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Examining the relationship between Vitamin A intake and weight management

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To the Graduate Council:

I am submitting herewith a thesis written by Jennifer Paige Eastep entitled "Examining the relationship between Vitamin A intake and weight management." I have examined the final electronic copy of 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.

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Examining the relationship between Vitamin A intake and weight management

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Abstract

Introduction: Treatments of retinoic acid, a metabolite of vitamin A (VA) are shown to induce hyperlipidemia in humans and animals, which may influence adiposity. To understand the role of VA in weight management, two studies, one in animals examining effects of VA combined with high-fat diet (HFD) on body mass (BM) gain and the second in humans comparing dietary VA between participants who were overweight/obese and who had and had not lost 10% of body weight by consuming a hypocaloric, low-fat diet, were conducted.

Methods: Male Sprague Dawley rats were fed a HFD containing sufficient VA (HF-VAS) or a HFD deficient in VA (HF-VAD) for eight weeks. BM, liver mass, white adipose mass, brown adipose mass and plasma glucose were measured. The groups were compared with student's t-test. For the second experiment, participants (51.9 ± 8.8 yrs, 35.0 $\pm$ 4.5 kg/m^2, 94.7% white, 59.6% female, 100% non-hispanic) in a lifestyle intervention were categorized as successful weight losers (loss of $\geq$ 10% body weight) or unsuccessful weight losers (loss of < 10% body weight) after 6 months of treatment on a hypocaloric, low-fat diet. Dietary intake, assessed by three, 24-hour dietary recalls was collected at 6 months. Dietary VA was compared between the groups using analysis of covariance.

Results: In the animal study, significant differences were detected in end day BM, liver mass and white adipose mass between the HF-VAS and HFD-VAD groups. No significant difference was detected in dietary VA consumed between participants who had successful 10% weight loss and those who did not have successful weight loss at 6 months.

Conclusion: Results from the animal experiment indicate that VA plays a role in adiposity in subjects consuming an obesogenic diet. No difference was detected in VA intake between subjects who successfully lost 10% weight and those who have not. This indicates dietary VA is not related to successful weight loss on a hypocaloric, low-fat diet. Additional studies are needed to understand the role VA plays in obesity development. Also, further investigation on how VA intake while consuming a hypocaloric low fat diet may affect weight loss is needed.

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Chapter I

Introduction

1.1 Background

Obesity, defined as excessive body fat content indicated by a BMI ≥ 30 kg/m^2, continues to be a nationwide problem with one third of Americans considered to be obese[1]. This epidemic predicts the development of various metabolic diseases[2], resulting in higher mortality rates and higher costs in health care[3]. Aside from this, obesity has been linked to decreased productivity in the workplace, which can indirectly increase healthcare costs[3]. The increases in mortality rates and costs of health care have become an urgent problem, which finding solutions must become priority.

Obesity is thought to be caused by positive energy balance, meaning that energy intake is greater than energy output[4]. Both genetic and environmental factors contribute to the development of obesity and its associated chronic metabolic diseases. Several genes, such as leptin and its receptor, responsible for monogenic obesity have been identified[5]. On the other hand, over nutrition seems to be an obvious cause of obesity development. However, the role of each micronutrient has not been revealed. Previous research indicates that VA may also play a role in the development of obesity[6]. This thesis includes two parts. The first one aims to investigate the contributions of VA intake on obesity development in rats fed a high-fat diet (HFD). The second portion studied the effects of VA intake on body weight alterations in participants during a hypocaloric, low-fat diet. The project seeks to answer the following questions: Does the presence of VA in the diet have an effect on weight gain while subjects are consuming a high-fat obesogenic diet? Is there a significant difference in the amount of dietary VA consumed by partcipants who successfully lost weight while consuming a hypocaloric, low-fat diet compared to those who did not in a clinical weight loss trial? The two experiments use

different models to answer the questions. The first experiment uses an animal model looking at weight gain on a high-fat diet, while the second experiment uses a human model looking at weight loss on a hypocaloric, low-fat diet.

Chapter II

Literature Review

2.1 Vitamin A (VA) and its homeostasis

2.1.1 The discovery of VA and provitamin A

VA, all-*trans* retinol ((2E, 4E, 6E, 8E)- 3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl) nona-2,4,6,8-tetraen-1-ol)), is a lipid soluble vitamin which exists in multiple isomeric forms[7]. All of these forms contain a 20 carbon structure with a methyl substituted cyclohexenyl ring and a tetraene side chain differing in the molecules attached to this side chain[7]. It was first identified in 1910s and later elucidated in 1930s as a lipid derived factor essential for the growth of animals[8,9].

VA activity was observed when rats fed a synthetic diet with lard or olive oil extracted with ether as the only fat source would lose weight and then die. In contrast, rats given certain lipid derived factors from foods such as eggs and butter survived and began to grow again[10]. Later on, its molecular identity as retinol was determined. VA activity can be derived from molecules that can be converted into retinol[11]. In the diet, these molecules are retinol esters (animal sources) and provitamin A carotenoids (plant sources)[12]. The majority of VA physiological activities are mediated by RA[13]. Retinol (an alcohol) is first reversibly oxidized into retinal (an aldehyde), and then irreversibly oxidized into RA. It has been recognized for its role as essential micronutrient for the general health of the individual. In the body, VA is essential for vision, growth, skin development and cell differentiation[14].

2.1.2 The discovery of carotenoids

Carotenoids were discovered as a result of the search for a medicinal agent, an anthelminthic to rid the body of parasitic worms, especially from the intestine[15]. Credit

for this discovery goes to Heinrich Wilhelm Ferdinand Wackenroder[15]. He published the results of his examination of carrots, with one of the purposes of that research being the search for the presence in the juice of the carrots of an effective anthelminthic[15]. This prompted Wackenroder to undertake chemical analysis of the juice in the attempt to identify the substance that was medically active[15]. The results of this work were published in 1831[15].

In humans, three carotenoids (beta-carotene, alpha-carotene, and beta-cryptoxanthin) have VA activity, and these and other carotenoids can also act as antioxidants[10]. In the body, provitamin A carotenoids are cleaved to form retinal, which is then reduced to retinol[12].

2.1.3 VA metabolism and its active metabolic intermediates

Retinol, the least potent form of molecules with VA activities, contains a hydroxyl group at carbon-15. Retinol can act as a precursor for the more active forms of molecules with VA activities. When retinol is oxidized to retinal the molecule contains an aldehyde group, which is further oxidized to RA containing a carboxylic acid group[7]. VA activity can be derived from molecules that can be converted into retinol[11]. The majority of VA activities are mediated by RA[13].

Enzymes responsible for the reversible oxidation/reduction reaction of retinol to retinal are termed dehydrogenases and exhibit properties as an alcohol dehydrogenase or a short- chain dehydrogenase reductase[16]. Enzymes facilitating the irreversible oxidation of retinal to RA are classified in the aldehyde dehydrogenase family[16]. Cytochrome P450 hydroxylase 26A (CYP26A) mediates modification of RA molecules to facilitate RA disposal. It is the primary CYP26 enzyme expressed in the liver, contributing

the largest amount of activity in the clearance of RA from humans[17]. This gene irreversibly oxidizes RA into more polar metabolites for excretion[17].

2.1.4 The conversion of carotenoids to VA

Of all known carotenoids, β-carotene is believed to be the most important in human nutrition[18]. The key step in the VA biosynthetic pathway is the oxidative cleavage of β-carotene into two retinal molecules by the enzyme β,β-carotene-15,15′-monooxygenase[18]. Subsequent oxidation yields retinoic acid which can then act as ligand for nuclear transcription factors. Beta-carotene can also undergo eccentric cleavage which can occur enzymatically or non-enzymatically[19]. This produces beta-apocarotenals and beta-apocarotenones, whose functions in mammals are unknown[19].

2.1.5 Digestion, absorption and storage of VA and carotenoids

Dietary VA comes from retinyl esters (animal sources) and provitamin A carotenoids (plant sources)[20]. Carotenoids consist of 40 carbons, conjugated double bonds, and may contain 1-2 cyclic structures at the end of their chain[7]. Pancreatic lipases hydrolyze retinyl esters into retinol and free fatty acids which then are absorbed into enterocytes. Retinol is then re-esterified into retinyl esters to be packaged in chylomicrons for delivery to other parts of the body through lymph circulation first and then blood circulation[20]. Provitamin A carotenoids are enzymatically cleaved to produce retinal which is then reduced into retinol[12,21]. This retinol produced is then converted to retinyl ester so that it can be incorporated into chylomicrons[22]. Dietary fat is essential for optimal absorption of VA because it acts as a facilitator for incorporation into chylomicrons[23]. Foods rich in VA include liver, milk, eggs, and those rich in provitamin

A include carrots, sweet potato and dark leafy greens[10]. The RDA for VA has been established for the different life stages to prevent deficiency and toxicity[24]. See Table1 below for RDA of VA.

Table 1 Recommended Daily Allowance of Vitamin A based on gender and age

Life Stage Group	Children 1-3 yr	Children 4-8 yr	Males 9-13 yr	Males 14-70 yr	Females 9-13 yr	Females 14-70 yr
VA (µg/day)	300	400	600	900	600	700

Physiological VA status is regulated through a network of enzymes and proteins which facilitate the transport, productions and catabolism of retinoids[25]. In this network, retinol is reversibly converted into retinal, and retinal is irreversibly converted into RA[26]. RA has been involved in the regulation of the expression levels of the enzymes in this system [13]. However, it is important to recognize that some of the physiological functions of retinal (such as vision) cannot be replaced through RA treatments[27].

