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Effect of dietary lipids on β -carotene absorption into micelles

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The University of Arizona, 1991

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EFFECT OF DIETARY LIPIDS ON β -CAROTENE
ABSORPTION INTO MICELLES

by

Carlos Enrique Montano

A Thesis Submitted to the Faculty of the
DEPARTMENT OF NUTRITION AND FOOD SCIENCE
In Partial Fulfillment of the Requirements
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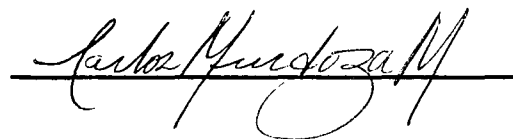
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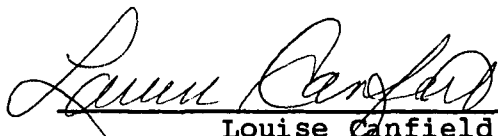
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Date

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ABSTRACT

As β -carotene is transported via micelles across the intestinal lumen for absorption, the factors that regulate uptake into micelles could also regulate at least in part β -carotene absorption in humans. Thus, we have studied the effect of different dietary lipids on β -carotene uptake into mixed micelles in vitro. Using this method, the effects of fatty acids of increasing degrees of unsaturation, vitamin A, cholesterol, and vitamin E on β -carotene uptake into micelles were studied. Uptake of β -carotene into micelles was stimulated by cholesterol and unsaturated fatty acids (uptake correlated with degree of unsaturation). β -Carotene solubilization into micelles was inhibited by vitamin A and vitamin E. These results suggest that the absorption of β -carotene may be partly regulated at the level of formation of intestinal micelles by the presence of different dietary components.

CHAPTER 1

INTRODUCTION

The carotenoids are one of the most important groups of natural pigments and are widely distributed in nature. The yellow, orange, and red colors of fruits and vegetables are mainly due to carotenoids. These compounds are chemically defined as a class of hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls), consisting of eight isoprenoid units (Davies, 1976). Considerable attention is currently being given to β -carotene as a possible preventive agent against cancer, coronary heart disease, and cataracts. Epidemiological evidence suggests an inverse relationship between the intake of β -carotene-rich vegetables and fruits and the incidence of cancer at various sites (Menkes et al., 1986; Winn et al., 1984; Ziegler et al., 1984). Animal studies have shown that β -carotene has a marked protective effect against a variety of carcinogens (Santamaria et al., 1983; Mathews-Roth and Krinsky, 1987), and in vitro experiments have revealed an antimutagenic (Belisario et al., 1985) and antitransformation activity (Som et al., 1984).

Dietary and plasma β -carotene levels (Gaziano et al., 1990; Riemersma et al., 1991) have also been found to

reduce the risk of heart disease. In addition, Esterbauer (1989) has proposed a protective role for carotenoids and vitamin E in atherosclerosis by preventing oxidation of low density lipoproteins and formation of cytotoxic compounds.

Furthermore, β -carotene is an important source of vitamin A, particularly in countries where vitamin A deficiencies are common. Malnourished children are significantly at high risk of death from respiratory and gastrointestinal disease if they are vitamin A deficient (Sommer, 1990).

Therefore, it is surprising that the mechanisms that regulate absorption and transport of β -carotene into and within cells are so poorly understood. This has been due to the lack of information on absorption and utilization of β -carotene. Little is known about stimulation or inhibition by other dietary components; e.g. dietary fat and other fat soluble vitamins. Thus it is not possible to predict the effectiveness of β -carotene when administered either as a drug or as a nutrient.

The study of intestinal β -carotene absorption has been hindered by the limitations of currently available experimental models. Our present understanding of β -carotene absorption and transport comes from studies in rats (Shapiro et al., 1984) or from dose response studies in humans (Brown et al., 1989; Dimitrov et al., 1988;

Jensen et al., 1986). There is no reliable animal model for study of β -carotene metabolism in humans. The rat is a poor model as it absorbs very little β -carotene (Ribaya-Mercado et al., 1989). Similarly, studies in humans are expensive and time consuming. Therefore, an understanding of β -carotene absorption and transport requires a simpler model.

As β -carotene is apparently transported via micelles across the intestinal lumen for absorption, the factors that regulate uptake into micelles might affect β -carotene absorption in humans. In this study the effect that different dietary lipids have on β -carotene solubilization into mixed micelles was investigated. For this purpose, an in vitro method for the preparation of mixed micelles containing known quantities of β -carotene was used (Canfield et al., 1990). The preparation is stable, reproducible, and simple to prepare. The procedure was modified from an earlier method (El-Gorab and Underwood, 1973) designed to simulate micelles formed in the lumen of the human small intestine. Using this model, the effects of (1) fatty acids of increasing degrees of unsaturation, (2) cholesterol, (3) vitamin A, and (4) vitamin E on β -carotene uptake into micelles were studied. It is expected that results gained in this study will contribute

to further knowledge of the nutritional variables that affect β -carotene absorption.

CHAPTER 2

LITERATURE REVIEW

2.1 Mechanism of Intestinal
Lipid Absorption

The process of lipid absorption can be divided into several phases: intraluminal digestion, micellar solubilization, permeation across the cell membrane, intracellular reesterification, chylomicron formation, and release of chylomicron from the cell into the lymphatics (Shiau, 1981).

Several dietary lipids such as triglycerides, phospholipids, and cholesterol esters are hydrolyzed by lipases in the lumen of the small intestine. The products of lipolysis, fatty acids and 2-monoglycerides, form liquid crystalline phases that are easily dispersed by bile salts into small bilayer vesicles and mixed bile salt micelles (Thomson and Dietschy, 1986). The micelles and vesicles are in rapid equilibrium with monomers of bile salts, fatty acids, and 2-monoglycerides. Lipolytic products appear to be absorbed as individual molecules by a mechanism that is poorly understood (Friedman and Nylund, 1980). Once inside, the enterocyte fatty acids are resynthesized into triglycerides in the smooth endoplasmic reticulum (Shiau, 1981).

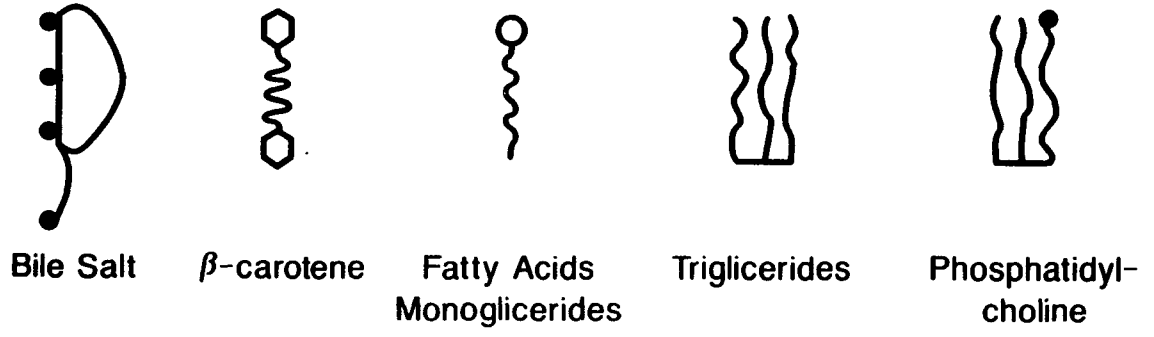
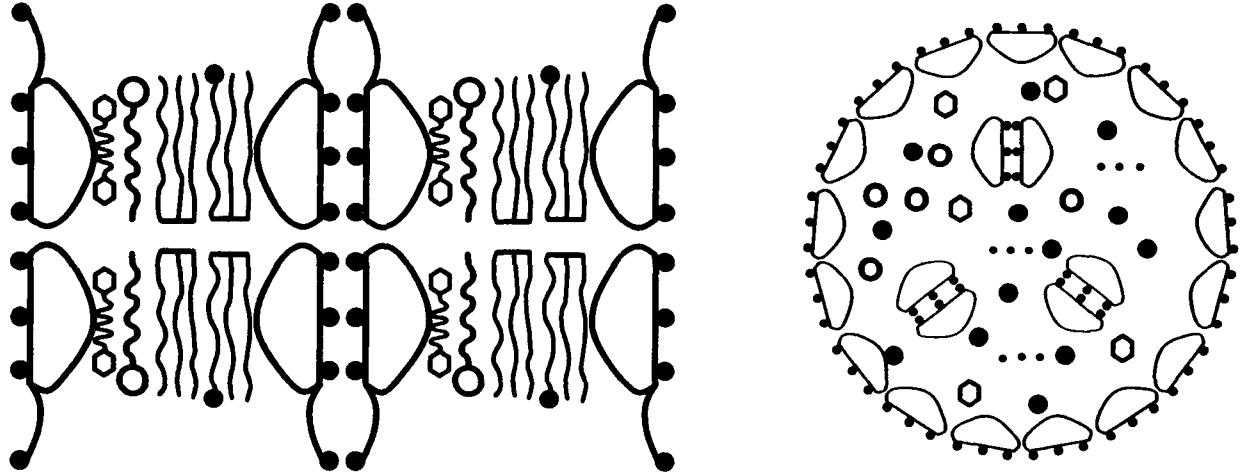
The newly resynthesized fat then undergoes processing whereby it is packed into small, phospholipid-coated fat droplets called chylomicrons, which are then secreted from the enterocyte and enter the lymphatic circulation (Meyer et al., 1986).

