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VARIATIONS IN CELLULAR LEVELS OF BETA-CAROTENE BEFORE AND AFTER SUPPLEMENTATION WITH BETA-CAROTENE

by

Ann-Marie Gilbert

B.Sc (Kines) Simon Fraser University, 1985

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

in the School of Kinesiology

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Simon Fraser University

1993

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APPROVAL

NAME: Ann-Marie Gilbert

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EXAMINING COMMITTEE:

CHAIR: Dr. N.M.G. Bhakthan

Dr. Miriam Rosin
Senior Supervisor

Dr. Allan Davison

Dr. Bruce Dunn

Dr. T. Greg Hislop
External Examiner
Division of Epidemiology, Biometry
& Occupational Oncology
Cancer Control Agency of B.C.

Date Approved: March 16/93

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Variations in cellular levels of beta-carotene before and after supplementation with beta-carotene

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ABSTRACT

Epidemiologic and experimental studies support a protective function for ß-carotene in carcinogenesis. Yet little is known about cellular levels of ß-carotene and the factors that modulate accumulation of this carotenoid in tissues. In this thesis, cellular levels of ß-carotene were examined, before and after 3 days supplementation with 90 mg ß-carotene per day, by collecting oral mucosa cells and assessing their ß-carotene content.

In Study 1, intra- and inter-individual variations in ß-carotene levels were examined in a Vancouver group by collecting samples on two occasions before supplementation and 7, 14 and 21 days after supplementation. Cellular levels of ß-carotene varied little within 62 individuals over a one week interval without supplementation: mean intra-individual variation was 1.22 ng/10^6 cells. Mean levels increased from 2.2 (± 2.52) to 6.80 (± 3.91) ng/10^6 cells in 52 individuals after supplementation. Inter-individual variation did not differ greatly before and after supplementation. ß-carotene levels before supplementation ranged from 0.29 to 15.2 ng/10^6 cells while after supplementation the range was 1.09 to 17.7 ng/10^6 cells. Samples obtained at day 7 after supplementation had the highest mean ß-carotene content: by day 14 ß-carotene levels had decreased by 3.46 ng/10^6 cells. ß-carotene levels approximated pre-supplementation levels by day 21. Presupplementation levels of ß-carotene did not determine the change in ß-carotene content with supplementation.

Four groups totaling 186 individuals from differing areas of B.C. (Vancouver, Lytton, Williams Lake and One Hundred Mile House) participated in Study 2 that examined the effects of alcohol, tobacco and diet on cellular levels of ß-carotene before and after supplementation. Cell samples were collected on day 0 and 7 days after supplementation (supplemental regimen as for Study 1). Initial

levels of ß-carotene did not differ between the study groups; however, day 7 ß-carotene levels of Williams Lake and One Hundred Mile House were significantly greater than Lytton and Vancouver ($p<0.001$). Females had higher ß-carotene levels than males, with post-supplementation levels of 8.92 compared to 8.12 ng/10^6 cells ($p<0.003$). Alcohol intake negatively affected cellular levels of ß-carotene. Individuals consuming greater than 250ml/week had 65% of the cellular ß-carotene of non-consumers after supplementation ($p<0.05$). Tobacco and alcohol interacted with both factors negatively affecting cellular levels of ß-carotene ($p<0.02$). Mean level of ß-carotene after supplementation among snuff users was 13.46 versus 7.15 ng/10^6 cells among non-users; however, this may be attributable to significantly higher carrot intake by snuff users (6.1 carrots per week) in comparison with non-users (1.86 carrots per week) ($p<0.005$). Carrot intake accounted for 18% of the variation in pre-supplementation levels of ß-carotene and 6.3% of the variation in post-supplementation levels of ß-carotene. Carrot intake was the best predictor of cellular levels of ß-carotene before supplementation whereas initial levels of ß-carotene along with consumption of tobacco, snuff and alcohol best predicted day 7 ß-carotene levels. Multiple linear regression accounted for 31% of the variance in day 7 ß-carotene levels. Responses to supplementation were consistent among a subgroup of individuals completing a second supplemental regimen 1 year later. Clearly, individuals respond differently to ß-carotene, thus dietary assessment of ß-carotene intake may not be an effective predictor of cellular levels of ß-carotene.

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I. LITERATURE REVIEW

I.1. Prospective use of ß-carotene in chemoprevention studies

"Chemoprevention" is the introduction of selected natural and synthetic substances into the diet for the purposes of reducing cancer risk (Malone,W.F., 1991). The low toxicity of ß-carotene and its putative anticarcinogenic activity make it an ideal agent to use in chemoprevention trials (Boone, C.W., et al, 1990, Mathews-Roth, M.M. 1988, Haveland-Smith, R.B., 1981, Heywood, R., 1985, Bendich, A., 1988). Intervention trials with ß-carotene are underway in an effort to reduce overall risk of cancer by preventing or reversing the carcinogenic process (Benner, S.E. et al, 1992). Among individuals taking supplemental ß-carotene, chemoprevention of cancers of the lung, oral cavity, skin and colon is being evaluated (Ziegler, R.G. et al, 1992, Malone, W.F., 1991, Greenwald, P. 1992). In addition to assessing cancer risk, these studies will evaluate the need for long term therapy and the optimal route of administration (Kelloff, G.J., et al, 1992). These studies will take between 2 and 30 years to conclude and will assess the ability of ß-carotene to *prevent* cancers.

As carcinogens cause damage to cellular components, including the DNA, it is probable that ß-carotene must be present within the cell to exert a protective effect. In the United States, the National Cancer Institute recommends intake of 5 or more servings of vegetables and fruits per day to reduce cancer risk (Zeigler, R.G., 1992). The impact of this quantity of dietary ß-carotene on cellular levels is unknown as little is known about cellular levels of ß-carotene or the accumulation of the carotenoid in human tissues. A specific mechanism for the uptake of ß-carotene in tissues has not been established, a distinct pattern of

accumulation by the tissues suggests that ß-carotene uptake is not entirely passive (Kaplan, L.A. et al, 1990, Schmitz, H.H. et al, 1991).

This study addresses the issue of accumulation of ß-carotene within cells by examining ß-carotene levels in oral mucosa cells before and after short-term supplementation. Intra- and inter-individual variation in ß-carotene levels and the effects of dietary ß-carotene, alcohol and tobacco on these levels are assessed.

I.2. Studies evaluating ß-carotene and cancer risk

The hypothesis that ß-carotene has chemopreventive activity has received considerable support from epidemiological studies in the past decade. Many research studies have evaluated ß-carotene and cancer risk and comprehensive reviews of these studies are available (Singh, V.N. and Gaby, S.K., 1991, Comstock, G.W. et al, 1992, Zeigler, R.G., 1989, Malone, W.F., 1991, Weisburger, J.H., 1991, Garewal, H.S., 1991). It is timely to summarize epidemiological research with ß-carotene.

Reports in the literature consistently indicate an inverse association between lung cancer risk and ß-carotene. Table 1 summarizes recent retrospective studies. Lung cancer risk decreases with increasing intake of green and yellow vegetables in Yunnan Province, China (Swanson, C.A., et al, 1992). Inverse associations between dietary intakes of ß-carotene, carrots or carotenes and lung cancer rates are reported in the literature (Harris, R.W.C., et al, 1991, Fontham, E.T., et al, 1988, Le Marchand, L., et al, 1989). These studies indicated that individuals with higher levels of serum ß-carotene compared to lower serum levels were not as likely to be lung cancer patients. It is interesting that analyses that controlled for smoking also report a protective effect of ß-carotene. Smokers have lower serum levels of ß-carotene; however, this effect may be in part due to lower intake of ß-carotene-containing foods. Since the association between dietary intake of ß-carotene and smoking is inverse (Le Marchand, L. et al, 1991), it follows that inhalation of smoke may alter appetite and result in decreased intake of ß-carotene.

Table 1 Retrospective studies evaluating the relationship between ß-carotene and lung cancer risk

Study population	Data collection	N	Association with lung cancer risk	Ref
United Kingdom, hospital based (males)	dietary carotene and serum ß-carotene	96 cases, 97 controls	inverse for dietary carotene inverse for serum ß-carotene	1
Louisiana, USA, hospital based	dietary carotenes	1253 cases, 1274 controls	inverse for carotene intake	2
Hawaii, USA, multiethnic origin	dietary ß-carotene intake	332 cases, 865 controls	inverse for ß-carotene intake or carrot intake	3
Hawaii, USA	carotene intake	325 cases, 627 controls	inverse for carotene intake	4
Yunnan Province, China	dark green leafy vegetables	428 cases, 1011 controls	inverse for vegetable intake	5

(1)Harris, R.W.C., et al, 1991, (2) Fontham, E.T., et al, 1988, (3)Le Marchand, L., et al, 1989, (4) Hinds, M.W., et al, 1984, (5) Swanson, C.A. et al, 1992.

The prospective studies (Table 2), that collected data before the development of cancer and measured cancer incidence or mortality in the following years, provide convincing evidence of an inverse relationship between serum ß-carotene and lung cancer risk. Individuals with higher serum levels of ß-carotene are not as likely to develop lung cancer as those individuals with lower levels of ß-carotene (Comstock, G.W. et al, 1991, Menkes, M.S, et al, 1986, Stahlein, H.B., et al, 1991, Stahlein, H.B., et al, 1984, Knekt, P., et al, 1990). In contrast, cancer rates among elderly individuals do not decrease with high levels of ß-carotene intake; but the authors suggest that unreliable food intake measures masked the protective effect of ß-carotene (Paganini-Hill, A., et al,

1987). In another study, age adjusted relative risk for lung cancer in men decreases for those consuming higher (relative to lower) levels of ß-carotene intake. The adjustment for smoking attenuates the relationship (Shibata, A. et al, 1992). A third study that adjusted for the effects of smoking indicates a significant elevation in lung cancer risk associated with a dietary intake of ß-carotene less than 3000 IU per day (Shekelle, R.B. et al, 1992).

Table 2 Prospective studies evaluating the association between ß-carotene and lung cancer risk

Study populations	Data collection	N	Association with lung cancer risk	Ref
Washington County, Maryland, USA	serum ß-carotene	99 cases, 196 controls	inverse for serum ß-carotene	5, 6
Basel, Switzerland	serum carotenes for cohort of 2874 males (80% ß-carotene) mortality assessment	2874 males, 68 cases	inverse for serum carotenes	7, 8
Finnish	serum ß-carotene for cohort of 36,265	152 cases, 284 controls	inverse for males no association for females	9
Hawaii, USA, multi-ethnic group	dietary ß-carotene for cohort of 11,894	53 lung cancers	no association	10

(5) Comstock, G.W. et al, 1991, (6) Menkes, M..S. et al, 1986; (7) Stahlein, H.B. et al, 1991, (8) Stahlein, H.B. et al, 1984; (9) Knekt, P. et al, 1990, (10) Paganini-Hill, A. et al, 1987,

Although retrospective and prospective studies indicate that ß-carotene may protect against the development of many cancers, a gathering body of evidence suggests that ß-carotene is specifically protective against certain cancers (Tables 3 and 4). Individuals with high serum ß-carotene or high intake of ß-carotene may be protected against oral, pharyngeal and esophageal cancers. Studies of bladder cancer show consistent protection by ß-carotene in case-control and cohort studies. ß-carotene intake and serum levels of ß-carotene correlate inversely with stomach cancer rates in 3 studies. Reports on the

association between cervical or prostatic cancer and ß-carotene conflict. Risk reduction by ß-carotene for solid organ tumours is inconclusive at this time.

Table 3 Prospective studies showing an association between ß-carotene and cancer risk for sites other than the lung

Study population	Data collection	Association with cancer risk	Ref
Hawaii, USA, multi-ethnic group	dietary ß-carotene	inverse for bladder	10
Washington County, Maryland USA	serum ß-carotene	inverse for bladder no association for colon	5, 6
Basel, Switzerland	serum ß-carotene	inverse for all cancer, stomach cancer	7, 8

In case-control studies it is difficult to determine whether ß-carotene levels in serum decrease as a result of the cancers or become lower because of decreased intake of ß-carotene. However, Smith et al (1991) approached this problem by assessing serum levels of ß-carotene in the families of cancer patients as well as from patients. Their results indicate lower serum levels of ß-carotene in families of cancer patients as well as the patients, thereby lending support to the hypothesis that lower serum ß-carotene in patients may result from lower dietary intake of ß-carotene. A third hypothesis explains lower serum levels of ß-carotene as a result of individual abilities to absorb and use ß-carotene at a cellular level.

Table 4 Retrospective studies showing an association between ß-carotene and cancer risk for sites other than the lung

Cancer site	Study population	Data collection	Association with cancer risk	Ref
bladder	Buffalo New York	carrot intake	inverse	11
oral	India	serum ß-carotene	inverse	12
oral and pharyngeal	North Carolina, USA	vegetable and fruit intake	inverse	13
esophageal	Milan, Italy	ß-carotene intake	inverse	14
	Milan Italy	ß-carotene intake	inverse	15
ovarian	Utah, USA	ß-carotene intake	inverse	16
	New York, USA	ß-carotene intake	inverse	17
cervical	New York, USA	serum ß-carotene	inverse	18
	USA	ß-carotene intake	n/a*	19
	USA	serum ß-carotene	n/a*	20
	Latin America	serum ß-carotene	inverse	21
	Netherlands	ß-carotene intake	positive	22
	Costa Rica	ß-carotene intake	inverse	23
breast	Italy	serum ß-carotene	n/a*	24
	Moscow	ß-carotene intake of post menopausal women	inverse	25
	Netherlands	ß-carotene intake	n/a*	26
prostate	Japan	ß-carotene intake	inverse	27
	Utah	ß-carotene intake	n/a*	28
	Hawaii	ß-carotene intake	n/a*	29
	Buffalo, New York	ß-carotene intake	inverse	30
other sites	New Zealand	serum ß-carotene	inverse for all cancers, specifically stomach, cervical,	31
	China	serum ß-carotene	inverse for stomach cancer	32
	Los Angeles, USA	ß-carotene intake	no effect for colon cancer	33
	Finland	serum ß-carotene	inverse for melanoma	34

* - no association

(11) Mettlin, C and Graham, G., 1979, (12) Ramaswamy, P.G., et al, 1990, (13) Winn, D.W., et al, 1984, (14) Valsecchi, M.G., 1992 (15) Negri, E. et al, 1992 (16) Slattery, M.L., et al, 1989, (17) Engle, A. et al, 1991 (18) Palan, P.R., et al, 1991, (19) Zeigler, R.G., et al, 1990, (20) VanEenwyk, J. et al, 1991, (21) Potischman, N., et al, 1991, (22) de Vet, H.C. et al, 1991, (23) Herrero, R., et al, 1991, (24) Marubini, E., et al , 1988, (25) Zaridze, D. et al, 1991, (26) Van'T Veer, P., et al, 1990, (27) Ohno, Y. et al, 1988, (28) West, D.W. et al, 1991, (29) Le Marchand, L. et al, 1991, (30) Mettlin, C., et al, 1989, (31)Smith, A.H., et al, 1991, (32) Chen, J. et al, 1992, (33) Peters, R.K. et al, 1992, (34) Kneckt, P. et al, 1991.

I.3. Three possible mechanisms for the chemopreventive action of ß-carotene

Although a detailed evaluation of the mechanisms by which ß-carotene exerts chemopreventive action is beyond the scope of this review, it is worthwhile to mention three prominent hypotheses. It is likely that some combination of these actions of ß-carotene account for most of its chemopreventive actions.

The first hypothesis for the role of ß-carotene as an anticarcinogenic agent is that ß-carotene acts as an antioxidant *in vivo*. Many carcinogens, both direct and indirect, act through free radical generation and propagation (Simic, M.G. 1988, O'Brien, P.J., 1988, Hochstein, P. and Atallah, A.S., 1988). ß-carotene is an excellent quencher of free radicals (Krinsky, N.I., 1990, Burton, G.W., 1989, Santamaria, L. et al, 1988) especially at low oxygen pressures, similar to that found at the cellular level. Therefore, ß-carotene may prevent carcinogens from damaging the DNA of the cell by acting as an antioxidant.

Second, ß-carotene's protective activity may result from its action as a vitamin A precursor. One molecule of ß-carotene cleaves in the intestinal mucosa to 2 molecules of retinol. Retinol has hormonal actions that contribute to the control of normal proliferation and differentiation of cells. As tumour cells do not proliferate or differentiate normally, (Boone, C.W., et al, 1990, Moon, R.C., 1989, Hennekens, C.H., et al, 1986), the supply of retinol from ß-carotene in the diet may restore normal rates of proliferation and differentiation and prevent tumour formation. In humans, the intestinal mucosa and the liver are capable of converting ß-carotene to retinol (Olsen, J.A., 1989) and up to 70% of

the ingested carotenoid may be converted to retinyl esters in the intestine (Goodman, D.S., 1984).

A third hypothesis is that ß-carotene may work to prevent or control cancer by enhancing various functions of the immune system. The use of ß-carotene in animal studies enhances T and B lymphocyte proliferative responses, increases macrophage and T-cell activities in tumour models, augments the lysis of tumour cells by natural killer cells, and protects phagocytic cells from autooxidative damage (Bendich, A. 1989, Bendich, A. and Shapiro, S.S., 1986). Parallel results are obtained in humans, with changes in surface antigen expression, T-cell function, increased helper/inducer T cells frequency, expression of cytokines, and expression of Ia and transferrin receptors occurring in individuals supplemented with ß-carotene (Garewal, H.S. et al, 1992, Prabhala, R.H., et al, 1991, Watson, R.R. et al, 1991, Mobarhan, S., et al, 1990, Prabhala, R.H. et al, 1989, Abril, E.R. et al, 1989, Alexander, M. et al, 1985).

I.4. Supplemental ß-carotene can protect against a wide variety of cellular damage

Supplementation with ß-carotene reduces photosensitivity of individuals with erythropoietic protoporphyria, a condition that renders the skin extremely sensitive to ultraviolet light. Therapeutic doses to reduce sensitivity vary between 30 and 180 mg/day (Mathews-Roth, M.M., 1976). ß-carotene accumulates in the skin (Lee, R., et al, 1975) and may act as a free radical scavenger. Plasma levels of ß-carotene in normal individuals decrease after controlled exposure to ultraviolet light (White, W.S., et al, 1988). The authors suggest that the ultraviolet light bleaches ß-carotene in the capillary beds of the

exposed tissues. Leukocytes from smokers ingesting 40 mg ß-carotene/day show reduced chemiluminesence after 6 weeks of supplementation. The authors suggest that ß-carotene scavenges oxidants generated by the myeloperoxidase/H_2O_2/halide system, thereby decreasing luminesence (Richards, G.A., et al, 1990). Repletion of ß-carotene with 15 or 120 mg ß-carotene/day (4 weeks) in individuals previously depleted of ß-carotene (2 weeks) causes a small decrease in lipid peroxidation levels in the blood (Mobarhan, S. et al, 1990). The two doses generate similar results: this may indicate that low doses of ß-carotene can achieve the same protection as high doses.

Supplementation with ß-carotene also reduces the frequency of early markers of carcinogenesis in humans. While vitamin A and retinoic acid are successful anticarcinogenic agents, they are also highly toxic (Kraemer, K.H., et al, 1988, Stich, H.F., et al 1988, Hong, W.K. et al, 1990). ß-carotene, the provitamin A carotenoid, is used in chemoprevention trials because of its low toxicity (Mathews-Roth, M.M., 1988, Heywood, R. et al, 1985, Bendich, A. 1988) and the evidence supporting its anticarcinogenic activity. Table 5 shows that ß-carotene, either alone or in combination with vitamin A, causes remission of oral leukoplakia and reduction of the frequency of micronucleated cells in exfoliated cell samples from the oral cavity. The latter endpoint quantifies chromosomal breakage occurring *in vivo* at this site.

Individuals ingesting ß-carotene and vitamin A concurrently enjoy greater benefits than with ß-carotene alone, as quantified by decreases in MNC and regression or prevention of leukoplakia. This suggests that these two agents can work synergistically. Garewal and colleagues report that 71% of individuals experience remission of leukoplakia with daily administration of 30 mg of

ß-carotene (Garewal, H.S., et al, 1990). These are better results than those obtained with twice weekly dosing at 180 mg ß-carotene and may indicate that daily dosing is more beneficial. Malaker reports that the regression of leukoplakia is greater among smokers as compared to non-smokers after ß-carotene treatment (Malaker, K. et al, 1991).

Table 5 Supplementation with ß-carotene reduces the frequency of early markers of cancer

Population	Supplementation	Results	Ref
Rural Filipinos chewing betel nut	150,000 I.U. ß-carotene + 50,000 I.U. vitamin A/week	Decrease in proportion of micronucleated cells (MNC) from 4.2 to 1.4%	1
Inuits chewing smokeless tobacco	180 mg ß-carotene/week	Reduction in frequency of MNC from 1.87 to 0.74 .	2
Fisherman from India chewing betel quid	(1) 180 mg ß-carotene/week	(1) *3 months* %MNC decrease from 4.0 to 1.1% *6 months* 14.8% remission of leukoplakia with ß-carotene	3, 4
	(2)180 mg ß-carotene + 100,000 I.U. vitamin A/week	(2) *6 months* - 27.5% remission of leukoplakia with ß-carotene + vitamin A.	
	(3) 200,000 I.U. vitamin A/week	(3) 57.1% remission of leukoplakias at 6 months.	
Patients with leukoplakia	30 mg ß-carotene/day	71% remission of leukoplakias at 6 months	5
Patients with oral leukoplakia in Italy	90 mg ß-carotene/day	44.4% remission after 4 months	6
Patients with , mucosal dysplasia in Canada	30 mg ß-carotene twice daily	44.4% remission of leukoplakia at 24 months	7

(1) Stich, H.F. et al, 1984, Stich, H.F. et al, 1985, (3) Stich, H.F. et al, 1988, (4) Stich, H.F. et al, 1988, (5) Garewal, H.S., et al 1990, (6) Toma, S., et al, 1992, (7) Malaker, K. et al, 1991.

Although ß-carotene effectively reduces the frequency of pre-malignant lesions of the oral cavity, ß-carotene supplementation is not effective in preventing recurrence of non-melanoma skin cancers, in reducing dysplasia or inducing regression of dysplasia in cervical tissues (De Vet, H.C.W., et al, 1991, Greenberg, E.R., et al, 1990). In light of these results, future research should determine the circumstances in which ß-carotene supplementation is beneficial to an individual.

I.5. ß-carotene content of foods

One way of relating the ß-carotene to food intake is to think of the supplement in terms of "carrot equivalents". A carrot that is 10 inches long with a 1 inch diameter has a mass of 140 ± 5.7 g (determined using 10 carrots meeting dimension requirements). Table 6 shows the estimated carrot weight required for the ingestion of specific amounts of ß-carotene.

Retinol equivalents (R.E.) or International Units of vitamin A (I.U.) are often used to indicate the ß-carotene content of foods and supplements. The following equations facilitate conversion between these units.

1 R.E. = 6 µg ß-carotene

1 IU = 0.6 µg ß-carotene

Table 6 ß-carotene content of carrots and equivalents to purified ß-carotene

mg of purified ß-carotene	carrots (grams)	carrots (ounces)	carrot equivalents*
15	197	6.9	1.4
30	394	13.8	2.8
45	591	20.7	4.2
60	788	27.6	5.6
90	1182	41.4	8.4
120	1576	55.2	11.3
150	1970	69.0	14.0
180	2364	82.8	16.8

* number of carrots (10 inches long with 1 inch diameter) to be ingested to obtain equivalent amount of ß-carotene

Table 7 illustrates the ß-carotene content of vegetables and fruits and shows the effects of processing on the ß-carotene content. In addition to losses of ß-carotene due to processing of foods, seasonal variations in ß-carotene content occur, with winter vegetables having significantly less ß-carotene than summer vegetables (Bureau, J.L., et al, 1986).