2.1.6 Mechanisms of VA and its metabolites functions

2.1.6.1 The vision cycle

Retinol metabolite, 11-*cis*-retinal, plays a critical role in vision. In the eyes, 11-*cis*-retinal is bound to opsin to form rhodopsin in rods. When light enters the eyes, 11-*cis*-retinal isomerizes to all-trans-retinal and dissociates from opsin. This results in a nervous signal along the optic nerve to the brain. A series of enzymatic reactions

converts all-*trans*-retinal back to 11-*cis*-retinal, which can then rebind to opsin to form rhodopsin to complete the vision cycle[10]

2.1.6.2 Mechanism of Retinoic Acid (RA)-mediated transcription

RA is found in multiple forms, with all-*trans* RA and 9-*cis* RA being significant in the body. RA regulates gene expression mainly through activating two nuclear receptor families, RA receptors (RARα, β and γ) and retinoid X receptors (RXRα, β and γ)[12,26]. RARs and RXRs are considered part of the nuclear receptor family, which are ligand-activated transcriptional activators critical for physiological processes[28,29]. RARs are activated by all-*trans* RA while RXRs are activated by 9-*cis* RA[26]. Activation occurs when RAR/RXR hetero-dimers or RXR/RXR homo-dimers bind to the RA responsive elements at the promoters of their downstream targeted genes[30,31]. It has been suggested that RXR can act as a universal dimerization partner for other families of nuclear receptors such as peroxisome proliferator activated receptors (PPARs), liver X receptor (LXR), thyroid hormone receptor (TR), and vitamin D receptor (VDR), indicating a complex transcriptional network which allows RA, to exert its biological activity.[32].

Chicken ovalbumin transcription factor II (COUPTF-II) nuclear receptors have DNA-binding abilities, meaning it can activate or inhibit gene expressing depending on the presence of ligands, corepressors or coactivators[33]. Deletion of COUPTF-II can alter processes such as angiogenesis and cardiovascular development, and therefore can be lethal[33]. It has been reported that COUPTF-II acts constitutively but its activity can be modulated by the presence of RA[34]. RA has been reported to release COUPTF-

II from the auto-repressed conformation, possibly affecting some of the metabolic activities regulated by COUPTF-II[34].

Hepatocyte nuclear factor 4 alpha (HNF4α) is a transcription factor responsible for several liver specific functions such as gluconeogenesis and lipid and bile synthesis[35]. It has been shown to direct 11% of genes actively transcribed in pancreatic islets[36]. Compared to the liver, it is expressed in lower amounts in other tissues such as intestine and kidneys[37]. The importance of the role it plays is indicated by the development of hepatic steatosis in HNF4α deficient mouse livers[35]. In humans, mutations in the HNF4α genes leads to maturity onset of type I diabetes, characterized by pancreatic β-cell dysfunction evidenced by loss of insulin secretion in response to glucose[38]. In hepatocyte, HNF4α maintains differentiation state, phenotype and also directs energy metabolism[38].

It has been observed that RA produced in intestines was found in the portal system of the liver[39], which indicates that plasma RA levels may play a role in signaling the body's retinoid status[13]. It has been demonstrated that retinoids regulate the expression of genes involved in hepatic glucose and lipid metabolism[40,41].

2.2 Hepatic metabolism of VA

The liver plays an essential role in energy metabolism. This is in part attributed to the regulation of expression levels of hepatic genes involved in glucose and lipid metabolism in response to hormonal and nutritional stimuli[42]. Obesity and other metabolic diseases such as diabetes have been linked to abnormal glucose and lipid metabolism in

the liver[43]. It is clear that metabolic abnormalities significantly impact hepatic functions and, thereby, contribute to morbidity.

2.2.1 Role of liver in the regulation of metabolic homeostasis

The liver is the most important site for retinoid storage in the body, accounting for uptake of 66-75% of all dietary retinoid absorbed in the intestines[44]. It accounts for 70-80% of all retinol binding protein (RBP) in circulation, making it the major site for RBP synthesis and secretion[22]. RBP maintains circulating levels of retinol to assure continuous delivery to target tissues under conditions of retinoid sufficiency[45]. The three RARs (α, β, γ) and three RXRs (α, β, and γ) are all expressed in the liver, making it an important target organ for retinoid actions[46].

2.2.2 Role of the liver in VA homeostasis and its metabolism

The majority of dietary retinol absorbed by enterocytes is secreted into the lymphatic system in the form of retinyl esters in chylomicrons, which contain dietary lipids, mostly triglyceride (TG) and cholesterol[22]. In circulation, 66-75% of chylomicron retinyl ester is removed by the liver while the remaining is removed by extrahepatic tissue such as adipose tissue, skeletal muscle, heart, spleen and kidney[22]. Most retinyl ester present in chylomicrons remains as the particles become chylomicron remnants in a process involving the lipolysis of TG and recruitment of apolipoprotein E (apo E) to the particles[22]. It is thought that chylomicron remnants are internalized solely by hepatocytes[47].

After chylomicron remnant retinyl esters have been up taken by hepatocytes, the retinyl esters are hydrolyzed by retinyl ester hydrolases[48]. In a retinoid sufficient state,

chylomicron remnant retinol is then transferred to hepatic stellate cells for storage[49,50]. In contrast, when dietary retinoids are insufficient more of the recently ingested retinol is secreted into circulation bound to RBP instead of being transported into storage.

Circulating retinol bound to RBP enters and leaves the liver multiple times before its elimination from the body. This process is called retinol recycling[51]. Studies in humans show that 14.3 mg/day of retinol passes through plasma compared with a disposal rate of 1.14 mg/day, which indicates that a large portion of retinol take up in organs and circulating tissues is recycled back into plasma and that only a minor portion is converted to active metabolites or degraded[52]. It is thought that this process of retinol recycling provides the liver an ideal means to sample and adjust the concentration of retinol available in plasma for peripheral tissue[53]. Total body retinol pool size can be estimated with deuterated retinol dilution technique and calculation of the change in total body retinol pool size can be achieved with the paired deuterated retinol dilution technique[54,55].

Hepatocytes play an essential role in hepatic retinoid metabolism and storage, accounting for some retinoid storage and facilitating mobilization of retinol from the liver[22]. These cells also contribute to retinoid activation to RA, to RA catabolism and to the excretion of retinoid catabolic products[22]. They have relatively high concentrations of retinol, retinyl ester, RBP, retinyl ester hydrolases, and enzymes that convert retinol to RA[22].

Mobilization of retinoids from the liver to target tissues in circulation is regulated in a process involving RBP[56]. RBP has a molecular weight of 21 kDa and has a single

binding site for one molecule of all-*trans* retinol[22]. This protein is synthesized in hepatocytes, but its expression has also been detected in other tissues, such as kidney and adipose tissue[22]. When retinol binds to RBP, the retinol-RBP complex enters the blood stream for transport to target tissues and its concentration is maintained at 2-3 μM. This complex circulates in the blood as a 1:1 molar complex with thyroxine hormone carrier transthyretin, making the complex less susceptible to filtration by the kidney[57,58]

2.2.3 Role of VA in the metabolism of glucose in the liver

Elevation of hepatic VA content was observed in diabetic patients as early as 1937[59]. Later, it was observed that rats fed a VA-deficient (VAD) diet had depleted hepatic glycogen content[60]. This study concluded that the depletion of hepatic glycogen was caused by the reduction of glycogenesis from trioses, rather than directly from glucose[60]. The depletion in VAD rats was not due to reduced energy intake, as pair fed rats with equal energy intake had higher hepatic glycogen content[60]. When rats were fed a diet with excess VA for 2 days, a dramatic increase in fasting hepatic glycogen content was observed[61].

It has been observed that insulin resistant humans and animals have elevated plasma RBP 4[62]. When mouse plasma RBP4 level was manipulated, the result was altered insulin sensitivity and expression levels of hepatic gluconeogenic genes[63]. A reduction of plasma retinol and RBP levels has been observed in patients with type I diabetes and also in streptozotocin-induced diabetic rats[64,65]. Insulin sensitivity was improved in insulin resistant ob/ob mice with administration of retinal[66].

Glucose is differentially metabolized in cells depending upon hormone and nutrition statuses[67]. For it to be utilized, it must undergo phosphorylation to become glucose 6-phosphate (G6P) through the action of hexokinases in the first step of glycolysis. In mammals, hexokinase is referred to as glucokinase (GK)[68,69]. Mutations altering the GK enzymatic activity are associated with maturity onset diabetes of the young[70]. Regulation of hepatic GK activity is accomplished by its binding to GK regulatory protein, phosphorylation by protein kinase A, and interaction with cytosolic GK-associated phosphatase[71-73]. Long term regulation of GK activity is achieved through transcription of GK gene (*Gck*)[74]. *Gck* is differentially regulated by an upstream promoter in pancreatic β-cells and a downstream promoter in hepatocytes[75]. In the liver, the cycle of fasting and re-feeding alters *Gck* expression. However the same cycle does not alter *Gck* expression in pancreatic β-cells[74]. It has been observed that in rat liver, *Gck* mRNA is induced by insulin and suppressed by glucagon[76-78].

In rat hepatocytes, all-*trans* RA has been shown to induce *Gck* expression without any additive effects on insulin mediated induction[79,80]. On the other hand, our research group has reported that retinoids synergize with insulin to induce *Gck* expression in rat hepatocytes[40]. In the liver of VAD rats, *Gck* activity and mRNA levels were lower compared to VAS controls. Also, treatment with RA induced *Gck* mRNA in rat hepatocytes[40].

Under conditions where dietary glucose is not available, the liver will produce glucose through gluconeogenesis. Phosphoenolpyruvate carboxykinase (PEPCK-C), the first rate-limiting enzyme of gluconeogenesis, converts oxaloacetate into phosphoenolpyruvate in the presence of GTP[81]. PEPCK-C expression and activity has

been detected in the liver, kidney and adipose tissues, where PEPCK-C may control gluconeogenesis, glyceroneogenesis and cataplerosis[81-84]. PEPCK-C is regulated by the transcription of its gene *Pck1* in different physiological conditions[81-83]. The hepatic expression of *Pck1* is induced by glucagon and suppressed by insulin[85], meaning the liver can produce glucose under fasting conditions. RA has been reported to stimulate *Pck1* expression, also two RA response elements (RARE) have been identified at *Pck1* promoter in hepatoma cells[86-88]. Lipophilic extract from rat liver was found to induce *Pck1* expression and attenuate insulin-mediated suppression of its transcription[89]. These lipophilic molecules were later identified as retinoids due their effects of insulin-induced *Gck* expression[40]. Of the two previously identified RARE in *Pck1* promoter, the proximal one was responsible for mediating the retinoid effect in hepatocytes[86,87,90,91].

