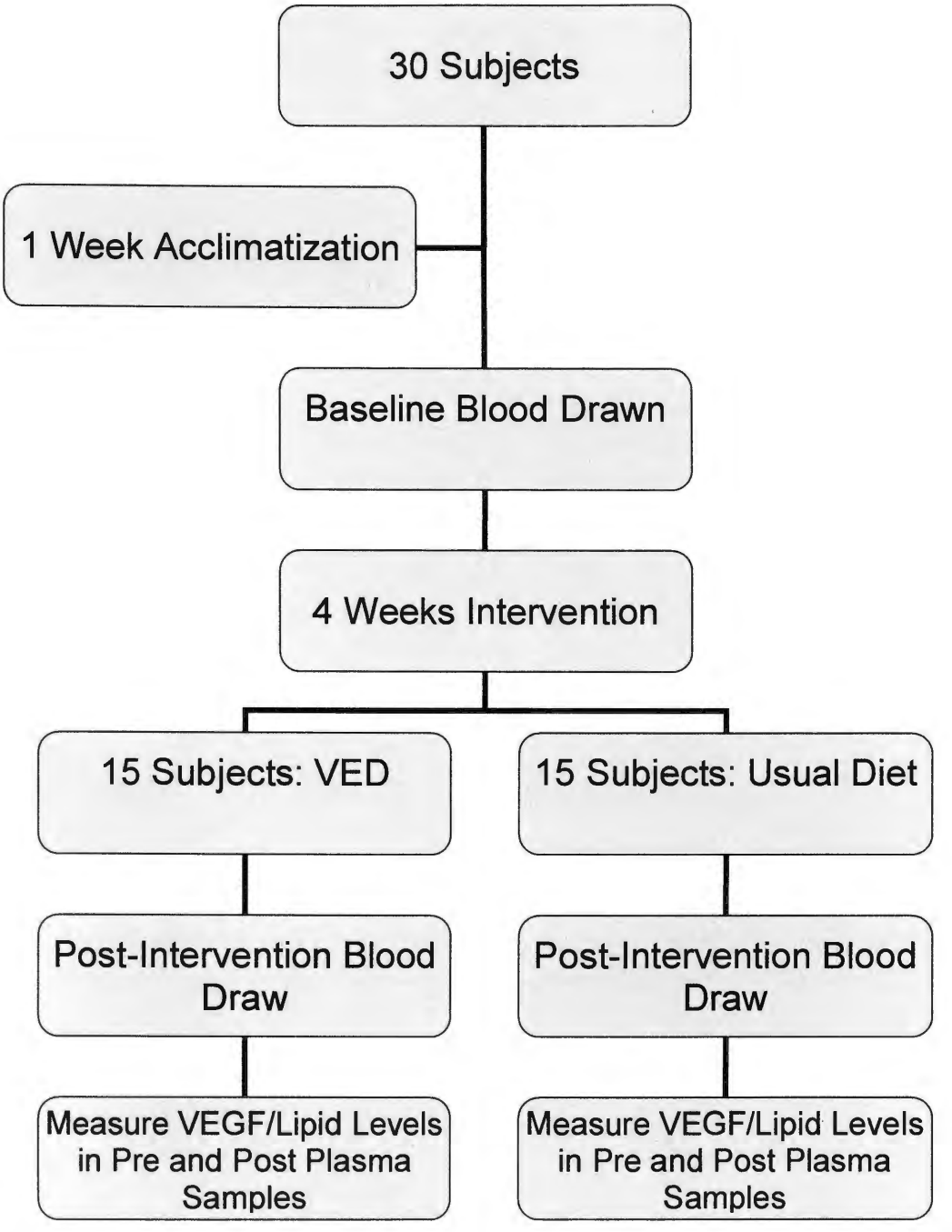

Figure 5. Flow diagram illustrating flow of study.

