ISBN 0-315-54901-7

FIELD TEST OF DARK ADAPTATION RESPONSE FOR VITAMIN A STATUS AND ZINC STATUS

BY

ROSEMARY WAVINYA MUTUNGI

A THESIS
PRESENTED TO
THE FACULTY OF GRADUATE STUDIES
UNIVERSITY OF MANITOBA

IN PARTIAL FULFILLMENT
OF THE REQUIREMENT FOR THE DEGREE OF
MASTER OF SCIENCE
IN
FOODS AND NUTRITION

FIELD TEST OF DARK ADAPTATION RESPONSE FOR VITAMIN A STATUS AND ZINC STATUS

BY

ROSEMARY WAVINYA MUTUNGI

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

TABLE OF CONTENTS:

ABSTRACT:

Forty-two adults, 4 males and 38 females, Inuit patients from communities in Northern Manitoba and Northern Territories participated in the study to test the performance of a recently proposed method to measure dark adaptation outside the laboratory with a group of people who can be expected to have vitamin A deficiency. A dark adaptation test which requires less than 20 minutes was carried out. The procedure may eliminate the need for blood sample analyses in all patients.

A diet history survey was carried out, to describe the vitamin A intake this of population group, for whom previous studies on vitamin A status were not conclusive. A 20 ml venous blood sample was drawn from each subject for determination of plasma retinol values and serum zinc and copper values. The serum zinc and copper status were described allowing a better interpretation of plasma retinol results. The haemoglobin and haematocrit count values were described thus providing a better understanding of their nutritional status in general.

No evidence of vitamin A deficiency was suggested by the dark adaptation test for there was no retinal impairment, nor the biochemical indicators or the diet nutrient intake. The mean dark adaptation times recorded were similar to times recorded in other

populations with adequate vitamin A status. The mean dark adaptation time to separate the white chips during dark adaptation was 43.0 $\pm$ 22.0 seconds while the mean time to identify the blue chips during dark adaptation was 213 $\pm$ 44.0 seconds. The dark adaptation time was not correlated to plasma retinol values and estimates of vitamin A obtained by diet history. However, dark adaptation blue chip test times were positively correlated with serum zinc values ($p=0.05$). This indicated an effect on the purkinje shift during dark adaptation. The effect of age on the perception of purkinje shift was significant ($P<0.05$).

The nutrient intake indicated a population that is not nutrient deficient, as for vitamin A (RE) mean intake was 1074 ug RE/day which was within range of the recommended intake for vitamin A.

The pregnant women had low serum zinc values compared to the sample group. The sample mean was 9.8 $\pm$ 2.1 umol/L with a range of 6.7-13.4 umol/L while the mean serum zinc value for pregnant women was 7.9 $\pm$ 1.3 umol/L and a range of 6.7-11.2 umol/L as compared to non-pregnant women whose mean was 10.4 $\pm$ 1.8 umol/L and a range of 7.8-13.4 umol/L. The vitamin A status of the sample group was adequate and there was no retinal impairment detectable by dark adaptation.

ACKNOWLEDGEMENT:

The author wishes to express her sincere thanks to Dr. Gustaaf P. Sevenhuysen, her thesis advisor, for his advice and guidance in the direction of this research and in the preparation of the manuscript. He was always there to answer questions and offer guidance with patience and sincere interest.

Gratitude is also extended to Dr. Bruce E. McDonald and Dr. Michael E. K. Moffatt members of the thesis committee for spending their valuable time reading rough copies of her thesis and for making suggestions for the improvement of this thesis. I am sincerely grateful for this.

I would also like to thank Dr. D. Parry of St. Boniface Hospital for his contribution in serum zinc and copper analyses, Ms Marilyn Latta for her technical expertise in plasma retinol analysis, Linda Neden for her statistical advise and suggestions, Ms Elizabeth Corner for frequently donating the blood sample for control analyses, Ms Gloria Penner for her encouragement and for making it easier for me to contact subjects at the boarding house, the Inuit community for participating in the study and staff of Northern Medical unit for their support.

Appreciation and thanks to all my friends and fellow graduate students for their encouragement.

Research for this thesis was supported by Kenya Government through the Kenya High Commission in Ottawa, Canada. I sincerely appreciated the financial support.

Last but not least, I wish to thank my family, my husband Richard Mutungi, my daughter Doreen Mutheu Mutungi, and my son Kevin Mutungi for their invaluable support and encouragement throughout. A special thanks for my husband for being there for me when I needed him, and to the children for their emotional support. Thank you.

Kwi Asyai makwa na Mbaitu ni na muvea na ndai nundu wa unumisya na kunenga vinya wa utethya wia uu. Ni na utanu kumumanyithya kana ni ninakethie na ne nukya kila naendie nthi ya kuasa kuvanda. Ni asanda na muvea munene kwenyu inyothe. Ndikolwa ni ivinda yiu na taniisye nenyu.

LIST OF TABLES:

LIST OF FIGURES:

LIST OF APPENDICES:

Chapter I

1.0 INTRODUCTION :

The human eye is capable of automatically adjusting to an extremely wide limit of light intensities. Dark adaptation occurs when the eye is exposed to a dark environment after previous exposure to a light environment (Pitt, 1981). The earliest clinical sign of hypovitaminosis A is a decrease in dark adaptation ability. Night blindness is a decrease in the retinal sensitivity to light under dim light conditions. Clinical manifestations of the deficiency of vitamin A occur when the deficiency is severe and long standing. As a result, serum vitamin A levels do not reliably indicate moderate vitamin A deficiency in individual subjects. A wide range of borderline serum retinol values exist where vitamin A dependent functions of the retina may or may not be affected (Carney and Russell, 1980; Russell et al., 1978; Russell et al., 1973). Serum retinol levels normally are reduced only when most of the retinol stored in the liver has been depleted. However, serum vitamin A can be affected by other physiological conditions such as lack of retinol binding protein, even when liver retinol stores are adequate (Pitt, 1981; Turley and Brewster, 1987).

Standard dark adaptation testing procedures are cumbersome and lengthy (45 minutes). In addition expensive equipment is required

which, is not suitable for use under field conditions. A simple inexpensive test for evaluation of dark adaptation is desirable, because vitamin A deficiency is prevalent in many developing countries and is associated with high mortality rates (Sommer et al., 1983; Sommer, 1984). It is also an important cause of night blindness which is a preventable nutritional problem (Carney and Russell, 1980; Solomon et al., 1982). Early detection of hypovitaminosis A and institution of prophylactic therapy programs could dramatically reduce the prevalence of xerophthalmia and vitamin A deficiency blindness among populations.

Chapter II

2.0 LITERATURE REVIEW:

2.1 DARK ADAPTATION:

Night blindness can be quantified by observing dark adaptation performance under test conditions. For the classical dark adaptation curve and rod-cone breakpoint see figure 1. The traditional dark adaptation is based on measurement of the final dark adapted threshold. The final dark adapted threshold is the minimum amount of light capable of stimulating the retinal receptors after minutes in an environment without light. The rod-cone breakpoint in the traditional dark adaptation has been shown to be useful for the diagnosis of sub clinical vitamin A deficiency. Poor dark adaptation may indicate abnormal vitamin A content of the body, since dark adaptation ability is influenced by vitamin A status in the body (Hecht and Mandelbaum, 1939). Consequently, they concluded in their study that, certain measurement of dark adaptation ability when carried out under properly standardized conditions, can be used as an aid in the determination of Vitamin A condition of the body. Diminution in the supply of vitamin A to the rod cells of retina results in impairment of the function of dark adaptation and this may be detected by rod scotometry and dark adaptometry. However, none of

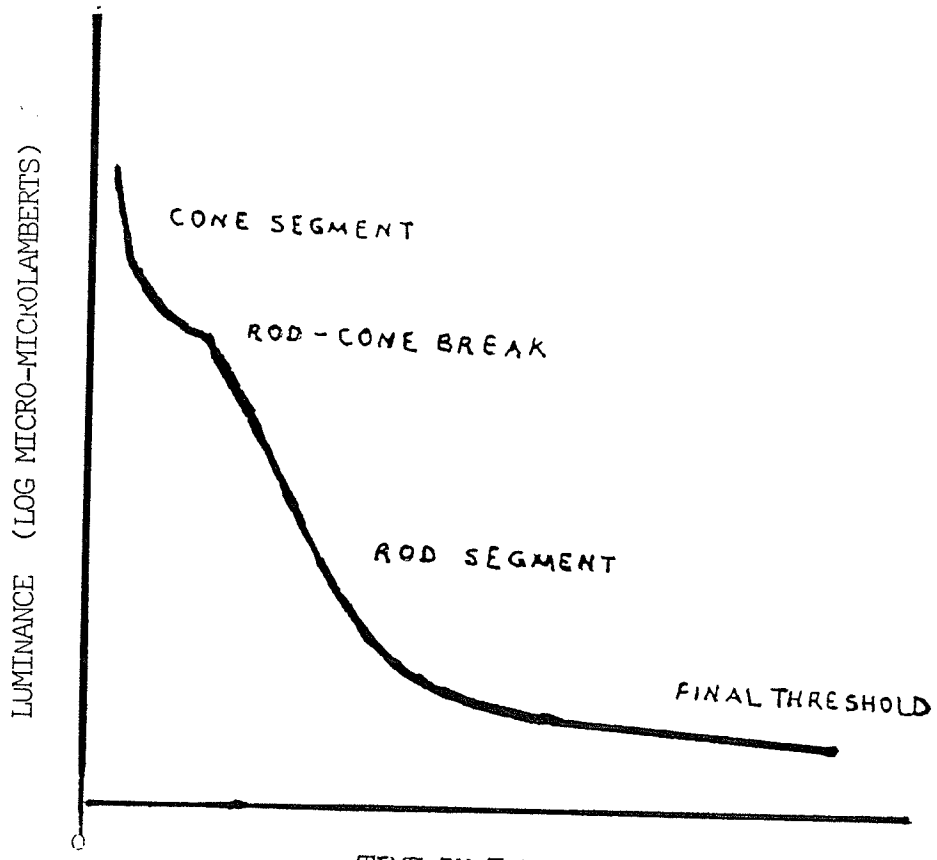


Fig.I., CLASSICAL DARK ADAPTATION CURVE.

these methods is applicable in all population groups, because a cooperative and responsive subject is essential. Also, the methods are not suitable for field studies because expensive and delicate equipment is required. The development of a simple but sensitive objective biophysical test of rod function applicable to all population groups susceptible to vitamin A deficiency would be a distinct advance in measuring the extend of the problem (Joint WHO/USAID Meeting, 1976). Such a method, the rapid dark adaptation test was developed by Thorton (1977), and validated by Vinton and Russell, (1981), and is applicable in all population groups for easy detection of vitamin A deficiency (Solomons, 1982).

The Rapid Dark Adaptation test (RDAT) (Thornton, 1977) requires inexpensive equipment and easy to use under field conditions was described by Thornton (1977). The Rapid Dark adaptation test measures the time required for subjects to observe the Purkinje shift during dark adaptation (Thornton, 1977), which is the change in spectral sensitivity of the retina that occurs at the rod-cone breakpoint during dark adaptation.

The change in sensitivity, also known as the Purkinje shift phenomenon, occurs when the peak wavelength sensitivity of the retina changes from red towards blue frequencies in the visual spectrum during the transition from day vision (photopic or cone mediated) to night vision (scoptic or rod mediated). Rods are

activated under scoptic lighting conditions (Vinton and Russell, 1981). The Purkinje Shift is perceived by subjects as an increasing brightness of blue objects under low-light (starlight) condition where colour vision does not operate. Therefore a blue object appears as a brighter shade of grey than would a red object. It is suggested that, in vitamin A deficiency, the time to the rod-cone breakpoint becomes prolonged by the delayed onset of predominantly rod mediated vision thus resulting in a prolonged dark adapted time (Patek and Haig, 1939; Hecht and Mendelbaum, 1939; Russell et al., 1973). The Rapid Dark Adaptation Test, based on the Purkinje Shift as an indicator of the rod-cone breakpoint, has been proposed as a tool to identify preclinical Vitamin A deficiency (Thorton, 1977; Carney and Russell, 1980; Solomons et al., 1982; Russell et al., 1973; Russell et al., 1978).

Russell et al., (1973), investigated 13 patients, 9 women and 4 men, between the ages of 21 to 75 years. All patients had normal visual acuity and were without eye diseases. There were no signs or symptoms of vitamin A deficiency except for one patient # 5, who complained of difficulty with driving at night. Seven controls were selected from patients referred to the ophthalmology clinic for a variety of problems that were not associated with dark adaptation function. Their visual acuity was from 20/25 to 20/100. Dark adaptation was studied on modified Goldman-Weeker adaptometers. The subject fixated on a 2 mm light

located 15 degree above the centre of test light with retinal subtense of 1 degree. The stimulus consisted of light flashes of one second interval of darkness. A tracking method was used whereby luminance of light was increased or decreased depending on the response of the subject. All tests were preceded by an instructional period and initial trial. Each subject was initially adapted to diffused white light of 3.13 log millilambert of luminance for 10 minutes after which dark adaptation threshold measurement were started immediately. Dark adaptation was usually complete after 35 to 40 minutes.

The final threshold was defined as the average of the three ascending and three descending thresholds obtained after 35-40 minutes in dark (an "ascending threshold" was the intensity at which the subject first saw the test light as its luminance was increased; a"descending threshold" was the intensity at which the subject ceased to see the test light as its luminance was lowered). This final threshold attained in 40 minutes was the measure of retinal function used.

All thirteen patients showed abnormally high final threshold ranging from 5.20 to 6.07 log picolamberts, with a mean of 5.55. The mean for the seven control was 4.9 and a range of 4.6-5.2 log picolamberts. Hence Russell et al., (1973), concluded that, quantitative dark adaptation tests are potentially useful clinical tools for recognising subclinical vitamin-A deficiency.

The dark-adaptation test is, therefore, able to uncover the initial functional deficit which is a functional abnormality that is readily reversible.

2.2 VITAMIN A DEFICIENCY:

In the adult human, Vitamin A deficiency results in impairment of retinol rod function, dark adaptation and in lowered blood levels of the vitamin. If the deficiency is prolonged conjunctival and corneal xerosis, stromal vascularization and corneal ulceration may ensue (Sauberlich et al., 1974).

Vitamin A has a multiplicity of functions, but only a role in vision is well understood. The photosensitive pigments in the eye consist of a metabolite of retinol (retinal) attached to a protein known as scotopsin. The pigment can not be produced or replaced at normal rates when vitamin A is deficient in tissues. Continuous break down and regeneration produce a shift in chemical balance. Hence the retina becomes less sensitive to light, resulting in night blindness. The epithelial tissues normally bathed in mucous or other secretions but during vitamin A deficiency they become dry, keratinized and susceptible to infection. This change called Keratinizing Metaplasia, has a disastrous effect on the cornea and conjunctiva and can cause permanent blindness (Bureau of Nutritional Sciences 1975b; Pratinindhi et al., 1987; Patek and Haig, 1939; Joint WHO/USAID

Meeting, 1976; Sommer et al., 1980; Sommer, 1984).

One function of vitamin A has been well defined biochemically-namely, its interaction with various opsins of the retina to form visual pigments. On exposure to light, the 11-cis form of retinaldehyde is isomerized to the all-trans form, which in turn triggers off a series of chromophoric changes in the complex. Concomitantly ion transport and membrane potential are affected. The latter may well give rise to nerve impulse that are sensed in the brain as vision (Joint WHO/USAID Meeting, 1976; Sklan, 1987). The greatest variability is in the times required to cure night blindness. The visual pigments are composed of Vitamin A aldehyde (retinene) joined to specific protein of the rods and cones called opsins. The amount of visual pigment that can be formed in the normal retina are limited by vitamin A and by the opsins (fig. 2) (Dowling and Wald, 1958).

During dark adaptation, a large amount of vitamin A (retinol) is converted into rhodopsin and reconversion of retinene and opsin into rhodopsin also takes place. Because of these reconversions, the visual receptors (rods) become so sensitive that even a minute amount of light causes excitation (fig. 3). Note the sensitivity of the retina is very low on first entering the darkness, but within 1 minute the sensitivity has increased ten-fold, that is, the retina can respond to light of one tenth the previously required intensity. At the end of 20 minutes the

sensitivity has increased about 6000-fold, and at the end of 40 minutes it has increased about 25,000-fold. The regeneration of rhodopsin in the retina is dependent on vitamin A. In night blindness, the retina contains less rhodopsin than a normal retina and the rate of regeneration of rhodopsin is much slower, and this condition can reflect on dark adaptation ability of individuals (Guyton, 1986). Vitamin A is the precursor of the visual pigment of the rods and cones in the retina. It seems reasonable to suppose that on a diet deficient in this factor the retina eventually synthesizes subnormal amount of visual pigment, with the corresponding decline of visual sensitivity that constitute night blindness.

Investigations conducted by Medical Research Council of Great Britain commonly referred to as Sheffield Study have been considered the most extensive and best controlled study on vitamin A and carotene in humans. Sixteen healthy volunteers were fed complete diets except for deficiency in vitamin A and carotene for a period ranging from 6 to 25 months. During eight months on the deficient diet, plasma vitamin A level fell in part of the subjects. Plasma carotenoids disappeared in 3 weeks. One person failed to manifest any decrease in his plasma vitamin A level after 22 months on the vitamin A deficient diet. The symptoms which appeared in vitamin A deficient subjects were increased intensity in dark adaptation, dryness of skin and eye discomfort. However, only 3 subjects had dark adaptation changes

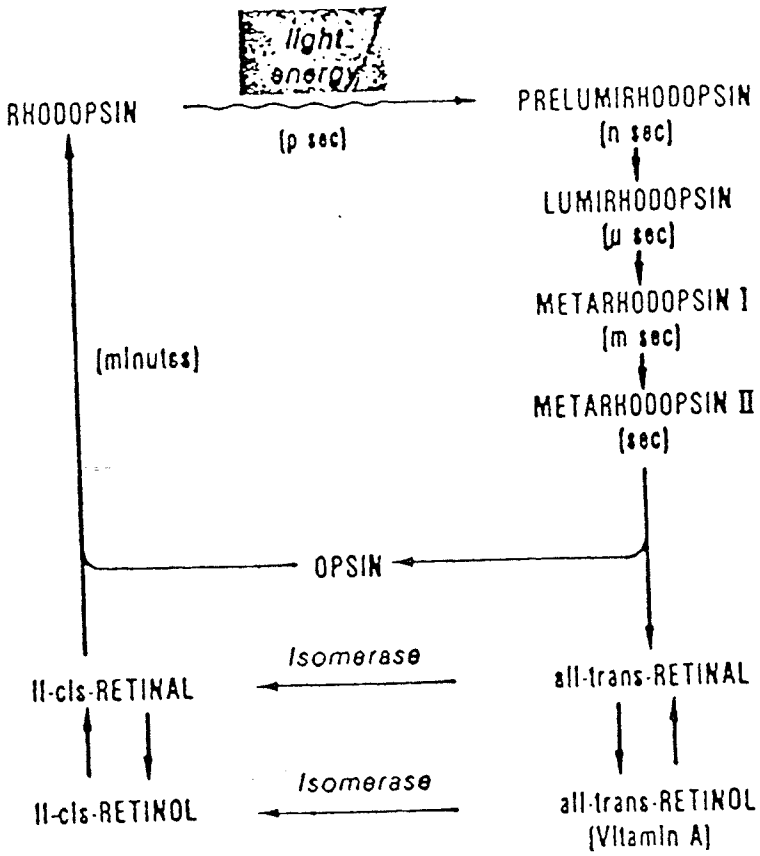


Fig. 2., Photochemistry of the rhodopsin-retinal-vitamin A visual cycle (Guyton, 1986).

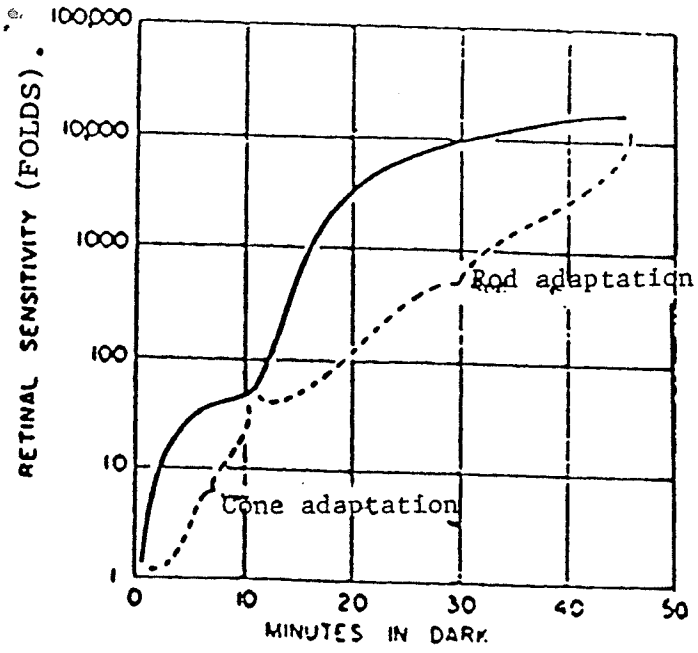


Fig.3., Sensitivity of the retina in dark.