2.2.4 Role of VA in the lipid metabolism

The liver and adipose tissues, in response to dietary intake and stored energy, regulate TG and fatty acid (FA) homeostasis in the body[92,93]. FAs are stored in adipose tissue as TGs in the postprandial stage[94]. TG in chylomicrons and very low density lipoprotein (VLDL) is converted to free fatty acids (FFA), monoglycerides and diglycerides through hydrolysis by lipoprotein lipase (LPL) for entry and storage in adipose tissues. Under fasting or starvation condition, FFAs are released from adipose tissue though lipolysis by lipases in adipocytes[95]. These FFAs can then be used by heart, liver and skeletal muscle for energy. In the liver, FFAs can be used for ketogenesis and glycerol for gluconeogenesis. Other organs, like the brain, can then utilize ketone bodies and glucose for energy.

Instances of metabolic abnormalities are often associated with changes in hepatic lipid metabolism. For instance, patients or animals with obesity and type 2 diabetes were reported to have excessive hepatic lipogenesis[43]. Insulin stimulates lipogenesis and inhibits lipolysis to regulate FA and TG homeostasis[96]. Patients and animals with obesity and type 2 diabetes experiencing hyperinsulinemia were determined to have excessive hepatic production of FAs and TGs. This happens because the liver can stimulate the expression of lipogenic genes, a process mediated by sterol regulatory element binding protein 1c (SREBP-1c), which is an insulin-induced transcription factor[97,98]. In the liver of mice, activation of liver X receptor (LXR) and RXR induced the expression of the SREBP-1c gene (*Srebp-1c*)[99]. In *Srebp-1c* promoter, the insulin responsive elements have been identified as two liver X receptor elements (LXRE) and one sterol regulatory element (SRE)[100]. Retinoids induced SREBP-1c expression and maturation, resulting in activation of the promoter activity of fatty acid synthase gene (*Fas*)[101,102]. In rat hepatocytes, retinoids synergized with insulin to induce expression of *Srebp-1c* mRNA in a dosage and time dependent manner[41]. The two LXREs responsible for the insulin induced *Srebp-1c* expression in rat hepatocytes were determined to be RAREs in its promoter[41,100].

Patients with acne treated with isotretinoin (13-*cis* RA) developed hypertriglyceridemia[103]. Healthy subjects given isotretinoin had elevated plasma apo C-III level. In adult hepatocytes, apo C-III was induced RXR specific agonist[104]. Patients with acute promyelocytic leukemia were observed to have gained weight and elevated levels of plasma TG and cholesterol after treatment of all-*trans* RA[105,106]. Rats consuming a VAD diet for 3 months after weaning were reported having lower plasma

TG and cholesterol, hepatic phospholipid contents, and hepatic synthesis of FA and phosphatidylcholine compared to controls fed a VAS diet[107]. This may be due to the combination of VA deficiency and hypoinsulinemia, because insulin secretion is impaired in rats fed a VAD diet[108]. Rats fed with all-*trans* RA and 13-*cis* RA and with 9-*cis* RA and RAR specific agonist induced hypertriglyceridemia[109,110]. This is attributed to the elevated production of VLDL in the liver and reduced uptake of TG in adipose tissue and skeletal muscle. In vitro experiments show that rat hepatocytes treated with RA increased the incorporation of lipid precursors into FA and TG. Reduced TG uptake was associated with reductions of LPL activities in adipose tissue and skeletal muscle[109]. Elevated plasma TG was reported for Sprague-Dawley and Zucker diabetic fatty rats treated with a RXR specific agonist[111]. This was attributed to a decrease in LPL activities in adipose tissue and skeletal muscle[111]. The reduction was likely mediated by a RXR-induced protein responsible for posttranslational processing of PLP since there were no effects of RXR activation on the mRNA level of LPL[111]. It is important to note that the role of hepatic lipogenesis in the increase of plasma TG was not clearly defined in these studies. In obese and diabetic mice, it has been reported that RXR sensitized insulin and reduced TG levels[112]. All-*trans* RA has been reported to repress obesity and insulin resistance through activation of PPAR β/δ and RAR[113]. Hepatic lipid metabolism was altered in mice with liver specific knockout of RXRα[114]. Of note, RXRα knockout mice have elevated expression of RARβ and RARγ, which implies the changes of RA signaling as RARβ can be up-regulated with treatment of RA[115].

These observations imply the roles of VA in lipid metabolism, probably through multiple mechanisms[12]. Treatment of RA may have (1) increased lipogenesis in the

liver, (2) reduced PLP activity in skeletal muscle and adipose tissue, and/or activated signal transduction pathways and transcription of RAR/RXR downstream targeted genes[116]. The two RAREs/LXREs on *Srebp-1c* are mediating insulin response and sensing nutrition VA status, meaning the retinoid and insulin signaling pathways can converge on the same sites in *Srebp-1c* promoter and synergize to induce its expression, resulting in the expression of its downstream lipogenic genes[12]. Because SREBP-1c is the dominating regulator for hepatic FA biosynthesis, it has been concluded that one of the ways that retinoids regulate lipid homeostasis is through regulation hepatic expression of *Srebp-1c* [12,97]. Looking at the effects of RA on insulin-mediated expression of *Srebp-1c* and *Pck1* in rat hepatocytes, it has been suggested that RA may be one of the possible factors leading to hepatic insulin resistance[12,41,91].

2.2.5 Role of VA intake in obesity development

Previous research indicates that VA plays a key role in the development of obesity. Patients with promyelocytic leukemia treated with all-*trans* RA had observed increases in BM and plasma cholesterol[105]. Excess of isotretinoin (13-*cis* RA) given to patients for the treatment of acne vulgaris has been shown to elevate blood TG and liver enzymes without any change in dietary intake[117,118]. It has been demonstrated that Zucker fatty rats fed a VAD diet gained significantly less weight than their VA sufficient counterparts[6]. It has been observed that patients with metabolic syndrome had significantly lower levels of plasma VA than their healthy counterparts[119]. Interestingly, research has demonstrated the use of VA in the treatment of obesity. Obese mice treated with *all-trans* RA experienced weight loss and improved insulin responsiveness as a result of the all-trans

RA inducing the expression of PPAR β/δ and RAR target genes involved in regulating lipid homeostasis[113].

The impact of VA on the mechanisms of obesity development is not well understood. A greater understanding of the role of VA in energy metabolism and obesity development may lead to more effective treatments for obesity and metabolic diseases.

2.3 Methods to assessing VA

2.3.1 Measuring VA in plasma

Methods have been established to quantify VA in plasma. Plasma samples first undergo an extraction process to remove the fat soluble vitamins and carotenoids. This extraction provides an extract suitable for reversed-phase liquid chromatography analysis with absorbance detection[120]. Below is Table 2 for normal retinol plasma levels.

Table 2 Reference Intervals for normal retinol plasma levels in humans based on age

Life Stage	Reference Interval
0-1 month	0.18-0.50 mg/L
2 months-12 years	0.20-0.50 mg/L
13-17 years	0.26-0.70 mg/L
18 years and older	0.30-1.20 mg/L

2.3.2. Measuring VA in the tissues

For establishing tissue concentration of VA, there is far less data than for plasma concentration[10]. Research indicates greater concentrations in tissue than plasma in rats[10]. For instance, it has been reported that normal rats have tissue concentrations ranging 40-580 pmol/g (kidney, liver, lung and pancreas) compared to a plasma concentration of 8-16 pmol/ L[10].

2.4 Role of western diet in the development of metabolic diseases

2.4.1 High-Fat Diet (HFD)

2.4.1.1 The definition of HFD and current status in population

With one third of Americans being considered obese, obesity has reached epidemic level in the United States[1]. This is a concern of public health as obesity leads to the development of various metabolic diseases[2], which result in higher mortality rates and higher costs in health care.

One of the dietary styles contributing to the obesity epidemic is the Western Diet (High-fat content), which is characterized by a high intake of red and processed meats, eggs, refined grains and sugars, and energy derived from fat, mainly saturated fatty acids, which can be as high as 35% of energy intake[121,122]. The Dietary Guidelines published by the USDA recommends that dietary fat provide 20-35% of energy, emphasizing consumption of n-3 polyunsaturated fatty acids (PUFA) while limiting the intake of saturated and trans fatty acids[123]. It has been reported that the fat intake of an average adult is 33% of energy intake[124]. However, it is important to note that adults

may consume more total kcal than recommended so fat intake would still be higher than recommended.

2.4.1.2 Studies using HFD

The consequences of consuming a HFD have been documented in human and animal subjects. The HFD pattern has been positively related to BMI and has been linked to increased risk for type 2 diabetes, coronary heart disease and colon cancer[122]. The intake of a HFD not only increases risk for these diseases, but also reduces functions of the immune system[121]. Additionally, high fat diet consumption may induce inflammatory pathways in individuals[121].

The detrimental effects of HFD consumption have been studied in animals extensively. For example, mice fed a HFD (45% energy from fat) for 12 weeks developed obesity, hyperinsulinemia, hyperglycemia, and hyperleptinemia[125]. High fat feeding alters mechanisms for digestion and absorption. Mice fed a HFD demonstrated significant differences in transcript levels of pancreatic enzymes compared to controls[125]. Sprague Dawley rats placed on a HFD for 10 weeks demonstrated greater oxidative stress (measured by serum levels of urinary-8-epi-prostaglndin-F2α and glutathione peroxidase) than controls fed a normal chow diet[126].