For this study, plasma VEGF was measured in both the experimental and control groups using a Human VEGF ELISA Kit, which is an *in vitro* enzyme-linked immunosorbent assay that quantitatively measures human VEGF levels in serum. Standard protocol for this kit (Ray Biotech, Inc, Norcross, GA) is as follows: an antibody specific for human VEGF is coated on a 96 well plate, into which samples and standards are pipetted. Any VEGF present in the wells binds to the pre-coated antibody on the plates. A biotinylated anti-human VEGF antibody is added to each well after washing to remove any unbound VEGF, followed by treatment with HRP-conjugated strepatvidin. In the final step, TMB substrate solution is added to the wells, and the well solutions change color to the degree that VEGF is bound. Once a stop solution is added, the solutions are read using an ELISA reader to determine VEGF concentration in each well.

All assay reagents and samples were brought to room temperature before use. All samples and standards were assayed in duplicates. For optimal readings, each plasma sample was diluted five-fold with kit assay diluent A before being pipetted into the antibody-coated wells. The standard solution was started at a dilution of 6000 pg/mL, then diluted with assay diluent A as outlined in the product protocol. Finally, the standard solutions were diluted again to match the dilution of the samples.

One hundred microliters of each standard and sample were pipetted into the antibody-coated wells of the microplate, then were allowed to incubate at room temperature for 2.5 hours. The standard and sample solutions were then discarded, and each well was washed four times with assay wash buffer solution. One hundred microliters of prepared biotinylated antibody were then added to each well, followed by

an incubation period of one hour. After removal of the solution following incubation, each well was again washed with wash buffer solution four times. One hundred microliters of prepared streptavidin solution were then added to each well, followed by a 45-minute incubation. The solution was discarded and each well as washed five times with wash buffer solution. Finally, 100μL of TMB one-step substrate reagent were added to each well, followed by a 30 minute incubation period with minimal exposure to light. Fifty microliters of stop solution were added to each well immediately before well content absorbencies were read at 450nm using an ELISA reader.

Once the absorbance of each sample was recorded, the percent difference between duplicates was calculated. Any duplicates with >10% variation were re-read or repeated. The mean absorbance of duplicates with ≤10% variation was calculated for each set of standards and samples. Standard curve values were taken and plotted on a line graph, and a second order polynomial was used to form the line of best fit. Experimental and control VEGF values were then determined using the equation for the standard line of best fit.

Statistical analysis was performed using SPSS version 15 for Windows. A factorial ANOVA test was used to determine the combined change in serum VEGF over time in both the control group and the experimental group. This was followed by Simple Effects Analysis to determine significance between baseline and post-intervention values separately for control group and VED group serum VEGF concentrations. Simple Effects Analysis was also used to determine individual differences between serum VEGF

levels in control and experimental groups, pre- and post-intervention. A p-value of <0.05 was used to indicate a significant change in VEGF over time.

Serum lipid samples were also measured both at baseline and post-intervention using standard protocol for eight of the 15 control samples and nine of the 15 VED samples. A smaller sample size had to be used for the serum lipids due to a shortage of plasma for lipid testing for some samples. Standard protocol was used to determine concentrations of various serum lipids, described in greater detail below.

To determine the triglyceride (TG) content of serum samples, standard, control, and VED samples were incubated with reagent for five minutes at 37 degrees Celsius, then read within 60 minutes at 500nm using an ELISA reader. TG was then calculated based on absorbance readings.

To measure high density lipoprotein cholesterol (HDLc) in serum, HDLc was first separated from LDLc and VLDL. Samples were mixed with precipitation buffer, incubated for ten minutes, and centrifuged for ten minutes. The remaining supernatant was the HDLc fraction and was transferred into new test tubes. Standard, control, and VED sample supernatants were incubated with reagent for five minutes at 37 degrees Celsius, then read within 60 minutes at 500nm using an ELISA reader. HDLc was then calculated based on absorbance readings.

Total serum cholesterol (TC) was determined by incubating standard, control, and VED samples with reagent for five minutes at 37 degrees Celsius, then read within 60 minutes at 500nm. TC was then calculated based on absorbance readings.

Low density lipoprotein (LDL) was calculated using the Friedewald Formula (LDLc = TC – HDLc – [TG/5]).

Statistical analysis of lipids was performed using SPSS version 15 for Windows. A factorial ANOVA test was used to determine the combined change in serum lipids over time in both the control group and the experimental group. This was followed by Simple Effects Analysis to determine significance between baseline and post-intervention values separately for control group and VED group serum lipid concentrations. Simple Effects Analysis was also used to determine individual differences between serum lipid levels in control and experimental groups, at baseline and post-intervention. A p-value of <0.05 was used to indicate a significant change in lipids over time.

