Effects of lecithin in salad dressing on the plasma appearance of fat-soluble micronutrients consumed in salads: contributions of chylomicrons and large VLDL

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Effects of lecithin in salad dressing on the plasma appearance of fat-soluble micronutrients consumed in salads: contributions of chylomicrons and large VLDL

by

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ABSTRACT

Provitamin A carotenoids, tocopherols, and phylloquinone, as the major fat-soluble micronutrients in salad vegetables, play essential roles in maintaining various physiological processes, such as cell differentiation and proliferation, normal organogenesis, and blood clotting. The non-provitamin A carotenoids, particularly lutein, zeaxanthin, and lycopene are important for maintaining ocular health and preventing chronic diseases. The intestinal uptake of fat-soluble micronutrients involves both simple diffusion and receptor-mediated transport. Various exogenous factors are able to affect the uptake process, e.g. food matrix and processing, dietary fat and fiber, and nutrient species and stereoisomers. Transferring fat-soluble micronutrients into the blood circulation depends on the normal synthesis of apolipoproteinB (apoB) and apoB-containing lipoprotein, which is promoted by the presence of sufficient lipids and suppressed by insulin. Overall, dietary fat is an elementary factor regulating the absorption of fat-soluble micronutrients.

Lecithin may influence the bioavailability of fat-soluble micronutrients. The role of the large very-low-density lipoprotein (VLDLA) plasma fraction in postprandial fat-soluble micronutrient transport is not clearly defined. Therefore, we conducted a human study to investigate the effects of the lecithin/oil ratio in salad dressing on the absorption of: 1) carotenoids, phylloquinone, and tocopherols from salad vegetables; 2) retinyl palmitate formed in the intestine from the provitamin A carotenoids. An additional objective was to investigate the origin of plasma chylomicrons and VLDLA and their roles in the transport of the absorbed fat-soluble micronutrients. Healthy women (n = 12) each consumed three salads with salad dressings containing: 1) 4 g soybean oil and 0 g hydroxylated soy lecithin...
(Solec® 8120, Solae, St. Louis, MO); 2) 3.8 g soybean oil and 0.2 g Solec® 8120; or 3) 3.2 g of soybean oil and 0.8 g Solec® 8120. The order in which the salads were consumed was randomly assigned according to a Williams Latin square design; salads were separated by ≥ 2 weeks. Blood was collected at baseline and 2, 3.5, 5, 7, and 9.5 h postprandially. Chylomicrons and VLDLA fractions were analyzed by HPLC with coulometric array electrochemical detection. ApoB-48 and apoB-100 contents in chylomicrons and VLDLA were determined by ELISA. There were no significant differences among the salad dressings in the resulting AUC values of the fat-soluble micronutrients in the chylomicron and VLDLA fractions. The exception was a decrease in the AUC value of phylloquinone in the chylomicron fraction when the salad dressing containing 0.8 g was compared with that containing 0 g Solec® 8120 (P < 0.02). The AUC values for α-carotene, β-carotene, lycopene, retinyl palmitate, and phylloquinone were substantially higher in the VLDLA than in the chylomicron fraction (P < 0.05). Both chylomicron and VLDLA fractions contained apoB-48 and apoB-100. ApoB-48 and apoB-100 were predominantly found in the VLDLA fraction (P < 0.0001). The AUC value of apoB-100 in VLDLA was significantly higher than that of apoB-48 in chylomicrons (P < 0.0001). We concluded that the hydroxylated soy lecithin in the amounts added to the salad dressing did not enhance the absorption of carotenoids, retinyl palmitate, phylloquinone, or tocopherols. The majority of the newly absorbed carotenoids, retinyl palmitate, and phylloquinone, as well as enterogenous (apoB-48) and hepatogenous (apoB-100) lipoprotein particles, were contained within large VLDL. Thus this lipoprotein subfraction has a major role in the transport of newly absorbed carotenoids and fat-soluble vitamins.
CHAPTER I.

GENERAL INTRODUCTION

Salad consumption, as a major contributor to vegetable consumption in the United States, is associated with a greater likelihood of meeting the recommended intakes for various nutrients, including carotenoids and vitamin E (Su & Arab, 2006). Although it has been reported that the bioavailability of nutrients, including provitamin A carotenoids, is low in raw vegetables (Rock et al., 1998), the absorption of fat-soluble micronutrients from vegetables is able to be improved by increasing the fat content of a meal (Dimitrov et al., 1988; Gijsbers et al., 1996). We previously reported that carotenoid absorption was higher after the consumption of salads with full-fat than with reduced-fat salad dressing, whereas no absorption of carotenoids was observed when salads were consumed with fat-free salad dressing (Brown et al., 2004). The consumption of 150 g avocado or 24 g avocado oil with vegetable salads resulted in similarly enhanced α-carotene, β-carotene, and lutein absorption compared with the consumption of avocado/avocado oil-free salads (Unlu et al., 2005). More recently, we found that the absorption of carotenoids, tocopherols, and phylloquinone increased linearly with increasing amounts of co-consumed fat from salad dressings (Agustiana et al., 2010). However, it is not recommended to have excessively high fat intakes to achieve high bioavailability of fat-soluble micronutrients. To further enhance the absorption efficiency, one strategy is to find a lipid that is more efficient than triacylglycerol in increasing the bioavailability of fat-soluble micronutrients.
The intestinal absorption of fat-soluble micronutrients involves the release from food matrix, emulsification with lipid droplets in the stomach, and incorporation into mixed micelles (Yonekura et al., 2007). Lecithin, as a structural component of mixed micelles and an excellent emulsifier, is likely to enhance the absorption of dietary lipids and lipophilic nutrients. Soybean lecithin was reported to increase the lymphatic absorption of triglyceride in rats (Nishimukai et al., 2003). Higher concentration of curcumin, a hydrophobic constituent of the spice turmeric, was observed in rat plasma and liver when curcumin was formulated with soybean lecithin compared with unformulated curcumin (Marczylo et al., 2007). However, few human studies have addressed the effects of the dietary lecithin on the bioavailability of fat-soluble micronutrients.

The utility of postprandial appearance of β-carotene and retinyl palmitate in chylomicrons and large very low density lipoproteins (VLDLA) as an indicator of intestinal β-carotene absorption have been studied well in humans (Hu et al., 2000; Paetau et al., 1997; Li et al., 2010). In our previous studies, after subjects consumed a β-carotene dose with a fat-rich meal, we observed postprandial appearances of β-carotene and retinyl palmitate in the plasma chylomicron fraction coincided in time and magnitude with their appearance in VLDLA (Hu et al., 2000; Paetau et al., 1997). Although it is known that the VLDLA fraction is a vehicle for assessing intestinal absorption of β-carotene and its major bioconversion product, retinyl palmitate (Hu et al., 2000; Paetau et al., 1997; Borel et al., 1997; Li et al., 2010), there has been little systematic study of the role of VLDLA in the transport of other newly absorbed fat-soluble
micronutrients, such as α-carotene, lycopene, lutein, tocopherols, and phylloquinone. The postprandial appearance of retinyl palmitate in VLDLA was hypothesized to be attributed to intestinal VLDL (Borel et al., 1997), but could also reflect their appearance in chylomicron remnants (Zheng et al., 2006). However, VLDLA have long been regarded as liver-derived lipoproteins (Karpe et al., 1994; Brodsky et al., 2008). Apolipoprotein B-48 (apoB-48) and apolipoprotein B-100 (apoB-100) are reliable biomarkers of lipoprotein particles of intestinal and hepatic origin, respectively (Lemieus et al., 1998; Phillips et al., 1997; Chan J, 1992). Therefore, an investigation of apoB-48 and apoB-100 in the plasma VLDLA fraction after a single meal can provide new insight regarding the origin of VLDLA.

The objectives of the present study were to investigate the effects of the lecithin/oil ratio in salad dressing on the absorption of carotenoids, phylloquinone, and tocopherols from salad vegetables and retinyl palmitate from intestinal metabolism of the provitamin A carotenoids. An additional objective was to investigate the origin of VLDLA and the roles of plasma chylomicrons and VLDLA in the transport of the absorbed fat-soluble micronutrients.

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CHAPTER II.

LITERATURE REVIEW

Physiological Functions of Carotenoids, Phylloquinone and Tocopherols

Carotenoids

Carotenoids are hydrophobic pigments synthesized by photosynthetic organisms. They provide plants with deep yellow, orange and red colors. Other non-photosynthesizing organisms only can obtain carotenoids by dietary intake. Carotenoid accumulations in tissues can pigment animals such as birds and fish. Carotenoids can be divided into two categories: provitamin A carotenoids and non-provitamin A, according to whether they can be converted into provitamin A (von Lintig et al., 2010).

The provitamin A carotenoids include α-carotene, β-carotene and β-cryptoxanthin. β-Carotene can be catalyzed by β-carotene 15,15'-monooxygenase 1 (BCMO 1) to from two retinal molecules (von Lintig et al., 2010), whereas α-carotene and β-cryptoxanthin are only able to generate a single retinal molecule because they contain one non-hydroxylated β-ring (Davis et al., 2008). The retinal is subsequently reduced to retinol (vitamin A) by retinal reductase, and a minor proportion of the retinal is irreversibly oxidized to retinoic acids by retinal dehydrogenases. Sufficient vitamin A and its derivatives (retinal and retinoic acid) are indispensible for normal immune system, cell differentiation, normal organogenesis, and eye
health (Sommer et al., 2012). Vitamin A deficiency, which is a widespread health problem in the developing world, causes more than a million cases of death and blindness every year (Sommer et al., 2012, Sommer et al., 1981). Increasing studies indicated that high consumption of carotenoid-rich fruits and vegetables was linked with a decreased occurrence of cancers (Thomson et al., 2013; Reiss et al., 2012; Takata et al., 2013; Jung et al., 2013). It had been hypothesized that β-carotene might prevent cancer development. In contrast, supplementation trials showed that supplementation with β-carotene increased, rather than decreased, incidence of lung cancer in cigarette smokers (The ATBC Cancer Prevention Study Group, 1994; Albanes et al., 1995; Omenn et al., 1996). However, recently, Pham et al. (2013) reported BCMO1, which was regulated by β-carotene uptake, had capabilities of inhibiting invasiveness of colon cancer cells and expressions of two neoplasia-related metalloproteinases (MMP7 and MMP28). In several animal studies, α-carotene indicated higher tumorigenesis prevention capability than β-carotene in the liver, lung, colon, and skin (Murakoshi et al., 1992, Narisawa et al., 1996). β-Cryptoxanthin is rich in Satsuma mandarin orange. It has been shown that Satsuma mandarin juices have a chemopreventive effect on chemically induced tumorigenesis in rat colon and lung (Tanaka et al., 2000, Kohno et al., 2001). Iskandar et al. (2013) recently reported that nicotine-induced emphysema and lung tumor volume and multiplicity were reduced when mice were dosed with β-cryptoxanthin supplement. Meanwhile, β-cryptoxanthin supplementation increased expressions of tumor suppressors including sirtuin, p53, and retinoic acid receptor (RAR)-β (Iskandar et al., 2013). Besides suppressing oncogenesis, β-cryptoxanthin has been
demonstrated to play a vital role in preventing bone loss by regulating various gene expressions that are involved in osteoblastic bone formation and osteoclastic bone resorption. The dietary intake of β-cryptoxanthin was associated with maintaining bone homeostasis in animal models as well as in menopausal women (Yamaguchi et al., 2012).

The non-provitamin A carotenoids, particularly lutein, zeaxanthin, and lycopene are important for maintaining many physiological processes (Sommer et al., 2012). Lutein and zeaxanthin are selectively accumulated in the primate retina and form the macular pigment. They are well-known xanthophylls because of their blue light filtering and antioxidant activities. It has been shown that lutein and zeaxanthin may play vital roles in inhibiting various ocular diseases, including age-related macular degeneration (AMD), photophobia, and cataracts (Kalariya et al., 2012). In addition to maintaining ocular health, increasing studies suggest that lutein has protective effects on immune system and inflammation. Lutein can prevent various ocular inflammation induced by endotoxin, laser, and streptozotocin. In vitro studies indicate that lutein is able to inhibit NF kappa-B activation and iNOS (inducible nitric oxide synthase) and COX-2 (prostaglandin-endoperoxide synthase 2) expressions (Kijlstra et al., 2012).

Lycopene, which is abundant in tomatoes and processed tomato products, contains 11 conjugated double bonds. Therefore, as one of most powerful antioxidant, its singlet-oxygen-quenching ability is about two times higher than that of β-carotene and 10 times higher than that of α-tocopherol (Di Mascio et al., 1989). Because of the antioxidant property, lycopene was hypothesized to be able to prevent chronic diseases. Recent studies show lycopene may exert
suppressive effects on various chronic diseases, including cancer and cardiovascular disease by reducing reactive oxygen species (ROS) levels (Giovannucci et al., 1999, Rao et al., 2002, Palozza et al., 2010a, Palozza et al., 2010b). Various redox molecules were modulated by lycopene such as antioxidant response elements (ARE), ROS-producing enzymes, small-GTPases, mitogen-activated protein kinases (MAPK), nuclear factor-κB (NF-κB), activator protein-1 (AP-1), as well as redox-sensitive proteins including p53, the Bcl-2 family proteins, and the Ku proteins (Palozza et al., 2012).

Tocopherols

Vitamin E, one of major fat-soluble antioxidants, is composed of eight compounds including α-, β-, γ-, and δ-tocopherols, as well as α-, β-, γ-, and δ-tocotrienols. α- Tocopherol, as the most potent form of vitamin E, constitutes more than 90% of vitamin E in the human body (Gohil et al., 2008; Brigelius-Flohe et al., 1999; Burton et al., 1990), whereas γ-tocopherol is the predominant form of vitamin E in the diet (Rigotti et al., 2007). The α-tocopherol synthesized by plants only have one stereoisomer, \textit{RRR}, while synthetic α-tocopherol, found in supplements and fortified food, contains equimolar concentrations of 2\textit{R}-stereoisomeric and 2\textit{S}-stereoisomeric forms, i.e. \textit{RRR}, \textit{SRR}, \textit{RSR}, \textit{SSR}, \textit{RRS}, \textit{SRS}, \textit{RSS}, and \textit{SSS}-α-tocopherol. The recommended daily allowance (RDA) for vitamin E is 15 mg per day for adult males and females, which is supposed to maintain the plasma α-tocopherol concentration as 12 μmol/L (Johnson et al., 2003). Novotny et al. (2012) recently report that 4 mg of \textit{RRR}-α-tocopherol
intake can maintain the plasma $\text{RRR-}\alpha$-tocopherol concentrations at 23 mmol/L, which suggest the actual vitamin E dietary requirement may be less than the current. The $\alpha$-tocopherol, as a peroxyl radical scavenger, protects erythrocytes from oxidative damage, which prevents peroxide-induced hemolysis. Therefore, hemolysis-induced anemia is a common symptom in patients with vitamin E deficiency (Niki et al., 2012). Moreover, vitamin E deficiency also can cause muscular weakness, neurological dysfunction, and reproductive failure (Burton et al., 1990; Kayden et al., 1993; Traber et al., 1996).

$\alpha$-Tocopherol is significantly associated with cell proliferation and apoptosis by affecting cell signaling and gene expression. These activities may relate to the specific distribution of $\alpha$-tocopherol in plasma membrane, i.e., it tends to incorporate into lipid rafts and associate with alterations in raft-related signaling pathways. In vascular smooth muscle cells, $\alpha$-tocopherol can inhibit protein kinase activity by dephosphorylation (Boscoboinik et al., 1991; Ricciarelli et al., 1998). Although the mechanism details have not been clarified, protein phosphatase 2A has been shown to be involved in this process (Boudreau et al., 2002). $\alpha$-Tocopherol also can prevent the proliferation of vascular smooth muscle cells, which is not mainly caused by its antioxidant property because $\beta$-tocopherol did not exert this inhibition effect (Tasinato et al., 1995). Tasinato et al. (1995) reported that the antiproliferative effect was correlated with inhibition of protein kinase activity via gene expression alteration. Moreover, $\alpha$-tocopherol has been found to protect various cell types of the vascular wall from cholesterol oxide-induced
mitochondrial dysfunction and apoptosis (Vejux et al., 2009; Royer et al., 2009; Mignet-Alfonsi et al., 2002; Lizard et al., 2000).