Preventing the intake or reducing the absorption of dietary fat has been shown to be beneficial to control the development of metabolic diseases. Reducing fat absorption may alter the outcomes of HFD feeding. Patients with obesity receiving120 mg three times/day of Orlistat(a drug that acts as a lipase inhibitor, preventing the digestion and then, absorption of dietary fat) for one year had significant reduction of body weight in

association with decrease in levels of vitamin E (VE) and β-carotene, but not VA and vitamin D (VD), compared with those receiving placebo, indicating the essential role of fat absorption in the maintenance fat-soluble vitamin homeostasis[127]. On the other hand, a 12-week treatment of Orlistat (120 mg three times daily) significantly reduced the plasma levels of VA and VE in addition to the reductions of body weight and fat mass in patients with obesity[128].

2.4.2 Lipophilic vitamins in HFD conditions

Based on their solubility in aqueous solution, vitamins are classified as either hydrophilic or lipophilic. Fat soluble or lipophilic vitamins are VA, VD, VE, and vitamin K (VK). They can be stored in the body. Historically, those lipophilic vitamins were identified due to the symptoms caused by their deficiencies in animal and human studies such as growth cessation in animals for VA and rickets for VD.

Upon intake, lipophilic vitamins are digested, absorbed and embedded with dietary fat, and transported with chylomicrons through circulation. The major component of dietary fat, triacylglycerol in chylomicrons is delivered and stored into peripheral tissues due to the action of lipoprotein lipase in the body. It seems to be possible that the amount of dietary fats and their compositions may affect functions, availability and metabolism of lipophilic vitamins, which in turn may affect body health. Here, we try to summarize the current understanding and progress of human and animal research work regarding the effects of HFD intake on functions and availability of lipophilic vitamins.

VD is incorporated into micelles and enters enterocytes through passive diffusion. After absorption, VD is then packaged in chylomicrons in enterocytes and delivered throughout the body through lymph circulation. VD synthesized in the skin is

transported through the body by VD-binding protein (DBP). Once VD reaches the liver, either through chylomicron remnants removal or DBP, it is hydroxylated to 25-Hydroxycholcalciferol (25-OH-D_3)[10,129].

Within the small intestine, dietary VE is incorporated into micelles. The pancreas secretes esterases that act on VE esters to yield free VE which then enters enterocytes through passive diffusion. In enterocytes, VE is packaged in chylomicrons and delivered to the rest of the body through lymph circulation[130].

In intestine, dietary VK is incorporated into mixed micelles comprising dietary lipids, bile salts, and products of pancreatic lipases.[131] Dietary VK is then absorbed into enterocytes through active transport. VK is then incorporated into chylomicrons which will enter lymph circulation for delivery to other parts of the body[131]. Chylomicrons travel through circulation, are embedded into peripheral tissue and deposit cargo due to the action of lipoprotein lipase. Chylomicron remnants are eventually taken by the liver where VK is incorporated into VLDL which re-enters circulation and can be up taken by osteoblasts.[131]

Dietary intervention in humans can cause the change of plasma levels of lipophilic vitamins. In participants of a randomized, double-blind, placebo-controlled clinical trial, daily intake of 8.8 grams (g) of plant stanol esters for 10 weeks did not change serum levels of VA, VD and γ-tocopherol, but reduced the levels of total and LDL cholesterol, carotenes and α-tocopherol[132]. A 2.6 g/day dose also significantly reduce total cholesterol (LDL), total TG, and carotenes levels, but not VA and VE[133]. In a moderately hypercholesterolemic population, sitostanol ester (3 g/day) for a year

significantly reduced the plasma levels of α-tocopherol and carotenes in association with the reduction of cholesterol, but not VA and VD levels[134]. On the other hand, short term (2-7 days) intake of HFD in human, did not affect the plasma levels of cholesterol, TG, carotenes and VE[135].

The effects of VA in HFD feeding have been explored in animal models. Supplementation of additional VA in HFD caused further increase of plasma TG levels and expression levels of genes for adipocyte differentiation in rats already fed a HFD[136]. On the other hand, in male mice, a supplementation of VA (20IU /g of diet) in a HFD increased plasma levels of IL-18 and macrophage inflammatory protein-1 (MIP-1γ), which occurred in a RALDH1 (*Aladh1* for gene)-dependent manner[137].

Adverse effects of excessive intake of VA on human and animals have been nicely summarized[138]. Male Sprague-Dawley rats fed on a high-fat diet supplemented with large doses of chitosan have reduction of liver VA and VE, but not VK, levels, demonstrating the effects of dietary components on the fat soluble vitamin statuses in the body[139]. It has been shown that dietary fat and fiber contents affect the VA storage in the liver and conversion of β-carotene into retinol in Mongolian Gerbils[140]. VA supplementation down-regulates leptin mRNA in adipose tissue in mice and RA stimulates UCP3 mRNA in muscle[141].

For VD to become activated, parathyroid hormone stimulates the kidneys to release the enzyme 1α-hydroxylase, which converts 25-OH-D_3 to 1α,25-Dihydroxycholecalciferol (1α,25-$(OH)_2D_3$)[142]. DBP binds to 1α,25-$(OH)_2D_3$ so that it is carried to target tissues throughout the body where it can be a ligand for VD receptor

(VDR). 1α,25-$(OH)_2D_3$ enters cells and binds to VDR causing VDR to form a heterodimer with RXR which acts as a transcription factor to regulate gene expression[10].

There is an association of reduced serum 25-hydroxyvitamin D (25D) concentrations with the development of human obesity[143]. However, the effect of VD supplementation on body weight reduction seemed to be uncertain[143]. In children (6-10 year-old), a slight lower value of serum 25D (drop from 26 ng/ml of nonobese to 23 ng/ml of obese) was also associated with obesity[144]. A recent randomized controlled study using seven doses of VD (400–4800 IU/d) in both lean and obese female subjects showed that the rise of blood VD levels is inversely related to the body fat mass, and the normalization of blood VD levels is not associated with reduction of body weight in obese subjects[145]. At this time there appears to be no published research investigating the effect of a HFD and VD intake and its plasma level in participants.

Both male and female VD receptor knockout mice have a lean phenotype and demonstrate resistance to HFD-induced obesity regardless whether they are in the C57BL/6J or CD1 outbred background[146-148]. On the other hand, transgenic expression of human VDR driven by aP2 promoter in adipose tissue resulted in gains of BM and body fat mass in mice[149]. This phenotype is associated with the elevation of only plasma cholesterol level and changes of expression levels of genes for fuel metabolism in fat tissues and the skeletal muscle[149].

When SD rats were fed a diet containing low fat (LFD, 10% energy from fat) or HFD (45% energy from fat) with normal VD or depleted VD (VDD), rats in HFD-VDD

group had higher nonalcoholic fatty liver disease (NAFLD) activity Score (NAS) than rats in HFD-VD group. On the other hand, rats in LFD groups are protected to the development of NAFLD[150]. The elevation of NAS in rats of HFD-VDD group is associated with change of the expression of genes for hepatic inflammatory and oxidative stress, suggesting the protective role of VD to the development of NAFLD[150]. However, feeding HFD (40% energy from fat) in rats for 12 weeks per se did not change serum 1,25-dihydroxyvitamin D(3) level, whereas supplementation of lactose (10% of the diet weight) significantly reduce VD level and HFD-induced BM gain[151].

VE is a collective term for tocopherols and tocotrienols. These molecules differ in their side chains; tocopherols have a phytol side chain while tocotrienols have an unsaturated side chain. Only four tocopherols and four tocotrienols meet human VE requirements[10], but in the human body α-tocopherol is the most effective[152].

VE is of significant interest because it is a lipid soluble antioxidant. It serves as a radical scavenger that protects polyunsaturated fatty acids in membranes and lipoproteins against lipid oxidation. Alpha tocopherol scavenges a radical through donating a hydrogen with the resulting alpha tocopherol radical reacting with ascorbate to return to its reduced state.[152]

It has been observed that overweight subjects (BMI > 27) taking an antioxidant supplement (1g vitamin C/800 IU VE) with a high-fat low-carbohydrate diet (63.6 ± 1.6% calories as fat) for 8 days had a trend of lower C-reactive protein (30% reduction compared to baseline). One the other hand, subjects in the placebo group had a trend for higher levels of CRP (50% increase compared to baseline[153].

Several studies have been done in animals to determine the effects of VE in HFD condition. Feeding of VE did not affect the plasma lipid levels in lean or obese mice fed a HFD[154]. The HFD-induced activation of JNK in rat skeletal muscle was attenuated by including antioxidants including vitamin C and VE in Wistar rats[155]. Male C57BL/6J mice fed a HFD (70% energy from fat) with elevated VE content had elevation of α-tocopherol in the liver and adipose tissues, which might be protective against lipid peroxidation[156]. Supplementation of VE (α-tocopherol) or VD (D3) in HFD significantly reduced the plasma level of IL-6, but not IL-10, in HFD-fed male Swiss mice[157]. Tocotrienol supplementation also reduced damages of feeding high-carbohydrate and high-fat diet to the heart and liver in Wistar rats[158]. A 6-week VE (α-tocopherol) treatment at a dose of 100 mg/kg daily via oral gavages significantly reduced the memory impairment induced by High-fat and High-carbohydrate diet in rats, probably through reduction of oxidative stress in the hippocampus[159]. Specific increase of VE content in mitochondria using a mitochondria-targeted VE derivative, MitoVit E (conjugated with triphenylphosphonium cations) has been shown to reduce hepatic oxidative stress and inhibit fat deposition in mice[160].

When mice were fed a HFD supplemented with α-tocopherol (VE, 0.9 g/kg of diet) and 1,25(OH)2 vitamin D3 (0.05 mg/Kg of diet) for 8 weeks, they had lower plasma levels of IL-6, indicating reduction of inflammatory response[157]. It was suggested that these two vitamins inhibit IL-6 production from adipocytes[157]. HFD induced obese SD rats supplemented with 350mg/kg diet of DL-α-tocopherol acetate exhibited significantly less BM and fat weight[126].