Table 7 ß-carotene content of fresh and processed foods

Food	Processing	ß-carotene content*
sweet potato	fresh	8288
	boiled	6380
	steamed	6400
carrot	fresh	7293
	10 min steamed	5834
spinach	fresh	6000
tomato	fresh	500
broccoli	fresh	1000
brussel sprouts	fresh	540
	cooked (30 min)	450
squash (butternut)	fresh	3464
	cooked (40 min)	2280
peach	fresh	46
	canned	26
nectarine	fresh	103

*(µg of ß-carotene/100g of food)

Condensed from: Khachik,F., et al, 1986, Bushway, R.J., 1986, Premachandra, B.R., 1976, Elkins, E.R., 1979, Bauernfeind, J.C., 1972., van der Pol, P.R., 1979, Gross, J., 1991.

I.6. Absorption of ß-carotene and some factors that influences its use in humans

Absorption of ß-carotene occurs in the gastrointestinal tract with the aid of esterases, lipases and proteases. Bile salts emulsify ß-carotene with fats in the intestine and ß-carotene passively moves into the intestinal cell (Simpson, K.L., Chichester, C.O., 1981). The oxygen requiring enzyme carotene 15,15'-dioxygenase in the intestinal cell may split ß-carotene into 2 molecules of

vitamin A (Bauernfeind, J.C., 1972). Estimates indicate that carotenoids supply up to 50% of the vitamin A in a North American's diet (Bendich, A., 1988). Enhanced conversion of ß-carotene to vitamin A occurs in a vitamin A deficient individual. Bile salts and the presence of protein and fat in the diet augment ß-carotene absorption into the intestinal cell (Bauernfeind, J.C. 1972, Simpson, K.L., Chichester, C.O., 1981). Any ß-carotene not converted to vitamin A is packaged into chylomicrons, which are absorbed into the lymph system, transferred to the circulatory system and transported to the liver.

Absorption of ß-carotene increases when ingested with a meal rather than with water. Inclusion of a second meal four hours after ingestion of ß-carotene further increases absorption of the carotenoid (Henderson, C.T. et al 1989). The meal may enhance ß-carotene uptake by stimulating bile secretion since the bile salts aid in the solubilization of ß-carotene. In addition, dietary lipids enhance absorption of ß-carotene from a supplement. Henderson and co-workers report a high correlation between serum triglycerides and serum ß-carotene when ß-carotene is ingested with dietary fat (Henderson, C.T., et al 1989). Cooney reports a positive association between the response to 1 week supplementation with 30 mg ß-carotene daily and dietary intake of fat (Cooney, R.V., et al 1991). Ingestion of supplemental ß-carotene with a high fat diet (~ 65 g fat) shortens the time to peak plasma concentrations from 15 days down to 5 days as compared to a low fat diet (~ 6 g fat) (Dimitrov, N.V., et al, 1988). The lipids form micelles that enfold the ß-carotene, allowing it to pass through the intestinal cells with chylomicrons (Henderson, C.T., et al 1989). This may explain why triglycerides and ß-carotene appear in the serum at the same time frame.

The efficiency of ß-carotene absorption depends on the dose of ß-carotene and the form of the carotenoid. Supplementation with 30 mg of ß-carotene produces a peak serum response only 1.6 times greater than a 12 mg supplement. Thirty mg of purified ß-carotene produces a serum response that is 5 to 7 times greater than the response with 30 mg of ß-carotene from carrots (Brown, E.D., et al, 1989). Chronic (daily for 6 weeks) ingestion of purified ß-carotene also produces a greater response than chronic ingestion of food sources of ß-carotene (Micozzi, M.S. et al, 1992). The efficiency of absorption of ß-carotene from supplements (99%) is greater than that from carrots (81%) (Rao, C.N., 1970). Inclusion of pectin in the diet reduces the response to purified ß-carotene by 50% (Rock, C.L., 1992). These results are evidence for the inhibitory effects of fibre on absorption of ß-carotene, both from ß-carotene containing foods and from purified supplements. Rock (1992) proposed that the pectin, a similar fibre to that found in carrots, inhibits ß-carotene absorption by delaying gastric emptying and interfering with micellar formation.

Serum levels of ß-carotene increase within 3 to 5 hours of ingesting a single food or supplemental source of ß-carotene (Henderson, C.T., 1989, Comstock, G.W. 1988). ß-carotene levels may remain elevated for 24 hours or longer, with higher doses of ß-carotene delaying peak time (Brown, E.D., et al, 1989). Thus serum levels of ß-carotene are responsive to recent intake of purified ß-carotene. Serum levels of ß-carotene peak after 1.5 to 4 weeks ingestion of 180 mg daily (Mathews-Roth, M.M., 1990). Differences in bioavailability between purified ß-carotene and ß-carotene from carrots were apparent in the timing of the peak response: pure ß-carotene peaks earlier than ß-carotene from carrots (Brown, E.D., et al, 1989). The latter study reports a poor serum response to ß-carotene in the form of carrots and concludes that

normal dietary intake of carotenoid-containing foods does not substantially affect serum levels of ß-carotene.

I.7. Transport of ß-carotene in humans

ß-carotene levels in chylomicrons rise significantly 3 hours after the ingestion of a ß-carotene-containing meal (Johnson, E.J., 1992). Increases in ß-carotene content of LDL's (low density lipoproteins) occur later and are sustained over a period of 2.5 days (Johnson, E.J., 1992). ß-carotene also associates to a lesser degree with very low density lipoproteins and high density lipoproteins (Parker, R.S., 1989). LDL's account for 75-80% of the ß-carotene in serum. Specifically, ß-carotene associates with LDL's of density between 1.019-1.063 g/ml (Goodman, D.S., 1984, Krinsky, N.I., et al, 1958).

I.8. Serum and tissue levels of ß-carotene and some factors which influence these levels in humans

Table 8 shows serum levels of ß-carotene in different study groups. The serum levels range from 18 to 40 µg/dl. The standard deviations shown for Stryker et al (1988) and Henderson et al (1989) are 82 to 100% of the mean serum levels of ß-carotene. This suggests considerable variation in serum ß-carotene levels for these study groups.

Table 8 Serum levels of ß-carotene in differing study populations

Definition of study group	Serum level of ß-carotene(mean)	Reference
Hospital staff	16 µg/dl	Henderson, C.T. et al 1989
Patients with history of nonmelanoma skin cancer	22.5 ± 18.5 µg/dl	Nierenberg, D.W. et al 1989
Men in MRFIT study	30.5 µg/dl	Russell-Briefel, R. et al, 1985
Men and women attending Pigmented Lesion clinic	men 18.5 ± 19.5 µg/dl women 31.8 ± 27.7µg/dl	Stryker, W.S, et al 1988
Older adult men and women of Washington County	men 29.3 µg/dl women 36.5 µg/dl	Comstock, G.W. et al, 1988

Table 9 presents ß-carotene levels of ß-carotene in organs and tissues of the human body. Tissue stores of ß-carotene reflect months of intake while serum levels indicate recent days or week of dietary ß-carotene (Parker, R.S., 1989).

Table 9 Levels of ß-carotene in human tissues

Tissue	ß-carotene concentration (µg/g tissue)	Reference
adrenal	5.04 *	Kaplan, L.A., et al 1990
	2.99	Stahl, W. et al, 1992
liver	2.46	Schmitz, H.H., et al, 1991
	1.62	Stahl, W. et al, 1992
	0.98 *	Kaplan, L..A.., et al 1990
	21.88§	Mathews-Roth, M..M.., 1976
	11.92§§	Mathews-Roth, M..M.., 1976
	0.06	Dimitrov, N.V., 1990
	0.08**	Dimitrov, N.V., 1990
testes	2.34*	Kaplan, L.A. et al 1990
	1.44	Stahl, W. et al, 1992
pancreas	0.67 *	Kaplan, L.A. et al 1990
adipose tissue	0.62	Parker, R.S., 1988
	0.20 *	Kaplan, L.A. et al 1990
	0.20	Stahl, W. et al, 1992
ovary	0.53*	Kaplan, L.A. et al 1990
breast	0.379	Nierenberg, D.W., et al 1992
kidney	0.19	Schmitz, H.H. et al 1991
	0.17	Kaplan, L.A. et al 1990
skin	0.147	Nierenberg, D.W., et al 1992
lung	0.13	Schmitz, H.H. et al 1991
	0.0616	Nierenberg, D.W., et al 1992
colon	0.0925	Nierenberg, D.W., et al 1992

* median levels are reported
§ - Rothmund- Thompson patient supplemented with 30 mg ß-carotene per day
§§ - Erythropoietic protoporphria patient supplemented with 180 mg ß-carotene per day
** - sample taken from one individual after 4 days supplementation with 150 mg ß-carotene per day

The concentrations of ß-carotene in organs and tissues differ greatly (Schmitz, H.H., et al 1991, Kaplan, L.A. et al 1990). Cameron and co-workers

report no correlation between ß-carotene levels in two types of exfoliated cells, oral mucosa cells and urogenital tract cells (Cameron, L.C. et al, 1989). Among the organs studies, ß-carotene concentrations are highest in the liver, adrenals and testes. These tissues have a high density of LDL receptors. As LDL's transport ß-carotene, the uptake of ß-carotene is likely passive through LDL receptor mediated mechanisms (Kaplan, L.A., et al, 1990). One piece of research suggests that LDL's preferentially load ß-carotene over other carotenoids (Kaplan, L.A., 1987).

ß-carotene is the predominant carotenoid in adipose tissue (Parker, R. S., 1988). In this study group, inter-subject variation in ß-carotene was 39-fold. Adipose tissue may absorb unlimited amounts of ß-carotene. However, until the ability of adipose stores to release ß-carotene into circulation is determined, the benefits of adipose stores of ß-carotene to other body tissues are questionable.

ß-carotene concentrations of liver samples from individuals with and without long term ß-carotene supplementation illustrate the responsive nature of hepatic cells to serum levels of ß-carotene Mathews-Roth, M.M., 1976). In contrast, Dimitrov (1990) reports that hepatic levels of ß-carotene do not change significantly after 4 days of supplementation. This may indicate a time lag between ingestion of the supplement and attainment of peak concentrations of ß-carotene in tissues.

In the autopsy studies by Kaplan (1990), and Schmitz (1991), a wide range of ß-carotene levels is shown for each tissue (data range not shown). Samples of oral mucosa and urogenital tract cells also show a wide range of ß-carotene levels before and after supplementation (Cameron, L.C. et al 1989). These differences may represent individual abilities to absorb and uptake

ß-carotene. Extreme differences in dietary intake also affect cellular levels of ß-carotene as shown by the mean levels of ß-carotene reported for the vegetarian groups and alcoholics of Table 10 (Stich, H.F., et al, 1986a).

Table 10 ß-carotene levels in exfoliated cells from different study groups

(values are presented in ascending order of mean ß-carotene levels)

Study group	Cell type	Cellular level ($ng/10^6$ cells)	N	Reference
Heavy alcohol drinkers	oral	0.08	56	Stich H.F. et al, 1986a
Lab personnel, Vancouver	oral	1.08	61	Stich H.F. et al, 1986a
Female lab personnel, Vancouver	oral	2.9**	22	Stich H.F. et al, 1986b
Filipino women, reverse smokers	oral	1.79∇ 4.29∇∇	28 34	Stich H.F. et al, 1986b
Hare Krishna members	oral	1.36	55	Stich H.F. et al, 1986a
Seventh Day Adventists	oral	1.39	28	Stich H.F. et al, 1986a
Female nurses in Vancouver (median value)	urogenital oral	0.6 4.1§ 1.6 9.9§	10 10	Cameron L.C. et al, 1989
Lab personnel, Hawaii With prebrush 1 week prior to sampling	oral	1.6 18.0§§ 6.0 7.7§§	10 4	Cooney R.V. et al, 1991

** - ß-carotene levels estimated from data on graph and mean value calculated. Mean value is after supplementation with 90 mg ß-carotene for 4 days. Samples were obtained 14 days after administration of last sample.-

∇ - mean ß-carotene level after supplementation with 180 mg ß-carotene weekly for 4 months

∇∇ - mean ß-carotene level after supplementation with 180 mg ß-carotene + 100,000 IU vitamin A weekly for 4 months

§ - mean ß-carotene level after supplementation with 180 mg ß-carotene twice weekly for four weeks

§§ - mean ß-carotene level after supplementation with 30 mg ß-carotene daily for seven days, sampling obtained at day 8.

Serum levels of ß-carotene increase 10-fold after 2 months supplementation with 20 mg ß-carotene daily (Albanes, D. et al, 1992). The wide range of responses to this supplementation is illustrated by the magnitude

of the standard deviation: it is 50% of the mean post-supplementation level of ß-carotene (Albanes, D., et al, 1992). Individual responses to ß-carotene supplementation may contribute to the wide range of tissue levels of ß-carotene. In one study, post-supplementation serum levels of ß-carotene vary 3 to 4-fold after one dose of 30 mg ß-carotene (Brown, E.D., et al 1989). In the second study, after eight weeks of daily supplementation with 15 or 45 mg ß-carotene, serum levels of ß-carotene in 15 individuals show 7 - and 5 - fold differences respectively (Dimitrov, N.V. et al, 1988). In both of these studies, dietary restrictions prevent error due to extra intake of ß-carotene. Exfoliated cells also show a wide range of ß-carotene levels after 4 weeks of ß-carotene supplementation (180 mg ß-carotene twice weekly), as shown in the 1.6-fold variation in urogenital cell levels and 3-fold variation in oral mucosa cell levels (Cameron, L.C. et al, 1989). Another study, involving four days supplementation with ß-carotene (90 mg/day) results in 3.4-fold differences in serum levels of ß-carotene and 3.5-fold differences in cellular levels of ß-carotene in oral mucosa cells (Stich, H.F., et al, 1986b). A third study of ß-carotene levels in exfoliated cells suggests that the method for cell collection is in part responsible for the increase and variation in ß-carotene levels of oral mucosa cells after supplementation (Cooney, R.V., et al, 1991). Among 4 unsupplemented individuals that donated cells on two occasions, cellular levels of ß-carotene increase from a mean of 1.6 to 6.0 ng/10^6 cells. Therefore an investigation of the impact of brushing on cellular levels of ß-carotene is needed to resolve this issue.

I.9. Some factors to be considered when examining tissue levels of ß-carotene

Season of sample collection

The season of sampling affects serum levels of ß-carotene wherein summer levels are higher than winter levels (Nierenberg, D.W., et al 1989). In contrast, serum levels of ß-carotene do not show a seasonal effect when collected in spring, summer and fall (Russell-Briefel, R., et al 1985). In a third study, serum samples collected in September, October and November show no differences in ß-carotene levels based on the month in which blood samples were collected (Comstock, G.W., et al 1988).

Dietary supplements

One study reports a correlation between multi-vitamin supplement use and serum levels of ß-carotene even though the supplements did not contain ß-carotene at the time of the study (Comstock, G.W., et al, 1988). A rationale for this suggests that vitamin supplement users may ingest a healthier diet than individuals not taking supplements. A second study indicates that regular ingestion of vitamin supplements elevates serum ß-carotene levels of smokers to the levels of non-smokers (Stryker, W.S., et al 1988). A third study reports a high correlation between preformed vitamin A in the diet and serum levels of ß-carotene. The authors suggest that conversion of ß-carotene to retinol decreases in the presence of preformed vitamin A, thereby sparing ß-carotene for absorption (Stryker, W.S., et al, 1988). After supplementation with ß-carotene (180 mg/week) or ß-carotene in combination with vitamin A (180 mg ß-carotene

+ 100,000 I.U. vitamin A), individuals receiving only ß-carotene had levels of 1.79 ng/10^6 cells at the end of the 4 months while those ingesting both supplements had ß-carotene levels of 4.29 ng/10^6 cells in oral mucosa cell samples (Stich, H.F., et al 1986b).

Smoking

Cigarette smokers have lower levels of serum ß-carotene than non-smokers (Ito, Y. et al, 1991, Russell-Briefel, R., et al 1985, Nierenberg, D.W., et al, 1989, Stryker, W.S., et al 1988, Comstock, G.W., et al 1988). A confounding factor is that dietary intake of ß-carotene is also lower in smokers; however, this difference may not be sufficient to explain the differing serum ß-carotene levels (Cade, J.E. et al, 1991, Stryker, W.S., et al 1988). Additionally, the response in serum to dietary ß-carotene among smokers is less than that of non-smokers (Stryker, W.S. et al, 1988, Bolton-Smith, C. et al, 1991, Coates, R.J. et al, 1991). Cigarette smoking may affect serum levels of ß-carotene by decreasing the bioavailability of ß-carotene or increasing clearance of ß-carotene by affecting hepatic enzymes. Smoking reportedly alters metabolism by raising levels of glucose, cortisol and adrenal androgens. Also, smoking may decrease levels of uric acid, albumin and leukocyte vitamin C while also altering the HDL/LDL cholesterol ratio (Nierenberg, D.W., et al 1989).

Alcohol intake

Intake of alcohol has a negative relationship with serum levels of ß-carotene (Ito, Y. et al, 1991, Russell-Briefel, R., 1985, Stryker, W.S., et al 1988). Synergism between alcohol and smoking is apparent in a study that shows lower

serum levels of ß-carotene in alcohol-consuming smokers than in non-drinking smokers (Stryker, W.S., et al, 1988). The results of a 1 week supplementation study indicates a negative association between serum responses and alcohol consumption (Cooney, R.V., et al, 1991). After 1 year of ß-carotene supplementation, serum responses are lowest among those individuals consuming the highest levels of alcohol (greater than 24 drinks per week) (Constantino, J.P., et al 1988).

Body composition

In the literature, the percentage of body fat correlates negatively with plasma ß-carotene among adolescents and adults (Herbeth B., et al, 1991, Albanes, D. et al, 1992, Nierenberg, D.W., et al 1989, Stryker, W.S., et al 1988). Obese individuals have the lowest levels of serum ß-carotene. One theory proposed for this relationship is that the large adipose content of an obese person dilutes the total body pool of ß-carotene. Analyses of the relationship between percentage of body fat and the response to ß-carotene supplementation provide conflicting results. Henderson and co-workers report no correlation between serum levels of ß-carotene and body fat index after supplementation with a single dose of ß-carotene, (Henderson, C.T., et al 1989). However, in a second study, Constantino reports an inverse correlation between serum levels of ß-carotene and body fat index after a year long supplementation study (Constantino, J.P., et al 1988). In a third study, high responses to supplementation correlate positively with lean tissue mass (Nierenberg, D.W., et al 1991).

Gender and age

Gender may play a role in determining serum levels of ß-carotene. Baseline levels of ß-carotene are higher in females than males in two recent studies (Nierenberg, D.W., et al, 1989, Comstock, G.W., et al, 1988). Furthermore, responses to supplementation are greater in females than males (Nierenberg, D.W. et al, 1991).

ß-carotene levels in serum increase with the age of the individual in one study (Nierenberg, D.W., et al 1989). No relationship between the age of an individual and serum levels of ß-carotene is shown in a similar study (Russell-Briefel, R., et al 1985). In a third study, younger males have greater serum responses to ß-carotene supplementation than older males (Sugerman, S.B. et al, 1991). The age effect may result from differences in body composition and serum lipids that occur as an individual becomes older.

Dietary ß-carotene

Dietary intake of ß-carotene is the best predictor of serum ß-carotene levels among adults (Albanes, A.D. et al, 1992, Russell-Briefel, R., 1985, Nierenberg, D.W., et al, 1989) and is a strong determinant of serum ß-carotene among adolescents (Herbeth, B., et al 1991). Carrot intake increases serum ß-carotene levels (Ito, Y. et al, 1991). The limiting factor in obtaining a good correlation between dietary ß-carotene and serum ß-carotene is the accurate measurement of dietary ß-carotene (Russell-Briefel, R., et al 1985).

The relationship between ß-carotene levels before and after treatment with ß-carotene

Two studies report that plasma ß-carotene before supplementation is the factor most positively associated with response to supplementation (Nierenberg, D.W. et al, 1991, Albanes, D. et al, 1992). In contrast, in another study ß-carotene levels in serum before and after the ingestion of a single supplement are not related (Brown, E.D. et al, 1989). This relationship warrants further investigation.

Prediction of response to supplementation

The response to ß-carotene supplementation in serum for a year long study is best predicted using a model that includes alcohol intake, body mass index, baseline serum ß-carotene level and high density lipoprotein level. The equation accounts for 22% of the variation in ß-carotene response (Constantino, J.P., et al 1988).

I.10. Summary of literature review

Evidence from the literature indicates a protective role for ß-carotene in carcinogenesis. Studies of serum levels of ß-carotene identify several factors that act to determine responses to dietary and supplemental ß-carotene. The impact of these factors on tissue levels of ß-carotene is not known. However, knowledge of these factors will be vital to the understanding of epidemiological data and the planning of future intervention trials. The research in this thesis

addresses the issue of possible variations in cellular levels of ß-carotene and assesses some of the factors that influence tissue levels of ß-carotene.

I.11 Research objectives

1. To quantitate ß-carotene levels in oral mucosa cells of a local Vancouver population in order to establish:

   a. intra-individual variation in the absence of supplementation;
   b. inter-individual variation in the absence of supplementation;
   c. inter-individual variation in the accumulation and depletion of ß-carotene after short-term supplementation with ß-carotene; and
   d. the relationship between unsupplemented and supplemented ß-carotene levels.

2. To study unsupplemented and supplemented levels of ß-carotene in different study groups to determine:

   a. whether populations differ in these levels;
   b. whether dietary ß-carotene, alcohol and tobacco play a role in determining these levels; and
   c. the consistency, in individuals, over a time interval of 1 year of unsupplemented and supplemented ß-carotene levels and responses to supplementation.

I.12 Approach

The first objective was addressed in Study 1 using individuals from Simon Fraser University. No-one in this study group used tobacco products. Two samples of oral mucosa cells, obtained 7 days before and on the first day of supplementation, were used to determine unsupplemented levels of ß-carotene. Following this, the accumulation and depletion of cellular ß-carotene was assessed by administering ß-carotene supplements (90 mg ß-carotene per day) on each of days 0, 1 and 2 and determining the ß-carotene content of cell samples obtained 7, 14 and 21 days after initiation of supplementation.

Study 2 involved individuals from four populations in British Columbia that were chosen for their differing dietary, alcohol and tobacco consumption patterns. A Vancouver group consisted of staff and students from Simon Fraser University, partly from Study 1; the other three groups were composed of native Indians from Lytton, One Hundred Mile House and Williams Lake areas. ß-carotene supplements were ingested on each of days 0, 1 and 2 (90 mg/day) and oral mucosal cell samples were collected at day 0 and day 7. The impact of dietary ß-carotene, alcohol and tobacco consumption on cellular levels of ß-carotene in unsupplemented and supplemented individuals of these four groups was examined.

A portion of each of the Vancouver, Lytton and Williams Lake groups completed a second supplementation regimen a year later. The unsupplemented and supplemented cellular levels of these individuals in the first and second years were compared to determine the consistency of individual responses to ß-carotene.

II. MATERIALS AND METHODS

II.1. Study populations

Individuals from four different populations were asked to participate in studies described in this thesis. A Vancouver group consisted of staff and students of Simon Fraser University and was composed of individuals from a variety of ethnic origins. The other three populations were composed of native Indians from Lytton, Williams Lake and One Hundred Mile House. The characteristics of each study group are detailed in the Results sections.

In the Vancouver population, individuals were informed of the nature of cell sampling and ß-carotene supplementation either in a group (class) setting or individually. In the remaining three populations, the researcher went 'door to door' and informed individuals of the nature of the cell sampling and ß-carotene supplementation and asked them to participate. All individuals participated on a volunteer basis. Participation rates as a percentage of each population were: Vancouver - 65%; Lytton - 4%; Williams Lake - 12.6% and One Hundred Mile House - 7%.