**Phylloquinone**

Phylloquinone, also named vitamin K₁, is the major form of vitamin K in the diet synthesized by plants. Another dietary form of vitamin K is menaquinones (vitamin K₂) which are synthesized by bacteria. The best sources of phylloquinone are green vegetables since phylloquinone is correlated with photosynthetic tissues (Shearer et al., 1996). The Adequate Intake (AI) for vitamin K is 120 μg/day for men and 90 μg/day for women, while no RDA has been established for phylloquinone (Institute of Medicine, 2001). Phylloquinone is an essential cofactor of γ-carboxylase, an enzyme catalyzing the γ-carboxylation of glutamate residues in proteins. This vitamin K-dependent γ-carboxylation has been found to be a post-translational modification process and enables the proteins, referred to as vitamin K-dependent proteins, to bind to calcium and interact with phospholipids, which are required by blood clotting and bone mineralization (Shearer et al., 2009).

Four vitamin K-dependent plasma proteins are indispensable for blood clotting. They are the coagulation cascade zymogens: factor II (prothrombin), factor VII, factor IX, and factor X, which are all synthesized in the liver. These coagulation cascade zymogens catalyze the formation of fibrin, an insoluble fiber network essentially required in blood clotting (Shearer et al., 2009). On the other hand, three vitamin K-dependent plasma proteins are involved in
anticoagulation including protein C, protein S, and protein Z (Broze et al., 2001; Furie et al., 1992). Warfarin, an anticoagulant, can inhibit the synthesis of vitamin K by interfering with the reducing of oxidized vitamin K to the reducted vitamin K.

Osteocalcin (bone Gla protein) was the first extrahepatic vitamin K-dependent protein found in bone. It is secreted by osteoblasts and contains three Gla residues, which facilitate the binding of mineral hydroxyapattites (Booth et al., 2012). Knockout mice with insufficient osteocalcin have been found to have increased bone density (Ducy et al., 1996). Matrix Gla protein (MGP) is another vitamin K-dependent protein secreted by osteoblasts, as well as chondrocytes and vascular smooth muscle cells. It is related to the organic matrix and calcium mobilization. Extensive arterial calcification has been found in MGP deficient mice, which suggests MGP is an important calcification inhibitor (Luo et al., 1997). The inhibition activity may be due to its binding to bone morphogenetic protein-2 (BMP-2). BMP-2 is a growth factor that converts preosteoblast into bone-forming cells (Abedin et al., 2004).

The vitamin K-dependent protein Gas6 has been shown to be associated with cellular growth by binding to Axl receptor, a tyrosine-protein kinase receptor. Gas6 can activate Axl tyrosine phosphorylation and enhance cell migration and growth. Li et al.(1996) reported that γ-carboxylated Gas6 has growth factor effects on Schwann cells and smooth muscle cells in the central nervous system, and lack of γ-carboxylated Gas6 may be associated with the pathogenesis of Alzheimer’s disease.
Mechanism of Carotenoid, Phylloquinone, and Tocopherol Absorption

Absorption of fat-soluble micronutrients from vegetables into the blood circulation starts with the mechanical and enzymatic break down of plant matrices by which carotenoids and fat-soluble vitamins are transferred from the food matrix into the lipid droplets in the stomach. The carotenoids and fat-soluble vitamins are then transferred from the lipid droplets into self-assembled intestinal dietary mixed micelles. Micelles are composed of phospholipids, bile salts, dietary lipids, and their hydrolysis products, including monoglycerides, diglycerides, and free fatty acids. Bile salts, the salt form of bile acids, are synthesized in the liver and secreted by the gallbladder. Bile salts and phospholipids exhibit amphipathic properties and reversibly form the exterior of micelles at neutral pH, and lipophilic compounds such as monoglycerides, diglycerides, free fatty acids, cholesterols, as well as fat-soluble micronutrients are incorporated into micelle hydrophobic interior. After incorporations into micelles, the fat-soluble micronutrients are uptaken by the enterocytes, incorporated into chylomicrons and transferred into the blood circulation via the lymphatic system. Although the mechanism of fat-soluble micronutrient absorption by the enterocytes is not completely understood, it has been considered to involve simple diffusion and receptor-mediated transport.

Simple diffusion

Carotenoid absorption has long been thought to occur by simple diffusion. In unanesthetized rats, β-carotene absorption rate linearly responded to its concentration in the
perfusate from 0.5 to 11 μmol/L (Hollander et al., 1978). Also, the β-carotene absorption was enhanced when simple diffusion was facilitated by increasing perfusate hydrogen ion concentration and perfusate flow rate, as well as by adding different types of fatty acids (Hollander et al., 1978). The concentration dependence of β-carotene uptake also has been found in the rat small intestinal cells (Scita et al., 1992) and Caco-2 cells (Garrett et al., 1999). The former showed no saturation up to 25 μmol/L β-carotene, and the latter indicated that β-carotene content increased when culture medium contained 2 to 27 μmol/L β-carotene. Similarly, intestinal absorption of tocopherols is also assumed to be by simple diffusion. Intestinal perfusion studies in rats have shown significantly higher intestinal permeability for α-tocopherol compared with γ-tocotrienol, which mainly contributes to the higher bioavailability of α-tocopherol (Abuasal et al., 2012).

**Receptor-mediated transport**

Although intestinal absorption of fat-soluble micronutrients has been considered for a long time as a passive process, it has recently been revealed that several lipid transporters are involved in mediating carotenoid and fat-soluble vitamin intestinal uptake. The first identified transporter is the scavenger receptor class B type I (SR-BI), which is a single-chain transmembrane glycoprotein expressed at the brush border membrane of enterocytes from the duodenum to the colon (Lobo et al., 2001). SR-BI is able to bind lipoproteins and facilitate the selective uptake of lipophilic compounds including cholesterol, phospholipids, and triglyceride
hydrolysis products (Terpstra et al., 2000; Hauser et al., 1998; Bietrix et al., 2006). It has been identified that SR-BI plays a role in regulating the intestinal absorption of α-carotene, β-carotene, lycopene, lutein, zeaxanthin, and vitamin E (van Bennekum et al., 2005; During et al., 2005; Moussa et al., 2008; Reboul et al., 2005; Reboul et al., 2006).

Cluster determinant 36 (CD36) is another scavenger receptor involved in fat-soluble micronutrient uptake. Similar to SR-BI, CD36 is a single chain-membrane glycoprotein found in the brush border membrane of the duodenum and jejunum (Terpstra et al., 2000). It has been reported that CD36 plays a key role in fatty acid uptake in the intestine, adipose tissue, and muscle. In CD36-null mice, intestinal lipid secretion and catabolism rate of chylomicrons in the blood were reduced after a high-fat intake (Drover et al., 2005). Although lipid secretion was decreased, the accumulation of chylomicrons in the blood masked this phenomenon and resulted in postprandial hypertriglyceridemia. It was assumed that CD36 regulated secretion and clearance of intestinal lipoproteins. van Bennekum et al. (2005) firstly reported that CD36 was associated with β-carotene uptake in transfected COS-7 cells and mouse brush border membrane vesicles. Sakudoh et al. (2010) further showed that the selective delivery of carotenoid depended on a CD36-related protein, Cameo2, in Bomyx mori. In addition, genetic variants in CD36 are associated with plasma lutein content in humans (Borel et al., 2011). More recently, CD36 was shown to facilitate the uptake of lycopene and lutein in mouse adipocytes and adipose tissue cultures (Moussa et al., 2011).
Niemann-Pick C1-like1 (NPC1L1) is a polytopic protein expressed in various human tissues, such as the liver and brush border membrane of the intestine (Davies et al., 2000; Altmann et al., 2004; Davies et al., 2005; Garcia-Calvo et al., 2005). It is the major cholesterol and phytosterol transporter in the intestine, which contributes 60% of dietary cholesterol uptake (Altmann et al., 2004; Davies et al., 2005; Davis et al., 2004). NPC1L1 also has been shown to interact with \( \alpha \)-tocopherol and \( \gamma \)-tocotrienol and play a role in their absorption (Narushima et al., 2008; Abuasal et al., 2010). Further NPC1L1 kinetic studies demonstrated significant lower \( V_{\text{max}} \) and \( K_m \) values for \( \gamma \)-tocotrienol compared to \( \alpha \)-tocopherol. The role of NPC1L1 in carotenoid absorption is not completely clear (During et al., 2005). Ezetimibe, an inhibitor of cholesterol transporter, was firstly shown to have an inhibition function in absorption of \( \alpha \)-carotene, \( \beta \)-carotene, \( \beta \)-cryptoxanthin, lycopene, lutein, and zeaxanthin in Caco-2 cells (During et al., 2005). However, the following study demonstrated neither ezetimibe nor an anti-human NPC1L1 antibody reduced lycopene uptake in Caco-2 cells (Moussa et al., 2008). Additional studies are thus worthwhile to investigate these discrepancies.

On the basolateral side of enterocytes, another transmembrane transporter ATP-binding cassette-A1 (ABCA1) is expressed. It mediates basolateral efflux of tocopherols. Besides incorporating into chylomicrons, newly absorbed tocopherols also can be secreted in nascent HDL. This process depends on ABCA1 (Reboul et al., 2009; Anwar et al., 2007). Reboul et al. (2009) reported that no secretion of \( \alpha \)-tocopherol in chylomicrons was observed when lipids are absent, and Anwar et al. (2007) suggested tocopherols were mostly incorporated into HDL in the
absence of oleic acid. In humans, ABCA1 also is found in the retinal pigment epithelium. Its gene variant is associated with retinal pigment optical density level response to lutein supplementation (Yonova-Doing et al., 2013). In chicks that have a recessive mutation in ABCA1 resulting in a severe HDL deficiency, the plasma lutein content was only 9% of control level (Connor et al., 2007). It is very likely that ABCA1 may also be involved in lutein efflux through enterocytes.

**Distribution of Carotenoids, Tocopherols, and Phyloquinone in Plasma Lipoproteins**

In humans, carotenoids are mostly transported in the low-density lipoproteins (LDL) and high-density lipoproteins (HDL). The hydrocarbon carotenoids are different from the xanthophylls in the relative distribution between LDL and HDL. The former (e.g., α- and β-carotene) are mainly detected in LDL, whereas the latter (e.g., zeaxanthin and lutein), which are more polar, are found in both HDL and LDL (Stahl et al., 2002). Recently, increasing studies emphasized the importance of HDL in the transport and retinal uptake of lutein (Loane et al., 2010; Mutungi et al., 2009; Wang et al., 2007). Moreover, lutein has been reported to exchange between human very low-density lipoprotein (VLDL) and HDL, whereas no exchanges were observed in α-carotene, β-carotene, and lycopene (Tyssandier et al., 2002).

The major carriers of tocopherols are HDL and LDL with similar proportions, and less than 20% of tocopherols are carried in VLDL and other lipoproteins (Perugini et al., 2000). The
transfer of α-tocopherol between circulating lipoproteins occurs either spontaneously or with assistance of plasma phospholipid transfer protein (PLTP) (Kostner et al., 1995). Spontaneous transfer is slow and only can account for little portion of the high exchange/transfer rates that have been demonstrated in vivo. PLTP has the ability to transfer various amphipathic/hydrophobic compounds, including phospholipids, diacylglycerides, unesterified cholesterol, and tocopherols, between lipoproteins in the blood circulation (Tzotzas et al., 2009).

Similar to carotenoids and tocopherols, phylloquinone is mainly distributed between LDL and HDL with approximately equally proportion, and less amount of phylloquinone is carried by IDL (Schurgers et al., 2002; Lamon-Fava et al., 1998).

Factors Influencing the Bioavailability of Carotenoids, Tocopherols and Phylloquinone Absorbed from Vegetables

The bioavailability of fat-soluble micronutrients is influenced by multiple factors. These factors can be classified as host-related factors or dietary factors (Yonekura et al., 2007). The digestion and absorption of fat-soluble micronutrients are tightly associated with the health status of individuals, which include gut health, nutritional and physiological status, lifestyle, and genotype. This review is mainly focused on dietary factors that affect the bioavailability of fat-soluble micronutrients in vegetables.
Food matrix and food processing

Vegetables, especially green leafy vegetables, are the primary sources of fat-soluble micronutrients, including carotenoids, tocopherols, and phylloquinone in the U.S diet (Eitenmiller et al., 1995; Murphy et al., 1990; Maras et al., 2004). However, the bioavailability of fat-soluble micronutrients is rather low in raw vegetables. One of the reasons is that the fat-soluble micronutrients embedded in the matrix of vegetables are more difficult to be released compared with those in supplements. To improve their bioavailability, food processing, e.g. heating and mechanical treatments, is often applied, which facilitates the release of fat-soluble micronutrients from the food matrix by disrupting the plant cell wall and organelles. Rock et al. (1998) reported that β-carotene plasma concentration was higher when women consumed thermally processed and pureed carrots and spinach versus raw carrots and spinach. Similarly, lycopene AUC (area under the curve) value in chylomicrons was significantly higher when tomato paste was consumed compared with fresh tomatoes (Gärtner et al., 1997). Moreover, food processing, including boiling, cooking, and microwaving, increased the phylloquinone content in carrots, spinach, as well as tomatoes (Damon et al., 2004).

In the matrix of vegetables, carotenoids are located either in the photosynthetic organelles (green leafy vegetables), or as part of semi-crystalline membrane-bounded solids (carrots and tomatoes) (Castenmiller et al., 1998; van het Hof et al., 1998). The differences in food matrix microenvironment are also critical to carotenoid absorption. It has been demonstrated that the
solubilization of β-carotene from raw carrot juice to oil phase was substantially higher than that from raw spinach (Rich et al., 2003).

**Dietary fat**

Besides food processing, the absorption of fat-soluble micronutrients from vegetables is also improved by increasing the fat content of a meal (Stahl, 2002). The fat intake is able to stimulate bile and pancreatic enzyme secretion and facilitate micelle formation. Brown et al. (2004) reported that no absorption of carotenoids was observed when salads were consumed with fat free salad dressing, and the carotenoid absorption was higher after the consumption of salad with full-fat (28 g canola oil) than with reduce-fat dressing (6 g canola oil). Likewise, serum phylloquinone concentration was higher when boiled spinach was consumed with 25 g butter *versus* without added fat (Gijsbers et al., 1996). In addition, it has been demonstrated that the consumptions of vitamin E-fortified apples with low-fat (2.4 g fat) and regular-fat (11 g fat) breakfasts increase the maximal plasma concentration and AUC value of α-tocopherol by 2- and 3-fold, respectively (Bruno et al., 2006). However, increasing fat content seems to affect the bioavailability of different carotenoid species to a different extent, in particular, it has stronger influence on the highly lipophilic carotenes (α-carotene, β-carotene, and lycopene) compared with less lipophilic xanthophylls (e.g., lutein and zeaxanthin). The β-carotene and lutein, released from the digesta of spinach puree containing 10% corn oil, showed no significant difference in micellerization in an *in vitro* digestion model (Ferruzzi et al., 2001). However, in
the presence of relatively low amount of fat (2-3.5%), the micellerization of lutein extremely exceeded that of β-carotene after an *in vitro* simulated digestion (Garrett et al., 1999; Chitchumroonchokchai et al., 2004; Garrett et al., 2000). It suggests that, with insufficient dietary fat, the micellerization of the more lipophilic carotenes is limited, whereas the less lipophilic xanthophylls seem to be more freely released from the food matrix to oil phase and mixed micelles.