VK is essential in blood clotting and bone formation. Quinone oxidoreductases reduce VK to vitamin K hydroquinone. Vitamin K hydroquinone serves as a cofactor for vitamin K gamma-carboxylase, which catalyzes the carboxylation of certain glutamic acid residues, resulting in their activation in blood clotting and bone formation. A reduced VK molecule is converted to vitamin K epoxide and then converted back to VK by vitamin K epoxide reductase[161]. This reduction and reoxidation of VK coupled with glutamic acid carboxylation is referred to as the VK cycle[10]. Because VK is recycled in the body, human deficiency of VK is rare[161].

Rats fed a HFD diet (45% more energy from fat) rich in corn oil had lower plasma VK level than those fed a low fat diet even more VK was present in the HFD, indicating the HFD feeding on the plasma level of fat-soluble vitamins[162]. Additionally, the liver VK levels was reduced in rats fed a low-fat diet with fish oil, but not that fed a HFD (already lowered) with fish oil[163].

2.5 Investigating weight loss in participants who are overweight and obese

2.5.1 Current recommendations for obesity treatment

For the treatment of obesity in adults, comprehensive lifestyle interventions have been recommended[164]. This consists of diet and physical activity goals, as well as behavioral therapy. Dietary goals to achieve weight loss for adults who are overweight and obese should be individualized and include a 500 to 750 Calories/day deficit[164]. Physical activity goals aim to gradually increase the amount of physical activity to at least 30 minutes of moderate to vigorous intensity physical activity on as many days as possible. Behavior therapy assists with behavior modification strategies, which are

implemented to change participants' dietary intake and physical activity. Recommended behavior modification strategies to assist in changing diet and physical activity include stress management, self-monitoring, problem solving, stimulus control, cognitive restructuring, time management and social support[164].

2.6.2 Weight loss interventions in adults

Typically weight loss interventions in adults include dietary changes, physical activity goals, and behavior modification. A low-Calorie diet is currently recommended to aid in weight loss[164]. The Dietary Guidelines, provided by the USDA, give standard recommendations for dietary changes. For adults, it is recommended that dietary intake consists of 45-65% carbohydrates, 10-35% protein, and 20-35% fat[123]. Many investigations on weight loss will limit participant dietary fat consumption to 30% or less of calories from fat[165-167]. Current weight loss interventions in adults do not included prescriptions for VA intake so it is not well understood how VA intake may be related to weight loss.

2.6 Conclusion

Obesity has reached epidemic levels with one third of this country's population considered to be obese[1]. This condition increases the risk of development of several metabolic diseases[2], resulting in higher mortality rates and costs in health care[3]. This problem is multifactorial, with energy imbalance, genetics and environmental factors playing a role[4-5].

Previous research indicates that VA may also play a role in the development of obesity[6]. It is clear that VA plays a role in glucose and lipid metabolism in both animals

and humans[59-65, 92-116]. While VA intake has been shown to impact obesity development, research investigating VA and obesity development in adults is limited, with most of the research looking at the effects of excessive VA intake[105, 117-118].

One of the contributions to the obesity epidemic in this country is the Western dietary pattern, characterized by a HFD. Consuming a HFD has been linked to increases in BM and risk of metabolic diseases[121-125]. The current research on lipophilic vitamin in HFD has focused mostly on their protective roles in HFD feeding conditions[129-153].

Current recommendations for treatment of obesity in adults include dietary changes, physical activity goals and behavior therapy[164]. A low-Calorie diet is currently recommended to aid in weight loss, with many weight loss interventions limiting fat intake to 30% or less of calories consumed[164-167]. Currently, weight loss investigations in adults do not involve interventions for VA intake. It is not clear how VA intake may be related to weight loss in adults.

2.6.1 Specific aims

This thesis includes two parts. The project seeks to answer the following questions: Does the presence of VA in the diet have an effect on weight gain while a high-fat, obesogenic diet is consumed? Is the presence of VA in the diet related to successful weight loss when a hypocaloric, low-fat diet is consumed? This thesis project incorporated two different models to investigate the impact of VA in obesity. The first is an animal model, where animals were fed a high fat diet with the goal of weight gain during dietary treatment. The second model looked at adults, where participants' fat and energy intake

were restricted with the goal of achieving weight loss. The two experiments examined the following aims:

1. Investigate the contributions of VA intake on obesity development in rats fed a high-fat diet (HFD).
2. Investigate the effects of VA intake on body weight alterations in participants during a hypocaloric, low-fat dietary intervention.

For this thesis project we hypothesized that VA intake will have an effect on obesity development when an obesogenic HFD is consumed. We also hypothesized that achievement of 10% weight loss would be related to a lower dietary VA intake when weight loss was due to consumption of a hypocaloric, low-fat diet.

It is important to note the use of different models for the experiments in this thesis. Because one model investigated weight gain and the other investigated weight loss, two different mechanisms (weight loss and weight gain), as well as two different diets, high-fat and low-fat, were examined.

Chapter III

Effects of Vitamin A in the Body Mass Gain in Sprague-Dawley Rats

3.1 Introduction

Obesity has become epidemic in this country with one third of Americans considered to be obese[1]. This epidemic predicts the increased incidence of various metabolic diseases[2], resulting in higher mortality rates and higher costs in health care[3]. The increases in mortality rates and costs of health care have become an urgent problem, so finding solutions must become priority.

One of the dietary styles contributing to the obesity problem is the western diet (High-fat content), which is characterized by a high intake of red and processed meats, eggs, refined grains and sugars, and energy derived from fat, mainly saturated fatty acids, which can be as high as 35% of energy intake[121,122]. The Dietary Guidelines has recommended that dietary fat provide 20-35% of energy[124]. It has been reported that the fat intake of an average adult is 33% of energy intake[124], but it is important to recognize that adults may consume more total kcal than recommended so fat intake would still be higher than recommended.

Lipophilic vitamins share the same routes of digestion and absorption as lipids. Previous research has established the impact of lipid digestion and absorption on lipophilic vitamins. Obese patients receiving120 mg of Orlistat three times/day (a drug that acts as a lipase inhibitor, preventing the digestion and then, absorption of dietary fat) for one year had significant reduction of body mass (BM) in association with decrease in levels of vitamin E (VE) and β-carotene, but not vitamin A (VA) and VD (VD), compared with those receiving placebo, indicating the essential role of fat absorption in the maintenance fat-soluble vitamin homeostasis[127]. Additionally, a 12-week treatment of Orlistat (120 mg three times daily) was shown to significantly reduce

the plasma levels of VA and VE in addition to the reductions of body weight and fat mass in patients with obesity[128]. Because VA shares the same routes of digestion and absorption as lipids, we hypothesize that VA intake will have an effect on obesity development in subjects consuming an obesogenic high-fat diet (HFD).

Here, we compared the growth current and plasma parameters of rats fed a HFD with sufficient amount of VA (HF-VAS) and a HFD without VA (HF-VAD) diet for eight weeks.

3.2 Materials and methods

3.2.1 Animals and diets

Male Sprague Dawley (SD) rats were bred at the University of Tennessee at Knoxville (a breeding colony since 2012). Our previous investigations of VA in energy metabolism have used Zucker fatty rats as a model for obesity[6,91] Zucker fatty rats develop obesity because they express a missense mutation in the extracellular domain of all leptin receptor isoforms[168]. For this investigation SD rats were used so that the findings can be applied to other strains of rats (protocol# 1582).

Animals were kept in the Animal Facility for the Nutrition Department at the University of Tennessee. Facility temperatures are maintained at 70-73 ºF, the humidity ranges from 45-65% and has a light and dark cycle in 12 hour increments (with the lights on from 6:00 a.m.-6:00 p.m.) Two to three rats were placed in a single cage and fed ad libitum. Cages were cleaned twice a week and given a fresh enrichment (either a piece of PVC pipe or a paper cone). They were housed in colony cages and fed either a high fat diet that is HF-VAD (0 IU/g VA) or HF-VAS (22.1 IU/g VA) after weaning (three weeks

of age) for a total of eight weeks. This period of eight weeks will be sufficient to induce VA deficiency.[8] The HF-VAS diet had 60% energy from fat. Average fat intake is close to 30% total calories from fat, so the fat content in the experiment is double the average fat intake. The HF-VAD diet was identical to the HF-VAS differing only in VA content. Diets for this experiment will be ordered from Harlan.

BM and food intake (in grams) were recorded weekly[6]. BM was measured for each rat to the nearest 0.01 gram with a calibrated scale. Food intake was measured by weighing the amount of feed given at the start for the week then subtracting the amount remaining at the end of the week and dividing the total by the number of rats that consumed the diet to get each rat's weekly intake. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Tennessee at Knoxville (865-946-2574).

3.2.2 Collection of plasma and tissue samples

At the end of eight weeks of dietary treatment, plasma glucose was measured with an assay kit (Procedure No. 1070) from STANBIO Laboratory (Boerne, Texas). Rats were euthanized with CO_2 and spinal cord dislocation in accordance with regulations. Hepatic blood was collected and centrifuged at 3,000 rpm for 30 minutes to collect plasma. Liver, white adipose, brown adipose were collected, weighed, snapped frozen in liquid nitrogen and stored at -80˚C.

Before euthanization, glucose level of the tail tip whole blood was measured using a glucose meter (Roche; Tucson, AZ). Tail blood (less than 10 ul) was collected with 25 G needle. Once rats were euthanized, hepatic blood was collected from the

vena cava, then the liver and white adipose were collected. Brown adipose was collected from the back of the neck. Tissues were weighed on a calibrated scale to the nearest 0.01 gram, wrapped in aluminum foil and frozen with liquid nitrogen. Plasma and tissue samples were then stored at -80 º C before used.

Measuring glucose from hepatic blood was accomplished with the technique described Trinder et al[169]. With this method, glucose is oxidized in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under influence of peroxidase with phenol and 4-aminoantipyrine to form a red-violet quinine complex. The intensity of the color is proportional to glucose concentration. Absorbance at 500 nm wavelength is then measured with a spectrometer from Glomax (Madison, WI). The glucose reagent contains phosphate buffer (200 mmol/L), phenol (4 mmol/L), 4-Aminoantipyrine (0.2 mmol/L), glucose oxidase (>15 KU/L) and peroxidase (>1.2 KU/L).