Due to a small sample size, Spearman's rho correlation was used to identify the correlation between change in serum VEGF over time and change in serum lipids over time. Baseline values were subtracted from post-intervention values to determine the change over time. SPSS version 15 for Windows was used.

CHAPTER IV

RESULTS

Serum samples from participants consuming a usual diet (control) and a diet rich with foods high in vitamin E (VED) were analyzed for VEGF concentration. There were no significant differences in serum VEGF levels between the control group and the VED group at baseline. Post-intervention, serum VEGF was significantly lower in the VED group than in the control group (see Table 2 and Figure 6).

Table 2. Comparison of Control and Vitamin E Diet Group Serum VEGF

VEGF (pg/mL)	Control Group (n=15) Mean ± SD[a]	Vitamin E Diet Group (n=15) Mean ± SD[a]	p-Value Simple Effects Analysis
Baseline	27.3 ± 3.0	26.1 ± 3.7	0.367
Post-Intervention	26.9 ± 2.8	21.6 ± 3.4	<0.001
Difference	0.37 ± 0.32	4.52 ± 0.71	<0.001
Percent Change	1.5	17.2	N/A

[a]SD = Standard Deviation

Change in Serum VEGF at Baseline versus Post Intervention

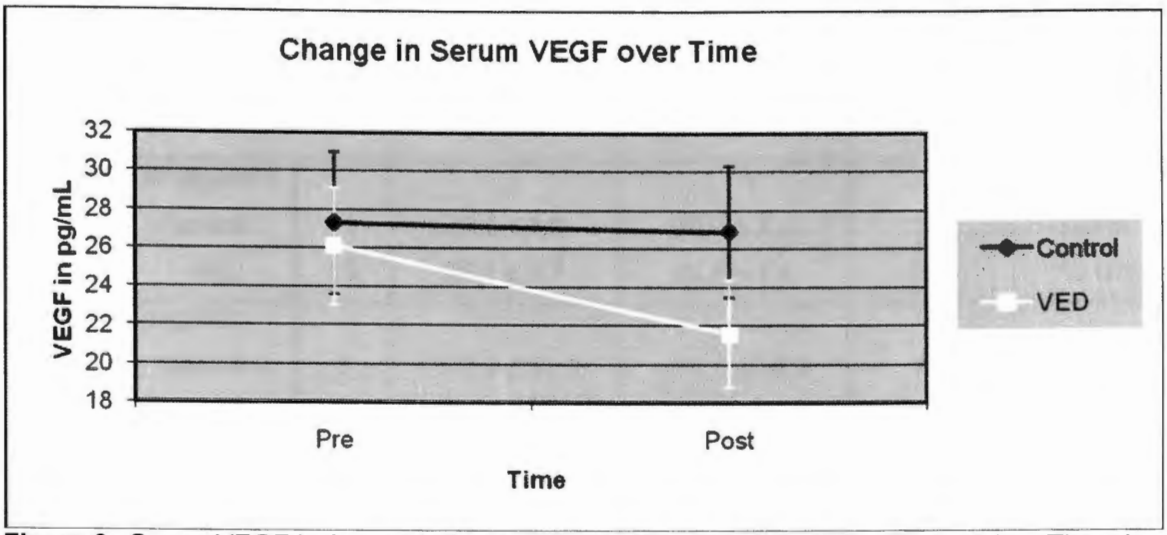

Figure 6. Serum VEGF before and after 4 weeks of dietary vitamin E supplementation. There is no significant difference between the control and VED groups at baseline (p=0.367). VEGF significantly decreased over time in the control group (p=0.015). VEGF also had a significant decrease in the VED group (p<0.001). Post intervention, serum VEGF was significantly lower in the VED group than the control group (p<0.001).

A significant difference was found in serum VEGF levels between baseline and post-intervention in the control group (p=0.015), with a 1.2 pg/mL decrease in serum VEGF over time. Serum from study participants who had consumed the VED for four weeks also had significantly lower VEGF levels compared to baseline levels (p<0.001). Post-intervention serum VEGF was 5.3 pg/mL lower than baseline levels in this group (see Table 2). TC, LDLc, HDLc, and TG were also compared before and after intervention. There was a significant difference in serum TC between control baseline and post-intervention blood draws (p = 0.039). No further significant differences were found in any blood lipids before or after intervention (see Table 3).