2.1.1 Micellar Structure and Function

Bile salts are secreted by the liver and enter the intestine through the biliary tract (Carey and Small, 1970). At low concentrations, bile salts exist as monomers in solution. However, when the critical micellar concentration of bile salts is reached, bile salt monomers begin to form aggregates referred to as micelles (Mazer et al., 1980). Micelles consisting only of bile salts are referred to as simple micelles. Solubilization of other lipids into simple micelles results in the formation of mixed micelles (Fig. 1).

Solubilization of lipolytic product by bile salt micelles forms a structure in which the hydroxyl groups of the bile salt molecules surround the aqueous phase, or when they are in the interior of the micelle, their hydroxyl groups face each other (Mazer et al., 1980). Fatty acids and mono-glycerides orient in the micelle with their polar head groups in contact with the aqueous phase and their hydrocarbon tails in the interior of the micelle (Fig. 1). The nonpolar center becomes the core that can solubilize

Fig. 1. Structure of a typical mixed micelle containing β -carotene. -- Bile salt molecules orient with their hydroxyl groups facing the aqueous phase, or when they are in the interior of the micelle, facing each other. Fatty acids and monoglycerides orient in the micelle with their polar head groups in contact with the aqueous phase, and their hydrocarbon tails in the interior of the micelle. The hydrophobic β -carotene molecules are found in the interior of the micelle.



additional lipids to form mixed micelles. Since the polar groups of bile salts are exposed to the aqueous phase, the entire structure becomes water soluble (Salle, 1978).

During the digestion of a fatty meal, the major micellar solutes are fatty acids and monoglycerides (Thomson and Dietschy, 1986). Their hydrocarbon chains are in rapid motion, primarily due to rotations around the carbon-carbon bonds which are maximal in the terminal carbons. Thus the middle of a mixed micelle is a dynamic liquid-like environment that does not exist in simple micelles made up only of rigid bile salt molecules (Carey and Small, 1970). Increased amounts of dietary fat in the intestine provide increased amounts of lipolytic products, which, when solubilized in bile salt micelles, increase the capacity of micelles to dissolve secondary solutes such as cholesterol, vitamin A, vitamin E, and carotenoids. In the absence of micellar solubilization, fat-soluble vitamins are not absorbed, and vitamin deficiency occurs (Mazer and Carey, 1983).

Once dispersed into mixed micelles, relatively high concentrations of secondary solutes can be transported to the microvillus membrane, where they may be efficiently absorbed. Therefore, mixed micelles serve as a delivery vehicle for otherwise insoluble molecules and increase

their bioavailability at the absorptive surface (Patton et al., 1984).

2.1.2 Lipid Uptake into the Enterocyte

It has been observed that mixed micelles are not absorbed as intact structures (Borgstrom et al., 1963). Thus, dissociation of lipolytic products from bile salt micelles must take place. This occurs by a mechanism that is not completely understood.

The intestinal uptake of lipolytic products has been thought to be mediated by a non-energy dependent passive process (Shiau, 1981). Lipolytic products cross, by simple diffusion, the lipid matrix of the cell membrane, then are transported through the cytosol bound to proteins called fatty acid binding proteins (FABPs). FABPs play a major role in transporting fatty acid from the brush border membrane to the smooth endoplasmic reticulum, where fatty acids are re-esterified to triglyceride (Potter et al., 1989).

2.1.3 Chylomicron Formation and Secretion

Within epithelial cells, the resynthesized triglycerides, together with cholesterol esters, phospholipids, and various apoproteins, form chylomicrons. These lipoproteins are released into the intercellular space by a

reversed pinocytosis, move across the basement membrane, and finally into the lymphatics (Sabesin and Frase, 1977).

2.2 Absorption and Metabolism of β -Carotene

β -Carotene absorption into the body can be divided in three stages: (1) incorporation into micelles, (2) absorption into the intestine, and (3) incorporation into secreted lipoprotein (Krinsky et al., 1958).

2.2.1 Incorporation into Micelles

Most studies favor the concept that a micellar phase is the preferred physiological state for most efficient intestinal absorption of the lipid soluble vitamins (Hollander, 1981). If sufficient bile is present, β -carotene is incorporated into fat micelles. El Gorab et al. (1973) studied the in vitro incorporation of β -carotene and retinol into micelles. They observed that 3-5% of β -carotene was solubilized by their micellar preparation; the solubility of retinol was 7-9 times greater than that of β -carotene. The authors concluded that the difference in uptake is due to a higher polarity of retinol, which renders it more soluble inside the micelle.

Bile stimulates the absorption of β -carotene from the gastrointestinal tract (Deuel, 1955). Since bile emulsifies lipids, its action in stimulating carotenoid absorption has generally been attributed to this property.

However, Olson (1964) reported that β -carotene that is dispersed in a micellar solution with a non-ionic detergent is not absorbed and converted into retinol esters by the intestine unless bile or sodium glycolate is also present. It was suggested in this study that bile not only disperses β -carotene in the intestinal lumen, but also stimulates formation of retinol by enhancing the absorption of β -carotene by means of an interaction at the membrane of intestinal mucosal cells.

El Gorab et al. (1975) also confirmed the observation that micellar solutions significantly enhanced β -carotene uptake. They observed in rat-everted gut sacs that β -carotene cleavage, and conversion to retinyl esters, occurred only in the presence of bile salt solutions.

2.2.2 Absorption into the Intestine

The mechanism by which fat soluble vitamins and other lipids are taken from the micellar phase into the mucosal cells is not clear. It has been assumed (Hollander, 1981) that after collision of micellar particles with the membrane of the villus cell, the components of the mixed micelles, including the fat soluble vitamins, pass through the lipid phase of the mucosal cell membranes to reach the cytoplasm. According to Hollander and Ruble (1978), absorption of physiological concentrations of

β -carotene into the intestine takes place by passive diffusion. It has been observed by Hollander (1981) and by others (Olson and Hayaishi, 1965; Olson, 1964; Olson, 1969) that 10% to 15% of dietary β -carotene may be taken up into mucosal cells. Absorption occurs in the mucosal cells lining the small intestine. Once inside the cell, β -carotene travels to the lateral surface of the cell and is incorporated into chylomicrons for transport to the liver. In the intestine, β -carotene can also be cleaved in the center of the molecule by the enzyme 15-15 dioxygenase to retinol (Carey, 1982). Recent work has shown the presence of apo-carotenals in rat (Tang et al., 1991) and human (Wang et al., 1991) intestinal mucosa homogenates after incubation with β -carotene. Handelman et al. (1991) also found members of the apo-carotenal series in the reaction products formed from β -carotene under three different oxidizing conditions. Detection of these compounds provides direct evidence that eccentric cleavage of β -carotene does occur.

Several factors have been considered as controlling mechanisms in β -carotene conversion to retinol. Dietary protein intake affects this process, which appears to be associated with the production of 15-15-dioxygenase (Kamath and Arnich, 1973).

2.2.3 Chylomicron Formation

β -Carotene is incorporated into chylomicrons and enters the lymphatic system (Krinsky et al., 1958). Labeled β -carotene has been given to patients to determine how much is absorbed (Goodman et al., 1966). It has been concluded that approximately 25% of the absorbed β -carotene remains intact and associated with chylomicrons in the lymph tissue, whereas about 75% is converted to vitamin A in the mucosal cells. Most of the vitamin A is esterified and is also incorporated in the lymphatic system (Krinsky et al., 1958).)

The lymph empties into the subclavian vein via the thoracic duct. From there, chylomicrons move to the liver and other tissues (Meyer et al., 1986). Chylomicrons are mostly triglycerides, and they become smaller as lipoprotein lipase removes most of the fat from them. They eventually become smaller particles, called chylomicron remnants (Shiau, 1981). There may be some uptake of carotenoids by muscle or adipose tissue as the chylomicron moves toward the liver. This is not known for certain. Once at the liver, chylomicrons are taken up by endocytosis (Friedman and Nylund, 1980).

2.2.4 Storage and Transport

Liver was found to contain the highest concentration of β -carotene among several tissues in humans, ranging

from 10- to 30-fold greater than lung or kidney (Schmitz et al., 1991).

For the most part, it is mobilized into the blood stream in very low density (VLDL) or low density lipoproteins (LDL) (Krinsky et al., 1958).

The distribution of β -carotene after its absorption and transport has been studied in rats (Shapiro et al., 1984). Using three different doses of β -carotene, these investigators showed that after two weeks at the lowest dose level (0.002% β -carotene in the diet), β -carotene accumulation was found only in the heart, kidney, and lung. When the highest dose was used, the highest organ concentration of β -carotene was found in the liver, followed by the adrenals and the ovaries. In humans, it has been found that the absorbed β -carotene is stored in several tissues, such as adipose tissue, and organs such as liver, adrenals, skin, testes, and ovaries (Goodman, 1985).

2.3 Human Studies

Jensen and coworkers (1986) measured serum β -carotene concentration in 17 adults after single ingestion of either one or three carrots. They observed that plasma β -carotene level increased after carrot ingestion and peaked after about 5 h. It has been found that maximum β -carotene concentrations occurs 24-48 h after dosing 30 men with a single dose of pure β -carotene (30 mg) or

carrots (270 g). Plasma response to pure β -carotene was greater than the response to a similar amount of β -carotene in carrots (Brown et al., 1989).

Dimitrov and coworkers (1988) measured β -carotene in plasma in 61 healthy individuals after they ingested various β -carotene doses of either 15 or 45 mg daily. Administration of β -carotene produced a great deal of variation in plasma levels.