For glucose measurement, glucose reagent stored at 2-8 º C, was removed from 4°C refrigerator and allowed to warm at room temperature for 30 minutes. Plasma samples were removed from storage and placed on ice to thaw. For each standard, sample and control, 1.0 mL reagent was added to cuvettes and warmed to 37ºC for 5 minutes. 10µL of each sample was added to its respective cuvette, mixed gently and returned to 37ºC incubation. After five minutes of incubation, absorbance of samples was measured. Glucose for each sample was calculated as

$$\text{Glucose}\left(\frac{\text{mg}}{\text{dL}}\right) = \frac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} * 100 \text{Glucose}\left(\frac{\text{mg}}{\text{dL}}\right) = \frac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} * 100$$

3.2.3 Statistics

Data was analyzed using independent-samples *t* test, as seen in previous research[6,40,149]. Student *t* test was conducted to compare means of food intake, liver mass, white adipose mass, brown adipose mass, and blood glucose. Differences were considered statistically significant at $p < 0.05$.

For comparing BM over time, a Bonferroni correction was used to calculate the p-value to determine if differences were considered statistically significant. BM for the groups was measured at baseline and then once a week for the eight week dietary treatment. The Bonferroni correction was calculated as 0.05 divided by 9 (total time points BM was measured). Differences in BM over time were considered statistically significant at $p < 0.0056$.

3.3 Results

Rate of BM gain was significantly less in rats fed HF-VAD starting at 7 weeks

To determine the effects of VA status on the gain of BM in SD rats, they were fed a HF- VAS or a HF-VAD diet for 8 weeks after weaning. Animals were weighed once a week for eight weeks. There was no significant difference in starting BM between HF-VAS and HF-VAD groups. As shown in Figure 1, rats fed a HF-VAD diet grew at a similar rate as those fed a HF-VAS diet did for the first 6 weeks. Starting at week seven, there was an observed significant difference in BM gain between the HF-VAS and HF-VAD groups, with the HF-VAD group weighing less. This trend continued for the remainder of the diet treatment, with increasing significance. This trend can be seen in Figure 1. The BM of both groups continued to rise throughout the 8 week study

period. Of note, without the Bonferroni correction, significant difference in BM was detected at week 6 of dietary treatment.

Figure 2 shows the weekly food intake of the rats fed the perspective diet. Of note, the total food intake for both groups was not significantly different. These results show that SD rats fed a HF-VAD were able to gain BM for 8 weeks. It was not until week seven that the rate of BM gain began to differ significantly between HF-VAD and HF-VAS. This suggests that the VA storage of SD rats at weaning is sufficient to support the BM gain for 6 weeks without any significant reduction in the rate of BM gain. These results demonstrate that the deficiency of dietary VA slowed down the rapid BM gain of SD rats fed HFD.

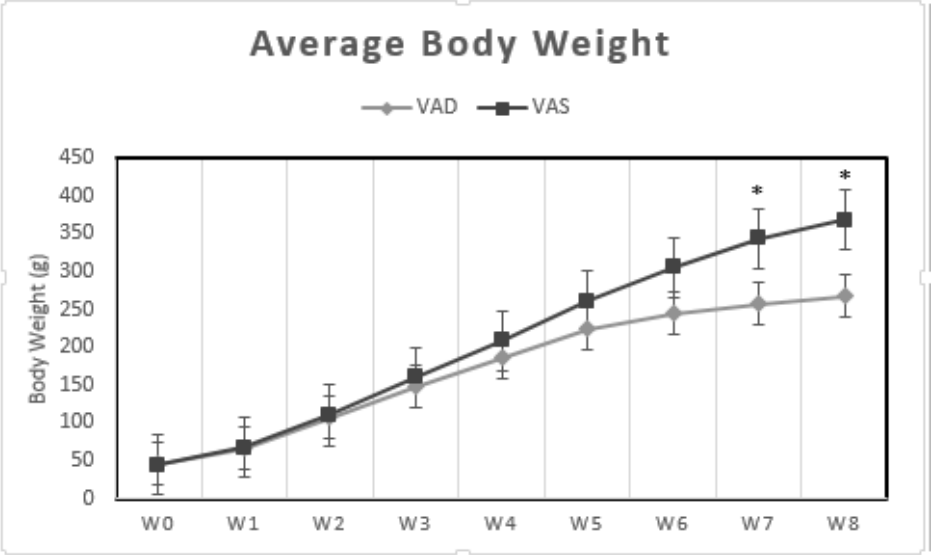

Figure 1 Comparison of Body Mass gain over 8 weeks of Sprague-Dawley rats receiving a high-fat diet and vitamin A sufficient diet (HF-VAS) and Sprague Dawley rats receiving a high-fat and vitamin A deficient diet (HF-VAD)(* for comparing the two dietary groups, all $p < 0.0056$). VAD- Vitamin A Deficient; VAS- Vitamin A Sufficient

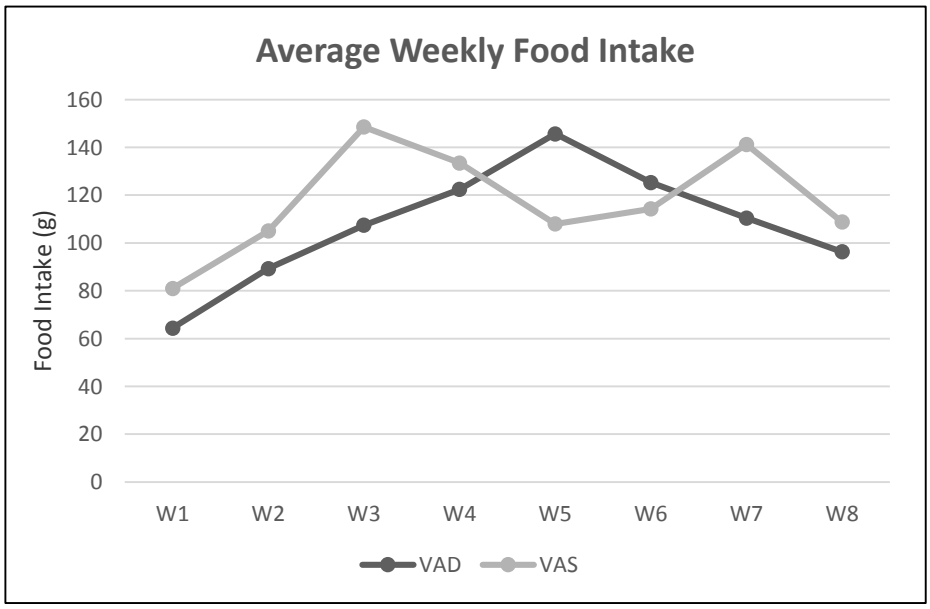

Figure 2 Total amount of diet consumed over 8 weeks of Sprague Dawley rats fed a high-fat and vitamin A sufficient diet (HF-VAS) and Sprague Dawley rats fed a high-fat and vitamin A deficient diet (HF-VAD)

VA deficiency resulted in morphological and metabolic changes in SD rats

The effects of VA deficiency on SD rat metabolism were analyzed after they had been fed a HF-VAD or a HF-VAS for 8 weeks. As seen in Table 1, the initial BM of the SD rats (3 weeks after birth as weaning) fed a HF-VAD diet was similar to the HF-VAS groups. After 8 weeks of diet treatment, HF-VAD group had significantly lower BM (267.5 ± 32.7 g vs. 386.4 ± 37.7 g). This demonstrates the reduced somatic growth and VA deficiency as shown previously[8]. At the end of the eight weeks, liver, white adipose and brown adipose were collected and weighed. There was an observed significant difference between the groups for liver and white adipose weight (all $p<0.05$), with the HF-VAD group having less mass for liver and white adipose. While there was not a significant difference for brown adipose mass ($p = 0.070$). All of these results demonstrate the impact of VA deficiency in SD rats after 8 weeks on the HF-VAD diet.

Table 3 Comparison of measures for Sprague Dawley rats receiving a high-fat vitamin A sufficient (HF-VAS) diet and Sprague Dawley rats receiving high-fat vitamin A deficient (HF-VAD) diet

Diet	**HF-VAD(mean±SD) (n)**	**HF-VAS(mean±SD)(n)**
Start day BM (g)	45.5 ± 6.5 (9)	44.7 ± 3.43 (6)
End day BM (g)	267.5 ± 32.7 (9)	386.4 ± 37.7 (6)*
Liver mass (g)	9.01 ± 1.42 (9)	14.88 ± 1.91 (5)*
Liver/BM ratio	1:29.7 (9)	1:25.9 (5)
White adipose mass (g)	2.16 ± 0.91 (7)	3.8 ± 1.25 (6)*
White adipose/BM ratio	1:123.87 (7)	1:101.68 (6)
Brown adipose mass (g)	0.31 ± 0.099 (7)	0.45 ± 0.13 (6)
Plasma glucose (mg/dl)	479.8 ± 179.2 (5)	434.5 ± 41.9 (4)
Plasma cholesterol (mg/dl)	530.6 ± 12.3 (5)	537.5 ± 17.5 (4)
Plasma triglyceride (mg/dl)	60.4 ± 30.8 (5)	70.0 ± 16.3 (4)

Note: BM-body mass, HF-VAD-high fat vitamin A deficient, HF-VAS- high fat vitamin A sufficient

3.4. Discussion

The growth curves of SD rats fed a HF-VAD or a HF-VAS diet demonstrate that VA content in the diet significantly impacted obesity development (BM gain) while consuming an obesogenic diet. Lack of VA in the diet began to reduce the rate of BM gain starting at week 7 for rats consuming the HF-VAD diet. The rats fed a HF-VAD diet still gained BM at a slower rate for the remaining week of dietary treatment. This difference could not be explained by any change in total energy intake because both HF-VAD and HF-VAS groups consumed similar total calories for the 8 week dietary treatment. Our previous experiments have shown that Zucker Lean and Zucker Fatty rats consuming a normal chow diet deficient in VA would reach a peak BM and then would start to lose BM with continued dietary treatment[6] in contrast to the current experiment where the VAD group still gained BM throughout the 8 weeks of dietary treatment. A possible explanation for this difference is the high caloric content of the HF-VAD. Another reason for the difference could be that VA storage in SD rats differs from VA storage in Zucker Lean and Zucker Fatty rats.