Table 3. Comparison of baseline and post-intervention serum VEGF, TC, LDLc, HDLc, and TG for control and VED groups[a]

	N[b]	Baseline Mean ± SD	Post-Intervention Mean ± SD	Percent Change	p-Value Simple Effects Analysis
Serum VEGF (pg/mL)					
Control	15	27.3 ± 3.0	26.9 ± 2.8	1.5	0.015
VED	15	26.1 ± 3.7	21.6 ± 3.4	17.2	<0.001
Serum TC (mg/dL)					
Control	8	179.9 ± 37.3	171.5 ± 36.6	4.7	0.039
VED	9	175.4 ± 22.7	168.9 ± 19.2	3.7	0.110
Serum LDLc (mg/dL)					
Control	8	102.4 ± 33.3	95.5 ± 30.0	6.7	0.136
VED	9	99.0 ± 30.8	93.3 ± 25.9	5.8	0.191
Serum HDLc (mg/dL)					
Control	8	61.1 ±19.1	58.7 ± 15.7	3.9	0.853
VED	9	63.4 ± 12.9	62.3 ± 12.8	0.2	0.796
Serum TG (mg/dL)					
Control	8	80.0 ± 45.0	79.4 ± 27.0	0.1	0.894
VED	9	98.1 ± 37.8	95.5 ± 35.9	2.7	0.450

[a]Lipid values come from another part of this larger study (conducted in our laboratory) entitled "Effects of Dietary Sources of Vitamin E on Serum Lipids and Vitamin E Concentration."
Definitions of abbreviations: VEGF = Vascular Endothelial Growth Factor; TC = Total Cholesterol; LDLc = Low Density Lipoprotein Cholesterol; HDLc = High Density Lipoprotein Cholesterol; TG = Triglycerides.
[b]Sample size for serum VEGF is 15 for both control and experimental groups. A shortage of plasma for some samples led to inability to find serum lipid concentrations in all study participants, leading to a reduced sample size for lipid data (N=8 for control group, N=9 for VED group).

Spearman's rho correlation was used correlate the change in serum VEGF over time with serum lipids. Results are displayed in Table 4. No significant correlations were found between VEGF and any of the blood lipids measured.

Table 4. Spearman's rho correlation of serum VEGF correlated with serum TC, LDL, HDL, and TG over time[a]

	TC p-value (r-value)	**LDL** p-value (r-value)	**HDL** p-value (r-value)	**TG** p-value (r-value)
VEGF	0.651 (-0.118)	0.448 (-0.197)	0.502 (-0.175)	0.478 (0.185)

[a]Definitions of abbreviations: VEGF = Vascular Endothelial Growth Factor; TC = Total Cholesterol; LDLc = Low Density Lipoprotein Cholesterol; HDLc = High Density Lipoprotein Cholesterol; TG = Triglycerides.

CHAPTER V

DISCUSSION

The primary hypothesis of this study was that dietary vitamin E (15mg/day) would decrease serum VEGF levels in human adults over time. In the present study, there was a significant decrease in VEGF over the course of four weeks in both the control and VED groups. The decrease seen in the VED group (4.5 pg/mL), however, was significantly greater than the decrease seen in the control group (0.37 pg/mL). This slight decrease in serum VEGF concentration seen in the control group post-intervention may have been due to a placebo effect, with control participants (knowing that they were participating in a nutritional study) unconsciously selecting a few more "healthy" foods than normal. Many "healthy" foods are rich in vitamin E, such as leafy greens and nuts. Control and VED groups were not significantly different at baseline ($p=0.37$).