Stich et al. (1986) reported high variability in β -carotene levels in human mucosal cells after oral administration of β -carotene. There is no clear explanation why bioavailability of β -carotene shows such individual variations. It is possible that absorption of β -carotene varies with diet (Jayarajan et al., 1980), vitamin A status (Goodman, 1985), or hormones (Curran-Celentano et al., 1985).

2.4 Effect of Dietary Components on β -Carotene Absorption

2.4.1 Fat

β -Carotene utilization depends substantially on the presence of fat in the gastrointestinal tract (Cornwell et al., 1962). The amount and type of fat in the diet may therefore affect bioavailability of the carotenoids.

There are several reports in the literature indicating that dietary fat is an important factor determining

how much β -carotene is absorbed. Some of these studies have used experimental animals; some have used humans.

Rats were shown to suffer a failure in the absorption and utilization of carotene on a low-fat diet (Ahmad, 1931). Incorporation of 10% fat in the diet resulted in almost complete utilization of carotene. A similar conclusion was reached by Kemmerer and Fraps (1938), who noted increased absorption of carotene by rats when the provitamin was fed in a diet containing cottonseed oil compared to a diet containing no fat. Shaw and Deuel (1944) observed a positive correlation between the amount of fat in the diet and carotene absorbed by rats.

A study done in Africa a number of years ago by Roels et al. (1958) found that in a village area where more than enough carotenoids were consumed for vitamin A activity, vitamin A deficiency still occurred, leading to blindness in children. The solution was not to give more β -carotene, but to give an additional 18 g/day of olive oil. The cause of vitamin A deficiency was probably lack of sufficient fat in the diet for the absorption of carotenoids.

Cornwell (1962) reported that aqueous and lipid dispersions of β -carotene are readily absorbed when administered with different test meal regimens. They observed that aqueous dispersions of β -carotene were poorly absorbed

by fasting subjects. On the other hand, aqueous dispersions of β -carotene administered with lipid test meals showed the highest serum carotene increments.

More recently, the effect of the amount of dietary fat on β -carotene absorption was studied in healthy volunteers after receiving various doses of β -carotene supplements (Dimitrov et al., 1988). It was found that absorption of β -carotene was affected by dietary fat concentration. Individuals placed on a high fat diet (65 g/d) showed significant increases in plasma β -carotene as compared with those placed on a low fat diet (6 g/d).

According to Hollander and Ruble (1978), increased β -carotene absorption in the presence of fatty acids may be caused by the ability of fatty acids to expand micelles and to stimulate the intracellular cleavage of β -carotene to retinal. Accelerated cleavage, which would decrease intracellular concentration of β -carotene and increase the effective concentration gradient for uptake, would cause a faster disappearance rate of β -carotene from the intestinal lumen.

Type of fat in the diet can also affect the absorption of β -carotene. Deuel et al. (1950) found a greater growth response in vitamin A deficient rats when margarine was used as a vehicle for β -carotene supplementation as oppose to cottonseed oil. The conclusion of the study was

that margarine, which contains hydrogenated fatty acids, was less oxidized than cottonseed oil; therefore, higher amounts of unoxidized β -carotene were available to form retinol.

Other studies have observed a negative effect of nondigestible oils on β -carotene utilization. Mineral oil, which is rich in saturated fatty acids, was reported to have a deleterious effect on the absorption of vitamin A in growing rats (Rowntree, 1931). Dutcher et al. (1941) found that the growth response of rats is impaired when butter containing β -carotene dissolved in mineral oil is fed to rats. According to these authors, mineral oil may act as a solvent for carotene, solubilizing endogenous carotene and depleting the ingested foods of their supply of this vitamin.

Several studies have compared the effect of saturated versus unsaturated fats on β -carotene absorption. Some studies found that polyunsaturated fats are better vehicles for β -carotene. For example, Sherman et al. (1941a) fed various natural oils to vitamin A deficient rats receiving β -carotene. Of the oils tested, polyunsaturated oils (soybean, cottonseed, and corn oil) gave the best growth response. On the other hand, the saturated fats, coconut oil, and butter fat had no appreciable effect

on growth. This study suggested that polyunsaturated fatty acids stimulate conversion of β -carotene to vitamin A.

Hollander and Rubler (1978) used a micellar perfusate to study the effect of different fatty acids on β -carotene absorption in rat jejunal and ileal intestinal loops. They found that the absorption rate was the highest when oleic acid was present in the perfusate. Separate additions of linoleic and linolenic acids caused β -carotene to be absorbed at a rate lower than that in the presence of oleic acid. Their proposed explanation for the lower β -carotene absorption produced by the polyunsaturated fatty acids is that these fatty acids may compete with β -carotene for sites on the fatty acid binding proteins (FABP). These proteins are necessary for transfer of fatty acids from the lipid cell membrane to the intracellular organelles (Ockner and Manning, 1974). FABP may also perform a similar function for β -carotene transfer through the intracellular aqueous cytosol. FABP has been shown to have a greater binding affinity for fatty acids with greater number of unsaturated sites (Clarke and Armstrong, 1989). Therefore, polyunsaturated fatty acids would have a greater binding affinity for FABP than for oleic acid and would be able to compete with β -carotene for binding to FABP. Alternatively, the polyunsaturated fatty acids may have caused increased oxidation of the β -carotene added to

the perfusate, explaining the lower β -carotene absorption observed in the presence of linoleic and linolenic acid.

Alam et al. (1989) compared the effect of lard (rich in saturated FA) and corn oil (polyunsaturated FA) on plasma β -carotene levels in a rat model. Rats were fed diets containing β -carotene or placebo, and either lard or corn oil for 13-16 weeks. In all animals receiving β -carotene, there was an increase in plasma β -carotene levels; however, plasma levels were significantly reduced in the group fed corn oil compared to the group fed lard. In addition, liver β -carotene levels were higher in the groups fed corn oil. The authors hypothesized that some of the β -carotene could have been utilized in the protection of the vulnerable double bonds of the polyunsaturated FA in the corn oil from oxidation. On the other hand, β -carotene may have been preferentially deposited in the liver to protect against potential peroxydative damage. In a follow-up study (Alam et al., 1990), the ability of different fats to promote β -carotene absorption in the rat were compared. Coconut oil (a saturated fat) gave the highest concentration in plasma, followed by safflower oil (polyunsaturated n-6). Menhaden oil (rich in polyunsaturated n-3 fatty acids) gave the lowest plasma concentration. In liver, safflower oil gave the highest concentration, followed by olive oil. Coconut oil gave the lowest

β -carotene liver concentration. It is possible that polyunsaturated fatty acids (PUFAs) increase absorption of β -carotene into the intestine by stimulating β -carotene solubilization into micelles. PUFAs may also facilitate transport of β -carotene from the intestine to the liver by increasing incorporation of β -carotene into chylomicrons. The lower plasma β -carotene levels in the presence of PUFAs could be related to the inhibitory effect of PUFAs on the synthesis of very low density lipoprotein (VLDL) in liver (McNamara, 1987). Thus, in the presence of PUFAs there would be less availability of lipoproteins to transport β -carotene in plasma.

2.4.2 Vitamin E

The presence as well as level of vitamin E in the diet has been reported to have a marked positive influence on the utilization of β -carotene. Moore (1940) was the first to report that the addition of tocopherol (0.1-2.0 mg/week) to a diet increased liver vitamin A reserves of the rat compared to animals fed a vitamin E depleted diet. Davis and Moore (1941) showed that vitamin E (1.0 mg/week) increased the time required to deplete vitamin A liver stores in rats fed diets with no vitamin A. The authors concluded that tocopherols act as antioxidants in the gastrointestinal tract.

Sherman (1941b) found that when rats were fed diets containing β -carotene plus 0.05 g of methyl linolate or methyl linolenate, the growth response was retarded. This study showed that 1 mg of α -tocopherol added to the diets daily restored the normal growth of rats. The study's conclusion was that in the absence of vitamin E, unsaturated fatty acids probably increased carotene oxidation.

Hickman et al. (1944) demonstrated that vitamin E enhanced the growth-promoting effect of vitamin A in the rat. These workers extended their work to β -carotene and found vitamin A activity of β -carotene to be influenced markedly by tocopherol intake of test rats. A daily dose of about 0.5 mg of mixed tocopherols yielded the optimal sparing action of carotene (0.79 ug added to the diets). Free tocopherols were more effective in this regard than their esters.

In another study, Hickman et al. (1944) reported that a mixture of tocopherols, distilled from edible oils, was slightly more active than any of the individual tocopherols in enhancing the synergistic effect of carotene utilization.

Level of vitamin E in the diet has also been shown to be important for β -carotene absorption. Burns et al. (1951) fed vitamin A depleted rats 0, 0.5, 1.0, or 2.0 mg of tocopherol/day with 1 mg of vitamin A acetate, or 1.0 mg

of β -carotene/day. At the 1 mg level of tocopherol, the growth response was as good in rats fed with β -carotene as with vitamin A. However, a daily dose of 2.0 mg of tocopherol significantly diminished the efficiency of utilization of β -carotene. The tocopherol did not influence the growth of rats fed vitamin A. In this study, carotene added to the diets was isolated from tomatoes. Since lycopene is the most abundant carotenoid in tomatoes, it is possible that the effect observed in this study was due to lycopene and not to β -carotene.

Hebert and Morgan (1953) found that 0.5 mg of tocopherol given daily in the diet of partially vitamin A depleted rats caused a significant increase in liver stores when 87-174 μ g of carotene were given in oil daily for 14 days. Above and below these levels of tocopherol, no effect was found. The tocopherol supplement had no significant effect when vitamin A was fed at levels of 35-129 μ g daily for 14-28 days.

