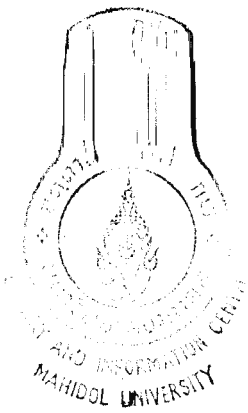

IMPACT OF DIETARY VITAMIN A INTERVENTIONS ON TOTAL BODY STORES IN THAI LACTATING WOMEN

VORACHART DHANANIVESKUL

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Thesis

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The contribution of a carotenoid rich foods to vitamin A (VA) status remains controversial. This study evaluated the efficacy of a provitamin A mid-day meal given 5 d/week for 3 months on changes in total body retinol stores (TBRS) by stable isotope dilution and in other VA status indicators among lactating women in Northeast Thailand. Seven hundred eighteen lactating women (1-12 month postpartum) were rapidly screened for low vitamin A intake and serum retinol below 0.87 umol/L (25 ug/dl). Eighty-five women were enrolled. They were matched by postpartum age, geographical area and randomized in a block fashion into 3 groups to receive one daily meal containing (A) dark green leafy and yellow/orange vegetables and fruits, (B) purified beta-carotene and (C) control (low carotenoid) vegetables and fruits. Fat content per menu was about 10 gm. The average beta-carotene content for the 12 weeks period in group A, B and C was 4.7, 3.6 and <0.05 mg/meal respectively. All subjects consumed over 95% of the food given. Prior to and following dietary intervention, vitamin A reserves were estimated by isotopic dilution method. In addition, maternal anthropometry, morbidity, and other indicators of VA status (MRDR at post-intervention only, serum and breast milk retinol and carotenoids, CIC and modified dark adaptometry) were examined. Twenty-four hour recall plus weighing method for one random day per week were collected throughout the study. 71 subjects completed the trial (n = 24,25 and 22). Data of habitual diet revealed that group A consumed about half of the amount of preformed vitamin A than other groups. Serum and breast milk beta-carotene increased most in group B, followed by group A but breast milk retinol increased more in group A compared to others (p=0.097). Serum retinol increased in all groups (from ~21 to ~46 ug/dl), reflecting seasonality. TBRS was comparable at baseline among the three groups (77, 81,95 mg, p=0.87). Mean (median) TBRS decreased by 15 (5), 3 (3) and 19 (14) mg in groups A, B and C (p=0.4). An inverse linear relation was detected between isotopic ratio at 3 day post-dosing and estimates of TBRS at both pre-intervention (r = -0.82, p<0.0001) and post-intervention (r = -0.79, P<0.0001). Thus, serum isotopic ratio of (D:H) 3 day post-dosing seems to be useful as an early indicator of TBRS. Serum and breast milk vitamin A can increase amidst decreasing TBRS in lactating women. Loss in TBRS may be prevented by daily beta-carotene supplements but less so with increased, short-term dietary beta-carotene intake. Longer interventions and more subjects are needed to show dietary effects on TBRS.

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คำสำคัญ : การเสริมวิตามินเอ/หญิงให้นมบุตร/เบต้า-แคโรทีน/การสะสมในร่างกาย/สารละลายอณูธรรมชาติ

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การเสริมอาหารที่มีแคโรทีนอยด์สูงเพื่อเปลี่ยนรูปเป็นวิตามินเอในร่างกายยังคงเป็นข้อถกเถียงกันอยู่ การศึกษานี้เป็นการประเมินประสิทธิผลของอาหารที่เป็นตัวตั้งต้นของวิตามินเอซึ่งถูกจัดให้กับหญิงให้นมบุตรภาคตะวันออกเฉียงเหนือของประเทศไทยในช่วงเวลา 5 วัน/สัปดาห์นาน 12 สัปดาห์ เพื่อศึกษาถึงการเปลี่ยนแปลงของการสะสมวิตามินเอในร่างกายโดยการใช้วิธีตรวจวัดจากสารละลายอณูธรรมชาติของวิตามินเอและการตรวจวัดภาวะวิตามินเอโดยวิธีต่างๆ หญิงให้นมบุตร 718 คน (1-12 เดือนหลังคลอด) ถูกคัดเลือกเพื่อเข้าร่วมโครงการจากการสำรวจการบริโภคอาหารวิตามินเอปริมาณต่ำและการตรวจวัดค่าวิตามินเอในเลือดที่มีค่าน้อยกว่า 0.87 ไมโครโมลต่อลิตร (25 ไมโครกรัมต่อเดซิลิตร) พบว่ามีเพียง 85 คน ที่อยู่ในเกณฑ์การคัดเลือกและถูกนำมาจัดกลุ่มโดยอาศัยเกณฑ์การสุ่มจากอายุเด็กหลังคลอด, สภาพภูมิประเทศของผู้เข้าร่วมโครงการเป็น 3 กลุ่ม โดยแบ่งตามอาหารที่ได้รับวันละหนึ่งมื้อคือ (A) กลุ่มที่ได้รับอาหารที่มีเบต้า-แคโรทีนในรูปของผักใบเขียวและผลไม้สีเหลืองส้ม, (B) กลุ่มที่ได้รับอาหารที่มีเบต้า-แคโรทีนสังเคราะห์ และ (C) กลุ่มควบคุมที่ได้รับอาหารจากผักและผลไม้ที่มีเบต้า-แคโรทีนต่ำ ซึ่งมีค่าเฉลี่ยของเบต้า-แคโรทีนคือ 4.7, 3.6 และน้อยกว่า0.05 มิลลิกรัมต่อมื้อตามลำดับ อาสาสมัครรับประทานอาหารที่แจกให้มากกว่า 95% ในช่วงของการเสริมอาหารจะทำการตรวจวัดปริมาณสำรองของวิตามินเอในร่างกายโดยวิธีการใช้สารละลายอณูธรรมชาติ มีการตรวจวัดภาวะของแม่ที่ให้นมบุตรต่างๆคือ ตรวจร่างกายทั่วไป ภาวะการเป็นโรค และตัวชี้วัดภาวะวิตามินเอต่างๆ [MRDR (ช่วงหลังการเสริมอาหารเท่านั้น) ปริมาณวิตามินเอและเบต้า-แคโรทีนในเลือดและในน้ำนมแม่ ตรวจวัดเยื่อบุตาขาว การปรับการมองเห็นในที่มืด] สำรวจการได้รับอาหารจากแบบสำรวจอาหารย้อนหลัง 24 ชั่วโมงและชั่งน้ำหนักอาหารโดยสุ่ม 1 วันต่อสัปดาห์ มีอาสาสมัครที่ตรวจวัดครบกระบวนการศึกษาทั้งสิ้น 71 คน (n = 24, 25 และ 22 คน) ข้อมูลของอาหารที่รับประทานเป็นประจำพบว่า กลุ่ม A ได้รับอาหารที่มีวิตามินเอประมาณครึ่งหนึ่งของกลุ่มอื่น เบต้า-แคโรทีนในเลือดและน้ำนมแม่มีการเพิ่มขึ้นมากที่สุดในกลุ่ม B รองมาคือกลุ่ม A แต่วิตามินเอในน้ำนมแม่เพิ่มสูงขึ้นในกลุ่ม A อย่างเกือบมีนัยสำคัญทางสถิติ (p=0.097) ปริมาณวิตามินเอในเลือดเพิ่มสูงขึ้นทุกกลุ่ม (จาก~21 ถึง ~46 ไมโครกรัมต่อเดซิลิตร)และเป็นภาพสะท้อนถึงช่วงการเปลี่ยนแปลงตามฤดูกาลด้วย การสะสมวิตามินเอในร่างกายถูกเปรียบเทียบในช่วงต้นระหว่าง 3กลุ่ม (77, 81, 95 มิลลิกรัม) ค่าเฉลี่ย (ค่ามัธยฐาน) ของการสะสมวิตามินเอในร่างกายลดลงดังนี้ 15(5), 3(3) และ 19(14) มิลลิกรัมในกลุ่ม A, B และ C (p=0.4) และพบความสัมพันธ์ในทางตรงข้ามระหว่างค่าอัตราส่วนสารละลายอณูธรรมชาติ ณ วันที่ 3 หลังการให้กับการประมาณการของการสะสมวิตามินเอในร่างกายทั้งในช่วงก่อนเสริมอาหาร (r=-0.82, p <0.0001)และหลังการเสริมอาหาร (r=-0.79, p=0<0.0001) ซึ่งเป็นประโยชน์ในการประมาณการเบื้องต้น

มีต่อหน้าต่อไป →

ของการสะสมวิตามินเอในร่างกายได้ วิตามินเอในเลือดและน้ำนมแม่สามารถเพิ่มขึ้นในขณะที่มีการลดลงของการสะสมวิตามินเอในร่างกายของหญิงให้นมบุตร ซึ่งการสูญเสียการสะสมวิตามินเอในร่างกายสามารถที่จะป้องกันได้โดยการเสริมอาหารที่มีเบต้า-แคโรทีนในแต่ละวันจะทำให้การสะสมวิตามินเอในร่างกายเพิ่มขึ้นได้แม้ว่าจะเป็นการได้รับการเสริมอาหารในระยะสั้น การเสริมอาหารในระยะยาวและจำนวนอาสาสมัครที่มากกว่าการศึกษานี้เป็นสิ่งจำเป็นต่อการศึกษาผลของการสะสมวิตามินเอในร่างกาย

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CHAPTER I

INTRODUCTION

Vitamin A deficiency (VAD) results in an impairment of vision in dim light and a series of defect of epithelial tissue of the eye leading to blindness. Subclinical VAD also contributes to increased risk of childhood morbidity and mortality (1). In 1995, WHO estimates that at least 3 million children manifest clinical signs of VAD annually while approximately 250 million of children under five years of age are at risk of deficient vitamin A status (2). In the areas where VAD is a public health problem, a mix of interventions is required to treat and control health consequences of VAD. Periodic distribution of high dose vitamin A capsules is recommended for treatment and prevention of xerophthalmia among the high risk groups. Fortification of foods which are widely consumed by the high risk population, if properly conducted, can be highly effective and sustainable in preventing VAD. In several countries where local vitamin A foods are or can be made available, dietary modification to improve intakes of these foods should be considered as part of a long-range plans.

Thailand is classified by the World Health Organization as a country with a moderate level of subclinical deficiency (3). A 1990 survey in Northern and Northeastern Thailand, for example, indicated that approximately one-fifth of preschool children in these regions experience subclinical vitamin A deficiency as evidenced by low liver stores (estimated by the relative dose response or RDR) and abnormal conjunctival epithelium (examined by conjunctival impression cytology or CIC) (4) . A different picture, however, emerged from the lower region of Southern

Thailand where case of infant xerophthalmia and keratomalacia in preschool children were reported in 1991 and 1992 (5) . The eruption of infant xerophthalmia in the lower southern region in 1992 called for a mix of intervention strategies, beginning with periodic supplementation of vitamin A, followed by the fortification of sweetened condensed milk and recommendations for increased consumption of vitamin A rich foods (6). Since then, a vitamin A supplementation program has been launched and targeted at infants and preschool children in high risk areas . This intervention in coordination with food-based dietary intervention efforts have results in no new reported cases of clinical vitamin A deficiency. Several small scale surveys in northeast Thailand indicated an existence of subclinical state of vitamin A deficiency among young children, pregnant and lactating women (7). Recent dietary surveys in 1989 and 1991 of lactating women in the northeast Province of Ubonrajthanee showed the average vitamin A intakes to be 253 and 333 RE/day representing 25 and 33 % of Thai RDAs, respectively (Viriyapanich T, unpublished observations). These data suggested that a considerable proportion of lactating women in rural northeast are at-risk of vitamin A deficiency and may benefit from dietary intervention program . Since vitamin A rich foods, especially vegetables and fruits are available or can be grown in most communities, the at-risk population should benefit from food-based strategy to promote consumption of these foods. Increasing production and consumption of locally available carotenoids- rich foods represents an appropriate long-term strategy to prevent chronic, subclinical vitamin A deficiency and promote reproductive and child health . Rural communities depend primarily on plant sources rich in provitamin A carotenoids (dark green leafy vegetables and yellow/orange, fruits and vegetables) for their vitamin A supply . However, the bioavailability and bioconversion of

provitamin A carotenoids are influenced by a number of factors such as chemical structure, food matrix in which a carotenoid is incorporated, cooking methods, dietary fat, nutrient status (vitamin A, protein, zinc) of the host, genetic factors and interactions. Earlier studies which linked the improvement of vitamin A status to the increase in intake of fruits and vegetables have been criticized in regard to research design and methods (8). The more recent study in Indonesian lactating women indicating lack of response of serum and breast milk vitamin A to a routine diet of dark green leafy vegetables created doubts as to the efficacy of these foods in the prevention programs of VAD (9). These findings could be due to poor responsiveness of presently used biochemical indicators or the closer-to-normal vitamin A status of the studied population. Circulating levels of vitamin A are not considered a reliable index of vitamin A status due to a homeostatic control over a broad range of body stores and reflect body stores only when these are very low or very high. Therefore, an estimation of total body reserve of vitamin A offers a good index of vitamin A status. Recently, the isotope dilution technique has been developed to assess body stores of vitamin A (10). The method is based on the equilibration of newly absorbed vitamin A, labeled with isotopic tracers with the existing body pool of vitamin A after which the body pool can be calculated by a dilution equation. This technique offers an opportunity to pursue further the question concerning the efficacy of provitamin A carotenoids on vitamin A nutriture. Therefore, this randomized trial is designed to evaluate the efficacy of consuming provitamin A rich foods on total body vitamin A stores and other indices in lactating women of rural northeastern Thailand.

CHAPTER II

OBJECTIVES

Specifically, this study aims to-

1. Assess the efficacy of daily meals containing provitamin A-rich vegetables and fruits given five days per week for twelve weeks in improving total body vitamin A stores and other aspects of vitamin A status in lactating women.

2. Test the ability of stable isotope dilution to detect changes in apparent total body vitamin A stores and breast milk concentrations of vitamin A in response to known, varied dietary intakes of beta-carotene; and

3. Evaluate the sensitivity and specificity of other common indicators of vitamin A status against estimates of total body stores, and changes in body stores of vitamin A in lactating women.

CHAPTER III

LITERATURE REVIEWS

3.1 Nomenclature, chemistry and units

Vitamin A is a generic descriptor for compounds containing the biological activity of retinol. The compound is soluble in fat and fairly heat stable but easily destroyed by oxidation, particularly when exposed to light and heat in a humid atmosphere in the absence of reducing agents or other stabilizers (11). The major derivatives of vitamin A include the ester (retinyl ester), the aldehyde (retinal) and the acid (retinoic acid) form. The interconversion of these derivatives is shown in Figure A (12).

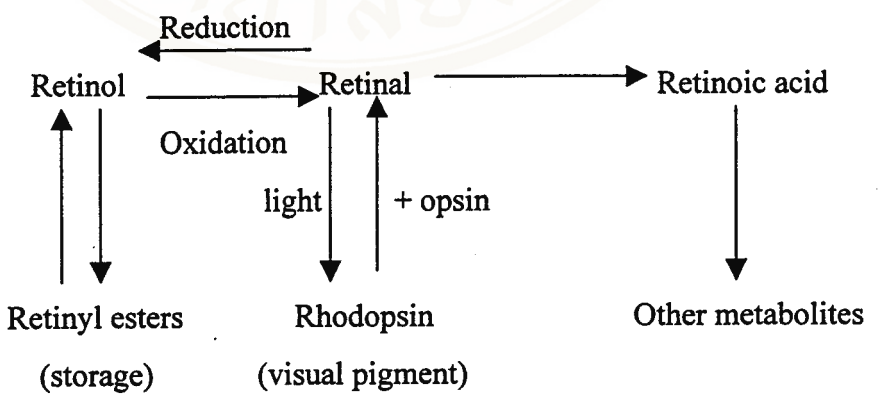


Figure A. Interrelationship between retinol, retinal, retinyl esters and retinoic acid.

Vitamin A is found in nature to a large extent in two basic groups:

i) preformed vitamin A or retinoids, which include retinol isomers and esters, retinal and dehydroretinol, can be found in animal foods and therefore absorbed only from animal tissue.

ii) those of provitamin A which refers to carotenoid precursors that can be converted by the body into retinol, are found primarily in plant foods, the most biologically active compound of these carotenoids is all-trans β-carotene (11,12).

Biological activity of vitamin A is conventionally expressed as retinol equivalents (RE) and commonly earlier as International units (IU). Interconversion of these units among different forms of vitamin A is shown below (13):

1RE = 1 ug retinol

= 6 ug all-trans beta-carotene

= 12 ug other provitamin A carotenoids

= 3.33 IU vitamin A activity from retinol

= 10 IU vitamin A activity from beta-carotene

3.2 Metabolism of vitamin A and carotene

3.2.1 Digestion and absorption

Most of the preformed vitamin A in the diet is in the form of retinyl esters. After the ingestion of food, preformed vitamin A of animal tissues and the provitamin A carotenoids of vegetables and fruits are released from proteins by the

action of pepsin in the stomach and various proteolytic enzymes in the small intestine (12). The upper intestine is the major site of lipid hydrolysis. Dietary fat and protein and their hydrolytic products stimulate, through the secretion of the hormone cholecystokinin, the secretion of bile. This emulsifies lipids and promotes the formation of micelles which have lipophilic groups on their inside and hydrophilic groups on their outside. In this way the absorption of fat is facilitated. Bile salts stimulate pancreatic lipase and other esterases that hydrolyze retinyl esters in intestinal mucosal cells (enterocytes). Retinol, the product of the hydrolysis, is well absorbed (70-90%) by mucosal cells.

Provitamin A carotenoids pass into the mucosal cells unchanged. A proportion of each, along with non-provitamin carotenoids, passes through unchanged into the lymph and the blood. The remainder undergoes clevage of the molecule by a specific enzyme 15,15'-dioxygenase within the intestinal mucosal cell (14), and is reduced mainly to retinol by retinaldehyde reductase, and finally is esterified to retinyl palmitate and other similar esters. Some carotenoids may remain unchanged and appear in the chylomicra together with retinol esters and some free retinol, and are transported through the lymph into the general circulation. A simplified schematic outline of these main metabolic pathways is shown in Figure B.