Furthermore, unprocessed vegetable consumption seems to require more fat to achieve the optimal absorption of fat-soluble micronutrients. As mentioned above, the chylomicron carotenoid content was higher when salad vegetables were consumed with 28 g canola oil than with either 6 g or 0 g canola oil (Brown et al., 2004). Similarly, the addition of avocado or avocado oil significantly increased the absorption of α-carotene, β-carotene, and lutein from salad and salsa (Unlu et al., 2005). In contrast, when α- and β-carotene supplements were consumed with a meal containing 3 g or 36 g dietary fat, no significant differences in plasma α- and β-carotene contents were observed between the low- and high-fat groups (Roodenburg et al., 2000).

The fat composition also plays a role in the bioavailability of fat-soluble micronutrients. Long-chain triglycerides are able to promote chylomicron formation, whereas medium-chain triglycerides diminish chylomicron formation. Therefore, the presence of long-chain triglycerides in both the first and second meals significantly enhanced carotenoid absorption compared with the presence of medium-chain triglycerides (Borel et al., 1998). Several studies
also have shown the degree of unsaturation in fatty acids can affect carotenoid absorption. The absorption of β-carotene was higher when a β-carotene supplement was ingested with beef tallow than with sunflower oil (Hu et al., 2000). Also, micellar oleic, but not linoleic acid, have been reported to enhance β-carotene absorption and its cleavage into retinol in rats (Raju et al., 2006). Furthermore, Huo et al. (2007) reported that the efficiency of lutein uptake by Caco-2 cells was significantly higher from that of micelles generated during the in vitro digestion of salad in the presence of triolein (monounsaturated fatty acid) compared with trioctanoin (saturated fatty acid), while the uptake of β-carotene, α-carotene and lycopene were not affected by the degree of unsaturation of the fatty acids in the added triglycerides. Few human studies have investigated the role of the degree of unsaturation of fatty acids in the absorption of lutein, α-carotene, and lycopene. It is likely that the degree of unsaturation in fatty acids may play a role in the bioavailability of carotenoids. According to the in vitro study of Huo et al. (2007), lutein may have a different response to the degree of unsaturation of fatty acids in vivo as compared with β-carotene, α-carotene, and lycopene.

**Phospholipids and other lipids**

The absorption of fat-soluble micronutrients involves gastric oil phase and dietary mixed micelles. Phospholipids, as one of the components of mixed micelles and an emulsifier for oil, have been studied for their bioavailability enhancing effects. They commonly exist in both fresh and processed food matrixes, especially phosphatidylcholine, which is also the major form of
phospholipids in bile. Under normal conditions, most dietary and biliary phosphatidylcholine is hydrolyzed by the action of phospholipase A$_2$ to produce lysophosphatidylcholine. It has been shown that lysophosphatidylcholine, a common component in soy lecithin, can enhance the bioavailability of β-carotene, lutein, and α-tocopherol in rats, mice, and Caco-2 human intestinal cells (Lakshminarayana, 2006; Sugawara, 2001; Baskaran, 2003; Koo, 2001). Moreover, the acyl chain length of phospholipids is also a critical factor influencing their effects on the bioavailability of fat-soluble micronutrients (Yonekura, 2006). Yonekura et al. (2006) reported that medium-chain phosphatidylcholine and medium-to-long-chain lysophosphatidylcholine increased β-carotene uptake, whereas long-chain phosphatidylcholine inhibited and short-chain phosphatidylcholine as well as short-to-medium-chain lysophosphatidylcholine had no effects on the β-carotene uptake.

Sucrose polyesters are often used as nonabsorbable fat replacers because they cannot be hydrolyzed by gastric and pancreatic lipases. They are able to partition fat-soluble micronutrients from mixed micelles and consequently decrease the nutrient uptake (Weststrate and van het Hof, 1995, Koonvitsky et al., 1997, Schlagheck et al., 1997). However, due to the relatively less lipophilic nature of xanthophylls, their absorption is less inhibited by sucrose polyesters when compared to β-carotene (Schlagheck et al., 1997; Broekmans et al., 2003).

Plant sterols and stanols have been reported to lower plasma cholesterol levels in humans (Nguyen et al., 1999). However, they can compete with fat-soluble micronutrients for the incorporation in mixed micelles. In the presence of β-sitosterol, β-carotene absorption was
diminished to approximately 50% in Caco-2 cells, and both of them were completely dissolved (Fahy et al., 2004). Also, the plasma TRL β-carotene content was reduced by 50% when human subjects consumed a single meal containing free or esterified plant sterols (Richelle, 2004).

**Dietary fiber**

Dietary fiber, especially soluble fiber, has been considered to have an inhibitory role in the absorption of lipophilic compounds, such as cholesterol (Brown et al., 1999). Similarly, as hydrophobic bioactive food components, the absorption of carotenoids was diminished by some types of fibers in humans (Riedl et al., 1999; Rock et al., 1992), rats (Zanutto et al., 2002), chicks (Erdman et al., 1986), and Mongolian gerbils (Deming et al., 2000). The mechanism of the inhibitory effects of fiber on carotenoid absorption has not been completely elucidated. Such mechanisms might include: 1) influencing the pancreatic enzyme activity and consequently inhibiting micelle formation; 2) slowing the diffusion of micelles to the enterocytes by increasing the viscosity and volume of the intestinal contents; 3) diminishing second meal effects by altering enterocyte renewal (Riedl et al., 1999). On the other hand, in one human study, volunteers were given a supplement containing carotenoids and α-tocopherol with or without various types of fiber, and significantly lower AUC values for carotenoids were observed in the fiber groups, whereas no differences were found for α-tocopherol AUC values (Riedl et al., 1999). Animal studies suggested that the effects of dietary fiber on vitamin E absorption might
be apparent only if the fiber intake reached an “effective level” (De Lumen et al., 1982; Schaus et al., 1985).

**Species and stereoisomers of fat-soluble micronutrients**

As mentioned above, dietary lipids do not affect all carotenoid species to the same degree. Increasing the fat content of the meal efficiently enhances the absorption of the more lipophilic carotenes, whereas the transfer of the less lipophilic xanthophylls from the oil phase to micelles occurs much more easily in low-fat dietary conditions (Yonekura et al., 2007). Thus, different carotenoid species may require different amounts and/or types of lipids for optimal absorption. Within one carotenoid species, the bioavailability of different stereoisomers may be different. The *cis*-isomer of lycopene has been shown to be more bioavailable than *all-trans* lycopene, which may be due to the shorter length of the *cis*-isomer which facilitates micellerization and uptake by intestinal cells (Boileau et al., 2002; Failla et al., 2008). However, the preferential uptake of the *all-trans* β-carotene, rather than the *cis*-isomers, was observed in several human studies (Gaziano et al., 1995; Stahl et al., 1993; You et al., 1996). Moreover, the α-tocopherol constitutes more than 90% of vitamin E in the human body (Gohil et al., 2008; Brigelius-Flohe et al., 1999; Burton et al., 1990), even though γ-tocopherol is the predominant form of vitamin E in the diet (Rigotti et al., 2007). The limited bioavailability of γ-tocopherol is caused by its low affinity of α-tocopherol transfer protein (α-TTP), which is only 9% compared with RRR-α-tocopherol (Hosomi et al., 1997). Likewise, the high α-TTP affinity of RRR-α-
tocopherol also contributes to its 2-fold higher plasma concentration after a single dose or 8 consecutive daily doses when compared to all-rac-α-tocopherol (Burton et al., 1998).

**Apolipoprotein B and Apolipoprotein B-containing Lipoproteins**

Apolipoprotein B (apoB) is a large plasma protein that binds to the surface of lipoproteins and plays an essential role in the assembly of triglyceride-rich lipoproteins. ApoB occurs in two isoforms, apolipoprotein B-48 (apoB-48) and apolipoprotein B-100 (apoB-100). They are encoded by the same gene on chromosome 2 (Welty et al., 1999; Veniant et al., 1999). In humans, apoB-48 is secreted by the intestine as a result of a post-transcriptional mRNA editing catalyzed by the apoB editing complex 1 (apoBEC-1). This enzyme converts codon 2153 to a stop codon (UAA) that truncates the polypeptide at 48% of its full length. The full length apoB, apoB-100, is primarily synthesized by the liver and contains 4536 amino acids.

Unlike all other apolipoproteins, apoB cannot exchange between lipoproteins, and it remains with the apoB-containing lipoprotein through its entire metabolic process. Therefore, apoB-48 and apoB-100 are regarded as reliable markers of intestinal and hepatic origin lipoprotein particles, respectively (Phillips et al., 1997; Karpe et al., 1994; Chan et al., 1992). ApoB-containing lipoproteins have long been referred to as chylomicrons and VLDL (Lemieus et al., 1998; Karpe et al., 1994). Chylomicrons are lipoprotein particles of intestinal origin, which transport newly absorbed lipids and fat-soluble nutrients. VLDL is synthesized by the liver, and transports endogenous lipids. VLDL can be converted to intermediate-density
lipoproteins (IDL) and LDL by the action of lipoprotein lipase. However, data from cell culture, rat liver perfusion, and human studies demonstrate that the liver has the capability of producing a wide spectrum of apoB-100 containing lipoproteins from VLDL down to LDL (Marsh et al., 1976). Recently, apoB-48 has also been found in lipoproteins with various sizes and densities, including VLDL, IDL, and LDL (Campos et al., 2005; Cartwright et al., 2001; Zheng et al., 2006; Johanson et al., 2004; Mero et al., 2000; Battula et al., 2000). By labeling apoB-100 and apoB-48 with trideuterated leucine, Zhang et al. (2006) reported that the liver and intestine were capable of secreting apoB-100- and apoB-48-containing lipoproteins, respectively, with a range of sizes from chylomicron-sized down to IDL-sized particles. The production rate of apoB-100 in the liver was higher than that of apoB-48 in the intestine (Zheng et al., 2006). Among the apoB-containing lipoproteins, this review will focus on chylomicrons and VLDL.

**ApoB synthesis and apoB-containing lipoprotein assembly**

As mentioned above, apoB transcriptional regulation is tissue-specific. In enterocytes of all mammals, apoB-48 is synthesized as the 48% of apoB-100 fragment due to the action of apoBEC-1. With the absence of apoBEC-1 in human liver, only apoB-100 is assembled into VLDL. ApoB translation is mainly regulated by 5' and 3' untranslated regions (UTRs) of apoB mRNA. In the 5' UTR of apoB mRNA, about 76% of the bases are either guanine or cytosine. The GC-rich regions are able to form stable secondary structures (Kozak et al., 1991). The 3' UTR of apoB mRNA is rich in adenine and uracil, and the AU-rich regions can also form
secondary structures that may play a role in the regulation of mRNA stability (Day and Tuite, 1998; Pontrelli et al., 2004). Several studies have investigated the effects of 5' and 3' UTRs in the translation of apoB by binding the UTRs with a luciferase reporter gene or apoB15 cDNA (Pontrelli et al., 2004; Sidiropoulos et al., 2005, 2007). The results suggested that the 5' UTRs facilitated apoB translation, but, in contrast, the 3' UTR had an adverse impact on the apoB translation by reducing the stability of apoB mRNA (Pontrelli et al., 2004; Sidiropoulos et al., 2005, 2007). Furthermore, insulin can also negatively affect apoB translation by the action of insulin-sensitive factor. The 110 kDa insulin-sensitive factor is able to bind the first 64 nucleotides of the 5' UTR that has been demonstrated to be the response region of apoB mRNA to insulin, and the binding subsequently induces reduction in apoB translation (Sidiropoulos et al., 2005, 2007). This process is assumed to be associated with phosphoinositide 3 kinase and mTOR pathways, because the binding of the insulin-sensitive factor to the 5' UTR and the insulin inhibition of apoB translation were diminished when HepG2 cells were treated with either phosphoinositide 3 kinase inhibitor (wortmannin) or mTOR inhibitor (rapamycin) (Sidiropoulos et al., 2007).

After translation of a signal peptide consisting of 24-27 amino acids, the nascent apoB chain and ribosomes are transferred to the rough endoplasmic reticulum (ER) (Boerwinkle and Chan, 1989). With sufficient lipid ligands, the nascent apoB is translocated into the ER lumen via a proteinaceous channel, known as the translocon (Chen et al., 1998). The translocon is primarily composed of the Sec61 protein and binds to ribosomes with high affinity (Alder et al.,
Following translocation, apoB translation continues. During and after translation, apoB is lipidated by the microsomal transfer protein (MTP) and subsequently forms a pre-VLDL (Olofsson et al., 2000; Gordon et al., 2000; Shelness et al., 2005). MTP is a membrane-associated heterodimer of protein disulfide isomerase (PDI) and a 97 kDa polypeptide subunit that improves the delivery of phospholipids, cholesteryl esters, and triglyceride to apoB (Hussain et al., 2003; Shoulders et al., 2005). Individuals with a mutation of the MTP gene have abetalipoproteinemia, which is characterized by a deficiency of VLDL formation (Di Leo et al., 2005). Although MTP plays an indispensable role in VLDL formation, a recent study implies that MTP is not required for apoB translocation in McA-RH7777 cells (Dashti et al., 2007). In addition to MTP, apoB co-translationally and post-translationally interacts with various molecular chaperones, including cyclophilin, PDI, BiP (an Hsp70 chaperone), Grp94 (an Hsp90 chaperone), calnexin, and calreticulin (chaperone-like lectins) (Chen et al., 1998; Tatu et al., 1997; Rashid et al., 2002; Stillemark et al., 2000; Zhang et al., 2003; Qiu et al., 2005). Most of the molecular chaperones may be associated with apoB posttranslational modification or degradation, or facilitate insertion of apoB into multiprotein complexes; however, the complete mechanism of each chaperone effect on apoB is not clear (Brodsky et al., 2008).

Following the formation of pre-VLDL, the primordial lipoprotein particles are packed and transported by coatomere Protein II (COP II) to the Golgi apparatus (Gurkan et al., 2006). This process is stimulated by a GTPase known as Sar1. Mutations in Sar1 are responsible for chylomicron retention disease, which is characterized by chylomicron deposition in intestinal
cells (Jones et al., 2003). Likewise, Sar1 also plays an essential role in apoB export from ER in rat hepatoma McA-RH7777 cells (Gusarova et al., 2003). In the Golgi apparatus, the primordial lipoprotein particles were further lipidated to achieve VLDL density, by which VLDL is formed. The final VLDL lipidation is mediated by phospholipase D1 and extracellular signal regulated kinase 2 (ERK2). They facilitate the formation of VLDL by increasing the production of cytosolic lipid droplets that deliver additional lipids into the Golgi apparatus.

**ApoB degradation**

**ER-associated degradation**

ER-associated degradation (ERAD) is a cellular pathway that recognizes misfolded secretory proteins in the ER and subsequently induces their ubiquitin-dependent degradation by the proteasome (Brodsky et al., 2007). The ERAD is able to prevent the development of cytotoxic aggregates caused by concentrated misfolded proteins. When the concentration of misfolded proteins increases rapidly in the ER and exceeds the capability of ERAD, the unfolded protein response (UPR) is activated by transmembrane UPR sensors (Schroder et al., 2005; Ron et al., 2007). The UPR stimulates the enhancement of ER chaperone synthesis, ERAD efficiency, as well as other degradation pathways. In addition to ER quality control, the ERAD also has the ability to regulate metabolic processes. When lipid loading is limited, which may be caused by low MTP activity or low lipid availability, apoB is targeted for ubiquitination and
subsequently degradation by the proteasome during translation process (Benoist et al., 1997; Dixon et al., 1991; Zhou et al., 1998).