A vitamin E –rich diet appeared to decrease serum VEGF in this study, with an average decrease of 17 percent. In the VED group, the difference in VEGF between baseline and post intervention averaged at 4.5 pg/mL. This significant decrease establishes a downward trend in serum VEGF with dietary vitamin E. It is possible that with an extended period of intervention with dietary vitamin E, serum VEGF would have been further reduced. Further, the relatively small degree of change in serum VEGF concentration over time (four weeks) seen between groups in this study may be explained by the fact that all study subjects were healthy, with the absence of a hypoxic state and

the presence of a normal blood lipid profile. For example, the hypercholesterolemic group in the Trapé et al. study had a 192 percent higher serum VEGF concentration than the control group (29). As stated by Ferrara et al., it is in the hypoxic state that VEGFR expression is upregulated, which leads to both sequestration and increased expression of VEGF (1). Without excessive serum VEGF available, vitamin E-induced downregulation was not likely in healthy subjects as it would be in those with hypoxic or hypercholesterolemic conditions (13).

Although some studies suggest that reduction or inhibition of serum VEGF is helpful in heart disease (6,7,41-42), other studies indicate that a decrease in serum VEGF is not always a desirable outcome for heart disease therapy. The potential power of vitamin E to downregulate VEGF may be less advantageous in some forms of ischemic heart disease as evidenced by studies that show a positive influence from VEGF in improving vascular ischemic conditions. Henry et al. found that an increase in VEGF significantly correlated with improved angina in a study called the VIVA trial (8). Hamada et al. found that upregulation of VEGF in hypoxic conditions (such as in congenital heart disease) led to greater endothelial tube formation, signifying increased cardiac perfusion in humans (38). VEGF administration significantly improved arterial repair after injury in mice according to a study by Hutter et al. (39). Sarkar et al. found improvements in myocardial perfusion and relief of heart disease symptoms, such as reduced chest pain during exercise and an absence of congestive heart failure symptoms with VEGF gene administration in humans, though results were not statistically significant (19).

In this study, we hypothesized that dietary vitamin E would decrease serum VEGF levels in healthy adults with a normal serum lipid profile. This is different from the study conducted by Rodriguez et al., which showed that vitamins C and E decrease serum VEGF in hypercholesterolemic conditions. Pigs treated with a high cholesterol diet had an increase in serum VEGF associated with hypercholesterolemia. When pigs were fed the same diet supplemented with vitamins C and E (alpha-tocopherol form), there was no such increase in serum VEGF levels. No information was given on how serum triglyceride concentrations were affected by antioxidant treatments in this study. Control (or healthy) pigs did not receive vitamin supplementation, however, so it is not possible to determine whether antioxidant vitamin supplementation reduces serum VEGF only in hypercholesterolemic conditions or if it also reduces VEGF in healthy conditions (14). A study by Nespereira et al. supports the idea that vitamins C and E may lower VEGF levels only in hypercholesterolemic conditions, at least in mice. These researchers found that vitamin C and alpha-tocopherol caused a decrease in aortic VEGF and VEGFR-2 in apo-E deficient (hypercholesterolemic) mice but not in wild type (healthy) mice (13). Further, a study conducted on healthy pig kidneys by Daghini et al. suggests that antioxidant vitamins C and E (alpha-tocopherol form) actually promote angiogenesis in healthy conditions (22). These studies differ from our study, however, in that they were conducted in animals rather than humans. Additionally, they used only the alpha-tocopherol form of vitamin E. The present study suggests that a vitamin E-rich diet (without isoform differentiation) does decrease serum VEGF in humans with a normal lipid profile.

In healthy individuals with a normal lipid profile, VEGF is not elevated and therefore does not pose a risk for forming hemiangiomas as it has been reported of doing when it is overexpressed or administered in excess, such as in heart disease or ischemic treatment (43,46,49,50). According to Celletti's studies, lowered VEGF in atherosclerosis would likely be beneficial, preventing exacerbation of atherosclerotic plaque growth (6-7).

A positive correlation between cholesterol and VEGF in human blood has been established by Trapé et al. Hypercholesterolemic human subjects (ten male, eight female) in the Trapé study had significantly higher serum VEGF (272.9 ± 207.5 ng/L) than control subjects (142.0 ± 57.7 ng/L), with a strong positive correlation between serum cholesterol levels and serum VEGF. Further studies need to be conducted to determine whether dietary vitamin E reduces VEGF in hypercholesterolemic humans. In the present study, all subjects had a normal blood lipid profile, and moderate reduction in serum VEGF was observed after four weeks of consumption of 15mg dietary vitamin E per day. In light of the correlation found between VEGF and cholesterol levels in the blood in the study by Trapé et al., there could be beneficial discoveries in studies observing the effect of dietary vitamin E on VEGF in hypercholesterolemia and the subsequent correlation between VEGF and cholesterol over time. Thus, further research could involve a study exploring the effects of vitamin E on serum VEGF levels in hypercholesterolemic conditions.