All these studies seem to indicate that moderate levels of vitamin E (0.5-1.0 mg) in the diet stimulate conversion of carotene to vitamin A. However, higher levels would have no effect or even have deleterious effects. Johnson and Baumann (1948) found evidence of a depressing effect of high levels of tocopherol (1.0-10.0

mg/day) on the liver stores and growth of their carotene fed-animals.

In a more recent study, Alam et al. (1990) observed an inhibitory effect of vitamin E on β -carotene metabolism. They fed two groups of rats polyunsaturated fatty acid-rich diets, containing 0.2% β -carotene. The diet of one of the groups was supplemented with a high level (0.2% of the diet) of α -tocopherol. After six weeks, the concentration of β -carotene was measured in plasma and liver. Plasma and liver levels of β -carotene were reduced by one-half in the group supplemented with vitamin E compared to the nonsupplemented. It is possible that vitamin E competes with β -carotene for solubilization into micelles, or competes for uptake into the intestine, thus reducing the amount of β -carotene available for transport to the liver. Vitamin E may also compete with β -carotene for transport inside very low (VLDL), and low density lipoproteins (LDL), explaining the low plasma β -carotene levels reported in the presence of vitamin E.

2.4.3. Other Carotenoids

β -Carotene utilization in animals is influenced by the presence of other carotenoids in the diet. For instance, it has been shown that xanthophylls inhibit utilization of carotene by rats and chicks. Kemmerer et al. (1947) showed that in vitamin A deficient rats,

0.9 mg of xanthophylls, when fed with 0.6 mg of β -carotene, dissolved in cottonseed oil, decreasing the utilization of carotene for liver storage of vitamin A about 20%. In the same study it was reported that 0.5 mg of vitamin E did not increase utilization of the carotene from spinach as measured by vitamin A liver storage, possibly due to the interference of xanthophylls in spinach. Presence of these compounds may account in part for the low utilization of carotene in many vegetables compared to the utilization of pure formulations of carotene.

Vavich and Kemmerer (1950) found that the level of carotene in the diet is also important. They studied the effects of xanthophylls on utilization of carotene for vitamin A liver storage in chicks, the effect of the level of xanthophylls on carotene utilization, and the effect of the level of carotene on the depressing action of xanthophylls. They found that neither 300 μ g nor 600 μ g of xanthophylls reduced storage of vitamin A in the liver of chicks fed 65 μ g of β -carotene daily. However, both levels of xanthophylls markedly reduced storage of vitamin A when 130 μ g of carotene were fed. These results may indicate that at high levels of carotene in the diet, there exists competition with other carotenoids for intestinal absorption.

2.4.4 Vitamin A

The administration of high doses of vitamin A has been reported to lower serum carotenoid values in poultry (Mattson and Deuel, 1943) and cattle (Jacobson et al., 1950), egg yolk carotenoid values in poultry (Dua et al., 1966), and milk fat carotene content in cattle (Blaxter et al., 1946). The depressing effect of vitamin A on carotene levels has not been clearly explained by the studies mentioned above. Mattson et al. (1943) suggested that the deleterious effect of vitamin A on carotene was due to development of a nonspecific enzyme system capable of destroying the carotenoids. Dua et al. (1966) proposed that vitamin A interferes with absorption of carotenoids from the intestinal tract; since vitamin A and carotenoids are structurally related, they may compete for absorption from the intestine.

It is important to mention that all these reports measured total carotenoids in different tissues; therefore, there is no information regarding the effect of vitamin A on individual carotenoids in animals.

In humans there is only one study of simultaneous feeding of vitamin A and β -carotene (reported by Stich et al., 1986). In this study individuals were fed high (180 mg) levels of β -carotene with and without vitamin A, and the amount of β -carotene in exfoliated mucosal cells

from the inside of the mouth was measured. Vitamin A given simultaneously with β -carotene resulted in a significant increase of β -carotene in buccal mucosa. Although their study provides evidence of enhancement, it does not provide specific information about intestinal events.

2.4.5 Protein

Qualitative or quantitative protein deficiency appears to impair the utilization of carotene. Berger et al. (1962) discovered that feeding a protein-free diet decreased but did not prevent carotene conversion to vitamin A.

Type of dietary protein may affect utilization of carotene. Fraps (1946) and El Gindi et al. (1953) found that substitution of a high-quality protein (casein) for a low-quality protein (gluten) in the ration of experimental rats increased the apparent digestibility of carotene and storage of vitamin A in liver.

The mechanism of this effect is not clear. Olson (1969) pointed out that the level of a number of key enzymes in utilization of carotene and in absorption and transport of vitamin A is depressed by protein deficiency. They included β -carotene 15,15-dioxygenase, pancreatic retinyl ester hydrolase, and retinol binding protein. These findings are of particular significance since

protein-calorie malnutrition and vitamin A deficiency are common in many parts of the world.

2.5 Effect of Hormones on β -Carotene Metabolism

The thyroid gland is believed to have some role in absorption of β -carotene. Johnson and Baumann (1947) demonstrated that less vitamin A accumulated in the liver and kidneys of rats rendered hypothyroid with thiouracil than in normal rats receiving equivalent amounts of β -carotene. Livers of hyperthyroid rats also contained more vitamin A than did those of normal controls.

Cama and Goodwin (1949) demonstrated that in the case of the rat, absorption of carotene is increased, as measured by plasma levels, when thyroxine is given, and is decreased after the thyroid antagonist, thiouracil, is administered. Chanda (1956) found that increased conversion of dietary carotene to vitamin A in the intestine was responsible for the increase in blood vitamin A that occurs when chickens are given thyroxine.

It has also been shown that thyroid hormones also have an effect on the carotene and vitamin A composition of milk. Chanda and Owen (1952) reported that thyroxine increased and thiouracil decreased the carotene and vitamin A content of cows milk.

These studies suggest that the thyroid gland is active in improving the availability of dietary carotene. The question to be answered is whether this effect occurs as a result of an increased absorption of β -carotene, or whether it might be ascribed to a more efficient conversion of carotene to vitamin A. Cama and Goodwin (1949) hypothesized that the beneficial effect of thyroid hormone in reducing the loss of carotene in the feces is due to increased absorption of β -carotene. They concluded that, although the bulk of evidence suggests that the thyroid functions in carotene metabolism by increasing absorption of the provitamin, one cannot completely rule out the possibility that thyroid hormone has some influence on dioxygenase activity.

In conclusion, this literature review shows that the absorption and subsequent metabolism of carotenoids is affected by other nutrients present in the diet and by thyroid hormones. However, it is important to note that most studies presented here were done several years ago, when the major interest on the carotenoids resided in their pro-vitamin A properties. Therefore, the majority of investigations were carried out measuring absorption of vitamin A rather than the appearance of the intact molecule of carotene in different tissues (Appendices A through C). This point is important since in recent years it has been

proposed that β -carotene and other carotenoids have biological actions of their own, independent of their ability to be metabolized to vitamin A. Therefore, new studies are needed to distinguish those factors that stimulate or inhibit absorption of carotene from those that affect their conversion to vitamin A.

Another limitation of previous studies is that their methods of analysis could not distinguish between individual carotenoids. Therefore, in many cases results were expressed as total carotenoids instead of specific compounds such as β -carotene, cryptoxanthin, lycopene, etc. This could be relevant since each of these compounds may have different biological properties. In this respect, it is also necessary to conduct similar studies with new analytical techniques, such as high-performance liquid chromatography, to determine the effect of diet on β -carotene and other individual carotenoid absorption.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Bile salts (sodium glycolate, glychochenodeoxycholate, glycodeoxycholate, taurocholate, taurochenodeoxycholate, and sodium taurodeoxycholate), fatty acids (stearic, oleic, linoleic, linolenic, and arachidonic acid), cholesterol, α -tocopherol, and Krebs-Ringer bicarbonate buffer were purchased from Sigma Chemical Co. (St. Louis, MO). β -carotene and retinol were purchased from Fluka Chemika-Biochemika (Buchs, Switzerland). Hexane, methanol, and tetrahydrofuran (THF) were purchased from Fisher Scientific (Fair Lawn, NJ). All solvents were HPLC grade. Organic solvents used for HPLC were filtered through an 0.45- μ m fluoropore filter (Millipore, Bedford, MA).

3.2 Methods

A bile salt stock solution containing 30 mM sodium glycolate, 30 mM sodium glychochenodeoxycholate, 15 mM glycodeoxycholate, 10 mM sodium taurocholate, 10 mM sodium taurochenodeoxycholate, and 5 mM sodium taurodeoxycholate was prepared, mixing all the bile salts at 37°C in distilled, deionized water (Milli Q, Millipore) to provide a

final bile salt concentration of 0.1 M. Stock solutions were stored at -20°C until use.

Stock solutions of β -carotene (1.85 mM) were prepared daily from crystalline β -carotene in HPLC grade THF. Concentrations and purity were verified spectrophotometrically.

Individual stock micellar reagents of triolein, mono-olein, phosphatidylcholine, oleic, linoleic, linolenic, and arachidonic acid, typically 150-200 mM, were prepared in HPLC grade hexane and maintained in darkened glass vials under argon at -20°C.

Retinol (3.50 mM) and α -tocopherol (2.5 mM) solutions were prepared by dissolving crystalline retinol or α -tocopherol in HPLC grade ethanol. Concentrations and purities were verified spectrophotometrically. Solutions were stored under argon at -20°C.