3.2.2 Transport

Absorbed vitamin A of dietary origin is transported as an ester in chylomicra and ultimately taken up by hepatocytes of the liver. The mobilization of vitamin A from liver stores and its delivery to peripherol tissue is a highly regulated process. A

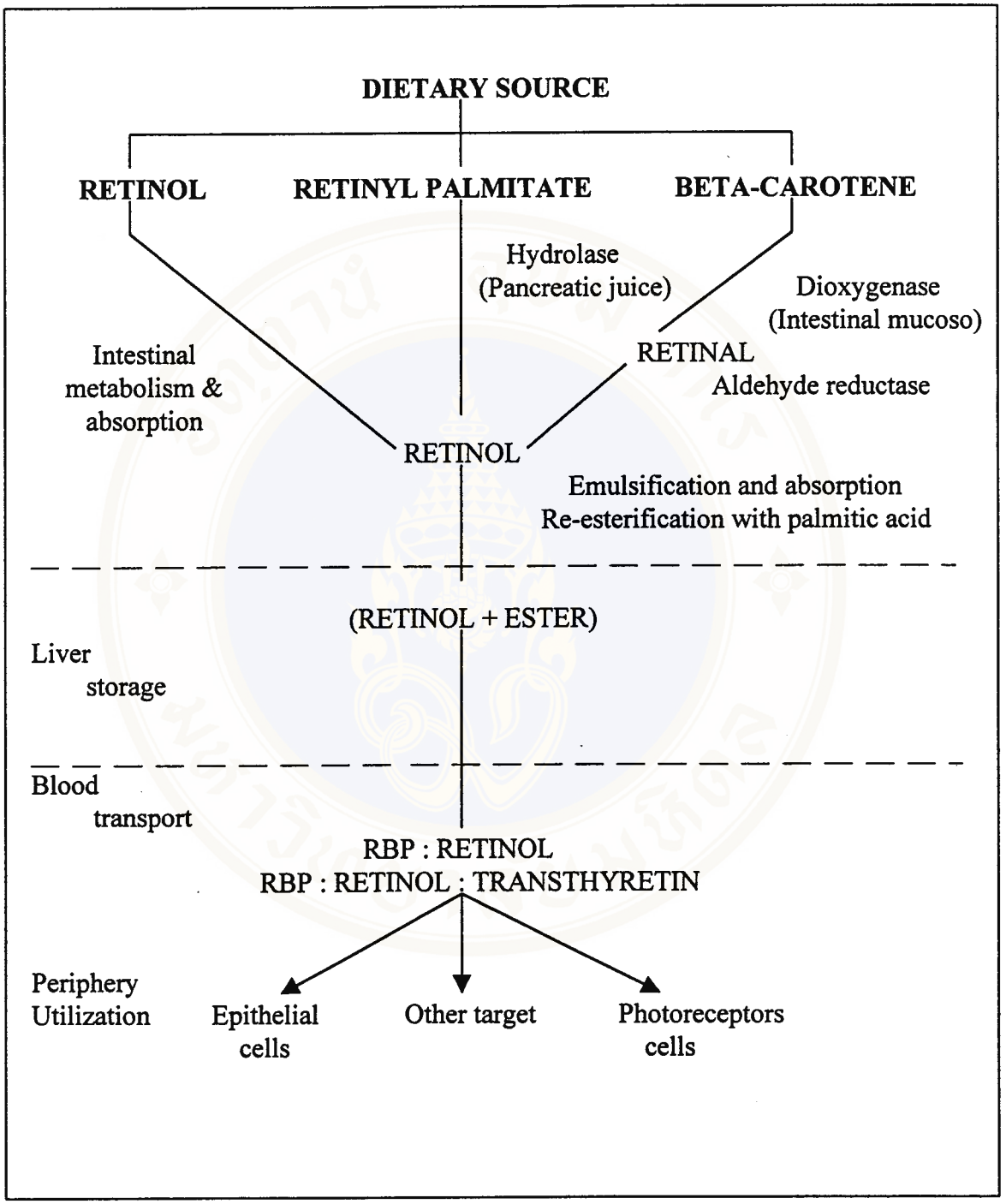


Figure B. Schema of vitamin A metabolism (15).

precursor (pre RBP) of a specific retinol-binding protein (RBP) is synthesized in liver parenchyma cells. Prior to release from the liver, the retinyl esters (storage form) are hydrolyzed to free retinol which then combine with an apo-retinol binding protein (RBP). The resultant holo-RBP is secreted into the plasma where it combines with pre-albumin and attaches to a specific receptor on the cell membrane, with retinol passing into the cell. Once in the cell, retinol is quickly bound by specific binding proteins in the cell cytosol. This combination helps to stabilize and protect vitamin A delivering to the target tissue. Vitamin A mobilization from the liver and its transport to the tissue are tied to rate of apo-RBP production and release in the liver, while RBP secretion in turn is blocked by retinol deficiency. Therefore, RBP plays a highly important role in the regulation of vitamin A function and metabolism (12, 16, 17).

3.2.3 Storage

The circulating retinyl esters are almost completely taken up by the liver. Normally, about 90% of vitamin A in the body is found in the liver (16).

Retinyl ester in the chylomycra are stored in vitamin containing globules (VAG) within the parenchyma cells. Within the liver, two types of cells are mainly responsible for vitamin A storage, the hepatocytes and the lipocytes. Under normal physiological conditions, the lipocytes, which are also called stellate cells, have been estimated to contain approximately 80% of the total vitamin A of the liver. Much of the retinol initially taken up by the hepatocytes is transferred to the non parenchymal cell fraction within 1 to 2 hours of update (17). A complex of retinol with cellular RBP is transported to the storage site and stored as lipoglycoprotein complex. Associated

with the complex is a tightly bound retinyl ester hydrolase which, in the presence of intracellular apo-RBP, hydrolyses retinyl ester and transfers the retinol to apo-RBP (18). Liver storage of vitamin A is affected by many factors: obviously by the rate of vitamin A uptake and the release rate of stored vitamin A from the liver in addition to other dietary and hormonal factors (19).

3.2.4 Excretion

Vitamin A is secreted in various forms in both urine and feces. Under normal physiological conditions, approximately 5-20% of ingested vitamin A is not absorbed from the intestinal tract, hence is excreted within 1-2 days into feces. The efficiency of enteric absorbtion of vitamin A is high (i.e.,80-95%), with 30-60% of the absorbed amount being deposited in esterified form in the liver. The balance of absorbed vitamin A is catabolyzed and is released in the bile or plasma, where they are removed by the kidney and excreted in the urine. About 30% of the biliary metabolites are reabsorbed from the intestine in an enterohepatic circulation back to tha liver, but most are excreted in the feces with intact carbon chains are excreted in the feces, whereas the shortened-chain, acidic metabolites are excreted in the urine. The relative amounts of vitamin A metabolites in the urine and feces, vary with vitamin A intake and the hepatic vitamin A reserve (20).

3.3 Function of vitamin A

3.3.1 Vision

The best elucidated function of vitamin A is in the visual process, especially in dim light. The retina of the eye has two kinds of photoreceptor cells: Rod cells contain the photosensitive protein, rhodopsin, are activated by darkness, whereas cone cells which are activated by light, contain one of three iodopsins. When a photon of light strikes the rod cells, opsin and retinal are liberated from rhodopsin pigment. The atom in the retinal molecule are rearranged to form trans-retinal. For visual activity in the dark, rhodopsin is regenerated; all trans-retinals converted to 11-cis retinal which combines with opsin to form rhodopsin. A deficiency of vitamin A delays this process and results in delayed adaptation to dim light or night blindness. A simplified visual cycle in the rod cells of retina is shown in Figure C.

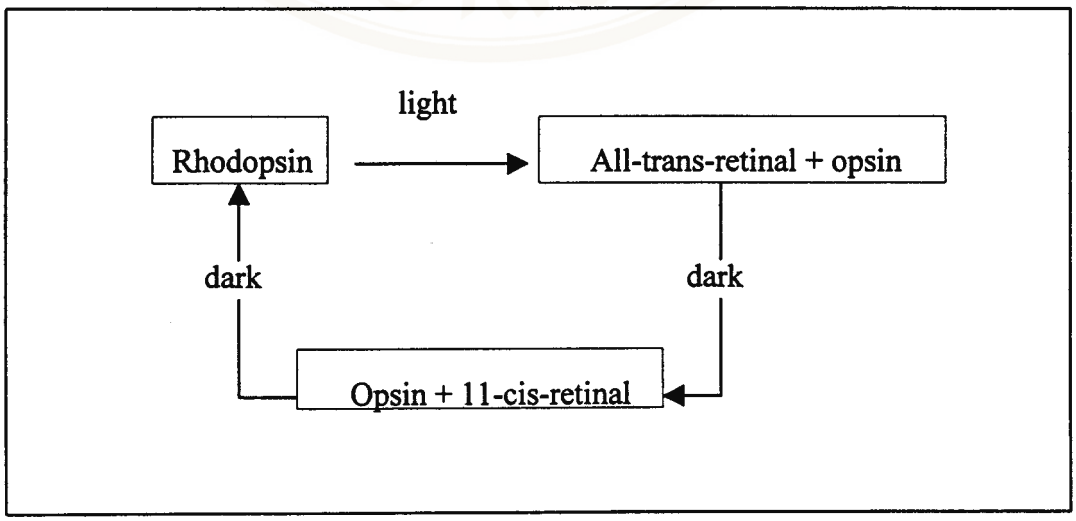


Figure C. Simplified visual cycle in the rod cells of retina (21).

3.3.2 Cellular differentiation

Vitamin A plays a role in the maintenance of healthy epithelial cells in the mucous membranes lining all openings into the interior of the body, i.e., alimentary, respiratory, and genito-urinary tracts, as well as the eyes. It is well documented that vitamin A-deficient individuals experience replacement of normal mucus-secreting cells by cells that produce keratin, particularly in the conjunctiva and cornea of the eye, the trachea, the skin and other ectodermal tissues (22). These epithelial cells cease to differentiate and lose their secretory function. The undifferentiated cells are flattened and multiply at an increased rate, so that the cells pile up on one another and the surface becomes keratinized. The cornea is of particular interest in as much as its ulceration and ultimately its destruction. In vitamin A-deficient children this is a major cause of blindness (12). Thus, prevention of vitamin A deficiency in children, pregnant and lactating women are essential in the program implementation.

3.3.3 Other functions

Vitamin A also plays roles in growth, reproduction and in the body's humoral as well as cellular immune responses. The precise mechanism of vitamin A in these processes, however, remain unclear. There is a posibility that vitamin A may affect nitrogen metabolism in the cell such that the amino acid patterns in tissues and plasma of vitamin A-deficient subject are altered. These changes together with keratinization of the taste buds resulting in loss of taste, might explain the depression of appetite and the reduction in food intake (23). The lack of protective mucus in the affected mucosae also leads to an increased susceptibility to infections (24). Adequate

supplies of retinol are necessary for spermatogenesis, maintenance of the testicular and vaginal epithelia, and for the proper development of the fetus (24).

3.4 Deficiency signs

Vitamin A deficiency or Hypovitaminosis A may be due to a dietary lack of vitamin A or provitamin A, or to poor absorption. Among the various clinical manifestations, those of the eye and skin are preeminent.

3.4.1 Animals

Vitamin A deficiency has been diagnosed in many animals. There are many signs that can occur earlier such as reduced appetite, weight loss, a reduction in goblet cell number and the reduced synthesis of some glycoproteins, ultimately vitamin A deficiency affects almost all tissues of the body. The major causes of death are infection, twisting of the intestine, and urinary blockage (22).

3.4.2 Man

The major signs of vitamin A deficiency in man relate more to external, readily diagnosed changes than to abnormalities of tissue or cellular structure. The earliest symptom is night blindness, the inability to see normally in dim light, which is noted clinically by abnormal dark adaptation. The other major sign of vitamin A deficiency is Xerophthalmia, which includes various stages of conjunctival xerosis, Bitot's spot, corneal xerosis and ulceration. As another sample, changes in skin have also been observed, most specifically follicular hyperkeratosis. Although the signs of vitamin A

deficiency have occasionally been noted among seemingly healthy adults, the most serious and dramatic manifestations are seen in very young children. In these cases, vitamin A deficiency is almost invariably associated with protein-calorie malnutrition, a low intake of fats, gastrointestinal distress and respiratory disease. Apart from clinical signs of deficiency, a marginal vitamin A status has been associated in children with increased morbidity and mortality, a decreased growth rate, and inefficient iron utilization (22). Major eye signs and classification are summarized in Table 1.

Table 1. Clinical classification of xerophthalmia (WHO, 1982) (24).

Classification		Description
X1A	- Conjunctival xerosis	drying of the conjunctiva
X1B	- Bitot's spot with conjunctival xerosis	an extention of X1A, foamy or cheesy patches forming on the conjunctiva
X2	- Corneal xerosis	a hazy or granular surface pebby dryness apparent on the cornea
X3A	- Corneal ulceration with xerosis	less than 1/3 of corneal surface affected
X3B	- Corneal ulceration/keratomalacia	more than 1/3 of corneal surface affected
XN	- Night blindness	maladaptation to dim light
XF	- Xerophthalmia fundus	retinal lesions
XS	- Corneal scars	scarring associated with previous xerophthalmic condition

The prevalence survey are needed to indicate the magnitude of vitamin A deficiency as a public health problem and to provide information of use in defining a suitable strategy for reducing its prevalence. The criteria for determining whether vitamin A deficiency is a significant public health problem are shown in Table 2.

Table 2. Criteria for assessing the public health significance of Xerophthalmia and vitamin A deficiency, based on the prevalence among children less than 6 years old in the community (WHO, 1982) (24).

Criteria	Minimum prevalence
Clinical (primary)	
- Night blindness (XN)	1.0%
- Bitot's spot (X1B)	0.5%
- Corneal xerosis and/or ulceration/ keratomalacia (X2+X3A+X3B)	0.1%
- Xerophthalmia - related corneal scars (XS)	0.05%
Biochemical (supportive)	
- Serum retinol (vitamin A less than 0.35 μmol/l(10 μg/ml))	10%

3.5 Dietary sources and recommended requirement

3.5.1 Dietary sources

Preformed vitamin A is found mostly in animal products. The best food sources are liver, milk, butter, egg yolk and fish liver oils. The best levels are found in

animal/fish livers and fish oils. The amount of vitamin A in the animal sources increase with the age of animal and varies with the species. Significant quantities of preformed vitamin A are also present in milk, whole eggs or other dairy and egg products. Chicken meat and other meats are poor sources of vitamin A.

Provitamin A carotenoids, represent important sources of vitamin A because of their widely available and inexpensive aspects. Provitamin A carotenoids are found in both plant and animal products. The most important sources of carotene are generally found in deep yellow, yellowish-red and dark-green fruits and vegetables. Color intensity, however, is not necessarily a reliable indicator of biologically active carotenoids. The carotenoid content of vegetables vary depending on the variety and the maturity of vegetables (25). Foods such as sweet potato can vary in color from white to deep orange depending on variety, and the total carotenoid content range from less than 100 μg, to more than 1600 μg per 100 gm. In ripening fruit, the decrease in chlorophyll is often accompanied by an increase in carotenoid content, making mature fruit a better source. In developing countries, the majority of dietary vitamin A is derived from carotenoids in fruits and vegetables (11).

Nearly 50 carotenoids are precursors of vitamin A, but most do not occur in significant amounts in the human diet. Generally, for a compound to be active it must have an unsaturated central chain and a substituted beta-ring. Among the carotenoids, beta-carotene is the most abundant in our foods with the most vitamin A activity; 6 μg of beta-carotene, or 12 μg of other carotenoids with vitamin A activity and cryptoxanthin, was equivalent to 1 μg of retinol. Table 3 and Table 4 showed the lists of some common carotenoids that contain vitamin A activity (25).

Table 3. Vitamin A activity of some carotenoids (25).

Carotenoid	Activity(%)
β-carotene	100
α-carotene	50 - 54
3,4- dehydro-carotene	75
β-carotene 5',6'-monoepoxide	21
β-carotene 5',8'-monofuranoxide	50
3-Hydroxy-carotene (Crytoxanthin)	50 - 60
4-Hydroxy-carotene (Isocrytoxanthin)	48
5,6-Dihydroxy-caroyene	Active
β-apo-2'-carotenal	Active
β-apo-8'-carotenal	36 - 72
β-apo-10'-carotenal	Active
β-apo-12'-carotenal	120
Anhydroletein	21
Citranaxanthin	44
Carotene epoxides	25
Echineone	45 - 52
Canthaxanthin	±
Lycopene	Inactive
Lutein	Inactive
Fucoxanthin	Inactive
Neoxanthin	Inactive
Violaxanthin	Inactive

Table 4. Vitamin A activity of various naturally occurring carotenoids (26).

Carotenoid	% vitamin A activity [a]
All-*trans*-β-carotene	17
β-*apo*-8'-carotenal	12
3-*keto*-β-carotene	9
All-*trans*-α-carotene	8 - 9
9-*cis*-β-carotene	6
15-cis-β-carotene	6
β-carotene-5,6-epoxide	3 - 4
β-cryptoxanthin	8 - 10
Lutein	0
Canthaxanthin	0
Lycopene	0

[a] : Calculated on a weight basis relative to all-trans-retinol. Activity of carotenoids is based on the average absorption of carotenoids assumed to be about 1/3 relative to that of all-trans-retinol and is based on 1 mol β-carotene being 1 mol retinol.

3.5.2 Recommended requirements of vitamin A.

The level of recommended intake is the amount sufficient to prevent vitamin A deficientcy among various age groups. Safe estimates of vitamin A intakes are based on the amount needed

(i) to correct impaired dark adaptation, abnormal electroretinograms and follicular hyperkeratosis among vitamin A depleted subjects ;

(ii) to increase the concentrations of retinol in the plasma of depleted subjects to normal range and

(iii) to maintain a normal plasma of retinol in well nourished subjects.

Increased of vitamin A intake is necessary for cellular growth of fetuses during pregnancy and to maintain maternal liver reserves during secretion of vitamin A in the breast milk. Recommended intakes for various age groups in Thailand are shown in Table 5 and recommended intakes of pregnant and lactating women from various committees are shown in Table 6.

Table 5. Recommended dietary allowances for vitamin A (Thai RDA, 1989) (27).

	Age	**RDA**(RE)
	(months)	
Infants	under 3	breast feeding
	3 - 5	375
	6 - 11	420
	(years)	
Children	1 - 3	390
	4 - 6	400
	7 - 9	500
Boys	10 - 12	600
	13 - 19	700
Girls	10 - 19	600
Men, Women	> 19	700
Pregnant		+ 200
Lactating 0 - 5 mo. postpartum		+ 400
> 5 mo. postpartum		+ 320

Table 6. Recommended dietary allowances (RE) for Female (28).

	FAO/WHO(1988)		NRC(1989)	DGE(1991)	DRV Panel(1991)[3]	SCF(1992)
	Basal[1]	Safe[2]	(USA)	(Germany)	(U.K.)	(European Community)
Adolescents & adults	**270**	**500**	**800**	**800**	**600**	**600**
Pregnancy	**370**	**600**	**800**	**800**[4]	**700**	**700**
Lactating	**550**	**950**	**1300**	**1800**	**950**	**950**

[1] Basal requirements were defined as the minimal amount needed to prevent clinical signs of deficiency in nearly all health persons in a population.

[2] Safe level of intake additionally ensures a suitable body reserve of vitamin A for times of low intake and stress in order to sustain health for prolonged periods.

[3] Dietary intake recommendations in United Kingdom have traditionally been lower than those in most other European countries and the USA. The values approach those of developing nations.

[4] Starting with week 14 of pregnancy 1100 μg RE are recommended.

3.6 Nutrition during Lactation and Vitamin A deficiency.

Lactation is a remarkable process during which the maternal body produces a secretion that provides no immediate benefits to the mother but can totally sustain the offspring. All mammals produce milks with different compositions, each one specific to the needs for growth and development of their offspring. Regardless of a woman's intention to breast feeding, her body prepares for lactation from the first moments of pregnancy: the mammary gland begins its maturation process with the development of the alveolar ductal system and the lacteal cells so that the breast is ready to produce milk upon delivery of the infant. The woman's hormonal balance during pregnancy contributes to the preparation of the breast and promotes accumulation of energy stores, but it suppressed the production of milk until the birth of the infant.

Vitamin A deficiency in lactating woman occur when the store of vitamin A in her liver is finished and she does not eat enough to cover her needs. Thus, her child or

children may be depleted of vitamin A, energy, protein and other nutrients. Undernourished children and their mothers often eat little fat or oil, which make vitamin A deficiency more likely. Fat and oil help the absorption of vitamin A so, when the diet is low in fat, little vitamin A is absorbed. In the children, signs of vitamin A deficiency often occur when they are recovering from infection, or during treatment for PEM (protein-energy malnutrition). This is because children grow rapidly at these times and vitamin A needs increase. They may have night blindness and low stores of vitamin A, which is especially important among pregnant or lactating women. The babies may be born with low stores of vitamin A and may not get enough from breast milk. Babies who are not breast fed or who stop breast feeding early and who are given dilute bottle feeds or other foods which contain little vitamin A may develop vitamin A deficiency. Vitamin A deficiency in lactating women cause xerophthalmia and lead to increased risk of infection for their offspring because:

(i) Resistance to infection is decreased. The cells which line the gut and respiratory tract secrete less mucus. Bacteria can stick to the cells and cause infection more easily.