This study was approved by the Ethics Review Committee of Simon Fraser University. Appropriate informed consent was obtained from each individual before initiation of the study.

II.2. Collection and storage of buccal mucosa cells

Cellular levels of ß-carotene were determined in samples of exfoliated buccal mucosa cells. Prior to sample collection, participants were instructed to

rinse their mouths vigorously with tap water to remove food debris. The buccal mucosae were brushed with a soft toothbrush to loosen cells and the mouth rinsed again with 30 ml of water. This rinse was collected in a 50 ml plastic centrifuge tube. An additional 20 ml of water was added to the tube and cells adhering to the toothbrush were collected by agitating the brush in the rinse. Cell samples were immediately placed on ice and protected from light. Mucous and food debris were later removed by straining the samples through two layers of cheesecloth. Cell pellets were obtained by allowing the samples to settle for at least two hours or by centrifuging at 1000 G for 5 minutes. Cell pellets were transferred into coded 1 ml plastic vials and placed in liquid nitrogen until processing. All procedures were performed under yellow light to minimize the decomposition of ß-carotene.

II.3. Processing of cell samples

Cell samples were thawed, brought to room temperature and the weight of each sample ascertained. To accomplish this, the samples were transferred to preweighed 1.5 ml vials. Water was removed by repeated centrifuging followed by aspiration of the liquid with a 100 µl syringe. The vials were reweighed and the sample weight calculated by subtracting the weight of the vial. Cell counts were estimated using tabulated values for predetermined cell counts for wet weights (Appendix 1).

Extraction of ß-carotene involved the following procedure. 200 µl of 10 mg/ml protease from *Streptomyces griseus* (freshly prepared in 0.05 M phosphate buffer, pH = 7.5), was added to the cell pellet and the tube was vortexed for 1 minute to ensure mixing. Samples were placed in a heating block

at 37° C for one hour. Tubes were vortexed four times during the hour to enhance cell digestion. 200 µl of 5% KOH in methanol was added and the tubes were placed in a 50° C heating block for an additional hour. This step furthered cell digestion and saponified lipids. Tubes were removed from the heating block and allowed to cool. One drop of phenol red (1% in methanol) was added to each tube to stain the alkaline phase. 750 µl of hexane was added and the sample vortexed vigorously for 1 minute, then centrifuged for one minute (12,000 G). The majority of the lower layer was removed and discarded at this time to prevent contamination of the organic phase with alkaline components of the aqueous phase. A second centrifuging for 5 minutes ensured complete separation of the organic and aqueous layers. 600 µl of the organic phase was withdrawn and placed in a new, labeled tube. The sample was centrifuged under reduced pressure using a Savant spin evaporator which completely evaporated the solvent. The dried sample was dissolved in 30 µl of anhydrous ethanol, vortexed and then centrifuged to concentrate the sample in the tip of the tube.

II.4. Quantification of ß-carotene in cell samples

High pressure liquid chromatography (HPLC) was used to quantify the ß-carotene content of the samples. This system used a Perkin Elmer Series 2.1 HPLC pump with the flow rate set at 0.4 ml/min; running solvent of 96% methanol, 4% methylene chloride, a Vydac 201 TP reversed phase column with an internal diameter of 2.1 mm and a length of 150 mm with 5 µm packing (custom packed by Alltech, U.S.A., obtained through Mandel Scientific, Canada); a Kratos SF 769Z detector using a tungsten lamp set at 450 nm, (time constant = 5.0 seconds, detector range = 0.04 absorbence units), and an integrator that

reported the area of the chromatographic peaks. The system was calibrated twice before each day's chromatography with 30 µl of methanol containing 1 ng of ß-carotene.

Ten microliters of water was drawn into a 50 µl syringe. The entire 30 µl of sample was subsequently loaded into the same syringe and this was injected into the HPLC system. Water was included with sample injection for two reasons: (1) it ensured that the sample was flushed from the syringe, and (2) the relative lower polarity of water decreased the solvent power of the ethanol and prevented peak smearing. The column separated the ß-carotene in the samples from other carotenoids and into two peaks, the all-trans and cis stereo-isomers. These peaks were summed to calculate the total peak area or height of the injection. The total peak area was used in a ratio with the calibration peak area to calculate the nanograms of ß-carotene in each injection.

$$(1 \text{ ng of ß-carotene in calibration injection}) \times \frac{\text{peak area of sample injection}}{\text{peak area of calibration injection}} = \text{ng of ß-carotene in sample injection} \quad (1)$$

The ß-carotene content of each sample was calculated as follows:

$$(\# \text{ of ng of ß-carotene in sample injection}) \times \frac{750\mu l \text{ hexane added}}{600\mu l \text{ hexane extracted}} = \text{ng in sample} \quad (2)$$

$$\frac{(\text{ng in sample})}{(\text{number of cells in sample})} \times 10^6 = \text{ng}/10^6 \text{ cells} \quad (3)$$

ß-carotene concentration is expressed in nanograms per million cells (ng/10^6 cells).

II.5. Technical variation

Analytical error due to processing of samples, extraction of ß-carotene from the sample and error of the HPLC system is summed as technical variation and was previously estimated in the same lab using identical methods and equipment (Cameron, L.M. 1987). In brief, thirteen individuals donated cell samples to a sample pool which was divided into 10 sample aliquots. The ß-carotene content of these aliquots was determined. Mean ß-carotene content was 1.6 ng/10^6 cells ± a standard deviation of 0.4 with a range of 1.1 to 2.2 ng/10^6 cells.

II.6. ß-carotene supplements

ß-carotene was supplied by Hoffman-LaRoche (Canada) as water-dispersible beadlets, composed of ß-carotene, starch, dextrose, gelatin and trace amounts of alpha-tocopherol and ascorbate palmitate. Beadlets were packaged by Quest Vitamins, Vancouver to produce capsules containing 30 mg of ß-carotene.

II.7. Rationale for cell sampling interval

The sampling interval was designed to assess the effects of the ingested ß-carotene on cellular levels of ß-carotene in the oral mucosa. To observe this it was advantageous to collect cells that had been present since the beginning of the oral supplementation. It is generally accepted that the process from cell division in the basal layer to exfoliation in the top layer takes approximately 5 to 7 days; therefore it was prudent to wait a minimum of 7 days to observe the

effects of supplementation on cellular levels of ß-carotene (personal communication M.Rosin). Subsequent collections of samples were also timed at 7 day intervals to maintain consistency.

II.8. Design of short-term supplementation studies

In Study 1, samples of oral mucosa cells were collected on two occasions prior to supplementation to determine cellular levels of ß-carotene in unsupplemented individuals. This occurred 7 days before supplementation and on the first day of supplementation before ingestion of the supplement. After supplementation with 90 mg ß-carotene for each of three days, samples of buccal mucosa cells were collected 7, 14 and 21 days after the initiation of supplementation to quantify the accumulation and depletion of cellular ß-carotene.

In Study 2, supplements of ß-carotene (90 mg/day) were ingested by each participant on each of days 0, 1 and 2. Unsupplemented levels of ß-carotene were determined on one sample of cells obtained on day 0 prior to ingestion of the first supplement. Post-supplementation levels of ß-carotene in oral mucosa cells were determined from samples collected at day 7.

In Study 1 and 2, the participants did not ingest ß-carotene supplements, apart from those in the supplemental regimen, either before or during the study. Dietary restrictions were not imposed.

II.9. Acquisition of dietary and lifestyle information

Two questionnaires were used to collect dietary information. The first was filled out by individuals in the Vancouver group for Study 2 (Appendix 2). The questionnaire queries the weekly frequency of carrot, spinach and broccoli consumption. These foods were chosen for their high content of ß-carotene and availability in grocery stores on a year round basis. The questionnaire also queried age and gender as well as alcohol, tobacco and snuff use.

A second dietary questionnaire was developed for estimating dietary intake of ß-carotene-containing foods in the Lytton, Williams Lake and One Hundred Mile House study groups during the Year 2 supplementation studies of Study 2 (Appendix 3). Preliminary data indicated that these groups did not consume diets that were similar to the Vancouver study group. These individuals did not have access to the same abundance of grocery items and thus their diets were limited in variety. In addition a greater proportion of their diet was obtained through hunting and fishing. This interviewer-based questionnaire contains an extensive list of foods which may be present in the diet of individuals from the study groups of interior B.C as well as the Vancouver-based group. Weekly frequency of intake of ß-carotene-containing foods was calculated from these questionnaires. A total of thirty-nine individuals from the Lytton, Williams Lake and Vancouver study groups completed the questionnaire.

A lifestyle questionnaire was completed by an interviewer to determine alcohol and tobacco usage for all individuals in the Lytton, Williams Lake and One Hundred Mile House groups. Age and gender were also recorded on this questionnaire (Appendix 4).

II.10. Statistical analysis

Student's t-test and analysis of variance tests were employed to determine whether there were significant differences between population means. The level of significance was set at $p<0.05$. When significant differences were detected, Sheffe's F-test was used to determine the direction of the difference. Factorial analysis of variance was used to assess the impact of independent variables on the dependent variable. Simple linear and multiple stepwise regression analyses were used to assess the relationships between diet and lifestyle factors and cellular levels of ß-carotene before and after supplementation.

III. RESULTS

Study 1

III.1. Objectives of Study 1

The objective of Study 1 was to quantitate ß-carotene levels of a local population and establish intra-and inter-individual variation in these levels before and after short-term supplementation with ß-carotene. Because both alcohol and tobacco usage are known to affect serum levels of ß-carotene (Russell-Briefel, R. et al 1985, Stryker, W.S., et al 1988) and may affect cellular levels, in this study we looked for a population that did not consume large quantities of alcohol or use tobacco products. A pilot study indicated that the Vancouver group fit these restrictions.

III.2. Population characteristics

The population group of Study 1 consisted of staff and students of Simon Fraser University. The dietary questionnaire of Appendix 2 was employed to determine the following information about the study group. Sixty-two individuals began the study: 27 females and 35 males. The mean age was 26.4 years (S.D.= 7.52). Sixty-six percent of the study group consumed alcohol with a mean weekly alcohol consumption of 81.6 ± 77.1 ml ethanol per week. This was approximately equal to five drinks per week. Beer was the most frequently consumed alcoholic beverage. Mean carrot intake was 3.2 ± 4.3 servings per week. A ß-carotene index was calculated by totaling the weekly frequency of

carrots, spinach and broccoli servings. The ß-carotene index was 5.0 ± 4.7 servings per week. This group did not use tobacco.

III.3. Compliance

Samples of oral mucosa cells were collected from 62 individuals on two occasions prior to supplementation. Fifty-five individuals ingested 90 mg ß-carotene on three consecutive days and fifty-three (83%) donated cell samples at day 7 after supplementation. At days 14 and 21, samples were collected from 55 and 34 individuals respectively. On the day before sampling participants were reminded by phone or message to come to the lab for brushing and cell collection. On sampling days, participants were sought out and encouraged to complete the sampling program. Despite these efforts the study population at day 21 was 55% of the population originally enrolled in the study. The individuals that did not complete the study were contacted after the study and asked to identify why they were not able to complete the study. The most common reasons were (1) unable to attend the date of cell sampling (2) did not come to SFU campus on the sampling day and (3) forgot the sampling date. These are common problems for a study involving consecutive sampling. The lack of daily contact (except when ingesting the supplement) may have enhanced participant dropout by distancing the participants from the study.

Given that cell samples were not obtained from all individuals for each sampling date, each analysis involves only those individuals for which complete data was available. Therefore, the analysis of unsupplemented individuals includes 62 data sets; analysis of the effect of supplementation at day 7 includes

53 data sets and analysis of accumulation and depletion of ß-carotene over 21 days involves 34 data sets.

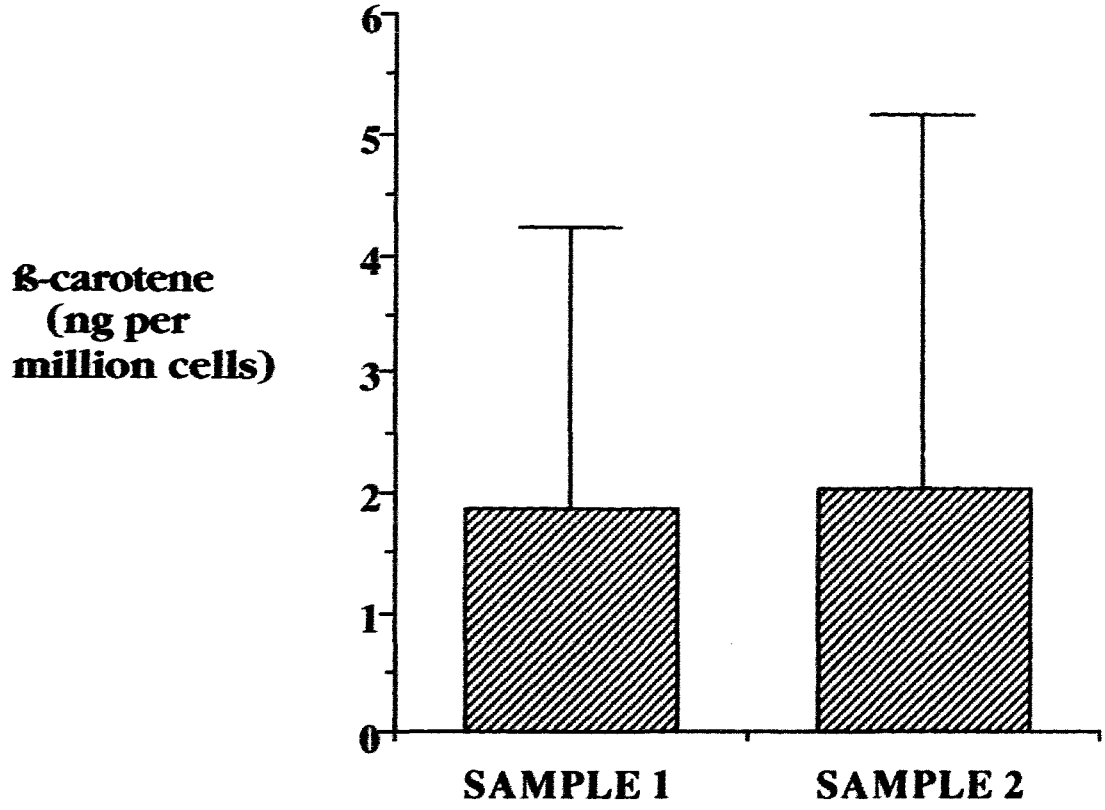
III.4. Intra-individual variation in ß-carotene levels

III.4a. Mean ß-carotene levels in cell samples from unsupplemented individuals

Two samples of oral mucosa cells were collected from 62 participants to determine ß-carotene levels in unsupplemented individuals. Figure 1 diagrams the means ± S.D. of the two sample sets. Sample 1 mean was 1.88 ± 2.33 ng/10^6 cells and Sample 2 mean was 2.03 ± 3.13 ng/10^6 cells. A paired t-test indicated that the two sample sets were significantly different ($p < 0.05$). The absolute differences between Samples 1 and 2 ß-carotene levels were calculated for each individual: the mean of these differences was 1.22 ± 2.55 ng/10^6 cells, which was equal to 66% of the mean Sample 1 value.

The increase in the mean unsupplemented ß-carotene level can be attributed to increases in ß-carotene content of 12 and 13 ng/10^6 cells by two individuals and increases between 4 to 5 ng/10^6 cells by three individuals. Sixty-nine percent of the study group showed a decrease in ß-carotene content. Of the decreases in ß-carotene content measured in unsupplemented individuals, three individuals showed a decrease of 1 ng/10^6 cells and one individual each showed a decrease of 2, 4 and 6 ng/10^6 cells. Eighty-two percent of individuals had changes between 1 and -1 ng/10^6 cells.

Figure 1 **ß-carotene levels in consecutive samples of oral mucosa cells**



Two samples (Sample 1 and Sample 2) of oral mucosa cells were collected from 62 individuals on 2 consecutive dates separated by an interval of 7 days. The bars represent sample means (±S.D.) of the two sample sets, measured in ng/10^6 cells.

III.4b. Correlation between ß-carotene levels in two samples obtained 7 days apart from unsupplemented individuals

The scatterplot of Figure 2 shows the correlation between cellular levels of ß-carotene in Samples 1 and 2. Simple linear regression calculates the relationship between ß-carotene concentrations in Samples 1 and 2 as:

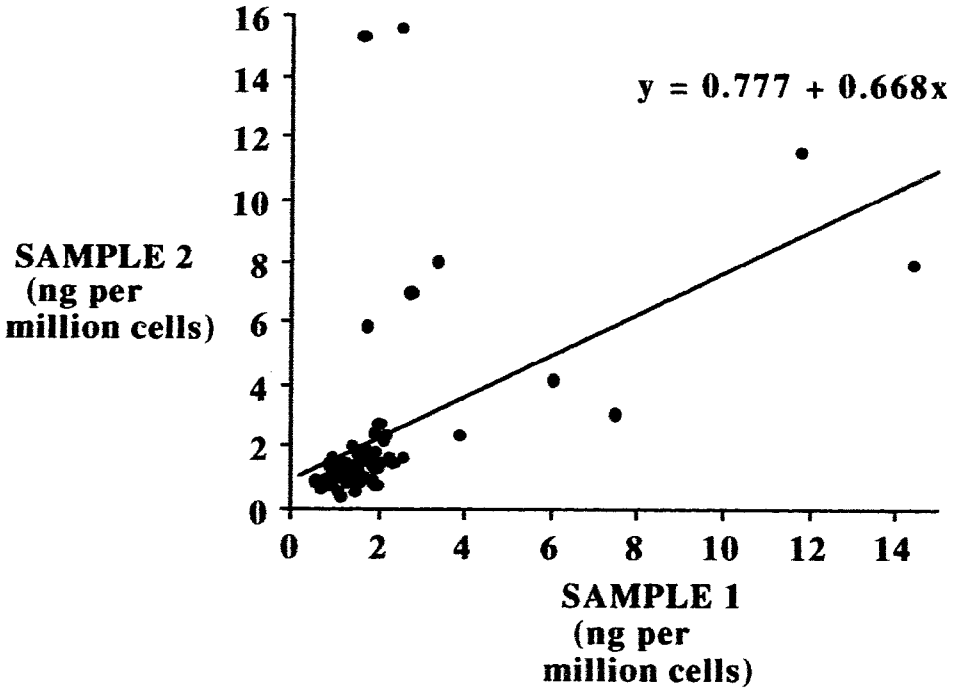
(Sample 2 ß-carotene) = 0.777 + 0.668 * (Sample 1 ß-carotene) (4)

The correlation between ß-carotene levels in the two samples is strong, positive and statistically significant with R = 0.497 ($p<0.001$).

III.4c. The effect of removing potential outliers on the correlation

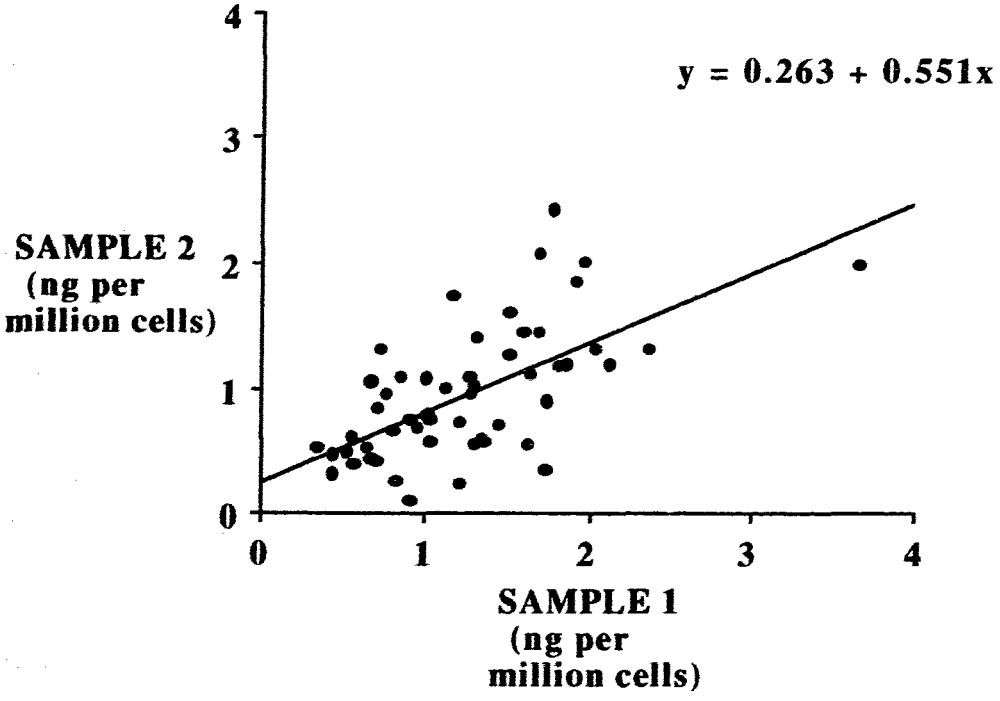
ß-carotene levels of 8 unsupplemented individuals were substantially higher in one sample than in the other sample. These individuals are visible in the top left and the bottom right portions of Figure 2. These values could be considered potential outliers and for comparative analysis can be removed according to the methods of Dixon (Sokal, R.R. and Rohlf, F.J. 1981). This method used a ratio $[r_n = \frac{Y_1 - Y_{mean}}{s}]$ to compare the suspected outlier (Y_1) value with the mean value (Y_{mean}) and produced a computed value r_n, where n is the sample size, s is the standard deviation. The computed value r_n is then compared with a critical value from the statistical table of values by Grubbs (Sokal, R.R. and Rohlf, F.J. 1981).

Figure 2 Correlation between pre-supplementation levels of ß-carotene in two samples of oral mucosa cells.



Two samples of oral mucosa cells were collected over an interval of 7 days. The scatterplot illustrates the correlation between ß-carotene levels in the two samples. The line of regression and the equation are shown. Units of Sample 1 and Sample 2 are ng/10^6 cells.

Figure 3 The effect of removing outliers on the correlation between ß-carotene levels in two consecutive samples of oral mucosa cells.



Sample collection is described in Figure 2. Outliers were removed according to Dixon (Sokal, R.R. and Rohlf, F.J., 1981). The scatterplot illustrates the correlation, line of regression and regression equation between ß-carotene levels in samples of oral mucosa cells from unsupplemented individuals. Note: scale is different from Figure 2.

The effect of removing potential outliers on the relationship between unsupplemented ß-carotene levels is shown in Figure 3. The mean of the differences between the samples was 0.4 ng/10^6 cells, approximately 25% of Sample 1 mean. Linear regression calculates the new relationship to be:

(Sample 2 ß-carotene) = 0.263 + 0.551 * (Sample 1 ß-carotene) (5)

The smaller y-intercept value indicates a relationship with an intercept closer to the origin (0,0). The correlation between the two sets of samples improves by removing the potential outliers and the new calculated R = 0.628 with the same level of significance ($p<0.001$).

It was decided to utilize the most conservative approach in future analysis using this data. Therefore potential outliers were not removed for the remainder of the analysis.