Cholesterol stock solutions (51.7 mM) were prepared in benzene. Krebs-Ringer bicarbonate buffer was prepared by combining 15 mM sodium bicarbonate with 1 mM calcium chloride and adjusting the pH to 7.4 with NaOH. The final buffer sodium ion concentration was 0.14 mM.

3.2.1 Micelle Preparation

Stock solutions of micellar reagents were combined to provide a micellar solution with the following final concentrations: triolein (1.13 mM), mono-olein (2.50 mM),

phosphatidylcholine (0.68 mM), β -carotene (300 μ M), and 15 mM of one of the different fatty acids: stearic, oleic, linoleic, linolenic, or arachidonic acid (Table 1).

In a separate series of experiments, linolenic acid containing micelles were prepared with addition of 330, 600, and 1200 μ M of either cholesterol, α -tocopherol, or retinol.

Micellar solutions were evaporated under argon just until dryness. Bile salt were added to provide a final bile salt concentration of 12 mM, and Krebs-Ringer buffer was added to provide a final sodium concentration of 0.15 mM.

This preparation was sonicated in glass flasks for 2 hr at 37°C in a bath sonicator (Sonicor Instruments, Copiague, NY), with occasional shaking. The resultant turbid mixture was transferred to precoated Beckman Ultra Clear Ultracentrifuge tubes (Beckman Instruments, Fullerton, CA) and centrifuged (Beckman model L3-50) at 4°C for 18 hr at 106,000 \times g using a 50 Ti rotor (Beckman). The tubes were precoated according to the procedure of Holmquist (46) using a solution of polyvinyl alcohol, and isopropanol, to prevent the adsorption of β -carotene to the tube walls. Following centrifugation, the clear aqueous solution containing the micelles was removed for further

Table 1. Micelle composition. -- Different compounds used to prepare micelles, as described in the methods section.

	mM	%
Triolein	1.13	3.60
Mono-olein	2.50	7.90
Fatty acid	15.00	47.50
Phosphatidylcholine	0.68	2.20
Bile salts	12.00	38.00
β -Carotene	0.30	0.91

analysis. Solubilization of β -carotene in the lower aqueous phase was taken to be evidence of micellar formation.

3.2.2 Spectrophotometric Analysis

β -Carotene, retinol, and α -tocopherol concentrations were determined spectrophotometrically (Beckman, Model Du-60) using absorption maxima of 460, 325, and 292 nm, respectively (Fig. 2). Extinction coefficients used for β -carotene, retinol, and α -tocopherol were 2620, 1850, and 585 ($E_1\%$).

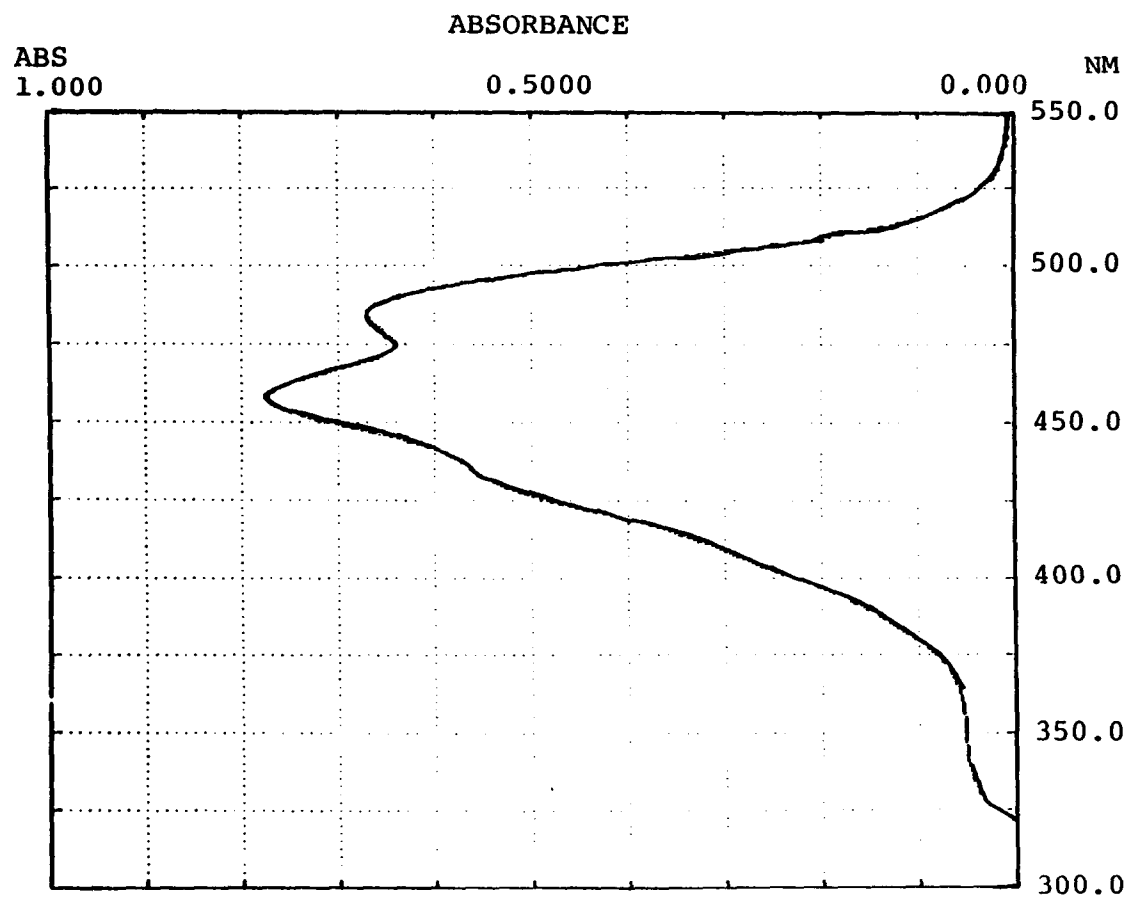
Cholesterol was determined by enzymatic analysis, using a commercial kit (Boehringer Mannheim, Indianapolis, IN).

3.2.3 HPLC Analysis of Micelles

β -Carotene was extracted from micelles with 4 volumes of hexane:ethanol (3:1, v/v) containing 0.25 g/l of butylated hydroxytoluene (BHT). Samples were vortexed 2 min and the lipid phase separated by centrifugation for 10 min at 1000 rpm in a microcentrifuge (Weaton, Millville, NJ). The upper hexane layer containing the lipids was aspirated with a Pasteur pipette, dried under nitrogen, gently layered with argon, and stored at -20°C until analysis.

Samples were resuspended in methanol:THF (80:20 v/v) containing 0.25 g/l BHT. HPLC analysis was performed

Fig. 2. Spectrophotometric scan of a typical micelle containing β -carotene, showing maximum absorption of β -carotene at 460 nm. -- Micelles were prepared according to the procedure described in the methods section and spectra were recorded from 250-550 nm. Absorption due to micelles prepared without β -carotene was subtracted. Quantification of β -carotene was accomplished using an extinction coefficient of 2620 ($E_1\%$).



using a Waters Model 510 pump, a Milton Roy Programmable Detector Model SM 4000, a 50 μ l loop, and Waters Maxima 820 version 3.02 system controller (Waters Assoc., Milford, MA). A Waters Resolve reversed-phase C18 column 10 micron (4.6 mm ID X 100 mm) in a 8 mm X 10 cm Radial Pak cartridge holder was used for all analyses. The running solvent was methanol:THF (90:10 v/v) containing 0.25 g/l BHT at a flow rate of 2.1 ml/min.

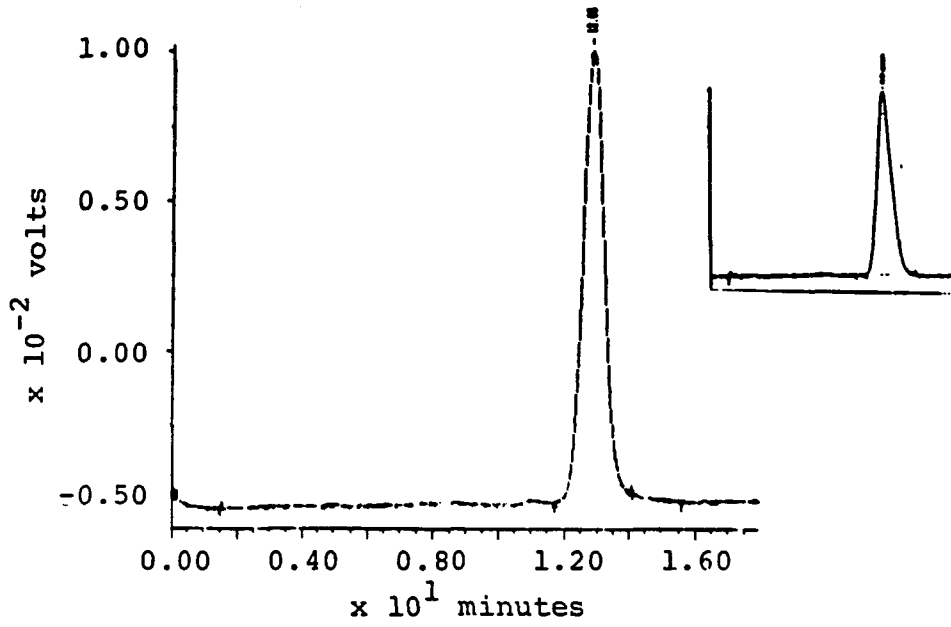
β -Carotene was identified at 452 nm, retinol at 325 nm, and α -tocopherol at 292 nm. β -Carotene, α -tocopherol, and retinol were determined by comparison with external standards prepared in the HPLC running phase solution (Fig. 3). All standards were prepared fresh for each experiment (concentrations of each standard were verified by measuring their absorbance spectrophotometrically and calculating concentration based on extinction coefficient).