(ii) Immunity against infection is decreased. The white blood cells can not fight infection so well (29).

The lack of vitamin A or vitamin A deficiency in rural area may be a hidden problem of public health significant. The primary cause is insufficient intake of vitamin A rich foods. The foods which are available may be to expensive for poorer families to buy or farming families may sell vitamin A rich foods such as eggs and milk. If families can not get vitamin A rich foods, children are likely to have vitamin A

deficiency. Another cause in North-east Thailand is the local practice of Taboos among pregnant and lactating women. Restriction of vitamin A rich foods such as dark green leafy vegetables, eggs, liver and fat in the diet of pregnant may lead to low birth weight (LBW) babies who were born with low store of vitamin A. Among lactating women, poor dietary intake may lead to reduction in quality and quantity of breast milk.

3.7 Assessment for vitamin A status.

Assessment of vitamin A status can be evaluated by clinical, biochemical and physiological procedures as follows.

3.7.1 The clinical assessment

The clinical diagnosis which is most commonly used, focus on ocular signs like conjunctival xerosis, Bitot's spots and corneal lesions. The classification of such sign, adapted by the WHO, is given earlier in Table 1. The criteria for community diagnosis to determine whether vitamin A deficiency based on xerophthalmia is a significant public health problem is shown in Table 2.

Although the clinical assessment is simple and shows good correlation with deficient level of serum vitamin A, it requires skill and well-trained personnel as well as a large sample size for community assessment. In addition, the ocular symptoms represent a late stage of vitamin A deficiency (30).

3.7.2 Biochemical assessment

a) Liver

The concentration of vitamin A in the liver is a relatively good measure of vitamin A status since over 90% of total body reserves of vitamin A is found in the liver. However, liver specimen need to be collected by biopsy and require a complicated procedure for extraction and analysis. Thus, determination of liver vitamin A is not recommended for a routine or community survey.

b) Serum

The most common biochemical test to assess vitamin A status in the community has been the determination of serum vitamin A levels. Recently, the world health Organization (WHO) has suggested that the levels of serum vitamin A in population groups can be interpreted as shown in Table 7 (31).

Table 7. Criteria for interpreting status based on vitamin A concentrations.

Level	Vitamin A	
	(μmol/l)	(μg/dl)
Deficiency	<0.35	<10
Low	0.35 - <0.70	10 - 19
Acceptable	0.70 - ≤1.75	20 - 50
High	>1.75	>50

The assessment by serum vitamin A reflects to a certain extent an individual vitamin A status. However, serum levels of vitamin A of <0.35 umol/l and <0.70 μmol/l were used to describe populations with deficient and low vitamin A status, respectively. Presumably, this occurs when blood levels are below homeostatic set-points that respond to improvement in vitamin A status. There is no direct evidence of the serum cut-off value where functional consequences, morbidity/mortality effects, begin to occur. For that reason, and for the sake of consistency, the cut-off value of ≤0.70 μmol/l has been retained to indicate a low vitamin A status. The prevalence level designating an important public health problem is lowered to 10% from the previous level of 15%. Table 8 shows the prevalence of serum values of vitamin A ≤0.70 μmol/l in children ≥1 year to indicate a public health problem (31).

Table 8. Prevalence of serum values of vitamin A <0.70 μmol/l in children 1 year (WHO 1996).

Level of importance as a public health problem	**Prevalence**
Mild	≥2 - ≤ 10%
Moderate	>10 - <20%
Severe	≥20%

c) The Relative Dose Response (RDR) and The Modified Relative Dose Response (MRDR).

Over 90% total body vitamin A stores are located in the liver. The widely used plasma retinol value is a poor reflector of vitamin A and is quite reliable only when plasma retinol concentration is <10 μg/dl (0.35 μmol/l). Moreover, normal level of plasma retinol may exist despite low concentration of retinol in liver biopsy which indicates severe deficiency. In 1979, Underwood and co-workers studied the qualitative effects of administrating a small dose of vitamin A to rats with graded, preexisting reserves of vitamin A in the liver and called the test, the relative dose response (RDR) (30). RDR is used to detect a marginal vitamin A status both in individuals and in populations. The RDR assay has been validated in humans using direct measures of liver vitamin A (32, 33). The test was developed from the principle that during vitamin A deficiency and marginal status, apo-retinol binding protein (RBP) accumulates in the liver. After a small oral dose of vitamin A is administered, the holo-RBP is released into the serum from the liver and transported to target tissues. The response of the individual to this dose of vitamin A is measured in the serum 5 hours after administration of the dose. RDR value is measured in the serum as a percentage (A_5-A_0/A_5×100), where A_5 is the serum retinol concentration 5 hours after the dose and A_0 is serum retinol concentration before dosing. Theoretically, the value can be 0 - 100% . RDR value of 20% or higher is indicative of inadequate liver reserves (<0.07 μmol/g liver or < 20 μg/g liver). Negative values often occur among individuals who exhibit adequate vitamin A status.

The limitation of RDR is that protein energy malnutrition and liver dysfunction could interfere with its interpretation. Another drawback is that two blood samples are required. In some cultures, it is difficult to draw blood samples, particularly from children. Thus, the need to obtain two blood samples at a 5-hours interval can pose large logistic and cultural problems. In addition, the need to analyze two blood samples to obtain a single human value increases the expense and time of each assay (31).

To corect for RDR draw back, the modified relative dose response (MRDR) assay has been developed when only one blood drawn is needed. The principle is similar to that of RDR. A biologically active analog of retinol(R), 3,4-didehydroretinol (DR), is given instead of retinyl acetate. A single dose of 3,4-didehydroretinyl acetate in oil is administrated orally. DR is a naturally occurring from of vitamin A that is found predominantly in freshwater fish, but also to a small extent in mammalian tissues, including human. DR has approximately 40% of the biological activity of retinol. Like R, DR binds to accumulated apo-RBP in the liver, and is released into the serum as holo-RBP. A molar ratio of 3,4-didehydroretinol/retinol (DR/R) in serum is determined 4-6 hours after dosing. MRDR values theoretically can range from 0 to ∞ (31) .

Interpretation of the MRDR in children with DR/R ratios of ≥ 0.06 are judged to be in a marginal vitamin A status. The suggested cut off value of 0.06 was determined by comparative studies with the RDR and conjunctival impression cytology(CIC) techniques (34). A public health problem might be assumed to exist if > 20% of a population of preschool children show abnormal MRDR ratios (≥0.06). The

MRDR, like RDR, gives a good indication of an individual's vitamin A status and can be applied also in community assessment. It can be used for evaluation of the response to an intervention program. The effects of confounding factors, such as infectious disease and moderate protein-calorie malnutrition, on serum retinol concentrations are minimized. Sionce MRDR requires only one blood sample, the effects of storage on vitamin A stability and of sample extraction efficiency are also minimized.

d) Breast milk vitamin A concentration

Breast-milk vitamin A concentration is unique indicator of vitamin A status because it provides information about the vitamin A status of both the mother and the breast-fed infant. The mother's secretion of vitamin A into milk is directly related to her vitamin A status, particularly when her vitamin A status is inadequate. All infants are born with low stores of vitamin A and depend on vitamin A in early breast milk, as well as subsequent concentrations in mature breast milk, to accumulate and maintain adequate stores until complementary food provide significant additional amounts of vitamin A in keeping with the growing child's increasing requirements. Thus, the concentration of vitamin A in breast milk also serves as an indicator of an infant's likely vitamin A status.

Milk vitamin A content is very high in colostrum (the milk secreted in the first 4-6 days postpartum), and remains high in transitional milk (days 7-21 postpartum). Most of vitamin A in breast milk is in the form of retinyl palmitate in the milk fat. During a feed, the fat content of milk is quite variable for an individual mother, the most important source of variation being from the first milk expressed from a full

breast which is lowest in vitamin A, to the last milk expressed which has the highest content. This variation in milk vitamin A concentration is unrelated to the mother's vitamin A status and must be accounted for when using breast milk as an indicator to assess vitamin A status of individuals. However, for the purpose of assessing vitamin A status of population, assuming that sample are collected throughout the day and at varied periods following the last feed, it is not necessary to account for the sampling variation in milk fat because this variation will be randomly distributed among all samples provided that the collection period is randomly distributed. Where random sampling throughout the day is not possible, milk vitamin A values should be expressed relative to fat concentrations. Milk samples should be collected from mothers 1-8 months postpartum, which means that colostrum and transitional milk are avoided. During this period, breast milk proximate composition is relatively stable and is likely to provide the major source of dietary vitamin A for the infant, with complementary foods contributing little if any. When interpreted on a population basis, it is not necessary to control the time of day of sample collection or the time since the infant was last breast-fed (31).

In vitamin A sufficient population, average breast milk vitamin A concentrations range from 1.75-2.45 μmol/l, whereas in a vitamin A-deficient population, average values are below 1.4 μmol/l. A cut-off of ≤1.05 μmol/l, or ≤8 μg/g milk fat, is selected based on considerations of the dietary vitamin A requirement of infants and its usefulness in monitoring changes in the vitamin A status of mothers (31).

e) Isotope dilution technique

The most precise assessment of vitamin A "reserves" should be the direct measure of total body stores. This can be safely accomplished by the use of stable isotope dilution. Unfortunately, the deuterium labeled isotope needed for the test is not readily available; sophisticated and expensive laboratory instrumentation (HPLC and gas chromatography-mass spectrometry) is required, and is not usually accessible in Third World countries or Developing countries; and several weeks are needed between administering the isotope and collection blood samples in order for equilibrium to be achieved. Isotope dilution has not been used in field studies and is unlikely to prove practical under most routine field conditions (3).

Isotope dilution technique is an indirect, quantitative method for estimating total body retinol stores. This technique consists of administering a known dose of deuterium-labeled vitamin A orally and measuring the plasma isotopic ratio of [$^2H_4$] retinol to retinol after the dose has mixed fully with endogenous vitamin A body stores (~20 day in adults; 62). Total body retinol stores are estimated according to the principles of isotope dilution, a set of assumptions regarding retention of the dose of labeled vitamin A, the ratio of specific activities of vitamin A in plasma to that in liver, and the irreversible loss of vitamin A over time. Calculation of total body retinol stores (TBRS) were obtained by using the Furr equation (10).

total body retinol stores = F x dose x (S x a x [(1/D:H)-1]); where

F = factor for efficiency of storage of an orally administered dose of VA = 0.5;

Dose = the amount of mg RE of Isotope D4 = 7.83 mg; D8= 7.3 mg.;

S = the assumed ratio of [$^2H_4$] retinol to retinol in plasma to that in liver = 0.65;

a = the half-life of vitamin A turnover in the body; and

D:H = the isotopic ratio of [$^2H_4$] retinol to retinol measured in plasma.

In Thailand, Isotope dilution was used to assess total body stores of vitamin A in lactating women following dietary interventions. Deuterated retinyl acetate was administered to all subjects followed by blood collection at 0, 3, 28 and 42 days post dose (35). Isotopic concentrations were analysed via mass spectrometry. Isotope ratio was calculated and plugged into Furr Equation (10) to determine total body retinol stores

3.7.3 Physiological assessment

a) Rapid dark adaptation and Modified dark adaptation

Rhodopsin pigment produced by rod cells of the retina is responsible for the vision in the dark. The cycle of this photopigment bleaching by light and regeneration in the dark has been identified. Since rhodopsin is a complex of retinal and opsin, the regeneration of this pigment depends somewhat on the stores of vitamin A in the retina. Depletion of vitamin A then results in less rhodopsin being formed leading to delayed dark adaptation time.

In 1973, Russell et al. (36) reported the diagnosis of subclinical vitamin deficiency by modified Goldman-Weekers adaptometer of which threshold

measurement was started immediately and completed within 35-40 minutes. In 1977, Thornton (37) described a rapid dark adaptation test which measures the time of the so-called Purkinje shift, a phenomenon whereby the peak wavelength sensitivity of the retina shifts from the red toward the blue end of the visual spectrum during the transition from day vision (photopic or cone-mediated) to night vision (scotopic or rod-mediated). The test was based on the time required to sequentially separate white, red and blue poker chips placed on a black-top table in a dimly illuminated playroom. Vinton and Russell tested the method and recommended it for assessing vitamin A status in the field (38). Solomons et al. (39) applied this test to young children and found that the rapid dark adaptation test could potentially be used to complement biochemical determination in clinical settings and field surveys. Vannuchi et al. (40) found that the rapid dark adaptation test is probably not suitable for preschool children who are too young to perform this test accurately, and useful only to detect severe cases of vitamin A deficiency.

In Thailand, a simple test of measuring the time required for a child to locate a bag of cookies one-meter away in a dark room has been described (41). However, standardization of this test from one setting to another was a problem. Thus, a portable dark adaptometer has been developed by research scientists and an ophthalmologist affiliated with the Institute of Nutrition, Mahidol University. The basis for the test is to determine the elapsed time after the eye has been bleached with bright light to inactivate the rhodopsin complex up to the resynthesis of the pigment enough to allow vision in a fixed dim light intensity. It can be speculated that the rate of rhodopsin formation depends upon the amount of retinol store in the retina. The more retinol

stores a subject has, the faster her rate of rhodopsin synthesis and the shorter her vision restoration time (VRT) will be. The opposite is thus, expected for a subject with lower reserves of retinol (30).

In 1995, The rapid dark adaptometry is modified and based on light intensity detection. The modified dark adaptometry was also conducted, briefy, subject's eyes were patched with dark eyecover for 10 minutes prior to looking through the eyepiece onto a blank screen inside the instrument which bore a small hole for target light. At this time, the filter density wheel inside the instrument began to move slowly from complete darkness (blue filter number 5.0) towards sheer brightness (no filter or 0.0). Subjects were requested to verbally identify the point when they can see a spot of purple-blue light. The number of filter density was recorded. Subjects with impaired dark adaptation are expected to require more brightness (lower filter numbers) to see the same object as their normal peers. An earlier trial involving 28 INMU officers revealed the average reading as 2.8 ± 0.4, thus, a value representing roughly one SD below mean or 2.4 is used as a cut-off point for the time being (35).

b) Conjunctival impression cytology (CIC)

Vitamin A is essential for normal cellular differentiation particularly, the mucus secreting epithelium. In vitamin A deficiency, normal columnar epithelium transform to a stratified squamous type while Goblet cells, responsible for mucus production, disappear (42).

In 1977, Egbert et al. reported the use of cellulose acetate filter paper to collect conjunctival surface specimens, a process now called " impression cytology " (43). In 1981, Sommer et al. showed that biopsy specimens from children with mild vitamin A deficiency revealed generalized metaplasia throughout the bulbar conjunctiva (44). In 1984, Hatchell and Sommer detected the early conjunctival changes in vitamin A deficient rabbits using impression cytology (42). Specifically, they showed the progressive disappearance of goblet cells and the appearance of enlarged epithelial cells in vitamin A deficient rabbits. Later study in 1986 indicated that imprint specimens from children with early xerophthalmia showed a complete loss of goblet cells and the appearance of enlarged partially keratinized epithelial cells (45). Natadisastra et al. in 1987, suggested that impression cytology can detect physiologically significant vitamin A deficiency prior to clinical sign (46). Recent study showed that children with normal vitamin A stores had normal conjunctival impression cytology while children with depleted liver stores showed abnormal conjunctival impression cytology despite their seemingly-normal clinical ocular examination (47).

In summary, conjunctival impression cytology (CIC) appears to be a simple and practical methodology to detect early stage of vitamin A deficiency (42, 45). The advantages of CIC method are minimally invasive and inexpensive equipments, no blood is required and specimens remain stable sample under routine field conditions. Moreover, results of CIC were shown to correlate with serum and liver vitamin A concentrations. The limitation of this method is that the epithelial "memory" may cause persistent or recurrent abnormalities despite acutely normal liver and serum

levels (48). Cautions to be exercised with CIC are that it is more difficult to perform among children less than 3 years old in addition to the requirement of a clearly defind criteria of normal and abnormal as well as careful standardization of the readers (49). The criteria for interpretation of CIC are shown in Table 9.

Table 9. Criteria for Interpretation of conjunctival impression cytology

Result	Mucin spots	Goblet cells	Epithelial cells
NORMAL (NOR)	densely/covering >25% of sample	+/- (>5)	normal > abnormal
Borderline Normal (BON)	covering >25% of sample	+/- (≥5)	abnormal > normal
Borderline Abnormal (BAB)	diffusely covering <25% of sample	rare (<5)	abnormal,+/- small islands of normal cells
Abnormal (ABN)	diffusely covering <25% of sample	none	abnormal
Unreadable (UNR)	too few mucin spots or epithelial cells to read with confidence		

Ref:Assessment of vitamin A status by Impression cytology "Training Manual"ICEPO, 1988.

3.8 Review of Previous Studies on Impact of Dietary VA Interventions.

The studies on vitamin A and carotenoid-rich foods Interventions and the effect on vitamin A status.