III.5. Inter-individual variation in unsupplemented individual

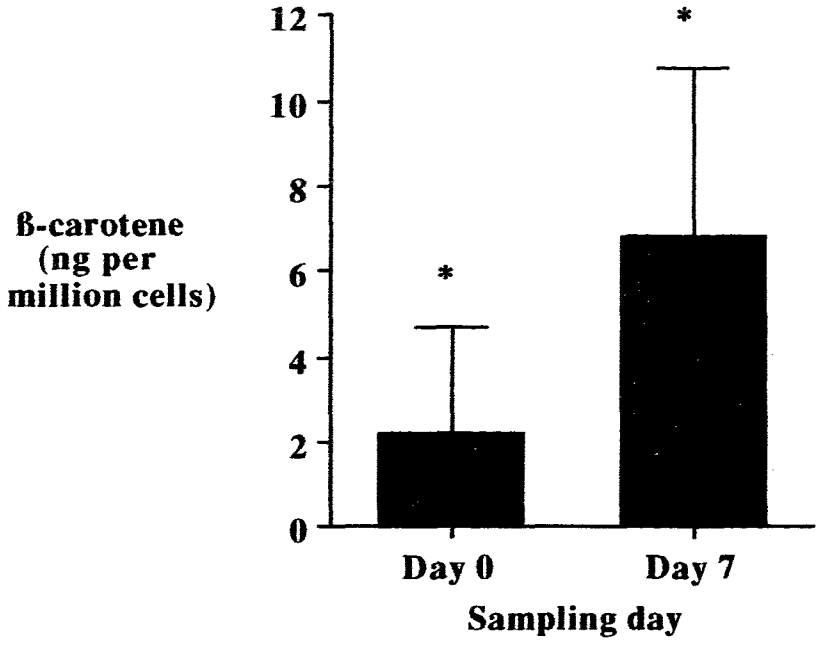
Inter-individual variation in ß-carotene levels of unsupplemented individuals is shown in Figures 1, 2 and 3. The standard deviation in Figure 1 at both sampling dates was larger than the mean ß-carotene content of the cell samples. In Figures 2 and 3 the wide range of ß-carotene levels in unsupplemented individuals is shown. ß-carotene levels ranged from 0.35 to 14.2 ng/10^6 cells at Sample 1 and 0.1 to 15.2 ng/10^6 cells at Sample 2.

III.6. Accumulation of ß-carotene as measured 7 days after the initiation of supplementation

Supplemental ß-carotene was ingested on days 0, 1 and 2 at a dose of 90 mg/day. No-one reported ill effects from the supplements or from the cell sampling. Cell samples were collected from fifty-three individuals that had complied with the supplemental regimen and from whom two unsupplemented cell samples were obtained. For the purpose of this study, a mean ß-carotene level was calculated from the ß-carotene levels in the 2 samples from unsupplemented individuals; this mean will be referred to as the day 0 ß-carotene.

The accumulation of ß-carotene in cell samples at day 7 is shown in Figure 4. Day 0 ß-carotene levels increased from a mean of 2.20 ± 2.52 ng/10^6 cells to a day 7 mean of 6.82 ± 3.91 ng/10^6 cells. The ß-carotene levels of the paired samples at days 0 and 7 were statistically different ($p<0.001$). The change in ß-carotene content by individuals ranged from 0 to 15.6 ng/10^6 cells with a mean of 4.59 ± 3.81 ng/10^6 cells

Figure 4 Accumulation of ß-carotene in cellular samples collected 7 days after supplementation with ß-carotene



Mean levels of ß-carotene at day 0 and 7 days after ingestion of 90 mg ß-carotene on each of days 0, 1 and 2. Day 0 ß-carotene levels are the mean values for each individual's two pre-supplementation levels of ß-carotene. Values shown are the mean ± S.D. for 53 individuals. * = $p<0.0001$

III.7. Correlation between pre- and post-supplementation levels of ß-carotene

Simple linear regression calculates the relationship between day 7 ß-carotene levels and mean day 0 ß-carotene levels as:

$$\text{Day 7 ß-carotene} = 5.45 + 0.61 * (\text{Day 0 ß-carotene}) \quad (6)$$

A moderate significant correlation was calculated between day 0 ß-carotene levels and day 7 ß-carotene levels with $R = 0.386$ ($p<0.005$). This indicates that the variation in day 0 ß-carotene levels accounts for 14.9% of the variation in day 7 ß-carotene levels.

III.8. Accumulation and depletion of ß-carotene 7, 14 and 21 days after the beginning of supplementation

Thirty-four individuals completed the supplementation regimen and donated cell samples at days 7, 14 and 21. The mean levels of ß-carotene at these times including day 0 are shown in Figure 5. For these 34 individuals the accumulation of ß-carotene was evident at day 7 with the mean increasing from 2.29 ± 2.60 ng/10^6 cells to 6.55 ± 3.9 ng/10^6 cells. Mean ß-carotene levels were statistically different at day 0 and day 7 ($p<0.001$). The mean accumulation in ß-carotene content was 4.26 ± 3.90 ng/10^6 cells by day 7. Depletion of ß-carotene occurred between day 7 and day 14 during which mean ß-carotene content decreased by 3.46 ± 3.54 ng/10^6 cells to a mean day 14 level of 3.09 ng/10^6 cells ($p<0.001$). Between day 14 and 21 ß-carotene content of cell

samples increased slightly with a mean of 0.30 ± 3.9 ng/10^6 cells but this change was not significant.

III.9. Inter-individual variation after supplementation

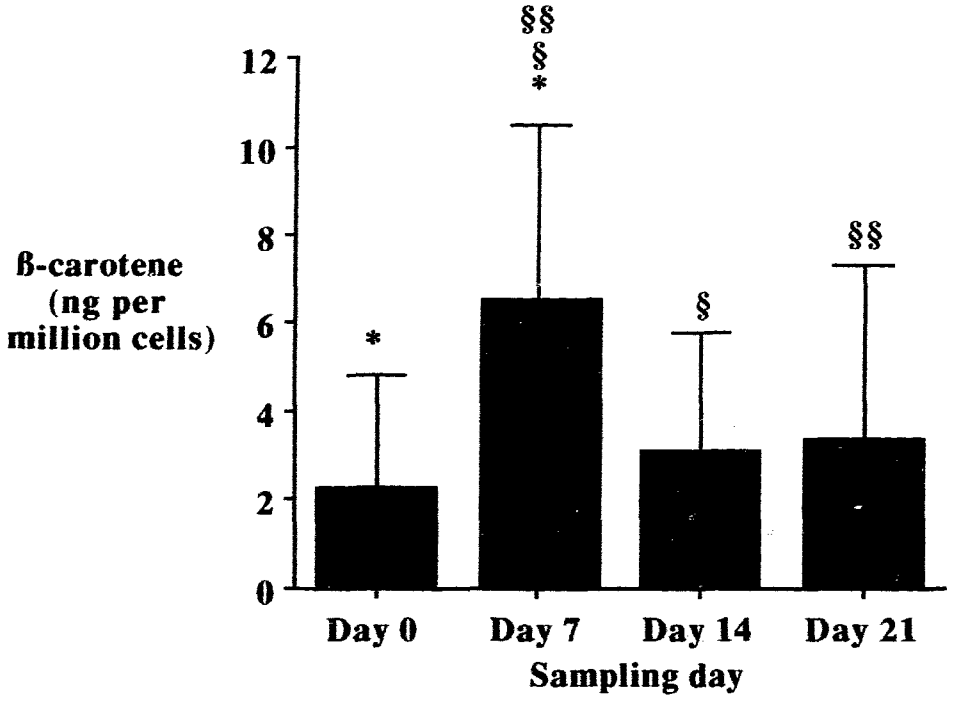
Substantial inter-individual variation was observed in cellular levels of ß-carotene after short-term supplementation. Table 11 lists the coefficients of variation for ß-carotene levels before and after supplementation. This relative comparison of variation is independent of the magnitude of the means and therefore clearly shows that the variation differed between day 0 and day 7. A decrease in variation is evident at day 7 while by day 21 the coefficient of variation shows that variation approached the day 0 level.

Table 11 A comparison of relative variation in cellular ß-carotene levels before and after supplementation

Day of cell sample collection	Coefficient of Variation
0	113.40
7	60.07
14	87.19
21	117.40

Figure 6 illustrates the inter-individual variation in ß-carotene levels by showing the wide range of ß-carotene levels observed throughout the study. Table 12 shows the minimum and maximum levels of ß-carotene observed through the 21 days of study.

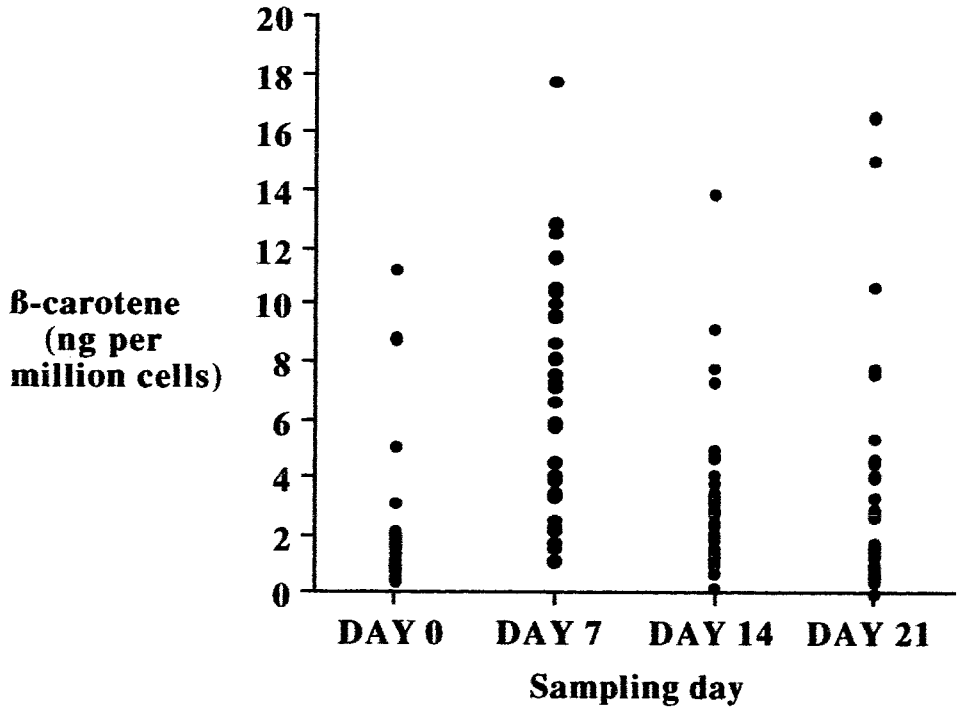
Figure 5 Accumulation and depletion of ß-carotene 7, 14 and 21 days after supplementation with ß-carotene



Bars represent the mean levels of ß-carotene (±S.D.) in samples of oral mucosa cells collected 0, 7, 14, and 21 days after supplementation with 90 mg of ß-carotene for each of three consecutive days (Days 0,1 and 2). Day 0 ß-carotene levels are the mean values for each individual's two pre-supplementation levels of ß-carotene. Data represents 34 individuals.

* $p<0.001$, § $p<0.001$, §§ $p<0.001$

Figure 6 Inter-individual variation in cellular levels of ß-carotene after supplementation



Each dot represents the ß-carotene level of one of the 34 individuals completing the 21 day study.

Table 12 Minimum and maximum levels of ß-carotene in cell samples

Day of cell sample collection	Minimum*	Maximum*	Median*
day 0	0.39	11.2	1.09
day 7	1.09	17.7	6.50
day 14	0.13	13.8	2.44
day 21	0.00	16.5	2.65

* ($ng/10^6$ cells)

III.10. A comparison of the effect of ß-carotene supplementation in individuals with low and high pre-supplementation levels of ß-carotene

The data of the study population was divided into four groups (quartiles) using day 0 ß-carotene levels as the criterion variable. Only individuals that had complete data sets were included. The mean post-supplementation levels of ß-carotene and the mean change in ß-carotene content of the lowest and highest quartiles were compared.

Figure 7 shows the mean (± S.D.) cellular levels of ß-carotene of the lowest and highest quartiles at days 0, 7, 14 and 21. Mean levels were visibly smaller in the lowest quartile throughout the 21 days. The pre-supplementation level in the highest quartile is greater than the day 7 level in the lowest quartile. Day 7 levels were significantly different between the lowest and highest quartiles ($p<0.05$) as were day 21 levels ($p<0.05$). Mean day 0 and day 7 ß-carotene levels

as well as day 7 vs. day 14 and day 7 vs. day 21 were significantly different in the lowest quartile ($p<0.001$). On none of the sampling days were ß-carotene levels of the highest quartile significantly different from each other.

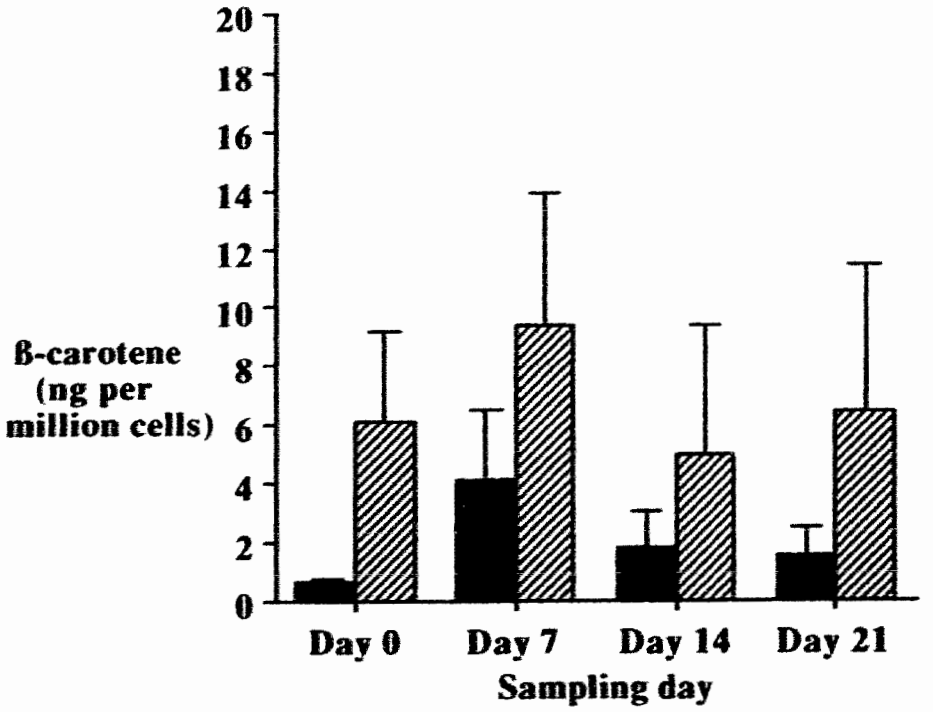
The change in ß-carotene content was calculated for each individual of each quartile and the mean of the change for each quartile is shown in column 3 of Table 13 along with the mean day 0 and day 7 ß-carotene levels of each quartile. The mean changes in ß-carotene content by the quartiles did not differ significantly.

Table 13 Mean change in ß-carotene content for each quartile

Quartile	Day 0 ß-carotene *	Day 7 ß-carotene*	Change at day 7*
Lowest	0.58 ±0.126	4.58 ±3.28	4.16 ±3.38
2nd	0.97 ±0.097	7.16 ±3.87	6.19 ±3.84
3rd	1.53 ±0.220	5.49 ±3.09	3.92 ±3.09
Highest	5.35 ±3.040	9.55 ±4.02	4.13 ±4.62

*** (ng/10^6 cells)**

Figure 7 The effects of ß-carotene supplementation on individuals with low and high pre-supplementation levels of ß-carotene



Mean pre-supplementation levels of ß-carotene (day 0) were used to divide the data into quartiles. Mean ß-carotene levels (± S.D.) throughout the 21 days of study are shown for the lowest (opaque bars) and highest (cross hatched bars) quartiles of pre-supplementation ß-carotene.

Study 2

III.11 Objectives

The objectives of Study 2 were to quantitate unsupplemented and supplemented levels of cellular ß-carotene in different study groups and to determine the effects of dietary ß-carotene, alcohol and tobacco on these levels. A subgroup of participants from these studies completed a second supplementation regimen 1 year later so that the consistency of supplemented ß-carotene levels and responses to supplementation could be assessed. The data also provided a unique opportunity to evaluate the relationship between unsupplemented and supplemented levels of ß-carotene across a range of ß-carotene levels.

III.12. Study populations

The population groups came from four areas of British Columbia, Canada. The Vancouver group was composed of 53 individuals from Study 1 and 44 additional individuals who were recruited during Study 2. The combined group consisted of 52 males and 45 females who attended or worked at Simon Fraser University. The remaining three groups were composed of native Indians living on reserve. The Williams Lake group resided in a small rural area within 80 km of Williams Lake and included 29 males and 22 females. The 100 Mile House group lived within 50 km of 100 Mile House and consisted of 11 males and 11 females. The Lytton group lived within 10 km of town and consisted of 19 males and 18 females. Table 14a summarizes the lifestyle characteristics that were assessed in this study for year 1 in the four groups.

Table 14a Characteristics of the four groups in year 1

Group	Age	Alcohol[a]	Alcohol intake[b]	Snuff[a]	Tobacco[a]	Carrot intake[c]	ß-carotene index[d]
Vancouver (N=97)	25.6±7.5	65%	96.73 ± 97.14	0%	0%	3.2±3.9	4.9±4.6
Williams Lake (N=51)	29.6±11.9	8%	183.11[e]	70.5%	19.6%	ND[f]	ND
100 Mile House (N=22)	25.7±0.3	55%	265.71 ± 299.30	45.5%	54.5%	ND	ND
Lytton (N=37)	30.1±10.4	70%	511.75 ± 444.57	13.5%	64.9%	ND	ND

a: percentage of each group that reported each habit
b: milliliters of ethanol per week for those individuals that drank.
c: Carrot intake is reported as the frequency of carrot consumption in one week.
d: ß-carotene index is the weekly sum of carrot, spinach and broccoli intake servings.
e: mean alcohol consumption for 4 individuals that consumed alcohol.
f: ND = not determined.

In the second year of study, a subgroup of individuals from each group participated in a second supplementation study that included a dietary analysis section. Efforts were made to contact previous participants: those that were available and willing to repeat the study were enrolled in year 2. In the three study groups that were contacted in year 2, a large proportion of the study group had either moved or was unavailable to repeat the study. Eight males and 5 females completed the supplementation study and the dietary analysis in the Vancouver group. In Lytton, 4 males and 6 females participated while in the Williams Lake group, 12 females and 4 males participated in the second study. The characteristics of each group are shown in Table 14b.

Table 14b Characteristics of the three groups in year 2

Group	Age	Alcohol[a]	Alcohol intake [b]	Snuff[a]	Tobacco[a]	Carrot intake [c]	ß-carotene index[d]
Vancouver (N = 13)	29.6 ± 8.8	77%	55.55 ± 39.17	0%	0%	1.9 ± 1.9	6.4 ± 4.9
Lytton (N = 10)	28.5 ± 8.1	90%	414.90 ± 323.49	10%	90%	2.2 ± 1.1	4.4 ± 3.2
Williams Lake (N = 16)	31.7 ± 13.3	0%	0	69%	13%	5.2 ± 7.0	6.6 ± 5.6

a: percentage of each group that reported each habit
b: milliliters of ethanol per week for those individuals that drank.
c: Carrot intake is reported as the frequency of carrot consumption in one week.
d: ß-carotene index is the weekly sum of carrot, spinach and broccoli servings.

III.13. Compliance

Cell samples at day 0 were collected from 207 individuals in year 1. At day 7 cell samples were collected from 186 individuals. The Vancouver study group decreased by 10%, the Lytton group by 25%; Williams Lake by 2% and the One Hundred Mile House group to 23% from their original numbers. As a whole, 89% of individuals completed the study in year 1.

Compliance was monitored in all study groups by observing the ingestion of the supplement by all participants. The Vancouver participants came to the research office to pick up their supplements while the researcher drove house to house to distribute supplements in the other groups. Participants were reminded in person and by phone call of the next sampling date and supplement distribution in the Vancouver group. This was done in person or by a third party in the other three groups. The distribution of supplements, collection of samples and procedure for reminding participants was time consuming in the Lytton, Williams Lake and One Hundred Mile House locations. This is reflected in the

lower number of individuals participating from each native group in comparison to the Vancouver group.

III.14. Mean pre-supplementation levels of ß-carotene in the different groups

Cellular ß-carotene was estimated in exfoliated oral mucosa cells of individuals prior to supplementation in each of four study groups (Vancouver, Williams Lake, 100 Mile House and Lytton) in year 1 and in three study groups (Vancouver, Williams Lake, and Lytton) in year 2. Presupplementation levels of ß-carotene are reported as day 0 ß-carotene and are the result of 1 sample obtained before ingestion of the supplement. Only individuals with samples at both day 0 and day 7 were included in the analysis. The data obtained is shown in Table 15 and presented in graphic form in Figures 8 and 9. Presupplementation levels of ß-carotene were not significantly different among the four groups in either year 1 or 2.

III.15. Mean cellular levels of ß-carotene after supplementation in the four study groups

The mean ß-carotene levels of the 4 study groups after supplementation are shown in Table 5 and graphed in Figures 8 and 9. In each population and for both years, mean day 7 levels of ß-carotene were significantly greater than day 0 ß-carotene levels ($p<0.001$). Mean ß-carotene levels in the groups were compared after supplementation and were shown to differ significantly in year 1 ($p<0.001$) and year 2 ($p<0.001$). In year 1, multiple comparisons showed that the Vancouver group differed significantly from the Williams Lake group as well as

the 100 Mile House group and the Lytton group differed significantly from the Williams Lake and 100 Mile House groups ($p<0.001$). In year 2, the Vancouver and Lytton groups differed significantly from the Williams Lake group ($p<0.001$). The Vancouver and Lytton groups did not differ significantly from each other on any sample occasion.