The net white fat mass was significantly lower in HF-VAD subjects than HF-VAS subjects. It has been demonstrated that rats fed a VAD diet had a loss of carcass fat, along with a reduction of BM[170]. These findings indicate that VA status plays a role in adiposity.

The net liver mass was significantly lower in HF-VAD subjects than HF-VAS subjects. On the other hand, there was no significant difference found in liver/BM ratio between SD rats fed a HF-VAD diet and SD rats fed a HF-VAS diet. A possible

contribution to the lower liver mass observed in the HF-VAD rats compared to the HF-VAS rats is depleted hepatic glycogen content. It has been observed that rats fed a VA-deficient diet had depleted hepatic glycogen content[60]. In that study it was determined that the depletion of hepatic glycogen was caused by the reduction of glycogenesis from trioses, rather than directly from glucose[60]. It was shown that the depletion in VAD rats was not due to reduced energy intake, as pairfed rats with equal energy intake had higher hepatic glycogen content[60]. This is comparable to the findings in the current study, as shown in Figure 2, SD rats fed a HF-VAD diet had equal total energy intake for the 8 week diet treatment as the SD rats receiving a HF-VAS diet.

No significant difference was detected in the plasma glucose levels between the HF-VAD and HF-VAS groups. Previous research from our lab showed a significant difference in the plasma glucose of Zucker Fatty rats fed a normal chow VAD compared to their VAS counterparts[6]. Differences in results could possibly be attributed to the use of a different strain of rat. SD rats may have greater storage of VA at weaning than Zucker Fatty rats and therefore may not have the same impact from a VAD diet on glucose metabolism. Alternatively, this difference may be caused by the presence of high fat content in the diets, which probably provide enough fatty acid for the muscle to use. This may spare glucose and prevent the drop of glucose in HF-VAD rats.

Additionally, no significant difference was found in the plasma cholesterol and plasma triglycerides between the HF-VAD and HF-VAS groups. Previous research from our lab showed a significant difference in the plasma triglycerides of Zucker Fatty rats fed a normal chow VAD compared to their VAS counterparts[6]. An explanation for this difference could be the high fat content of the diet treatment used in this experiment.

3.5 Conclusion

The current research on lipophilic vitamins in HFD has focused on their protective roles in HFD feeding conditions. How HFD affects homeostasis of lipophilic vitamins remains to be an open question. Our current findings support the assertion that VA status plays a key role in adiposity development in HFD conditions

Chapter IV

Comparison of Dietary Vitamin A Consumption in Participants with 10% Body Weight Loss and Those without 10% Weight Loss in a Randomized Clinical Trial

4.1 Introduction

Obesity continues to be a serious national problem with one third of the population considered to be obese[1]. This condition increases the risk of development of various metabolic diseases[2] such as diabetes and hyperlipidemia, resulting in higher mortality rates and higher costs in health care[3]. Additionally, obesity has been linked to decreased productivity in the workplace, which can indirectly increase healthcare costs[3]. The increases in mortality rates and costs of health care have become an urgent problem, which finding solutions must become priority.

The problem of obesity is thought to be caused by positive energy balance, meaning that energy intake is greater than energy output[4]. While over nutrition seems to be an obvious cause of obesity development, the role of individual micronutrients has not been revealed. Previous research indicates that vitamin A (VA) may play a role in the development of obesity[6]. It has be demonstrated that patients with promyelocytic leukemia treated with all-*trans* retinoic acid (RA) experienced increases in body mass (BM) and plasma cholesterol[105]. While this demonstrates the effects of excess VA on BM gain in humans, it is currently not understood how dietary VA plays a role in weight loss, and its role when a hypocaloric, low-fat diet is consumed.

This project aims to investigate the effects of VA intake on BM alterations in participants during dietary interventions. This will be achieved by comparing dietary VA intake between participants who successfully lost 10% weight and those who did not at 6 months in a lifestyle intervention. Based on previous research, we hypothesized that participants who achieved 10% weight loss will have consumed significantly less dietary

VA than those who did not achieve 10% weight loss while consuming a hypocaloric, low-fat diet.

4.2 Methods

4.2.1 Study design

A secondary data analysis as conducted using data collected from a previous published lifestyle intervention trial which examined a reduced variety dietary prescription[165]. In the original study, 202 participants who were overweight and obese were randomly assigned 1 of 2 conditions: Limited Variety (LV) or Lifestyle. Both of the treatment conditions were given a standard low-calorie, low-fat diet prescription (1200 kcal/day for participants weighing less than 200 lbs at baseline and 1500 kcal/day for participants weighing greater than 200 lbs at baseline, with fat being restricted to 30% of energy intake), a physical activity prescription consisting of 200 minutes of moderate-intensity physical activity per week and walking 10,000 steps per day, and a cognitive behavioral intervention to aide in dietary and physical activity behavior changes[165]. The LV was also given a limited variety prescription, designed to decrease the number of non-nutrient-dense, energy-dense foods (NND-EDF) (for example ice cream, cookies, chips) consumed to only 2 selected by the participants[165]. The NND-EDFs also included modified versions such as reduced sugar and sugar free ice cream. Participants in the Lifestyle group did not receive the limited variety prescription.

Participants participated in the intervention for 18 months, which provided 48, 60 minute group sessions. These sessions, modeled after lessons used in the Diabetes Prevention Program, covered lessons on behavioral and cognitive skills to aide with

dietary and physical activity behavior changes. During the first 6 months, which was considered the weight loss phase, participants had weekly sessions. Months 7-18 were considered the maintenance phase so the participants had two sessions a month[165]. Measures were taken at baseline, 6, 12, and 18 months.

At the end of the study it was found that the LV group consumed less variety of NND-EDFs and less overall energy intake daily from these foods than the Lifestyle group at 6, 12, and 18 months[165]. While the LV group consumed less total energy than the Lifestyle group at 6 months, there were no significant differences between the groups in energy intake at 12 and 18 months[165]. Weight loss did not differ significantly between groups and at 18 months both groups lost approximately 9% of body weight[165].

The current study was a secondary data analysis. The time point examined was month 6. For analysis, the participants were divided into two groups, successful weight loss at 6 months (loss of 10% or more of body weight), and unsuccessful weight loss at 6 months (loss of less than 10% of body weight). Weight loss of 5-10% in overweight and obese subjects has been shown to improve risk factors for diseases related obesity[171]. The dependent variables that were investigated are VA intake. Variables for VA included Total Vitamin A Activity International Units, Beta Carotene provitamin A carotenoid, Total Vitamin A Activity Retinol Equivalents, Beta Crytoxanthin provitamin A Carotenoid, Alpha Carotene provitamin A carotenoid.

4.2.2 Participants

In the initial study, participants were recruited from Providence, RI and Knoxville, TN. Eligibility criteria included aged 21-65 years, a BMI between 27 and 45 kg/m^2, and

the ability to walk 2 blocks[165]. Participants were excluded if they reported a heart condition, chest pain, or loss of consciousness; were taking weight loss medications or participating in another weight loss intervention; had undergone bariatric surgery; were pregnant or lactating, or less than 6 months post-partum or planning to become pregnant during the study; were allergic to foods being used in the taste-test measures conducted during the study; or were consuming less than 5 different types of NND-EDFs[165]. For the current secondary data analysis, only participants with complete dietary data and weight measurements at baseline and 6 months were included in the analyses.

4.2.3 Measures

Measures were collected by trained research staff blinded to the randomization assignment at baseline and 6 months in a research setting. Demographic measures, such age and race, were collected through a self-report survey at baseline[165]. Of note, all of the measures collected for participants were not collected at the same time of the year. The study collected measures from 6 different cohorts over time.

Anthropometrics

Participant height was measured at baseline and weight measures were taken at baseline and 6 months to calculated BMI. Height measurements were recorded to the nearest millimeter at baseline using a stadiometer[165]. Weight was measured with a calibrated scaled, while wearing light street clothes, and recorded to the nearest 0.05 kg[165]. For this study, percentage weight loss was used to assess weight loss. Percentage weight loss was calculated as:

$$Weight\ loss\ \% = \frac{Weigh\ (6\ months) - Weight(baseline)}{Weight\ (baseline)} * 100\%$$

Dietary intake

Looking at participant dietary intake, three random 24-hour dietary recalls were collected by phone during a 1 week period at 0 and 6 months. Recalls were conducted by interviewers from the Cincinnati Center for Nutritional Research and Analysis trained and blinded to the intervention and interventions assignment[165].

The Nutrition Data System Software for Research developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN was used to collect and analyze the 3-day food records. The multiple-pass method was used to collect the data. There are 5 phases for this method of recall of dietary data. In the first phase, the participant tells the reviewer all of foods and beverages that were consumed. The second phase occurs when the reviewer reviews the list with the participant. In the third phase, the interviewer receives detailed information of the foods and beverages consumed. In the fourth phase, participants were given 2-dimensional models of food portions sizes to assist with accurate reporting. In the fifth phase the reviewer reviews the entered information with the participants to ensure completeness.

Variables for dietary intake were calculated as means of the three days. Variables include VA as Total Vitamin A Activity International Units, Beta Carotene provitamin A carotenoid, Total Vitamin A Activity Retinol Equivalents, Beta Crytoxanthin provitamin A Carotenoid, Alpha Carotene provitamin A carotenoid.

4.2.4 Statistics

Independent t-tests and chi-squared tests were conducted to compare demographics and anthropometrics measures between participants included in the secondary data analyses and those not included. A significant difference was found between those included and those not included in the analyses for education, which was entered as a covariate in the remaining analyses.