Study			
Author	Design	Results	Conclusions
Roels et al., 1958; Ruanda	**Subjects:** boys aged 9-16 y **Treatment:** Gr1: 200 g raw grated carrots (19 mg carotene) per day (n=5) Gr2: 200 g raw grated carrots (19 mg carotene)+18 g olive oil per day (n=4) Gr3: 28 mg carotene)+18 g olive oil per day (n=4) Gr4: 18 g olive oil (n=4) Gr5: placebo (n=4) **Supplements were supplied in two meals** **Duration of interventions:** 31 days after 8 days on basal diet	*Change in serum retinol and carotene:* Gr1: 36->51 µg/dl, 43->84 µg/dl Gr2: 32->53 µg/dl(n.s.), 48->335 µg/dl Gr3: 35->56 µg/dl, 64->501 µg/dl Gr4: 37->39 µg/dl(n.s.), 52->47 µg/dl(n.s.) Gr5: 33->38 µg/dl(n.s.), 49->43 µg/dl(n.s.) Changes in serum retinol and carotene levels in Gr2 and 3 were different from those in other groups($p<0.07$ and $p<0.001$ resp). The range of changes in serum retinol within groups was large (8-46 µg/dl)	After consumption of carrots or purified carotene, both with fat, serum retinol and carotene levels increased. After consumption of carrots without fat the increase in serum retinol was not statistically significant.
Pereira & Begum, 1968; India	**Subjects:** children aged 2-5 y **Treatment:** 30 g cooked green leafy vegetables (1.5-2.25 mg β-carotene per day; n=29) **Fat content of supplement:** no information given **Duration of intervention:** 3 months	*Change in serum retinol:* After vegetable consumption, 22->31 µg/dl After intramuscular injection of VA, decrease in all groups, including the placebo group	-Increase in serum retinol after consumption of vegetables -Lack of an effect of intramuscular injection of VA is difficult to explain
Muhilal et al.,1977; Indonesia	**Subjects:** children aged 3-5 y **Treatment:** Gr1: dark green leafy vegetables (1.9 mg β-carotene per day; n=32/39) Gr2: salt fortified with vitamin A (300 RE/d; n=43/61) **Fat content of supplement:** no information given **Duration of intervention:** 75 days	*Change in serum retinol and β-carotene:* Gr1: 20->21 µg/dl (n.s.), 16->32 µg/dl (resp.) Gr2: 19->23 µg/dl, 19->32 µg/dl *Change in haemoglobin:* Gr1: no changes (n=39) Gr2: 10.5->11.5 g/dl (n=61)	-No increase of serum retinol level after consumption of dark green leafy vegetables -Increase in β-carotene in both groups could be due to consumption of other foods rich in β-carotene ,outside control of investigators
Devadas et al., 1978; India	**Subjects:** children aged 4-5 y **Treatment:** Gr1-4: 40-75 g green leafy vegetables (1.2 mg β-carotene per day)(n=15 per group) Gr5: β-carotene in powdered tablet (1.2 mg/d; n=15) Gr6: no intervention (n=15) **Fat content of supplement:** no information given **Duration of intervention:** 2.5 months	*Change in serum retinol:* Gr 1, 14->21 µg/dl Gr 2, 12->20 µg/dl Gr 3, 13->21 µg/dl Gr 4, 13->22 µg/dl Gr 5, 14->22 µg/dl Gr 6, 12->10 µg/dl (n.s.)	Serum retinol increased as much in the groups that received vegetables as in the group that received purified β-carotene

The studies… .—(*continued*)

Study			
Author	Design	Results	Conclusions
Jayarajan et al., 1980; India	**Subjects:** children aged 2-6 y **Treatment:** All groups received 40 g spinach (1.2 mg β-carotene per day) , 50 g cooked rice, and Gr1: no oil (n=26) Gr2: 5 g groundnut oil (n=22) Gr3: 10 g groundnut oil (n=22) **Duration of intervention:** 4 weeks (serum retinol also measured until 6 wks after the end of the intervention)	*Change in serum retinol:* Gr 1, 20->24 µg/dl Gr 2, 21->29 µg/dl Gr 3, 21->29 µg/dl -It increased most in subjects with an initial level <20 µg/dl -Serum retinol was maintained up to 6 wks after supplementation	-Increase in serum retinol after 40 spinach daily. -Increases were greater when 5-10 g oil was added. -The increase in serum retinol was maintained for at least 6 wks after supplementation.
Devadas et al., 1980; India	**Subjects:** children aged 3-5 y **Treatment:** 1st month basic diet with virtually no vitamin A for all subjects, then 2 months Gr1: basic diet (n=5) Gr2: 142 g papaya (1.2 mg β-carotene daily; n=8) Gr3: 30 g amaranth (1.2 mg β-carotene daily; n=5) Gr4: vitamin A (300RE/d; n=10) **Fat content of supplement:** no information given; daily diet contained 5-7 g fat **Duration of intervention:** 2 months	*Change in serum retinol:* Gr 1, 13->13 µg/dl (n.s.) Gr 2, 13->29 µg/dl Gr 3, 14->29 µg/dl Gr 4, 13->35 µg/dl Change in haemoglobin level: Gr 1, 9.2->9.8 g/dl Gr 2, 9.2->10.9 g/dl Gr 3, 8.0->10.2 g/dl Gr 4,8.9->10.8 g/dl	Serum retinol increased as much after consumption of papaya as after consumption of amaranth and it increased most after vitamin A
Charoenkiatkul et al., 1986; Thailand	**Subjects:** children of pre-school age who has been in an orphanage>6 months,where they were given vitamin supplements **Treatment:** Gr1: in wet season, 2 weeks no intervention, 2 weeks cooked ivy gourd (1.1 mg β-carotene per day; n=15) Gr2: in cool, dry season, 2weeks cooked ivy gourd (1.2 mg β-carotene per day), 2 weeks vitamin supplement (450 RE/day; n=15) **Fat content of supplement:** ivy gourd supplement contained 0.2-0.4 g fat per portion - cooked ivy groud was given at 11 am, 4 pm by mixing with their habitual diet (~6 g fat) **Duration of intervention:** 2 weeks per treatment	*Change in serum retinol and β-carotene:* Gr 1: 2 weeks without intervention: serum retinol and β-carotene decreased from 39 to 25 µg/dl and from 44 to 27 µg/dl, respectively; 2 weeks vegetables: serum retinol increased from 25 to 49 µg/dl and β-carotene from 27 to 106 µg/dl Gr 2: 2 weeks vegetables: Initial serum retinol level of 35 µg/dl unchanged, serum β-carotene increased from 36 to 87 µg/dl; 2 weeks supplements: serum retinol increased from 35 to 48 µg/dl and β-carotene decreased from 87 to 57 µg/dl	The authors ascribe the increase in serum retinol levels in Gr 1 and the lack of increase in Gr 2 during first two weeks to discontinuation of vitamin supplements. They conclude that 30 g of vegetables can maintain vitamin A status.

The studies… .—(*continued*)

Study			
Author	Design	Results	Conclusions
Dimitrov et al., 1988	**Subjects:** Male & Female aged 21-63 y **Treatment:** 15 M+F, 45 mg β-carotene daily for 30 day; 11 M+F, 15 mg β-carotene daily for 8 wk; 9M+F,45 mg β-carotene daily with high fat(>63 g fat) diet for 5 day followed by 16 day normal fat diet.	-Absorption of β-carotene was affected by dietary fat concentration. -Yellowing of the skin occasionally occurred during daily dosing with 45 mg β-carotene without evidence of toxicity.	-The observed individual variation in bioavailability of β-carotene raises questions regarding clinical use of this micronutrient. -The determination of target plasma β-carotene concentrations is essential for effective use of this compound in prevention or treatment.
Amatayakul et al., 1989; Northern Thailand	**Subjects:** Clinically healthy, nonpregnant, nonlactating women aged 18-35 y **Treatment:** oral contraceptives with and without multivitamin supplements through 13 cycles, test by RDR test after 13 cycles.	-Serum level of vitamin A were significantly increase above baseline in all users of OC throughout 13 cycles of observation. -Serum levels of OC users were significantly above those of the control group at all time during treatment.	Based on the RDR test, which indirectly evaluates liver stores of VA reduced below a critical level, and found little evidence that long term ingestion of estrogen-containing Ocs by a group of Thai women of low socioeconomic status caused a physiologically significant deterioration in their VA status.
Furr et al. 1989	**Subjects:** 11 hospitalized patients aged 22-87 y **Treatment:** estimated by measuring the dilution of a 45-mg oral dose of tetradeuterated retinyl acetate (99% pure); the ratio of $^{2}H_{4}$-retinol:^{1}H-retinol in plasma was measured by GC-MS; liver biopsy samples teken at surgery were directly analyzed for VA.	The correlation coeficient between calculated and measured liver vitamin concentrations for 10 subjects was 0.88, and the Spearman's rank correlation coefficient was 0.95 (p<0.002).	Total body reserves of VA in humans can be estimated validly in the marginal and satisfactory ranges by a benign, relatively noninvasive procedure.
Hussein et al., 1989; Egypt	**Subjects:** boys aged 6-13 y **Treatment:** Gr1: megadose vitamin A (200,000 IU) (n=7) Gr2: spinach (3.7 mg β-carotene per day; n=4), with 10 g oil Gr 3: grated carrots (2.4 mg β-carotene per day; n=2), no information about fat content **Duration of intervention:** 40 days (21 servings in Gr 2 and 3)	*Change in serum retinol:* In all 3 groups from 17 to 34 µg/dl *Change in serum carotene:* Gr 1, with 17% Gr 2, with 42% Gr 3, remained unchanges	-Serum retinol increased as much after 21 servings of spinach and carrots as after a megedose of vitamin A -It is clearly why serum b-carotene increased in the spinach group and in the group given a megadose of vitamin A, but remained the same in the carrot group

The studies… .—(*continued*)

Study			
Author	Design	Results	Conclusions
Hussein et al., 1990; Egypt	**Subjects:** boys aged11-13 y **Treatment:** subjects were provided with different amounts of carrots, carrot juice and cooked spinach leaves (n=71) **Fat content of supplement:** no information given; daily diet provided 35-50 g fat **Duration of intervention:** 2 weeks	*Change in serum retinol and carotene:* Carrots: 150 g/day resulted in an increase of serum retinol, but 30, 50 or 75 g/day did not Serum carotene levels remained unchanged at all dosages Carrot juice: 30 or 45 ml/day did not change serum retinol or carotenelevel Spinach: 150 or 280 g/day increased serum carotene but not serumretinol	-An increase in serum carotene was not always accompanied by an increase of serum retinol nor did the opposite occur -It is not clear what amounts of which supplements were given to whom and for how many days.
Stoltzfus et al., 1993; Indonesia	**Subjects:** 153 Indonesian mothers 1-3 wk post-partum **Treatment:** received either a capsule of 312 μmol of vitamin A as retinyl palmitate or a placebo	1.Milk retinol conc. of the vitamin A group were higher than those of placebo. 2. Prevalences of low serum retinol conc. of the vitamin A group were less than those of placebo.	High dose vitamin A supplementation of lactating mothers is an efficacious way to improve the vitamin A status of both mother and breast-fed infant.
Duncan et al. 1993	**Subjects:** Weanling male Sprague-Dawley rats (wt~50 g, n=36) with liver vitamin A ranging from 1.4 to 23,000 nmol **Treatment:** received an intravenous dose of [^{3}H] retinol-labeled plasma.	There was a sigmnificant effect of dietary group on terminal body weight($p<0.05$). The liver VA levels were related to dietary intake of VA. Liver vitamin A ranged from a low value of <1.5 nmol to 23,000 nmol in a rat of each group.	-This modified isotope dilution technique is a sensitive and reliable method for assessing VA status in rats over a wide range of vitamin A nutriture. -This method could be modified for use in humans.
Bulux et al., 1994; Guatamala	**Subjects:** 67 Guatemalan children aged 7-12 y **Treatment:** Gr 1, placebo (n=17) Gr 2, retinyl palmitate (1000 RE in oil; n=17) Gr 3, β-carotene in carrots (50 g of carrots; cooked in 10 g absorbed vegetable fat; 120 μg β-carotene / carrot 1 g ; n=17) Gr 4, purified β-carotene supplements (6.3 mg of 10% β-carotene mixed into o rice-based beverage; n=16) **Duration of intervention:** 20 days	-Plasma concentrations of β-carotene were increased by 0.59±0.65 and 0.6±0.67 μmol/L after supplementation with purified β-carotene capsules for 10 and 20 d,respectively. -Addition of cooked carrots to the diet resulted in no significantly changed in plasma β-carotene	-Plasma concentrations of β-carotene and retinol before initiation of study were not different among the treatment group. -Short-term administration of β-carotene capsules (6 mg/d) produced a threefold increase in plasma β-carotene by 10 d. -Equivalent β-carotene intake in carrots resulted in no significant changes in plasma β-carotene. The differences may reflect lower bioavailability of β-carotene from foods as compared with capsules.

The studies… .—(*continued*)

Study			
Author	Design	Results	Conclusions
De Pee et al., 1995; Indonesia	**Subjects:** 175 anemic breastfeeding women in West Java, Indonesia **Treatment:** Gr 1, stir-fried local dark green leafy vegetable(n=57) Gr 2, wafer enriched with β-carotene, Iron vitamin C and folic acid (n=62) Gr 3, non enriched wafer as a control group **Duration of intervention:** 12 weeks	During the intervention, the enriched-wafer group had a serum retinol increased of 38%,while breast milk retinol increased 67%. These increases were significantly greater than those for women in the control or vegetable groups.	β-carotene was very poorly absorbed from dark green leafy vegetables, but absorption from the enriched wafers was good and suggest that the approach to combat vitamin A deficiency by increasing the consumption of leafy green vegetables should be re examined.
Tanumihardjo et al., 1996; Indonesia	**Subjects:** 25 lactating women aged 15-40 y from suburban village in West Java, Indonesia **Treatment:** starting at 1-3 mo after delivery were determined at three monthly intervals (times 1, 2 and 3) during lactation and then again (time 4) after they had ingested vitamin A capsules (8.4 μmol, 8000 IU) daily **Duration of intervention:** 35 days	-The mean MRDR ratio in these women rose from 0.084±0.047 (time 1) to 0.099±0.045 (time 2) and then to 0.100±0.054 (time 3) but after supplementation the mean MRDR ratio fell to 0.040±0.021 (time 4) ($p<0.0001$). -Mean serum retinol concentrations at the first three times were 0.94±0.23, 0.87±0.20 and 0.80±0.20 μmol/L, but then rose to 1.10±0.31 μ mol/L at time 4 ($p<0.04$)	-MRDR values better distinguished the vitamin A ststuses of the women than did serum retinol concentrations. -Iron status may also have improved marginally from time 1 to time 4, but most of the increase appeared before the vitamin intervention.

CHAPTER IV

SUBJECTS AND METHODS

A. Phase 1 Identification of Lactating women with suboptimal vitamin A status

1. Study Site and Subject Selection

The study protocol was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Mahidol University. The first screening covered the district of Trakarnpeutphol, Kud Khao Pun and Khemaraj Ubonrajthanee Province NE Thailand. The area were chosen from demographic and health profile of infants, preschool children and pregnant women. Blood samples were obtained from 96 lactating women in September 1996 (mid-rainy season). All subjects showed serum retinol above 1.05 μmol/L (30 μg/dl). The next screening was conducts in Najaluay District, about 95 kilometers South of Ubon city. There are 6 subdistricts altogether with 54 villages. The screening of all lactating mothers in Najaluay district, using anthropometry and dietary vitamin A intake were conducted during the beginning of January, 1997. 718 Lactating mothers at one to twelve month postpartum were covered. Those who consumed less than 80% of Thai RDA (28) for lactating women (<800 RE / day) were subjected to blood collection for serum retinol analysis (n=521) during January and February, 1997. Eighty-seven lactating mothers who showed serum retinol less than 0.87 μmol / L (25 μg / dl) were eligible for the study

in March, 1997. The study objectives, procedures, expected benefits and risks were explained to these women and all of them (n=29) signed the consent form and agreed to be enrolled into the intervention trial. The study diagram with number of subjects at each stage is shown as Figure D.

B. Phase 2 Isotope Preparation and Baseline Assessment

Each subject underwent a blood collection (day 0) followed by an oral administration of Vitamin A acetate (10, 19, 19, 19-D4 and 10, 14, 19, 19, 19, 20, 20, 20-D8) which were purchased from Cambridge Isotope Laboratories (Woburn, MA). The isotope capsules were prepared by the method of M. Haskell (personal communication) at the John Hopkins University, Center for Human Nutririon. Briefly, the procedure is as follows: 10 ml of hexane: ethanol (50:50) was added to approximately 1 gm of isotope. Concentration was determined based on Beer's Law from the absorbance measured at 325 nm using a spectrophotometer (UV 160U, Shimadzu, Columbia, MD). The isotope solution was gradually dried under nitrogen. The amount of corn oil required to reach the desired concentration was added to the dried residue while mixing. The corn oil mixture was placed under nitrogen to evaporate any residual solvents. The absorbance was read at 325 nm and final concentration was determined. The amount of isotope-oil mixture necessary to provide 9.0 mg of 10, 19, 19, 19-D4 retinyl acetate or 8.4 mg of 10, 14, 19, 19, 19, 20, 20, 20-D8 was added to each gelatin capsule. The capsules were stored at -20°C and shipped on dry ice to Institute of Nutrition, Mahidol University.

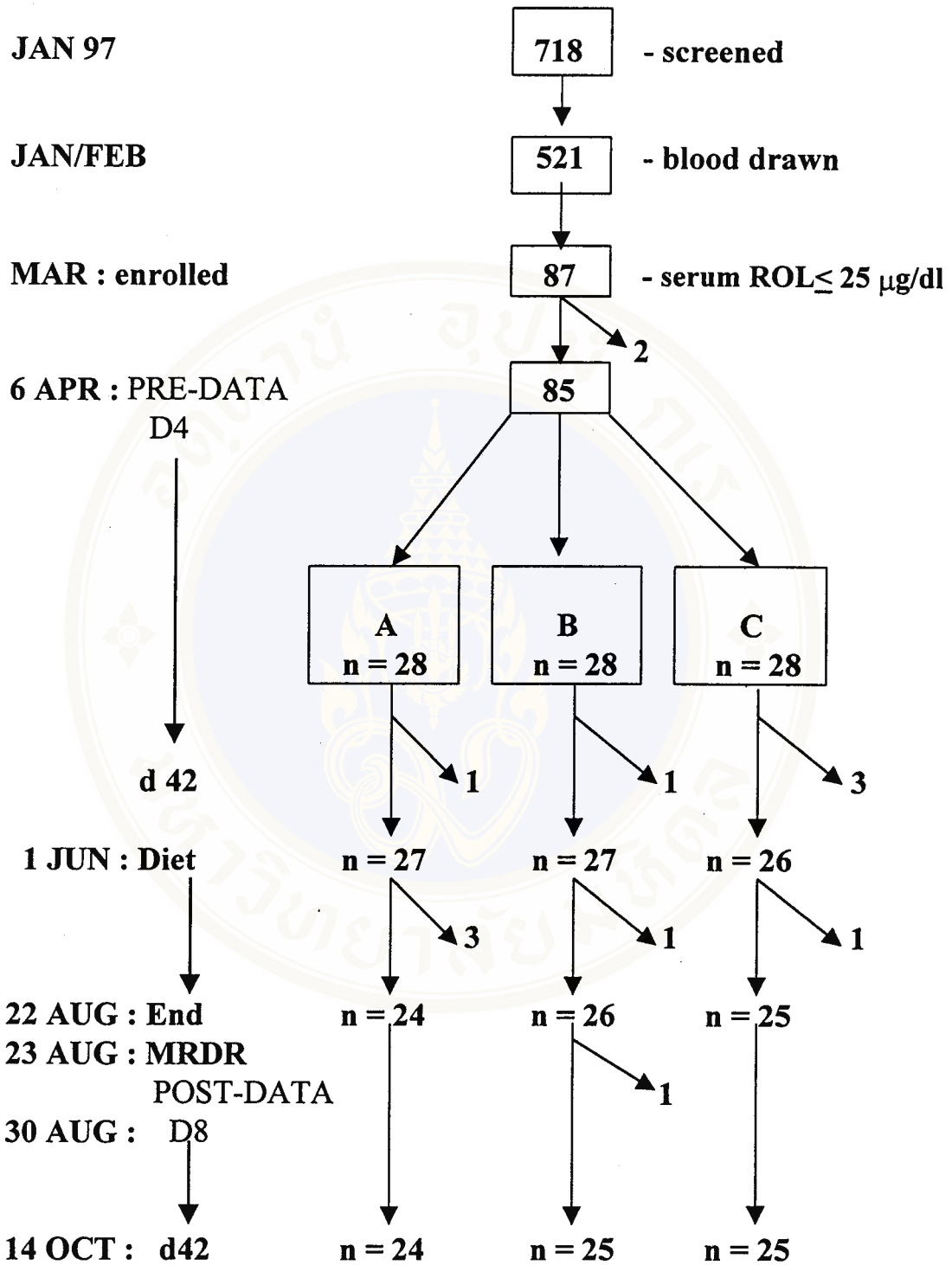


Figure D. The subject selection and withdrawal during study period at Najaluay district, Ubonrajthanee province

Prior to the trial, total body stores of vitamin A were assessed by isotopic dilution technique. Each subject underwent a blood collection (day 0) followed by an oral administration of 9 mg deuterated retinyl acetate (10, 19, 19, 19-D4) in an oil based gelatin capsule. The capsule was consumed together with local sausages (50 gm) which provided about 15 gm fat to facilitate absorption of the isotope. Blood were drawn on day 3 (all subjects), day 28 (half n) and day 42 (half n) and serum separated and kept frozen for later determination of isotope dilution. Subjects are to be rank-ordered according to their fractional disappearance (10) of tracer on day 3 of the post-dosing "window" during which plasma disappearance curve is sensitive to stores. Estimation of liver stores is based on Furr equation (8) in relation to the levels of D4 tracer in serum on day 28 and the fractional catabolic rate derived from the terminal slope calculated for the interval between day 28 and day 42. On day 42, the baseline assessment were conducted in lactating mothers on the followings:

1. Anthropometric measurements (weight, height, mid upper arm circumferences),

2. Physical examination (blood pressure, fever, etc.),

3 Morbidity over the past two weeks,

4. Biochemical assessment i.e., serum and breast milk retinol and carotenoids (beta-carotene, lutein, cryptoxanthin, zeaxanthin, and lycopene) fat in breast milk,

5. Functional assessment i.e., conjunctival impression cytology (CIC) and modified dark adaptometry. CIC was conducted according to the method described earlier (11). Modified dark adaptometry by light intensity detection (INMU) was also

conducted, briefly, mother's eyes were patched with dark eyecover for 10 minutes prior to looking through the eyepiece onto a blank screen inside the instrument which bore a small hole for target light. At this time, the filter density wheel inside the instrument began to move slowly from complete darkness (blue filter, Kodak, number 5.0) towards sheer brightness (no filter or 0.0). Subjects were requested to verbally identify the point when they can see a spot of purple-blue light. The number of filter density was recorded. Subject with impaired dark adaptation are expected to require more brightness (lower filter numbers) to see the same object as their normal peers. An earlier trial involving 28 INMU officers revealed the average reading as 2.8 +/- 0.4, thus a value representing roughly one standard deviation below mean of 2.4 or less is used as a cut-off point for the time being.