Table 15 Mean pre-supplementation and day 7 levels of ß-carotene in the four study groups

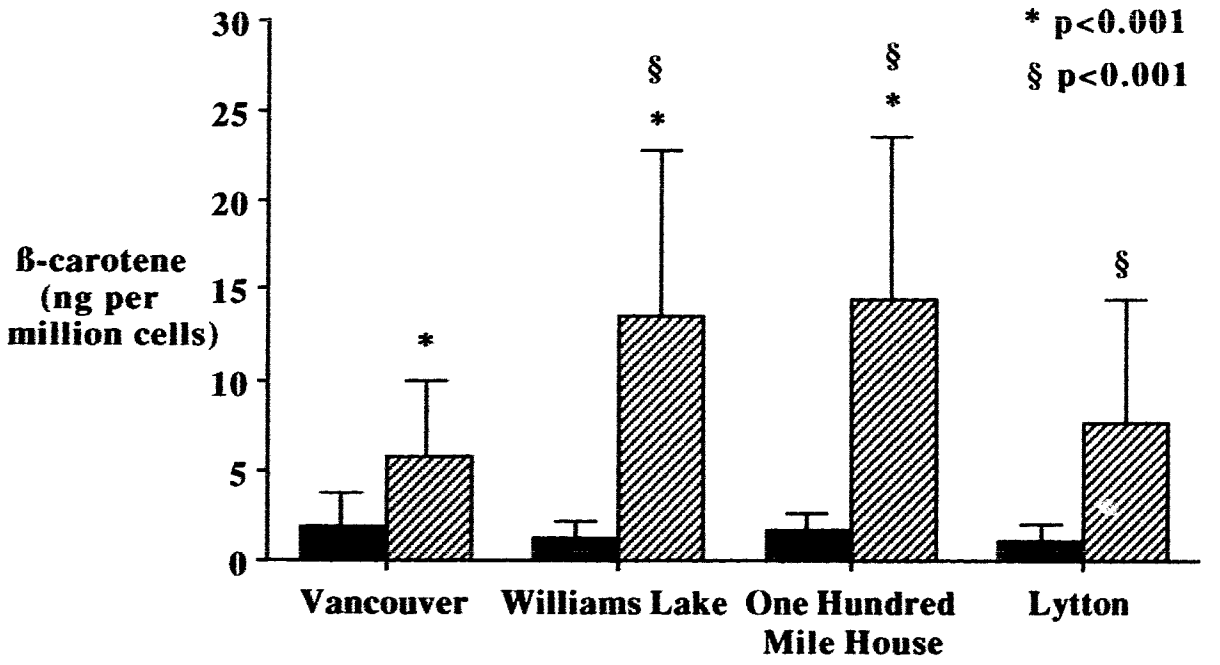
	YEAR 1		YEAR 2	
Study group	**Day 0§**	**Day 7§**	**Day 0§§**	**Day 7§§**
Vancouver	1.71 ± 2.03	5.73 ± 4.22* N = 88	1.08 ± 0.62	2.97 ± 1.51** N = 13
Lytton	1.02 ± 0.98	7.71 ± 6.84‡ N = 33	1.46 ± 0.77	3.62 ± 2.41## N = 10
Williams Lake	1.26 ± 0.91	13.57 ± 9.34*, ‡ N = 48	2.26 ± 5.42	10.73 ± 6.7**## N = 16
One Hundred Mile House	1.65 ± 0.89	14.48 ± 9.22*, ‡ N = 17		

ß-carotene levels shown are ng/10^6 cells

Results of 1 way ANOVA test for significant differences between means and multiple comparison procedure using Sheffe's test. Symbols indicate levels of significance for differences between means of the following groups:

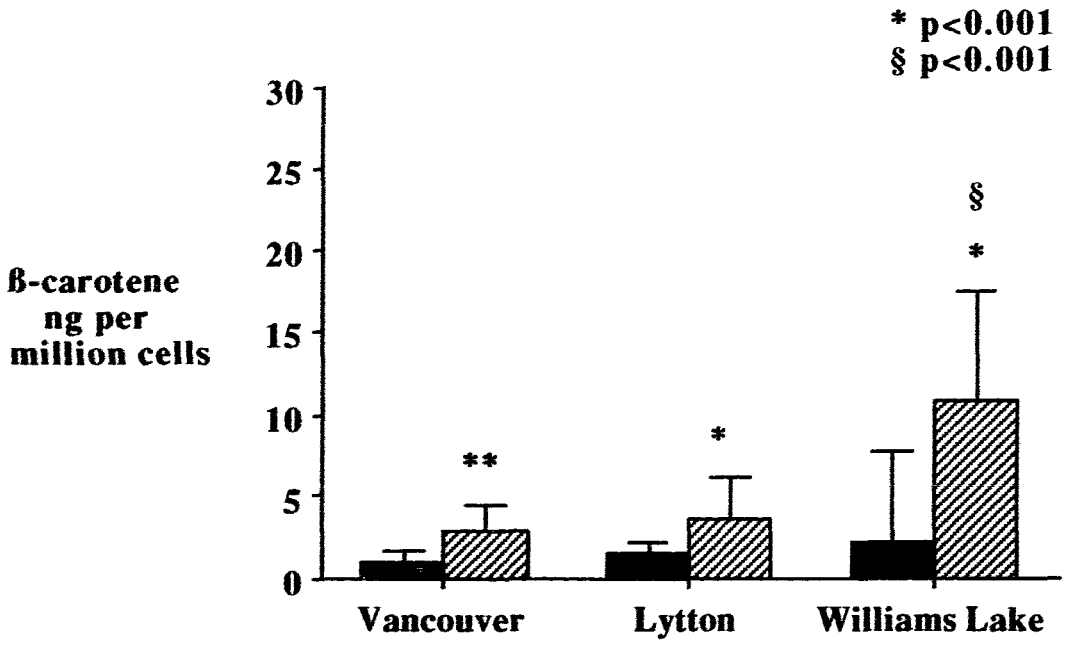
§ $p<0.001$ for day 0 and day 7 levels of all four groups
§§ $p<0.001$ for day 0 and day 7 levels of all four groups
* $p<0.001$ for day 7 levels between study marked groups
‡ $p<0.001$ for day 7 levels between study marked groups
** $p<0$ 001 for day 7 levels between study marked groups
$p<0.001$ for day 7 levels between study marked groups

Figure 8 Mean ß-carotene levels of four study groups before and after supplementation in year 1



Mean ß-carotene levels ± S.D. in samples of oral mucosa cells from individuals of the 4 study groups before (opaque bars) and after (cross-hatched bars) supplementation with ß-carotene (90 mg/day for each of three days). Buccal mucosa cells were sampled before and 7 days after the initiation of supplementation.

Figure 9 Mean ß-carotene levels of three study groups before and after supplementation in year 2.



Mean ß-carotene levels ±S.D. in the oral mucosa of individuals from 3 study groups before (opaque bars) and after (cross-hatched bars) supplementation with ß-carotene (90 mg/day for each of three days). Buccal mucosa cells were sampled before and 7 days after the initiation of supplementation.

III.16 Inter-individual variation in ß-carotene levels before and after supplementation

Individual responses to ß-carotene supplementation were assessed in 186 individuals. ß-carotene levels ranged from 0.00 to 11.2 ng/10^6 cells before supplementation and from 0.34 to 41.8 ng/10^6 cells after supplementation. The data were sorted in ascending order of post-supplementation levels of ß-carotene (Figure 10). The post-supplementation levels show a continuum across all populations.

III.17. Correlations between day 0 and day 7 levels of ß-carotene

Correlations between pre-supplementation levels of ß-carotene and day 7 levels were assessed for all individuals together and by study group. Three correlations of statistical significance were calculated.

In year 1, a significant correlation was calculated between day 0 and day 7 ß-carotene levels when all groups were included ($R= 0.17$, $p<0.05$). The linear regression equation was:

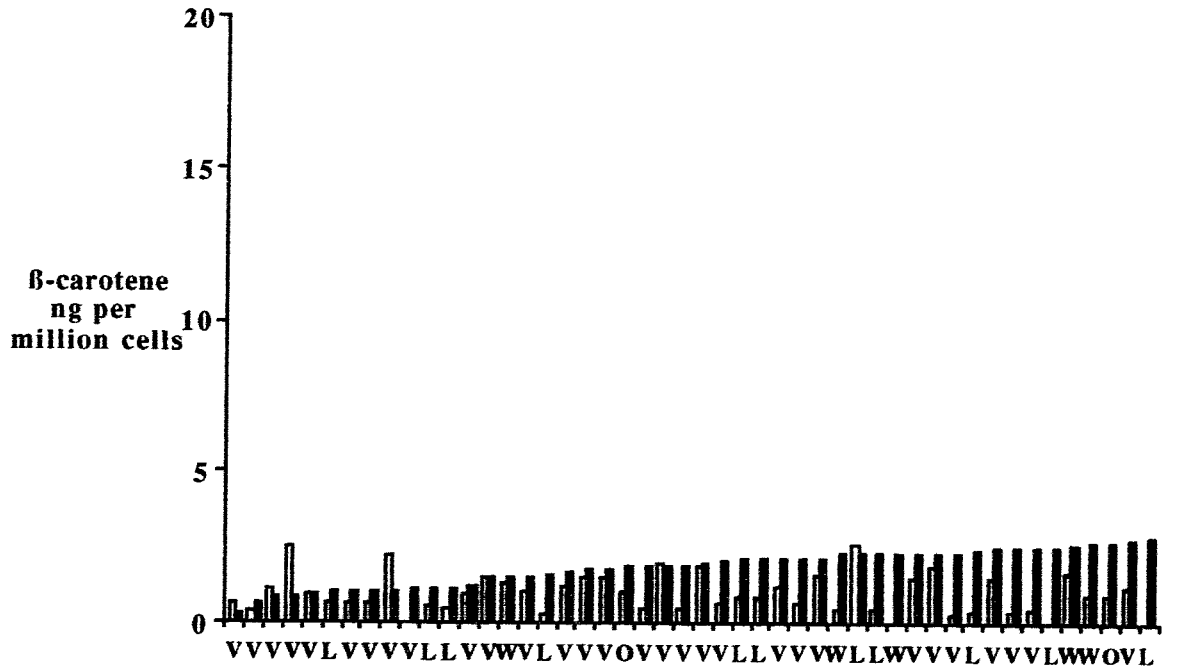
$$\text{Day 7 ß-carotene} = 7.54 + 0.80 * \text{Day 0 ß-carotene} \quad (7)$$

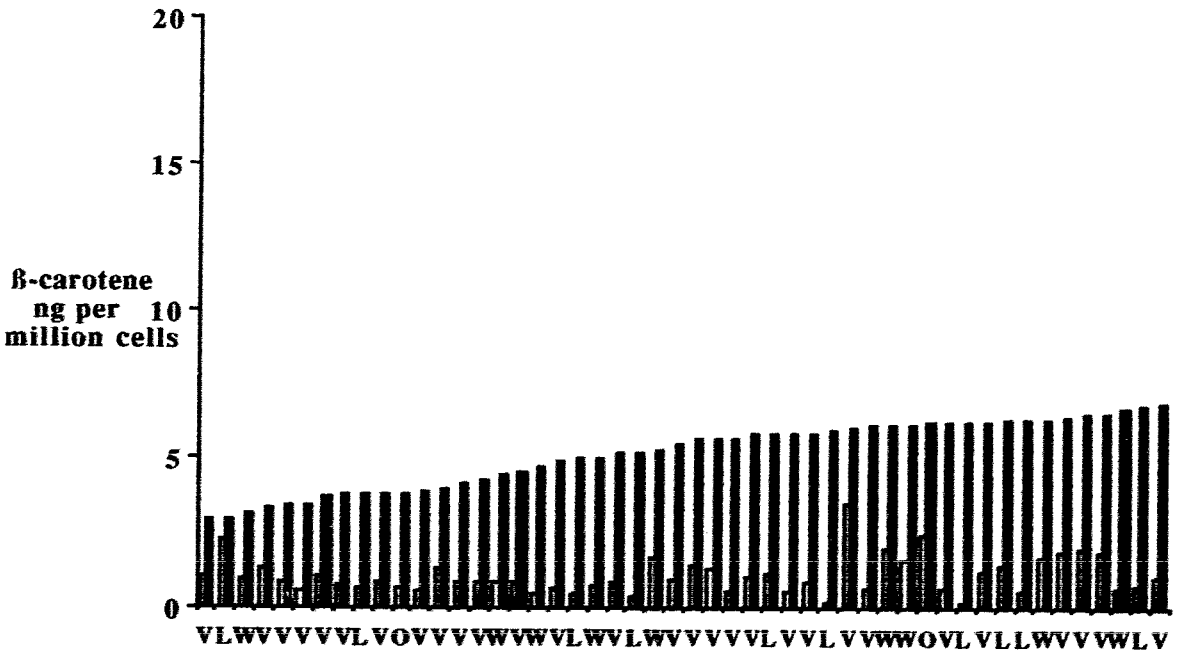
Second, the Vancouver group in year one showed a correlation of $R = 0.48$ ($p < 0.001$) between day 0 and day 7 ß-carotene levels with the following regression equation:

$$\text{Day 7 ß-carotene} = 4.05 + 0.96 * \text{Day 0 ß-carotene} \quad (8)$$

Figure 10 ß-carotene levels in 186 individuals before (open bars) and after (closed bars) supplementation with ß-carotene.

Each individual ingested ß-carotene supplements for three consecutive days at a dose of 90 mg/day. Samples of oral mucosa cells were collected and analyzed for ß-carotene content on day 0 (before supplementation) and 7 days after initiation of supplementation. The bars represent ß-carotene levels in cell samples before (open bars) and after (closed bars) supplementation. Individuals from each population are indicated with the following abbreviations: V = Vancouver, W = Williams Lake, L = Lytton, O = One Hundred Mile House. *-* indicates individuals with ß-carotene levels after supplementation ranging from 20 -42 ng/10^6 cells


20
15
ß-carotene
ng per
million cells
10
5
0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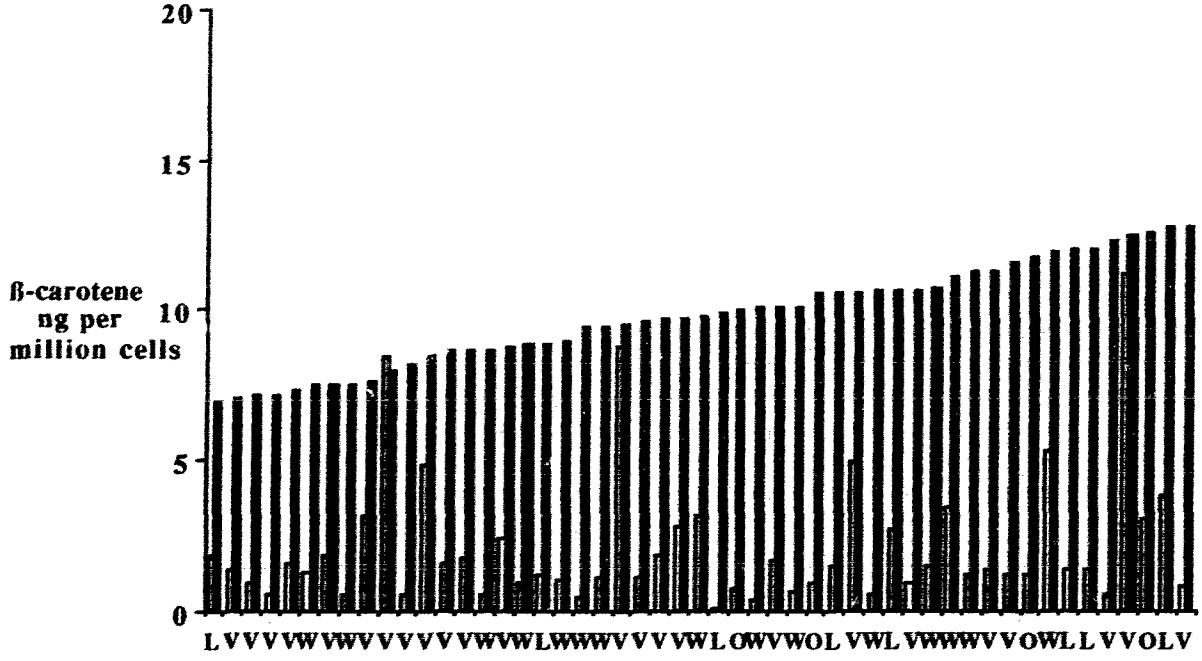
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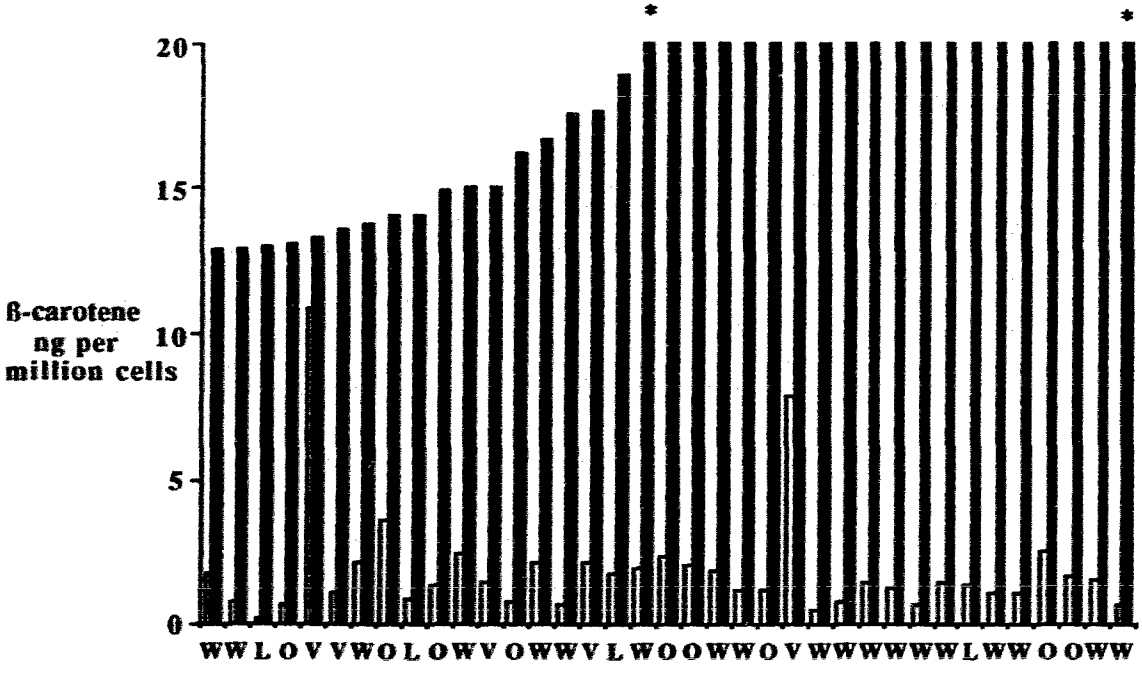
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Third, the day 0 and day 7 ß-carotene levels of the Williams Lake groups in year 2 were significantly correlated with R = 0.75, ($p < 0.001$). The regression equation was:

$$\text{Day 7 ß-carotene} = 3.77 + 7.18 * \text{Day 0 ß-carotene} \quad (9)$$

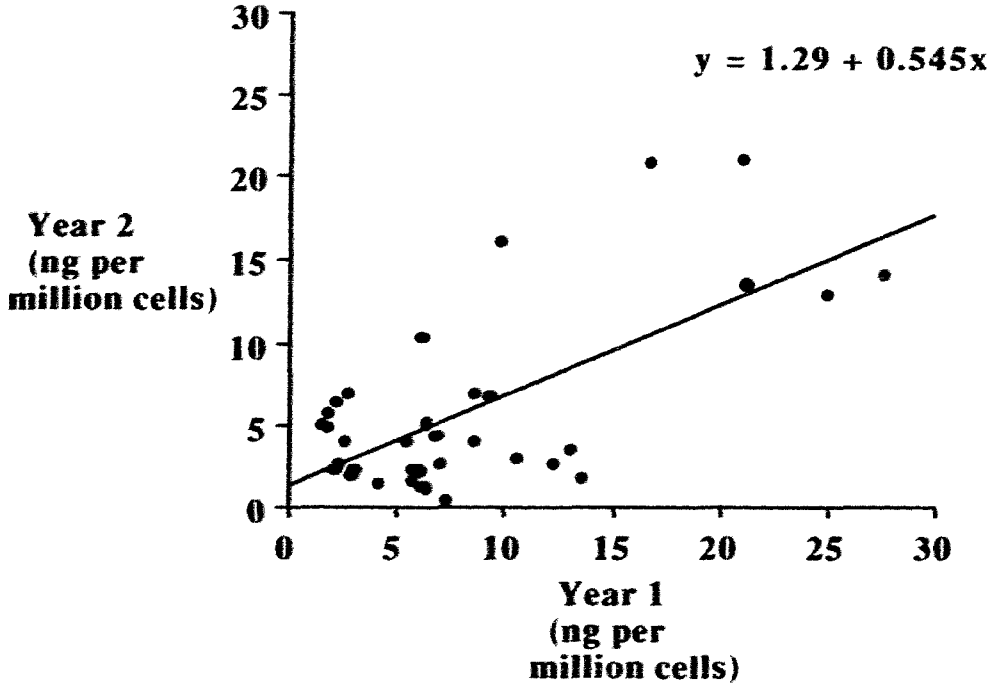
III.18. Consistency of ß-carotene levels over a 1 year time interval

ß-carotene levels before supplementation and at day 7 after supplementation were compared for those individuals that participated in the repeat study of year 2. The pre-supplementation levels of ß-carotene were not significantly different in year 1 and 2. Presupplementation levels of ß-carotene were not correlated between year 1 and 2.

Figure 11 shows that post-supplementation levels of ß-carotene were significantly correlated in year 1 and 2 ($R = 0.74$, $p<0.001$). However, day 7 levels of ß-carotene differed significantly among individuals in years 1 and 2 ($p<0.005$).

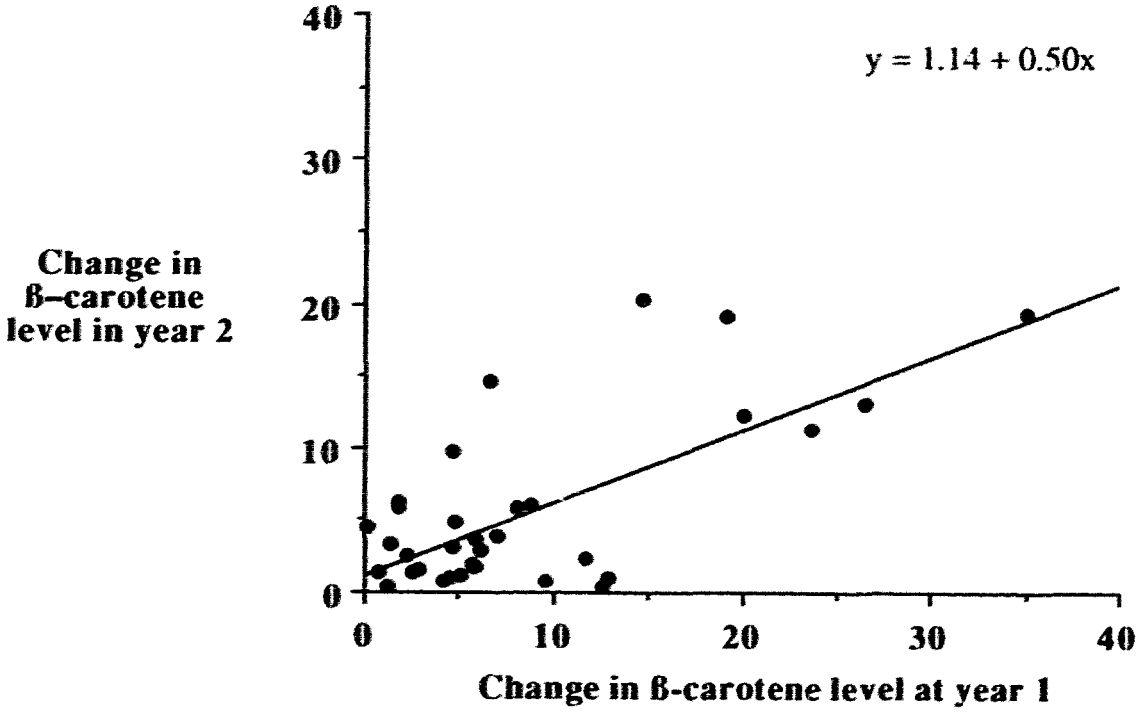
The change in ß-carotene content from pre-supplementation levels was calculated for each individual in each year. In Figure 12 the strong correlation between individual changes in ß-carotene levels for years 1 and 2 is shown ($R = 0.69$, $p<0.001$). The responses to supplementation were also significantly different in years 1 and 2 ($p < 0.005$). A comparison of the responses to ß-carotene supplementation for year 1 and 2 is seen in Figure 13. The mean difference between ß-carotene levels at day 7 in years 1 and 2 was -2.82 ± 5.34 ng/10^6 cells.