3.2.4 Statistical Analysis

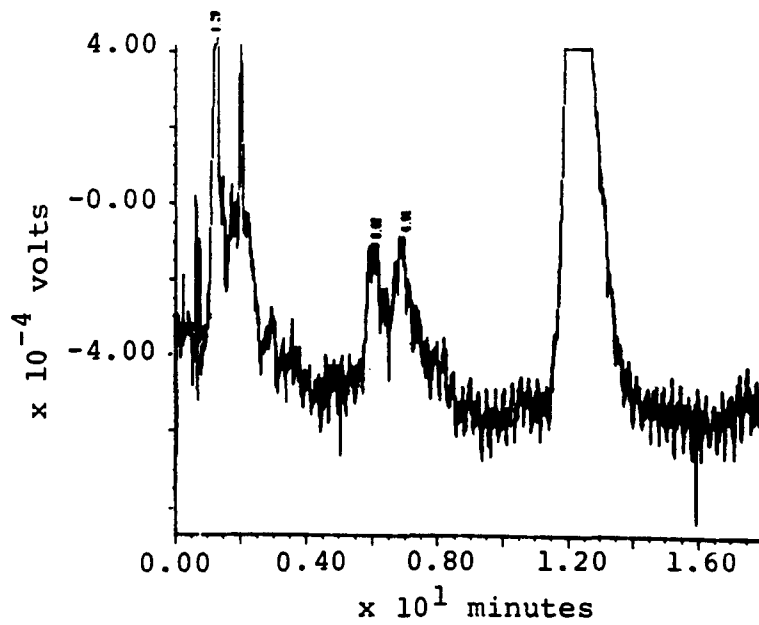
One-way ANOVA and multiple comparison tests were used to determine statistical significance ($P < 0.05$) among different treatments on an IBM PC with the statistical package STATA.

Fig. 3. HPLC elution profile of β -carotene extracted from micelles. -- Panel A shows a peak detected at 452 nm corresponding to β -carotene. The insert shows the elution profile of authentic β -carotene (4 $\mu\text{g/ml}$). Panel B shows peaks with absorption at 325 nm (100X magnification) that may represent breakdown products of β -carotene.

PANEL A



PANEL B



CHAPTER 4

RESULTS

4.1 Effect of Fatty Acid Composition on β -Carotene Uptake into Micelles

To determine the effect of different fatty acids on β -carotene incorporation into micelles, micelles were prepared with fatty acids of increasing levels of unsaturation. We found that incorporation of β -carotene into micelles was stimulated by unsaturated fatty acids, and that the extent of incorporation increased with degree of unsaturation of the fatty acid present in the micelle. The lowest percent incorporation (0.63%) was observed in the micelles containing stearic acid (18:0), and the highest incorporation (6.00%) was obtained in those micelles containing linolenic acid (18:3). β -Carotene uptake into micelles containing linoleic (18:2), linolenic (18:3), or arachidonic acid (20:4) were significantly higher ($p < 0.05$) than β -carotene uptake in micelles containing stearic acid (18:0) (Fig. 4 and Table 2). Three other experiments were conducted and yielded similar results.

Fig. 4. Effect of micellar fatty acid composition on β -carotene uptake into micelles. -- Mixed micelles of different fatty acid composition were prepared using one of the following fatty acids: stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), or arachidonic (20:4). Each experimental point represents the mean of 3 replicates, from a single experiment. Results are expressed as percent (%) incorporation of β -carotene into micelles. * = significantly different than micelle containing stearic acid (18:0) at $P < 0.05$.

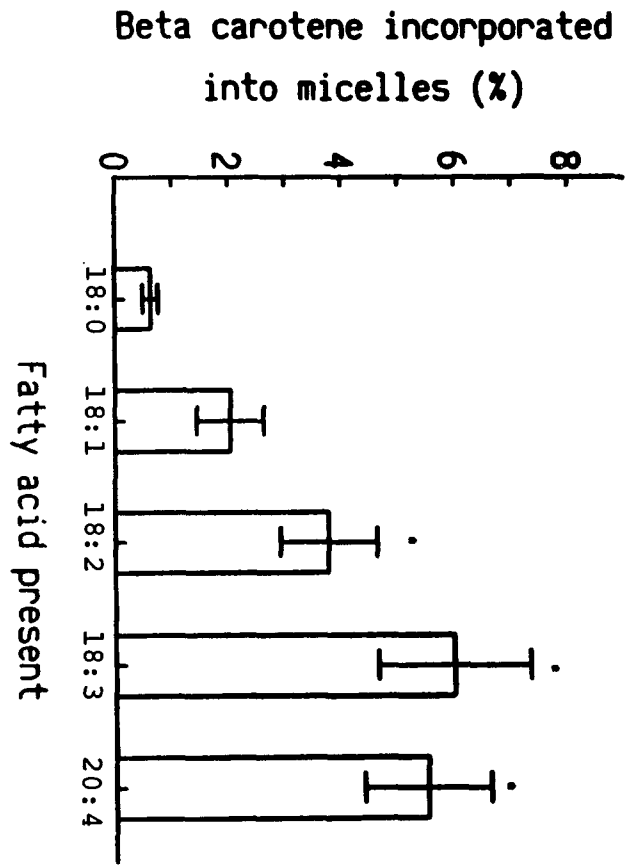


Table 2. Effect of different fatty acids on β -carotene uptake into micelles.

FA	% Uptake	Std. Dev.
18:0	0.63	0.18
18:1	2.00	0.47
18:2	3.70	0.91
18:3	6.00	1.25
20:4	5.53	1.01

4.2 Effect of Retinol (Vitamin A) on β -Carotene Incorporation into Micelles

The effect of retinol on β -carotene uptake into micelles was studied in micelles prepared as described in Table 1, with addition of increasing concentrations of retinol (300, 600, 1200 μ M), corresponding to β -carotene:retinol ratios of 1:1, 1:2, or 1:4. We found that addition of 300 μ M of retinol to micelles (β -carotene:retinol=1:1) significantly reduced ($p < 0.05$) β -carotene uptake into micelles (from 1.01 to 0.81). Addition of retinol in ratios of 1:2, and 1:4 did not show statistically significant ($p < 0.05$) inhibition beyond that observed with the 1:1 ratio (Fig. 5, Panel A, and Table 3). Incorporation of retinol decreased from 38.17 to 27.05% as increasing concentrations of retinol were added to the initial mixture (Fig. 5, Panel B).

4.3 Effect of Cholesterol on β -Carotene Uptake into Micelles

Incorporation of β -carotene into micelles was studied in micelles containing increasing concentrations of cholesterol. Micelles were prepared according to procedures shown in the methods chapter and with added cholesterol concentrations of 300, 600, or 1200 M, corresponding to β -carotene: cholesterol ratios of 1:1, 1:2, and 1:4. β -Carotene uptake into micelles was significantly greater ($p < 0.05$) in micelles containing cholesterol than in

Fig. 5. Effect of vitamin A (retinol) on β -carotene uptake into micelles. -- Micelles were prepared according to the procedure described in methods. Increasing concentrations of retinol were added, corresponding to a β -carotene:retinol ratio of 1:1, 1:2, or 1:4. Micelles with no retinol were prepared as a control. Panel A shows the percent incorporation of β -carotene uptake into micelles as a function of retinol added. Points represent mean of 3 replicates of one representative experiment. * = significantly different than control micelle (with no retinol) at $P < 0.05$. Panel B shows the percent incorporation of retinol into micelles with increasing concentrations of retinol in the incubation mixture. Points represent the mean of 3 replicates.