For participants included in this secondary analysis, independent t-tests and chi-squared tests were used to compare demographics and baseline anthropometrics, and baseline dietary intake of selected VA variables between those who had successful weight loss at 6 months and those who did not have successful weight loss at 6 months. No significant differences were found between the two groups for baseline measures.

To determine if there was a significant difference in the amount of dietary VA consumed at 6 months between participants with successful weight loss and participants who did not have successful weight loss, a mixed analysis of covariance (ANCOVA) was conducted, with weight loss % (10% weight loss at 6 months and less than 10% weight loss at 6 months) as the between-subjects variable and time (6 months) as the within-subjects variable, and education as a covariate.

All analyses were conducted using SPSS Statistics 22.0, with alpha level set at 0.05.

4.3 Results

Baseline participant characteristics

Baseline characteristics for those included in the analyses (n= 113) compared to those that were excluded from the analyses (n=89) are shown below in Table 4. No significant difference was found in weight, BMI, gender, race, ethnicity, marital status, age, and group assignment. A significant difference was found between these groups for education, with those included in the analyses having a higher percentage of having some college education or higher than those not included in the analyses (91.2 % vs. 83.1 %). Therefore, education was entered as a covariate in all subsequent analyses.

Baseline characteristics for the successful weight loss and unsuccessful weight loss groups are shown in Table 5 below. Participants were 51.9 ± 8.8 years, predominately white (94.7%), female (59.6%), with some college education (91.2%), married (75.2%) and not Hispanic (100%), and were obese (BMI = 35.0 ± 4.5). No significant differences were detected for baseline characteristics and self-reported dietary intake of VA between the groups.

Comparison of self-reported dietary intake of VA at 6 months between groups

There was no significant difference found for self-reported dietary intake of any of the VA variables at 6 months between participants with successful weight loss and participants with unsuccessful weight loss. See Table 6 below for comparison of means of dietary VA variable values for participants.

Table 4 Baseline characteristics for participants included vs. those not included in secondary analyses

Variable	Not Included (n=89)	Included (n=113)
Age (y)	51.9 ± 8.8	51.9 ± 8.8
Weight (lb)	218.9 ± 38.1	223.3 ± 40.2
BMI (kg/m^2)	34.4 ± 3.9	35.0 ± 4.5
Female (%)	55.1	59.3
White (%)	88.8	94.7
Non-Hispanic (%)	98.9	100
Some college education or higher (%)*	83.1	91.2
Married (%)	71.9	75.2
Lifestyle assignment (%)	48.3	52.2

Note: *Significant at p<0.5

Table 5 Baseline characteristics and self-reported dietary intake of vitamin A for participants with successful weight loss and participants with unsuccessful weight loss (M± SD)

Variable	Successful weight loss (n = 66)	Unsuccessful weight loss (n = 47)
Age (y)	53.3 ±8.3	50.0 ±9.2
Weight (lb)	220.6 ± 40.6	227 ± 39.8
BMI (kg/m^2)	34.7 ± 4.6	35.5 ± 4.4
Female (%)	59.1	59.6
White (%)	93.9	95.7
Non-Hispanic (%)	100	100
Some college education or higher (%)*	92.4	89.4
Married (%)	70.0	83.0
Lifestyle assignment (%)	48.5	57.4
Beta Carotene provitamin A carotenoid (mcg)	3724.0 ± 2984.2	2815.6 ± 2632.9
Total Vitamin A Activity Retinol Equivalents (mcg)	1304.6 ± 770.9	1006.9 ± 528.8
Total Vitamin A Activity Retinol Activity Equivalents (mcg)	959.0 ± 571.2	748.5 ± 362.4
Beta Crytoxanthin provitamin A carotenoid (mcg)	149.0 ± 175.5	97.8 ± 110.8
Alpha Carotene provitamin A carotenoid (mcg)	692.7 ± 737.7	472.8 ± 511.5
Total Vitamin A Activity International Units (IU)	8956.2 ± 5897.4	6802.5 ± 4692.8

Note: y-years old; lb- pounds; kg-kilograms; m- meters; mcg-microgram; IU-International Units

Table 6 Comparison of means of dietary vitamin A between participants with successful 10% weight loss and participants with less than 10% weight loss (M± SD)

Variable	**Successful weight loss (n = 66)**	**Unsuccessful weight loss (n = 47)**
Beta Carotene provitamin A carotenoid (mcg)	3625.54± 3094.07	3109.14± 3171.55
Total Vitamin A Activity Retinol Equivalents (mcg)	1022.07± 579.31	989.20± 590.79
Total Vitamin A Activity Retinol Activity Equivalents (mcg)	688.91± 341.65	701.83± 364.68
Beta Crytoxanthin provitamin A carotenoid (mcg)	124.28± 125.26	122.48± 119.17
Alpha Carotene provitamin A carotenoid (mcg)	619.82± 595.45	555.37± 556.15
Total Vitamin A Activity International Units (IU)	7848.80± 5501.16	7129.18± 5542.82

Note: mcg- microgram; IU- International Units

4.4 Discussion

The purpose of this investigation was to examine the relationship between dietary VA and weight loss in participants in a lifestyle intervention. This was accomplished by comparing the amount of VA consumed between participants who successfully lost 10% weight and those who did not while consuming a hypocaloric, low-fat diet. Since no significant difference was detected in amount of VA consumed between the groups, the results indicate that the amount of VA consumed in a weight loss intervention where participants are experiencing a negative energy balance and restricting fat did not have a relationship with weight loss.

There is limited human research investigating the effect of VA on obesity and the previous research on the relationship between VA intake and obesity in humans has mostly focused on the effects of excessive VA intake, and VA influence on weight gain, rather than loss[105,117-118]. There is a need for further investigation on how VA intake impacts weight loss.

In contrast to the first experiment for this thesis project, this experiment used a human model where the goal for the participants was weight loss rather than weight gain, as seen in the animal experiment. The diets also differed in that the participants were instructed to follow a hypocaloric, low-fat diet as opposed to the obesogenic, high-fat diet provided to the animal subjects. Dietary fat is essential for absorption of VA, and restriction of fat may have played a role in the results of this experiment. Even if participants consumed VA rich foods, if they did not also consume adequate fat, then the VA would not have been absorbed to exert its physiological effects.

One limitation of this study would be that the sample was homogenous in race and ethnicity. Another limitation would be the use of self-reported dietary data. The sample of those included in the analysis was also greatly reduced due to participants being excluded from the analysis from lack of complete dietary data. Also, it is important to note the large variation of average VA intake (based on a large standard deviation) for participants in both groups. Additionally, since the study design was a secondary data analysis conclusions on cause and effect cannot be made.

4.5 Conclusion

Our findings indicate that VA intake does not play a role in weight loss. Since the weight loss intervention called for a restriction on fat intake, absorption of VA may have been impacted. If VA absorption was impaired, it would not be able to carry out its physiological activities.

It is important to note the contrast between the experiments used for this thesis project. The first experiment used an animal model to examine the relationship between VA intake and weight gain (obesity development) in HFD feeding conditions, while the second experiment used a human model to look at the relationship between VA intake and weight loss with a diet that was hypocaloric and low-fat. Even if the results of the animal experiment indicate that VA plays a role in weight gain while consuming an obesogenic HFD, this does not mean that VA plays a role in weight loss when consuming a diet that is hypocaloric and low-fat. It is possible that fat content in the diets for these experiments played a role in the results we have seen. The relationship between VA intake and weight management may depend on the fat content

of the diet. Another explanation for the results could be the negative energy balance state used in this experiment. It is possible that VA does not influence weight management when a hypocaloric diet is implemented.

Chapter V

Discussion and Future Perspectives

5.1 Conclusion

This thesis project incorporated two different models to investigate the impact of VA in obesity. The first was an animal model, where animals were fed a high-fat diet with the goal of weight gain during dietary treatment. The second model looked at humans, where participants' fat and energy intake were restricted with the goal of achieving weight loss during the intervention. Because one model investigates weight gain and the other investigates weight loss, two different models (weight loss and weight gain), and two different diets (high-fat and low-fat) were examined.

We have known that vitamin A (VA) status affects plasma lipids levels, which is supported by the results of the animal experiment. The current research on lipophilic vitamin in high-fat diet (HFD) has focused mostly on the protective roles of lipophilic vitamins in HFD feeding conditions. How HFD affects homeostasis of lipophilic vitamins remains to be an open question. Knowing the essentiality of lipophilic vitamins for general healthy, it can be easy to assume that their uptake is helpful in a variety of dietary conditions. However, their uptakes share the same route as dietary lipids. Mutual effects of dietary lipophilic vitamins and other lipid molecules on each other's digestions, absorption and functions may exists. Our current findings support the assertion that VA status plays a key role in adiposity in HFD conditions. It is feasible to assume that transport, storage and metabolism of these lipophilic vitamins alter with the change of diets or metabolic states of a subject or population. Additional studies are needed to understand the impacts of dietary components on the homeostasis of lipophilic vitamins. Additional biochemical studies are need to analyze all the samples collected. The expression levels of glucose and lipid metabolic enzymes in the

metabolic active tissues and organs, such as the liver, adipose tissue and muscle will certainly reveal the metabolic changes introduced by the elimination of VA in a diet of high-fat setting.

Since our current findings showed no significant difference of VA intake between participants who had successfully lost 10% weight and those who had not, this indicates that intake of lipophilic vitamins was not related to weight loss. It has been established that excessive VA intake can contribute to BM gain in participants without changes in caloric intake, but it is not known if VA intake plays a role in weight loss when a hypocaloric, low-fat diet is consumed. While the results of the animal experiment indicate that VA plays a key role in weight gain in HFD conditions, the relationship (if any) between VA intake, fat intake, and weight loss remains to be an open question. Additional studies are needed to understand the role VA plays in the process of weight loss. The amount of fat consumed could have affected the results of the current experiment. Dietary fat is required for VA absorption. It is possible that the effects of VA on weight management may depend on the fat content of the diet consume.

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Vita

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