Figure 11 Correlation between post-supplementation levels of ß-carotene in year 1 and year 2



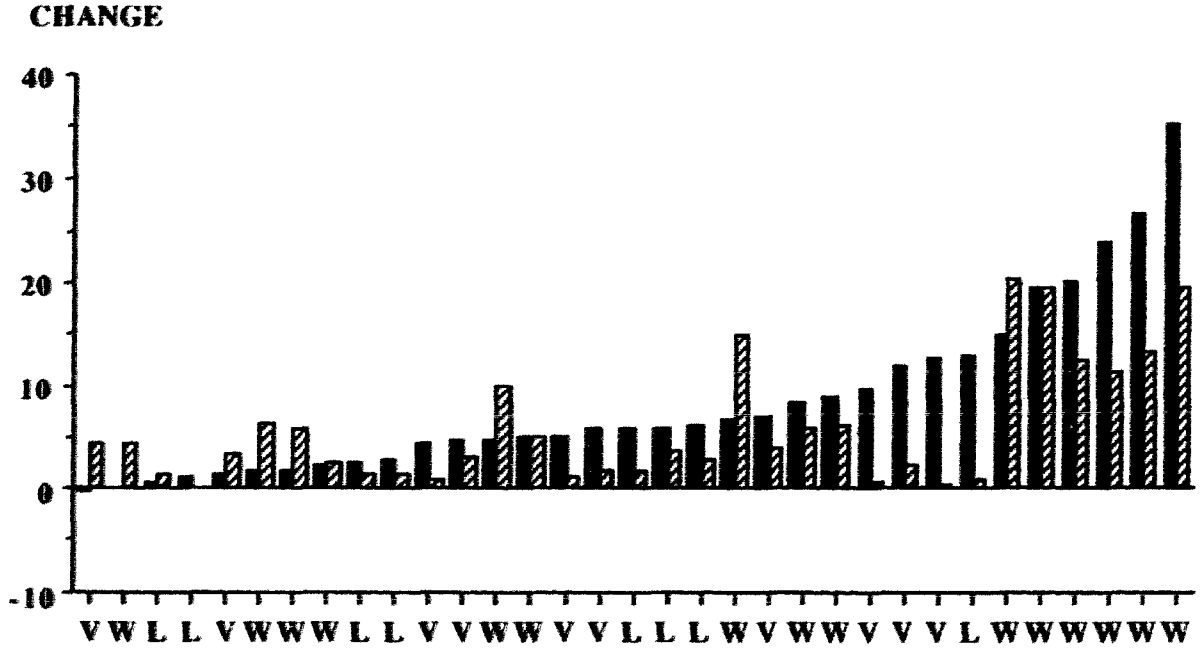
Cell samples were obtained from 37 individuals that had completed the second supplementation study in year 2. The correlation between the cellular levels of ß-carotene after supplementation between year 1 and 2 is shown with the linear regression equation.

Figure 12 Correlation between responses to ß-carotene supplementation in year 1 and year 2.



Cell samples were obtained from 37 individuals that completed supplementation studies in year 1 and year 2. The change in ß-carotene content from is the difference between pre and post-supplementation levels of ß-carotene. The correlation and the line of regression are shown.

Figure 13 Responses to ß-carotene supplementation by individuals in repeat supplementation studies



The changes in ß-carotene content (ng/10^6 cells) for year 1 (opaque bars) and year 2 (cross-hatched bars) were calculated and are presented in ascending order of the change in year 1. Individuals from each group are identified with: V = Vancouver; L = Lytton and W = Williams Lake.

III.19. Effects of gender, alcohol intake, tobacco or snuff use on levels of ß-carotene in cells.

Dietary and lifestyle questionnaires of Appendices 2, 3 and 4 supplied data for analysis of variance (ANOVA). The data from the groups were pooled and divided using the different levels of the factors gender (male or female), alcohol use (yes or no), tobacco use (yes or no) and snuff use (yes or no). Interactions between the independent variables on cellular levels of ß-carotene were analyzed by comparing the means of the subgroups using ANOVA. Complete data was available for 176 of the 186 individuals in year one and 36 of 39 individuals in year 2.

III.19a. Presupplementation levels of ß-carotene

No significant main effects were shown on the pre-supplementation levels of ß-carotene (Table 16). Alcohol intake was further divided into three categories of intake: (1) non-drinker, (2) social drinker = 2 drinks per day or < 250 ml per week of ethanol (personal communication B. Dunn) and (3) < 250 ml ethanol per week or greater than 2 drinks per day). An interaction between the level of alcohol intake and tobacco use affected pre-supplementation levels of ß-carotene ($p<0.002$) (Table 17).

Table 16 Effects of gender, alcohol, tobacco and snuff on pre-supplementation levels of ß-carotene in years 1 and 2.

Source	Year 1 p	Year 2 p
Gender	0.67	0.17
Alcohol	0.59	0.52
Gender*alcohol	0.99	0.77
Tobacco	0.25	0.75
Gender*tobacco	0.99	0.16
Alcohol*tobacco	0.002	0.06
Gender*alcohol*tobacco	0.96	÷
Snuff	0.50	0.17
Gender*snuff	0.62	0.71
Alcohol*snuff	0.93	÷
Gender*alcohol*snuff	0.61	÷
Tobacco*snuff	0.74	÷
Gender*tobacco*snuff	0.73	÷
Alcohol*tobacco*snuff	0.91	÷

÷ - insufficient data for this calculation.

Table 17 Effect of alcohol and tobacco on pre-supplementation levels of ß-carotene

Alcohol	Nondrinker	Intake < 250ml/week	Intake > 250 ml/week
Tobacco			
Yes	N = 21 1.32	N = 12 1.19	N = 18 0.69
No	N = 73 1.52	N = 70 1.57	N = 2 5.14

* ($ng/10^6$ cells)

III.19b. Cellular levels of ß-carotene after supplementation

Table 18 presents the results of the factorial ANOVA test on day 7 cellular levels of ß-carotene. Day 7 ß-carotene levels were positively influenced in individuals that were female with mean ß-carotene levels of 8.92 ng/10^6 cells among females and 8.12 ng/10^6 cells among males ($p<0.0025$).

Table 18 Effects of gender, alcohol, tobacco and snuff on post-supplementation levels of ß-carotene in years 1 and 2

Source	Year 1 p	Year 2 p
Gender	0.0025	0.95
Alcohol	0.02	0.06
Gender*alcohol	0.78	0.04
Tobacco	0.29	0.35
Gender*tobacco	0.39	0.83
Alcohol*tobacco	0.0091	0.69
Gender*alcohol*tobacco	0.32	‡
Snuff	0.0001	0.41
Gender*snuff	0.030	0.09
Alcohol*snuff	0.39	‡
Gender*alcohol*snuff	0.35	‡
Tobacco*snuff	0.66	‡
Gender*tobacco*snuff	0.92	‡
Alcohol*tobacco*snuff	0.33	‡

‡ - insufficient data for this calculation.

Snuff use in year 1 was associated with higher ß-carotene levels, with means of 13.46 ng/10^6 cells among users and 7.15 ng/10^6 cells among non-users ($p<0.0001$). Dietary intake of carrots and the ß-carotene index were assessed among snuff users and non-users using data collected in year 2. Carrot intake was significantly higher among those individuals that used snuff with means of

6.10 carrots per week versus 1.86 carrots per week by non users ($p<0.005$). Snuff users also had higher ß-carotene levels than non-users in year 2 although this was not statistically significant (means of 8.72 ng/10^6 cells versus 5.31 ng/10^6 cells among non-users).

In year 1, those that did not consume alcohol had higher day 7 ß-carotene levels than those that did consume alcohol when alcohol intake was stratified into three groups ($p<0.05$) (Figure 14). As alcohol intake increased, day 7 ß-carotene levels decreased.

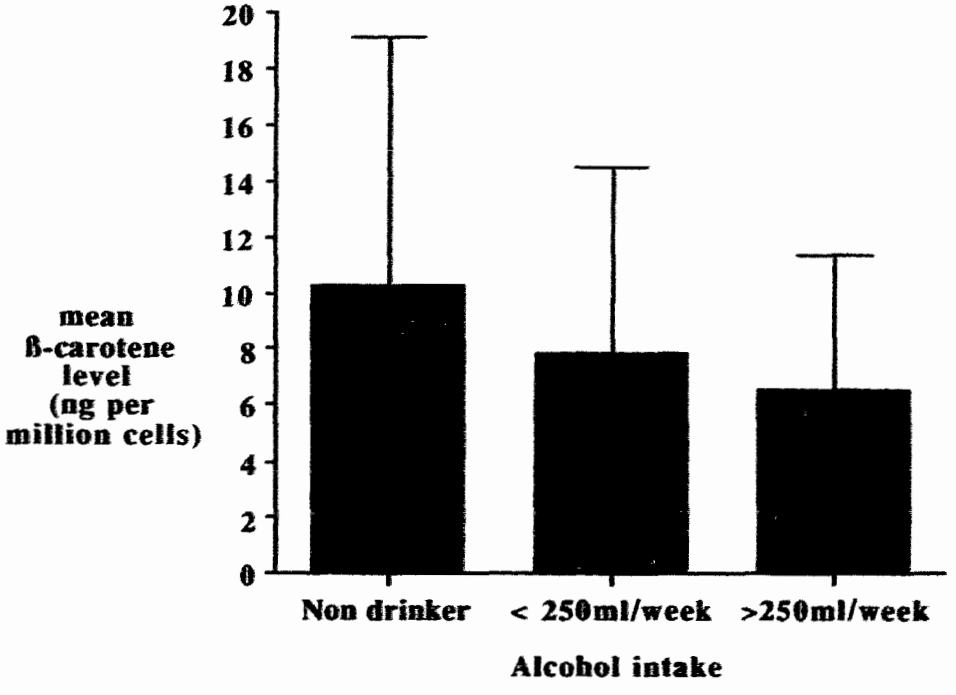
Table 19 shows the interaction between gender and snuff use in year 1, indicating that females using snuff had the highest ß-carotene levels at day 7 ($p<0.03$). The negative impact of alcohol on day 7 levels of ß-carotene is evident in Table 20 ($p<0.009$).

Table 19 The effect of gender and snuff use on post-supplementation levels of ß-carotene

Snuff	No	Yes
Gender		
Males	N = 68 6.52	N = 30 12.12
Females	N = 63 7.58	N = 15 16.90

* (ng/10^6 cells)

Figure 14 Mean ß-carotene levels after supplementation within individuals that are grouped by alcohol intake



The study group was divided into groups based on alcohol intake. Non-drinkers were one group (N = 89), those that were social drinkers (2 drinks per day or < 250 ml ethanol per week) were the second group (N = 76) and the third group were those that drank more that 2 drinks per day (< 250 ml ethanol per week), (N = 19). Error bars show standard deviation.

Table 20 The effect of alcohol and tobacco use on post-supplementation levels of ß-carotene*

Tobacco	Yes	No
Alcohol		
Yes	N = 26 8.53	N = 67 7.14
No	N = 16 13.24	N = 168 9.42

* (ng/10^6 cells)

In year 2, the interaction between gender and alcohol was significant, with the highest ß-carotene levels observed among women that did not consume alcohol ($p<0.04$).

III.19c. Effects of gender, alcohol, snuff and tobacco on cellular levels of ß-carotene in the native study groups

The native groups differed from the Vanccuver study group by ethnic origin, cultural influences and socio-economic status. Because of these differences, the effects of gender, alcohol, tobacco and snuff on cellular levels of ß-carotene were examined among native individuals as a group using ANOVA. Sufficient data was available only for year 1. Analysis was completed on 98 individuals at pre-supplementation and 89 individuals at post-supplementation (Table 21). Significant interactions were shown for gender*alcohol at pre-supplementation and gender*alcohol*snuff as well as gender*tobacco*snuff at post-supplementation.

Table 21 Effects of gender, alcohol, tobacco and snuff on cellular levels of ß-carotene in native Indian individuals (year 1)

Source	Presupplementation p	Postsupplementation p
Gender	0.47	0.99
Alcohol	0.62	0.78
Gender*alcohol	0.04	0.38
Tobacco	0.06	0.36
Gender*tobacco	0.85	0.66
Alcohol*tobacco	0.45	0.55
Gender*alcohol*tobacco	0.55	0.09
Snuff	0.39	0.25
Gender*snuff	0.28	0.32
Alcohol*snuff	0.35	0.75
Gender*alcohol*snuff	0.86	0.02
Tobacco*snuff	0.72	0.28
Gender*tobacco*snuff	0.69	0.01
Alcohol*tobacco*snuff	0.37	0.36

The effects of gender and alcohol on mean pre-supplementation levels of ß-carotene in native individuals are shown in Table 22. The interaction between gender, alcohol, snuff and tobacco and the effects on post-supplementation levels of ß-carotene are shown in Tables 23 and 24. Overall, positive influences on cellular ß-carotene at day 7 were being female, a nondrinker and a snuff user.

Table 22 Effects of alcohol intake on pre-supplementation levels of ß-carotene among males and females of the native Indian groups

	Alcohol	
Gender	**Yes**	**No**
Males	N = 22 0.86	N = 32 1.42
Females	N = 16 1.11	N = 28 1.25

* (ng/10^6 cells)

Table 23 Effects of alcohol and snuff use on post-supplementation levels of ß-carotene among males and females of the native Indian groups

Alcohol	**Yes**		**No**	
Snuff	**No**	**Yes**	**No**	**Yes**
Gender				
Males	N = 10 8.3	N = 11 8.4	N = 10 10.2	N = 19 14.3
Females	N = 10 9.2	N = 4 17.9	N = 14 10.2	N = 11 16.5

* (ng/10^6 cells)

Table 24 Effects of tobacco and snuff use on post-supplementation levels of ß-carotene among males and females of the native Indian groups

Tobacco	Yes		No	
Snuff	**No**	**Yes**	**No**	**Yes**
Gender				
Males	N = 10 8.3	N = 11 8.4	N = 10 10.2	N = 19 14.3
Females	N = 10 9.2	N = 4 17.9	N = 14 10.2	N = 11 16.5

* (ng/10^6 cells)

III.20. Effect of dietary ß-carotene on cellular levels of ß-carotene

The relationship of dietary ß-carotene to cellular ß-carotene was examined first by grouping individuals into categories of intake for each of carrots and ß-carotene index (sum of weekly intake of carrots, spinach and broccoli). The categories were determined by dividing the study group into thirds and assigning the intake level that fit each category: (1) less than 2 servings per week, (2) between 2 and 5 servings per week and (3) greater than 5 servings per week. Tables 25 and 26 show the positive relationships between intake of carrots or ß-carotene-containing foods and cellular levels of ß-carotene before and after supplementation.

Table 25 Relationship between dietary intake of carrots and cellular levels of ß-carotene

Carrot intake (weekly frequency)	Day 0* Mean cellular level of ß-carotene ($ng/10^6$ cells)
weekly servings < 2	1.20 ± 0.95
2 ≤ weekly servings < 5	1.52 ± 0.99
2 ≤ weekly servings	3.22 ± 3.78

* $p<0.001$

Using linear regression, correlation coefficients and regression equations were calculated for the relationship between cellular levels of ß-carotene and carrot intake. The correlation between day 0 levels of ß-carotene and carrot intake had a calculated correlation of R = 0.43, ($p<0.0001$) accounting for 19% of the variation in day 0 ß-carotene levels, and a regression equation of:

Day 0 ß-carotene = 1.02 + 0.23(carrot intake) (10)

Day 7 levels of ß-carotene showed a modest correlation with carrot intake with R = 0.27, ($p<0.05$) that accounted for only 7.5% of the variation in Day 7 ß-carotene levels, and a regression equation of:

Day 7 ß-carotene = 4.70 + 0.29(carrot intake) (11)

The ß-carotene index was also related to cellular levels of ß-carotene at day 0 (Table 17). The correlation had a calculated R = 0.43, accounting for 19% of the variation ($p<0.0001$), with a regression equation of:

Day 0 ß-carotene = 0.77 + 0.197(ß-carotene index) (12)

Table 26 Relationship between dietary ß-carotene and cellular levels of ß-carotene

ß-carotene index (total weekly servings of carrots, spinach and broccoli)	Day 0§ Cellular level of ß-carotene ($ng/10^6$ cells)
weekly servings < 2	1.14 ± 0.98
2 ≤ weekly servings < 5	1.21 ± 0.77
5 ≤ weekly servings	2.71 ± 3.04
	Day 7§§ Cellular level of ß-carotene ($ng/10^6$ cells)
weekly servings < 2	3.76 ± 2.40
2 ≤ weekly servings < 5	5.35 ± 4.19
5 ≤ weekly servings	6.96 ± 4.55

§ $p<0.002$

§§ $p<0.03$

The impact of dietary ß-carotene on cellular levels of ß-carotene was diminished after supplementation. This was evident in the correlation between day 7 ß-carotene and ß-carotene intake of R = 0.25, ($p<0.05$), accounting for 6.5% of the variation. The linear regression equation was:

Day 7 ß-carotene = 4.45 + 0.231(ß-carotene index) (13)

III.21. Prediction of cellular levels of ß-carotene

One purpose of Study 2 was to determine the best equation for predicting ß-carotene levels in cells. The contributions of each of the independent variables age, alcohol intake, snuff use (yes/no), snuff amount, tobacco use

(yes/no), tobacco amount and gender (males/female) were used in all regression analysis. Carrot intake (per week) and ß-carotene intake index (sum of carrot, spinach and broccoli intake) were used in regression analysis for the Vancouver group in year 1 and with all groups in year 2. Although all variables were entered into the equation, the model only included the best subset of independent variables to predict the significant relationship.

The coefficients used to create the best predictive equation for all individuals in year 1 are shown in Table 27. The variables that were entered into the equation were: age, gender, tobacco use, snuff use, alcohol intake, day 0 ß-carotene level, snuff amount and tobacco amount. $R = 0.55$, accounting for 30% of the variation in day 7 ß-carotene levels ($p < 0.001$).

Table 27 Coefficients for the regression equation predicting day 7 ß-carotene levels in year 1 for all groups

Variable	Coefficient
Intercept	5.21
Day 0 ß-carotene	0.95
Tobacco	-4.43
Snuff	8.75
Snuff*alcohol intake	-0.007

The equation was:

$$\text{Day 7 ß-carotene} = 5.21 + (0.95 * \text{Day 0 ß-carotene}) - (4.43*\text{Tobacco use}) + (8.75*\text{Snuff use}) - (0.007*(\text{Snuff use}*\text{Alcohol intake}) \quad (14)$$

Table 28 shows the coefficients for the variables involved in predicting day 7 ß-carotene in year 1 within the Vancouver group. The correlation was

significant with R = 0.50, accounting for 25% of the variation in day 7 ß-carotene levels ($p<0.0001$).

Table 28 Coefficients for the regression equation predicting day 7 ß-carotene levels in year 1 (Vancouver group)

Variable	Coefficient
Intercept	2.87
Day 0 ß-carotene	1.88
Alcohol*day 0 ß-carotene	-0.005
Alcohol*Carrrot intake	0.003
Day 0 ß-carotene*Carrot intake	-0.054

The equation was:

Day 7 ß-carotene = 2.85 + (2.00*Day 0 ß-carotene) +

(0.003*(Alcohol intake * Carrot intake)) -

(0.05 *(Day 0 ß-carotene*Tobacco use)) -

(0.005(Day 0 ß-carotene*Alcohol intake)) (15)

Table 29 shows that among the native Indian groups, the regression equation in year 1 included only alcohol intake and snuff use, accounting for 15% of the variation in day 7 ß-carotene levels ($p < 0.005$).

Table 29 Coefficients of the regression equation predicting day 7 ß-carotene levels in year 1 (Native groups)

Variable	Coefficient
Intercept	6.05
Alcohol intake	-0.01
Snuff use	4.7

The same modeling analysis was used to predict day 7 ß-carotene levels in year 2; the results are shown in Table 30. The relationship accounted for 36% of the variation in day 7 ß-carotene levels and was moderately significant

($p<0.05$). Regression equations were not statistically significant when calculated for the Vancouver or native Indian groups separately.

Table 30 Coefficients of the regression equation for predicting cellular ß-carotene levels after supplementation in year 2 (All groups)

Variable	Coefficient
Intercept	3.79
Day 0 ß-carotene	2.73
Snuff amount	-2.84
Day 0 ß-carotene * Snuff use	7.18
Gender	-3.00
Gender * Snuff use	98.6

IV. DISCUSSION

IV.1 Overview of the Discussion

The studies discussed in the thesis have shown that populations, and individuals within populations, respond differently to ß-carotene supplementation. These differences can be identified by examining ß-carotene levels in exfoliated cells. The research findings will be discussed in the following order:

(1) Intra-individual variation in ß-carotene levels of unsupplemented individuals and probable contributors to this variation.

(2) Inter-individual variation in ß-carotene levels of unsupplemented and supplemented individuals.

(3) Population differences in cellular ß-carotene levels.

(4) Consistency of individual responses to supplementation regimens completed in year 1 and year 2.

(5) Some of the factors that may affect cellular levels of ß-carotene.

(6) Limitations of Studies 1 and 2.

(7) Proposal for future studies involving ß-carotene.

IV.2 Intra-individual variation in ß-carotene levels of unsupplemented individuals and probable contributors to this variation

Study 1 presents novel information on intra-individual variation in ß-carotene levels by comparing two sets of cell samples from unsupplemented individuals. The ß-carotene levels in the two samples are significantly correlated and individual differences amount to a mean value of 1.22 ng/10^6 cells or 66% of the Sample 1 mean. After removal of outliers, intra-individual variation decreases to 0.4 ng/10^6 cells. This information is important since it is necessary to have an estimate of the natural variability in ß-carotene levels within individuals in order to assess the impact of a supplementation regimen on cellular ß-carotene levels. The low levels of within-person variation present in Study 1 made detection of the supplement in cell samples very simple. The mean change in ß-carotene content with supplementation is 4.6 ng/10^6 cells while intra-individual variation is 1.22 ng/10^6 cells.

Intra-individual variation in cellular ß-carotene levels can result from 2 components: technical and biological sources. Each of these components should be less than the total 66% intra-individual variation estimated in Study 1. The discussion in this section focuses on some probable causes of technical and biological variation as well as error as contributors to intra-individual variation.

The technical components of intra-individual variation that contribute to the 66% variation in Study 1 include variability introduced during processing of the sample, extracting the ß-carotene from cells, injecting the ß-carotene into the HPLC system and reporting of the ß-carotene content with the integrator. An additional variable involves the variation inherent in using an estimate of cell

count (from the wet weight table of Appendix 1) rather than doing the cell count. Cameron (1987) estimated of the technical variation of the procedures used to quantify cellular ß-carotene content by creating a pooled cell sample from which ten aliquots were prepared, weighed and analyzed for ß-carotene content. (Cameron, L.C., 1987). Technical variation amounts to 25% of the mean ß-carotene content of the samples or 0.4 ng/10^6 cells. This estimate of technical variation can be applied to the current studies because Study 1 used identical processing procedures to those described by Cameron (1987). It is interesting that the magnitude of technical variation is equal to the intra-individual variation of the study group without outliers.

The introduction of error or a bias between Sample 1 and 2 may account for the decrease in cellular ß-carotene experienced by the majority of individuals. However, the collection of cell sample 2 was identical to the collection of the first sample. Samples were quickly removed from light sources and placed on ice until processing. Cell samples from both Samples 1 and 2 were coded and the tubes were mixed to blind the researcher to the origin of the sample being processed. This protocol may be rigorous enough to rule out bias as a source of the small decrease in cellular ß-carotene.

Biological factors may contribute to the 66% intra-individual variation in ß-carotene levels of Study 1. Some probable hypotheses to account for the variation in ß-carotene levels within an individual include: (1) an increased rate of cellular turnover that could alter ß-carotene content of Sample 2; (2) differences in ß-carotene intake before Sample 1 and between Sample 1 and 2; and (3) oxidation of ß-carotene from freshly exposed cells. The following discussion illustrates the underlying mechanisms by which these changes would alter ß-carotene levels in cells.

Brushing the oral mucosa may increase cellular turnover and decrease cellular levels of ß-carotene in the second sample. Brushing a tissue is irritating and may stimulate cellular proliferation (Tannock, I.F., and Hill, R.P., 1987) thereby decreasing cellular levels of ß-carotene in a second sample.

A change in dietary intake of ß-carotene between samples may cause ß-carotene levels in the samples to differ. Ingestion of a single dose of 29 mg ß-carotene in the form of carrots (equivalent to 3 carrots according to Table 5) produces peak plasma responses in 2 days. As these levels remain elevated for 4 to 7 days (Brown et al, 1989), this dietary change may increase cellular levels of ß-carotene. Decreases in dietary intake between Samples 1 and 2 might decrease the ß-carotene content of Sample 2. Individuals showed both increases and decreases in ß-carotene levels between Samples 1 and 2, supporting this hypothesis.