**Polyunsaturated fatty acid-induced degradation**

The apoB degradation can also happen after the ER exit of pre-VLDL, known as PERPP (post-ER presecretory proteolysis). Dietary polyunsaturated fatty acids (PUFAs) are able to trigger PERPP and then decrease VLDL levels (Fisher et al., 2001). *In vitro* and animal studies have shown that in the presence of PUFAs, apoB is damaged by lipid peroxides, and aggregates are formed and subsequently degraded by autophagy (Pan et al., 2004). This process most likely occurs in the Golgi apparatus, because pre-VLDL requires further addition of lipids in the Golgi in which VLDL may be exposed to PUFAs (Brodsky et al., 2008).

**Insulin-induced degradation**

Insulin, as an essential peptide hormone, is involved in lipid metabolism by inhibiting hormone-sensitive lipase, promoting storage of triglycerides, as well as diminishing VLDL production in the liver. Acute increases in insulin reduce apoB synthesis and VLDL secretion in HepG2 cells, rat and human hepatocytes, and rats (Patsch et al., 2004; Sparks et al., 1986; Pullinger et al., 1989; Salhanick et al., 1991; Durrington et al., 1982; Chirieac et al., 2000). These inhibitory effects of insulin primarily depend on PI3K (phosphatidylinositol 3-kinase) activity (Phung et al., 1997). Insulin is able to activate PI3K by tyrosine phosphorylation of
insulin receptor substrates. The activated PI3K subsequently: 1) inactivates protein phosphatase 1B (PTP1B), which increases the expression of a cysteine protease ER60 and consequently promotes apoB degradation; 2) suppresses the activities of FoxO1 and Foxa2, two transcription activators of MTP, and then MTP expression is reduced; 3) decreases phosphatidylinositol biphosphate (PIP2) that is responsible for activating phospholipase D1 and ARF-1, two factors required in pre-VLDL formation (Vergès et al., 2010). The PUFAs-induced PERPP may also play a role in the effects of insulin on the production of apoB-containing lipoproteins since it has been observed that PUFA-rich diets increase insulin sensitivity (Riserus et al., 2008; Galgani et al., 2008).

**ApoB-100 synthesis in human intestine**

It is universally known that human apoB-48 is primarily secreted by the intestine and apoB-100 is the predominant isoform in human liver. However, the detection of apoB-100 in the human intestine by using an apoB-100 cDNA probe (Knott et al., 1985) raises a question: Can the human intestine synthesize apoB-100? Several *in vitro* studies reported Caco-2 cells were able to produce apoB-100 (Hughes et al., 1987; Lee et al., 1988). A human intestinal organ culture study indicated apoB-100 represented 16 ± 3% of the intestinal apoB mRNA, whereas apoB-100 only contributed 3-5% of newly synthesized intestinal apoB (Hoeg et al., 1990). The synthesis of apoB-100 may be associated with age. Immunoprecipitates of 11-week fetal intestine showed major radioactivity incorporation into apoB-100 and little incorporation into apoB-48. The
intestine from 16 week fetuses incorporated radioactivity into both apoB-100 and apoB-48, whereas adult intestine immunoprecipitates only contained radioactivity in apoB-48 (Glickman et al., 1986). Another immunoprecipitate study, in which intestinal biopsy samples were obtained from 6 children aged 3 months to 8 years, reported radioactivity primarily in apoB-48, but a relatively small amount was detected in apoB-100 (Levy et al., 1990). In the same study, apoB-100 was found in thoracic duct lymph chylomicrons donated by a 5-year old boy with severe hypertension (Levy et al., 1990). This finding was consistent with studies of Kane et al. (1980) and Ruf et al. (1999), where apoB-100 presented in chylomicrons from thoracic duct lymph samples obtained from five normolipidemic patients and a patient undergoing thoracic surgery, respectively. However, hepatic lymph contributes about 25 to 50% of lymph flowing through the thoracic duct (Ohtani et al., 2008). The detection of apoB-100 in thoracic duct lymph might be caused by contamination of hepatic apoB-100-containing large VLDL presented in the thoracic duct lymph and being collected with chylomicrons. To examine this possibility, Lock et al. (1983) investigated apoB isoforms in chylomicrons isolated from a male patient with chyluria. Chyluria is a medical disorder with symptoms of chyle urine and lymph channel blocking. The apolipoprotein composition in urine chylomicrons was determined by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie Blue staining. Lock et al (1983) concluded that the apoB-48 is virtually the only apoB in chylomicrons. However, a weak band in the apoB-100 size position was observed in urine chylomicrons isolated after an overnight fasting. Although the intestine has the ability to
synthesize apoB-100, it tends to choose apoB-48 to form chylomicrons. Mice with apobec-1 gene knock out, whose intestine secreted only apoB-100, had a reduction of chylomicron secretion into the lymphatic system and triglyceride accumulation in the enterocytes after being fed a large dose of lipids (Lo et al., 2008). The synthesis rate of apoB-100 in human intestine still needs to be quantified, and whether it is regulated by diet and insulin is not clear.

Factors affecting apoB secretion and apoB-containing lipoprotein assembly

Dietary fat

As mentioned above, dietary PUFAs (polyunsaturated fatty acids), particularly the n-3 fatty acids enriched in fish oil, are able to reduce VLDL levels by PUFAs-induced post ER nonproteasomal degradation. A VLDL apoB-100 kinetics study reported that triglyceride level was decreased in 10 normal male subjects after consuming a daily fish oil supplement for four weeks. The triglyceride reduction is due to a decreased production rate of VLDL apoB-100 (Bordin et al., 1998). In addition, dietary fat may affect VLDL particle size (Stacpoole et al., 1991). When normal subjects consumed very low fat diets, more apoB-100 was secreted with VLDL/IDL-like particles, whereas high fat diets increased larger VLDL particle secretion. Also, the fractional clearance rate of VLDL/IDL was increased in both normal and familial hypercholesterolemic subjects after they consumed a very low fat diet.
Aging

Dietary fat is not the only factor regulating apoB and apoB-containing lipoprotein secretion. The magnitude of apoB-100 in VLDL, IDL, and LDL has been positively correlated with age in normolipidemic male subjects (Millar et al., 1995). ApoB-100 kinetic analyses also revealed that aging was linked with an increased production of VLDL and a decreased fractional clearance rate of apoB-100 in IDL and LDL in the fed state. This is supported by an earlier study with radiolabeled LDL apoB, which reported that the LDL apoB fractional catabolic rate was higher in young male subjects (20-39 y) compared with old male subjects (60-80 y) (Ericsson, 1991). Besides aging, postmenopausal status has been shown to result in greater postprandial lipid responses in women (Cohn et al., 1988; van Beek et al., 1999; Masding et al., 2003). In sequential dietary studies recently conducted at Reading University, postprandial data from 98 healthy UK female adults were pooled and analyzed (Jackson et al., 2008). The investigators reported that triglyceride absorption and maximal triglyceride concentration were augmented in postmenopausal women, and the most evident difference in triglyceride absorption was observed between the younger and the older premenopausal women. Normal apoB secretion depends on the availability of lipids; therefore, it could be hypothesized that menopausal status might affect apoB and apoB-containing lipoprotein metabolism by regulating the postprandial lipid responses.
**Gender**

In several kinetic studies, a gender difference in lipoprotein metabolism was noted. Women have been shown to have higher triglyceride-rich lipoprotein and LDL-apoB fractional catabolic rates than men, whereas no significant differences are found in production rates (Matthan et al., 2008; Watts et al., 2000). In addition, gender differences observed in younger subjects were more significant than in older subjects (Stanhope et al., 2011; 2009). The younger male adults exhibited greater fasting triglyceride and apoB responses after 2 weeks of sugar consumption, whereas the responses in postprandial triglyceride and apoB were comparable between the genders (Stanhope et al., 2011). It was suggested that the gender difference in lipoprotein metabolism may reflect VLDL clearance rate rather than its secretion rate.

**ApoB and apoB-containing lipoprotein kinetics**

Kinetic studies of apoB-containing lipoproteins began with chylomicrons by labeling the triglyceride component. Chylomicron triglyceride was found to be cleared from the blood within minutes in healthy subjects, whereas the clearance rate was relatively slow in subjects with coronary heart disease (Nestel et al., 1964; Grundy et al., 1976). By labeling chylomicrons with either retinyl palmitate or iodinated protein components, subsequent studies reported the half-time of chylomicrons in blood was as short as 15-30 min (Berr et al., 1984; Cortner et al., 1987; Karpe et al., 1997; Schaefer et al., 1978; Stalenhoef et al., 1984; Schaefer et al., 1986). Although, in the early stages of kinetic investigations, radioiodinated apoB-48 and apoB-100
were shown to be depleted within 15 min and 30 min, respectively, in normal subjects
(Stalenhoef et al., 1984), the apoB-48 and apoB-100 were radiolabeled in vitro after isolation
from plasma. To avoid radiation hazards and exogenous labeling, amino acid precursors labeled
with stable isotopes are now widely used. When apoB-48 and apoB-100 were endogenously
labeled with stable isotopes, the catabolism of chylomicrons was found to be significantly slower
than it had been assumed. Several studies reported that apoB-48 had similar fractional turnover
rate to apoB-100 in triglyceride-rich lipoproteins, with an average fractional catabolic rate (FCR)
of four to six per day and an average residence time of 4-6 h (Lichtenstein et al., 1992; Welty et
al., 1999; Welty et al., 2000; Batista et al., 2004; Welty et al., 2004;). The FCR difference was
observed in the two major VLDL subfractions (large and small VLDL); i.e., the FCR of large
VLDL is significantly faster than that of small VLDL, but no consistent differences were found
in the production rate (PR) between the two subfractions (Marsh et al., 2002). Moreover, the
postprandial PR of apoB-100 in VLDL is about twice as great as that in the fasting condition
(Marsh et al., 2002).

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CHAPTER III.

EFFECTS OF LECITHIN IN SALAD DRESSING ON THE PLASMA APPEARANCE OF FAT-SOLUBLE MICRONUTRIENTS CONSUMED IN SALADS:

CONTRIBUTIONS OF CHYLOMICRONS AND LARGE VLDL

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Running head: PLASMA APPEARANCE OF FAT-SOLUBLE MICRONUTRIENTS

Abbreviations used: VLDLA, large very-low-density lipoprotein subfraction; ApoB-48, apolipoprotein B-48; ApoB-100, apolipoprotein B-10
ABSTRACT

Background: Lecithin may influence the bioavailability of fat-soluble micronutrients. The role of the large very-low-density lipoprotein (VLDLA) plasma fraction in postprandial fat-soluble micronutrient transport is not clearly defined.

Objectives: This study investigated the: 1) effects of the lecithin/oil ratio in salad dressing on the absorption of carotenoids, phylloquinone, and tocopherols from salad vegetables and retinyl palmitate from intestinal metabolism of the provitamin A carotenoids; 2) roles of the plasma chylomicron and VLDLA fractions in the transport of the absorbed fat-soluble micronutrients.

Design: Healthy women (n = 12) each consumed three salads with salad dressings containing: 1) 4 g soybean oil and 0 g hydroxylated soy lecithin (Solec® 8120, Solae, St. Louis, MO); 2) 3.8 g soybean oil and 0.2 g Solec® 8120; or 3) 3.2 g of soybean oil and 0.8 g Solec® 8120. The salads were separated by ≥ 2 wk. Blood was collected at baseline and 2, 3.5, 5, 7, and 9.5 h postprandially. Fat-soluble micronutrients in the lipoprotein fractions were analyzed by HPLC with coulometric array electrochemical detection. Apolipoprotein (apo) B-48 and apo B-100 contents in chylomicrons and VLDLA were determined by ELISA.

Results: There were no differences among the salad dressings in the area under the curve (AUC) values for the fat-soluble micronutrients in the chylomicron or VLDLA fractions. The exception was a decrease in the AUC for phylloquinone in the chylomicron fraction when the salad dressing containing 0.8 g was compared with that containing 0 g Solec® 8120 (P < 0.02). The AUC values for α-carotene, β-carotene, lycopene, retinyl palmitate, and phylloquinone were
substantially higher in the VLDLA than in the chylomicron fraction ($P < 0.05$). ApoB-48 and apoB-100 contents were higher in the VLDLA fraction than in the chylomicron fraction ($P < 0.0001$).

**Conclusions:** The hydroxylated soy lecithin in the amounts added to the salad dressing did not enhance the absorption of fat-soluble micronutrients. The majority of the absorbed fat-soluble micronutrients and enterogenous (apoB-48) and hepatogenous (apoB-100) lipoprotein particles were contained within the large VLDL fraction. Thus this VLDL subfraction has a major role in the transport of newly absorbed carotenoids and fat-soluble vitamins.

**INTRODUCTION**

Salad consumption is a major contributor to vegetable consumption in the United States (1). Although the bioavailability of carotenoids and fat-soluble micronutrients is low in raw vegetables (2-5), the absorption is able to be improved by increasing the fat content of a meal (6, 7). Because high fat intakes are not recommended, one strategy is to find a lipid that is more efficient than triacylglycerol in increasing the bioavailability of fat-soluble micronutrients. Lecithin, as a structural component of mixed micelles and an excellent emulsifier, is likely to enhance the absorption of dietary lipids and lipophilic nutrients. Soybean lecithin was reported to increase the lymphatic absorption of triglyceride in rats (8). Also, after 10 days of lutein consumption with either phosphatidylcholine or lysophosphatidylcholine, the lutein contents in rat plasma, liver, and eyes were significantly higher than those in which lutein was consumed
without phospholipids (9). However, it has not been addressed the effects of dietary lecithin on the bioavailability of carotenoids and fat-soluble vitamins in humans. The exception was one study which showed that multiple doses of an oral preparation of phylloquinone containing lecithin and glycocholic acid maintained phylloquinone status in infants at least equal to that of an intramuscular injection (10).

In our previous studies, after the consumption of a β-carotene dose with a fat-rich meal, we observed that the postprandial appearance of β-carotene and retinyl palmitate in the plasma chylomicron fraction coincided in time and magnitude with their appearance in large very low density lipoproteins (VLDLA) (11, 12). Although the VLDLA subfraction has been used as a vehicle for assessing the intestinal absorption of β-carotene and its major bioconversion product, retinyl palmitate (11-14); there has been little systematic study of the role of VLDLA in the transport of other newly absorbed fat-soluble micronutrients. The postprandial appearance of retinyl palmitate in VLDLA was hypothesized to be attributed to intestinal VLDL (13), but could also reflect the appearance of retinyl palmitate in chylomicron remnants (15). However, VLDLA have long been regarded as liver-derived lipoproteins (16, 17). Apolipoprotein B-48 (apoB-48) and apolipoprotein B-100 (apoB-100) are reliable biomarkers of lipoprotein particles of intestinal and hepatic origin, respectively (18-20). Therefore, an investigation of apoB-48 and apoB-100 in the plasma VLDLA fraction after a single meal could provide new insight regarding the origin of VLDLA.
The objectives of the present study were to investigate the effects of the lecithin/oil ratio in salad dressing on the absorption of fat-soluble micronutrients from salad vegetables. An additional objective was to investigate the origin of VLDLA and the roles of the plasma chylomicron and VLDLA fractions in the transport of the absorbed fat-soluble micronutrients.