6. Dietary assessment - dietary intake was assessed weekly by 24 hr recall plus weighing technique on one random day per week throughout six weeks of isotope equilibration period. Comparison of preformed vitamin A intake was done by calculation of preformed VA Index score such as follows:

$$\text{Preformed VA Index Score} = \sum_{i=1}^{12} \frac{[\text{Preformed VA Inyake (RE)}]_i \times w_i}{500}$$

where w = week of study; $w_1=1, w_2=2, w_3=3, \ldots, w_{12}=12$

500 = cut off point of MSDA score to screen subjects with low VA intake

C. Phase 3 Dietary Intervention

Subjects were matched according to month postpartum, geographical areas and serum retinol concentrations and then, randomly assigned on a block fashion to one of

three dietary treatment groups. Subjects were given one meal per weekday, 5 days per week for 12 consecutive weeks. The meal consisted primarily of processed meat (local beef and pork sausages), steamed or blanched vegetables, chili dip, juice or fruits in syrup and glutinous rice. Fat content averaged 10 gm. per meal. There were no detectable amount of retinol in the meal. The three dietary regimen differing in sources and average content of beta-carotene over the 12 weeks period are:

Group	**β-carotene (mg./meal)**
A. Dark green leafy, yellow/orange vegetables and fruits	4.7 +/- 2.6
B. Purified B-carotene (water miscible, Roche) (served in orange drink)	3.6 +/- 0.1
C. Control vegetables and fruits : low in pro VA carotenoids	< 0.05

Although the menu of group A were first planned to provide β-carotene around 3.6 mg/meal, large variation of B-carotene contents in vegetables and fruits due to varieties, soil condition, seasonality resulted in an average intake of 4.7 mg/meal over a 12 week period. The meal were prepared at INMU, kept frozen at -20°c and transported to the field station in Najaluay distric in a dry ice container. All foods were stored frozen at the field station and delivered by the field workers to subjects at their cottage in the rice fields. Foods were weighed before and after consumption. Aliquots of each food menu were sampled for analysis of carotenoid profile. Dietary intake was assessed weekly by 24 hr recall plus weighing method on one random day per week for 24 weeks of study period (6 weeks equilibration pre-

intervention + 12 weeks intervention + 6 weeks equilibration post-intervention). In addition, weekly history of morbidity as recorded in subject health cards, medications, smoking and alcohol consumption were recorded to adjust for possible influences on dietary intake and vitamin A stores and status.

D. Phase 4 Post-Intervention Assessment

At the end of dietary intervention (12 weeks), subjects were assessed for anthropometric measurements, modified relative dose respone (MRDR), serum and breast milk retinol and carotenoids, serum α-1 acid glycoprotein, CIC and modified dark adaptometry (INMU). To prevent the interference of 3,4-didehydroretinol or vitamin A2 with deuterated retinyl acetate, MRDR was conducted about 5 days earlier, using the protocol described elsewhere (11a). Briefly, a standard dose of 2.5 mg of A2 was given orally to each subject followed by a single venous blood collection 4-6 hours later. Serum was analyzed for retinol and 3,4-didehydroretinol via HPLC. MRDR ratio of A2/A of > 0.06 depicts depleted hepatic stores. For the isotope dilution assessment at post-intervention, a subsequent dose of 8.4 mg deuterated retinyl acetate(10, 14, 19, 19, 19, 20, 20, 20-D8) was administered to all subjects followed by blood collection at 3, 28 and 42 days post-dose. As done in the baseline, subjects were evaluated weekly for dietary intake and recent morbidity.

E. Phase 5 Subject Withdrawal

Throughout the study period, a total of 13 subjects dropped out from the project as shown in Fig 1. The major causes of withdrawal include migration (n=6), stop breast feeding (n=3), child death (n=1) and illness (bladderstone, pneumonia, disease of opticociliary, n=3). As a result, a total of 74 subjects completed the study with the sample size of group A, B and C of 24, 25 and 25 respectively.

F. Phase 6 Laboratory Analysis and Calculations

Serum and breastmilk retinol and carotenoids were analyzed by HPLC (52, 53). Breastmilk' s fat was analyzed by modified Mojonnier method (54). β-carotene in foods were determined by HPLC (53, 55). The ratio of enriched deuterated retinol to retinol in plasma were determined using method of Handelman et al (56) utilizing gas chromatography mass spectrometry. Detailed procedures were described in Appendix 1.

Calculation of total body retinol stores (TBRS) were obtained by using the Furr Equation (10):

Total body stores (in millimoles retinol) = F x dose x (S x a x [(H:D) –1])

= [(0.5x mg RE of isotope D4 or D8) x (0.65 x (Exp-0.005) x (days since dose)] x [(1/isotope ratio-1)]

- mg RE isotope D4 = 7.83 mg; D8 = 7.3 mg

- day since dose = 28 or 42

- isotope ratio = as sent by M. Haskell.

Where F is a factor that represents the efficiency of storage of an orally administered dose, taken as 0.5; dose is the oral dose of labeled retinol in 7.83 mg D4 and 7.3 mg D8, H:D is the ratio of H to deuterated retinol in the plasma after the equilibration period (28 and 42 d in this study) and –1 corrects TBRS for the contribution of the administered dose to the total body pool. S is the ratio of specific activities of retinol in serum to that in liver, considered to be 0.65 while "a" is the fraction of the absorbed dose of deuterated retinol remaining in the liver reserves at the time of blood sampling. It was introduced into the equation as a correction to the H-D ratio because of the loss of labeled vitamin A by catabolism and because of replacement by absorbed unlabeled dietary vitamin A over time; a is based on the half-life of vitamin A in the liver e.g. 140 d and is assumed to be independent of the size of liver stores and that it is time variant; $a = e^{-kt}$ where k = -0.005 and t is time in days since the isotope was administered (day 28 & day 42)..

G. Phase 7 Statistical Analysis

Data for variables are reported in mean plus and minus standard deviations. Medians as well as range of minimum to maximum values were given for variables with non-normal distribution. Changes in biochemical indicators were calculated by subtracting pre-intervention values from the post-intervention ones. Differences

within groups (before versus after) were determined by paired t-test. Differences between the three dietary intervention groups were examined by analysis of variance (ANOVA). Analysis of changes of total body retinol stores was done by analysis of covariance (ANCOVA) to adjust for baseline differences. For index with non-normal distribution such as nutrient intake data, the Kruskal-Wallis test was used to detect changes between treatments while difference of intake within groups was determined by Wilcoxon's matched-pairs signed-ranks test. Pearson's product-moment correlation and Spearman's rank correlation analyses were use to correlate the isotopic ratio (D:H) at day 3 post-dose with the calculated values of TBRS by using the Furr equation (10) as above with the isotopic ratio at day 28 or day 42 postdosing.

The computer package SPSS for Windows version 7.5 (SPSS Inc., Chicago, USA) was used for all statistical analyses and a P value of <0.05 was considered significant.

CHAPTER V

RESULTS

5.1 Basic Profile

Basic characteristics of the three intervention groups are shown in Table 10. At baseline mothers' age ranged from 15 to 47 years old with postpartum period ranging from 2 to 18 months. Anthropometric measurements (weight, height, mid-upper arm circumferences) are comparable among the three groups at pre-and postintervention. Likewise, the majority of subjects have BMI within the normal range (18.5-24.9 kg/m2).

Table 10 Basic Characteristics of Lactating women during Pre-and Post intervention [1]

	A (n=24)		B (n=25)		C (n=25)[1]	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Age (yrs)	**26.9±6.9**	**27.4±6.9**	**25.4±5.2**	**25.9±5.2**	**25.0±4.9**	**25.5±4.9**
Postpartum Period (mo)	**13.0±3.0**	**13.5±3.0**	**13.2±3.7**	**13.7±3.7**	**12.7±3.5**	**13.2±3.5**
Weight (kg.)	**49.3±5.6**	**48.8±6.3**	**51.7±6.6**	**50.5±6.5**	**53.9±10.7**	**53.7±10.2**
Height (cm.)	**152.7+6.5**	**152.3±6.3**	**151.9±6.2**	**151.9±6.1**	**153.5±4.2**	**153.4±4.3**
BMI (kg/m^2)	**20.8±2.6**	**21.0±2.3**	**22.4±2.4**	**21.8±2.2**	**22.8±4.3**	**22.8±4.2**
MUAC (cm.)	**24.9±2.0**	**25.3±1.9**	**26.0±1.8**	**25.9±2.3**	**26.0±3.4**	**26.1±3.2**

[1] values in mean +/- standard deviation;

Dietary groups: A= dark green leafy and yellow/orange vegetables

B= purified β-carotene

C= control

5.2 Physical Examination (Blood pressure, fever, etc.)

Physical examination was done at pre and post intervention. All mothers append healthy and were given health card which monitor and cover their health care throughout the study.

5.3 Morbidity

Few mothers were ill during the study. The illness was primarity due to upper respiratory infection, chickenpox, skin allergy and diarrhea (Table 11). There appear to be no differences among morbidity episodes of the three groups. It was noted that slightly fewer mothers were ill during post intervention.

Table 11 Morbidity episodes recorded in Health cards during Pre-Post Intervention

Group	A		B		C	
	Pre	Post	Pre	Post	Pre	Post
Upper Respiratory Infection	2	-	1	2	1	-
Chickenpox	-	-	1	-	2	-
Skin Allergy	-	-	1	-	-	-
Enterritis	1	-	-	-	-	-
Diarrhea	1	-	-	-	3	-
Cystitis	-	-	-	-	-	1

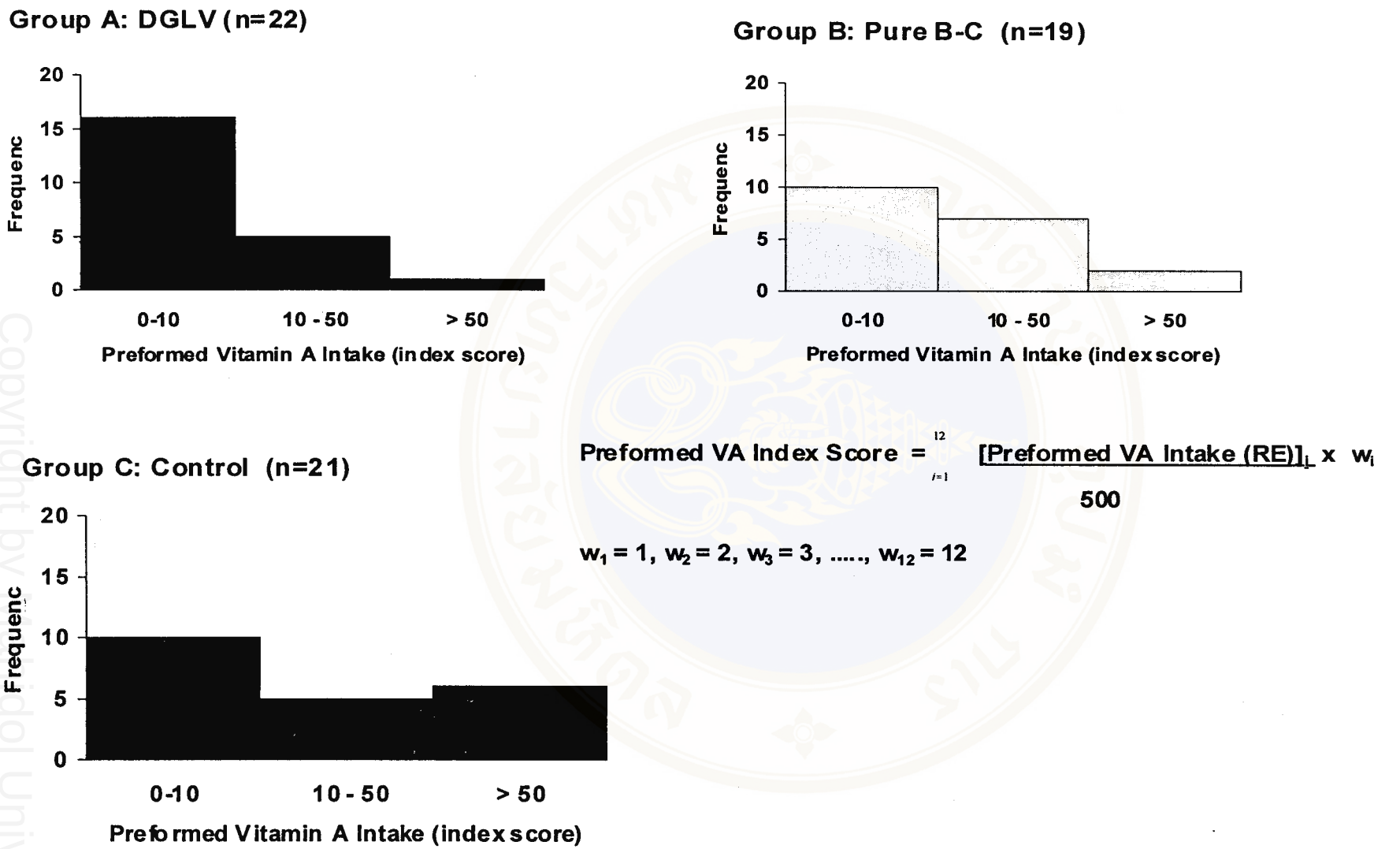
5.4 Dietary Intake

Dietary intake of lactating women was assessed on a weekly basis by 24 hour recall plus weighing method on one random day per week for 24 weeks of the study (6 weeks isotope equilibration at pre-intervention + 12 weeks during intervention + 6 weeks isotope equilibration at post-intervention). Food weight before and after consumption indicated that subjects consumed over 95% of the food given in all three groups. Energy intake at baseline averaged around 1580 kcal and slightly increased to 1670 kcal during intervention, then dropped to 1520 kcal post-intervention. Energy distribution of carbohydrate: protein: fat was 82 : 14 : 4, 82 : 13 : 5, and 83 : 13 : 4, respectively. Majority of protein intake came from plant sources with the ratio of animal: vegetable protein of 1:2. At each period of the study, namely, pre-, during and post-intervention, there were no significant differences of the macronutrient intakes among the three intervention groups although a slight decrease was observed at the end of intervention. Data are attached as Appendix 2.

The intervention meal provided energy intake in the range of 288 to 308 kcal. Fat content averaged about 10 g per meal in the form of local sausages. Based on chemical analysis, no preformed vitamin A was detected in the intervention meal. As mentioned earlier, group A received beta-carotene from vegetables and fruits, in mean +/- standard deviation of 4750$\pm$262 μg per meal. Beta-carotene intake in group B averaged 3600$\pm$115 μg while group C received less than 0.05 μg of beta-carotene per meal.

Dietary vitamin A intake as shown in Table 12, indicated that group A consumed less than half of the amount of preformed vitamin A (livers, eggs, whole fishes and whole small green frogs) compared to group B and C, at baseline and during intervention period. Consumption of preformed vitamin A of subjects in group A was significantly less than the control groups at both times. When preformed vitamin A intake were calculated as index score over the 12 weeks of intervention period (Fig. E), it was apparent that group C consumed the most preformed vitamin A followed by group B and lastly, group A. Since intervention meals contain no detectable amount of preformed vitamin A, this must come from the habitual or background diet which reflected seasonal availability of animal origin of vitamin A foods. The consumption of preformed vitamin A of group B and C fell dramatically at the end of intervention period to the same levels as group A. Significant increase in the consumption of beta-carotene in group A and B over C were due to dietary intervention. Although beta-carotene intake at home among the three groups were comparable at the beginning of intervention, the consumption of beta-carotene rich foods declined remarkably in all three groups at post-intervention when subjects had to work in the rice fields.

DISTRIBUTION OF PREFORMED VITAMIN A INTAKE



$$\text{Preformed VA Index Score} = \sum_{i=1}^{12} \frac{[\text{Preformed VA Intake (RE)}]_i \times w_i}{500}$$

$w_1 = 1,\ w_2 = 2,\ w_3 = 3,\ \ldots..,\ w_{12} = 12$

Fig. E Dietary intake of preformed vitamin A (retinol) as calculated by Index score during intervention

Table 12 Dietary vitamin A intake at pre-, during and post-intervention [1]

	A (n=24)	B (n=25)	C (n=25)
Pre-intervention			
Retinol (RE)	34 [a]	59 [ab]	67 [b]
	(13 – 1216)	(6 – 1106)	(16 – 628)
Beta-carotene (μg)	613	1072	790
	(170 – 2377)	(88 – 2896)	(24 – 2150)
Total VA (RE) [2]	159 [a]	265 [b]	210 [ab]
	(50 – 1612)	(81 – 1378)	(50 – 902)
During-intervention			
Retinol (RE)	38 [a]	61 [ab]	101 [b]
	(3 – 394)	(8 – 642)	(8 – 691)
Beta-carotene (μg)	3089 [a]	2271 [b]	453 [c]
	(2221 – 5061)	(1760 – 3330)	(128 – 2066)
Total VA (RE)	549 [a]	479 [a]	169 [b]
	(383 – 998)	(354 – 1076)	(50 - 859)
Post-intervention			
Retinol (RE)	37	37	36
	(2 – 416)	(0.5 – 436)	(4 – 193)
Beta-carotene (μg)	217	177	197
	(56 – 1054)	(13 – 1104)	(24 – 1451)
Total VA (RE)	89	86	84
	(18 – 491)	(9 – 574)	(21 – 382)

[1] medians with range of minimum – maximum values in parentheses with different superscript letters within the same ROWS are significantly different, $p < 0.05$, Kruskal-Wallis test

[2] Calculated by retinol RE + ug beta-carotene/6

5.5 Biochemical Assessment

5.5.1 Serum retinol and carotenoids

Lactating women entered the study with serum retinol below 0.87 μmol/L (25 μg/dl) during screening and baseline period. following dietary intervention, significant increase of serum retinol to an average of 1.7 μmol/L (48 μg/dl) were

observed in the 3 treatment groups. There appeared to be no significantly differences of serum retinol concentrations among the 3 groups during pre and post intervention period. The average value of serum retinol for each period and each groups are shown in Table 13. Distribution of serum retinol at pre- and post-intervention are shown in Figure F, indicating a comparable shift of circulating retinol concentrations towards a satisfactory range (> 0.70 μmol/L or 20 μg/dl). The majority of the subjects (~60%) showed serum retinol between 1.4-2.1 μmol/L (40-60 μg/dl). At baseline, about 43 % of lactating women manifested serum retinol below 0.70 μmol/L (20 μg/dl) while at post-intervention, none of the subjects except two (7%) in group B had low serum retinol.