A third hypothesis suggests that brushing of the oral mucosa increases the ß-carotene content of subsequent cell samples by getting rid of older, oxidized cells containing little ß-carotene. Cooney and co-workers suggest that latter hypothesis to explain the increase in ß-carotene content observed between 2 samples from 4 unsupplemented individuals obtained 8 days apart (Cooney, R.V., et al, 1991). The data from Study 1 does not support this theory. It is noteworthy that serum levels of ß-carotene are different between Hawaii groups with 1 and 2 samples before supplementation. As brushing the oral mucosa is not likely to affect serum levels of ß-carotene, this suggests that other factors may have affected ß-carotene levels in the Hawaii groups.

Depletion of ß-carotene from oral mucosa cells may occur through oxidative processes after the first sample is obtained. Removal of upper cells

layers would expose lower layers to higher concentrations of oxygen than normal. As ß-carotene is labile to oxygen and light (Krinsky, N.I., 1989, Burton, G.W., and Ingold, K.U., 1984), this would result in oxidative loss of ß-carotene. In this hypothesis, the ß-carotene content of second and subsequent samples would decrease. Some individuals do show lower levels of ß-carotene in the second sampling.

IV.3 Inter-individual variation in ß-carotene levels of unsupplemented and supplemented individuals

Inter-individual variation, also known as variation between individuals, is also the sum of technical and biological variation. Studies 1 and 2 quantified inter-individual variation in cellular ß-carotene levels in unsupplemented and supplemented individuals. In both studies, a naturally occurring wide range of ß-carotene levels is present in each of the study groups. In contrast, retinol levels in serum do not show a similar wide range in ß-carotene levels, either in unsupplemented or supplemented individuals (Tangney, C.C., et al, 1987). The discussion of inter-individual variation concentrates first on unsupplemented individuals and then on supplemented individuals, including comparisons to reported inter-individual variation in ß-carotene levels in other tissues.

ß-carotene levels of unsupplemented individuals in Study 1 vary by 124% (1.88 ± 2.33 ng/10^6 cells) of the mean at Sample 1 and by 154% (2.03 ± 3.13 ng/10^6 cells) at Sample 2 (as estimated by the standard deviation). In Study 2, ß-carotene levels vary between individuals by 106% of the mean. If the estimated technical variation accounts for only 25% of the inter-individual variation, (as estimated by Cameron, 1987), then biological variation may account

for a large component of the variation. Biological variation is the sum of those factors which influence ß-carotene bioavailability in humans and will be addressed later in the discussion.

Table 31 shows inter-individual variations in different tissue levels of ß-carotene as well as the inter-individual variations in ß-carotene levels of Studies 1 and 2. For comparative purposes, the ß-carotene content of cell samples has been converted to µg ß-carotene/g wet weight of cells with the use of Appendix 1. Each tissue and study group presents a unique range of ß-carotene levels. The overall ß-carotene level in exfoliated cells is the smallest of all tissues mentioned. However, it is similar to other sites in that there is a wide range of ß-carotene present. Tissue such as the adrenals, liver and adipose cells may have higher ß-carotene levels because of high concentrations of LDL receptors that participate in ß-carotene delivery to the cell (Kaplan, L.A. et al, 1990). Differences in LDL receptor density may explain the relatively low levels of ß-carotene in exfoliated cells. Additionally, the short life span of the exfoliated cells may limit the time for accumulation of ß-carotene.

Despite the wide range of pre-supplementation levels of ß-carotene, supplementation with ß-carotene increases the concentration of ß-carotene in most individuals of both studies. Supplemented levels of ß-carotene are not limited by the amount of ß-carotene present in the tissue before supplementation. This information is important from a chemoprevention viewpoint: individuals at high risk for cancer may be able to reduce their risk by elevating ß-carotene levels with supplements regardless of their pre-supplementation level of ß-carotene.

Table 31 Inter-individual variation in ß-carotene levels of serum and human tissues (unsupplemented)

Tissue	Range of ß-carotene levels	Difference between individuals	Reference
serum	12.00 - 131 µg/dl	11-fold	Tangney, C.C. et al 1987
	4.00 - 23.5 µg/dl	6-fold	Henderson, C.T. et al, 1989
oral mucosa cells	0.002 - 0.148 µg/g	74-fold	Cameron, L.C. et al, 1989
	0.002 - 0.376 µg/g	188-fold	Study 1
	0.00 - 0.277 µg/g	277-fold	Study 2
urogenital tract cells	0.002 - 0.087 µg/g	44-fold	Cameron, L.C. et al, 1989
lung	0.05 - 1.5 µg/g	30-fold	Schmitz, H.H. et al, 1991
adipose tissue	0.34 - 35.1 µg/g	103-fold	Parker, R.S. 1989
	0.05 - 2.37 µg/g	47-fold	Kaplan, L.A. et al, 1990
kidney	0.05 - 1.5 µg/g	30-fold	Schmitz, H.H. et al, 1991,
	0.05 - 1.09 µg/g	22-fold	Kaplan, L.A. et al, 1990
liver	0.43 - 10.4 µg/g	24-fold	Schmitz, H.H. et al, 1991,
	0.21 - 3.94 µg/g	18-fold	Kaplan, L.A. et al, 1990
adrenals	0.94 - 158.7 µg/g	169-fold	Kaplan, L.A. et al, 1990

Day 7 levels of ß-carotene vary by 16-fold in Study 1: the standard deviation at day 7 was 60% (4.59 ± 3.81 ng/10^6 cells) of the mean value. In Study 2, all 4 groups show ranges in ß-carotene levels that overlap. This data may indicate that each individual responds differently to ß-carotene supplementation. Assuming that technical variation remains constant, this suggests that biological factors within each individual affect the use of the supplement.

Less inter-individual variation is apparent in serum than cellular levels of ß-carotene of individuals receiving supplementation with ß-carotene. Dimitrov evaluated peak serum levels of ß-carotene in a study in which participants ingest

a single dose of 150 mg ß-carotene (Dimitrov, N.V., 1990). As calculated from the standard deviation, mean ß-carotene levels vary by 17% after supplementation (Dimitrov, N.V., 1990). Low and high fat diets affect the inter-individual variation during ingestion of 45 mg/day of ß-carotene for 5 days: ß-carotene levels vary by 22% of the mean with the low fat diet and 35% with the high fat diet. (Dimitrov, N.V., 1990). In a second study involving 4 months supplementation with 15 mg ß-carotene daily, between-subject variation was 5.6% of the mean ß-carotene level (Constantino, J.P., 1988). The values of 106% to 154% inter-individual variation after supplementation in Studies 1 and 2 suggest that inter-individual variation is greater in cells than in serum.

Kaplan reports differing tissue levels of ß-carotene as a function of LDL receptor density (Kaplan, L.A. et al, 1990). The wide range of supplemented ß-carotene levels in oral mucosa cells may suggest that individuals differ in the density of LDL receptors on oral mucosa cells.

IV.4 Responses to ß-carotene was consistent in repeat supplementation studies

The consistency of an individual's response to ß-carotene supplementation has not previously been assessed. This section discusses the consistency of unsupplemented and supplemented ß-carotene levels in samples obtained from the same individual at 1 year intervals. Study 2 shows high correlations between post-supplementation levels of ß-carotene and changes in ß-carotene content with supplementation (response) when assessed one year apart suggesting consistency in the response to supplementation.

Dietary and physiological factors may affect the bioavailability of ß-carotene (Dimitrov, N.V., 1990. Henderson, C.T. et al, 1989. Russell-Briefel, R. et al, 1985). The consistency of responses may indicate that these factors did not change over the 2 years. Stahlein reported a moderate correlation between serum levels of carotenes in samples collected 5 years apart (Stahlein, H.B., et al 1991).

Presupplementation levels of ß-carotene were assessed for consistency over a 7 day period and over a 1 year period. ß-carotene levels in the first samples correlate well with ß-carotene levels in samples collected 7 days later; however ß-carotene levels in samples collected in year 1 do not correlate with levels of ß-carotene in samples collected in year 2. Study 2 shows that unsupplemented cellular levels of ß-carotene correlate with dietary intake of carrots or the ß-carotene index therefore, differences in dietary intake of ß-carotene may affect pre-supplementation levels of ß-carotene. Although tissue levels of ß-carotene may represent accumulation of ß-carotene over months (Parker, R.S., 1989), the limited life cycle of a buccal mucosa cell suggests it would indicate dietary intake over a shorter term. This may explain why cellular levels of ß-carotene correlate over a 7 day period but not over a one year period. It is noteworthy that the differences in means between unsupplemented ß-carotene levels assayed 1 year apart are not statistical significant.

The season of sampling must be considered when examining the consistency of response to ß-carotene. For serum samples collected in opposing seasons, mean baseline levels of ß-carotene are reported to be different (Nierenberg, D.W., et al, 1989, Russell-Briefel, et al, 1985, Comstock, G.W., et al, 1988). Summer ß-carotene levels in serum also differ significantly from winter levels. Although samples of Study 2 are collected in the summer and early fall of

two separate years, no seasonal effect is evident. This agrees with the findings of Comstock who reports that samples collected in adjoining months do not show significant differences (Comstock, G.W. et al, 1988).

The magnitudes of responses to supplementation are lower in year 2 than in year 1. This may indicate the introduction of a bias. Some probable hypotheses to explain the lower ß-carotene levels after supplementation in year 2 are: (1) different batch of ß-carotene supplements in year 2, (2) old ß-carotene supplements used in year 2, and (3) change in HPLC protocol and standards , between year 1 and 2.

The use of different batches of ß-carotene supplements or old supplements would affect post-supplementation levels of ß-carotene in a systematic way. If the supplements are from different batches with differing levels of ß-carotene, i.e., old capsules with oxidized ß-carotene, then post-supplementation levels of ß-carotene would be less in year 2 than year 1. The age of the capsules and the lot number should be noted in future studies to prevent the possibility of this type of error. In addition the ß-carotene content of the capsules was not assessed before each supplementation study. To assess differences in ß-carotene content between batches, a sampling of the capsules could be analyzed and the ß-carotene content quantified. For better reproducibility, this should be done before the initiation of any supplemental study. Second, improvement of storage conditions may minimize losses of ß-carotene during storage. Capsules are stored in large canisters, sealed from light and at -20°C. A more rigorous storage protocol might include sealing the capsules in an atmosphere of nitrogen and storing them at -80°C.

Changes in HPLC protocol and ß-carotene standards over the 1 year interval between the 2 studies could be avoided if all samples were analyzed at the same time.

IV.5 Populations respond differently to ß-carotene supplementation

This study is the first to explore the effects of supplementation on study groups with different lifestyle characteristics. Although the mean ß-carotene levels for the 4 study groups are not significantly different prior to supplementation, they become different after supplementation. The groups from Williams Lake and One Hundred Mile House have the highest levels of ß-carotene while Lytton and Vancouver have the lowest levels of ß-carotene. When the supplementation is repeated 1 year later, the same trend in ß-carotene levels is apparent. The different lifestyle characteristics of the individuals in each group of Study 2 partially explain the differences in post-supplementation levels of ß-carotene. The factors that may influence these cellular levels are detailed later in the discussion. This section compares the supplementation responses of the 4 study groups to that reported for other groups.

The range of ß-carotene levels after supplementation agree with those previously reported. A study of lab personnel receiving 90 mg ß-carotene for 4 days shows a mean ß-carotene level of 2.9 ng/10^6 cells when assessed 14 days after delivery of the last supplement (Stich, H.F. et al, 1986b)(see Table 10). This value is similar to the day 14 level of Study 1. In contrast, post-supplementation levels of ß-carotene of Study 2 are higher that than those observed in Filipino women receiving 180 mg ß-carotene twice weekly for 4 months (Table 10,

Literature review). It is interesting that the ß-carotene supplementation of Study 1 and 2 create higher ß-carotene levels than those attained by the Filipino women ingesting ß-carotene (180 mg twice weekly) and vitamin A (100,000 IU) (Stich, H.F. et al, 1986b). One hypothesis to explain the difference in post-supplementation levels of ß-carotene by these different studies would include the reverse smoking habits of the women as well as differing dietary habits of Filipino and North American individuals as factors that influence cellular ß-carotene levels. This suggests that biological factors can have a great impact on post-supplementation levels of ß-carotene.

Supplementation of female nurses in Vancouver produces mean ß-carotene levels that are in the middle of the post-supplementation ß-carotene levels observed in Study 2 (Cameron, L.C., et al 1989). Their supplementation of 180 mg twice weekly for 4 weeks increases cellular levels of ß-carotene to 9.9 ng/10^6 cells. As the post-supplementation levels of ß-carotene are similar in the latter study to those of Studies 1 and 2, it would be worth exploring the idea of whether a 3 day supplementation study can achieve similar ß-carotene levels in individuals as a 4 week supplementation study. This would require a cross-over study design, wherein the same individuals would participate in the short-term and long-term studies with a washout period between the studies.

The change in ß-carotene content with supplementation by the Williams Lake and One Hundred Mile House groups is similar to that reported for a group of lab personnel in Hawaii (Cooney, R.V. et al, 1991). The supplementation protocol is different for the Hawaii group as it involves ingestion of 30 mg ß-carotene for 7 days and cell sampling on the eighth day (Cooney, R.V. et al, 1991). Mean post-supplementation levels of ß-carotene increase to 18.0 ng/10^6 cells from baseline levels of 1.6 ng/10^6 cells. Table 32 compares ß-carotene

levels in serum after supplementation. Each researcher uses different supplementation regimens and sampling protocols. However, it is interesting that mean serum levels of ß-carotene spread over a wide range of values, with long term supplementation producing the highest levels (Table 32).

Study 1 indicates that baseline levels of ß-carotene correlate well with post-supplementation levels. In contrast, there is no association between baseline levels of ß-carotene and post-supplementation levels of ß-carotene in Study 2. While mean day 0 levels of ß-carotene are not statistically different between the 4 study groups, post-supplementation levels of ß-carotene differ significantly. Stich (1986a) reports differing baseline levels of ß-carotene among vegetarians and alcoholics. These two groups represent dietary extremes: the first diet is replete with ß-carotene and the latter diet is virtually devoid of ß-carotene. The data of Study 2 show that none of the four groups represent dietary extremes in ß-carotene intake and this may account for the similarity in mean pre-supplementation ß-carotene levels.

Table 32 Serum levels of ß-carotene after supplementation

Study group	Serum ß-carotene (µmol/L)	Reference
Hawaii (without prescrape) 30 mg ß-carotene for 7 days	2.4	Cooney, R.V. et al, 1991
Hawaii (with prescrape) 30 mg ß-carotene daily for 7 days	5.0	Cooney, R.V., et al, 1991
300 men receiving 15 mg ß-carotene daily for 1 year	3.2	Constantino, J.P., et al, 1988
Peak ß-carotene levels in serum of four individuals per group ingested single doses of ß-carotene as follows: 15 mg 30 mg 45 mg 150 mg	 0.596 0.596 0.707 1.170	Dimitrov, N.V., et al, 1990
Peak ß-carotene level with diet containing: 16 g fat 94 g fat	 0.689 2.66	Dimitrov, N.V. et al, 1988

IV.6 Factors involved in accumulation of ß-carotene in tissues

Factors that influence ß-carotene absorption and its use in humans affect ß-carotene levels in cells. These factors include personal attributes and lifestyle habits. Study 2 considers the following factors: gender, age, alcohol intake, smoking, snuff use and dietary ß-carotene. The following section discusses the impact of these factors on cellular levels of ß-carotene in Study 2.

Gender

Gender affects cellular levels of ß-carotene such that females consistently have higher cellular levels of ß-carotene than males. Serum levels of ß-carotene among unsupplemented females are consistently higher than those in males of the same study group (Ito, Y et al, 1991, Nierenberg, D.W. et al, 1989, Comstock, G.W. et al, 1988, Stryker, W.S., et al, 1988, Kaplan, L.A., et al, 1987). After 1 year of supplementation with ß-carotene, women show the largest increases in serum ß-carotene (Nierenberg, D.W. et al, 1991). The reasons for differing ß-carotene levels and responses to ß-carotene supplementation by males and female have not been explored. As more studies involve both males and females in identical study protocols the underlying causes of these effects may become evident.

Age

The study groups in this thesis do not reveal an effect of age on either pre- or post-supplementation levels of ß-carotene. A wide range of ages participated in the studies in this thesis, from ages 15 to 70 years, but the mode value was 22 years. Involving a study population with individuals spread evenly throughout the age range may produce different results. In studies from other laboratories, increasing age is reported to correlate with serum ß-carotene levels (Nierenberg,D.W., 1989); however, no correlation between age and serum ß-carotene is found in a different study (Russell-Briefel, R., et al, 1985). In a third study, the response to supplementation by two study groups, mean ages 74 years and 24 years, is different with greater responses appearing in the younger age group. Causes of the differing responses may be differences in body composition and serum lipid patterns (Sugerman, S.B., 1991).

Dietary ß-carotene

Dietary intake of carrots or having a high ß-carotene index (sum of carrots, spinach and broccoli) positively affects cellular levels of ß-carotene of unsupplemented individuals. As the magnitude of the correlation is the same between carrot intake or the ß-carotene index, it is probable that carrot intake is the factor driving the relationship. Variation in carrot intake accounts for 18.5% of the variation in cellular levels of ß-carotene. This underscores the importance of other biological factors in determining cellular levels of ß-carotene. In a study of serum ß-carotene and diet, dietary intake of carrots correlates well with serum levels of ß-carotene (Ito, Y. et al, 1991). In other studies, indexes of dietary intake of ß-carotene adequately predict serum levels of ß-carotene in adults and adolescents (Cooney, R.V. et al, 1991, Russell-Briefel, R. et al, 1985, Nierenberg, D.W. et al, 1989, Herbeth, B., et al, 1991).

The correlations between carrot intake or ß-carotene index and day 0 ß-carotene ($R = 0.43$) for the Vancouver group are higher than that reported between dietary intake of ß-carotene and baseline serum ß-carotene (Russell-Briefel, R. et al, 1985). The latter reports that the accuracy of dietary analysis limits the magnitude of the correlation. While Russell-Briefel (1985) used a food frequency questionnaire containing 40 food items, the dietary analysis of year 1 used a questionnaire that determined the frequency of carrot, spinach and broccoli intake to assess dietary ß-carotene. The higher correlation obtained in the current study may indicate that other sources of ß-carotene are not significant contributors to ß-carotene intake in the Vancouver group.

The positive association between post-supplementation levels of ß-carotene in cells and dietary ß-carotene is significant. Dietary ß-carotene

accounts for 6% of the variation in supplemented in comparison to 18% of the variation in unsupplemented levels of ß-carotene in cells. Although this study does not determine the dose response between supplemental ß-carotene and cellular levels of ß-carotene, the ingested dose of ß-carotene increases the post-supplementation level of ß-carotene. Given that responses to identical doses of ß-carotene as food or purified supplements differ greatly, presumably because of the greater bioavailability of supplemental ß-carotene (Micozzi, M.S. et al, 1992, Brown, E.D. et al, 1989, Rao, C.N., et al, 1970), further research might explore the dose response relationship between supplemental ß-carotene and cellular levels of ß-carotene. In contrast to the current study, dietary ß-carotene was not related to serum ß-carotene levels after 2 months of supplementation (Albanes, D. et al, 1991), suggesting that serum levels of ß-carotene plateau after chronic supplementation. This raises a second issue of whether cellular levels of ß-carotene would also plateau after longer term supplementation.

Food frequency questionnaires collect dietary information quickly and avoid observer bias because participants fill out the questionnaires on their own. However, information may be lost if participants do not understand either the question or the format for the answer. To improve the questionnaire of year 1, it may be useful to provide answers or categories of intake for individuals to circle (i.e., less than once a week, etc.). This may minimize information loss and improve the correlation between estimates of dietary ß-carotene and cellular ß-carotene.

Estimating dietary intake of other food items in addition to ß-carotene-containing foods proves to be more difficult. The year 2 questionnaire is similar to a dietary history and estimates food intake over a long period of time. The disadvantages of this method of dietary assessment are the time requirement and

the learning curve of participants, occasionally meaning that the first dietary history must be discarded. Both of these problems are evident in the analysis of the year 2 dietary questionnaire. Furthermore, individuals in the native study groups appear to have difficulty in quantifying serving size and frequency of ingestion. Differences in educational and cultural factors may affect the recall of individuals thereby jeopardizing the validity of the data collection (Coates, R.J., et al, 1991). Although this method provides more detailed and accurate information about the usual diet of participants, it requires greater subject co-operation and more time. Fehily (1983) reports that there is a compromise between obtaining accurate dietary information and maintaining a good subject response rate (Fehily, A.M., 1983). Other issues associated with the questionnaire in year 2 relate to its administration by an interviewer. This introduces problems of over-reporting 'good' foods; under-reporting 'bad' foods; biases associated with revealing personal information to an interviewer; and issues of information confidentiality and anonymity (Gibson, R.S., 1987, NRC., 1986). It would be interesting to determine whether the short 3 item questionnaire used in year 1 would obtain a better estimate of dietary ß-carotene among the native groups of study 2 than the long questionnaire that was used in year 2.

Alcohol intake

Study 2 shows an inverse relationship between alcohol intake and unsupplemented and supplemented levels of ß-carotene in cells. Individuals that consume more than 2 alcoholic drinks per day have post-supplementation levels of ß-carotene that are 65% of levels among non-drinkers. While tobacco has no

main effect on its own, alcohol interacts with tobacco use, and the combination of theses factors decreases levels of ß-carotene in cells.

These results in cells agree with those found for the impact of alcohol intake on serum ß-carotene. Alcohol intake negatively affects serum levels of ß-carotene (Albanes, D., 1992, Ito, Y. et al, 1991, Russell-Briefel, R. et al, 1985, Stryker, W.S. et al, 1988). In another study involving 1 year of supplementation with ß-carotene, the lowest serum responses to the supplement are among those individuals consuming more than 3.5 drinks per day (Constantino, J.P. et al, 1988). In a third study of short term supplementation with ß-carotene, the serum response to supplementation is negatively associated with alcohol consumption (Cooney, R.V. et al, 1991). Alcohol may decrease the response to ß-carotene by interfering with the absorption of the supplement in the intestinal tract.

Tobacco use

Smokers do not show significantly lower pre- or post-supplementation levels of ß-carotene in Study 2 when compared to nonsmokers. However tobacco interacts with alcohol intake, lowering pre-supplementation levels of ß-carotene. The lower levels of ß-carotene in cells of these individuals may contribute to the increased cancer risk associated with tobacco and alcohol consumption. In the literature, cigarette smokers are reported to have lower serum levels of ß-carotene than non-smokers (Ito, Y., et al, 1991, Russell-Briefel, R., et al, 1985, Nierenberg, D.W. et al, 1989, Stryker, W.S. et al 1988). Tobacco use does not decrease post-supplementation levels of ß-carotene in Study 2. In agreement, smoking does not affect serum levels of ß-carotene among individuals receiving 15 mg ß-carotene/day for 1 year, suggesting that

supplementation may compensate for the ß-carotene lowering effect of smoking in serum. (Constantino, J.P., et al, 1988).

The correlation between serum ß-carotene and dietary ß-carotene is reported to decrease in smokers when compared to non-smokers (Coates, R.J. et al, 1991, Bolton-Smith, C. et al, 1991). A direct comparison to this finding was not available from the thesis data. In year 1, the dietary information includes only non-smokers, while in year 2, the correlation between diet and cellular ß-carotene is not significant either for smokers or non-smokers. Dietary intake of ß-carotene in year 2 is not lower among smokers when compared to non-smokers, in contrast to that reported in a study in which serum levels of ß-carotene among smokers and non-smokers were examined (Cade, J.E., et al, 1991, Stryker, W.S. et al, 1988).