Adopted from (Guyton, 1986).

of sufficient magnitude to serve as criteria to measure the ability of varying amount of retinol or carotene to cure them. The plasma vitamin A level in these subjects fell to less than 15 ug/100 mL (50 IU) at the onset of impaired dark adaptation. Of these three subjects, one was treated with 390 ug (1300 IU) of retinol per day which gradually restored his capacity to adapt to darkness (Hume and Krebs, 1949).

2.3 SERUM RETINOL AND DARK ADAPTATION:

Carney and Russell, (1980), did a study with 67 patients. They reported that a serum vitamin A levels equal to or greater than 40 ug per 100 mL predicted normal dark adaptation (retinal function) 95 % of the time, a serum vitamin A levels equal to or greater than 30 ug per 100 mL predicted normal dark adaptation 68 % of the time and a level equal to or greater than 20 ug per 100 mL predicted normal function 27 % of the time. Therefore, Carney and Russell (1980) concluded that in individual patients with serum vitamin A levels < 40 ug per 100 mL one can be sure of vitamin A sufficiency only if a normal dark adaptation response is elicited. In contrast, no significant correlation between RDAT score and plasma vitamin A level was observed in healthy Guatemalan children (Solomons et al., 1982). Serum retinol levels are reduced only when most of the retinol stored in the liver has been depleted (Bureau of Nutritional Sciences 1975b; Turley and Brewster, 1987; Pitt, 1981).

In their study, Sauberlich et al. (1974) reported impaired dark adaptation in one subject even though his plasma vitamin A was still at a level of 30-35 ug/100 mL. In the other subjects however, significant dark adaptation impairment was observed only after their plasma vitamin A levels had dropped below 30 ug/100 mL.

Incidence of abnormal dark adaptation attributed to vitamin A deficiency have been reported at different serum vitamin A levels. Sauberlich et al., (1974), reported abnormal dark adaptation at serum vitamin A level greater than 20 ug/100 mL, while Carney and Russell, (1980), reported abnormal dark adaptation at serum vitamin A level of less than 40 ug/100 mL but most studies report serum vitamin A levels equal to or less than 10 ug/100 mL to present a high risk of vitamin A deficiency (Joint WHO/USAID Meeting, 1976; Bureau of Nutritional Sciences 1975b; Sauberlich et al., 1974). However a low vitamin A level above that known to be almost invariably associated with deficiency (10 ug/100 mL) can not alone predict deficiency in an individual (Pilch, 1987). Sauberlich et al., (1974), reported onset of abnormal dark adaptation which was reversible on vitamin A treatment, in one patient whose serum vitamin A level ranged from 30 to 35 ug per 100 mL. Carney and Russell, (1980), pointed out that in individual patients with serum vitamin A level <40 ug per 100 mL one can be sure of vitamin A sufficiency only if a normal dark adaptation response is elicited. In their study,

among patients who had serum vitamin A levels between 30-39 ug per 100 mL, 6 of 18 had abnormal dark adaptation tests. Upon vitamin A therapy dark adaptation was normalized in all six within 1-4 weeks.

It is uncertain whether vitamin A levels greater than 40 ug per 100 mL can be safely interpreted as predicting normal vitamin A-dependent retinal function in otherwise normal individuals (i.e apparently health) to maintain normal rod and cone functioning or if it is higher than that which would be required. This uncertainty of accurate vitamin A requirement emphasises the unreliability of plasma retinol values as a measure of vitamin A adequency. Subsequently, since the High Performance Liquid Chromatography (HPLC), is recommended for retinol measurements (Kaplan et al., 1987). Kaplan et al., (1987) used the HPLC to develop reference ranges for plasma retinol by age and sex (see tables 1 and 2). In addition recent works of (Turley and Brewster, 1987) suggest plasma retinol concentration vary with age and sex of individuals with children having levels of 25 to 45 ug/100 mL and adults levels of 32 to 90 ug/100 mL. A strong correlation between age and gender was found using HPLC to assay serum retinol. Retinol levels were significantly higher in men than in women (Kaplan, 1987). Low plasma vitamin A concentration may not necessarily indicate depressed vitamin A stores. Vitamin A circulates in plasma complexed to retinol-binding protein and prealbumin. A deficiency in these proteins caused by

Table # 1.

Reference means for plasma retinol by age and sex:

Age-group (years)	Male (ug/L)	Female (ug/L	probability of gender difference.
18-24	520(230-760)	480(310-780)	<0.02
25-34	600(310-830)	530(320-840)	<0.007
35-44	660(430-990)	490(300-1000)	<0.003
45+	710(450-1030)	610(360-980)	<0.03
All	630(380-930)	530(320-800)	<0.001

*5th-95th percentiles in parentheses adapted from (Kaplan et al.,(1987).

**p statistics comparing log-transformed means.

Table # 2.

Plasma retinol percentiles reported for adults by age and sex (ug/L):

	Percentile points for plasma nutrient levels				
	10	25	50	75	90
Sexes combined	360	440	550	670	780
Males	430	510	620	720	810
Females	340	410	500	620	740

(Kaplan et al., 1987).

hypoproteinemia results in a decrease in plasma retinol even though hepatic stores of vitamin A remain adequate (Turley and Brewster, 1987). In zinc deficiency, retinol-binding protein synthesis is decreased and a similar situation occurs which results in decreased plasma retinol even though hepatic stores of vitamin A remain adequate. Supplying protein and zinc in each respective case, restores circulating retinol-binding protein and in turn vitamin A plasma levels return to normal (Turley and Brewster, 1987). A recent review points out the limited usefulness of serum vitamin A as an indicator of total vitamin A stores (Nutritional Review, 1987).

Favaro et al., (1986), reported the relationship between plasma vitamin A levels and the dark adaptation test response in 31 preschool children. The results of a rapid dark adaptation test were compared with the estimates of dietary intake of vitamin A (in 29 children) and with plasma levels of retinol (in 31 children) (Favaro et al., 1986). The coefficient of correlation (r) for the plasma vitamin A against RDAT was zero and was expressed as $y=18.670-0.002x$. However, intake of dietary vitamin A showed a positive and significant correlation ($p<0.001$) with plasma vitamin A levels.

2.4 ZINC AND DARK ADAPTATION:

When retinal tissue is deprived of vitamin A, both rod and cone function is impaired. The first clinical sign of vitamin A deficiency is night blindness, impaired dark adaptation. It has been proposed that low levels of vitamin A can be caused by impaired mobilization of the vitamin from the liver as a result of zinc deficiency (Solomons and Russell, 1980). Altermately the improvement in dark adaptation by intake of zinc supplements may be due to enhanced activity of previously depressed retinol dehydrogenase (Morrison et al., 1978).

Retinaldehyde must be supplied constantly to the rods for the formation of visual pigment (rhodopsin) and the prevention of night blindness. Illumination of the retina bleaches rhodopsin causing a release in retinaldehyde which is then reduced to retinol. To resynthesize rhodopsin retinol must be reoxidized to retinaldehyde by alcohol dehydrogenase which is a metalloenzyme of zinc (Morrison et al., 1978; Russell et al., 1978).

Solomon and Russell, (1980), reported a study which demonstrated that a single intraperitoneal injection of 10 mg of zinc sulfate (2.4 mg of zinc) produced an abrupt rise in plasma retinol and a reciprocal decline in hepatic retinol, on monitoring liver and plasma retinol levels during a 6-hr, post injection period, compared to saline-injected rats. This study

supported the hypothesis that the metabolic defect produced by zinc deficiency was a failure to mobilize hepatic vitamin A. The conclusion was that, mobilization of retinol from the liver stores to circulation would be achieved by the administration of zinc-sufficient diets to animals depleted of zinc (Solomon and Russell, 1980).

Russell et al., (1978), reported a case of two subjects who's dark adaptation threshold failed to respond to supplementation of vitamin A. These subjects had serum zinc levels below 65 ug/L (i.e < 10 umol/L). They responded to $ZnSO_4$ supplements of 220 mg/day when it was added to the regimen of daily vitamin A supplements as reflected by a drop in dark adaptation threshold. Dark adaptation normalized in both subjects within one week (fig. 3). The other two subjects who had elevated dark adaptation thresholds, failed to show any change in final dark adaptation until they were supplemented with 10,000 ug/dL daily of vitamin A oral supplement for 1 to 2 months. However this did produce increased plasma vitamin A levels of 26 and 56 ug/dL respectively, but final threshold remained elevated (Russell et al., 1978; Morrison et al., 1978; Solomons and Russell, 1980).

Morrison et al., (1978), reported on 6 patients with abnormal dark adaptation who responded to zinc replacement by a decrease in final dark adapted thresholds to normal (see table 3). Two of the six patients had been treated with vitamin A (10,000 IU/day)

for 2 to 4 weeks without satisfactory response. However, after addition of oral zinc sulfate (220 mg/day) for 1 to 2 weeks their final dark adapted thresholds returned to normal. Three other patients who were treated with zinc sulfate prior to vitamin A therapy also responded with normal dark adaptation tests (see table 3).

Smith et al, (1974) have demonstrated that weanling rats on zinc deficient diets had plasma retinol binding protein levels which were 25% of normal and hepatic levels which were 50% of normal. The low retinol binding protein concentrations in the liver of zinc-deficient rats is in contradiction to the elevated levels of hepatic retinol binding protein in vitamin A deficient animals. These observations suggest that zinc deficiency results in depressed hepatic synthesis of retinol binding protein, while vitamin A deficiency results in decreased secretion of the transport protein from the liver.

Table # 3.

Dark adapted thresholds (Unit:log lux) before and after supplementation with retinol and zinc.

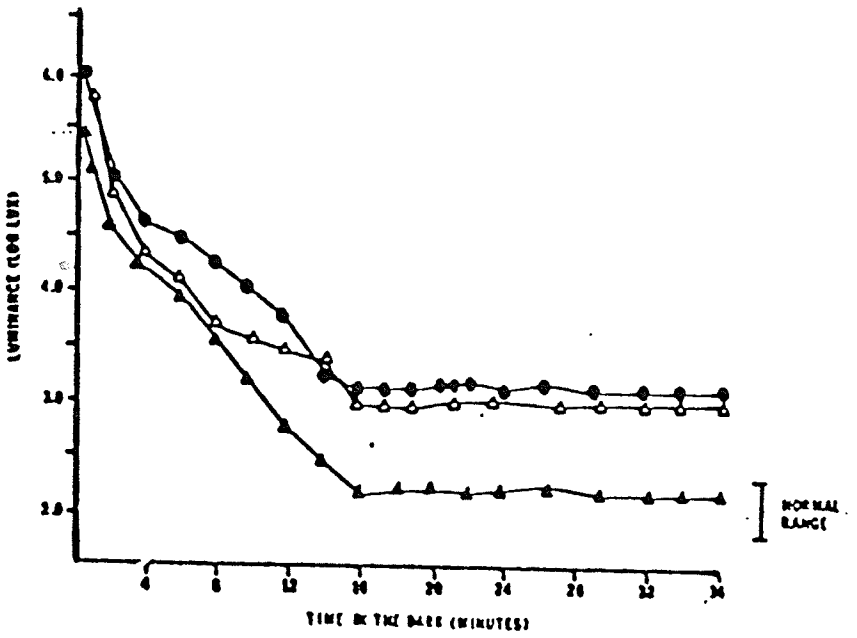
	THRESHOLDS	TREATMENT		
Patient	Initial before treatment	Vitamin A 10,000 IU (2-4 wk)	zinc sulfate 220 mg/day (1-2 wk)	Vitamin A and Zinc (2 wk)
1	3.2	2.6	2.1	
2	3.0	3.2	2.2	
3	3.1		2.2	
4	2.5		2.1	
5	4.3		3.1	
6	2.8			2.3

Normal dark adapted threshold for 6 age matched controls:2.1 $\pm$ 0.2 log lux (Morrison et al., 1978).

The dark adaptation curve of one of the subjects who's dark adaptation threshold normalized after zinc supplementation is illustrated in figure 4.

FIG 4. dark adaptation curve of one of the subjects who normalize their dark adaptation after zinc supplementation

from MORRISON ET AL., 1978.



weeks of oral vitamin A (10,000 IU/day); ▲——▲, after addition of oral zinc sulfate (220 mg/day). The bar represents the mean ± SD for 21 control subjects (Reproduced with permission from Reference 40.)

Fig.4., Dark adaptation curve of one of the subjects who normalized their dark adaptation curve after zinc sulfate supplementation. Adopted from (Morrison et al, 1978).

2.5 ZINC AND COPPER STATUS IN PREGNANCY:

Smith et al., (1988) undertook a study to look at the effect of pregnancy on zinc metabolism. They had 18 non-pregnant women, 16 early-pregnant women (13-17 weeks) and 16 late-pregnant women (28-34 weeks) in their study. Blood was collected at 0, 30, 60, 120, and 180 minutes after ingesting 100 g glucose to evaluate changes in variables of zinc nutriture. They observed that fasting plasma zinc concentrations decreased significantly as pregnancy progressed. The late-pregnant women had significantly higher erythrocyte zinc levels and greater 24-hour urinary zinc and glucose excretion in response to glucose challenge. Plasma zinc after glucose load in nonpregnant women exhibited a curvilinear response whereas no change was observed for the pregnant women. This lack of response by pregnant women may be related to their plasma zinc concentrations. Plasma zinc concentrations were found to decline with pregnancy. Smith et al., (1988) reported that late-pregnant women excreted 2.4 and 1.5 times more zinc than early-pregnant and non-pregnant women respectively.

Elevated serum copper and low levels of serum zinc have been documented as effects of oral contraceptives although the latter was not found as consistently as elevated copper concentration. Plasma copper level was observed to be significantly higher ($P<0.01$) for oral contraceptive users than for non-user of oral contraceptive (Crews et al., 1980).

2.6 SUMMARY:

It has been shown that vitamin A deficiency leads to night blindness, a decrease in retinol sensitivity to light under dim lighting conditions. However, serum retinol values have been found to be quite unreliable as an indicator of vitamin A status due to the wide range of values in which night blindness may or may not occur. It is therefore, more reliable to use serum retinol values coupled with other physiological indicators in assessing vitamin A status.

Retinal sensitivity can be assessed by use of the classical dark adaptation and rapid dark adaptation test measurements. However, due to the inconvenience and the expensive nature of the equipment required for classical dark adaptation, rapid dark adaptation has been proposed as a useful method for detecting vitamin A deficiency in the field. On the other hand, several factors, other than vitamin A deficiency have been shown to cause night blindness. Zinc deficiency results in the immobilization of vitamin A from liver stores, thus resulting in night blindness, in spite of the adequate liver stores of vitamin A. Since other factors appear to affect the zinc status, such as pregnancy, use of oral contraceptives, cirrhosis etc, it is important to investigate some of these other factors in conjunction with vitamin A status.

2.7 NUTRITIONAL STATUS OF THE INUIT POPULATION:

Following the Nutrition Canada Survey of 1970-1972, concern was expressed regarding the Vitamin A status of native Canadians in isolated communities. Firstly, diets reported by Inuit people during the survey were calculated to contain considerably fewer vitamin A active compounds than diets of either the national survey sample or the Indian survey sample. Though seasonal variation in intake could be expected, a low vitamin A status of Inuit people was assumed (fig. 5) (Bureau of Nutritional Sciences, 1975a and 1975b). It was noted that the interpretation of the data from 24-hour recall was especially difficult for vitamin A. The values, as expected varied over a wide range and the mean for each intake was 1.3 to 1.8 times the median (Bureau of Nutritional Sciences 1975b).

The survey also described lower serum retinol values for the Inuit than were present in the National or Indian samples. The median values were lower for all age groups, even though none of the values were found to be in the high risk category established for the survey, set at 10 ug/100 mL (see table 4).

No clinical cases of vitamin A deficiency were observed and the data did not confirm deficiency status for the Inuit population. However, the low Vitamin A intakes and serum retinol values below the national average suggested a risk of inadequate vitamin A status. Hence the Inuit population of Arctic Bay was

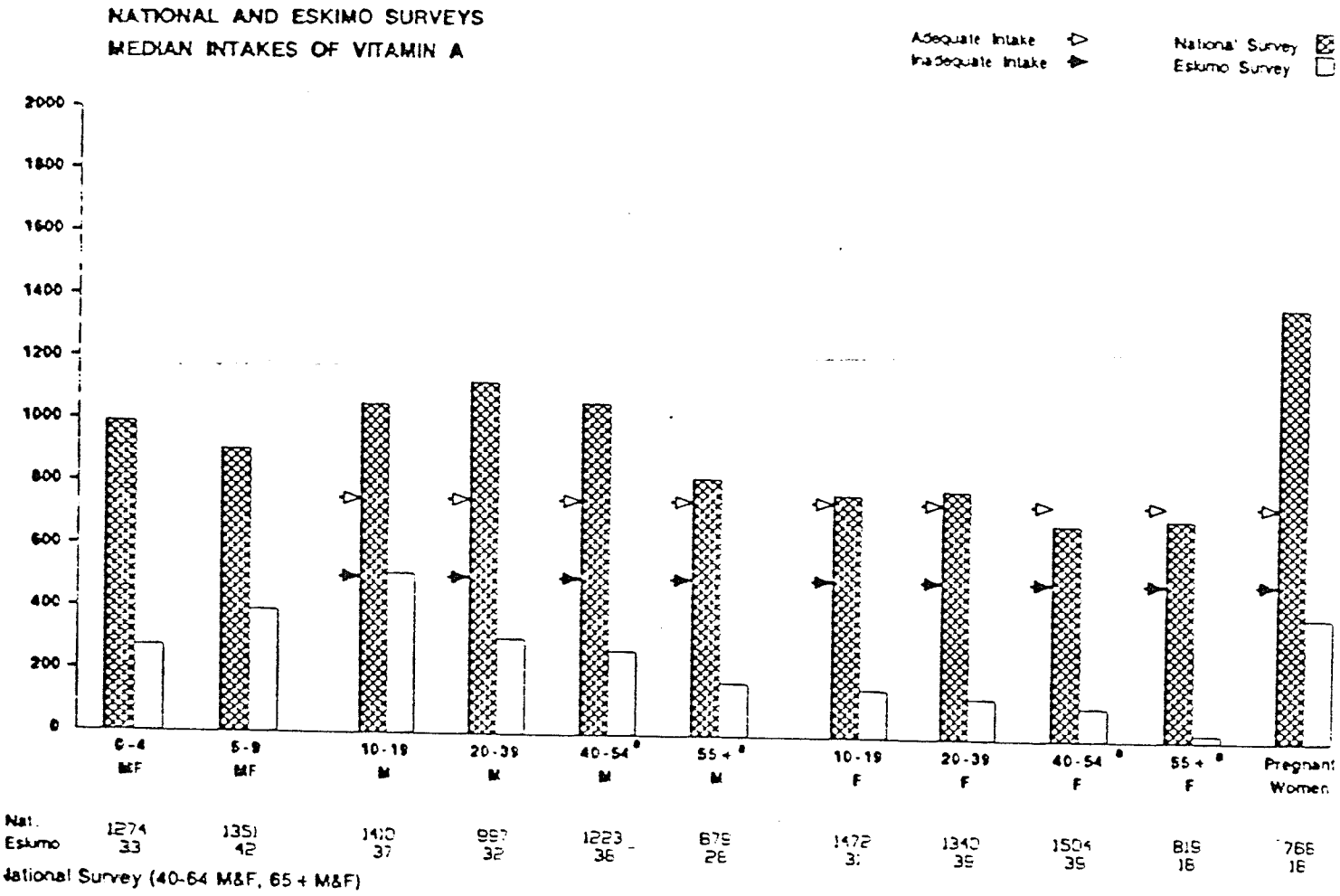


Fig. 5., National and Eskimo surveys median intakes of vitamin A. (Bureau of Nutritional Sciences, 1975a).

Table # 4.

Percentage of Subjects at "High Risk" by the Nutrition Canada Survey Criteria

	Nutrition Canada National Eskimo		Arctic Bay and Nanisivik				
			Inuit			Non-Inuit	
			1976	1978	1980	1978	1980
:r of specimens*	13,156	355	300	91	287	13	52
ıoglobin	0.7	1.9	0.7	6	0.7	0	0
HC	2.3	3.1	2	8.2	0.4	0	0
ransferrin Saturation	7	12	12	8	12	8	0
m Folate (<2.5)	13	58	9	-	10	-	0
m Folate (<4.0)†	-	-	-	-	33	-	-
min C	6	46	6	-	-	-	-
min A	0.02	0	0	0	1.3	0	0
lesterol	19	8	5	6	14	15	25
ıflavin	0.2	0	0.7	1.3	-	0	-
ıc	0.2	6.1	1.2	-	-	-	-

ıber of specimens — not all specimens were of adequate size for all tests, nor were specimens obtained from all subjects (e.g. infants and some l children).
ı Risk by the Montreal hospital laboratory criteria.

(Verdier et al., 1987).

Table # 5.