A final hypothesis of this study was to identify a decrease in serum VEGF concentrations with consumption of a vitamin E-rich diet, independent of serum lipids.

There were no significant correlations between the change in serum VEGF over time and the change in serum TC, LDLc, HDLc, and TG over time, in the control group or the VED group. Serum TC, LDLc, HDLc, and TG also did not significantly change over time, while serum VEGF levels moderately decreased. A significant decrease in TC ($p = 0.039$) was found in the control group; this may have been due to a small sample size, especially in light of the rather large standard deviations at baseline and post-intervention (refer to Table 2).

A small sample size is perhaps the greatest limitation to the study. While there were 15 subjects in both control and experimental groups, serum lipids were measured for only eight and nine subjects in control and experimental groups, respectively (serum VEGF was measured in all 30 subjects). This was due to a shortage of serum supply for lipid testing, caused primarily by repeated testing of some serum samples to obtain accurate serum VEGF data. A larger sample size, as well as a consistent sample size for each factor tested, would add to the strength of the study. An increased time of intervention would also add to its strength.

Another limitation of the study was the fact that all but two of the subjects were female. Having equal representation of both genders in a like study would provide better evidence for the population at large rather than women alone.

Certainty of vitamin E-rich food intake was another limitation of the study. While subjects were given vitamin E foods and were required to turn in a food diary, there is no way for researchers to verify that all of the vitamin E foods given to the subjects were indeed consumed by the subjects, even though they were instructed to return and obtain

more food if household members were eating from their supply. However, it is unlikely that this was much of an issue in that subjects chose the food items themselves in the questionnaire administered at the beginning of the study.

A final limitation of this study involves a lack of information on the actual amount of vitamin E consumed by each participant and how vitamin E intake may compare with other nutrients consumed. The study was designed to provide 15mg of dietary vitamin E for VED group participants. However, the actual amount of vitamin E consumed was not measured, nor were the quantities of other nutrients consumed measured. The study looks only at VEGF and serum lipid levels in volunteers consuming a vitamin E-rich diet, making the assumption that those consuming this diet are receiving greater amounts of vitamin E than those consuming their usual diets in the control group. Additionally, researchers in this study were unable to differentiate between isoforms of vitamin E consumed by members of the VED group (i.e. how much tocotrienol was consumed vs. how much tocopherol was consumed).

Overall, this study supports the idea that, in general, a diet rich in vitamin E may lower serum VEGF in healthy human adults. While a decrease in VEGF has yielded positive results in atherosclerotic conditions (6-7), no studies known to the author suggest that an increase or decrease in serum VEGF concentration in healthy adults has any protective benefit. Evidence supporting a decrease in serum VEGF concentrations with dietary intake of vitamin E in healthy individuals opens the door for further research to be conducted in individuals with atherosclerotic heart disease. In light of the results found in

the present study, dietary vitamin E may have potential as a future component of medical nutrition therapy in atherosclerosis.

CHAPTER VI

CONCLUSION

This study rejects the hypothesis that vitamin E has no effect on serum VEGF in human adults. Rather, the present study suggests that a vitamin E-rich diet may lead to a decrease in serum vascular endothelial growth factor. This is evidenced by a significantly lower VEGF concentration in the experimental group compared to the control group post-intervention but not at baseline. However, the decrease was small (17%), meaning that while there was a statistically significant difference in serum VEGF with intervention, it is difficult to determine whether this gradual downward trend is of practical significance.

This study suggests that vitamin E downregulates VEGF in healthy conditions, with a normal serum lipid profile. Further studies with a larger sample size could be conducted to observe the effect of vitamin E in lowering serum VEGF in conditions of high cholesterol and atherosclerosis, where uncontrolled expression of VEGF can occur.

Vitamin E was also found to reduce serum VEGF independent of serum lipids. A vitamin E-rich diet did not affect serum lipids in this study.

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