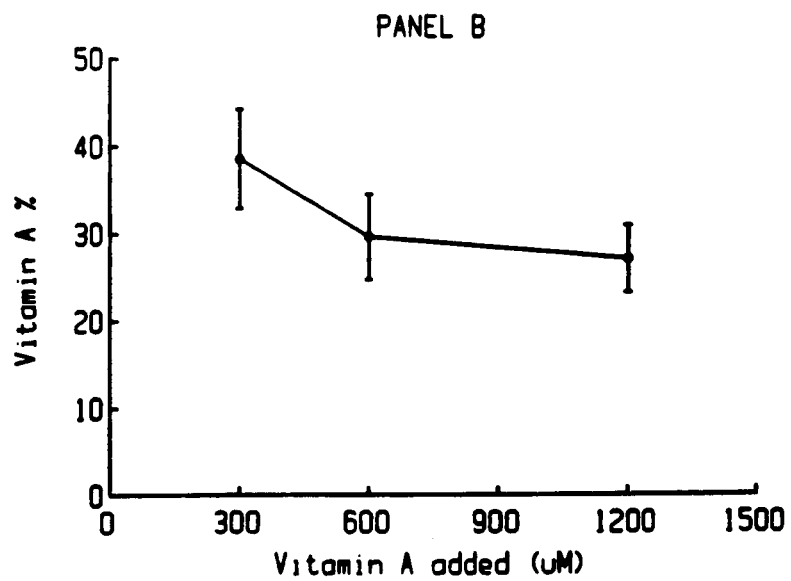
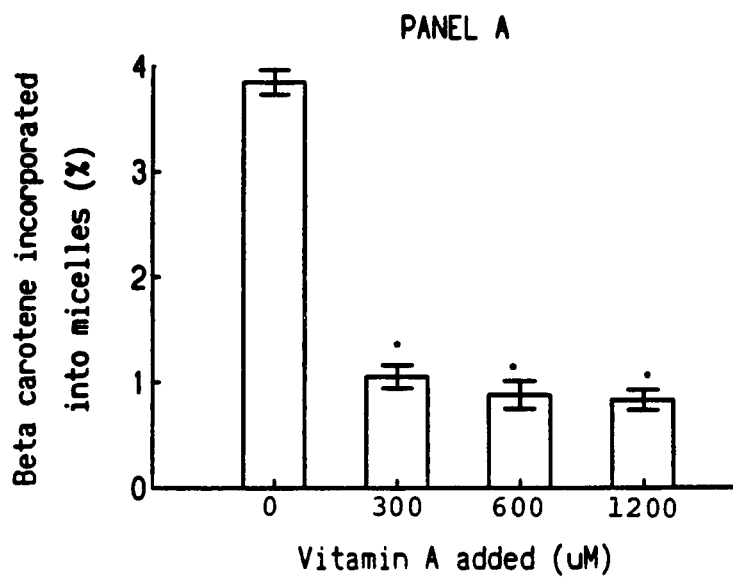
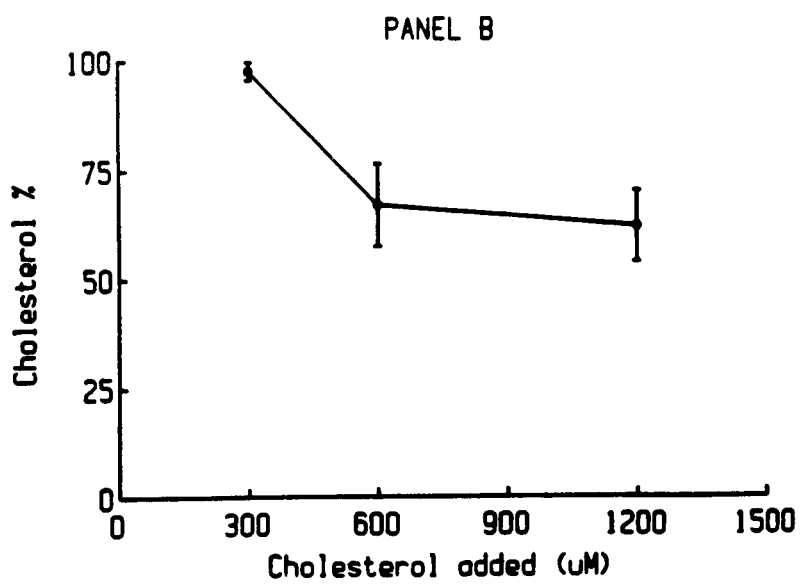
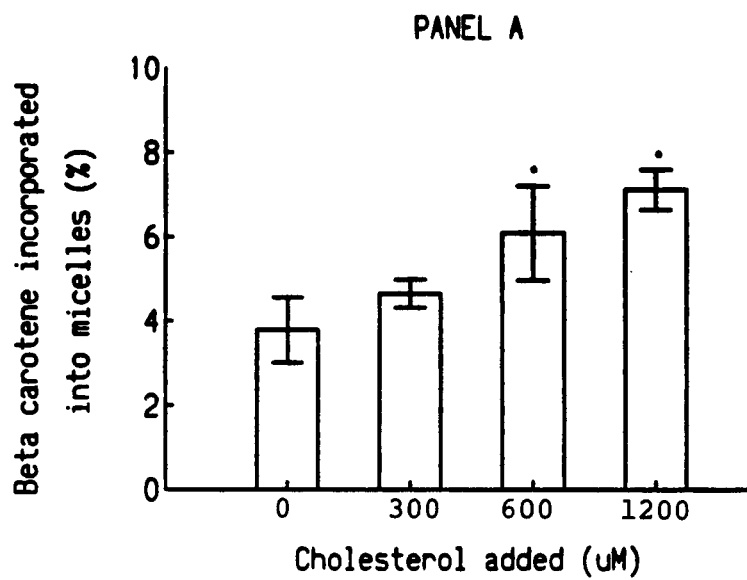


Table 3. Effect of dietary lipids on β -carotene uptake into micelles.

PUFAs	+	10-fold*
Vitamin A	-	4-fold
Vitamin E	-	3-fold
Cholesterol	+	2-fold

* As compared to micelles containing only β -carotene.

Fig. 6. Effect of cholesterol on β -carotene solubilization into micelles. -- Micelles were prepared according to methods; cholesterol was added at increasing concentrations (300, 600, 1200 μ M), corresponding to a β -carotene:cholesterol ratio of 1:1, 1:4, or 1:4. Micelles containing no cholesterol were prepared as a control. Panel A shows the percent incorporation of β -carotene in micelles containing different concentrations of cholesterol. Points represent mean of 3 replicates of one representative experiment. * = significantly different than control micelle (with no cholesterol) at $P < 0.05$. Panel B shows the percent incorporation of cholesterol into micelles with increasing concentrations of cholesterol in the incubation mixture. Points represents mean of 3 replicates.



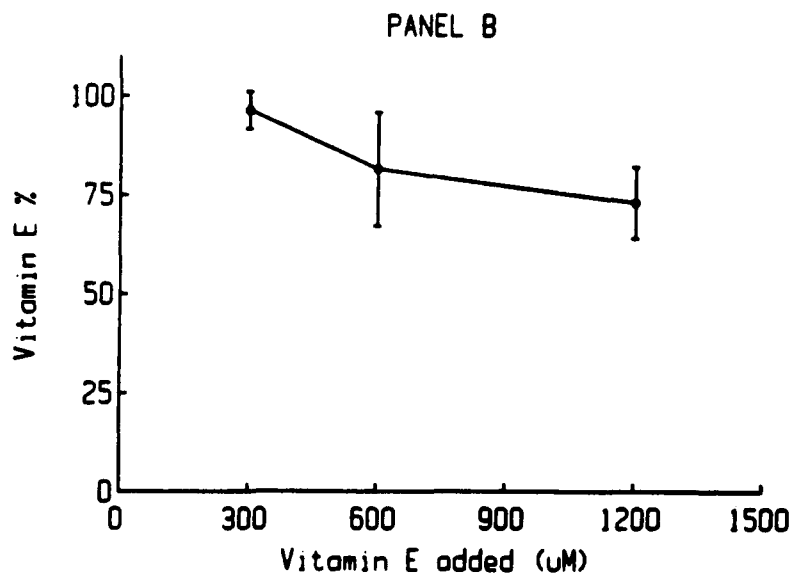
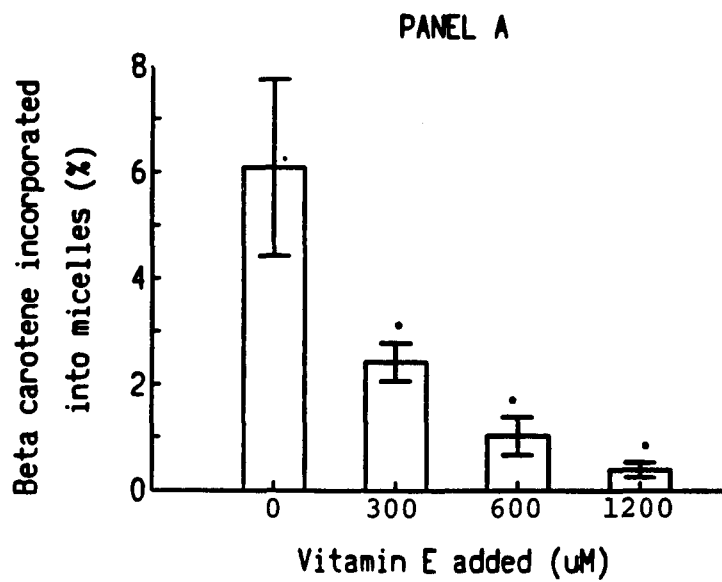
micelles with no cholesterol added (Table 3). The range of uptake was found to be 3.82% in the micelle with no cholesterol added, and 7.07% in the micelle with the highest concentration of cholesterol (Fig. 6, Panel A).

Figure 6, Panel B shows that the percent solubilization of cholesterol into micelles decreased from 97.48 to 61.33 as higher concentrations of cholesterol were added to the micelles.

4.4 Effect of α -Tocopherol (Vitamin E) on β -Carotene Solubilization into Micelles

To determine the effect of α -tocopherol on β -carotene incorporation into micelles, micelles were prepared by the procedure followed in previous experiments (Table 1) and by adding to the mixtures increasing concentrations of α -tocopherol (300, 600, or 1200 μ M), corresponding to β -carotene: α -tocopherol ratios of 1:1, 1:2, and 1:4. Addition of α -tocopherol to micelles significantly ($p < 0.05$) inhibited β -carotene incorporation into micelles. The uptake of β -carotene into micelles with no α -tocopherol added was 6.16%; addition of increasing amounts of α -tocopherol decreased incorporation of β -carotene to 0.39% (Fig. 7, Panel A). Uptake of α -tocopherol into micelles decreased from 98.01 to 73.40% as increasing concentrations of α -tocopherol were added to micelles (Fig. 7, Panel B).