Percentage of Values Below the Nutrition Canada Survey National Sample 5th Percentile

	Nutrition Canada Eskimo Report	Arctic Bay and Nanisivik				
		Inuit			Non-Inuit	
est	1972	1976	1978	1980	1978	1980
er of specimens*	355	300	91	287	13	52
:						
moglobin	17	13	26	11	15	0
matocrit	16	9	12	18	8	0
CHC	16	5	14	0.4	0	0
n	6	10	4	7	8	0
Transferrin Saturation	8	7	3	6	8	0
ıum Folate	42	3	-	4	-	0
amin C	33	5	-	-	-	-
amin A	22	30	33	29	0	12
:						
boflavin	1	4	9	-	0	-
dine	27	13	9	-	0	-
	Percentage of Values Above the Nutrition Canada Survey National Sample 95th Percentile†					
d:						
olesterol	5	3	4	8	0	18

mber of specimens — not all specimens were of adequate size for all tests nor were specimens obtained from all subjects (e.g. infants and some small dren).

(Verdier et al.,I987).

investigated over the Period between 1976 and 1981 (Verdier et al., 1987; Verdier and Eaton, 1987). Four separate surveys were carried out. The study concluded that the Inuit subjects had blood levels indicative of fewer deficiencies than Nutrition Canada Survey, with the exception of Vitamin A (Table 5 and 6). The data was compared with that reported in the Nutrition Canada Eskimo Report by showing the percentage of persons at risk of deficiency and percentage of persons having values below the fifth percentile and above 95th percentile of the national distribution. "High risk" was defined as the level at which there is "a high probability that a nutritional problem may exist". "Moderate risk" as "an average probability that a nutritional problem is present or developing". Because of the small number involved, the total population was used in the initial comparison (see table 6) (Verdier et al., 1987).

In all phases a higher percentage of subjects had values below the national 5th percentile and lower than the values seen in the Inuit during the Nutrition Canada Eskimo Survey. In the third phase, 3 subjects had values at "high risk" (< 10 ug/100 mL) (Verdier et al., 1987). The number of Inuit with low levels of serum vitamin A increased during the three phases of the study over the number observed in the Nutrition Canada survey(see table 5). The low serum vitamin A found in this study were similar to those reported in the Alaska Survey report (1956) which stated "Food sources of Vitamin A are rich and plentiful and yet plasma levels are often low". The only trend seen was an increasing

Table 2 -->Nutrition canada interpretive standards;---> table #6

number of subjects with extremely low serum vitamin A levels. Verdier et al. (1987) Conclusion was based on the observation that the distribution of serum retinol values was considerably lower than that of the national sample for Canadians investigated during the Nutrition Canada survey. About 30% of the sample was found to record serum retinol values below the Nutrition Canada national sample 5th percentile. Yet, only 1.3% of the Inuit subjects were found to have serum retinol values below 10 ug/100 mL, most of whom were children (Verdier et al., 1987).

In the dietary survey accompanying their study Verdier and Eaton (1987) found low intakes of milk and vegetables which are important sources of vitamin A for Southern Canadians. A similar pattern was therefore found in both the Nutrition Canada Survey and the Arctic Bay study, indicating few or no deficient individuals in spite of low apparent intakes of vitamin A containing foods.

2.8 **CONCLUSION:**

The health effects of lower than average vitamin A status found among Inuit population groups are unclear. Low vitamin A status has been associated with increased risk of mortality among children, as well as clinical symptoms related to epidermal function and lowered immune response (Sommer et al., 1980; 1983; 1984; 1987; Sommer, 1984). The low vitamin A status observed among Inuit populations may therefore affect the prognosis of medical care. In addition no results of serum retinol measurements are available for Inuit patients.

Chapter III

3.0 THE STUDY PROTOCOL:

3.1 THE PURPOSE OF THE STUDY:

The purpose of the research was to test the performance of a recently proposed method to measure dark adaptation for use outside the laboratory with a group of people who might be expected to have vitamin A deficiency. Reliability of the method depends on standardization of the test procedure, especially under the survey conditions. The outcome of the project could lead to a procedure for screening people for vitamin A status, without the need for blood sample analyses. The procedure requires less than 20 minutes and is interactive. It could find practical application in the clinical setting, in research and in field surveys (Solomons et al, 1982).

The study describes the vitamin A status of a population group for whom previous studies are not conclusive but indicated the possibility of vitamin A deficiency (Bureau of Nutritional Sciences, 1975a and 1975b). A dark adaptation procedure was used as part of testing for vitamin A status, with the expectation that this procedure can be used in the future as a screening tool for vitamin A status, preliminary to the more cumbersome blood analyses. In addition, zinc and copper status were described

allowing better interpretation of the plasma retinol results. The haemoglobin levels and the haematocrit count levels provided a better understanding of the subjects, nutritional status in general, since vitamin A status may influence the patients' response to a variety of infectious diseases (Sommer, 1984; Sommer, 1987). The dietary history questionnaire provided data for calculation of retinol equivalence, retinol concentration and total energy among other nutrients. This provided knowledge of the subjects dietary intakes of vitamin A and other nutrients.

3.2 **RESEARCH DESIGN:**

Inuit patients from Northern Communities of Manitoba and Northwest territories (Appendix I) who were attending medical care facilities in Winnipeg provided data to test the hypotheses. The patients who had just arrived and who had not spent more than 3-4 days in Winnipeg were considered to avoid the influence of vitamin A fortified foods provided at the Inuit boarding home in the study. A total of 50-100 subjects were expected to participate.

Plasma retinol levels, serum zinc levels, serum copper levels, haemoglobin levels, haematocrit count levels, rapid dark adaptation test times and dietary intakes of vitamin A were estimated for all subjects. Statistical analysis and any other interpretation of the data took expected values for these indicators into account in relation to gender, age, health status and pregnancy status.

3.3 HYPOTHESES:

1. Rapid dark adaptation measurement times are inversely related to plasma retinol values.
   - Dependent Variable -Rapid dark adaptation measurement times.
   - Independent Variable - Plasma retinol values.

2. Plasma retinol values are related to estimates of dietary Vitamin A obtained by diet history.
   - Dependent Variable - Plasma retinol values.
   - Independent Variable - Estimates of dietary Vitamin A.

3. Dark adaptation times are related to estimates of dietary vitamin A obtained by diet history.
   - Dependent Variable - Dark adaptation times.
   - Independent Variable - Estimates of dietary vitamin A.

4. Rapid dark adaptation test values show inverse relationship to serum zinc values.
   - Dependent Variable - Rapid dark adaptation test values.
   - Independent Variable - Serum zinc values.

Chapter IV

4.0 DATA COLLECTION.

4.1 SUBJECT CONTACT AND SELECTION:

All subjects were patients from Inuit Communities in Northern Manitoba and Northwest Territories (Appendix I) travelling to Winnipeg for medical care. The Northern Medical Unit keeps record of all the patients who come from the Northern Communities to Winnipeg for medical care. Some of these patients stay for 1-3 days, others stay for 1-3 weeks or longer, depending on their medical problems. Most pregnant women who come to Winnipeg for delivery stay in the city for at least three weeks or as long as several months, depending on gestation and due date. When these patients arrive in Winnipeg, they reside at the boarding house (5 East Gate), with friends or in hotels. The Northern Medical Unit has contact with each patient during the time they attend outpatient clinics or the time they are admitted to a hospital ward. The Northern Medical Unit is notified when they are discharged from the ward, or transferred to another hospital.

The investigator received the names of patients, a day before they arrived in Winnipeg. The investigator went to the boarding house every day of the study in the evening to meet with those

subjects who had arrived during the day to explain the study and ask for their participation in the research. An interpreter accompanied the investigator when necessary. Those who agreed to take part were asked to sign a consent form in the presence of a witness, who was either, a fellow patient from the North, a friend or the interpreter. For the subjects who signed the consent form, a meeting was arranged at the Health Sciences Centre, Department of Community Medicine, Health Sciences. The meeting took place within 72 hours (3 days) of the subject arriving in Winnipeg. Transportation was provided for the patient where necessary. Data was collected at this single contact with the subject.

4.2 PROCEDURES:

The study was carried out under the supervision of Department of Foods and Nutrition and the Department of Community Medicine. The study and methods were presented to the Keewatin Regional Health Board for approval by one of the doctors in Community Medicine. Prior approval for the Study was also obtained from the Faculty of Human Ecology, University of Manitoba in accordance with its procedures for research with human subjects.

Those subjects who agreed to participate in the research were requested to answer a diet history questionnaire which took about

1 hour. Next the Rapid Dark Adaptation Test was carried out which took about 20 minutes. Both of these procedures were carried out in one room at the Department of Community Medicine, Health Sciences. Following the diet interview and RDAT, the subjects were taken to the Haematology Laboratories of the Health Science Centre where a 20 mL blood sample was taken. The blood sample was drawn by a nurse or a technologist employed by the Haematology Laboratories. It was collected in two vacutainer tubes, a heparinized tube for plasma retinol analysis and a metal-free tube for the serum zinc and serum copper analyses. The subjects were returned to their place of residence whenever it was necessary.

The blood sample were refrigerated until later in the day when the investigator removed the plasma and the serum at the University of Manitoba Department of Foods and Nutrition laboratory. The plasma was then frozen at -20^{o}C until all subjects' data had been collected. The serum was stored at 0-4^{o}C for a period of less than two weeks then it was transported to St. Boniface Hospital Haematology Laboratories for zinc and copper analyses.

4.3 **ANALYSES:**

The plasma was analyzed for plasma retinol levels at the University of Manitoba Foods and Nutrition laboratory using

High-Performance Liquid Chromatography (HPLC) (Driskell et al 1982; Beiri et al 1979). Serum zinc and serum copper were determined at St. Boniface Hospital using Atomic Absorption Spectrophotometry (AAS) techniques (Kaplan and Pesce 1984; Teit 1986). The latter was supervised by Dr. D. Parry of St. Boniface Hospital Haematology Laboratories.

The retinol equivalents, total energy and retinol concentration of the individual diet histories were calculated together with other nutrients at the University of Manitoba using the Canadian Nutrient File (Health and Welfare, 1986) and University of Manitoba computer NAP programme. The dark adaptation times were used as recorded and an arithmetic mean of the best two attempts among three trials was calculated for each subject.

Chapter V

5.0 MATERIALS AND METHODS:

5.1 RAPID DARK ADAPTATION TEST:.

5.1.1 Equipment.

1. A standard dark light fixture on 4 ft stand.
2. One 7 1/2 Watt, bulb (120 volts).
3. A neutral density film (flashed density on film allowing 1% transmittance): Exposed x-ray film cut to aperture.
4. 18 plastic discs (poker chips) with reflective finish. Five were white, six were blue and seven were red (Reference disc from Munsell colour laboratories). Each disc had a diameter of 3.8 cm.
5. A dark non-reflective work surface: sheet of black felt to cover the table surface.
6. Two stop watches.
7. An absolutely dark room without light leaks for conducting the test, achieved by covering doors and windows with plastic and/or felt using tape.

Luminosity of the test room was reduced to 0.002 foot lambert, or 0.00068 cd/m^2, target brightness. This level of brightness was achieved by suspending the light and taking

readings directly under the fixture using an opticon model 550 Radiometer/Photometer by EG & G Eletro-optics Division, Ontario. The Rapid Dark Adaptation test was performed on a fully light adapted eye. The subjects were in a dark room that was brightly lit before beginning dark adaptation test.

The procedure was explained to individual subjects before the beginning of the dark adaptation test. During the explanation, the subject were shown a pile of colored discs which had been mixed together and requested to start separating them according to colour (white disc first followed by blue) once the lights were dimmed. He or she would then proceed to separate out the white discs and then signal as soon as they were separated. Subsequently the subject signaled to stop the test when the red and blue discs were separated. Throughout the period of the test, the investigator replaced any discs that were wrongly categorized to the unseparated pile in the middle of the working surface until all discs were correctly separated. The final time indicated the amount of time required to complete the test with 100% accuracy. All subjects were asked to repeat the light adaptation and the rapid dark adaptation test three consecutive times. This took about 20 minutes.

One stop watch timing was stopped when the subject had separated the white discs and signalled stop. The other stop watch timing was stopped at the end of the separation of the blue

and red discs. The times showed the time taken to do the separation for respective colors (white and blue) hence total time taken to complete the separation.

It was explained to the subject that under reduced lighting conditions he or she would not be able to distinguish colour and would only be able to separate the discs according to relative brightness. Individual subjects were then light adapted for one minute by fixating on a white hard paper surface which was placed on the work table. Stop watch timing was started at the same time as the light was dimmed down to 0.002 foot lambert or 0.0068 cd/m^2 the subject then proceeded to separate the discs.

5.2 DIET HISTORY INTERVIEW:

The diet history questionnaire enquired about the long term dietary intake of Vitamin A sources. The interview allowed open ended answers and food models were used to standardize responses on food amounts. Responses were intended to reflect the subjects eating pattern over the last month. Changes in eating pattern between seasons were included in the questioning. The diet history interview took about 1 hour with the help of an interpreter.

The retinol equivalence, total energy and retinol concentration were calculated among other nutrients from the diet

history responses using the Canadian Nutrient File (Health and Welfare, 1986) and the NAP programme of University of Manitoba.

5.3 DETERMINATION OF VITAMIN A IN PLASMA:

5.3.1 Procedure:

The procedure for determination of vitamin A using High-Performance Liquid Chromatography (HPLC) that was used in this was study similar to that of Driskell et al., (1982) except that tocopherol acetate was used as the internal standard instead of retinol acetate and methanol was used instead of ethanol to dissolve the sample prior to injection. Mobile phase composition was adjusted to optimize peak separation.

5.3.2 Extraction Method:

- Using a glass syringe type pipetter, 100 uL of internal standard was transferred into a 10 by 75 mm glass disposable tube.
- 100 uL of sample (plasma) was added and mixed on a vortex for 20 seconds (pipet tip was primed with sample by filling and emptying once before actually pipeting).
- 200 uL hexane was added and mixed on a vortex for 45 seconds.
- The mixture was then centrifuged for 3 minutes at approximately 2000 RPM (IEC model CS 3/4 speed).

- Using a 100 uL glass pipetter, about 150 uL of the hexane layer was transferred to a 1.5 mL microcentrifuge tube (pipetter was rinsed twice with hexane between samples).
- The hexane layer was then evaporated under nitrogen.
- Using a glass syringe pipetter the sides of the tubes were washed with 100 uL of methanol.
- The tubes were capped, mixed on vortex for 30 seconds and put in the refrigerator.

5.3.3 **Analysis Method:**

50 uL of the prepared sample were injected onto the HPCL column. The operating parameters were as follows:

COLUMN:	Ultrasphere OD, S 5.0 um, 250 mm x 4.6 mm
PRECOLUMN:	Alltech Pellicular C18
SOLVENT:	Methanol:Water 98:2
FLOW RATE:	2 mL/minute
DETECTOR:	UV at 280 nm. Range 0.01
INTEGRATOR:	Hewlett Packard 3390 A.

Integrator was set as follows:

ZERO	= 0 -0.8	0.00	INTG # = 9
ATT 2↑	= 3	2.00	INTG # = -9
CHT SP	= 0.2	5.00	PK WD = 0.16
TRSH	= 4	11.50	STOP
AR REJ	= 0		

5.3.4 Reagents:

Methanol:Water 98:2- HPLC grade methanol was mixed with HPLC grade water and filtered it through a 0.45 um Type FH millipore filter under a water aspirator vacuum. Degassed it under vacuum for 10 minutes while stirring.

Hexane - reagent grade or HPCL filtered as above.

Ethanol - anhydrous, filtered as above.

Methanol - HPCL grade filtered as above.

5.3.5 Standards:

Stock standards

1. Retinol 500 ug/mL- weighed 50 mg of retinol(Kodak # 5159) into a small beaker, dissolved in ethanol, transferred to 100 mL volumetric and brought to volume with ethanol.

   Tocopherol acetate - 500 ug/mL - weighed 50 mg of tocopherol acetate (Sigma T-3376) into a small beaker, dissolved in ethanol , transferred to a 100 mL volumetric and brought it to volume with ethanol.

2. Standards for determination of purity

   Retinol - 1 ug/mL - diluted 0.050 mL (using glass syringe pipetter) of 500 ug/mL retinol to 25 mL with ethanol.

Tocopherol acetate - 50 ug/mL - diluted 1.0 mL of 500 ug/mL tocopherol acetate to 10 mL with ethanol.

The retinol and tocopherol acetate standards were measured in a spectophotometer at 325 and 285 nm respectively to determine their purity and values in the standards curves were corrected accordingly

3. Standards for contamination checked by HPCL. Standards were prepared as indicated below and run on HPCL to determine retention time for each standard and to check standards for contamination.
   Retinol - 5 ug/mL - diluted 0.1 mL of 500 ug/mL retinol to 10 mL with methanol.

   Tocopherol acetate - 100 ug/mL - diluted 1.0 mL of 500 ug/mL tocopherol acetate to 5 mL with methanol.

4. Internal standard
   Tocopherol acetate 20 ug/mL dilute 1 mL of 500 ug/mL tocopherol acetate to 25 mL with ethanol.

5. Recovery checks standards
   Combined retinol and tocopherol - 20 ug/mL retinol and 1 ug/mL tocopherol-diluted 0.050 mL of 500 ug/mL retinol and 1.0 of 500 ug/mL tocopherol to 25 mL with ethanol.

Tocopherol acetate - 40 ug/mL -diluted 2.0 mL of 500 ug/mL tocopherol acetate to 25 mL with ethanol.

To determine recoveries, 50 uL of 40 ug/mL tocopherol acetate solution and 50 uL of combined retinol and tocopherol standards were added to a set of plasma samples instead of the 100 uL of internal standard added in step one of the extraction procedure. Another set of samples had 50 uL of 40 ug/mL tocopherol acetate and 50 uL of ethanol added. Recover was then calculated from the difference between the two set of results.

5.3.6 **Standard curve:**

Standards for calibration curve were prepared by combining different quantities of retinol with a finited amount of tocopherol acetate. After running the standards on the HPCL, peak area ratio for R/TA were determined and plotted against weight ratios which had been corrected for the purity of the standards.

5.3.7 **Calculations:**

Tocopherol acetate was added to the sample so that the final concentration was 0.5 ug/50 uL. Once the sample had been run the area of the retinol peak was divided by the area of the tocopherol acetate peak. The value thus obtained was applied directly to the standard curve or the previously calculated slope and intercept values to obtain a weight ratio of retinol/ tocopherol acetate. Multiplying the area ratio by 2000 resulted

in ug Retinol/dL plasma. It was not necessary to run standard every day once the calibration curve was established. However a standard was run every day before the samples were run.

Formula for calculation:

This is the slope and intercept.

$$\frac{\text{weight ratio} + 0.002}{13.80} \times 2000 = \text{ug/dL} \quad \text{---> R+0.002/6.9 -> ug R/dL}$$

5.4 SERUM ZINC AND SERUM COPPER ESTIMATIONS:

A venous blood sample was drawn by a technologist or a nurse using metal free vacutainer tubes and refrigerated until later in the day when the serum was extracted by the investigator and stored at 0-4^{o}C for periods of less than two weeks. The serum was then transported to St. Boniface Hospital Haematology Laboratories where it was analyzed for serum zinc and serum copper using Atomic Absorption Spectrophotometry under the supervision of Dr. D. Parry of St. Boniface Hospital.

5.5 HAEMOGLOBIN AND HAEMATOCRIT COUNT ESTIMATIONS:

A venous blood sample (whole blood) was used by the investigator to determine the subjects haemoglobin levels and the

haematocrit count levels. The haemoglobin was determined in duplicates using calorimetry. A standard of 12 mg/dL was used to standardize the haemoglobin absorbency. The standard was left to warm up to room temperature for 15-20 minutes and the venous blood was fully mixed and left to stand at room temperature. It was later mixed thoroughly but gently before using it for determination of the haemoglobin level. All samples were done in duplicate and repeated when there was a 10% difference between the duplicates. The cyanomethemoglobin reagent, Drabkin's solution and cyanomethemoglobin standard solution were used as the reagents (Sigma procedure No.525)

For heamatocrit determination whole blood was thoroughly mixed and then drawn into capillary tubes, capped and spun in a micro-centrifuge for 10 minutes. The tubes were placed on a readers grove and the heamatocrit percent was then read and recorded in the data record. Duplicates that differed by 10% or more were repeated.

Chapter VI

6.0 STATISTICAL ANALYSIS:

The Univariate procedure produces simple descriptive statistics (including quantiles) for numeric variables. The Univariate procedure provides great detail on the distribution of a variable. The following is included in the Univariate procedure: detail on the extreme values of variables, quantiles, median, several plots to picture the distribution, frequency tables and test that the data are normally distributed (SAS, 1985b).

The other statistical procedure used was the General Linear models (GLM) procedure which uses the method of least squares to fit general linear models. Among the statistical methods available in GLM are regression and partial correlation. GLM handles classification variables, which have discrete levels, as well as continuous variables, which measures quantities. In this study GLM was used for simple and multiple regressions. In multiple regression, the value of a dependent variable (also called a response variable) are describe or predicted in terms of one or more independent or explanatory variable (SAS, 1985a).