SUBJECTS AND METHODS

Subjects

Thirteen healthy, nonsmoking, normolipidemic women, aged 18-40 y, were enrolled in this study. One subject dropped out before completing the first study period due to a gastrointestinal problem that prevented her from consuming the test salad. The mean (± SD) age of the remaining 12 subjects was 27 ± 6.9 y; the mean (± SD) body mass index (BMI; in kg/m²) was 20.7 ± 3.0. The subjects were screened initially by using a standardized questionnaire that addressed health and lifestyle factors. At the time of interview, the applicants’ body weights and heights were measured and recorded. Those who met the eligibility criteria donated a fasting blood sample for a blood biochemistry profile, blood lipid profile, and complete blood count. The exclusion criteria included frequent consumption of alcoholic beverages (> 1 drink/d), current or recent (previous 12 mo) cigarette smoking, pregnant or planning to become pregnant during the study, current or recent (previous 4 mo) use of hormonal contraceptives, use of dietary supplements in the past 1 mo, current or recent (previous 1 mo) ingestion of plant sterols and/or medications known to affect lipid metabolism, body mass index (BMI) ≤ 18 and ≥ 30, known food allergy, and vegetarianism. The SCOFF questionnaire was used to access whether
applicants had a history of restrictive eating (21). Subjects who had 2 or more “yes” responses on the written questionnaire were excluded. All procedures involving human subjects were approved by the Iowa State University Institutional Review Board.

**Test salads**

The test salads consisted of 48 g romaine lettuce (Classic Romaine® Salad Mix, Dole Food Company, Thousand Oaks, CA), 48 g fresh spinach leaves (Spinach, Dole Food Company), 66 g fresh, shredded carrots (Shredded Carrots, Dole Food Company), and 85 g fresh cherry tomatoes (NatureSweet, San Antonio, TX). The total weight (247 g) equaled the 90th percentile of the quantity of salad (lettuce and other vegetables) that was eaten by adults per eating occasion in the 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII) (22). The prepackaged salad vegetables were special ordered to minimize and standardize the time spent in shipment. The cherry tomatoes, romaine lettuce, and spinach leaves were manually sorted to achieve uniform color intensity from week-to-week, and thereby minimize variation in the carotenoid/fat-soluble vitamin contents in the salads. The green color of the romaine and spinach leaves was used as an indicator of chlorophyll and, by extrapolation, carotenoid contents (23).

Representative samples of the spinach, romaine lettuce, cherry tomatoes, and carrots from the test salads consumed during each of the 6 weeks of the study were stored at -70°C. The salad dressings were supplied by Unilever R&D (Vlaardingen, The Netherlands). The salad dressings were prepared by combining 4 g of the lipid phase with 56 g of an aqueous salad dressing base formulation. The lipid phase (4 g total) in the form of phylloquinone- and tocopherol-stripped
soybean oil contained either: 1) 0 g; 2) 0.2 g; or 3) 0.8 g hydroxylated soy lecithin (Solec® 8120, Solae, St. Louis, MO). The phylloquinone and tocopherols were stripped from the soybean oil at Unilever R&D by using a standard short path distillation procedure. The total phospholipid contents of the lipid phases were analyzed by Spectral Service (Cologne, Germany). The 60 g serving of the salad dressing was chosen to simulate the typical intake of salad dressing for a person eating a salad as all or most of a meal. This amount of salad dressing was chosen to correspond with the amount of salad vegetables; thus, 60 g was the 90th percentile of the quantity of low-calorie and full-fat salad dressings reported by adults eating salad components at an eating occasion in the 1994–1996 CSFII (22). Each salad dressing was weighed directly onto the salad and tossed with the salad vegetables to ensure even distribution.

Before consuming the test salad, the subjects first drank 250 mL of water and then waited 15 min to allow for emptying of the stomach. Then, the test salads were consumed together with another 250 mL of water within a 30 min-period. Subjects were instructed to consume the entire salad and then to scrape the residual salad dressing from the bowl with a spatula to ensure that all of the test salad was consumed.

**Experimental design**

Each subject consumed three test salads that had identical vegetable composition and identical aqueous base phase in the salad dressing, but different composition of the lipid phase in the salad dressing: 4 g of soybean oil containing 0 g of Solec® 8120, 3.8 g of soybean oil containing 0.2 g of Solec® 8120, or 3.2 g of soybean oil containing 0.8 g of Solec® 8120. The
subjects consumed each of the three test salads according to the treatment orders of a Williams Latin square design (24). The 12 subjects were each randomly assigned to one of the 6 treatment orders, so that each of the 6 treatment orders was represented twice. The washout period between the test salads was ≥ 2 weeks.

Blood samples were collected from a forearm vein by a licensed medical technician using a butterfly needle blood collection set (BD Safety-Lok TM Blood Collection Set, Becton, Dickinson, and Co, Franklin Lakes, NJ). For days 1-3 of each study period, the subjects were instructed to avoid foods containing high amounts of carotenoids, phylloquinone, tocopherols, and vitamin A. A list of foods to avoid was provided. On day 4, the subjects consumed a weighed, standardized diet containing only minor amounts of carotenoids, tocopherols, phylloquinone, and vitamin A. On day 4, breakfast and dinner were consumed under supervision in the Iowa State University Nutrition and Wellness Research Center; the lunch and snacks were carried out. The nutrient contents of the diet were analyzed by Nutritionist Pro nutrition analysis software (Axxya Systems, Stafford, TX). The daily diet provided 2249 Kcals, 71.57 g protein, 48.10 g fat, and 380.53 g carbohydrate. The calculated carotenoid and fat-soluble vitamin contents of the daily diet were 11.23 μg β-carotene, 0.18 μg lutein (plus zeaxanthin), 0.05 β-cryptoxanthin, 2.97 mg vitamin E, 15.44 μg vitamin K, 56.96 μg RAE vitamin A. On day 5, subjects again reported to the Nutrition and Wellness Research Center. A 10-mL blood sample was collected at baseline after an overnight fast. The subjects consumed the test salad, and additional blood samples were collected at 2, 3.5, 5, 7 and 9.5 h after the test salad consumption.
Blood samples were transferred to 10-mL vacutainer tubes containing spray-dried K$_2$EDTA as anticoagulant (BD Vacutainer®, Becton, Dickinson, and Co). Blood samples were immediately placed on ice and then centrifuged (700 × g, 4° C, 30 min) to separate plasma. During these procedures, blood samples were protected from light. After the 5-h blood sample collection, the subjects consumed a low-fat lunch that was also low in carotenoids, tocopherols, phylloquinone, and vitamin A. On the basis of analysis with the Nutritionist Pro software, the morning snack and lunch were estimated to contain 0.32 g and 1.50 g fat, respectively.

**Triglyceride-rich lipoprotein (TRL) isolation**

Chylomicrons and large VLDL (VLDLA) were isolated from plasma by cumulative rate ultracentrifugation (25, 26). To form the density gradient, salt solutions were prepared, including a 1.006 g/mL sodium chloride stock solution at pH 7.0, and 1.020 g/mL and 1.065 g/mL potassium bromide (KBr) solutions prepared from the 1.006 g/mL sodium chloride stock solution. The densities were confirmed by using a digital density meter (DMA-48; Anton-Paar USA, Ashland, VA). Four milliliters of plasma were transferred to a centrifuge tube (Ultra Clear, Beckman Instruments, Inc, Spinco Division, Palo Alto, CA), and 0.14 g KBr/mL was added to adjust the density to 1.10 g/mL. The prepared salt solutions were overlaid on the plasma in order of highest to lowest density: 1) 3 mL of 1.0065 g/mL density solution; 2) 3 mL of 1.020 g/mL density solution; 3) 2 mL of 1.006 g/mL density solution. Because there were only six places in each Beckman SW 40i swinging bucket rotor, two ultracentrifuges (L8-70M, L-90K; Beckman Instruments Inc.) were used to centrifuge the plasma samples at 28,300 rpm (101,136 × g) for 43
min. The chylomicron fraction was removed and the tube was refilled with 1.006 g/mL density solution. The tube then underwent a second ultracentrifugation at 40,000 rpm (142,948 × g) for 67 min. The VLDLA fraction was then removed from the top of the centrifuge tube. The chylomicron and VLDLA fractions were stored at -70°C until analyzed. All procedures were performed under yellow light.

**ELISA assays of apolipoproteinB-48 and total apolipoprotein B**

The contents of apolipoprotein B-48 (apoB-48) and total apolipoprotein B (total apoB) in the chylomicron and VLDLA fractions were analyzed in triplicate by using commercial apoB-48 (Human ApoB-48 ELISA kit, Shibayagi, Gunma, Japan) and total apoB (Total Human Apolipoprotein B ELISA assay, ALeRCHECK, Inc. Portland, Maine) ELISA kits. A 175-µL aliquot of each chylomicron fraction and a 60-µL aliquot of each VLDLA fraction were analyzed by ELISA. The remainder of each lipoprotein fraction was used for fat-soluble micronutrient analyses by HPLC with coulometric array electrochemical detection (HPLC-ECD). The postprandial apoB-100 contents were obtained by subtracting the apoB-48 content from the total apoB content within each chylomicron and VLDLA sample. The calculated apoB-100 contents were accurate because the apoB-48 ELISA has no cross reactivity with apoB-100 (27, 28), and the total apoB standard was calibrated against an international reference material (29).

To evaluate the precision of the ELISA assays, we used a pooled plasma chylomicron and VLDLA fraction as a quality-control material. Excess plasma collected during the study was
combined and used to isolate the chylomicron and VLDLA fractions. The two fractions were then pooled, divided into multiple vials, and stored at -70°C. Three replicates of the quality-control material were routinely analyzed with each ELISA assay. The mean (± SD) apoB-48 and total apoB contents in the quality-control material were 0.945 ± 0.0386 mg/L and 7.59 ± 0.264 mg/L, respectively. For the apoB-48 ELISA assay, the inter-assay CVs were 5.89% and 7.07% in the chylomicron and VLDLA sample sets, respectively. For the total apoB ELISA assay, the inter-assay CVs were 7.16% and 6.36% in the chylomicron and VLDLA sample sets, respectively.

**HPLC-ECD analyses of carotenoids and fat-soluble vitamins in the plasma triacylglycerol-rich lipoprotein (TRL) fractions**

The contents of the α- and β-carotene, lutein, lycopene, retinyl palmitate, phylloquinone, and α- and γ-tocopherol in the plasma triacylglycerol-rich lipoprotein fractions were analyzed by HPLC with coulometric array electrochemical detection (HPLC–ECD). The retinyl palmitate was analyzed as the major bioconversion product of the α- and β-carotene consumed in the salad vegetables. Each chylomicron/VLDLA sample (~2 mL) was deproteinated by addition of an equal volume of methanol (Fisher Scientific, Chicago, IL), followed by two extractions with 4 mL of hexane (EMD Chemicals Inc. Gibbstown, NJ) containing 1 g/L butylated hydroxytoluene (BHT) (Fisher Scientific, Chicago, IL) to extract carotenoids, retinyl palmitate, phylloquinone, and tocopherols. The combined hexane layers were dried by using a speed vacuum evaporator (Model SPD 131 DDA, Thermo Electron Corporation, Milford, MA) with a universal vacuum
system (UVS 800 DDA, Thermo Electron Corporation), and redissolved in 30 μL methanol (EMD Chemicals Inc. Gibbstown, NJ) and 30 μL methyl-tert-butyl ether (MTBE) (Sigma, St. Louis, MO). A 25 μL aliquot was injected into the HPLC−ECD system. The carotenoids, retinyl palmitate, phylloquinone, and tocopherols were separated on a C30 Carotenoid Column (Waters, Milford, MA) (30). The mobile phase consisted of methanol (EMD Chemicals Inc., Gibbstown, NJ), MTBE (Sigma, St. Louis, MO) and aqueous ammonium acetate buffer (1.0 M, pH 4.6) (Sigma, St. Louis MO). The proportions in mobile phases A and B of methanol:MTBE:ammonium acetate were 95:3:2 (by vol) and 25:73:2 (by vol), respectively. The following gradient was used: 0-1 min, 100% mobile phase A; 1-4 min, linear gradient to 75% mobile phase B; 40-60 min, linear gradient to 100% mobile phase B. The flow rate was 1.0 mL/min. An ESA CoulArray system (Chelmsford, MA) was operated by using CoulArray software (CoulArrayWin 2.0) and consisted of a 542 auto sampler set at 4°C, two 582 solvent delivery modules, CoulArray thermal organizer, and a 16-channel 5600A CoulArray detector. The applied detector cell potentials included the predominant response potential for carotenoids (450 mV), retinyl palmitate (750 mV), and tocopherols (300 mV). For the simultaneous analysis of phylloquinone, reductive potentials (-800 mV) were applied to two detector channels, which were followed by two oxidative potentials (50 mV, 200 mV) on the subsequent two detector channels. The 50 mV channel was used for the quantification of phylloquinone. The detection limits for α-carotene, β-carotene, lycopene, lutein, phylloquinone, α-tocopherol, and γ-tocopherol were 0.010 μg/L, 0.008 μg/L, 0.046 μg/L, 0.046 μg/L, 0.027 μg/L, 3.872 μg/L, and 0.272 μg/L,
respectively. Trans-lycopene was the only lycopene isomer detected in the samples. All sample preparations and extractions were performed under yellow light.

**HPLC-ECD analyses of carotenoids and fat-soluble vitamins in the salad vegetables**

Representative samples of the spinach, romaine lettuce, cherry tomatoes, and carrots from the test salads consumed during each of the 6 weeks of the study were stored at -70°C and analyzed in duplicate. A modification of the method of Granado et al. (30) was used to extract and analyze carotenoids and tocopherols. Frozen vegetable samples were thawed at room temperature before being processed in a food processor (Handy Chopper Plus HC3000; Black & Decker Corp, Towson, MD). Briefly, carotenoids and tocopherols were extracted from 1 g of the vegetable homogenate with 12 mL of methanol containing 1 g/L BHT (Fisher Scientific, Chicago, IL) and 12 mL of tetrahydrofuran (THF) containing 1 g/L BHT (VWR, Boston, MA). An 0.5 mL aliquot of the methanol/THF extract was transferred to a screw-capped test tube and combined with 1.0 mL of 0.4 g/mL potassium hydroxide in methanol containing 0.1 M pyrogallol. Each tube was flushed with argon and tightly capped before vortexing for 3 minutes. Two mL of ultrapure water were added and the tube was vortexed again for 30 sec. Four mL of hexane/methylene chloride (5:1 by volume) was added and thoroughly mixed by vortexing for 60 sec. The sample was then centrifuged to separate the organic phase. The organic phase was evaporated to dryness under vacuum. The dried lipid extract was reconstituted with 1000 µL each of MTBE and methanol; a 25 µL aliquot was injected into the HPLC-ECD system. All procedures were carried out under yellow light.
The procedure for extracting phylloquinone from the vegetables was a modification of a method of Koivu et al. (31). Ten milliliters of 2-propanol were added to 1 g of homogenized vegetable sample. After vortexing for 2 min, 5 mL of hexane were added and the sample was vortexed for 2 min. The sample was then re-extracted using another 5 mL of hexane. Five mL of ultrapure water were added followed by vigorous shaking. Each sample was centrifuged for 5 min at 700 × g to separate the organic and water phases. For romaine lettuce and spinach, the total volume of the hexane layer was recorded and a 1 mL aliquot was evaporated to dryness under vacuum. Thus, the phylloquinone content in 1 g of lettuce or spinach was obtained by multiplying the content in 1 mL aliquot by the recorded total hexane volume. Because of the low contents of phylloquinone in cherry tomatoes and carrots, the entire hexane layer (about 10 mL) was dried down under vacuum. Each dried extract was reconstituted with 100 µL of MTBE and 100 µL of methanol. A 25 µL aliquot was injected into the HPLC-ECD system as described above.