Table 13 Serum retinol of lactating women during screening, pre- and post-intervention [1,2]

Period	Intervention Groups					
	A		B		C	
	μg/dl	μmol/L	μg/dl	μmol/L	μg/dl	μmol/L
Screening (Jan'1997)	20.3±4.4[a] (N = 29)	0.7±0.2[a] (N = 29)	20.4±3.7[a] (N = 29)	0.7±0.1[a] (N = 29)	20.5±2.9[a] (N = 29)	0.7±0.1[a] (N = 29)
Pre Intervention (Apr'1997)	20.3±5.8[a] (N = 28)	0.7±0.2[a] (N = 28)	21.2±5.5[a] (N = 28)	0.7±0.2[a] (N = 28)	21.8±7.1[a] (N = 29)	0.8±0.3[a] (N = 29)
Post Intervention (Aug'1997)	47.1±11.5[b] (N = 24)	1.6±0.4[b] (N = 24)	44.2±14.6[b] (N = 25)	1.6±0.5[b] (N = 25)	48.7±13.0[b] (N = 25)	1.7±0.5[b] (N = 25)

1 all values in mean +/- SD
2 Means with different superscript letters within COLUMNS are significantly different, $p<0.05$, ANOVA

For serum carotenoids, all three groups started off with similar levels. Serum beta-carotene increased significantly in group B who received the purified

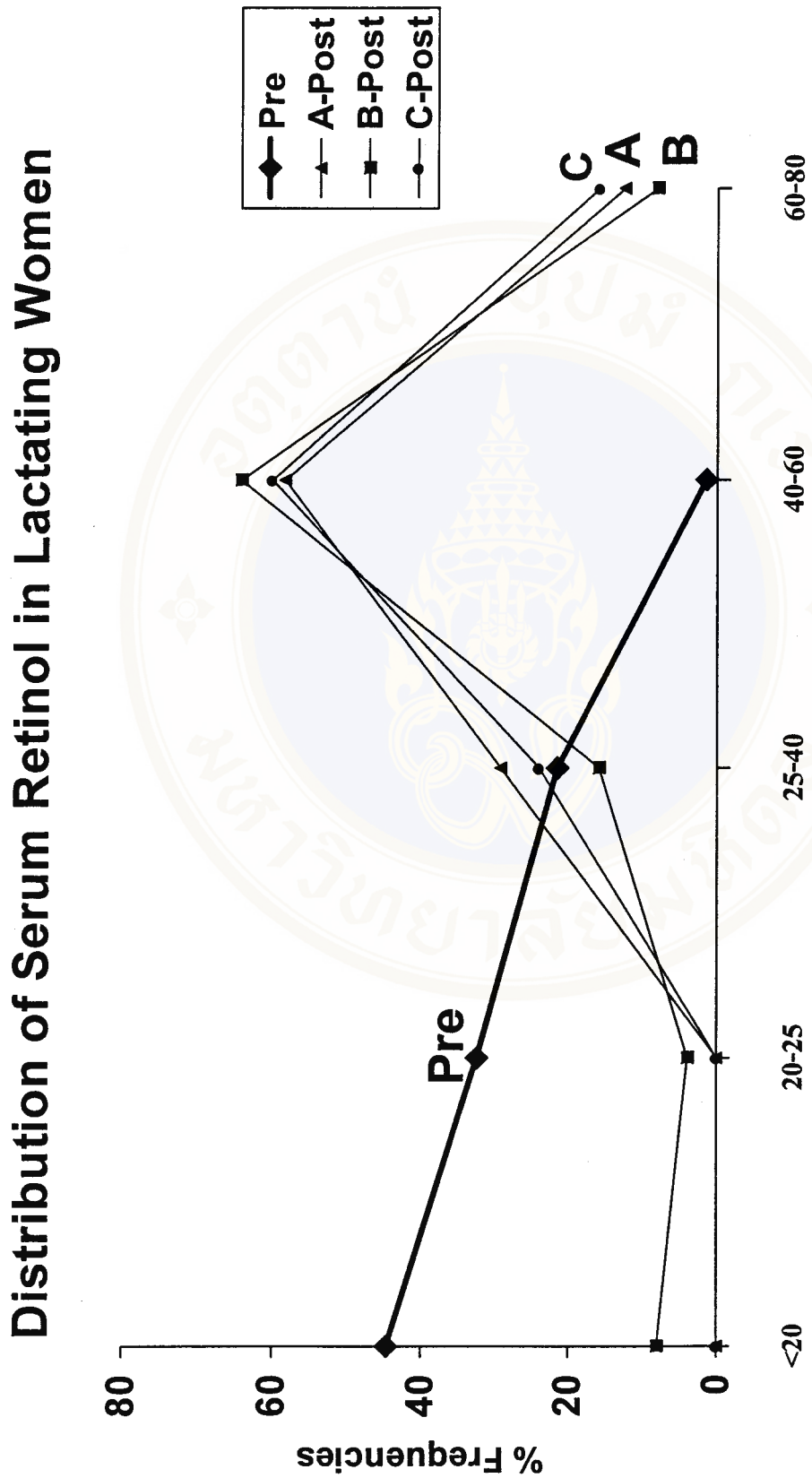


Fig. F Frequency distribution of serum retinol concentrations (µg/dl) in lactating women at pre-intervention (n=85) and post-intervention (n; = 24, 25, 25 for groups A, B and C)

beta-carotene compared to group A or C (Table 14). Of notice is the similar elevation of serum beta-carotene of group A and C at post-intervention. Increased fruit and vegetable consumption in group A was reflected through a significant increase of serum lutein concentration that were really biomarker of fruit and vegetable consumption. Other levels of serum carotenoids: lycopene, zeaxanthin, alpha-carotene were similar among the three groups.

Table 14 Serum Carotenoids(μmol/L)at Pre- and Post-Intervention (Mean±SD)[1]

Serum Carotenoids (μmol/L)	Intervention Groups					
	A (n=24)		B (n=25)		C (n=25)	
	Pre	Post	Pre	Post	Pre	Post
β-carotene	0.21±0.20	0.34±0.20 [a]	0.28±0.19	0.51±0.25 [b]	0.22±0.26	0.35±0.28 [a]
Lutein	0.42±0.28	0.69±0.23 [c]	0.46±0.22	0.45±0.25 [d]	0.37±0.24	0.49±0.31 [d]
Zeaxanthin	0.09±0.04	0.16±0.10	0.09±0.04	0.10±0.06	0.08±0.05	0.15±0.12
Lycopene	0.09±0.14	0.07±0.11	0.12±0.13	0.07+0.11	0.09±0.12	0.05±0.07
α-carotene	0.02±0.02	0.04±0.03	0.01±0.01	0.01±0.06	0.01±0.01	0.02±0.02

[1] Post value means with different superscript letters within the same ROWS are significantly different, $p<0.05$, ANOVA

5.5.2 Breast milk retinol and carotenoids

As shown in Table 15, the concentrations of breast milk retinol at baseline were comparable among the three groups. Since casual milk samples were collected, the values were also expressed in relation to gm of milk fat. During intervention, subjects receiving carotene-rich vegetables and fruits (group A) increase breast milk retinol more than others ($p=0.097$, ANOVA). Distribution

of breast milk retinol (μg/g milk fat) indicated a shift towards higher levels from baseline, more prominently in group A (vegetables and fruits) than groups C and B (Fig. G) Considering the cut-off level of <8 μg/g milk fat suggested by WHO, low breast milk retinol were detected in 14 % of all subjects at baseline. Surprisingly, 24 % of subjects in group B (purified beta-carotene), 4% in the control group and none in group A showed breast milk retinol below 8 μg/g milk fat in Table 16.

Table 15 Breast milk retinol concentrations in lactating women at Pre- and Post-Intervention (Mean±SD)[1]

Breast milk	Intervention Groups					
	A (n=24)		B (n=25)		C (n=25)	
	Pre	Post	Pre	Post	Pre	Post
Retinol (μmol/L)	2.1±1.0	3.1±1.7[2]	1.7±0.7	1.8±1.1[a]	1.9±1.1	2.4±1.4
Retinol (μg/dl)	39.5±10.3	59.8±34.7	33.4±7.6	42.2±14.3	40.7±12.8	65.4±29.4
Fat (g/dl)	3.0±1.3	2.9±1.6	3.0±1.4	3.6±1.4	3.8±2.1	4.1±2.0
Retinol (μg/g milk fat)	16.0±8.0	23.4±12.9	13.0±5.5	13.9±8.6	14.6±11.3	18.2±8.2

[1] Post value means with different superscript letters within the same ROWS are significantly different, p<0.05, ANOVA

[2] Post value means with in the same group is no significantly, p=0.097, Anova

Table 16 Number of subjects had the breast milk retinol concentrations at the cut-off level of <8 μg/g milk fat at Pre- and Post-Intervention

Breast milk retinol at the cut-off level of <8 mg/g milk fat (n (%))	Intervention Groups					
	A (n=24)		B (n=25)		C (n=25)	
	Pre	Post	Pre	Post	Pre	Post
	3 (12.5%)	0 (0)	4 (16%)	6 (24%)	6 (24%)	1 (4%)

Breast milk β-carotene in subjects receiving purified beta-carotene significantly increased compared to groups A and C at the end of intervention period (Table 17). Lutein level increased significantly in breast milk of group A

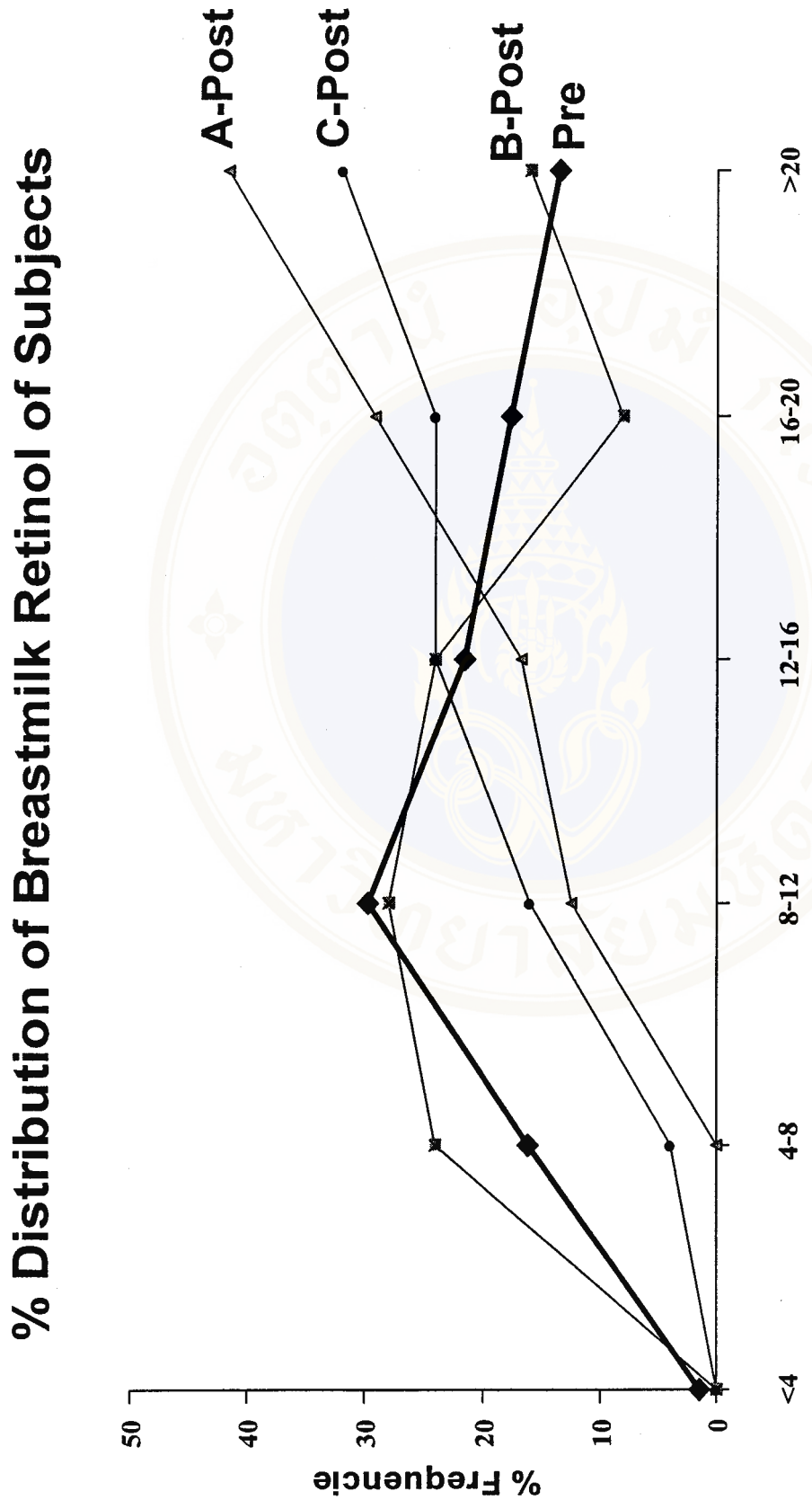


Fig. G Frequency distribution of breast milk retinol concentrations (µg/g milk fat) in lactating women at pre-intervention (n=85) and post-intervention (n; =24, 25, 25 for groups A, B and C)

who were given carotene-rich vegetables and fruits, following the same pattern in serum as shown earlier. Other carotenoids showed a wide variation with little changes after intervention.

Table 17 Concentrations of Breast milk Carotenoids at Pre- and Post-Intervention (Mean±SD)[1]

Breastmilk Carotenoids (μg/L)	Intervention Groups					
	A (n=24)		B (n=25)		C (n=25)	
	Pre	Post	Pre	Post	Pre	Post
β-carotene	5.3±5.2	9.7±4.9[a]	7.3±5.0	13.1±6.2[b]	5.8±6.5	6.4±6.7[a]
Lutein	37.8±22.4	62.1±19.6[c]	40.2±18.0	38.8±19.7[d]	31.4±21.7	41.7±26.1[d]
Zeaxanthin	10.7±5.6	18.0±11.1	11.5±5.4	10.0±6.6	10.3±6.8	16.6±12.7
Lycopene	1.6±2.4	1.5±1.8	2.4±2.2	1.7±2.6	1.8±2.7	0.6±0.7
α-carotene	0.7±1.1	1.2±0.8	0.4±0.4	0.8±0.8	0.4±0.4	0.8±1.0

[1] Post value means with different superscript letters within the same ROWS are significantly different, $p<0.05$, ANOVA

5.5.3 Total body stores

As shown in Table 18, total body retinol stores (TBRS) were calculated by using the isotopic ratio at day 28 and 42 post –dosing in the formula of Furr et al (10). At baseline, total body stores ranged widely from 0.03 to 1.2 mmol with mean values of 0.27-0.32 mmol. Following intervention, total body stores of vitamin A declined considerably, resulting in the mean values (mmol) of 0.18, 0.25 and 0.23 in group A, B and C respectively. The reduction of TBRS (post minus pre values) occurred the most in group C with the mean loss of 0.07 mmol or 19 mg followed by group A with the mean loss of 0.05 mmol or 15 mg and, lastly group B with the mean loss of 0.01 mmol or 3 mg. There were no significant differences among the mean or median values as well as changes of

TBRS among the three groups. The decline in TBRS was reflected also by more subjects (n=10) showing the liver stores below 0.07 μmol/g. At post intervention, most of the subjects showed MRDR in the adequate range except for 16 subjects who had MRDR values >0.06, reflecting deficient hepatic stores (Data not Shown).

As shown in Fig. H, regression analysis indicated a significant inverse linear relation between serum isotopic ratio (D:H) at day 3 post-dose and TBRS at both pre-intervention (Pearson's correlation r= -0.608, n=69, p<0.0001; Spearman 's rank correlation r = -0.820, p<0.0001) and post-intervention (Pearson's correlation r= -0.638, n=71, p<0.0001; Spearman's rank correlation r= -0.789, p<0.0001).

5.6 Functional Assessment

For conjunctival impression cytology (CIC), there were no significant differences among three groups at baseline and 12 weeks of intervention (Table 19). Nearly all subjects fell into normal (32 – 45%) or borderline normal (45 – 68%). Few were unreadable and none were detected as abnormal conjunctiva / epithelium. Modified dark adaptation test also revealed the values within normal range of 2.6-2.8 arbitrary units for light intensity (Table 20).

Table 18. **Total body retinol stores (TBRS) , Liver retinol concentrations of lactating women at pre- and post-intervention[1].**

	INTERVENTION GROUPS					
	A		B		C	
	PRE	POST	PRE	POST	PRE	POST
Total body retinol stores[2]	N = 24		N = 25		N = 22	
mg, mean±SD	77.2±86.8	50.0±33.2	81.3±79.8	70.6±50.3	91.5±80.6	67.1±35.3
median	48.8	39.98	56.9	56.4	56.5	63.4
(min – max)	(16.1-410.5)	(10.5-126.3)	(17.2-341.7)	(20.2-255.8)	(9.6-292.6)	(14.8-145.3)
m mol, mean±SD	0.27±0.30	0.18±0.12	0.28±0.28	0.25±0.18	0.32±0.28	0.23±0.12
median	0.17	0.14	0.2	0.2	0.2	0.22
(min – max)	(0.06-0.71)	(0.04-0.44)	(0.06-1.2)	(0.07-0.9)	(0.03-1.02)	(0.05-0.51)
Changes in TBRS						
mg, mean(median)	-	-15(-5)	-	-3(-3)	-	-19(-14)
m mol, mean(median)	-	-0.05(-0.02)	-	-0.01(-0.01)	-	-0.07(.0.05)

[1] means ± SD, unless specified otherwise, no significant differences among the three groups, ANCOVA.
[2] estimated by using the Furr equation (8)

Fig. H1

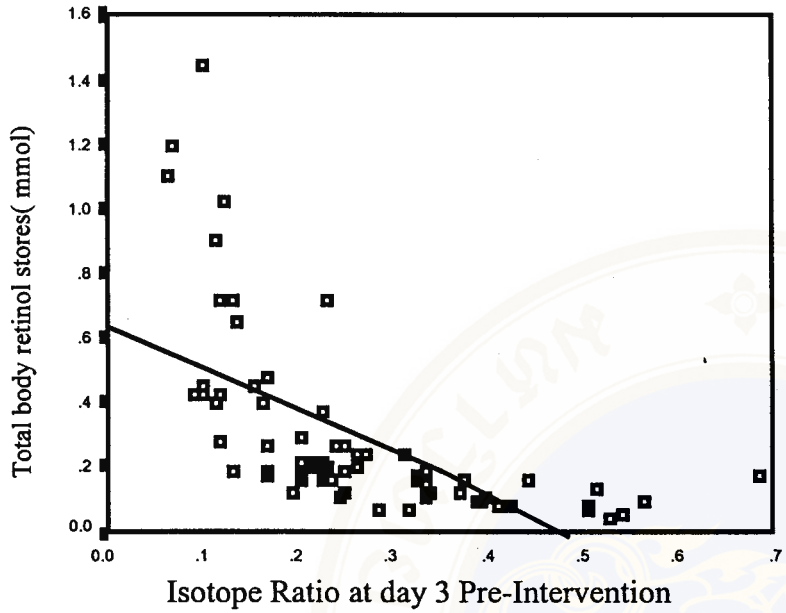


Fig. H2

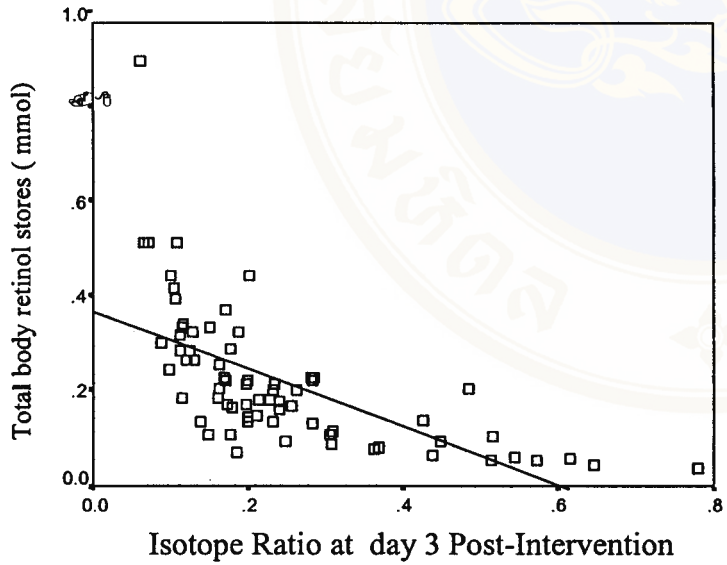


Fig. H Correlation between isotope ratio 3 days after oral dose of deuterated retinyl acetate versus values for total body retinol stores estimated by using total liver reserves in the Furr's equation(8). For all values at pre-intervention, (n=69), r = -0.608 (Pearson' s correlation) and r = -0.820 (Spearman' s rank correlation), p<0.0001 (Fig. 5a) and at post-intervention, (n=71), r = -0.638 and r = - 0.789 respectively, p<0.0001 (Fig. 5b)

Table 19 Conjunctiva Impression Cytology in Lactating women at Baseline and Post-intervention (n (%))

Conjunctiva Impression Cytology	Intervention Groups					
	A (n=24)		B (n=25)		C (n=25)	
	Pre	Post	Pre	Post	Pre	Post
Normal (NOR)	11 (45.8%)	14 (58.3%)	8 (32%)	14 (56%)	9 (36%)	11 (44%)
Borderline normal (BON)	11 (45.8%)	10 (41.7%)	17 (68%)	10 (40%)	15 (60%)	14 (56%)
Borderline abnormal (BAB)	0 0	0 0	0 0	0 0	0 0	0 0
Abnormal (ABN)	0 0	0 0	0 0	0 0	0 0	0 0
Unread (UNR)	2 (8.4%)	0 0	0 0	1 (4%)	1 (4%)	0 0

Table 20 Modified dark Adaptometry value in Lactating women at Baseline and Post-intervention (Mean±SD, Median (min-max))

Modified Dark Adaptometry	Intervention Groups					
	A (n=24)		B (n=25)		C (n=25)	
	Pre	Post	Pre	Post	Pre	Post
DA value Mean+SD Median (min-max)	2.7±0.2 2.8 (2.2-3.2)	2.6±0.2 2.6 (2.2-3.0)	2.8±0.2 2.8 (2.2-3.2)	2.7±0.2 2.8 (2.4-3.0)	2.8±0.2 2.8 (2.4-3.2)	2.7±0.2 2.8 (2.2-3.0)

5.7 Correlations & Sensitivity/Specificcity among VA indices

As shown in Table 21, comparison among various VA indices indicated significant correlations between breast milk β-carotene and serum β-carotene (r

= 0.97); TBRS and serum β-carotene (r = 0.6); TBRS and breast milk β-carotene (r = 0.59).