The statistical analyses were carried out in the following steps:

1. Descriptive statistics, including the arithmetic mean, standard deviation, standard error and range, were determined for the data collected for all the subjects.
2. Pearson's correlation coefficients was determined for each hypothesis to determine degree of association between the dependent and independent variables.
3. Linear statistics were calculated using small ranges where possible or when it was necessary.
4. For each hypothesis, the independent variable was used in a regression equation.
5. Multiple regression analysis was carried out to determine the influence of zinc status on the relationship between plasma retinol and dark adaptation test times.
6. Dietary information was categorized into high, medium and low nutrient intake, and used in analysis of covariance to determine the association of dark adaptation times with plasma retinol values, haemoglobin levels or haematocrit.

Chapter VII

7.0 RESULTS:

7.1 THE DESCRIPTION OF THE SAMPLE GROUP:

The experimental sample was made up of Inuit patients who came from Communities in northern Manitoba and Northwest Territories for medical care facilities in the Health Sciences Centre, Winnipeg (Appendix I). The sample consisted of 44 subjects who were informed about the study, and signed the consent form to participate in the study (Appendix A). The consent form was written in English and translated into the Inuit language for those who could not read English (Appendix B). The sample included patients with gastrointestinal problems, with metabolic systemic problems and pregnant women. Mean age was 32.0 $\pm$ 14.0 years, with a median of 29 years, and range 18-73 years.

Data was collected from all 44 subjects, who completed the rapid dark adaptation test, the dietary history questionnaire and provided 20 mL of venous blood in two different tubes, one for retinol analysis and the other for copper and zinc analyses (Record form, Appendix C; data, Appendix D).

Two subjects were dropped during statistical analysis of the

data because their dietary history questionnaires were not considered properly completed due to problems through the interpreter. A medical diagnosis was obtained for every subject (see table 19). One subject was omitted from the calculation of mean vitamin A intake (RE), because she reported a very high intake of foods that contribute vitamin A (RE) (Appendix G). The high intake of liver led to vitamin A intake more than 3.5 standard deviation above the mean, skewing statistical calculation in a way that did not represent the total sample.

7.2 RAPID DARK ADAPTATION TEST:

Three trials of the rapid dark adaptation test were carried out with each subject. The first trial was used as a learning experience and though recorded was not used for analysis. This trial thus provided standardized lighting conditions for a period of ten minutes before subsequent trials were conducted (Sevenhuysen, 1984). Thornton, (1977), judged that a time of more than three minutes to complete the rapid dark adaptation test was considered an abnormal dark adaptation time. In the present study the time taken to complete the test with 100% accuracy determined the individual's dark adaptation ability.

The mean time taken to identify the blue chips during dark adaptation was 213 $\pm$ 44 seconds, 19 of 42 (45%) subjects had time above the sample mean.

The mean time to separate out the white chips was 43 ± 22 seconds, with a very wide range from 19-117 seconds. The shortest trial of rapid dark adaptation test measurement times had a mean of 181 ± 39 seconds. Among the 42 subjects, 22 (52.4%) recorded their shortest trial of the last two trials above the mean time of the total sample's shortest time to dark adapt (see table 7).

7.3 DIETARY INTAKE:

From the responses to the dietary history questionnaire, retinol equivalence, total energy, and other selected nutrients, namely protein, carbohydrate, fat and iron were calculated using the Canadian Nutrient file (Health and Welfare, 1986) (see table 8).

The mean daily intake of selected nutrients, calculated from the dietary history questionnaire, showed the mean intake of vitamin A (RE) was 1074 ± 701 (S.D) RE/day. The mean intake was higher than the median (948 RE), which this means there were some reports of high intakes of rich sources of vitamin A among the subjects. However, both the mean intake and the median intake for vitamin A showed that the vitamin A (RE) intake for the sample population was within range of recommended intakes for adults. These data reflect a population consuming a diet adequate in vitamin A sources although their mean plasma retinol was in the low normal range (46.8 ± 14.3 ug/dl). This trend also was

observed by Verdier et al., (1987). However, in the present study 19% of the subjects had low vitamin A (RE) intake set at 0-500 RE/day.

7.4 PLASMA RETINOL:

Among the 42 subjects, 6 had plasma retinol levels below the normal range(see table 18). Of these 6 subjects, 4 were expectant, 1 had gastrointestinal problems and 1 had minor tests. All four male subjects in the sample had plasma retinol levels within the normal range (see table 1; Appendix D; E).

Among the 37 women, 14 were pregnant according to medical diagnosis. However, only 12 indicated they were pregnant to a question about their pregnancy status (see Appendix C; D). Among these 14 women, 4 had Plasma retinol levels below normal range set at (32.0-80.0 ug/dl), (see table 1; table 18; Appendix E). However they did not show significant functional impairment with any of the variables.

7.5 SERUM ZINC:

It was observed that there was a high incidence of low serum zinc levels among the subjects (see table 9). Twenty-two subjects (52%) had serum zinc values below normal, defined as 10.0-19.0 umol/L. None of the subjects had serum zinc above 19.0 umol/L.

Two pregnant women had low serum zinc values of 6.7 and 6.8 umol/L. However their dark adaptation performances, at 225 and 227 seconds respectively, were slightly higher than the sample mean, but still within the mean found in the literature for vitamin A sufficient individuals (Vinton and Russell, 1981).

One of the five male subjects had a low serum zinc level (8.9 umol/L). All other subjects with low serum zinc were women. According to doctor's diagnosis of the 22 subjects who had low serum zinc levels, 13 were expectant women, 5 had gastrointestinal problems, 2 had metabolic systemic problems and 4 had other minor medical problems. Several subjects were diagnosed with more than one medical problem. Hence there were more subjects diagnosed with low serum zinc levels than those reported (see table 18; Appendix D; E).

Table # 7.

Rapid Dark Adaptation Test Measurements Times (seconds) for 42 subjects:

Variables	Mean	Std.Dev.	Median	Range
Mean RDAT time for blue chips[a]	213	43	214	135-348
Mean RDAT time for white chip[a]	43	22	38	19-117
Shortest time[b]	181	39	182	107-300

[a] Mean test time was the average time of the second and third trials.

[b] Shortest test time was the shorter of the second and third trials.

Table # 8.

Diet History Nutrient Intake Estimate for 42 subjects:

Mean daily intakes of selected nutrients.

Variables	Mean	Std.Dev.	Median	Range
Protein (g)	118	67	103	192-330
Fat (g)	82	50	70	10-201
Carbohydrates (g)	297	156	289	24-623
Energy (KCal)*	2370	1127	2350	480-4810
Vitamin A (ug RE)**	1070	701	948	88-3876
Iron (mg)	24	25	19	5-164

**Rounded to 10 Kcal

*(N=41)

Table # 9.

Blood values of 42 subjects:

Variables	N	Mean	Std.Dev	Median	Range
Plasma Retinol	41*	46.8	14.3	43.5	23.1-79.3
Serum Zinc	42	9.8	2.1	9.6	6.7-13.4
Serum Copper	42	23.7	8.2	21.2	10.9-41.1
HB	42	13.6	2.5	13.9	7.0-18.9
Haematocrit %	42	39.0	6.4	39.0	23.0-57.0

Normal range

	female	males
Plasma retinol	32.0-80.0 ug/dl	38.0-93.0 ug/dl
Serum zinc	10.0-19.o umol/l	10.0-19.0 umol/l
Serum copper	12.0-20.o umol/l	12.0-20.0 umol/l
Haemoglobin level	11.0-16.0 ug/dl	13.0-18.0 ug/dl
Haematocrit count%	37.0-47.0 %	40.0-54.0 %

*One plasma retinol sample lost during storage.

(Henry and Winkelman, 1974).

7.6 HYPOTHESES:

7.6.1 Hypothesis 1.,

Rapid dark adaptation measurement times are inversely related to plasma retinol values.

- Dependent Variable - Rapid dark adaptation measurement times.
- Independent Variable - Plasma retinol values.
- Covariate - Age

Dark adaptation performance is defined in two ways:

(i) Ability to perceive white chips (white objects).

(ii) Ability to perceive blue chips (blue objects).

Table # 10.

Correlation of white chip test time with plasma retinol and age:

Parameters	PR>|T|	Estimate
Plasma retinol	0.2578	-0.59808
Age	0.2753	0.76094
Plasma retinol and Age interaction	0.5059	-0.01032

White chip test time:

There is no relationship between plasma retinol values and dark adaptation test times for the white chips (see table 10). However, the correlation was negative, which could be further examined for influence of other variables such as training, language (an interpreter was used when the subject could not communicate satisfactorily in English) and using a larger sample. Age seemed to influence the relationship, young individuals recorded shorter rapid dark adaptation times for white chips. The strong relationship of white chips times with plasma retinol and age interaction was primarily due to 3 elderly subjects in the sample. Therefore, the hypothesis that rapid dark adaptation white chips test times was inversely related to plasma retinol values was rejected.

Table # 11.

Correlation of blue chip test time with plasma retinol and age:

Parameters	PR>|T|	Estimate
Plasma retinol	0.4389	1.08742
Age	0.0832	3.93875
Plasma retinol and Age interaction	0.2429	-0.04870

Blue chip test times:

Plasma retinol does not appear to influence the rapid dark adaptation test times for blue chip (see table 11), but when dark adaptation test time for the blue chip was assessed against age, plasma retinol appeared to show a negative relationship with age. The purkinje shift appeared to be delayed in respect to age.

Since rapid dark adaptation measurement times and the separation of either white chip or blue chips ($p<0.05$), were not significantly correlated to plasma retinol values, the hypothesis that the observed "estimate" is equal to zero was therefore accepted. This meant that rapid dark adaptation measurement times were not inversely related to plasma retinol levels and the latter did not seem to influence RDAT. Therefore, the hypothesis that rapid dark adaptation blue chips test times was inversely related to plasma retinol values was rejected.

7.6.2 **Hypothesis 2.;**

Plasma retinol values are related to estimates of dietary Vitamin A intake obtained from diet history.

- Dependent Variable - Plasma retinol values.
- Independent Variable - Estimates dietary vitamin A intake.

Table # 12.

Correlation of plasma retinol with dietary intake of vitamin A and energy:

Parameters	PR>|T|	Estimate
Vitamin A intake	0.5208	0.00867
Energy intake	0.6789	0.00245
Vitamin A and Energy intake interaction	0.5359	-0.00003

Plasma retinol values were not significantly correlated to estimates of dietary vitamin A (see table 12). Since plasma retinol values were not deemed significantly correlated to estimates of dietary vitamin A ($p<0.05$), the hypothesis that the observed "estimate" is equal to zero was accepted. This means that, plasma retinol values were not related to estimates of dietary vitamin A. Therefore the hypothesis that plasma retinol values were inversely related to dietary vitamin A obtained from diet history was rejected (see other statistic on appendix F)

Table # 13.

The estimates of Dietary intakes categorized.

The nutrient	Categories	Range	N	(% of total)
Energy(Kcal)	Group1	0-1500	12	28.6
	Group2	1501-2500	13	31.0
	Group3	2501-3500	11	26.2
	Group4	Above 3500	6	14.3
Vitamin A(RE)	Group1	0-500	8	19.0
	Group2	500-1000	15	35.7
	Group3	1000-1500	11	26.2
	Group4	Above 1500	8	19.0

*Mean intakes see table 8.

The dietary data was categorized according to the Recommended Nutrient Intake (Bureau Nutritional Sciences, 1983). The recommended vitamin A intake for the adult women is 800 RE and for the adult men is 1000 RE. The recommended vitamin A intake for the pregnant women is increased with an additional 100 RE/day, therefore, the total recommended intake for the pregnant women is 900 RE/day (Bureau Nutritional Sciences, 1983). The dietary data for the subjects was categorized according to those subjects who fell below the recommended nutrient intake for vitamin A (RE), those within the category of the recommended nutrient intake, those above the recommended nutrient intake and those with a very high intake compared to the recommended daily intake for vitamin A (RE) (see table 13). There were 8 of 42 subjects (19%) ingesting less than the recommended vitamin A (RE) intakes, 15 of 42 (35.7%) with vitamin A (RE) intakes within the recommended intake, 11 of 42 (26.2%) with vitamin A (RE) intakes above the recommended intake and 8 of 42 (19.0%) with very high vitamin A (RE) intakes compared to the recommended intake (see table 13).

The mean daily intakes of selected nutrient (see table 8), were calculated with an exception of one subject. One subject was omitted due to her high intake of organ meats which are a rich source of vitamin A. Her inclusion in the total calculated mean would have biased the results of estimated daily intake of vitamin A. equivalence. The type of foods and amounts the subject

consumed are listed in appendix G. The rest of the foods for this subject were within conceivable amount of intake per month.

7.6.3 **Hypothesis 3.;**

Dark adaptation times are related to estimates of dietary vitamin A.

- Dependent Variable - Dark adaptation times.
- Independent Variable - Estimates of dietary vitamin A.

Table # 14.

Correlation of dark adaptation test times white chip with dietary intake of vitamin A and age:

Parameters	PR>|T|	Estimate
Vitamin A intake	0.9287	0.00075
Age	0.0025	1.16679
Vitamin A intake and Age interaction	0.9257	0.00003

White chip test time:

Dark adaptation test times for the white chips was not related to the estimates of dietary intake of vitamin A (see table 14). Therefore the hypothesis that the observed "estimate" is equal to zero was accepted. This means there was no relationship between dark adaptation test times for the white chips and dietary intake of vitamin A. Note that age showed a relationship with dark adaptation white chip test times as was observed previously.

Table # 15.

Correlation of dark adaptation test times blue chips and estimates of dietary vitamin A intake.

Parameters	PR>|T|	Estimate
Vitamin A intake	0.0754	0.03885
Age	0.0040	2.80640
Vitamin A and Age interaction	0.0465	-0.00173

Blue chip test times:

A correlation between impaired dark adaptation test times for the blue chips and dietary intake of vitamin A would be expected as was shown previously in adults (Vinton and Russell, 1981). However the purkinje shift did not seem to be related to the estimates of dietary Vitamin A. Therefore the hypothesis that dark adaptation times are related to estimates of dietary vitamin A obtained by diet history was rejected.

7.6.4 **Hypothesis 4.;**

Rapid dark adaptation test values show inverse relationship to serum zinc values.

- Dependent Variable - Rapid dark adaptation test values.
- Independent Variable - Serum zinc values.
- Covariate - Age

Table # 16.

Correlation of white chip test times with serum zinc and age:

Parameters	PR>|T|	Estimate
Serum zinc	0.77455	1.14005
Age	0.0597	2.14425
Serum zinc and Age interaction	0.4121	-0.09147

White chip test times:

The mean rapid dark adaptation for separating the white chips was not significantly correlated to serum zinc. Nevertheless, age appeared to have an effect on the relationship (p=0.06). However, the hypothesis that the observed "estimate" of the serum zinc parameter is equal to zero was accepted. There was no inverse relationship between dark adaptation white chip test times and serum zinc levels. Therefore the hypothesis that rapid dark adaptation test values show inverse relationship to serum zinc was rejected.

Table # 17.

Correlation of blue chip test times with serum zinc and age:

Parameters	PR>|T|	Estimate
Serum zinc	0.0503	17.08034
Age	0.0018	8.95850
Serum zinc and Age interaction	0.0066	-0.76746

Blue chip test times:

The rapid dark adaptation measurement times based on the purkinje shift appears to be affected by serum zinc levels depending on the age of the individual. Rapid dark adaptation test times showed a positive relationship with serum zinc values which appeared to be significantly influenced by age. This finding suggests that serum zinc levels are related to the perception of the purkinje shift. However, the hypothesis that the observed "estimate" of the serum zinc parameter is equal to zero has to be rejected. However the hypothesised relationship between dark adaptation measured by chip test times and serum zinc values, was an inverse one. Since a positive relationship was found a mechanism other than the hypothesised one must explain the observations.

This relationship was stronger when the age of the subjects is taken into consideration. This means that the expected rapid dark adaptation test times will be greater at higher levels of serum zinc for younger subject than the older subject.

Table # 18.

Serum zinc, serum copper and plasma retinol levels by diagnosis (Number of subjects).

		Serum zinc (umol/L)		Serum copper (umol/L)		Plasma retinol (ug/dl)	
Diagnosis	N	<10	>19	<12	>20	<32	>90
Pregnant	14	13	0	0	13	4	0
GI problems	7	5	0	0	3	1	0
Metabolic/systemic problems	5	2	0	0	1	-	-
Other	16	4	0	0	1	1	0
Total	42	24	0	0	18	6	0

7.7 HAEMOGLOBIN LEVELS AND HAEMATOCRIT %:

There was a higher incidence of low percent haematocrit among the subjects than the incidence of low haemoglobin levels. Fourteen subjects (33%), all females, had haematocrit values below the normal range. Only 4 of 42 subjects (9.5%), three females and one males, had haemoglobin levels below the normal range (Appendix D).

Nonetheless, the haemoglobin levels showed a normal distribution with 16 female subjects having haemoglobin levels below the 50th percentile. The 50th percentile was 13.5 ug/dL for the female subjects, and 14.5 ug/dL for the male subjects based on the set standard normal ranges. All the five men had haemoglobin levels below their 50th percentile. Similarly, haematocrit count showed a normal distribution (see statistic Appendix F). Two female subjects, both of whom were pregnant and 19 years of age had low haemoglobin levels (8.2 and 8.0 mg/dL) and low percent haematocrit (23.0 and 32.0 % respectively). These two subjects also were observed to have low serum zinc levels (7.9 and 7.2 umol/L respectively) and high serum copper levels (41.1 and 39.2 umol/L respectively). Their plasma retinol levels also were below the normal range for their specific sex-age reference group (see table 1 Kaplan et al., 1987). Their retinol levels were 30.0 and 29.8 ug/dL respectively. In spite of these values their dark adaptation times were below the mean times for the sample (see the tables 19 and 20), indicating that dark adaptation performance was not affected.

Table # 19.

Blood results for two subjects who's values were outside the normal range:

Subject	005	006
Sex	female	female
Age	19	19
HB	8.2	8.0
Haematocrit %	23.0	32.0
Serum zinc	7.9	7.2
Serum copper	41.1	39.2
Plasma retinol	30.0	29.8

*Both subjects were 8 moths pregnant.

* Normal ranges see appendix E.

Table # 20.

Dark adaptation measurements of subject #005 and 006:

Sub	White chip test time	Blue chip test time	RDAT shorter trial
005	36.5	135.0	107.0
006	26.5	207.0	203.0

*Sample mean=213 $\pm$ 44 seconds.(blue chip test time)

Table # 21.

Description of variables for pregnant and non-pregnant women (37 Subject).

	pregnant N=14	Non-pregnant N=23
Dark adaptation white chip test time(seconds)	Mean 37.0 Std 6.0 Range 26.0-45	Mean 47.0 Std 28.0 Range 19-117
Dark adaptation blue chip test time(seconds)	Mean 217.0 Std 43.0 Range 135-278	Mean 215.0 Std 46 .0 Range 135-348
Shorter trial of the two two trials recorded (seconds)	mean 193.0 Std 40.0 Range 107-242	Mean 180.0 Std 39.0 Range 120-300
Serum zinc(umol/l)	mean 7.9 Std 1.3 Range 6.7-11.2	Mean 10.4 Std 1.8 Range 7.8-13.4
Serum copper(umol/l)	Mean 33.3 Std 5.4 Range 23.1-41.1	Mean 20.6 Std 5.6 Range 12.5-33.2
Plasma retinol(ug/dl)	Mean 36.7 Std 7.2 Range 32.1-45.2	Mean 47 Std 12.3 Range 31.0-79.3
Protein(g)	Mean 134.5 Std 80.3 Range 46-329.9	Mean 107.7 Std 61.7 Range 23.1-290.8
Carbohydrate(g)	Mean 320.9 Std 152.0 Range 40.2-515.9	Mean 273.5 Std 146.8 Range 24-623.0
Energy (Kcal)	Mean 2638.3 Std 1253.3 Range 727.7-4814.6	Mean 2157.1 Std 1020.5 Range 477-4653.4
Vitamin A(ug RE)	Mean 1690 Std 1269.3 Range 88.0-4030	Mean 920.2 Std 537.9 Range229.4-2280.6

Table # 22.