**Statistical analyses**

Statistical analyses were performed by using SAS (version 9.2; SAS Institute Inc., Cary, NC). The outcome variables were the 0–9.5 h area under the curve (AUC) values in the plasma chylomicron and VLDLA fractions for carotenoids, phylloquinone, retinyl palmitate, tocopherols, apoB-48, apoB-100 and total apoB. The AUC values were calculated by the trapezoidal method using analyte content (expressed as the content in the chylomicron or VLDLA fractions isolated from 1 L plasma) as the y axis and time (h) as the x axis.
ANCOVA was used to estimate the differences among the salad dressings in the AUC values of the fat-soluble micronutrients in the chylomicron and VLDLA fractions. Visual inspection of the QQ plots of the residuals showed that log transformation of the AUC values eliminated unwanted curvature. Therefore, the AUC values were log transformed to achieve normal distributions and analyzed by mixed model analysis of covariance (ANCOVA) using treatment and study period as fixed factors, subject as random factor, and the log of the baseline (0 h) analyte content [log(baseline)] as a covariate. Log(baseline) was not a significant covariate for phylloquinone in chylomicron and VLDLA fractions. Therefore, these outcome variables were analyzed by a mixed model analysis of variance (ANOVA) with AUC as the dependent variable and treatment and study period as fixed factors and subject as random factor. The need for inclusion of treatment × study period and log(baseline) × treatment interaction terms was evaluated by using a custom model that included treatment and covariate as main effects and also these interaction terms. The log(baseline) × treatment interaction was not significant for any of the analytes in either lipoprotein fraction (chylomicron or VLDLA) and thus this interaction term was excluded from the model. There was a significant treatment × study period interaction for α-carotene and β-carotene in VLDLA; thus this interaction term was retained as an additional fixed effect in the model for these outcome variables. Posthoc multiple comparisons of least squares means were calculated by paired Student’s t-test using Tukey’s adjustment. \( P \) values < 0.05 were considered significant.
The baseline (0 h) contents of carotenoids, retinyl palmitate, and tocopherols in the plasma chylomicron fraction were compared with those in the VLDLA fraction by using paired Student’s t-tests, which indicated significant baseline differences between the two fractions ($P < 0.001$). Due to these baseline imbalances, ANCOVA with baseline as covariate became problematic. If log baseline values were used as a covariate, AUC values for each analyte in each of the two lipoprotein fractions were modeled as least squares means. To adjust for differences in baseline analyte content across subjects, the least squares mean for the AUC values with log baseline as a covariate was calculated at the average baseline value for that analyte across all subjects. However, this approach cannot adjust for the baseline differences between fractions. Thus, the incremental AUC (iAUC) values were calculated and used to compare the postprandial responses for each carotenoid or fat-soluble vitamin in the plasma chylomicron versus VLDLA fractions. The iAUC, which was defined as the area under the curve and above the baseline (0 h) value, was calculated by trapezoidal approximation. If the postprandial content of a carotenoid or fat-soluble vitamin in a lipoprotein fraction at a specific time point was lower than the baseline fasting content, then that area below the baseline was included as a negative contribution toward the total calculated iAUC value. The iAUC values in chylomicron and VLDLA fractions were compared by ANOVA with fraction and treatment as main factors and treatment × fraction interaction as random factor.

Repeated-measures ANOVA was used to compare differences between the baseline (0 h) and postprandial contents of carotenoids, fat soluble vitamins, apoB-48, and apoB-100 in the
plasma lipoprotein fractions at the individual time points, 2 h, 3.5 h, 5 h, 7 h, and 9.5 h. The repeated-measures ANOVA involved treatment and time as fixed factors and subject and subject × treatment as random factors. The least squares means were compared by post-hoc t-tests by using Tukey’s adjustment.

For the analytes that had significant postprandial increments, the times to reach maximal contents in the plasma lipoprotein fractions (tmax) were determined from visual examination of the data. The tmax values of each analyte were compared between fractions by ANOVA with fraction, treatment and treatment × fraction interaction as fixed factors. The least squares means were compared by post-hoc t-tests.

The AUC values of apoB-48 and apoB-100 were compared between fractions by ANOVA with fraction and treatment as main factors and treatment × fraction as random factor. The apoB-48 AUC value and apoB-100 AUC values were compared within each fraction by ANOVA with analyte and treatment as main factors and treatment × analyte as random factor. P values < 0.05 were considered significant.

RESULTS

Carotenoid, phylloquinone, and tocopherol contents in the test salads

The carotenoid, phylloquinone, and tocopherol contents in the test salads are presented in Table 1. Across the 6 wk of the study, the mean contents (± SEs) of total carotenoids (α-carotene, β-carotene, lutein, zeaxanthin, and lycopene), total tocopherols (α- and γ-tocopherols), and phylloquinone in the 247-g test salads were 30.660 ± 0.430 mg, 4.786 ± 0.202 mg, and 0.153
± 0.006 mg, respectively. Thus, the contents of the bioactive components of interest in the raw vegetables were remarkably consistent. The contents of α- and γ-tocopherol varied the most among all of the analytes in the salad vegetables. Tocopherol contents in vegetables are dependent on growing conditions, e.g., growing season, sunlight intensity, and soil state (32-34).

**Postprandial changes in carotenoid and fat-soluble vitamin contents in the plasma chylomicron and VLDLA fractions**

There were no significant differences among the three salad dressings in the AUC values for the carotenoids or fat-soluble vitamins in the postprandial chylomicron or VLDLA fractions. The exception was a decrease in the AUC value of phylloquinone in the plasma chylomicron fraction when the salad dressing containing 3.2 g soybean oil and 0.8 g Solec® 8120 was consumed compared with the salad dressing containing 4 g soybean oil and 0 g Solec® 8120 ($P < 0.02$, **Figure 1**). In contrast, the AUC values for phylloquinone in the VLDLA fraction were not different between the three salad dressings. No significant differences were observed among the salad dressings in the AUC values for apoB-48 and apoB-100 in the postprandial chylomicron and VLDLA fractions.

The iAUC values for each analyte between chylomicron and VLDLA fractions were compared. In this statistical model, the effects of the salad dressing treatments were not significant for any of the analytes, including phylloquinone. In contrast, in the previous statistical model (above), the chylomicron AUC value for phylloquinone was significantly lower when salad vegetables were consumed with the salad dressing containing 0.8 g Solec® 8120
compared with the salad dressing containing 0 g Solec® 8120. This discrepancy reflects the overwhelming predominance of the appearance of phylloquinone in the VLDLA fraction, no matter which of the three salad dressings was consumed. In other words, the postprandial distribution of phylloquinone between the chylomicron and VLDLA fractions was not affected by the addition of Solec® 8120 hydroxylated soy lecithin to salad dressing in the amounts used in the current study. The iAUC values for α-carotene, β-carotene, lycopene, retinyl palmitate, and phylloquinone were substantially higher in the VLDLA than in the chylomicron fraction (P < 0.05, Table 2). For example, β-Carotene, the major pro-vitamin A carotenoid in the salad vegetables, was primarily accumulated in the VLDLA fraction with mean (± SE) iAUC value of 58.805 ± 8.608 nmol•h/L, whereas its mean (± SE) iAUC value in the chylomicron fraction was 18.515 ± 2.055 nmol•h/L. In addition, significant increases from baseline in the α-carotene, β-carotene, lycopene, retinyl palmitate, and phylloquinone contents were observed in both the chylomicron and VLDLA fractions as early as 2 h after salad consumption (Figures 2 and 3). The mean (± SE) difference for tmax values of α-carotene, β-carotene, lycopene, retinyl palmitate, and phylloquinone in chylomicron fraction versus VLDLA fraction was -0.986 ± 0.505, -0.792 ± 0.492, -1.361 ± 0.868, -2.153 ± 0.592, -1.000 ± 0.6931, respectively. The tmax values of α-carotene, β-carotene, lycopene, retinyl palmitate, and phylloquinone were significantly lower in the chylomicron fraction than in the VLDLA fraction (P < 0.01), which means the maximal contents of these fat-soluble micronutrients were reached earlier in the chylomicron fraction compared with the VLDLA fraction (Figures 2 and 3).
In contrast with the other carotenoids, the mean iAUC value for lutein in the plasma VLDLA fraction was negative (-5.086 ± 2.918 nmol•h/L), even though there was a substantial amount of lutein in the salad vegetables (Table 1). The VLDLA lutein contents at 2, 3.5, 5, 7, or 9.5 h were not significantly different from the baseline (0 h) content (Figure 4). However, the mean iAUC value for lutein in the plasma chylomicron fraction was positive (2.608 ± 0.465 nmol•h/L), and the plasma chylomicron lutein contents at 3.5 h and 5 h were significantly higher than the baseline (0 h) content ($P < 0.05$). Thus, our data suggest that, unlike the other carotenoids, newly absorbed lutein was primarily transported by the plasma chylomicron fraction with little or no accumulation in the VLDLA fraction.

Under our study conditions, there were no postprandial increments in $\alpha$- or $\gamma$-tocopherols in either the plasma chylomicron or VLDLA fractions. The $\alpha$- and $\gamma$-tocopherol contents in the chylomicron fraction did not significantly change until 7 h; then significant decreases from baseline were observed at both 7 h and 9.5 h ($P < 0.01$) (Figure 4). In the VLDLA fraction, significant decreases in the $\alpha$- and $\gamma$-tocopherol contents were observed as early as 3.5 h ($P < 0.01$) (Figure 4).

Overall, after consumption of the test salads with salad dressings containing only 4 g of total lipids, we observed notable differences among the fat-soluble micronutrients in their postprandial appearances in the plasma lipoprotein fractions.

**Postprandial changes in apolipoprotein B contents in the plasma chylomicron and VLDLA fractions**
The plasma chylomicron and VLDLA fractions each contained both apoB-48 and apoB-100, which is consistent with other studies (15, 29, 35-38). The majority of the postprandial apoB-48 and apoB-100 was found in the VLDLA fraction (Figure 5). The mean (± SE) absolute AUC values for apoB-48 in the chylomicron and VLDLA fractions were 0.535 ± 0.076 mg•h/L and 7.518 ± 0.918 mg•h/L, respectively. The apoB-48 absolute AUC value in the VLDLA fraction was significantly higher than that in the chylomicron fraction (P < 0.0001). Also, the mean (± SE) apoB-48 content in the VLDLA faction at baseline (0 h) (0.778 ± 0.115 mg/L) was about 10 times higher than the maximal postprandial mean (± SE) content of apoB-48 in the chylomicron fraction (0.080 ± 0.015 mg/L, at 2 h) (Figure 5). Significant decreases in the apoB-100 content from baseline were observed at 7 h in the chylomicron fraction (P < 0.01) and at 5 h, 7 h, and 9.5 h in the VLDLA fraction (P < 0.01), and significant increases in the apoB-48 content from baseline were observed at 2 h and 3.5 h in the chylomicron fraction (P < 0.01) whereas no significant changes apoB-48 content were found in the VLDLA fraction (Figure 5). One lipoprotein particle only contains one type of apoB, and apoB-48 and apoB-100 are reliable biomarkers for intestine- and liver-derived lipoproteins, respectively (18-20). Thus, the changes in the apoB-48 and apoB-100 contents reflect the particle number changes in intestine- and liver-derived lipoproteins, which means, in the postprandial state, the particle number of chylomicron-sized enterogenous lipoproteins increased, and the particle number of chylomicron- and VLDLA-sized hepatogenous lipoproteins decreased. The mean (± SE) absolute AUC values for apoB-100 in the chylomicron and VLDLA fractions were 1.110 ± 0.130 mg•h/L and 60.705 ±
5.748 mg·h/L, respectively. As expected, the apoB-100 AUC value in the VLDLA fraction was significantly higher as compared with the chylomicron fraction ($P < 0.0001$). Moreover, the apoB-100 absolute AUC value was substantially higher than the apoB-48 AUC value in both the plasma chylomicron and VLDLA fractions ($P < 0.0001$). Thus, apoB-100 had greater postprandial appearance in both the chylomicron and VLDLA fractions than did apoB-48 after salad consumption.

**DISCUSSION**

In the current study, the apparent intestinal absorption of fat-soluble micronutrients from salad vegetables was measured based upon their postprandial appearances in the plasma chylomicron and VLDLA fractions. Chylomicrons have long been regarded as the major lipoproteins that transport newly absorbed fat-soluble vitamins and other fat-soluble bioactive food components. However, recently our group and others reported that measuring the appearance of retinyl esters in the VLDLA fraction is necessary to avoid underestimating total vitamin A absorption (13, 14). Retinyl esters are formed in the enterocytes from provitamin A carotenoids, incorporated into chylomicrons, and subsequently cleared into liver hepatocytes in which they are hydrolyzed to retinol (39). The liver secretes vitamin A back into the plasma primarily in the form of retinol bound to retinol-binding protein rather than in the form of retinyl esters with hepatogenous lipoproteins (40). Thus, in the VLDLA fraction, the great increment in retinyl palmitate content in the VLDLA fraction most likely reflected the quantity of newly absorbed retinyl palmitate that was formed in the enterocytes from the α- and β-carotene
consumed in the salad vegetables. Moreover, it has been shown that the appearance of β-carotene in plasma after a single oral dose is biphasic (41, 42). The early peak at 5-6 h post dosing represents the newly absorbed dietary β-carotene (43, 44), and the late and larger peak at 24-48 h post dosing represents hepatic secretion of β-carotene in liver-derived lipoproteins (12). In the current study, we observed that the contents of α- and β-carotene, lycopene, retinyl palmitate, and phylloquinone peaked at 3.5 h and 5 h in the chylomicron and VLDLA fractions, respectively (Figure 2 and 3). Thus, the increments in the carotenoid and phylloquinone contents in both lipoprotein fractions should reflect the quantities that were newly absorbed. However, their tmax values in the two fractions were not completely coincident; the later tmax within the VLDLA fraction than within the chylomicron fraction may reflect a precursor product relation.

Based upon the AUC values, the postprandial appearance of α-carotene, β-carotene, lycopene, retinyl palmitate, and phylloquinone in the VLDLA fraction greatly exceeded their appearance in the chylomicron fraction (Table 2; Figures 2 and 3) (P < 0.05). Thus, the majority of the newly absorbed fat-soluble micronutrients from the salad vegetables were contained within the large VLDL fraction in the context of this study in which the co-consumed salad dressing contained only 4 g of lipids. In our earlier study, when a large β-carotene dose (25 mg) was consumed with a 33-g fat load, Paetau et al. (12) reported that β-carotene was primarily accumulated within the plasma VLDL fraction and there was a somewhat lower accumulation in chylomicrons. In a subsequent study from our group, Hu et al. (11) showed that postprandial
accumulations of β-carotene and its major bioconversion product, retinyl palmitate, were of similar magnitude in the chylomicron and VLDLA fractions after a large (25 mg) dose of β-carotene was consumed with 60 g of either beef tallow or sunflower oil. These findings suggest that the distributions of β-carotene and retinyl palmitate in the chylomicron and VLDLA fractions may depend on the amount of co-consumed fat. When the meal is rich in fat, newly absorbed β-carotene and retinyl palmitate are incorporated into chylomicron and VLDLA fractions in similar proportion. When fat is limited, e.g. 4 g of total lipids in the current study, the VLDLA fraction contains the major transport particles for newly absorbed β-carotene and retinyl palmitate. Under the current study conditions, newly absorbed α-carotene, lycopene, and phylloquinone were primarily accumulated in the VLDLA fraction instead of the chylomicron fraction. This finding highlights the need for additional human studies regarding the effects of the fat content of the test meal on the distributions of these fat-soluble micronutrients in plasma lipoproteins, especially in chylomicrons and VLDLA particles. Our study highlights the importance of measuring fat-soluble micronutrients in the postprandial VLDLA fraction to avoid underestimating carotenoid (α-carotene, β-carotene, and lycopene), retinyl palmitate, and phylloquinone absorption.