Table 21 Correlation Between Total body retinol store (TBRS) and other Vitamin A Indices (Correlation coefficient(p value))

Vitamin A Indices	TBRS	Serum Retinol	Breast milk Retinol	Serum β-carotene	Breast milk β-carotene
TBRS	1.000	-0.078 (p=0.354)	0.0161 (p=0.849)	**0.5968 (p<0.005)[a]**	**0.5913 (p<0.005)[a]**
Serum Retinol		1.000	-0.0751 (p=0.373)	**-0.2728 (p=0.01)[b]**	**-0.1774 (p=0.034)[b]**
Breast milk Retinol			1.000	0.0815 (p=0.333)	0.0572 (p=0.498)
Serum β-carotene				1.000	**0.9751 (p<0.005)[b]**
Breast milk β-carotene					1.000

[a] Correlation coefficient with different superscript letters between TBRS and other indices are significantly correlated, p<0.05, Partial Correlation.

[b] Correlation coefficient with different superscript letters other indices are significantly correlated, p<0.05, Partial Correlation.

When TBRS is regard as reference index, a cut-off point was developed for TBRS based upon the critical level of 20 μg/g liver. Liver weight was determined from the assumption of 2.4% body weight (10). In this study, average bodyweight was 50 Kg leading to 1200 mg liver weight. The critical level of 20 μg/g liver is equivalent to 20x1200 = 24,000 μg or 24 mg of TBRS. From Table 22, when TBRS < 24 mg or > 24 mg are used as reference, serum retinol gave sensitivity of 62.5% and specificity of 56.25%. Inaddition, sensitivity and

specificity of breast milk retinol in relation to TBRS were 25% and 86.9%, respectively.

Table 22 Sensitivity and Specificity between TBRS (as reference standard) and Serum and Breast milk retinol

Vitamin A Indices	Total body retinol stores (TBRS)	
	Sensitivity	Specificity
Serum retinol	62.5%	56.25%
Breast milk retinol	25.0%	86.88%

CHAPTER VI

DISCUSSIONS

The controversy surrounding the efficacy trials of feeding carotene-rich diet to improvement of vitamin A status rests upon two main issues. First, that the population studied must be depleted or low in vitamin A status to begin with in order to detect the response to intervention. We chose to study lactating women because there is an increased demand for vitamin A at this stage. To identify the subjects, over 700 lactating women were screened for those with low vitamin A intake whose blood were collected for serum retinol analysis. Only lactating women with serum retinol concentration below 0.87 μmol/L (25 ug/dl) were eligible for the study. This cut-off value of <0.87 μmol/L was chosen based on our earlier studies in northeastern school children (58, 59) as well as data from the second National Health and Nutrition Examination Survey (60) to indicate subjects at possible risk for vitamin A deficiency and likely to respond to interventions.

The second issue involves the choice of indicators of vitamin A status in response to different interventions. Since vitamin A can be stored in the body, mainly in the liver and in smaller amount elsewhere (1), such reservoirs are able to maintain the levels of circulating retinol and keep key functional organs (retina, epithelium, immune systems etc) well supplied of vitamin A, especially during the time when dietary supply of preformed vitamin A or provitamin A carotenoids become scarce. Only when the body stores are lower than a critical level, will the blood supply and tissue levels of vitamin A start to decline. Therefore, dietary intervention at the time

when this homeostatic mechanism is in place, may contribute retinol to body stores amidst stable levels of retinol in the blood and other tissues. Such was the line of thought leading to this study which reexamined the question concerning the contribution of provitamin A carotenoids from vegetables as reported earlier in Indonesian lactating women.

Our dietary intervention was designed to compare the efficacy of β-carotene in foods with the synthetic form. To simulate the habitual diet, a combination of dark green leafy vegetables and yellow/orange fruits and vegetables were given rather than separating them as in the former Indonesia study (9). To facilitate absorption of β-carotene in foods, the intervention include about 10 gm of fat per meal.

Although hepatic concentration of vitamin A is considered the best indicator of vitamin A status, direct biopsy of the liver is not a feasible method for population study. The indirect biochemical assessment such as the dose response tests (57) are useful for identifying the at risk groups in need of intervention but can not provide quantitative estimates of vitamin A stores. Recently, isotope dilution technique has been proposed to quantitatively estimate total body reserves of vitamin A in humans (10). The technique has been shown to provide a very good estimate of liver reserves in Bangladeshi surgical patients despite a wide prediction interval for individual subjects (61). Further study on the kinetics of oral dose of deuterated retinyl acetate in healthy US subjects as well as Bangladeshi women (24-36 yr old) indicated the mean equilibration time of 16-17 days (62) which was not affected by the size of hepatic reserves. The most recent study in Guatemalan elderly (63) showed that

equilibration occurred by 20 day postdoing which is in agreement with the former report. Thus, in this study, the isotopic ratio on day 28 or day 42 after the dose was administered, should be well within the equilibrium part of the curve and can be used to calculate total body reserves of vitamin A using the Furr equation (10).

In agreement with the findings from 15 Guatemalan elderlies (63), we reported here a significant inverse linear relation between the isotopic ratio (D:H) at day 3 post-dose and the calculated total body stores of vitamin A. Our findings confirm the former proposal of Green et al.(64, 65) in their studies in rats to use day 3 as the "window of opportunity" to predict body stores of vitamin A. Through the collection of a pool of data from different studies in humans, including ours, up to date, a prediction equation can possibly be formulated which can shorten the length of the study remarkably and make the studies more feasible in the field settings when multiple blood collections at day 20 or beyond can be avoided.

Data on total body retinol stores showed a wide fluctuation at both pre- and post-intervention in all three groups. Their calculated stores at baseline: 0.25±0.20 mmol were about 2-3 times higher than the values reported for Bangladeshi adults (61, 62) of 0.10±0.11 mmol but were less than half of the values reported for the Guatemalan elderly of 0.78±0.06 mmol (63) and the US adults of 1.03±0.45 mmol (62). A decline of body stores were observed at post-intervention in all three groups. However, the group receiving purified beta-carotene seemed to lose the least with the mean (median) reduction in mg of –3 (-3) followed by those fed vegetables and fruits, -15(- 5), and control subjects, -19(-14). In regard to the MRDR which was conducted

at post-intervention only, 16 subjects (23.5%) were considered deficient (MRDR > 0.06). To enable the explanation of the decline of TBRS amidst the intervention, other biochemical indices must be considered to complete the picture.

Serum retinol concentrations increased significantly, in similar magnitude of about two folds, among the three groups at the end of intervention. One major factor is seasonality. As shown in Fig. I, data were obtained from lactating women in rural areas of Ubon province where no interventions were given, distribution of serum retinol concentrations during rainy season shift to the right where nearly all subjects had serum values beyond 1.05 μmol/L. On the other hand, during the dry season, serum retinol distribution regressed towards the lower end, enable us to identify a group of lactating women (n=87) whose serum retinol were below 0.87 μmol/L (<25 μg/dl) to enroll in this study. Despite the fact that subject enrollment began in the middle of dry season, the protocol for baseline isotope dilution required a six week of equilibration where multiple blood samples were collected (day 0. 3, 28, 42). Thus, by the time dietary intervention started, the rainy season was about to begin. At the end of intervention period (12 weeks), the time was mid-rainy season, the same period where we had observed the shift of serum retinol towards the satisfactory range. Therefore, one explanation for serum retinol pattern appears to be seasonal variation which reflects a combination of food availability, reduction in morbidity and infection and others. The next factor is dietary intake of these lactating women. Since we provided only beta-carotene as fruits and vegetables (group A) or purified form (group B), dietary data were collected weekly by 24 hr recall on a random day basis to assess nutrient intakes of the background diet. During intervention, macronutrient as well as beta-carotene intakes from habitual diet were similar among the three groups.

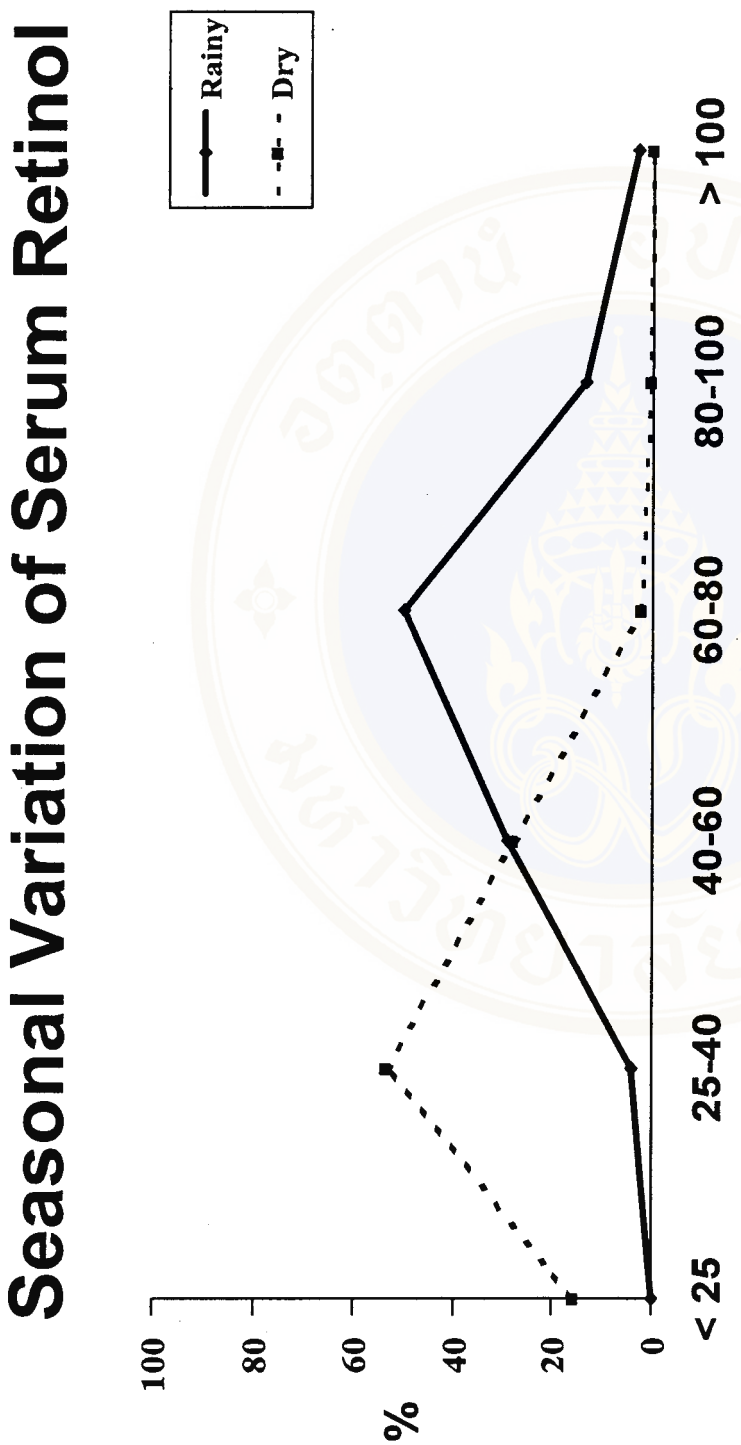


Fig. I Variation of serum retinol concentrations (μg/dl) in lactating women of Ubon province, NE Thailand during rainy & dry season.

However, lactating women in group A appeared to consume less than half of preformed vitamin A (retinol from animal origins) compared to the control group (Table 12, Fig E). This happened despite the fact that subjects were randomized into three groups by the village. The increased consumption of retinol foods in the control group can partially explain the rise in serum retinol as shown in Fig. J. It should be noted that the intervention ended during the time of rice planting, all lactating mothers moved to live and work in the rice paddy fields. This was when the quality of food intakes, especially animal and plant sources of vitamin A, decreased remarkably, priming them for the decline in body reserves of vitamin A followed by a regression of serum retinol toward the lower end when the dry season finally arrived where foods are scarce.

Serum and breast milk carotenoids appear to reflect the compliance of the intervention meal and dietary consumption of carotenoid rich foods in the background diet. Our data support this notion due to a very strong correlation ($r = 0.97$) between serum and breast milk beta-carotene. Increase in beta-carotene concentrations in milk may benefit the infants by some being converted to retinol (L.Canfield, personal communication).

Following intervention, breast milk retinol concentrations increased the most (and nearly reach a significant level if number of subjects are increased) in subjects receiving carotene-rich fruits and vegetables followed by the control and those given purified beta-carotene. The majority of subjects in all three groups showed the mean values above the cut-off values of 1.05 μmol/L or 8 μg/g milk fat as suggested by

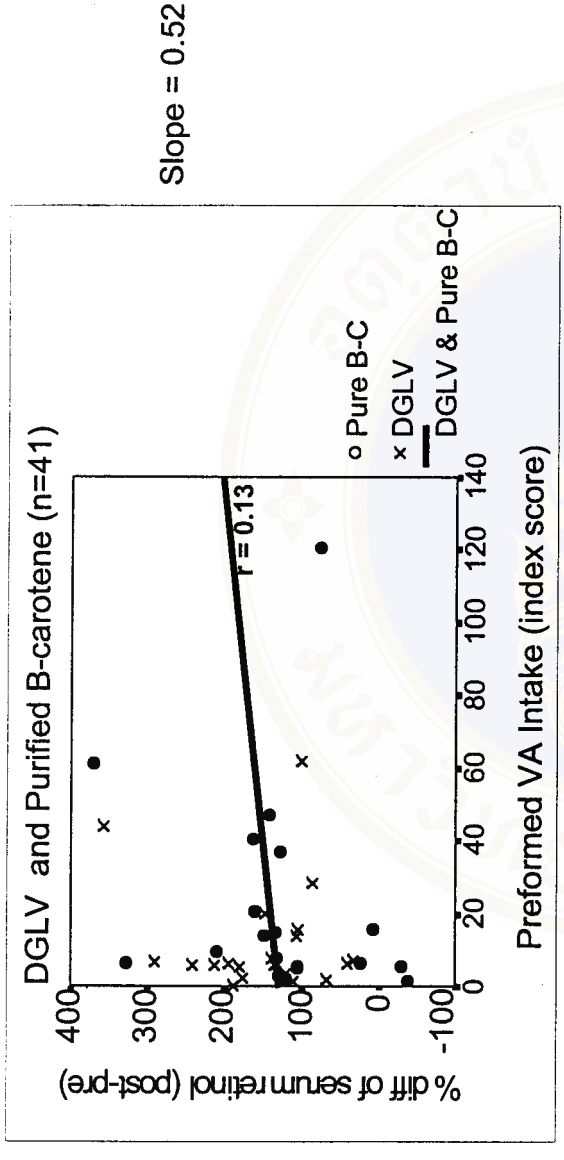


Fig. J1

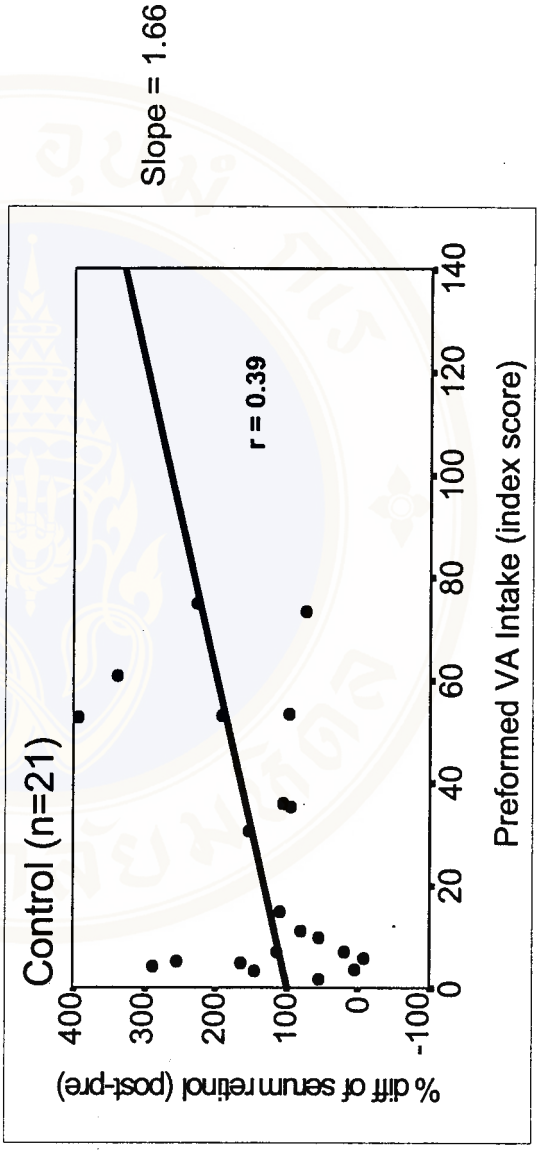


Fig. J2

Fig. J Correlation between preformed VA intake (index score as in Fig. 2) and % difference of serum retinol (post minus pre intervention values) among fruit-vegetables & purified β-carotene (7a) and control (7b).

WHO (57). Maintenance of both serum and breast milk retinol at the end of intervention must be at the expense of body vitamin A stores as shown by a decline of TBRS. However, considering the pattern of decline, although this did not reach a statistical significance between groups due to a limited sample size, the capacity of reducing the loss in median values of the purified beta-carotene group (-3 mg) followed closely by the vegetables and fruits group (-5 mg) in comparison to the control group (-14), seems to suggest the benefit of the intervention of beta-carotene in the first two groups as contributing to retinol stores. This speculation is in line with the findings from China (S. Yin, personal communication) which showed that feeding school children with green and yellow vegetables can sustain serum retinol as well as prevent the decrease in liver stores as observed in the control group.