Correlation of white and blue chip test times with retinol and zinc for pregnant and non pregnant women(37 subjects)

		Pregnant(n=14)		Non-pregnant(n=23)	
		Not significant		Not significant	
		R>|T|	Estimate	PR>|T|	Estimate
Correlation of white chip test time with serum zinc and age.	zinc	0.0527	23.47806	0.5546	-3.21750
	Age	0.0590	5.87533	0.3068	1.56945
	Inter.	0.0652	-0.75271	0.8835	-0.02231
		Not significant		Not significant	
Correlation of blue chip test time with serum zinc and age.	zinc	0.6689	23.63692	0.3859	10.32578
	Age	0.8219	-3.20210	0.0363	7.29291
	Inter.	0.5289	1.19546	0.0773	-0.60770
		Not significant		Significant	
Correlation of white chip test time with retinol and age.	Retinol	0.4756	1.30277	0.0368	-1.95094
	Age	0.7141	0.89984	0.6295	-0.48463
	Inter.	0.6384	-0.3314	0.0803	0.04114
		Not significant		Not significant	
Correlation of blue chip test time with retinol and age.	Retinol	0.7086	3.55919	0.8731	0.37281
	age	0.4284	10.48312	0.4182	2.15500
	Inter.	0.7100	-0.13824	0.7001	-0.02304

Chapter VIII

8.0 DISCUSSION:

8.1 RAPID DARK ADAPTATION:

In present study, correlations between plasma retinol and mean rapid dark adaptation measurement times, were found to be nonsignificant. Carney and Russell, (1980), also assessed the relationship between dark adaptation and serum retinol and concluded that serum vitamin A levels greater than 40 ug/100 mL can safely be interpreted as predicting normal vitamin A dependent retinal function. Hence, serum vitamin A less than 40 ug/100 mL would be taken as vitamin A sufficient only when a normal dark adaptation has been elicited. In the present study normal dark adaptation was elicited in spite of the low normal plasma levels observed among the subjects. Yarbrough and Dann, (1941) in reporting on a North Carolina study, concluded that a single measurement of dark adaptation could not be used as an indicator of avitaminosis A. In the present study, three measures of dark adaptation were used. Nonetheless, there is need to determine the precision of dark adaptation measurements due to the variations observed (Sevenhuysen, 1984). Favaro et al. (1986) found no correlation between rapid dark adaptation times and vitamin A status as determined by plasma retinol levels of

preschool children. A similar observation was reported by Solomons et al., (1982) with Guatemalan children.

There appears to be no functional impairment exhibited by the subjects in the present study in spite of some low plasma retinol values, i.e <40 ug/dL (Vinton and Russell, 1981). There were 17 subjects out of 42 (40.5%) who had serum retinol less than 40 ug/dL. It has been reported that, functional impairment occurs at serum retinol levels <30 ug/dL (Sauberlich et al., 1974; Carney and Russell, 1980). The plasma retinol levels in the present study ranged from 23.1-79.3 ug/dl. These levels are consistent with other reports; Favaro et al., (1986) did not find any significant correlation between plasma retinol and rapid dark adaptation test times in the preschool children whose plasma retinol ranged from 9.3-32.0 ug/100 mL. Similarly, Solomon et al., (1982), did not find a correlation between plasma retinol levels and the time for dark adaptation to darkness with children whose plasma retinol varied from 26.9-51.7 ug %. As in the above mentioned studies, in the present study all subjects completed the test in a time considered normal according to the results obtained by Vinton and Russell, (1981).

As shown graphically (fig. 6) there was no correlation between plasma retinol and dark adaptation. The possible explanation for this observation may relate to the fact that no real functional change occurred, and the low serum zinc values observed among the

subjects, most of whom were pregnant women, may be related to the normal low retinol values observed in the present study. Previous studies did not report on the status of pregnant women and though dark adaptation blue chip test times were significantly correlated with serum zinc values, it is possible that the low serum zinc values observed with pregnancy may be a normal physiological response, without accompanying functional impairments. However this needs further investigation.

The purkinje shift did not seem to be related to the estimates of dietary vitamin A intake. However, a correlation between impaired dark adaptation and vitamin A deficient would be expected as was shown previously in adults (Vinton and Russell, 1981). But, it is difficult to find a correlation in most studies because of the many factor that might influence vitamin A status. The body stores of the individual need to be established, and the day to day variation in intake of vitamin A food sources need to be considered, and other nutritional deficiencies that might affect vitamin A intake have to be verified.

Difference in food consumption are however, most likely to be a major factor in determining carotene levels but not retinol levels (Kaplan et al., 1987). Relationship with gender might also be related to life-style practices other than diet, such as smoking and alcohol usage, the absorption of the carotenes from the gut, or to the manner in which they are carried in the blood

(Kaplan et al., 1987). Although most dietary beta carotene is metabolized to retinol in the gut, a considerable amount is directly absorbed by passive mechanisms depending upon the vitamin A status of the individual.

8.2 DIETARY INTAKE:

The dietary data was calculated using the Canadian Nutrient File (Health and Welfare, 1983). The nutrient intake per day calculated from the dietary history questionnaire, showed a mean intake of vitamin A (RE) of 1070 $\pm$ 701 RE/day. The mean intake was higher than the median (948 RE). However both the mean intake and the median intake showed vitamin A intake of the population to be within the range of the recommended intake for adults. These results reflected a population consuming a diet rich in vitamin A sources although their mean plasma retinol was in the low normal range (46.8 $\pm$ 14.3 ug/dL). This trend was observed by Verdier et al., (1987). In calculation of mean vitamin A intake for the total sample group, one subject was left out because she reported a very high intake of organ meats which resulted in a very high intake of vitamin A retinol equivalent.

The estimated dietary intake for energy and retinol equivalents (RE) was categorized into four groups (see Table 10). Among the subjects, 35.7% fell in the group that had vitamin A intake between 500-1000 RE/day. This category was within the

recommended vitamin A intake which for women is 800 RE/day and 1000 RE/day for men. The recommended intake for pregnant women includes an additional 100 RE giving them a recommended intake of 900 RE/day. However there were two groups of 19% of the subjects on each side of the recommended nutrient intake category (see table 13). one of the groups was ingesting less than the recommended nutrient intake and the other group was ingesting much more than the recommended nutrient intake (Bureau of Nutritional Sciences, 1983). There is need for concern over the 19% of the subjects that were ingesting vitamin A below the recommended nutrient intake although they did not show impaired retinal function or abnormal dark adaptation.

The mean energy intake was 2370 $\pm$ 1127 Kcal and median was 2347 Kcal. When the estimated dietary intake for energy was categorized, 31.0% of the subjects ingested 1501-2500 Kcal per day which is within the accepted dietary intake for energy. However, 26.2 % of the subjects consumed between 2501-3500 Kcal. This high energy intake could have been as a result of a high energy intake among the pregnant women in the sample or an over estimate due to use of food frequency questionnaire which tends to overestimate in most cases. Fourteen percent of the subjects reported a very high energy intake (above 3500 kcal/day), which could lead to overweight. Further investigation is required to establish if the number of overweight cases in the population is increasing. Nevertheless, in this sample 28.6% of the subjects

reported low energy intakes, between 0-1500 kcal/day, with one subject having an extremely low energy intake (480 kcal/day). Moffatt (1988) reporting on nutritional problems of Native Canadians, indicated that Inuit women frequently have caloric intakes in the borderline range. In this study a similar observation was made with 28.6% consuming caloric intake on the borderline or even lower categorized as 0-1500 kcal (see table 13). It should be recognized that the methodology used to record what each subject had consumed over the period of a month may not have recorded all items and the possible influence of the method needs to be investigated further.

Generally the estimated nutrient intakes were higher than that would be expected but, within the range for dietary history estimates using a food frequency questionnaire which is known to overestimate intake. In conclusion, the nutrient intake of the population did not reflect any inadequacy in any of the selected nutrient intakes. The mean daily intakes were slightly higher but acceptable as a result of food frequency (see table 8; 13). However, there were a few low intake reported for each nutrient (see table 8 for the ranges and table 13 for the percentages).

8.3 **PLASMA RETINOL:**

The blood samples were analyzed for plasma retinol and serum zinc and serum copper (see Table 9). In calculating mean plasma

retinol, one subject was not included as the sample broke was accidentally during storage. The mean plasma retinol was in the low normal range (46.8 $\pm$ 14.3 ug/dL). The median was lower than the mean (see table 9). It is worthwhile investigating the cause of the low retinol in the pregnant women and in the one subject who had come for other medical tests, although they did not show impaired function by the dark adaptation test. As for the subjects with gastrointestinal problems, it has been reported in literature to result in low plasma retinol (Solomons et al., 1980). One subject had low plasma retinol (23.1 ug/dL) and also low serum zinc (7.5 umol/L), but recorded a normal dark adaptation blue chip test time (Vinton and Russell, 1981). Hence the subject did not show functional impairment and was within the times achieved by other investigators (see appendix **D**; see table 7).

In spite of the normal intake of vitamin A (RE) the sample exhibited a population with low normal vitamin A status, although this did not appear to affect the purkinje shift. There is need for further investigation on these aspects.

8.4 **SERUM ZINC:**

The serum zinc however, was observed to be low (mean 9.82 $\pm$ 2.1 umol/l). There were 22 subjects with low serum zinc values. The sample included 14 pregnant, women of these 13 showed low

serum zinc (mean 7.9 $\pm$ 1.3). It has been reported that, pregnancy decreases serum zinc due to the excess excretion of zinc (Taper et at., 1980; Smith,et al., 1974; Prasad, et al., 1982a and 1982b; King et al., 1987). The investigator suggests that the incidence of low zinc within the population needs to be investigated. The study was composed mostly of women with only four men, hence there is a possibility that other factors related to women such as oral contraceptives or pregnancy could be the contributing factor for the low serum zinc levels observed. However, only one female, aged 24 years, who reported taking oral contraceptives had low serum zinc and high serum copper levels (8.2 and 33.2 umol/L respectively).

It has also been reported that decreased serum zinc concentration may be a consequence of increased urinary clearance of zinc (Russell et al.,1978; Smith et al., 1988). Pregnant women in their late pregnancy have been reported to excrete 2.4 and 1.5 times more zinc than early-pregnant or non-pregnant women respectively (Smith et al., 1988). All the expectant women in this study were in their late pregnancy (eight months pregnant "between 32 and 34 weeks" or the third trimester) except two. The urinary zinc excretion of the pregnant women was not measured in this study. There is evidence that certain conditions do result in low serum zinc levels, such conditions include, pregnancy, in women taking oral contraceptives, alcoholic cirrhosis and other types of liver disease (Halsted and Smith, 1970). Therefore the

low serum zinc observed among these subjects requires further investigation.

Moreover low serum zinc levels or zinc deficiency has been shown to be associated with low plasma vitamin A levels. Low plasma vitamin A levels have been shown to be caused by impaired mobilization of vitamin A from the liver as a result of zinc deficiency (Solomons et al., 1980; Russell et al., 1978).

In the present study serum zinc was significantly positively correlated with rapid dark adaptation blue chip test times ($p=0.05$). This suggests that the purkinje shift is affected by serum zinc levels.

However a negative relationship was expected and the positive one is not explained by the theoretical model. A possible explanation of the relationship may include the following points:

(1) Non of the rapid dark adaptation test times where below the level which indicate vitamin A or zinc deficiency meaning that non of the test times indicated retinal impairment. Therefore the relationship may be spurious.

(2) There were a number of pregnant women whose metabolism may influence serum zinc values in a way different from a non-pregnant population. Hence the variable values may have changed in such a way as to lead to a positive relationship. It remains to be investigated whether a positive relationship will be

observed among population groups that show dark adaptation times indicative of vitamin A deficiency.

The association of serum zinc levels with perception of the purkinje shift appears more pronounced when age of the subjects is taken into account. The correlation of dark adaptation blue chip time is significant ($p<0.05$) for the interaction of serum zinc values with age of the subjects. These associations between serum zinc level and age need further investigation.

Solomon et al., (1978), reported no significant correlation between rapid dark adaptation test times and serum zinc levels among Guatemalan children. However, none of the children's serum zinc levels were below 70 ug/dL (<10 umol/L). In the present study, there were 22 subjects who had low serum zinc levels (< 10 umol/L) range 6.7-11.2 umol/L and a number of them were pregnant women (see table 17; 22). The only explanation for the lack of retinal functional impairment with such low serum zinc is a possibility of a low physiological serum zinc level which does not affect organ function in pregnant women (see tables 21; 22). However this needs to be investigated further.

8.5 HAEMOGLOBIN LEVELS AND HAEMATOCRIT COUNT %:

Haemoglobin levels (mg/dL) and Haematocrit count % reflect the general nutritional status of the population. In the present

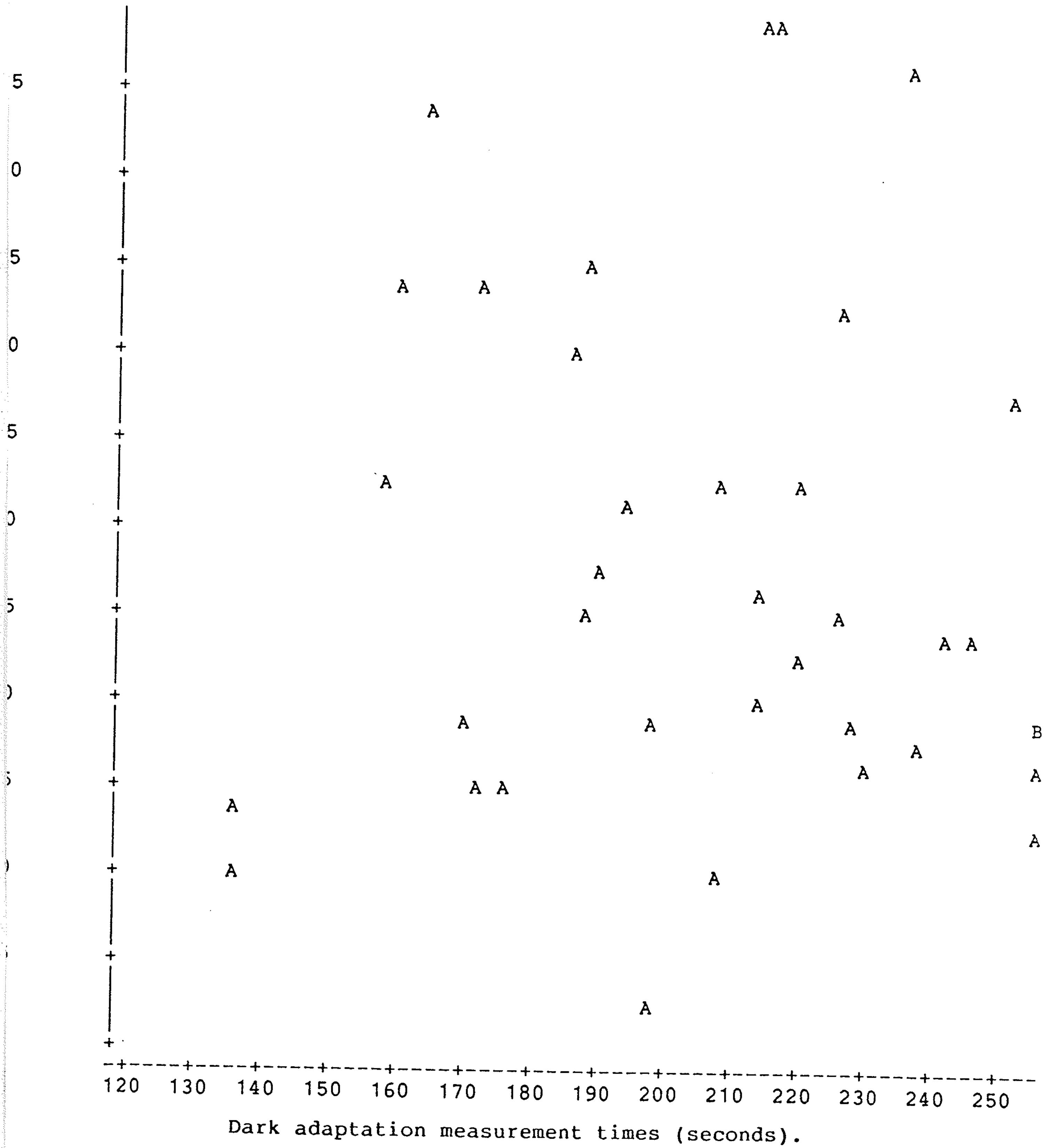
study haemoglobin levels and haematocrit count percent did not reflect a population at high risk of general nutritional deficiency (Verdier et al., 1987; Bureau of Nutritional sciences, 1975a; 1975b).

8.6 NUTRITIONAL STATUS OF PREGNANT WOMEN:

In this study there were 37 women, 14 of 38 were pregnant. Major differences in plasma constituents and nutrient intakes were observed between the pregnant and non-pregnant as was expected (see table 21). The pregnant women performed the dark adaptation white chip test times faster than the non-pregnant women (mean 37.0 $\pm$ 6.0 vs 47.0 $\pm$ 28.0 seconds), while there were no significant differences on dark adaptation blue chip test times and the shortest trial (see table 22). Pregnant women appeared to have significant low retinol values as compared to non-pregnant women. The mean plasma retinol values for pregnant women the mean was 36.7 $\pm$ 7.2 ug/dL and range 23.1-45.2 ug/dL while for non-pregnant women were 47.0 $\pm$ 12.3 ug/dL and the range 31.0-79.3 ug/dL (see table 21).

The plasma retinol values were compared to dietary intake of vitamin A and it was observed that pregnant women had a higher Vitamin A (RE) intake than the non-pregnant women. This might indicate that the low normal retinol values observed among the pregnant women to be more physiological than functional.

When dark adaptation blue chip and white chip were correlated with plasma retinol and serum zinc levels no significant relationship were found except for the retinol values of non-pregnant women and dark adaptation white chip test times which indicated a positive relationship. Although low serum zinc levels were observed among the pregnant women, those values alone did not appear to affect the purkinje shift.



TE: 1 OBS HAD MISSING VALUES

Fig 6., Plot of a correlation between plasma retinol and dark adaptation measurement time for blue chips.

Chapter IX

10.0 CONCLUSION:

Clinical signs of hypovitaminosis A cannot be used to detect early vitamin A deficiency. Moreover, plasma vitamin A levels are uncertain indicators of deficiency in individual subjects because of the wide range of "borderline values" in which vitamin A dependent rod function may or may not be interfered with (Carney and Russell; Solomons et al., 1982; Vinton and Russell, 1980). Nevertheless, dark adaptation test should not be used alone since, night blindness can be affected by other nutrients, among them zinc, riboflavin and vitamin C (Russell et al., 1982; Solomons et al., 1982). In the present study, the effect of vitamin A together with serum zinc and serum copper, was investigated. However no retinal impairment was observed despite the low normal plasma retinol values observed among the subjects. The plasma retinol values observed in this study were within the normal range (normal range for females = 32.0-80.0 ug/dL, and for male = 38.0-93.0 ug/dL) and did not appear to affect the purkinje shift. However, there was a significant correlation between serum zinc levels and dark adaptation blue chip test times. The low serum zinc levels observed among the sample population point to a possible problem in the population. The purkinje shift appeared to be significantly affected by serum

zinc levels thus prolonging dark adaptation times especially when age was taken into account.

The nutrient intakes revealed that the sample population consumed nutrients within the range of recommended intakes. Their vitamin A intakes was within the recommended intake (800 RE for women and 1000 RE for men). Although, the dietary vitamin A was within the recommended intakes, it showed no significant correlation with plasma retinol.

In the present study, serum zinc levels and the serum copper were found to be of concern. A high percentage (52%) of the sample population had serum zinc levels below the normal range (10.0-19.0 umol/L). At the same time a high percentage (52%) of the sample population had higher than normal serum copper levels (12.0-20.0 umol/L). Consequently, these biochemical parameters need to be investigated further.

In conclusion therefore, the rapid dark adaptation test could possibly complement biochemical determinations. It may also prove more quantitative as a screening test, but individual variation needs to be taken into account and the age effect needs to be assessed. More than one or two trials need to be carried out at any one time.

Chapter X

PROPOSED RESEARCH:

The sample recorded low mean serum zinc, high mean serum copper and a mean plasma retinol level within the normal range. However, finding no retinal impairment in the sample, the investigator proposes further research in rapid dark adaptation times with only expectant women in all the three trimesters, with the objective of verifying whether the low serum zinc observed is purely physiological and not functional. The cause of high serum copper levels observed in the sample population deserve a further investigation. A dietary intake also would need to be included in the study.

REFERENCES:

Alaska,an appraisal of the Health and Nutritional Status of Eskimos. Washington, D.C: A report by the Interdepartmental Committee on Nutrition for National Defence, August 1956.

Adelman, M. O., Dwyer, J.T., Woods, M., Bohn, E., and Otradovec, C. L., 1983. Computerized Dietary Analysis System: A Comparative View , J. Am. Dietet. 83:42.

Annesley, T., Glacherio, D. and Wilkeron, K.,1984. Analysis of Retinoids by High-Performance Liquid Chromatography using Programmed Gradient Separation, J. Chromatogr., 28:693.

Beiri, J. G., Tolliver, T. J., and Catignani, G. L., 1979. Simultaneous Determination of - Tocopherol and Retinol in Plasma or Red cells by High Pressure Liquid Chromatography, Am. J. Clin. Nutr., 32:2143.

Block, G., 1982. A review of Validation of Dietary Assessment Methods, Am. J. Epidemiol., 115:492.

Boron, B., Hupert, J., Barch, D. h. Fox, C. C., Friedman, H., Layden, T. J. and Mobarhan, S., 1988. Effect of Zinc Deficiency on Hepatic Enzymes Regulating Vitamin A Status, J.Nutr., 118:995.

Brenner, S., and Roberts, L. J. 1942. Effect of Vitamin A depletion in young Adults. Arch. Intern. Med., pp.474-482.

Brown, G. C., Felton, S. M., Benson, W. E., 1980. Reversible Nightblindness Associated with Intestinal by pass Surgery, Am. J. Ophathalmol., 110:552.

Bureau of Nutritional Sciences, 1983. Recommended Nutrient Intakes for Canadians, Health and Welfare Canada, Ottawa.