In contrast to the other carotenoids, newly absorbed lutein from the salad vegetables was primarily incorporated into the plasma chylomicron fraction (Figure 4). Paetau et al. (12) reported a rapid accumulation of canthaxanthin in chylomicron, VLDL and LDL fractions that began as early as 2 h post-dosing. Canthaxanthin, which was used as a model xanthophyll
carotenoid, has plasma kinetics similar to those of lutein \((45, 46)\). The xanthophyll distribution difference in postprandial lipoproteins might be caused by differences in fat content. In our current study, only 4 g of total lipids were consumed, whereas 33 g of fat were consumed in our previous study by Paetau et al. These findings seem to suggest potential effects of fat content on the lipoprotein distribution of xanthophyll carotenoids, and the fat effects may be different from those on the distributions of other carotenoids, such as \(\beta\)-carotene.

There were no postprandial increments in \(\alpha\)- or \(\gamma\)-tocopherols in either the plasma chylomicron or VLDLA fractions when the salad was consumed with the test salad dressings containing a total of only 4 g of soybean lipids (Figure 4). Reboul et al. \((47)\) reported that \(\alpha\)-tocopherol is not secreted in chylomicrons when lipids are absent. Similarly, Anwar et al. \((48)\) showed that tocopherols were mostly incorporated into high density lipoproteins (HDL) in the absence of oleic acid. In our previous study \((49)\), subjects consumed test salads containing amounts of salad vegetables that matched those in the current study. Significant increments in \(\alpha\)- and \(\gamma\)-tocopherol contents in chylomicrons were observed only when the test salads were consumed with salad dressings containing 8 g or 32 g soybean oil. Therefore, in the current study, the absence of newly absorbed \(\alpha\)- and \(\gamma\)-tocopherols in chylomicron and VLDLA fractions may be caused by the limited amount of co-consumed soybean oil and a resulting incorporation into HDL.

In humans, apoB-48 is secreted by the intestine and is a reliable marker of lipoprotein particles of intestinal origin \((18-20)\). ApoB-100 is primarily synthesized by the liver and is
regarded as a marker of lipoprotein particles of hepatic origin (17, 20). Chylomicrons and VLDLA are usually referred to as apoB-48- and apoB-100-containing lipoproteins, respectively (16). However, increasing evidence, including our current study, indicates that the chylomicron and VLDLA fractions each contain both apoB-48 and apoB-100 (15, 29, 35-38). The evidence suggests that chylomicon and VLDLA fractions as isolated from human plasma each consist of both enterogenous and hepatogenous lipoprotein particles. The chylomicrons and VLDLAs were isolated based upon their particle densities and sizes. Thus, the presence of apoB-48 and apoB-100 in both fractions shows that some postprandial apoB-48-containing lipoproteins have similar particle sizes with apoB-100-containing lipoproteins, as hypothesized by Nakano et al. (50). The AUC value of apoB-48 was substantially higher in the VLDLA fraction than in the chylomicron fraction ($P < 0.0001$). This finding implies that more enterogenous lipoprotein particles were contained in the VLDLA fraction, which can, at least partially, explain the greater postprandial appearance of $\alpha$-carotene, $\beta$-carotene, lycopene, retinyl palmitate, and phylloquinone in the VLDLA fractions as compared with the chylomicron fraction. Likewise, a higher AUC value for apoB-100 was observed in the VLDLA fraction than in the chylomicron fraction ($P < 0.0001$), which means that, as expected, more particles of hepatic origin were accumulated in the VLDLA fraction. We observed higher baseline contents of carotenoids, retinyl palmitate, and tocopherols in the VLDLA fraction. These higher baseline contents may be explained by the fact that plasma hepatogenous lipoprotein particles transport fat-soluble micronutrients, except retinyl palmitate, out of the liver (4).
The greater postprandial appearance of apoB-48 in the VLDLA fraction compared with the chylomicron fraction raises the question of the origin of the apoB-48-containing particles in VLDLA. Two hypotheses may explain their origin: 1) they are the remnants of chylomicrons; 2) chylomicrons are not the sole precursor for VLDLA and a significant proportion of apoB-48-containing VLDLA particles are directly secreted by the intestine. The first hypothesis is based on the metabolism of fat-soluble micronutrients (51-54). Fat-soluble micronutrients are taken up by the enterocytes, incorporated into chylomicrons, and transferred into the blood circulation via the lymphatic system (55). In the bloodstream, the chylomicron triacylglycerols are hydrolyzed by the action of lipoprotein lipase, yielding chylomicron remnants (56, 57). This hydrolysis process has been examined by labeling the triacylglycerol component of chylomicrons, and kinetic analyses showed it occurs within minutes (51, 52). The second hypothesis was obtained from a lipoprotein kinetics study by Zheng et al. (15) in which apoB-48 and apoB-100 were labeled by stable isotopes. The study indicated that the human intestine and liver are able to release various sizes of apoB-48 lipoproteins and apoB-100 lipoproteins, respectively, which range in size from chylomicrons- and VLDLs-, down to IDL-sized lipoproteins. This finding implies that intestinal and hepatic lipoproteins do overlap in particle size, which has been supported by several recent studies (50, 58). This hypothesis is also applicable to explain the origin of the apoB-100-containing particles in the chylomicron fraction in our study, i.e., they are secreted by the liver. We also observed that the contents of α- and β-carotene, lycopene, retinyl palmitate, and phylloquinone peaked significantly earlier in the chylomicron fraction compared
with the VLDLA fraction ($P < 0.01$). This finding can be explained by the first hypothesis, i.e.
the $t_{\text{max}}$ delay in the VLDLA fraction was attributed to the lipolysis of chylomicron
triacylglycerols. However, we only had 5 postprandial blood collection points. Thus, it is
possible that we missed the actual $t_{\text{max}}$ for both the fractions, and they might overlap each other.
Because a limited number of time points were applied in the current study, we cannot conclude
with certainty which hypothesis is more applicable to our data.

The apoB-100 AUC value was higher than the apoB-48 AUC value in both the chylomicron
and VLDLA fractions ($P < 0.0001$). Thus, the overall production of apoB-100 appears to be
much higher than that of apoB-48, which has been shown by other studies (15, 36). On the other
hand, the high content of apoB-100 may be caused by the delayed lipolysis of apoB-100-
containing lipoproteins that must compete for lipoprotein lipase active sites with apoB-48-
containing lipoproteins (51-54, 59). With the markedly higher apoB-100 content compared with
apoB-48, we may conclude that the major postprandial lipoprotein is apoB-100-containing
lipoprotein. We also observed significant decreases of apoB-100 content from baseline in both
the chylomicron and VLDLA fractions ($P < 0.01$), which is consistent with the finding in the
apoB-48/100 kinetic study by Zheng et al. (15) that the postprandial apoB-100 mass decreased in
the chylomicron and light VLDL fractions. The reduction in apoB-100 content suggests that the
number of hepatogenous lipoproteins decreases. Recently, it was reported that the major
increase in postprandial triacylglycerol was contained in apoB-100-containing lipoproteins
instead of in apoB-48-containing lipoproteins (59). Therefore, the decrease of apoB-100-
containing particle number may be compensated by the increase of particle size by which apoB-100-containing lipoproteins contain more fat-soluble micronutrients than apoB-48-containing lipoproteins. In the current study, the significant increase of apoB-48 content was observed only in the chylomicron fraction, whereas Campos et al. (29) reported a increase in both chylomicron and VLDL fractions but a decrease in LDL fraction after a high-fat (104 g) breakfast consumption. One explanation is that a highfat load stimulates the intestine to secrete more large VLDL-sized apoB-48 containing particles but less small LDL-sized apoB-48 containing particles in order to adjust the absorbed dietary fat (29). The apoB-48/100 postprandial content depends on its production rate and clearance rate both of which were not clear in the current study due to no stable isotope tracers were applied (15, 60).

In the current study, the addition of Solec® 8120, a commercial hydroxylated soy lecithin to salad dressing did not enhance the intestinal absorption of fat-soluble micronutrients from salad vegetables. The AUC value for phyloquinone in the plasma chylomicron fraction was decreased when the salad dressing containing 3.2 g soybean oil and 0.8 g Solec® 8120 was consumed compared with the salad dressing containing 4 g soybean oil and 0 g Solec® 8120 ($P < 0.02$, Figure 1). The decrease in the AUC value of phyloquinone may be attributed to the low postprandial contents of phyloquinone in the chylomicron fraction. In the chylomicron fraction, the maximal postprandial content of phyloquinone (0.23 ± 0.09 nmol/L) was about 20 and 5 times lower than those of β-Carotene (4.58 ± 1.27 nmol/L) and α-carotene (1.15 ± 0.36 nmol/L), respectively, when 3.2 g soybean oil and 0.8 g Solec® 8120 were consumed. The bioavailability
of phylloquinone varies not only with the consumed fat content but also with the consumed fat composition (5, 7). Jones et al. (61) reported that a polyunsaturated fatty acid-rich meal resulted in lower phylloquinone absorption compared with the other two meals that contained 2-fold lower polyunsaturated fatty acid. But no systematic studies have addressed the effects of lecithin composition and/or content on the absorption of phylloquinone. Moreover, in our study, phylloquinone was consumed from vegetables, whereas 20 μg of free $^{13}$C-labelled phylloquinone supplement were consumed with a fat-rich meal in the study by Jones et al (61). Further investigations are thus needed to thoroughly assess the roles of lecithin and polyunsaturated fatty acid × food matrix interaction in phylloquinone absorption. Lysophosphatidylcholine, a common component in commercial soy lecithins, was shown to enhance the bioavailability of β-carotene, lutein, and α-tocopherol in rats, mice, and Caco-2 human intestinal cells (9, 62-64). The acyl chain length of phospholipids is also a critical factor influencing the bioavailability of fat-soluble micronutrients (65). Yonekura and colleagues (65) reported that both medium-chain phosphatidylcholine and long-chain lysophosphatidylcholine increased β-carotene uptake. In our study, we may have observed enhanced bioavailability of the fat-soluble micronutrients in the salad vegetables if homogeneous long-chain lysophosphatidylcholine had been added to the salad dressings. However, pure lysophosphatidylcholine leads to lower quality salad dressings. Among the commercially-available lecithins, the hydroxylated soy lecithin in Solec® 8120 was determined to have the best product properties and taste profile. It also improved acid stability.
In the current study, the apoB-48 content in both fractions was analyzed by apoB ELISA assay. The assay uses a newly available apoB-48-specific monoclonal antibody that was raised against the apoB-48 C-terminal decapetide (KLSQILQTYMI) and calibrated by using recombinant apoB-48 antigen (27). The recovery of apoB-48 exceeds 90% (27). The ELISA assay values are highly correlated with values obtained by gel scanning, which has often been used to quantify apoB-48 (28, 16). However, compared with the ELISA assay values, the apoB-48 values are underestimated by 50% with the gel scanning method (28). In the current study, the intra- and inter-assay precision of the apoB-48 ELISA was evaluated by using a quality-control material. The inter-assay CVs and mean intra-assay CV were excellent (< 8%) in both the chylomicron and VLDLA samples. The high-recovery and sensitivity of the apoB-48 ELISA confirm that it is a reliable method to quantify apoB-48 content in plasma samples.

In conclusion, the addition of Solec® 8120 hydroxylated soy lecithin to salad dressing in the amounts used did not enhance the intestinal absorption of fat-soluble micronutrients from salad vegetables. This study showed that the plasma VLDLA fraction contained the major transport particles for α-carotene, β-carotene, lycopene, retinyl palmitate, and phylloquinone over a 9.5-h period after consuming these bioactive components in salad vegetables with only 4 g of total lipid in salad dressing. We showed that the plasma VLDLA fraction contains more enterogenous (apoB-48) and hepatogenous (apoB-100) lipoprotein particles as compared with the chylomicron fraction. In this study, the fat-soluble micronutrients were consumed as they...
naturally occur within salad vegetables. Thus, our experimental conditions were similar to real diets and the outcomes have more realistic and physiological meanings.

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### TABLE 1

Carotenoid, tocopherol, and phylloquinone contents in the vegetables in the test salads

<table>
<thead>
<tr>
<th>Component</th>
<th>Weight</th>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>Lutein</th>
<th>Lycopene</th>
<th>α-Tocopherol</th>
<th>γ-Tocopherol</th>
<th>Phylloquinone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot, grated</td>
<td>66</td>
<td>5.407 ± 0.318 (^2)</td>
<td>7.709 ± 0.372</td>
<td>0.211 ± 0.022</td>
<td>– (^3)</td>
<td>0.424 ± 0.019</td>
<td>0.056 ± 0.001</td>
<td>0.002 ± 0.000</td>
</tr>
<tr>
<td>Lettuce, romaine</td>
<td>48</td>
<td>1.394 ± 0.139</td>
<td>1.603 ± 0.121</td>
<td>–</td>
<td>0.281 ± 0.023</td>
<td>0.277 ± 0.021</td>
<td>0.075 ± 0.006</td>
<td></td>
</tr>
<tr>
<td>Spinach, leaf</td>
<td>48</td>
<td>2.172 ± 0.078</td>
<td>3.572 ± 0.171</td>
<td>–</td>
<td>1.576 ± 0.135</td>
<td>0.148 ± 0.008</td>
<td>0.071 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>Tomato cherry</td>
<td>85</td>
<td>1.200 ± 0.055</td>
<td>0.187 ± 0.014</td>
<td>7.068 ± 0.306</td>
<td>1.479 ± 0.065</td>
<td>0.544 ± 0.060</td>
<td>0.004 ± 0.000</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>247</td>
<td>5.407 ± 0.318</td>
<td>12.475 ± 0.566</td>
<td>5.574 ± 0.292</td>
<td>7.068 ± 0.306</td>
<td>3.760 ± 0.128</td>
<td>1.026 ± 0.041</td>
<td>0.152 ± 0.008</td>
</tr>
</tbody>
</table>

\(^1\) \(n = 6\). A representative sample of each vegetable from each of the 6 weeks of the study was analyzed in duplicate.

\(^2\) All values are means ± SEs.

\(^3\) – Not detected.
TABLE 2

Incremental area under the response curve (iAUC) values for carotenoids, retinyl palmitate, and phylloquinone in plasma chylomicron and VLDLA fractions after consuming the test salads.\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>(\alpha)-Carotene</th>
<th>(\beta)-Carotene</th>
<th>Lutein</th>
<th>Lycopene</th>
<th>Retinyl palmitate</th>
<th>Phylloquinone</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol · h/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chylomicrons</td>
<td>4.507 ± 0.655(^2)</td>
<td>18.515 ± 2.055</td>
<td>2.608 ± 0.465</td>
<td>0.913 ± 0.363</td>
<td>27.853 ± 3.982</td>
<td>1.482 ± 0.212</td>
</tr>
<tr>
<td>VLDL A</td>
<td>22.162 ± 3.524(^5)</td>
<td>58.805 ± 8.608(^5)</td>
<td>-5.086 ± 2.918(^3)</td>
<td>4.634 ± 1.147(^4)</td>
<td>113.382 ± 14.784(^5)</td>
<td>3.340 ± 0.504(^4)</td>
</tr>
</tbody>
</table>

\(^1\)\(n = 12\) women; There were no significant treatment differences and data were combined across the three salad dressing treatments.

The iAUC values in chylomicron and VLDLA fractions were compared by ANOVA with fraction and treatment as main factors and treatment × fraction interaction as a random factor.

\(^2\)All values are means ± SEs.