Comparison of biochemical indices of vitamin A status such as serum and breast milk retinol against estimate of total body stores (Table 22) indicated that serum retinol is more sensitive in detecting individuals with depleted vitamin A status. On the other hand, breast milk retinol showed a higher specificity in identifying lactating women with adequate vitamin A stores. This evidence supported the use of serum retinol to identify population at risk of vitamin A deficiency. However, our data showed that breast milk retinol appeared to distinguish better, the response to different dietary interventions.

From our study, it appears that carotenoid rich foods when routine consumed, can raise serum and breast milk retinol. However, it is important that dietary vitamin A or beta-carotene trials adequately measure seasonal changes in "background" dietary

intakes that could affect vitamin A status outcomes. Following this line of thought, future study should consider lengthen the intervention period into the mid-dry season (8-12 months) as well as increasing the number of subjects to avoid potential, unmeasurable confounders and to raise the chances of detecting changes in vitamin A status and total body vitamin A stores.

To apply these findings to program for lactating women, our study suggested that promoting consumption of provitamin A foods should be year-round. However, dietary intervention program should aim at dry season to buffer for food scarcity as well as increasing vitamin A demand of lactation. Where feasible, fortification of common food items with purified β-carotene should be useful. In the area where provitamin A foods are scarce or unavailable, fortified foods would be appropriate. Response to interventions among lactating mothers can be monitored or evaluated through the measurement of dietary intake together with breast milk retinol.

CHAPTER VII

CONCLUSIONS

In conclusion, this study addressed a controversial issue concerning the contribution of carotenoid-rich foods to vitamin A status as measured by biochemical and functional indices. Since the most precise assessment of vitamin A status is the measurement of total body stores, this study employed the use of isotope dilution technique to estimate vitamin A reserves. Lactation women (N=85) in Northeast Thailand who exhibited low serum retinol concentrations (<0.87 μmol or <25 μg/dl) were enrolled, they were randomly assigned to receive one daily meal containing either dark green leafy and yellow/orange vegetables and fruits or purified β-carotene or control (low carotenoid) vegetables and fruits. The meal was given 5 day per week for 12 consecutive weeks.

The results showed that:

Habitual Diet

At baseline, the fruit and vegetable group received lowed preformed vitamin A in the home diet. During intervention, β-carotene intake increased from dietary intervention in fruit and vegetable as well as purified β-carotene group. At the end of intervention, all groups showed lower vitamin A intake due to their engagement in working in the rice-fields. Other nutrients were comparable among groups throughout the study.

Vitamin A Status

Serum retinol increased similarly in all 3 groups after intervention and may reflect seasonal variation. Breast milk retinol shifted to higher values in fruit and vegetable group at post intervention. As a reflection of compliance, increases in serum and breast milk β-carotene were detected in purified β-carotene and fruit and vegetable groups. In addition, lutein as a biomarker for provitamin A foods increased only in fruit and vegetable group.

Total body retinol stores (TBRS) declined in all groups. The reduction in TBRS was highest in control group followed by fruit and vegetable group with the least decline in purified β-carotene group. In addition, significant inverse linear relation was noted between isotopic ratio at day-3 post-dosing and TBRS estimates. Moreover, a strong correlation was observed between serum and breast milk β-carotene while TBRS correlated modestly with serum as well as breast milk β-carotene. Using TBRS as reference standard, serum retinol showed a moderate sensitivity and specificity while breast milk retinol showed a high specificity but low sensitivity in detecting individuals with depleted vitamin A status.

Therefore, our data indicated that short-term increase in β-carotene rich foods had no effects on serum retinol and modestly increased breast milk retinol but may have prevented a slight decline in total body retinol stores, that may result from breast feeding.

Future Recommendations

- Explore the use of serum isotopic ratio at day 3 as an early indicator of TBRS,

- Refinement of isotope dilution technique as a ***"gold standard"*** of vitamin A status, and

- Future intervention studies should extend through the full seasonal cycle (e.g. 12 months) to evaluate long-term effects of diet on vitamin A status and body stores.

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APPENDIX 1

LABORATORY ANALYSIS

A. Determination of Carotenoids in foods

Procedure :

1. 10-20 g aliquot of the ground sample weight in the beaker or conical flask.
2. add 1 g solid magnesium carbonate (Ajax Chemicals, Auburn, Australia) to neutralize any organic acids and 2 g celite 545 (Fluka Chemie AG, Switzerland) as filter aid.
3. add 50 ml THF (J.T. Baker, Phillipburg, USA.)
4. homoginizing for 1 min using an ultra-turrax homogenizer (IKA, staufen, Germany).
5. filtered through Whatman no. 1 in a buchner funnel under vacuum.
6. repeat extraction 3 to 5 times or until no yellow colour in the liquid.
7. transferred to a rotary evaporator to dry at about 37°C.
8. dissolved with 50-100 ml CH_2Cl_2 (J.T. Baker, Phillipburg, USA.) and transferred to a separating funnel which had 100 ml 10% NaCl (E. Merck, Darmstadt,Germany).
9. mixed by careful shaking, separate the lower aqueous phase off to conical flask containing Na_2SO_4 (E. Merck, Darmstadt,Germany) for drying. Repeat until no colour in NaCl phase.
10. filtered through Whatman no. 1 in a buchner funnel under vacuum and wash with CH_2Cl_2
11. dry in a rotary evaporator to dry at about 37°C.

12. dissolved with small amount of CH_2Cl_2 (about 2-4 ml) by ultrasonic agitation and dilute to 10 ml with 1%THF in CH_3OH (J.T. Baker, Phillipburg, USA.) for inject to HPLC.

HPLC condition :

Injection volume : 20 µl Rheodyne injector

Column : Vydac 201 TP 54 (5 µm, 250 X 4.6 mm., Alltech Associates, Deerfield, Illinois, USA) replacement of metal frits with Hastalloy C 2.0 µm frits.

Mobile phase : 1% THF in CH_3OH flow isocratic 1.0 ml/min

Detector : 450 nm LC-UV Pye Unicam

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B. Serum extraction of retinol, vitamin A and β-carotene

1. 400 µl serum
2. add 400 ml of absolute ethanol and vortex for 30 sec.
3. add 750 ml of Hexane, vortex 30 sec. Centrifuge for 10 min. Transfer hexane level to a clean test tube
4. Extract again with same volume of hexane
5. Combine the hexane layers
6. Evaporate hexane layers to dryness under stream of nitrogen, do not allow to dry unattended for extended time.

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C. Extraction of Carotenoids in Human Milk

Procedure : Chemical extraction

1. 2.5 ml of milk sample
2. 2.5 ml ethanol
3. 3.75 ml 50% KOH (w/w), stir under N_2 at 25°C (RT) for 1 – 3 hrs
4. Extract 3X with 1.25 ml hexane (which had 1% BHT)
5. Combine hexane, dry and blow with N2
6. Dissolve with 100 µl CH_2Cl_2 (with 1% BHT) and dilute with 400 µl injection solvent (Mobile phase) in ultrasonic bath, and filter through 0.2 µm filter into injection vial cap immediately

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APPENDIX 2

NUTRIENT INTAKE

A. Intervention Meal – *one meal/wk day* (mean±SD)

Nutrients	Pre-VA Meal	Control Meal
Energy (Kcal)	288±45	308±52
Carbohydrate (g)	35±5	56±12
Fat (g)	10±2	10±2
Protein (g)	27±4	28±3
Protein-animal (g)	22±3	24±2
Protein-vegetable (g)	5±1	4±1
Animal VA (RE)	-	-
β-carotene (μg)	4750±262	>0.05
Total VA (RE)	792±42	-

(Purified β-carotene in orange juice = 3600 ± 115 μg β-carotene)

B. Macronutrients Intake at *Pre-, During* and *Post-intervention* among the 3 groups in mean ±SD, median (min-Max)

B1 Pre-intervention

Macronutrients	Intervention groups During-Intervention		
	A (N = 24)	B (N = 25)	C (N = 25)
Carbohydrate (g)	308±50 317 (230-392)	299±78 293 (196-538)	293±81 298 (132-481)
Fat (g)	14±7 12 (8-41)	14±5 14 (5-23)	15±5 14 (8-31)
Protein (g)	52±8 50 (37-69)	52±13 50 (28-83)	53±13 52 (32-79)
Protein-Animal (g)	17±6 14 (7-31)	18±5 19 (3-25)	20±7 18 (12-42)
Protein-Veg. (g)	35±6 36 (26-47)	34±9 34 (19-61)	33±9 33 (18-54)
Energy (Kcal)	1630±240 1609 (1206-2011)	1562±397 1494 (1030-2752)	1556±398 1600 (780-2427)

B2 During-intervention

Macronutrients	Intervention groups During-intervention		
	A (N = 24)	B (N = 25)	C (N = 25)
Carbohydrate (g)	319±55 315 (229-453)	310±47 314 (220-408)	316±63 320 (199-429)
Fat (g)	18±4 18 (11-25)	18±4 18 (10-27)	21±6 20 (13-40)
Protein (g)	51±9 49 (36-71)	52±8 52 (30-66)	53±11 52 (38-76)
Protein-Animal (g)	15±5 15 (6-24)	17±5 16 (6-25)	18±6 17 (10-30)
Protein-Veg. (g)	36±6 35 (25-52)	35±6 35 (24-46)	35±7 36 (21-49)
Energy (Kcal)	1672±267 1651 (1252-2322)	1646±231 1656 (1107-2104)	1698±327 1694 (1219-2312)

B3 Post-intervention

Macronutrients	Intervention groups at Post-intervention		
	A (N = 24)	B (N = 25)	C (N = 25)
Carbohydrate (g)	303±63 300 (209-458)	292±51 299 (190-395)	297±71 304 (165-409)
Fat (g)	13±6 12 (7-30)	12±4 11 (5-21)	13±6 11 (5-34)
Protein (g)	47±10 46 (32-73)	46±8 47 (25-65)	47±11 47 (24-68)
Protein-Animal (g)	12±6 11 (4-28)	13±5 13 (3-21)	13±7 12 (4-28)
Protein-Veg. (g)	35±8 34 (24-52)	33±6 33 (22-46)	34±8 35 (16-49)
Energy (Kcal)	1559±309 1531 (1069-2321)	1492±258 1510 (929-2025)	1523±361 1551 (838-2201)

C. Macronutrients and vitamin A : at Intervention day (feeding) and Non-Intervention day (no feeding) of During Intervention Period.(Mean±SD)

Nutrients	Lactating Women Groups		
	A N = 24	B N = 25	C N = 25
Energy ; feeding	1765±299	1720±238	1766±342
no feeding	1556±254	1574±276	1611±3343
Carbohydrate; feeding	332±61	320±49	324±63
no feeding	303± 53	302±56	305±691
Fat; feeding	21±3	22±4	24±7
no feeding	13±5 1	15±4 3	17±8 3
Protein; feeding	53±10	54±8	55±12
no feeding	48±9	50±10	51±11
A - Protein; feeding	17±5	19±6	20±7
no feeding	13±7	15±6	16±7 1
Veg-Protein; feeding	37±7	35±6	36±7
no feeding	35±6	35±7	34±8 1
Animal Vit A; feeding	65±83	149±235	194±287
no feeding	75±107	118±196	159±222
β-carotene; feeding	5071±984a	4111±398 b	570±415 c
no feeding	622±611 3	567±298 3	536±540 1
Total Vit A; feeding	910±195 a	834±268 a	289±302 b
no feeding	178±167 3	212±212 2	249±234

[1 2 3] No feeding day values mean with different superscript numbers within the same COLUMNS are significantly differences from feeding day values : 1 $p<0.05$; 2 $p<0.01$; 3 $p<0.001$, Friedman Test.

a bc mean with different superscript letters with in ROWS are significantly differences at $p<0.05$, Kruskal-Wallis Test

APPENDIX 3

INTERVENTION FOOD

3.1 Food Group from Vegetables and Fruits and β-cartene contents

Code	Name	Wt. Content (gm./pk.)	β-carotene content (mg./pk.)
A1	Ivy gourd, blanched	75	8.155
A2	Pumpkin + Carrot, steamed	80	4.019
A3	Chinese Cabbage, blanched	80	2.959
A4	Chinese Swamp Cabbage, blanched	80	1.397
A5	Pak Prang, blanched	80	1.650
A5N	Chinese Cabbage, blanched	80	As... A3
A6	Pak Khom (Amaranth), blanched	80	8.158
AS1	Pumpkin, in syrup (Pumpkin 70 gm.+ syrup 70 gm)	140	0.672
AS2	Papaya, in syrup (Papaya 70 gm.+ syrup 70 gm)	140	0.622
AW	Carrot juice 50%	200 ml.	2.179
B1, C1	Mustard white, blanched	80	None
B2, C2	Cabbage, blanched	80	>0.05
B3, C3	Cauli flower, blanched	80	None
B4, C4	Young Water Melon, boiled	80	>0.05
B5, C5	Sesbania flower, blanched	80	>0.05
BW	Orange juice with β-carotene	150	3.61
CW	Pineapple juice	150	none

3.2 Meat Product Group

M1	Processed pork "Moo yor"	41	gm./pk.
M2	Sausage with chilli	36	gm./pk
M3	Vienna sausage with garlic	40	gm./pk.
M4	North Thai processed pork "Sai Oau"	45	gm./pk.
M5	Frankfurtur sausage	42	gm./pk.

3.3 Menu Planning of β-carotene Intervention Foods for 12 Weeks of Subject group A, B and C at Najaluay District, Ubonrajthanee Province.

Subject "group A"

(β-cartene from dark -green or yellow-orange vegetables and fruits)

Week 1-4	1st day	A1 + M1 + CW
	2nd day	A2 + M2 + CW
	3rd day	A3 + M3 + AS2
	4th day	A4 + M4 + AS2
	5th day	A5 + M5 + AS1
Week 5-6	1st day	A1 + M1 + AW
	2nd day	A2 + M2 + AW
	3rd day	A3 + M3 + AS2 + AW
	4th day	A4 + M4 + AS2 + AW
	5th day	A5 + M5 + AS1 + AW

Week 7	1st day	A1 + M1 + AW
	2nd day	A2 + M2 + AW
	3rd day	A3 + M3 + AS1 + AW
	4th day	A4 + M4 + AS2 + AW
	5th day	A6 + M5 + AS1 + AW
Week 8	1st day	A1 + M1
	2nd day	A2 + M2
	3rd day	A3 + M3 + AS1
	4th day	A4 + M4 + AS2
	5th day	A6 + M5 + AS1
Week 9-12	1st day	A1 + M1
	2nd day	A2 + M2
	3rd day	A3 + M3
	4th day	A4 + M4
	5th day	A5n + M5

Subject "group B" (β-cartene from Purified β-cartene)

Week 1-12	1st day	B1 + M1 + BW
	2nd day	B2 + M2 + BW
	3rd day	B3 + M3 + BW
	4th day	B4 + M4 + BW
	5th day	B5 + M5 + BW

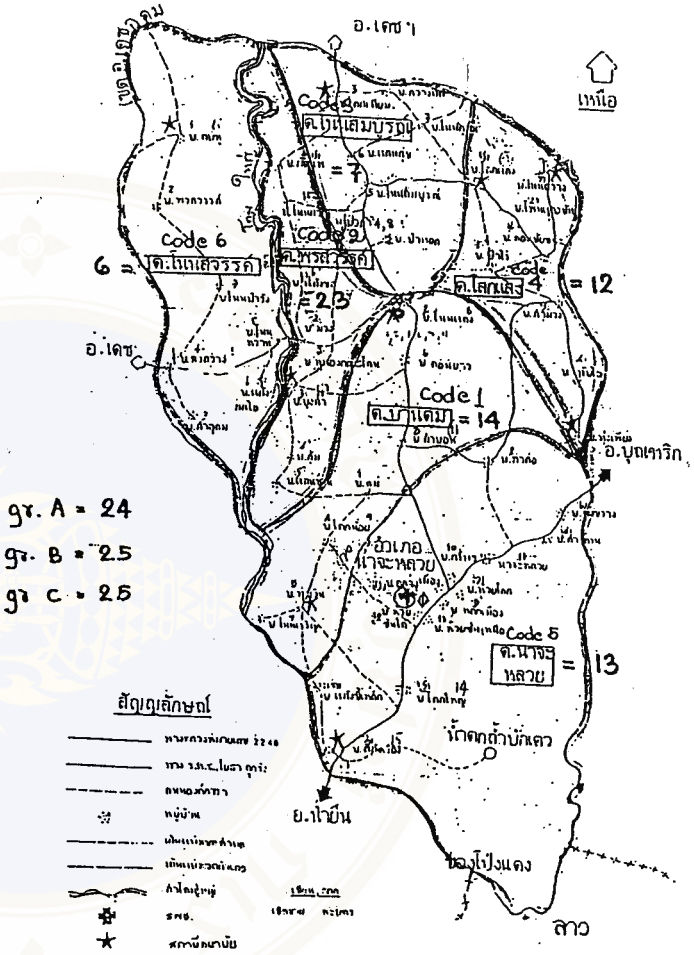
Subject "group C" (Non β-cartene food or Controlled group)

Week 1-12	1st day	C1 + M1 + CW
	2nd day	C2 + M2 + CW
	3rd day	C3 + M3 + CW
	4th day	C4 + M4 + CW
	5th day	C5 + M5 + CW

3.4 Distribution of β-carotene value for each month of Group A (β-cartene from dark -green or yellow-orange vegetables and fruits)

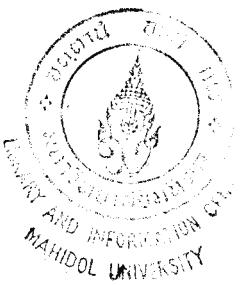
Month 1	β-carotene (mg/meal)	Month 2	β-carotene (mg/meal)	Month 3	β-carotene (mg/meal)
wk 1 d1	8.1546	wk 1 d1	10.3332	wk 1 d1	8.1546
d2	4.0186	d2	6.1972	d2	4.0186
d3	3.5811	d3	5.7597	d3	2.9594
d4	2.1188	d4	4.1974	d4	1.3971
d5	2.3224	d5	4.5010	d5	2.9594
wk 2 d1	8.1546	wk 2 d1	10.3332	wk 2 d1	8.1546
d2	4.0186	d2	6.1972	d2	4.0186
d3	3.5811	d3	5.7597	d3	2.9594
d4	2.1188	d4	4.1974	d4	1.3971
d5	2.3224	d5	4.5010	d5	2.9594
wk 3 d1	8.1546	wk 3 d1	10.3332	wk 3 d1	8.1546
d2	4.0186	d2	6.1972	d2	4.0186
d3	3.5811	d3	5.8102	d3	2.9594
d4	2.1188	d4	4.1974	d4	1.3971
d5	2.3224	d5	11.0084	d5	2.9594
wk 4 d1	8.1546	wk 4 d1	8.1546	wk 4 d1	8.1546
d2	4.0186	d2	4.0186	d2	4.0186
d3	3.5811	d3	3.6316	d3	2.9594
d4	2.1188	d4	2.0188	d4	1.3971
d5	2.3224	d5	8.8298	d5	2.9594
Total	80.7820	Total	126.1768	Total	77.9564
Mean±SD	*4.0±2.2*	Mean±SD	*6.3±2.6*	Mean±SD	*3.9±2.3*
	Total Mean±SD		*4.75±2.62*		

Ubonrajthanee Province



Najaluay District

APPENDIX 4 . Map of Ubonrajthanee Province and the study area (Najaluay District)



BIOGRAPHY

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