Bureau of Nutritional Sciences, 1975a. Nutrition Canada: The Manitoba Survey Report, National and Welfare, Ottawa.

Bureau of Nutritional Sciences, 1975b. Nutrition Canada:The Eskimo Survey Report, National and welfare, Ottawa.

Carney, E. A., and Russell, R. M., 1980. Correlation of Dark Adaptation Test Results with Serum Vitamin A Levels in Diseased Adults, J. Nutr., 110:552.

Chou, P. P., Zinc: In Methods in Clinical Chemistry. Pesce and Kaplan 1987. pp.596. The C. V. Mosby Commpany, Toronto.

Chow,F. I., and Omaye, S. T., 1983. Use of Antioxidants in the Analysis of Vitamin A and E in Mammalian Plasma by High Performance Liquid Chromatography, Lipids, 18:837.

Cohen, N., Rahman, H., Mitra, M., Spraque, J., Islam, S. De Regt Leemhuis, E. and Jail, M. A., 1987. Impact of Massive doses of Vitamin A on Nutritional Blindness in Bangladesh, Am. J. Clin. Nutr., 45:970.

Crews, M. G., Taper, L. J., and Ritchey, S. J., 1980. Effect of oral contraceptive agents on Copper and Zinc balance in Young Women, Am. J. Clin. Nutr., 33:1940.

De Leenheer, A. P., de Bevere, V. O. R. C., de Ruyter, M. G. M., and Claeys, A. E., 1979. Simultaneous Determination of Retinol and d-Tocopherol in serum by High-Performance Liquid Chromatography, J. Chromatogr., 162:408.

Dhanamitta, S., Stoecker, B., Manneechine, A., and Valyaesevi, A., 1983. Dark Adaptation Plasma Vitamin A and Urinary Calcium of Vitamin A supplemented children in North East Thailand, Nutr. Rep.Inter., 27:67.

Dowling, J. E., and Wald, G., 1958. Vitamin A deficiency and Nightblindness, Proc. Nat. Acad. Sci., 44:648.

Driskel, W. J., Neese, J. W., Bryant, C. C., and Bashor, M. M., 1982. Measurement of Vitamin A and Vitamin E in human serum by High-Performance Liquid Chromatography, J. Chromatogr., 231:439.

English, J. L., and Hambidge, K. M., 1988. Plasma and Serum Zinc Concentrations: Effect of Time between Collection and Separation, Clinica. Chimica. Acta., 175:211.

Favaro, R. M. D., de Souza, N. V., Vannucchi, H., Desai, I. and de Oliveira, J. E., 1986. Evaluation of Rose-Bengal Staining Test and Rapid Dark Adaptation Test for the Field Assessment of Vitamin A Status of preschool Children in Southern Brazil, Am. J. Clin. Nutr., 43:940.

Feldman, J. B., 1941. Light Threshold and its Clinical Evaluation, Arch. Opthalmol., pp 466-471.

Fisher, K. D., Carr, C. J., Huff, J. E., and Huber, T. E., 1970. Dark Adaptation and Night Vision, Feder. Proc., 29:1605.

Guyton, A. C., 1979. Photochemistry of Vision: In Test book of Medical Physiology, pp. 713-715. W. B. Saunders Commpany West Washington Square Philadelphia,PA 19105.

Hecht, S., and Mendelbaum, J., 1939. The relationship between Vitamin A and Dark Adaptation, J. A. M. A., 112:1910.

Henry, R., Cannon, D. C., and Winkelman, J. W. 1974. Clinical Chemistry: Principles and Technics. Second Edition. Haper & Row. Pul. Inc.

Holt, A.B., Spargo, R. M., Iveson, J. B., Faukner, G.s., and Cheek, D.B., 1980. Serum and Plasma Zinc , Copper and Iron Concectration in Aboriginal Communities of North Western Australia, Am.J. Clin. Nutr., 33:119.

Huber, A. M. and Gershoff, S. N., 1975. Effect of zinc deficiency on the oxidation of retinol and ethanol i n rats, J. Nutr., 105:1486.

Hume, C. E., and Krebs, H., 1949. Vitamin A Requirement of Human Adults, Med. Res. Counc. (Gr. Brit.), Spec. Rep. Ser. 264

Joint WHO/USAID Meeting, 1976. Vitamin A deficiency and Xerophthalmia, WHO Tech. Rep. Ser. 590, Geneva.

Kaplan, L.A., Stein, E. A., 1987. Reference range of retinol, Tocopherol, Lycopene and Alpha- and Beta- Carotene in Plasma by Simultaneous High-Performance Liquid Chromatographic Analysis, Clin. Physiol. Biochem., 5:297.

Karvetti, R., Knuts, L., 1981. Agreement between Dietary Interviews, J.Am. Dietet. Assoc., 79:654.

Kim, V., and Solomons, N. W., 1983. Performance of Genetically-Colorblind Individual on Rapid Dark Adaptation Test Based on Purkinje Shift, Perceptual and Motor Skill, 56:251.

King, J. C., Bronstein, M. N., Fitch, W. L., and Weininger, J., 1987. Nutrient Utilization During Pregnancy, Wld rev. Nutr. Diet., 52 pp 71-142.

Klevay, L. M., inHyg, S. D., Reck, S. J., Jacob, R. A., Logan, G. M., Munoz, J. M., and Sandstead, H. H., 1980. The Human requirement for Copper. 1. Health Men Fed Convectional, American Diets, Am. J. Clin. Nutr., 33:45.

McClean, S. W., Ruddel, M. E., Gross, E. G., DeGiovanna, J. J., and Peck, G. L., 1982. Liquid Chromatographic Assay for Retinol(Vitamin A) and Retinol Analogs in Therapeutic Trials, Clin. Chem, 28:693.

McLare, D., Oomen, H., and Escapini, H., 1966. Ocular Manifestations of Vitamin A deficiency in Man, Bull. WHO, 34:357

McLaren, D. S., Techalian, M., Khoury, G., Shirajian, E., 1965. Xerophthalmia in Jordan, Am. J. Clin. Nutr., 17:117.

Mejia, L. A., and Arroyave, G., 1983. Determination of Vitamin A in Blood.Some Practical Consideration of the Time of Collection of the Specimens and the Stability of the Vitamin, Am. J. Clin. Nutr., 37:147.

Menon, K., and Vijayaraghavan, K., 1980. Sequelae of Severe Xerophthalmia-a follow up Study, Am. J. Clin. Nutr., 33:218.

Moffatt, M. E. K., 1989. Nutritional Problems of Native Canadian Mothers and Children, Can. Fam. Physician, 35:377.

Morrison ,S. A., Russell, R.M., Carney, E. A., and Oaks, E. V., 1978. Zinc Deficiency: A cause of abnormal Dark Adaptation in Cirrotics, Am. J. Clin. Nutr. 31:276.

Mullen, B. J., Kratzler, N. J., Grivetti, L. E., Schutz, H. G. and Meiselman, H. L., 1984. Validity of a food frequency questionnaire for determination of Individual Food Intake, Am. J. Clin. Nutr., 39:136.

Niernberg, D. W. and Lester, D. C., 1985. Determination of Vitamin A and E in Serum and Plasma using a Simplified Clarification Method and High- Performance Liquid Chromatography, J. Chromatogr., 345:275.

O'Mara, P. A., Zwick, Beatrice, E. S., and Lund, D. J., 1982. Microprocessor-controlled Light-Emitting Diode Dark Adaptometer, Med & Biol. & Comput., 20:70.

Patek, A. J., and Haig, C., 1939. The Occurance of Abnormal Dark Adaptation and its relation to Vitamin A Metabolism in Patients with Cirrhosis of the Liver, J. Clin. Investigat.

Perlman, I., Baarzilai, D., Tamarhaim, and Schramek, A., 1983. Night Vision in case of Vitamin A deficiency due to malbsorption, Br. J. Ophthalmol., 67:37.

Pilch, S. M., 1987. Analysis of VitaminA data from the Health and Nutrition Examination Surveys, J. Nutr., 117:636

Pitt, G. J., 1981. The assessment of Vitamin A status, Proc. Nutr. Soc., 40:173.

Prasad, A. S., Dreosti, I. E., and Hetzel, b. s., 1982. Aplication of Recent Advances in Zinc Metabolism: in Current Topics in Nutrition and Disease Volume 7. Alan ,R. Liss, Inc., 150 fifth Avenue New York, Ny 10011.

Ripps, H., and Wheale, A., 1976. Visual Adaptation in: The Eye, 2a: 101, Academic Press Inc. New York, 2nd Ed.

Russell, R., Multack, R., Smith, V. C., Krill, A. E., 1973. Dark Adaptation Testing for Diagnosis of Sub-Clinical Vitamin A Deficiency and Evaluation Therapy, Lancet, 2:1161

Russell, R. M., Morrison, A. S., Smith, F. R., Oaks, E. V., and Carney, E. A., 1978. Vitamin A Reversal of Abnormal Dark Adaptation in Cirrhosis, Ann. Intern. Med., 88:622.

Sabrell, W. H. and Harris, R. S., 1967. The Vitamins, Vol. 1.

Sandberg, M. A., Rosen, J. B., and Berson, E. L. 1977. Cone and Rod Function in Vitamin A deficiency with Chronic Alcoholism and in Retinitis Pigmentosa, Am. J. Opthalmol., 84:658.

Sandstrom, B., Davdsson, L., Lundell, L., and Olbe,l., 1987. Zinc Status and Dark Adaptation in Patients Subjected to Total Gastrctomy: Effect of Zinc Supplementation, Hum. Nutr: Clin. Nutr., 41:235.

Sanz, D. C., and Santa-Cruz, M. C., 1986. Simultaneous Measurement of Retinol and Alpha- Tocopherol in Human Serum by High-Performance Liquid Chromatography with Ultraviolet Detection,** **

SAS User's guide: Statistics Version 5 Edition. 1985a. SAS Institute Inc box 8000 Cary, NC 27511 8000 U. S. A.

SAS User's guide: Basic Version 5 Edition. 1985b. SAS Institute Inc box 8000 Cary, NC 27511 8000 U. S. A.

Sauberlich, H. E., Hodges, R. E., Wallace, D. I., Kolder, H., Canham, J. E., Hood, J., Raica, N., JR. and Lowry, L. K., 1974. Vitamin A Metabolism and Requirements in the Human Studies with the use of Labeled Retinol, Vitam. Horm., 32:251.

Sevenhuysen, G. P., 1984. Sources of Measurement Variation in the Rapid Dark Adaptation Test, Nutr. Rep. Intern., 30:213.

Shanklin, D., Endres, J. M., and Sawicki, M., 1985. A Comparative Study of Two Nutrient Data Bases, J. Am Dietet. Assoc., 85:308.

Sigma Diagnostics™.,1984. Total Hemoglobin. Quantitative, Colorimetric Determination in Whole Blood at 530-550 nm. [Procedure No.525].

Sklan, D., 1987. Vitamin A in Human Nutrition, Prog. Food. Nutr. Sci., 11:39.

Smith, J. E., and Goodman, D. S., 1979. Retinol Binding Protein and the Regulation of Vitamin A Transport, Fed. Proc., 38:2504.

Smith, J. E., Brown, E. D., and Smith, J. C., 1974. The effect of Zinc deficiency on the Metabolism of Retinol-Binding Protein in rat, J. Lab. Clin. Med., 84:692.

Solomons,N. W., Russell, R. M., Vinton, N. E., Guerrero, A. M., and Mejia, L., Pinedo, O., 1982. Application of Rapid Dark Adaptation Test in Children, J. Pediatr. Gastroent. Nutr., 1:571.

Sommer, A., Hussain, G., Muhilal, Tarwotjo, I., Susanto, D., and Saroso, J. S., 1980. History of Night Blindness: A Simple Tool for Xerophthalmia Screening, Am. J. Clin. Nutri., 33:887.

Sommer, A., Hussain, G., Tarwotjo, I., and Susanto, D., 1983. Increased Mortality in Children With Mild Vitamin A Deficiency, The Lancet, 2:585.

Sommer, A., Katz, J., Tarwotjo, I., 1984. Increased Risk of Respiratory Disease and Diarrhea in Children with Pre-exiting Mild Vitamin A Deficiency, Am. J. Clin. Nutr. 40:1090.

Sommer, A., Tarwotjo, I and Katz, J., 1987. Increased risk of xerophthalmia following diarrhea and respiratory disease, Am. Clin. Nutr., 45:977.

Sommer, A., 1984. Vitamin A deficiency and Mortality Risk, The Lancet, 1:347.

Solomons, N. W., Russell, R. M., Vinton, N. E., Guerrero, A. M., Mejia, L., and Pinedo, O., 1982. Application of Rapid Dark Adaptation Test in children, J. Pediatr. Gastroent. Nutr., 1:571.

Solomons, N. W., and Russell, R. M., 1980. Interaction of Vitamin A and Zinc Implication for Human Nutrition, Am. J. Clin. Nutr., 33:2031.

Srikatia, S., 1975. Human Vitamin A deficiency, World Rev. Nutr. Diet., 20:184.

Stuff, J. E., Garza, C., Smith, E. O., Nichols, B. L., and Montandon, C. M.,1983. A comparison of dietary Methods in Nutritional studies, Am. J. Clin. Nutr., 37:300.

Sullivan, J. F., Jetton, M. M., Hahn, H. K., and Burch, R. E., 1980. Enhanced Lipid Peroxidation in Liver Microsomes of Zinc-deficient rats, Am. J. Clin. Nutr., 33:51.

Taper, L. J., Hinners, M. L., and Ritchey, S. J., 1980. Effect of Zinc Intake on Copper balance in Adult females, Am. J. Clin.Nutr., 33:1077.

Thompson, J. N., Erdody, P., and Maxwell, W. B., 1973. Simultaneous Fluorometric Determination Of Vitamin A and E in Human Serum and Plasma, Biochem. Med., 8:403.

Thornton, S. P., 1977. A Rapid Test for Dark Adaptation, Ann. Ophthalmol., 9:731.

Todd, K. S., Hudes, M., and Calloway, D., 1983. Food Intake Measurement: Problems and Approaches, Am. J. Clin. Nutr., 1983.

Turley, C. P., and Brewster, M. A., Vitamin A: In Methods in Clinical Chemistry, Pesce and Kaplan 1987, pp. 551., The C. V. Mosby Company, Toronto.

Verdier, P. C., Eaton, R. D. P., and Copper, B., 1987. A study of the Nutritional Status of an Inuit Population in the Canadian High Arctic Part 1. Biochemical Evaluation. Can. J. Pul. Healt., 78:229.

Verdier, P. C., and Eaton, R. D. P., 1987. A Study of the Nutritional Status of an Inuit Population in the Canadian High Arctic Part 2. Some Dietary Sources of Vitamin A and C., Can. J. Pul. Healt., 78:236.

Villard, L., and Bates, C. J., 1986. Dark Adaptation in Pregnant and Lactating Gambian Women: Feasibility of Measurement and Relation to Vitamin A Status, Hum. Nutr: Clin. Nutr., 40:349.

Vinton, N. E., and Russell, R. M., 1981. Evaluation of A Rapid Test of Dark Adaptation, Am. J. Clin. Nutr., 34: 1961.

Wallingford, J.C.and Underwood, B.A., 1987. Vitamin A Status needed to maintain Vitamin A concentration in nonhepatic Tissue of the Pregnant Rat, J. Nutr., 117:1410.

Walt, R. P., Kemp, C. M., Lyness, L., Bird, A. C., and Sherlock, S., 1984. Vitamin A Treatment for Night blindness in Primary Biliary Cirrhosis, Br. Med. J., 288:1030.

Warth, J. A., Prasad, A. S., Zwas, F., and Frank, R. N., 1981. Abnormal Dark Adaptation In Sickle Cell Anemia, Clin. Med. 98:189.

Werner, J. S. 1982. Development of Scotopic Sensitivity and the absorption Spectrum of the human Ocular, Opt. Soc. Am., 72:247.

Yarbrough, M. E., and Dann, W. J., 1941. Dark Adaptometer and Blood Vitamin A Measurements in a Northern Carolina Survey Nutrition, J. Nutr. 22:597.

Young, D. C., 1980. Dark Adaptation in Five year-old and Adults, Vision Research, 21:811.

APPENDIXES:

Appendix A:

RAPID DARK ADAPTATION TEST FOR VITAMIN A STATUS.

BACKGROUND INFORMATION FOR SUBJECTS (PATIENTS).

Dark adaptation is a reliable and reproducible indicator of vitamin A status in the individual. Identifying decreased ability to dark adapt is the first clinical sign of inadequate vitamin A status. A rapid dark adaptation test makes use of eye functions which operate after 3 to 4 minutes in a dark room. Under very low lighting you will not distinguish colour but you will be able to perceive increasing brightness of objects.

Although Vitamin A deficiency is rare, the Northern Communities have been reported to include individuals with low blood Vitamin A levels. Testing such individuals for dark adaptation function may show the relationship between Vitamin A status and performance of the field test. The research will study the performance of a standardized test for dark adaptation for identifying people at risk to have low Vitamin A values before other clinical symptoms become evident.

When you visit the hospital, we would like a technologist or a nurse to draw (20 ml) sample of your blood to measure your serum retinol, zinc and copper levels. We will then conduct a dark adaptation test in a dark room three consecutive times in a period of about 20 minutes. Finally, we will ask you questions about foods you usually eat.

We require your signed consent. If you choose not to participate it will in no way affect your future medical care. If you do participate we will give you follow up on the test results and we will not identify you as an individual in any publications resulting from this study.

Consent.

I_____(Father, Mother, or Legal guardian) agree to an interview on the hospital premises, a blood sample will be drawn on me or my child_____for the study of Vitamin A deficiency and its relationship with the Rapid Dark Adaptation Test. I understand that if I refuse to participate it will not affect the care I get, my child and my family, and that I have the right to withdraw at any time from the study.

_____(name).

_____(signature).

_____(witness).

Appendix B.

ᑐᒃ ᑭᓯᒥᐊᒍᑦ ᑕᒻᓇ ᑲᐅᔨᓴᒃᑕᐅᔪᑦ ᑲᓄᖅ Vitamin "A"-ᑲᑦᓯᐊᒪᓚᑕ ᑎᒻᒥᒍᑦ.

ᑐᒃ ᑭᓯᕙᓕᑦ ᒍᑎᒃᓴᒃ ᐃᓄᒡ ᓄᑦ (ᐊᓐᓂᐊᕕᒡᒥᑉ ᑐᓐᓄᑦ).

ᐃᒡᓗ ᑕᖅᑐᖅ ᑲᐅᔨᓴᕕᐅᑕᓂᖅ ᐊᒃ ᐳᒃ ᐃᓄᐃᑦ ᐃᔨᒡᓄᑦ ᐊᒪᓗ ᑕᒻᓇ ᑲᐅᔨᓴᕐᑕᐅᓂᖅ ᑐᑭᓯᕙᓕᑦ ᒍᑕᐅᓇᔭᒃᒪᑦ ᐃᓐᓄᐃᑦ ᑎᒡᒥᒥᑦ Vitamin "A"-ᑲᑎᐊᒪᓚᑕ. ᑕᒡ ᕙ ᑕᒻᓇ ᑐᒃ ᑭᓯᕙᓕᑦ ᒍᑎᒥᔭᐅᓇᔭᒃ ᑐᖅ ᑲᐅᔨᓴᕐᑕᐅᐸᑕ ᐃᓐᓄᐃᑦ ᐊᓂᐊᕕᒡ ᒎᓚᐅᔪᑦ, ᑕᐃᒪ ᐃᒡ ᓗᑕᖅ ᒍᑦ ᑕᖅᑐᒍᑦ ᐃᑎᖅ ᑎᑕᐅᓗᑎᒃ ᑲᐅᔨᓴᕐᑕᐅᑕᓚᔭᒪᑦᑕ. ᑕᐃᒪ ᑐᒃ ᑭᓯ-ᕙᓕᑦ ᒍᑎᒥᔭᐅᓇᔭᒃ ᑐᒃ ᓯᕗᖅ ᓚᖅ ᐸᒃ ᐃᓄᒃ ᐃᒡ ᓗᑕᖅ ᒍᑦ ᐃᑎᖅ ᑎᑕᐅᒍᒥ ᐃᒡ ᓗᑕᖅ ᒥᒃ ᑕᖅᑐᖅᒥᒃ ᑕᖅᓴᒡᒥᑦ ᐸᑦ ᑕᐃᒪ Vitamin "A"-ᓕ ᓇᒻᒪᒋᓐᒪᑦ. ᑲᐅᔨᔭᐅᓇᔭᒃ ᐳᒃ. ᐃᔨᒍᑦ ᓇᒻᒪᓯᐊᓚᓯᐊᒥᐊᒪᑦᑕ ᑕᐅᑐᒃ ᓂᒍᑦ ᑕᖅᑐᒍᑦ 3-4-ᒥᓂᑦ ᐱᐊᓂᒃ ᑲᓕᑦ ᐃᔨᒍᑦ ᑕᐅᑐᓯᐊᓚᔭᒃ ᒪᑦᑕ ᐃᒡ ᓗᑕᖅᒥ ᑕᖅᑐᒥ. ᑕᐃᒪ ᑲᐅᔨᒪᒍᓯ ᐃᒡ ᓗᑕᖅᒥ ᑕᖅᑐᒍᑦ ᐃᑎᖅ ᑐᓐᓂ ᑲᑦ ᓚᓂᒃ ᑕᐅᑐᒃ ᓇᕐᓐᒪᑦ ᑭᓯᐊᓂᓚ ᐱᖅᑯᑎᑦ ᑕᒃ ᑯᓐ ᓇᓚᓗᐊᒃ ᓗᑎᒃ. ᐱᖅᑯᑎᑦ ᑲᐅᔨᒪᓇᓚᓗᐊᓗᑎᒃ ᑕᖅᑐᒍᑦ ᐃᑦ ᑎᖅ ᑐᓐᓂ ᐃᒡ ᓗᑕᖅ ᒍᑦ.