\(^3,4,5\)Significantly different from chylomicrons, \(^3\)\(P < 0.05\), \(^4\)\(P < 0.01\), \(^5\)\(P < 0.001\).
FIGURE 1. Mean (± SE) changes from baseline (fasting) in the content of phylloquinone in the plasma chylomicron fraction after 12 women each consumed test salads with salad dressing containing 0 g, 0.2 g or 0.8 g Solec® 8120 hydroxylated soy lecithin. A significant decrease ($P < 0.02$) in the chylomicron phylloquinone AUC values was observed when the salad dressing containing 0.8 g Solec® 8120 lecithin was consumed compared with the salad dressing containing 0 g Solec® 8120.
FIGURE 2. Mean (± SE) changes from baseline in the contents of α-carotene, β-carotene, and lycopene in the plasma chylomicron (A) and VLDLA (B) fractions. Repeated-measures ANOVA with Tukey’s adjustment was used to compare differences between the baseline and postprandial values of α- and β-carotene and lycopene at 2 h, 3.5 h, 5 h, 7 h, and 9.5 h, respectively. Treatment and time were included in the model as fixed factors and subject and subject × treatment interactions as random factors. *P < 0.05, **P < 0.01, ***P < 0.001.
FIGURE 3. Mean (± SE) changes from baseline in the contents of retinyl palmitate and phylloquinone in the plasma chylomicron (A) and VLDLA (B) fractions. Repeated-measures ANOVA with Tukey’s adjustment was used to compare differences between the baseline and postprandial values of retinyl palmitate and phylloquinone at 2 h, 3.5 h, 5 h, 7 h, and 9.5 h, respectively. Treatment and time were included in the model as fixed factors and subject and subject × treatment interactions as random factors. ***P < 0.001
**FIGURE 4.** Mean (± SE) changes from baseline in the contents of lutein, α-tocopherol and γ-tocopherol in the plasma chyomicron (A) and VLDL (B) fractions. Repeated-measures ANOVA with Tukey’s adjustment was used to compare differences between the baseline and postprandial values of lutein and tocopherols at 2 h, 3.5 h, 5 h, 7 h, and 9.5 h, respectively. Treatment and time were included in the model as fixed factors and subject and subject × treatment interactions as random factors. *P < 0.05, **P < 0.01, ***P < 0.001
FIGURE 5. Mean (± SE) changes from baseline in the contents of apoB-48 and apoB-100 in the plasma chylomicron (A) and VLDLA (B) fractions. Repeated-measures ANOVA with Tukey’s adjustment was used to compare differences between the baseline and postprandial values of lutein and tocopherols at 2 h, 3.5 h, 5 h, 7 h, and 9.5 h, respectively. Treatment and time were included in the model as fixed factors and subject and subject × treatment interactions as random factors. **P < 0.01  The AUC values of apoB-48 and apoB-100 were compared between fractions by ANOVA analysis with fraction and treatment as fixed factors and treatment × fraction as random factor. The apoB-48 AUC value and apoB-100 AUC value were compared within each fraction by ANOVA analysis with analyte and treatment as main factors and treatment × analyte as a random factor. The apoB-48/apoB-100 AUC value in the VLDLA fraction was significantly higher than that in the chylomicron fraction (P < 0.0001). The apoB-100 AUC value was substantially higher than the apoB-48 AUC value in both the plasma chylomicron and VLDLA fractions (P < 0.0001).
Salad consumption is a major contributor to vegetable consumption in the United States. Although it is associated with a greater likelihood of meeting the recommended intakes for various nutrients, the bioavailability of fat-soluble micronutrients, including carotenoids, tocopherols and phylloquinone, is low in raw vegetables, especially when co-consumed fat is limited. To achieve high absorption of fat soluble micronutrients and meanwhile to avoid having excessively high fat intakes, we were looking for a lipid that is more efficient in increasing the bioavailability of fat-soluble micronutrients compared with triacylglycerol. Lecithin was a potential candidate, because it is a structural component of mixed micelles and an excellent emulsifier, and soybean lecithin was reported to enhance the absorption of diet triacylglycerol and curcumin, a lipophilic constituent of the spiceturmeric.

Chylomicrons have long been regarded as the major transport particles for newly absorbed fat-soluble micronutrients. Therefore, their postprandial appearance in chylomicrons has been utilized as an indicator of intestinal absorption. However, in previous studies, we observed that postprandial appearances of β-carotene and retinyl palmitate in the plasma VLDL fraction coincided with that in the chylomicron fraction in time and magnitude, which implied the importance of VLDLA as a vehicle for assessing intestinal absorption of β-carotene and its major bioconversion product, retinyl palmitate. Few human studies have addressed the origin of VLDLA and the role of VLDLA in the transport of other newly absorbed fat-soluble micronutrients, such as α-carotene, lycopene, lutein, tocopherols, and phylloquinone.
We conducted a human study to investigate the effects of the lecithin/oil ratio in salad dressing on the absorption of fat-soluble micronutrients, as well as the roles of plasma chylomicrons and VLDLA in the transport of the absorbed fat-soluble micronutrients. There were no significant differences among the salad dressings in the resulting AUC values of the fat-soluble micronutrients in the chylomicron and VLDLA fractions. The exception was a decrease in the AUC value of phylloquinone in the chylomicron fraction when the salad dressing containing 0.8 mg hydroxylated soy lecithin (Solec® 8120) was consumed compared with the salad dressing containing 0 g Solec® 8120 ($P < 0.02$). The AUC values for $\alpha$-carotene, $\beta$-carotene, lycopene, retinyl palmitate, and phylloquinone were substantially higher in the VLDLA than in the chylomicron fraction ($P < 0.05$). Both chylomicron and VLDLA fractions contained apoB-48 and apoB-100. ApoB-48 and apoB-100 were mainly found in the VLDLA fraction ($P < 0.0001$). The AUC value of apoB-100 was significantly higher than that of apoB-48 within each of the two fractions ($P < 0.0001$).

We concluded that the hydroxylated soy lecithin in the amounts added to the salad dressing did not enhance the absorption of carotenoids, retinyl palmitate, phylloquinone, or tocopherols. Over a 9.5-h period after consuming these bioactive components in salad vegetables with only 4 g of salad dressing, the majority of the newly absorbed $\alpha$-carotene, $\beta$-carotene, lycopene, retinyl palmitate, phylloquinone, as well as enterogenous (apoB-48) and hepatogenous (apoB-100) lipoprotein particles, were contained within large VLDL. Thus this lipoprotein subfraction has a major role in the transport of newly absorbed carotenoids and fat-soluble vitamins.

In this study, the fat-soluble micronutrients were consumed as they naturally occur within salad vegetables. Thus, our experimental conditions were similar to real diets, and the
outcomes may reflect the actual distribution of fat-soluble micronutrients in postprandial triacylglyceride-rich lipoproteins, i.e., the majority of newly absorbed fat-soluble micronutrients is contained in the VLDLA fraction instead of the chylomicron fraction. This finding highlights the importance of measuring fat-soluble micronutrients in the postprandial VLDLA fraction to avoid underestimating carotenoid (α-carotene, β-carotene, and lycopene), retinyl palmitate, and phylloquinone bioavailability. Meanwhile, the findings of apoB-48 and apoB-100 in both the chylomicron and VLDLA fractions further support the hypothesis that apoB-48 and apoB-100 containing lipoproteins overlap in density. Although the study design does not allow us to conclude with certainty the origin of VLDLA-sized apoB-48 containing lipoproteins, our findings indicated the chylomicrons and VLDLA can not be simply regarded as enterogenous (apoB-48) and hepatogenous (apoB-100) lipoprotein particles, respectively. In contrast, they are the mixtures of intestine- and liver-derived particles. Thus, hepatic and intestinal lipoproteins should be separated by apoB isoform (apoB-48 or apoB-100) instead of particle size/density.

Besides these major findings, we also observed some interesting phenomena that may inspire future studies and provide new insights regarding the mechanism of fat-soluble micronutrient absorption:

1) Under the current study conditions, newly absorbed β-carotene and its major bioconversion product, retinyl palmitate, were primarily contained into the VLDLA fraction instead of the chylomicron fraction. In contrast, our previous studies reported that the β-carotene was primarily secreted into the VLDLA fraction followed by a slightly lower accumulation in chylomicron fraction when β-carotene dose (25 mg) was consumed with a meal containing 33 g of fat, and postprandial appearances of β-carotene and retinyl palmitate
in VLDLA fraction were of similar magnitude of those in chylomicron fraction after consumption of β-carotene (25 mg) with a fat-rich (60 g) meal. These findings suggest the distributions of β-carotene and retinyl palmitate in chylomicron and VLDLA fractions may depend on the fat content in the co-consumed meal. When the meal is rich in fat, newly absorbed β-carotene and retinyl palmitate are incorporated into chylomicron and VLDLA fractions with similar proportion. When the fat is limited, VLDLAs become the major transport particles of newly absorbed β-carotene and retinyl palmitate. This hypothesis requires further human studies in which fats with different amounts are consumed with identical meals and the distributions of fat-soluble micronutrients in postprandial lipoproteins are simultaneously investigated. These studies can also provide an insight regarding the effect of fat content on the distributions of other fat-soluble micronutrients in the postprandial lipoproteins.

2) In contrast to the other carotenoids, newly absorbed lutein was mainly incorporated into the chylomicron fraction after the salad consumption, whereas no increments were observed in the VLDLA fraction. This finding highlights the difference of intestinal absorption between lutein and hydrocarbon carotenoids (e.g. α-carotene, β-carotene, lycopene). Canthaxanthin has similar kinetics to those of lutein and has been shown to accumulate in chylomicron, VLDL and LDL fractions as early as 2 h post-dosing after the consumption of fat-rich (33 g) meal. It seems to suggest potential effects of fat content on the lipoprotein distribution of lutein, and the effects may be different from those on the distributions of other carotenoid, such as β-carotene. Moreover, ABCA1, a transmembrane transporter mediating nascent HDL secretion from the intestine, is associated with plasma lutein content and retinal pigment optical density. When co-consumed lipids are limited,
tocopherols are mostly incorporated into nascent HDL through ABCA1. Whether the analogous pathway exists in lutein intestinal absorption could be an interesting topic. Further investigations into the lutein appearance in the whole spectrum of lipoproteins with various fat contents would be worthwhile as it may help to clarify the absorption and transport mechanism of lutein.

3) Lutein had the strongest correlation with apoB-48 in the both chylomicron and VLDLA fractions compared with the other fat-soluble micronutrients (Table 3). The correlation coefficients between the relative contents of apoB-48 and each fat-soluble micronutrient were calculated, where the relative content is defined as the value after baseline correction (posprandial content - baseline). It is not clear that whether lutein is tightly associated with apoB-48 in molecular level. But it has been reported that lutein is able to activate ERK1/2 (extracellular-signal-regulated kinases) that facilitate the formation of apoB-48 containing lipoproteins. More in vitro and in vivo studies are needed to further explore the potential linkage between apoB-48 and lutein.

4) We found both chylomicron and VLDLA fractions contain apoB-48 and apoB-100. Additional kinetics analysis is required to conclude which of the following hypotheses is more applicable to our data: 1) apoB-48 containing VLDLAs are the remnants of chylomicrons; 2) chylomicrons are not the sole precursor for VLDLAs, and a significant proportion of apoB-48 containing VLDLA are directly secreted by the intestine. Two models can be designed: 1) Model 1 provides for the direct secretion of apoB-48 and apoB-100 into chylomicron and VLDLA fractions and allows apoB-48 and apoB-100 to leave the plasma at any compartment; 2) Model 2 used chylomicrons as the sole precursor for VLDLA. Simulation, Analysis, and Modeling Software (SAAM) can be used to find the best fit model.
TABLE 3. Correlation coefficients between the analytes and apoB-48 responses in chylomicron and VLDLA fractions.$^{1,2}$

<table>
<thead>
<tr>
<th></th>
<th>In Chylomicrons</th>
<th>In VLDLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutein</td>
<td>0.734</td>
<td>0.534</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>0.493</td>
<td>0.295</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.523</td>
<td>0.262</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.589</td>
<td>0.240</td>
</tr>
<tr>
<td>Retinyl palmitate</td>
<td>0.614</td>
<td>0.350</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>0.452</td>
<td>0.407</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>0.464</td>
<td>0.468</td>
</tr>
<tr>
<td>Phylloquinone</td>
<td>0.591</td>
<td>0.264</td>
</tr>
</tbody>
</table>

$^{1}$ Data were combined across the 3 salad dressing treatments.

$^{2}$ All analytes were significantly correlated with apoB-48 in chylomicron and VLDLA fractions, $P < 0.001$. 
APPENDIX

TABLE 4. Hydroxylated soy lecithin (Solec® 8120) composition:

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Weight-%</th>
<th>Mol-%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>15.14</td>
<td>24.81</td>
</tr>
<tr>
<td>1-LPC</td>
<td>/*</td>
<td>/*</td>
</tr>
<tr>
<td>2-LPC</td>
<td>3.74</td>
<td>9.17</td>
</tr>
<tr>
<td>PI</td>
<td>8.19</td>
<td>12.37</td>
</tr>
<tr>
<td>LPI</td>
<td>2.19</td>
<td>4.84</td>
</tr>
<tr>
<td>PS-Na</td>
<td>/*</td>
<td>/*</td>
</tr>
<tr>
<td>LPS</td>
<td>/*</td>
<td>/*</td>
</tr>
<tr>
<td>SPH</td>
<td>/*</td>
<td>/*</td>
</tr>
<tr>
<td>PE</td>
<td>0.66</td>
<td>1.15</td>
</tr>
<tr>
<td>LPE</td>
<td>/*</td>
<td>/*</td>
</tr>
<tr>
<td>APE</td>
<td>12.83</td>
<td>16.35</td>
</tr>
<tr>
<td>PG</td>
<td>1.02</td>
<td>1.7</td>
</tr>
<tr>
<td>DPG</td>
<td>0.69</td>
<td>1.27</td>
</tr>
<tr>
<td>PA</td>
<td>5.55</td>
<td>10.23</td>
</tr>
<tr>
<td>LPA</td>
<td>1.11</td>
<td>3.25</td>
</tr>
<tr>
<td>Other</td>
<td>9.07</td>
<td>14.86</td>
</tr>
<tr>
<td>Sum</td>
<td>60.19</td>
<td>100</td>
</tr>
</tbody>
</table>
**TABLE 5. Salad dressing aqueous base formulation:**

<table>
<thead>
<tr>
<th>Salad Dressing base formulation</th>
<th>wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultra pure reversed osmosis water</td>
<td>71.1458</td>
</tr>
<tr>
<td>Sugar</td>
<td>10.0000</td>
</tr>
<tr>
<td>Salt, refined</td>
<td>2.0000</td>
</tr>
<tr>
<td>Keltrol 521 CP kelco</td>
<td>0.5000</td>
</tr>
<tr>
<td>Waxy corn starch, E1422, Cluster S4R4</td>
<td>1.5000</td>
</tr>
<tr>
<td>Spirit vinegar 12%</td>
<td>8.0000</td>
</tr>
<tr>
<td>Citric acid monohydrate E330</td>
<td>0.1800</td>
</tr>
<tr>
<td>EDTA dissolvine</td>
<td>0.0075</td>
</tr>
</tbody>
</table>
TABLE 6. Amount of lipid and the salad dressing (SD) base on the salad

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hydroxylated soy lecithin (g)</th>
<th>Soybean oil (g)</th>
<th>SD base (g)</th>
<th>Total (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>4.00</td>
<td>56.00 g</td>
<td>60.00</td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
<td>3.80</td>
<td>56.00 g</td>
<td>60.00</td>
</tr>
<tr>
<td>3</td>
<td>0.80</td>
<td>3.20</td>
<td>56.00 g</td>
<td>60.00</td>
</tr>
</tbody>
</table>
I would like to take this opportunity to express my thanks to those who helped and supported me in the past four and a half years. First and foremost, I’d like to express my deep appreciation to my major professor Dr. Wendy White, who instructed, helped and supported me throughout the entire research. Her insights and passions on research always inspired me to pursue my dreams in the research field.

I am also grateful to my committee members: Dr. Don Beitz, Dr. Stephanie Clark, Dr. Matthew Rowling, and Dr. Chong Wang. Thank them for providing valuable guidance and thoughts in my POS meeting.

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