Fig. 7. Effect of vitamin E (α -tocopherol) on β -carotene incorporation into micelles. -- Micelles were prepared as described in the methods section, with increasing concentrations of α -tocopherol (300, 600, 1200 μ m), which correspond to a β -carotene: α -tocopherol ratio of 1:1, 1:2, or 1:4. In addition, micelles containing no α -tocopherol were prepared as a control. Panel A shows the % incorporation of β -carotene into micelles with increasing concentrations of α -tocopherol. Points represent the mean of 3 determinations of one representative experiment. * = significantly different than control micelle (with no α -tocopherol) at $P < 0.05$. Panel B shows the percent incorporation of α -tocopherol into micelles with increasing concentrations of α -tocopherol in the incubation mixture. Points represent mean of 3 replicates.



The average incorporation of β -carotene (5.00%) into mixed micelles was much lower than the incorporation of vitamin A (37.00%), cholesterol (97.00%), and vitamin E (98.00%) (Table 4).

Table 4. Incorporation of different lipids into micelles.

	Uptake %	Std. Dev.
β -Carotene*	5.53	1.96
Vitamin A	38.17	6.41
Vitamin E	98.01	5.10
Cholesterol	97.48	4.67

* Separate addition of 300 g of each lipid to micelles with the composition described in the methods section.

CHAPTER 5

DISCUSSION

The data presented here demonstrates that uptake of β -carotene into mixed micelles is significantly affected by various dietary lipids. Since β -carotene solubilization is the first step in β -carotene absorption, these findings suggest a mechanism by which other dietary lipids may affect β -carotene absorption (Olson, 1964).

In each of the experiments presented here, we observed that the average incorporation of β -carotene (5.0%) into mixed micelles was much lower than the incorporation of vitamin A (37.0%), cholesterol (97.0%), and vitamin E (98.0%) (Table 2). Similarly, studies with laboratory animals (Olson and Hayaishi, 1965; Shapiro et al., 1984) and humans (Brown et al., 1988; Dimitrov et al., 1988) have shown that only a small percent (5-25%) of the β -carotene administered is absorbed. Poor uptake of β -carotene into micelles may be a function of the nonpolar properties of β -carotene (El-Gorab and Underwood, 1973). Bile salts are less efficient in solubilizing nonpolar than polar lipids (Carey and Small, 1970). In addition, the packing of nonpolar lipids into micelles is less efficient (Salle, 1978). Nonpolar molecules, such as β -carotene, are

apparently confined to the hydrophobic core of the micelle (Fig. 1). On the other hand, more polar compounds such as retinol, cholesterol, and tocopherol are able to accommodate their hydrocarbon structures in the hydrophobic core and their hydrophilic portion facing the aqueous phase, resulting in increased solubilization into micelles. Other properties of lipids may also affect their solubilization into micelles, such as the formation of ionic bonds with bile salts. Vitamin E is known to undergo oxidation-reduction reactions that would facilitate the formation of ionic bonds with bile salts. This property may also account for the higher incorporation of vitamin E into micelles compared to β -carotene. This study suggests that the low β -carotene uptake into micelles may explain at least in part the low absorption of this compound in vivo.

β -Carotene incorporation into micelles was greater in the presence of unsaturated fatty acids (Table 3). β -carotene uptake into micelles containing polyunsaturated fatty acids (PUFAs) was significantly higher ($p < 0.05$) than uptake into micelles containing a saturated (18:0) or a mono-unsaturated (18:1) fatty acid. Incorporation of β -carotene into micelles containing linolenic acid (18:3) was 10-fold higher than uptake into micelles containing stearic acid (18:0) and 3-fold higher than micelles containing oleic acid (18:1). PUFAs are known to increase

the fluidity and permeability of the micelle membrane (Salle, 1978); these structural features may enhance the ability of the micelle to solubilize additional lipids such as β -carotene. In contrast to the work presented here, studies in vivo have shown an increased β -carotene absorption in the presence of saturated or mono-unsaturated fatty acids (Alam et al., 1989; Alam et al., 1990) compared with PUFAs. In vivo, PUFAs may increase β -carotene oxidation during uptake into the intestinal cell or stimulate β -carotene bioconversion to vitamin A. Shiau (1981) has suggested the possibility that PUFAs may influence the synthesis of chylomicrons or very low density lipoproteins within the mucosal cells and affect, in this way, transport of fat soluble vitamins. The difference in our results and those of others (Alam et al., 1989; Alam et al., 1990) may also be explained by the fact that we studied only micellar solubilization. Other investigators have measured the presence of β -carotene in plasma and other tissues involving various regulatory steps at the level of intestinal absorption and transport of β -carotene to different organs. Each of those steps may be affected differently by other compounds absorbed from the diet.

Cholesterol stimulated the uptake of β -carotene into micelles 2-fold. Cholesterol is known to expand the size of micelles and increase their ability to solubilize

other fat soluble compounds (Mazer et al., 1983). Therefore, β -carotene appears to be more efficiently absorbed into micelles containing cholesterol. We are aware of no other studies on the effect of cholesterol on β -carotene absorption.

Addition of the two fat soluble vitamins, retinol and α -tocopherol, into mixed micelles, significantly reduced β -carotene uptake into micelles (Table 4). Addition of retinol to micelles in equimolar amounts with β -carotene reduced uptake of β -carotene 4-fold. Addition of the same concentration of α -tocopherol reduced β -carotene incorporation into micelles 3-fold. In various animal models, retinol and α -tocopherol were shown to lower plasma carotenoid values (Alam et al., 1990; Dua et al., 1966; Jacobson et al., 1950; Mattson and Duel, 1943). It has been proposed that different lipid soluble compounds compete with each other for solubilization inside micelles (El-Gorab et al., 1975; Hollander, 1981). Therefore, it seems likely that β -carotene competes with both retinol and α -tocopherol for solubilization into the limited space of the micelle.

CHAPTER 6

SUMMARY

In this study we have produced micelles containing β -carotene using a standard protocol that is reproducible and simple to prepare. We observed that the amount of β -carotene incorporated into micelles is affected by the presence of other dietary lipids. Uptake was stimulated by cholesterol and poly-unsaturated fatty acids (uptake correlated with degree of unsaturation). These lipids alter the size, fluidity, and permeability of micelles, probably rendering them more capable of solubilizing higher amounts of β -carotene.

We also showed that vitamins A and E decreased β -carotene uptake into micelles, suggesting that β -carotene competes with vitamins A and E for solubilization inside the micelles. Our results suggest that absorption of β -carotene in humans might in part be regulated at the level of formation of intestinal micelles by the presence of different dietary components.

We expect that the findings of this study will contribute to the understanding of the nutritional variables that affect β -carotene absorption, information

that could be useful in designing more efficient vehicles
for β -carotene supplementation.

APPENDIX A

EFFECT OF FAT ON β -CAROTENE ABSORPTION

Type of Fat	Model Studied	Response	Author
Margarine vs cottonseed oil	Vitamin A def. rats	+ Growth response	Deuel, 1941
Mineral oil	Growing rats	- Growth response	Rowntree, 1931
(Soy, corn oil) vs (Coconut, butter)	Vitamin A def. rats	+ Growth No effect	Sherman, 1941
18:0, 18:1, 18:2, 18:3,	Perfusate intestinal loops	18:1 > 18:0 > 18:2 > 18:3 β -carotene in loops	Hollander, 1978
Corn oil, lard	Rats	Plasma β -C: Lard > corn Liver β -C: Corn > lard	Alam, 1989
Coconut, safflower, menhaden, olive	Rats	Plasma β -C: Co > Sa > Ol > Me	Alam, 1990

+ Means increased levels
- Means decreased levels

APPENDIX B

EFFECT OF VITAMIN A ON β -CAROTENE ABSORPTION

Supplement Added	Model Studied	Response	Author
B-C (As alfalfa) + vitamin A (9300 IU/Kg diet)	Poultry carotenoids	- Serum	Mattson, 1943
Vitamin A (4,400 IU/Kg diet)	Poultry	- Egg yolk carotenoids	Dua, 1966
B-C (non-specified) Vitamin A (240,000 IU)	Cattle	- Milk carotenoids	Blaxter, 1946
180 mg β -C + Vitamin A	Humans	+ Buccal mucosal β -C levels	Stich, 1986

+ Means increased levels

- Means decreased levels

APPENDIX C

EFFECT OF VITAMIN E ON β -CAROTENE ABSORPTION

Supplement Added	Model Studied	Response	Author
β -C (100 IU/week) vitamin E (0.1-2.0 mg/week)	Rats	Liver + vitamin A stores	Moore, 1940
Vitamin A deficient diet + 1 mg/week vitamin E	Rats	Time to + deplete vitamin A stores	Davis, 1941
PUFAs + 1 mg/week vitamin E + β -C (non specified)	Vitamin A deficient rats	+ Growth	Sherman, 1941
Vitamin E (0.5 mg/day) β -C (0.79 μ g/day)	Vitamin A deficient rats	+ Growth	Hickman, 1944
Vitamin E: 0.5, 1.0, or 2.0 mg/day + β -C 1 mg/day	Rats	Growth + 0.5, 1.0 - 2.0	Burns, 1951
Vitamin E (0.5, > 1.0 mg/day) β -C (174 μ g/day)	Vitamin A deficient rats	Liver vitamin A stores + 0.5 mg - > 1.0 mg	Herbert, 1953
Vitamin E (2.0 or 10 mg/day) β -C (44 μ g/day)	Rats	- Liver vitamin A stores	Johnson, 1948
PUFAs + vitamin E (2% of diet) β -C (2% of diet)	Rats	- Liver and - Plasma β -C levels	Alam, 1990

+ Means increased levels

- Means decreased levels

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