ᐃᑦᓚᓂ Vitamin "A"-ᑲᑎᐊᒥᑐᑲᓚᐃᑐᓚᓗᐊᒃ ᑎᓗᒍ ᐃᓄᒡ ᓂᒃ, ᑭᓯᐊᓂ ᑲᐅᔨᓴᓚᐅᔭᑲᓚᓚᑕ ᐊᐅᓯᐊᒥᒪᓚᑕ ᐃᓚᒋᑦ ᐊᐅᒡ ᒥᒡ ᑯᑦ Vitamin "A"-ᒥᑦ ᓇᒻᒪᒥᑦ ᐸᒡ ᒪᑦᑕ. ᑕᐃᒪᓚ ᑭᓯᐊᓂ ᑲᐅᔨᓴᕐᑕᐅᓗᓯ ᑐᑭᓯᔭᐅᓯᐊᓂᓴᐅᓇᔭᒃ ᐳᓯ ᓱᒃ? Vitamin "A"-ᓯ ᓇᒪᒋᓐᓂᖅ ᑲᒃ ᒪᓚᑦ. ᑕᒻᓇ ᑐᒃ ᑭᓯᕙᓕᑦ ᒍᑕᐅᓇᓗᒃ ᑐᒃ ᐃᒃ ᑲᓚ-ᓂᖅ ᑲᐸᓕᖅ ᓚᔭᒃ ᒪᑦ ᑕᐸ ᑯᓄᒡ ᐃᓄᒡ ᓄᑦ ᓱᒃ? Vitamin "A"-ᒥᑦ ᓇᒻᒪᒋᓂᖅ ᑲᒃ-ᒪᒡᓚᑕ ᐊᒪᓗ ᑯᒃ ᓴᓇᒃ ᓯᒥᓚᐅᒃ ᑎᓗᒍ ᑐᒃ ᑭᓯᔭᐅᑲᒡ ᓂᓴᐅᓇᔭᒃ ᒪᑦ.

ᑕᐃᒪᓚ ᐅᕙᒍᑦ ᑲᐅᔨᓴᒃ ᑎᑎᔨᑎᒍᑦ Vitamin "A"-ᑲᑎᐊᒪᓚᐸᓯ ᐃᓚᐸᓯᒡ ᓂᒃ ᐊᐅᒡᑕᐅᑎᑎᓇᔭᒃ ᐳᒍᑦ ᐊᓐ ᓂᐊᕕᒡ ᒥᑎᓗᓯ ᓇᔪᒡᐊᓗᓐ ᓂᑦ ᐅᕙᓗᕙ ᐊᐅᒡᔭᐃᔨᑦ ᓇᔪᒡᐃᑦ ᐊᓐ ᓂᐊᕕᒥ (20 ml)-ᑐᒥᒃ ᑲᐅᔨᓴᓚᒃ ᓴᒥᓐ ᓂᒃ ᐊᐅᒡ ᒥᒃ ᐱᖅᓯᕕᒥᓇᔭ-ᒪᑎᑦ ᑲᐸᒎᑦ. ᐊᒪᓗ ᑕᐃᒪ ᐃᒡ ᓗᑕᖅ ᒎᑦ ᑕᖅᑐᒎᑦ ᐃᑎᖅ ᑎᑕᓚᔭᓚᐸᑎᒋᑦ ᐱᖅᓚᓯ-ᐃᒃ ᑕᓗᑎᑦ ᐊᒃ ᑯᓐ ᓂᐅᓂᒡᑦ ᓇᒻᒪᑲᑎᒥᑎᐊᒥᐊᒃ ᐸᓗᒥᑦ 20-ᒥᓂᑦ ᐱᐊᓂᒃ ᐸᑦ ᐱᐊᓂᓚᖅ-ᓗᑎ. ᑭᒡᒍᓚᖅ ᐸᒥᒃ ᓚ ᐊᐱᖅ ᓯᓚᖅ ᓚᔭᓚᐸᑎᒋᑦ ᓱᓐ ᓇᓂᒃ ᓂᖅ ᒥᕙᒡ ᒪᓚᐱᑦ ᐃᓚᐸᓯᒡ ᓂ.

ᐊᕐᒍᒥᒃ ᐃᓚᒡ ᓂᑦ ᐱᔭᒥᐊᑲᓚᐸᑕ. ᑕᐃᒪ ᑲᐅᔨᓴᕐᑕᐅᓗᑭᓯᐊᓂᖅ ᒍᕕᑦ ᐊᐅᒥᓚ-ᐅᓗᑎ ᐱᔭᒥᐊᑲᒃ ᐳᑎᑦ. ᐊᒪᓗ ᑐᒃ ᑭᓯᓯᒪᓯᐊᒥᐊᑲᒃ ᐳᑎᑦ ᐊᕐᒥᑲᓗᐊᒃ ᑎᓗᑎᑦ ᐸᓐ ᓂᐊ-ᕕᒡ ᒥ ᑲᐅᔨᓴᒃ ᑕᐅᓂᖅ ᒥᕙᒃ ᑕᐃᑦ ᓱᓐ ᓂᓚᕐᑐᖅ ᐱᑦ ᓯᐸᑕᐅᑐᐃᓐ ᓇᓚᔭᑐᑎᑦ ᐱᖅᑯᓯᑎᒃ ᒪᑦ ᓚᓗᒥᑦ. ᐊᒪᓗ ᑕᐃᒪ ᐊᕐᓂᖅ ᒍᕕᑦ ᑲᐅᔨᒪᔭᒪᒋᒍᑎᑦ ᑲᓐ ᓄᐃᓚᐅᒪᓚᑦ ᑲᐅᔨᓴᓚᒃ ᓴᑦ ᐊᐅᑦ ᐱᓚᐅᒃ ᑕᒍᑦ. ᑐᒃ ᑭᓯᓯᒪᓯᐊᒥᐊᑲᒥᒍᑎᓗ ᑭᑦ ᑐᒥᐊᓄᑦ ᐅᖅᑳᓗᑭᒥᐊᓚᒥᓇᐸᑕ ᑲᐅᔨᓴᕐᑕᐅᓚᐅᓂᖅ ᓚᓗᑎᑦ ᐃᒡ ᕕᑐᐊᒃ ᐱᖅᓚᖅ ᒥᔨᒥᓚᐅᑕᐃᑦ ᓗ ᑲᐅᔨᒪᓇᔭᓚᐸᑎᒃ.

ᐅᕙᖓ ______________________ (ᐊᑦ ᑕᑕᐅᔪᖓ, ᐊᓐ ᓇᓇᐅᔪᖓ, ᐅᕙᓗᓐ ᓂᑦ ᒥᐊᓂᖅ-ᓯᔨᐅᔪᒃ) ᐊᕐᓯᒪᒍᖓ ᐊᑉ ᐱᖅ ᓯᑕᐅᓂᐅᑉ ᒥᒃᓴᓐ ᓄᑦ ᐊᓐ ᓂᐊᕕᐅᑉ ᐱᖅᑯᓯᑯᑎᒋᑦ ᒪᓚᑦ ᓯᒋᑦ. ᑕᐃᒪ ᐊᐅᒥᑕᐅᓂᐅᑉ ᒥᒃᓴᓐ ᓄᑦ ᐊᕐᐳᖓ ᐊᒪᓗ ᓄᑦ ᑕᓕᓕ ᐊᕐᒍᑎᒥᕙᓕ ᐃᒥᓂᒃ ᐊᕐᒍᑎᓂᖅ ᐊᔭᖅ ᒪᑦ ᐅᒃ ᑭᐅᑭᓗᐊᒃ ᒪᑦ ______________________ ᑐᒃ ᑭᓯᕙᓕᑦ ᒍᑎᓴᒥᒃ Vitamin "A" ᒥᒃ ᓴᓐ ᓄᑦ ᑐᒃ ᑭᓯᓯᒪᐸᓗᓕᓗ ᐊᕐᒥᑲᓗᐊᒃ ᑎᓗᖓ ᐊᓐ ᓂᐊᕕᒃ ᒥ ᑲᐅᔨᓴᕐᑕᐅᓂᖅ ᒥᕙᒃ ᑕᖓ ᓱᓐ ᓂ-ᓚᕐᒥᒪᑦ ᐃᒃ ᑲᔭᑕᐅᑎᐊᑐᐃᓇᓂᐊᒃ ᒪᑦ ᑕᒻᓇ ᓄᑦ ᑕᓕᒃ ᐊᒪᓗ ᐊᕐᓚᐅᓕᓗᐊᒃ ᓗᖓ ᑭᐸᒃ ᓯᒍᒪ ᐊᑦ ᑎᓕ ᐱᑐᐃᓐ ᓇᒥᐊᑲᓚᐸᑯ ᑲᐅᔨᓴᑕᓯᐊᒥᓚᖅ ᒍᒪ ᑕᐸᓯᒪ ᒥᒃ ᓴᓐ ᓄᑦ.

______________________________ (ᐊᑦ ᑎᑦ)

______________________________ (ᐊᑦ ᑎᓚᐅᕕᒃ)

______________________________ (ᐊᕐᑎᓗᑎᑦ ᑕᐅᑐᒃ ᑎᒥᓚᐅᒃ ᑲᑦ)

Appendix C:

RAPID DARK ADAPTATION TEST.

SUBJECT DESCRIPTION. DAY'S DATE _____

SUBJECT NUMBER _____

NAME _____

AGE _____

SEX (M) or(F) _____

WHEN DID YOU ARRIVE IN WINNIPEG? _____

TYPE OF MEDICAL PROBLEM _____

_____

_____

ON MEDICATION NO OR YES _____

IF YES WHAT? _____

_____

ARE YOU EXPECTING? NO OR YES _____

IF YES GIVE GESTATION AGE _____

ARE YOU ON BIRTH CONTROL PILLS?
NO OR YES _____

IF YES WHAT? _____

WHEN DID YOU LAST EAT? _____

TIME THE BLOOD SAMPLE WAS DRAWN _____

TIME THE BLOOD WAS CENTRIFUGED _____

Appendix C: cont.

RDAT TIMES: END OF WHITE: END OF BLUE AND RED:

FIRST TIME: _____

SECOND TIME: _____

THIRD TIME: _____

MEAN OF THE LAST TWO TIMES: _____

HAEMOGLOBIN LEVELS _____

HEAMATOCRIT COUNT _____

PLASMA RETINOL LEVELS _____

SERUM ZINC LEVEL _____

SERUM COPPER LEVEL _____

DIET HISTORY:
RETINOL EQUIVALENCE _____

TOTAL ENERGY _____

RETINOL CONCENTRATION _____

Appendix D:

BLOOD AND RDAT DATA:

SUB	S	A	HB	HMTC	MRW	MRDAT	SRDAT	SZC	SCP	PRET	PRE
003	1	38	18.9	54.0	46.0	214.0	207.0	10.6	17.7	79.3	N
004	1	21	12.8	38.0	19.0	186.0	137.0	12.0	15.5	60.2	N
005	1	19	8.2	23.0	36.5	135.0	107.0	7.9	41.1	30.0	Y
006	1	19	8.0	32.0	26.5	207.0	203.0	7.2	39.2	29.8	Y
007	1	41	13.9	37.0	56.0	237.0	190.0	9.6	19.2	37.4	N
008	1	38	16.0	38.0	60.0	157.0	150.0	12.8	20.4	53.0	N
009	1	68	12.5	36.0	117.0	348.0	300.0	7.8	23.7	49.2	N
010	1	19	14.0	45.0	28.0	213.5	182.0	7.8	16.5	46.3	N
011	1	46	12.1	34.0	34.0	171.5	145.0	9.6	24.2	34.7	N
012	2	34	7.0	42.5	30.0	193.0	161.0	11.2	21.3	51.4	N
013	1	40	13.8	40.0	27.5	172.0	144.0	13.4	17.3	63.9	N
014	1	24	14.1	39.5	27.5	225.0	150.0	8.2	33.2	62.0	N
015	2	32	16.7	45.5	29.5	216.0	165.0	8.9	15.0	78.4	N
016	1	28	14.2	39.0	32.5	175.0	155.0	8.6	14.2	35.5	N
017	1	19	14.2	33.0	37.5	255.0	180.0	8.0	32.0	38.6	N
018	1	33	11.8	31.5	40.0	227.5	190.0	6.8	31.5	38.5	Y
020	1	30	16.0	29.0	34.5	225.0	190.0	6.7	29.0	45.2	Y
021	1	23	15.3	39.0	45.0	197.5	185.0	8.2	30.3	39.0	Y
022	1	21	12.5	38.5	72.5	256.5	183.0	10.0	15.9	38.9	N
023	1	21	14.2	41.0	52.5	255.0	250.0	8.3	17.5	36.2	N
024	1	29	12.6	36.5	33.0	213.0	182.0	10.0	12.5	39.4	N
025	1	36	14.7	41.0	30.0	252.5	190.0	12.4	19.8	57.0	N
027	1	62	18.0	57.0	57.5	282.5	229.0	10.2	27.9	31.0	N

Appendix D: cont.

028	1	73	13.2	42.0	109.5	208.0	195.0	10.8	22.3	52.9	N
029	2	40	15.0	45.0	40.0	163.5	141.0	12.4	19.2	73.8	N
030	1	29	11.2	33.0	41.5	245.5	190.0	7.8	38.3	43.4	Y
031	1	37	13.9	41.0	34.0	220.0	170.0	11.9	21.1	52.9	N
032	1	22	12.2	33.0	27.5	152.5	130.0	7.0	40.6	.	Y
033	1	65	14.4	40.0	112.5	255.0	210.0	11.1	22.1	32.4	N
034	1	18	12.7	39.5	29.0	169.0	135.0	11.4	14.3	38.4	N
035	1	18	11.7	36.0	37.5	135.5	120.0	8.2	20.5	34.2	N
036	1	34	13.4	40.0	37.5	187.5	170.0	12.6	32.2	45.2	N
038	1	34	17.4	45.0	32.5	188.0	166.0	12.9	17.4	64.4	N
039	1	19	15.3	42.0	24.5	220.0	180.0	11.7	17.7	43.0	N
042	1	31	13.9	39.0	31.0	271.0	242.0	8.2	30.2	31.3	Y
043	2	23	16.6	47.0	41.5	235.0	215.0	12.7	16.1	75.8	N
044	2	21	16.8	48.0	45.0	160.0	125.0	13.2	10.9	63.6	N
045	1	20	11.9	34.0	38.0	198.0	195.0	7.0	23.1	23.1	Y
046	1	28	15.7	44.0	38.0	189.0	181.0	8.9	19.4	48.0	N
047	1	27	10.8	30.0	40.5	229.5	217.0	7.5	33.1	36.0	Y
048	1	31	13.2	36.0	39.0	277.5	240.0	11.2	32.2	43.5	Y
049	1	25	12.2	35.0	44.5	242.0	224.0	9.7	30.9	43.6	Y

Appendix E:

Meaning of abbreviations:

SUB = The code number of the subject -->ID= 003-049

S = Sex Female or Male.Categorized, Female(1) and Male(2)

A = Age: The respective age of the subjects in years.

HB = Haemoglobin level in gm/dl (Normal=F -> 11-16 gm/dl)
(=M -> 13-18 gm/dl)

HMT = Haematocrit count % (normal=F -> 37-47%)
(=M -> 40-54%)

MRW = Mean rapid dark adaptation time white chips in seconds

MRDAT = Mean rapid dark adaptation Test times in seconds

SRDAT = Rapid dark adaptation test shortest time in seconds

SZC = Serum zinc in umol/l (normal = 10.0-19.0 umol/l)

SCP = Serum copper in umol/l (normal = 12.0-20.0 umol/l)

PRET = Plasma retinol level in ug/dl (normal 32-90 ug/dl)
(F-> 32-80 ug/dl)
(M-> 38-93 ug/dl)

PRE = Pregnancy status categorized as Pregnant(Y) or (N).

Appendix F:

Statistics

SAS

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE INFORMATION

NUMBER OF OBSERVATIONS IN DATA SET = 42

DENT VARIABLE: PRETINOL

E	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
	3	116.76040492	38.68346864	0.17	0.9143	0.014155	31.6626
	38	8054.11359108	223.74093308		ROOT MSE		PRETINOL MEAN
CTED TOTAL	38	8171.88400000			14.95830824		47.82000000

E	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE III SS	F VALUE	PR > F
	1	0.00183281	0.00	0.9877	1	94.08248078	0.42	0.5208
	1	28.33387323	0.13	0.7240	1	38.88803280	0.17	0.6768
ENER1	1	87.41480278	0.39	0.5369	1	87.41480278	0.39	0.5369

ETER	ESTIMATE	T FOR H0: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
CEPT	45.65119788	3.07	0.0041	13.32437594
	0.00687313	0.65	0.5208	0.01337468
	0.00346307	0.42	0.6768	0.00587803
ENER1	-0.0000031	-0.63	0.5369	0.00000482

Pretinol=Plasma retinol values
Vital =Vitamin A (dietary intake)
Enerl =Energy intake

UNIVARIATE

VARIABLE=HMTC

MOMENTS

N	42	SUM WGTS	42
MEAN	39.0357	SUM	1639.5
STD DEV	6.38347	VARIANCE	40.7487
SKEWNESS	0.372366	KURTOSIS	1.28597
USS	65669.7	CSS	1670.7
CV	16.3529	STD MEAN	0.984991
T:MEAN=0	39.6305	PROB>|T|	0.0001
SGN RANK	451.5	PROB>|S|	0.0001
NUM ^= 0	42		
W:NORMAL	0.978513	PROB<W	0.7

QUANTILES(DEF=4)

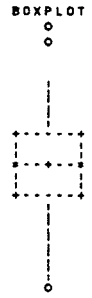
100% MAX	57	99%	57
75% Q3	42.125	95%	53.1
50% MED	39	90%	46.55
25% Q1	34.75	10%	31.65
0% MIN	23	5%	29.15
		1%	23
RANGE	34		
Q3-Q1	7.375		
MODE	33		

EXTREMES

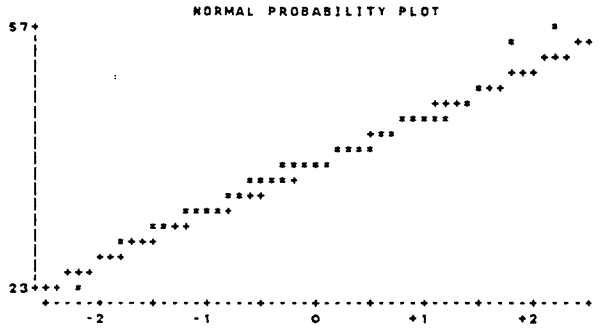
LOWEST	HIGHEST
23	45.5
28	47
30	48
31.5	54
32	57

```
STEM LEAF                            #
  56 0                               1
  54 0                               1
  52
  50
  48 0                               1
  46 0                               1
  44 00005                           5
  42 005                             3
  40 000000                          6
  38 00500055                        8
  36 00050                           5
  34 000                             3
  32 0000                            4
  30 05                              2
  28 0                               1
  26
  24
  22 0                               1
     ----+----+----+----+
```

BOXPLOT



NORMAL PROBABILITY PLOT



FREQUENCY TABLE

VALUE	COUNT	PERCENTS CELL	PERCENTS CUM
23	1	2.4	2.4
28	1	2.4	4.8
30	1	2.4	7.1
31.5	1	2.4	9.5
32	1	2.4	11.9
33	3	7.1	19.0
34	2	4.8	23.8
35	1	2.4	26.2
36	3	7.1	33.3
36.5	1	2.4	35.7
37	1	2.4	38.1
38	2	4.8	42.9
38.5	1	2.4	45.2
39	3	7.1	52.4
39.5	2	4.8	57.1
40	3	7.1	64.3
41	3	7.1	71.4
42	2	4.8	76.2
42.5	1	2.4	78.6
44	1	2.4	81.0
45	3	7.1	88.1
45.5	1	2.4	90.5
47	1	2.4	92.9
48	1	2.4	95.2
54	1	2.4	97.6
57	1	2.4	100.0

*HMTC = Haematocrit count values.

UNIVARIATE

ABLE=HB

MOMENTS

	42	SUM WGTS	42
	13.6429	SUM	573
DEV	2.5027	VARIANCE	6.26346
NESS	-0.474012	KURTOSIS	0.858708
	8074.16	CSS	256.803
	18.3444	STD MEAN	0.386174
AN=0	35.3262	PROB>|T|	0.0001
RANK	451.5	PROB>|S|	0.0001
A= 0	42		
RMAL	0.865296	PROB<W	0.372

QUANTILES(DEF=4)

100% MAX	18.8	99%	18.8
75% Q3	15.3	95%	17.91
50% MED	13.8	90%	16.77
25% Q1	12.2	10%	10.82
0% MIN	7	5%	8.03
		1%	7
RANGE	11.8		
Q3-Q1	3.1		
MODE	13.8		

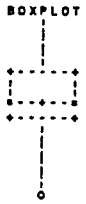
EXTREMES

LOWEST	HIGHEST
7	15.7
8	15.8
8.2	17.4
10.8	18
11.2	18.8

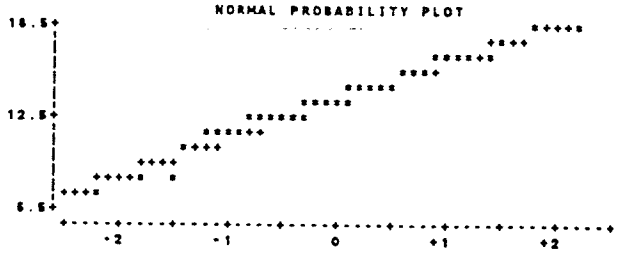
```
 LEAF                  #
 09                    2
  8                    1
 00678                 5
 0337                  4
 0122247               7
 2248899               7
 12255678              8
 2788                  4
 8                     1

 02                    2
 0                     1
----+----+----+----+
```

BOXPLOT



NORMAL PROBABILITY PLOT



FREQUENCY TABLE

VALUE	COUNT	PERCENTS CELL	CUM
7	1	2.4	2.4
8	1	2.4	4.8
8.2	1	2.4	7.1
10.8	1	2.4	9.5
11.2	1	2.4	11.9
11.7	1	2.4	14.3
11.8	1	2.4	16.7
11.9	1	2.4	19.0
12.1	1	2.4	21.4
12.2	2	4.8	26.2
12.5	2	4.8	31.0
12.6	1	2.4	33.3
12.7	1	2.4	35.7
12.8	1	2.4	38.1
13.2	2	4.8	42.9
13.4	1	2.4	45.2
13.8	1	2.4	47.6
13.9	3	7.1	54.8
14	1	2.4	57.1
14.1	1	2.4	59.5
14.2	3	7.1	66.7
14.4	1	2.4	69.0
14.7	1	2.4	71.4
15	1	2.4	73.8
15.3	2	4.8	78.6
15.7	1	2.4	81.0
16	2	4.8	85.7
16.8	1	2.4	88.1
16.7	1	2.4	90.5
16.8	1	2.4	92.9
17.4	1	2.4	95.2
18	1	2.4	97.6
18.8	1	2.4	100.0

HB= Haemoglobin values.

GENERAL LINEAR MODELS PROCEDURE

NDENT VARIABLE: AVL

	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
CE	3	11605.70499773	3868.56833258	17.20	0.0001	0.575876	34.6864
L	38	8547.41404989	224.93194868		ROOT MSE		AVL MEAN
R	41	20153.11904762			14.99773145		43.23809524
ECTED TOTAL							

	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE III SS	F VALUE	PR > F
ICE	1	7.38230413	0.03	0.8572	1	24.04879876	0.11	0.7455
NC	1	11443.63152183	50.88	0.0001	1	847.50738563	3.77	0.0597
NC*AGE	1	154.68111177	0.69	0.4121	1	154.68111177	0.69	0.4121

METER	ESTIMATE	T FOR HO: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
ERCEPT	-7.03709204	-0.21	0.8370	33.96453326
NC	1.14003433	0.33	0.7455	3.46661847
	3.14425308	1.94	0.0597	1.10466316
NC*AGE	-0.09147295	-0.83	0.4121	0.11030612

= Dark adaptation test for white chips.
nc= serum zinc values

GENERAL LINEAR MODELS PROCEDURE

ENDENT VARIABLE: AV2

RCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
EL	3	27422.18215251	9140.72738417	6.92	0.0008	0.353424	17.0300
OR	38	50167.96070463	1320.20949223		ROOT MSE		AV2 MEAN
RECTED TOTAL	41	77590.14285714			36.33468718		213.35714286

RCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE III SS	F VALUE	PR > F
NC	1	2586.77823494	1.96	0.1697	1	5398.03316119	4.09	0.0503
NC*AGE	1	13947.10067329	10.56	0.0024	1	14793.19314232	11.21	0.0018
	1	10888.30324427	8.25	0.0066	1	10888.30324427	8.25	0.0066

AMETER	ESTIMATE	T FOR HO: PARAMETER=0	PR > |T|	STD ERROR OF ESTIMATE
ERCEPT	5.05181488	0.06	0.9514	82.28515728
NC	17.08034456	2.02	0.0503	8.44695691
NC*AGE	-8.95849925	3.35	0.0018	2.67624410
	-0.76745718	-2.87	0.0066	0.26723631

V2 = Dark adaptation test for blue chips.
zinc = serum zinc values

Appendix G:

The types of foods and amount consumed by one subject who had excessive vitamin A (RE) intake per day took.

Foods	Amount/month
Goose meat	168 gm
Caribou frozen meat	304 gm
Caribou liver	3808 gm*
Caribou heart	104.5 gm
Caribou tongue	66.5 gm
Sausage	125 gm
Arctic char	895 gm
Chicken eggs	2
Goose eggs	1
Whole milk White	237 ml
Yogurt-fruit	175 gm

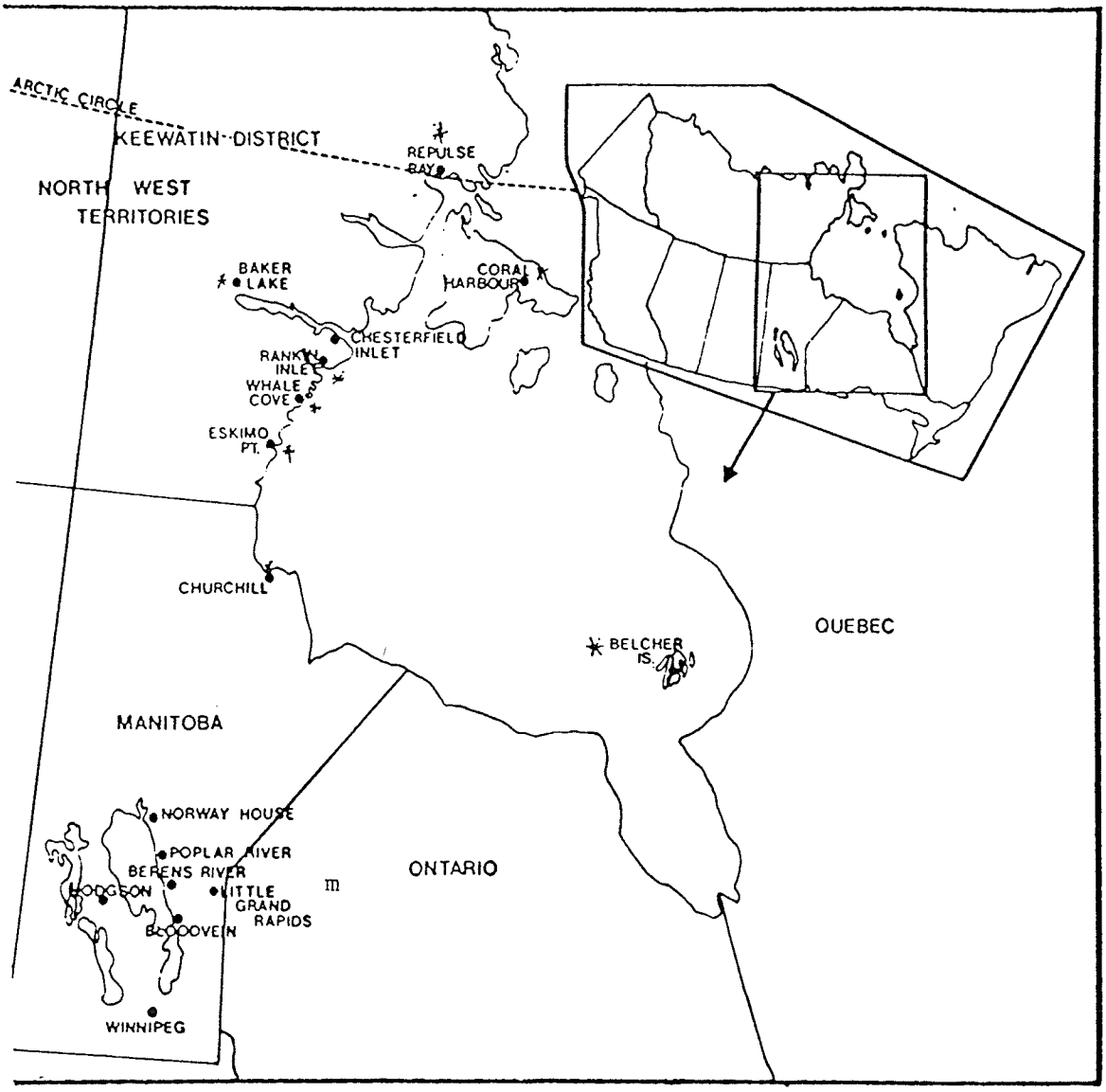
*such high amount of liver would result in quite high amount ofVitamin A Intake(RE).

Appendix H.

OBS	ID	PROT	FAT	CHO	ENER	ZINC	IRON	VITA
1	3	95.970	81.618	524.702	3061.23	5.2397	21.6436	1067.3
2	4	32.712	61.215	409.218	2152.20	2.3861	14.5316	347.5
3	5	156.811	127.617	475.676	3674.50	21.3978	24.3861	987.3
4	6	183.484	76.904	337.230	2778.40	9.2900	27.8203	3875.9
5	7	23.076	17.825	98.523	640.19	2.9918	5.4456	538.0
6	8	55.527	44.220	116.431	1064.84	5.9027	11.2045	506.6
7	9	43.188	35.004	141.815	1055.80	5.6402	8.8454	505.6
8	10	98.071	108.868	381.485	2815.86	13.0025	19.8776	1817.9
9	11	280.783	123.502	623.034	4853.37	14.9843	41.8373	1658.8
10	12	163.552	200.849	574.102	4748.32	17.6857	29.3334	1248.0
11	13	125.483	106.293	245.887	2455.62	11.0674	23.3814	834.9
12	14	192.281	145.671	309.574	3344.56	7.3208	32.2150	2280.6
13	15	202.837	162.235	267.820	3456.55	16.1527	34.7787	1446.8
14	16	81.814	83.854	272.207	1971.46	7.4300	17.8851	718.0
15	17	177.635	158.876	505.259	4032.68	11.8042	25.8638	1367.0
16	18	188.288	125.588	515.864	3860.78	10.4742	38.3342	1477.8
17	20	46.553	24.815	74.411	727.72	1.8563	7.0706	88.0
18	21	141.766	142.205	336.838	3177.07	11.0441	17.4417	1783.6
19	22	136.310	25.483	207.608	1611.47	1.8811	12.9402	353.7
20	23	75.556	68.036	359.139	2208.04	4.7832	16.8486	727.4
21	24	107.827	57.667	119.898	1427.69	5.7267	13.7266	348.6
22	26	57.343	42.523	226.578	1469.84	4.5373	12.4815	706.9
23	27	180.182	47.518	231.937	2082.18	2.9978	40.7690	374.1
24	28	69.561	9.229	24.407	477.03	1.7669	7.5449	973.7
25	29	192.423	20.187	71.188	1277.28	2.2850	20.5238	1202.3
26	30	84.283	36.224	203.231	1486.58	11.4882	23.2530	22222.1
27	31	328.858	192.044	457.866	4814.58	11.3880	72.4005	1852.8
28	32	150.784	71.505	381.846	2678.73	10.7096	23.5314	1272.2
29	33	85.051	28.435	40.182	787.38	2.8401	21.6287	2128.8
30	34	128.151	149.046	289.703	3008.08	12.1418	14.8145	1480.8
31	35	82.465	53.895	288.124	1857.38	6.7312	10.0377	598.1
32	36	93.598	143.253	389.207	3092.84	12.2817	19.2187	467.1
33	38	107.176	61.309	78.806	1226.05	6.8702	12.8726	948.0
34	39	46.992	55.252	268.547	1761.80	5.4832	11.8877	229.4
35	42	187.526	104.879	188.150	2378.89	13.8825	19.2844	1187.0
36	43	107.783	87.366	371.582	2780.52	7.8828	20.0221	577.4
37	44	78.752	83.168	527.751	3142.00	8.0389	14.8489	1170.4
38	45	58.886	52.006	471.548	2488.63	8.2036	12.0180	946.6
39	46	56.861	72.473	372.280	2368.64	8.1293	16.4461	4030.7
40	47	71.803	37.978	185.626	1378.54	4.1007	13.5740	1381.9
41	48	83.087	44.328	291.548	1767.04	5.5346	18.4859	922.4
42	49	139.394	87.780	256.528	2324.70	7.4745	20.8132	858.6

Nutrient intake per day for 42 subjects.

Appendix I.



A representative map of the area where the subjects came from.

Appendix J.

Diet history questionnaire. Some examples of the kind of questions asked.

SUBJECT NUMBER_____

RAPID DARK ADAPTATION TEST STUDY.

FOOD FREQUENCY INTERVIEW QUESTIONAIRE (DIET HISTORY).

PARTICIPANT_____

DATE_____

HELLO MY NAME IS ROSEMARY.

I AM DOINNG A STUDY ON DARK ADAPTATION TEST AND I WILL BE LOOKING AT THE FOODS THAT PEOPLE EAT, ESPECIALLY THOSE WHICH ARE SOURCES OF VITAMIN A AND HOW THESE MAY AFFECT PEOPLES' DARK ADAPTATION AND THEIR HEALTH. I WOULD LIKE TO ASK YOU SOME QUESTIONS ABOUT THE FOODS YOU EAT.

FOR EXAMPLE, I WOULD LIKE TO KNOW WHAT YOU HAVE BEEN EATING AND DRINKING OVER THE PAST MONTH, I WILL BE TALKING ABOUT MANY DIFFERENT KINDS OF FOODS SUCH AS: MEATS, FISH BREADS AND CEREALS.

OF THE FOODS I WILL TALK ABOUT, I WILL ASK YOU TO TELL ME WHICH FOODS YOU REMEMBER EATING DURING THE LAST MONTH. THEN I WILL ASK YOU HOW MANY TIMES YOU ATE THOSE FOODS, WHETHER IT WAS

EVERY DAY,

EVERY WEEK, OR

EVERY MONTH.

LET'S BEGIN BY TALKING ABOUT MEATS:

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	W	M	AMOUNT IN MLS/GMS	CODE	COMMENT
MEATS	Beef roast/steak/stew								
I would like to ask about meats. Method of cooking? Do you add fat when cooking? How much do you eat? How many times? Do you add gravy/sauce/ ketchup/ mustard/ mayonnaise etc? Do you usually eat the skin?	steakttes fried								
	ground -lean/regular								
	Plain hamburgers								
	Cheese hamburgers								
	Double hamburgers								
	Single hamburgers								
	Pork, chops fried								
	roast								
	Chicken, fried								

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	W	M	AMOUNT IN MLS/GMS	CODE	COMMENT
SPECIALTY MEATS	Organ Meats-Kidney								
From what animals?	Liver								
How often do you eat them?	Heart								
How much do you eat?	Tongue								
Method of cooking?	Tripe								
	Other								
	Prepared Meats								
Number of slices?	Bologna								
Size of slices?	Canned								
Size of sausage	Sausages								
method of cooking?	Weiners								

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	W	M	AMOUNT IN MLS/GMS	CODE	COMMENT
FISH	Shellfish(fresh)								
Now I would Like to ask you about fish. PLease tell me what type of fish you usually eat? How much do you eat? Method of cooking? When are they available? Was fat added when cooking? Was a sauce/ gravy added before eating? How often do you take them?	Pickerel								
	Whitefish								
	Pike								
	Sucker								
	Cisco								
	Lake trout								
	Sturgeon								
	Smoked fish								
	Other								
	Canned - Salmon								

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	W	M	AMOUNT IN MLS/GMS	CODE	COMMENT
EGGS	Chicken								
Do you usually eat eggs?									
How many?	Duck								
How often?									
Method of cooking?	Goose								
Do you usually add fat									
when cooking egges?	other								
Do you add anything to									
the eggs before you eat									
them?									
Do you eat any wild									
birds?									
How long are they									
available?									

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	W	M	AMOUNT IN MLS/GMS	CODE	COMMENT
DAIRY PRODUCTS	White milk - Whole								
Do you usually drink any kind of milk?	2% or 1% or Skimmed								
	NFDM								
How much?									
How many times?	Canned								
	Other								
	Unspecified								
	Yogurt - plain(%B.f)								
	fruit(%B.f)								
Regular or sugar free?	Chocolate milk -store								
	hommade( )								
Added milk/cream?	Pudding - chocolate								

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	W	M	AMOUNT IN MLS/GMS	CODE	COMMENT
BREADS AND ROLLS	Bread - White								
Now I would like to talk about breads and cereals	Homemade bread( )								
What kind of bread do you usually eat?	Brown (60%, 100%)								
How much?	Buns - Hot Dog								
How often do you eat it?									
Was any butter/margarine added?	Hamburger								
Was any Jam/jelly/ honey added?	Other								
	Muffins								
Name if a sandwich (filings)	Pancake/waffles								
	Other								
Did you add peanut butter?	Bannock								
Bannock recipe?									
Fried?	PASTA								

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	NO.OF. SERVINGS W	NO.OF. SERVINGS M	AMOUNT IN MLS/GMS	CODE	COMMENT
RICE	Rice _ White								
Do you usually eat rice?									
How much do you eat?	Instant								
Do you add fat?									
How often do you eat it?	Brown								
Do you usually add gravy									
or sauce?	Wild Rice								
	Other								
	CEREALS								
Which hot cereal do you									
usually eat?	Hot - oatmeal								
Do you add sweetener?	Cornmeal								
Do you add Milk/cream?	Cream of wheat								
Do you add fat?	Other								
What kind of cold cereals	Cold - Oatmeal								
do you usually eat?									

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	NO.OF. SERVINGS W	NO.OF. SERVINGS M	AMOUNT IN MLS/GMS	CODE	COMMENT
FRUITS	Berries -gooseberries								
During summer season do you eat any berries? How much do you eat? How many times? Do you add milk/cream? Do you add sweetener? Do you eat them with anything else? Please tell me if you usually eat any other fruits or drink any fruit juice?	saskatoons								
	blueberries								
	chokecherries								
	strawberries								
	raspberries								
	juice or Others								
	Apricots / Cantaloup								
	Apple, fresh, with skin								
	canned or Juice								
	Fresh grapes or plums								

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	W	M	AMOUNT IN MLS/GMS	CODE	COMMENT
VEGETABLES	Potatoes - boiled								
Do you usually eat potatoes?	baked or mashed								
Which Method of cooking?									
How much do you eat?	Panfried/french fried								
Do you add fat gravy?									
How many times?	Beet greens								
Add any milk / cream / butter?	Pumpkin								
	Broccoli								
Do you eat the skins?	Carrots								
Fresh/frozen/canned?	Peas								
Do you add fat?	Corn								
Do you add gravy/ sauce?	Turnip or onion								
	Lettuce								

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	W	M	AMOUNT IN MLS/GMS	CODE	COMMENT
SOUPS	Cream, made with milk								
What kind of soup do you usually eat?	with water								
Method of cooking?	Other								
How much do you take?									
How many times?									
Do you usually eat crackers with the soup?	Noodle, canned								
	Dry								
	Other, commercial								

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	W	M	AMOUNT IN MLS/GMS	CODE	COMMENT
BEVERAGES	Coffee - Instant								
Now I would like to talk about drinks	Perked								
Do you add any sweetener?	Other								
	Tea								
Regular or diet?	Soft drinks - Cola								
How much do you drink? How frequently do you drink it?	Other								
	Alcohollic - Beer								
	Wine								
Rye/Gin/Rum/Vodka How much? How often?	Distilled								
	Whisk								

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	W	M	AMOUNT IN MLS/GMS	CODE	COMMENT
DESSERTS	Ice Cream - Chocolate								
The last group of foods that I would like to ask about are desserts and snack foods.	Vanila								
	Other								
What do you usually eat for dessert?	Ice Cream bar/sandwich								
	Cake - Brownies								
With Icing?	Chocolate								
Do you add anything to the ice cream?	White								
How much?	Other								
How often?	Pie								
	Cookies - Commercial								
	Homemade								

QUESTION	ITEM	DESCRIPTTION	MODEL	NO.OF. SERVINGS D	NO.OF. SERVINGS W	NO.OF. SERVINGS M	AMOUNT IN MLS/GMS	CODE	COMMENT
SNACK FOODS	Cheese curls								
Do you usually eat any kind of candy?	Chocolate bar( )								
How many or how much?	Candy - gumdrop								
How many times?	jelly type / Caramel/								
	toffee / hard								
	Potato chips( )								
Do you use a dip?	Taco chips								
	Other								
	Nuts- Peanuts/almonds								
	Mixed /Pistachio								
	popcorn								