Snuff use

Presupplementation levels of ß-carotene do not differ significantly between snuff users and non-users. Stich reports lower levels of ß-carotene in serum samples from snuff users as compared to non-users (Stich, H.F., et al, 1985). In another study, snuff users do not differ significantly in their serum levels (Kaugers, G.E., et al, 1991). In the current study, snuff users as a group have the highest mean post-supplementation levels of ß-carotene in year 1 and 2. However, snuff users also report higher levels of carrot intake than non-users. Therefore it is possible that differences in ß-carotene levels after supplementation reflect dietary differences rather than an effect of snuff on cellular ß-carotene.

Best prediction of cellular levels of ß-carotene after supplementation

The best prediction of post-supplementation levels of ß-carotene includes pre-supplementation levels of ß-carotene in cells, tobacco use, snuff use and the interaction between snuff use and alcohol intake. This equation accounts for 30% of the variation in post-supplementation levels of ß-carotene. The best predictive equation for serum response to supplementation includes alcohol intake, body mass index, baseline serum ß-carotene and HDL levels and accounts for 22% of the variation in ß-carotene levels after supplementation (Constantino, J.P. et al, 1988). Variability in serum levels of ß-carotene after ingestion of a single dose of ß-carotene cannot be predicted with a regression equation including age, body fat, initial serum ß-carotene, serum cholesterol and triglycerides (Johnson, E.J., et al, 1992). From this data it is apparent that factors

other than those mentioned in the thesis and in the literature affected the bioavailability of ß-carotene in humans.

IV.7 Limitation of these studies

This thesis collected pilot data on cellular levels of ß-carotene and factors that may influence these levels. As with any studies the interpretation of the results are limited by potential error or bias in sample collection. Potential sources of bias in these studies are (1) selection of study populations, (2) recruitment of individuals within each study group and a potential source of error in data collection includes the dietary questionairre used in years 2 of Study 2.

The selection of study populations with distinctly different habits and personal attributes was essential to answer the questions posed in Study 2. However, the factors motivating individuals to participate likely differed between the study groups. The Vancouver group of Study 2 was composed of students enrolled in a fitness oriented undergraduate Kinesiology course and employees of the Kinesiology department. Motivation in this group may have been interest in research and the possible health benefits from participation in the study. Among the native groups of Study 2, individuals were not motivated by health concerns or research interests to participate in the research study. In contrast to the Vancouver group, most individuals had to be convinced that the research project was worthy of their time and effort. Discussion with these individuals revealed that their lifestyles were not fitness oriented nor were they health conscious. Therefore the motivation of individuals to participate in the study was not identical in the 4 study groups.

The presentation of the study to potential participants was modified to accommodate the different backgrounds of the individuals and this may also have biased the type of individuals that participated. In Study 1, the approach for the Vancouver group was " To assess the ß-carotene levels in cells after supplementation." This was done either on an individual basis or in a group setting. In the group setting, peer pressure to participate may have interfered and enrolled individuals in the study that may not have been interested in participating. It may be that those individuals pressured into participation were those individuals that did not complete either the supplemental regimen or the sampling regimen. In the native populations of Study 2, individuals were first asked to answer a questionnaire about their tobacco and alcohol consumption patterns. Those individuals that did not answer the questionnaire were not asked to participate in the study. Subsequently, individuals were asked to participate "in a research study that involves taking ß-carotene capsules for three days and brushing the mouth using a soft toothbrush." These two different approaches that were used in the different study groups may have biased recruitment from the study populations.

Three of the groups in Study 2 were composed of native Indian individuals while the fourth group was a mix of ethnic origins. Factors other than those assessed in Study 2 may contribute to the unique levels of ß-carotene observed in the 4 study groups. The cultural, social and economic differences of these groups limit the applicability of the data to the general population.

During the explanation of the study, individuals may have become sensitized to sources of ß-carotene as they were told that ingesting the supplement would be equivalent to ingesting large quantities of carrots. This may be a potential source of error. This sensitization could have altered

ß-carotene levels if individuals changed their normal ß-carotene intake during the course of the study. To avoid this effect in future studies, either a prescribed diet should be followed by all individuals or the normal dietary intake of ß-carotene-containing foods could be assessed on a weekly basis using food frequency questionnaires. Another possibility would be to include a group ingesting placebo capsules and to assess differences between the placebo and treatment groups.

The collection of lifestyle information involved two different questionnaires: use of the different questionnaires and their administration may have affected the accuracy of the data collected. First, a questionnaire asking about alcohol intake, tobacco use and dietary ß-carotene intake collected information from the Vancouver group (Appendix 2). A second questionnaire was one that our lab had used with success in many different study populations to assess tobacco and alcohol intake (M. Rosin, personal communication). It was used with the native groups (Appendix 3). Furthermore, participants from the Vancouver group answered the questionnaire of Appendix 2 by themselves while the questionnaire for the native groups (Appendix 3)was administered by the interviewer. The different format and administration of the questionnaires may have biased the data collection.

Dietary information was obtained using two different formats. All individuals of the Vancouver group were queried about their weekly intake of carrots, broccoli and spinach in a self-administered questionnaire in year 1 (Appendix 2). A subset of individuals from each of the Vancouver, Lytton and Williams Lake groups answered a detailed interviewer-based food frequency questionnaire in year 2 (Appendix 4). The completion of this questionnaire proved to be labor intensive (up to 2 hours per individual). Visual aids such as

cups, bowls, plates were used to help the individual to quantify serving size. All individuals had difficulty in quantifying serving size however this problem was most apparent in the Lytton and Williams Lake groups. Therefore the dietary information from Lytton and Williams Lake reported only on the weekly frequency of food intake. For this reason the analysis of diet was separated for year 1 and year 2 to eliminate any bias that may have been introduced by the different dietary questionnaires.

IV.8 Recommendations for future studies involving ß-carotene

These studies show low levels of intra-individual variation in cellular levels of ß-carotene therefore, the effects of supplementation with ß-carotene are easily observed in samples of cells obtained 7 days after the initiation of supplementation. A wide range of ß-carotene levels is present both before and after supplementation. As the responses to repeat supplementation are similar, this suggests that individuals respond differently and uniquely to ß-carotene. Dietary intake of ß-carotene is a powerful predictor of ß-carotene levels in unsupplemented individuals while gender, tobacco use and alcohol intake are shown to affect cellular levels of ß-carotene.

The results of the thesis provide direction for a number of research projects. First, they question whether serum levels and cellular levels of ß-carotene respond differently to intake of the carotenoid. These differences need to be explored as they may provide insight into the factors controlling ß-carotene levels in serum and cells. As carcinogens cause damage in cells, it is relevant to know the ß-carotene levels present in cells. Furthermore, the

relationship between ß-carotene levels in exfoliated cells and those in other tissues needs to be explored: the issue is whether exfoliated cells can be used as a surrogate for the accumulation of ß-carotene in other tissues. This information would be valuable for the evaluation of cancer risk of an individual. These studies show that individuals respond differently to ß-carotene so an estimate of dietary intake of ß-carotene may not be sufficient for the evaluation of cancer risk. Therefore, future studies of ß-carotene might include the assessment of ß-carotene in exfoliated cells, serum and organ tissues (surgical tissue samples or autopsy samples) both in unsupplemented and supplemented individuals.

Second, alcohol intake negatively affects cellular responses to ß-carotene supplementation. Further examination of this effect should include individuals with alcohol intakes spread evenly across the range. Third, the effect of smoking on cellular levels of ß-carotene warrants further exploration. As tobacco and alcohol are major contributors to cancer risk, it is important to assess their effects on ß-carotene levels in target tissues and the potential chemopreventive effects of long term supplementation with ß-carotene.

Fourth, the dietary questionnaire of year 2 was extensive and therefore took a long time to complete. The validity of using a long questionnaire is in doubt. The current knowledge of ß-carotene content in foods makes it possible to design an efficient questionnaire to assess ß-carotene intake. It will always be necessary to include other items of interest but the cost in time versus benefits in additional information must be weighed carefully in each study. Fifth, dietary intake with the supplement should be closely monitored or controlled to completely delineate those factors which influence the bioavailability of ß-carotene in humans.

Last, in both Study 1 and 2, the post-supplementation levels of ß-carotene were assessed by determining ß-carotene levels in one sample of oral mucosa cells obtained 7 days after initiation of supplementation. Study 1 shows that by day 14 ß-carotene levels had passed their peak. However, individual differences in responses to supplementation may be explained by differences in the time to peak ß-carotene content. It is possible that some individuals reach their peak level in 5 days while other reach their peak in 10 days. As Studies 1 and 2 only observe ß-carotene levels at 7 day intervals, individual differences in time to peak ß-carotene levels could not be assessed. A future study should include an assessment of the variability in the amount of time required to reach peak ß-carotene levels.

V. APPENDICES

V.1 Appendix 1 - A method for estimating cell counts using sample wet weights.

	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
10	***0.2***	***0.22***	***0.24***	***0.26***	***0.28***	***0.305***	***0.33***	***0.358***	***0.385***	***0.483***
20	***0.44***	***0.465***	***0.49***	***0.52***	***0.545***	***0.57***	***0.60***	***0.625***	***0.65***	***0.68***
30	0.71	0.735	0.76	0.79	0.82	0.85	0.875	0.90	0.93	0.96
40	***0.99***	***1.02***	***1.05***	***1.08***	***1.1***	***1.14***	***1.17***	***1.2***	***1.23***	***1.26***
50	1.3	1.34	1.38	1.42	1.46	1.50	1.54	1.58	1.62	1.66
60	1.7	1.75	1.79	1.84	.188	1.92	1.97	2.02	2.07	2.12
70	2.17	2.22	2.27	2.33	2.38	2.44	2.50	2.56	2.62	2.69
80	2.75	2.81	2.87	2.94	3.0	3.06	3.12	3.19	3.26	3.33
90	3.4	3.48	3.55	3.63	3.71	3.8	3.89	3.97	4.05	4.13
100	4.21	4.31	4.40	4.5	4.6	4.7	4.8	4.9	5.0	5.09
110	5.18	5.27	5.36	5.45	5.55	5.65	5.75	5.85	5.95	6.05
120	6.16	6.27	6.37	6.47	6.57	6.67	6.77	6.87	6.97	7.07

The values in this table are cell counts for wet weights of samples. The cell counts in bold italics were counted and weighed. These values were used to create the remainder of the table. The X and Y axis (in bold) represent the wet weights of samples in units of mg (Y axis) and 10th of mg (X axis). The inner table presents the cell counts (in 10^6 cells) for each corresponding wet weight. For example, a sample weighing 53 mg corresponds to a cell count of 1.42×10^6 cells. The italicized values were measured and are the basis upon which the remainder of the table was calculated.

V.2 Appendix 2 - Diet and lifestyle questionnaire (Study 1)

Lifestyle Questionnaire

Name Date Location

Age Gender

Are you a smoker? Cigarettes? Cigar? Pipe? Other?

Brand Name

Packs/Day

Do you use chewing tobacco or snuff?

Brand?

Frequency (per day)

Can you estimate your daily/weekly alcohol consumption?

i. daily (type and quantity)

ii. weekly (type and quantity)

How much of each food item did you eat in the past seven days?

Carrots: total number of carrots eaten

Spinach: total quantity of spinach eaten

Broccoli: total amount of broccoli eaten

Do you currently take any vitamin supplements?

Type

Frequency

Do you take any medication regularly?

Please specify

V.3 Appendix 3 - Lifestyle questionnaire (Study 2)

1986/87 Survey on Snuff and Smoking Habits Date

Interviewer	Location
Name	Place of Birth
Date of Birth	Ethnic Origin
Profession	Length of time at current profession
Snuffing Pattern (Current)	Smoking pattern (current)
Type of Tobacco	Type (cigarettes)
Number of dips/day	Number of cigarettes/day
Number of cans per week	Proportion of each cigarette smoked
How long per dip	Number of years smoking
Number of years of snuffing	
Swallowing or spitting	
Estimate amount of snuff	
Tobacco chewing pattern	Chewing gum
Type of tobacco	Type
Number of plugs per day	How much per day
Number of cans/pouches per week	
How long per plug	Soft drinks
Swallowing or spitting	Type
Number of years chewing	Number of bottles per day
Estimate amount of plug	
Brushing teeth	with toothpaste daily
	without toothpaste non-daily
Coffee Type	Tea Type
Number of cups per day	Number of cups per day
Beer Type	Wine Type
Quantity per day	Quantity per day
Liquor Type	Lysol
Quantity per day	Shaving lotion
	Rubbing alcohol
	Chinese cooking wine

V.4 Appendix 4 - Dietary questionnaire (Study 2)

Food Frequency Questionnaire

Subject ID_____ Gender_____

Date of Birth_____Occupation_____

	Frequency		Amount	
	No	Unit	No	Unit
Dairy products	( )	( )	( )	( )
Homo milk	( )	( )	( )	( )
2% milk	( )	( )	( )	( )
Skim milk	( )	( )	( )	( )
Chocolate milk	( )	( )	( )	( )
Evaporated milk	( )	( )	( )	( )
Butter milk	( )	( )	( )	( )
Sour cream	( )	( )	( )	( )
Yogurt	( )	( )	( )	( )
Ice cream	( )	( )	( )	( )
Half/Half cream	( )	( )	( )	( )
Cream cheese	( )	( )	( )	( )
Swiss cheese	( )	( )	( )	( )
Cheddar cheese	( )	( )	( )	( )
Eggs	( )	( )	( )	( )
Meats				
Ground beef (lean or regular)	( )	( )	( )	( )
Beef (type)	( )	( )	( )	( )
Deer (type)	( )	( )	( )	( )
Cariboo (type)	( )	( )	( )	( )
Pork (type)	( )	( )	( )	( )
Ham	( )	( )	( )	( )
Bacon	( )	( )	( )	( )
Wieners/sausages	( )	( )	( )	( )
Luncheon meats (type)	( )	( )	( )	( )
Liver (type)	( )	( )	( )	( )
Organ meats (type)	( )	( )	( )	( )
Poultry (type and part)	( )	( )	( )	( )
Ducks/geese/other	( )	( )	( )	( )
	( )	( )	( )	( )
Fish (type)	( )	( )	( )	( )
	( )	( )	( )	( )

Food Frequency Questionnaire (1987)

	Frequency		Amount	
	No	Unit	No	Unit
Vegetables				
Asparagus	( )	( )	( )	( )
Avocado	( )	( )	( )	( )
Beans (type)	( )	( )	( )	( )
	( )	( )	( )	( )
Beet greens	( )	( )	( )	( )
Broccoli	( )	( )	( )	( )
Brussel sprouts	( )	( )	( )	( )
Cabbage	( )	( )	( )	( )
Carrots	( )	( )	( )	( )
Corn	( )	( )	( )	( )
Endive	( )	( )	( )	( )
Kale	( )	( )	( )	( )
Lettuce (type)	( )	( )	( )	( )
Mustard greens	( )	( )	( )	( )
Peas	( )	( )	( )	( )
Peppers (type)	( )	( )	( )	( )
Pumpkin	( )	( )	( )	( )
Squash (type)	( )	( )	( )	( )
Sweet potatoes	( )	( )	( )	( )
Tomatoes	( )	( )	( )	( )
Ketchup	( )	( )	( )	( )
Tomato soup	( )	( )	( )	( )
Tomato juice	( )	( )	( )	( )
Turnip greens	( )	( )	( )	( )
Fruits				
Apricots	( )	( )	( )	( )
Apples	( )	( )	( )	( )
Banana	( )	( )	( )	( )
Cantaloupe	( )	( )	( )	( )
Pink grapefruit	( )	( )	( )	( )
Orange	( )	( )	( )	( )
Orange juice	( )	( )	( )	( )
Nectarines	( )	( )	( )	( )
Papayas	( )	( )	( )	( )
Plums	( )	( )	( )	( )
Prunes	( )	( )	( )	( )
Grapes	( )	( )	( )	( )
Raisins	( )	( )	( )	( )
Berries (type)	( )	( )	( )	( )
	( )	( )	( )	( )

	Frequency		Amount	
	No	Unit	No	Unit
Breads and cereals				
Breakfast cereal (hot) (type)	( )	( )	( )	( )
Breakfast cereal (cold) (type)	( )	( )	( )	( )
Noodles (type)	( )	( )	( )	( )
	( )	( )	( )	( )
Other pasta (specify)	( )	( )	( )	( )
	( )	( )	( )	( )
Rice (type)	( )	( )	( )	( )
Bread (specify white or brown)	( )	( )	( )	( )
Muffins (type)	( )	( )	( )	( )
Fruit pie (type)	( )	( )	( )	( )
Carrot cake	( )	( )	( )	( )
Cookies (specify)	( )	( )	( )	( )
Sweet roll/doughnuts	( )	( )	( )	( )
Pizza (specify toppings)	( )	( )	( )	( )
Beverages				
Tea	( )	( )	( )	( )
Coffee	( )	( )	( )	( )
Soft drinks	( )	( )	( )	( )
Fruit drinks	( )	( )	( )	( )
Other	( )	( )	( )	( )
Condiments				
Margarine	( )	( )	( )	( )
Butter	( )	( )	( )	( )
Whipping cream	( )	( )	( )	( )
Mayonnaise	( )	( )	( )	( )
Mustard	( )	( )	( )	( )
Sugar	( )	( )	( )	( )
Fast food				
Cheese snacks (specify)	( )	( )	( )	( )
Potato chips	( )	( )	( )	( )
Pretzels	( )	( )	( )	( )
Licorice	( )	( )	( )	( )
hard candy	( )	( )	( )	( )
French fries	( )	( )	( )	( )
Take out food (specify)	( )	( )	( )	( )
Vitamin supplements (specify)	( )	( )	( )	( )
	( )	( )	( )	( )
	( )	( )	( )	( )

VI. REFERENCES

Abril, E.R., Rybski, J.A., Scuderi, P., Watson, R.R., 1989, ß-carotene stimulates human leukocytes to secret a novel cytokine, Journal of Leukocyte Biology, 45: 255-261.

Albanes, D., Virtamo, J., Rautalahti, M., Haukka, J., Palmgren, J., Gref, C.G., Heinonen, O.P., 1992, Serum ß-carotene before and after ß-carotene supplementation, European Journal of Clinical Nutrition, 46(1):15-24.

Alexander, M., Newmark, H., Miller, R.G., 1985, Oral ß-carotene can increase the number of OKT4+ cells in human blood, Immunology Letters, 9:221-224.

Bauernfeind, J.C., 1972, Carotenoid vitamin A precursors and analogs in foods and feeds, Journal of Agriculture and Food Chemistry, 20(3), 456-473.

Bendich, A., 1988, The safety of ß-carotene, Nutrition and Cancer, 11:207-214.

Bendich, A., 1989, Carotenoids and the immune response, Journal of Nutrition, 119: 112-115.

Bendich, A., Shapiro, S.S., 1986, Effect of ß-carotene and canthaxanthin on the immune responses of the rat, Journal of Nutrition, 116, 2254-2262.

Benner, S.E., Lippman, S.M., Hong, W.K., 1992, Current status of chemoprevention of head and neck cancer, Oncology, 6(8):61-66.

Bolton-Smith, C., Casey, C.E., Gey, K.F., Smith, W.C., 1991, Antioxidant vitamin intakes assessed using a food-frequency questionnaire: correlation with

biochemical status in smokers and non-smokers, British Journal of Nutrition, 65(3):337-346.

Boone, C.W., Kelloff, G.J., Malone, W.E., 1990, Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trials: A review, Cancer Research, 50:2-9.

Brown, E.D., Micozzi, M.S., Craft, N.E., Bieri, J.G., Beecher, G., Edwards, B.K., Rose, A., Taylor, P.R., Smith, J.C., 1989, Plasma carotenoids in normal men after a single ingestion of vegetables or purified ß-carotene, American Journal of Clinical Nutrition, 49:1258-1265.

Bureau, J.L., Bushway, R.J., 1986, HPLC determination of carotenoids in fruits and vegetables in the United States, Journal of Food Science, 51(1):128-130.

Burton, G.W., 1989, Antioxidant action of carotenoids, Journal of Nutrition, 119:109-111.

Bushway, R.J., 1986, Determination of alpha and ß-carotene in some raw fruits and vegetables by high performance liquid chromatography, Journal of Agriculture and Food Chemistry, 34:409-412.

Cade, J.E., Margetts, B.M., 1991, Relationship between diet and smoking, Journal of Epidemiology and Community Health, 45(4):270-272.

Cameron, L.C., 1987, Kinetics of ß-carotene accumulation and retention in exfoliated cells from supplemented individuals, MSc thesis for Faculty of Graduate Studies, Dept of Pathology, U.B.C.

Cameron, L.M., Rosin, M.P., Stich, H.F., 1989, Use of exfoliated cells to study tissue specific levels of ß-carotene in humans, Cancer Letters, 45:203-207.

Chandler, L.A., Schwartz, S.J., 1987, HPLC separation of cis-trans carotene isomers in fresh and processed fruits and vegetables, Journal of Food Science, 52(3): 669-672.

Chen, J., Geissler, C., Parpia, B., Li, J., Campbell, T.C., 1992, Antioxidant status and cancer mortality in China, International Journal of Epidemiology, 21(4):625-635.

Coates, R.J., Eley, J.W., Block, G., Gunter, E.W., Sowell, A.L., Grossman, C., Greenberg, R.S., 1991, An evaluation of a food frequency questionnaire for assessing dietary intake of specific carotenoids and vitamin E among low income black women, American Journal of Epidemiology, 134(6):658-671.

Comstock, G.W., Bush, T.L., Helzlsouer, K., 1992, Serum retinol, ß-carotene, vitamin E and selenium as related to subsequent cancer at specific sites, American Journal of Epidemiology, 135(2):115-121.

Comstock, G.W., Helzlsouer, K.J., Bush, T.L., 1991, Prediagnostic serum levels of carotenoids and vitamin E as related to subsequent cancer in Washington County, Maryland, American Journal of Clinical Nutrition, 53:260s-264s.

Comstock, G.W., Menkes, M.S., Schober, S.E., Vuilleumier, J.P., Helsing, K.J., 1988, Serum levels of retinol, ß-carotene and alpha-tocopherol in older adults, American Journal of Epidemiology, 127(1): 114-123.

Constantino, J.P., Kuller, L.H., Begg, L., Redmond, C.K., Bates, M.W., 1988, Serum level changes after administration of a pharmacologic dose of ß-carotene, American Journal of Clinical Nutrition, 48: 1277-1283.

Cooney, R.V., Bertram, J.S., Hankin, J.H., Kolonel, L.N., Miyake, A., Billings, K., Bourne, W., 1991, Relationship between dietary, serum and tissue levels of carotenoids, Cancer Letters, 61:81-87.

De Vet, H.C.W., Knipschild, P.G., Willebrand, D., Schouten, H.J., Sturman, F., 1991, The effect of ß-carotene on the regression and progression of cervical dysplasia: a clinical experiment, Journal of Clinical Epidemiology, 44(3): 273-283.

Dimitrov, N.V., Meyer, C.M., Ullrey, D.E., Chenoweth, W., Michelakis, A., Malone, W., Boone, C., Fink, G., 1988, Bioavailability of carotenoids, American Journal of Clinical Nutrition, 48: 298-304.

Dimitrov, N.V., Ullrey, D.E., 1990, Bioavailability of carotenoids, in "Carotenoids: Chemistry and Biology", Eds N.I. Krinsky et al, Plenum Press, New York.

Dixon, W.J., 1950, Analysis of extreme values, Ann Math Stat, 21:488-506, in "Biometry", Eds, R.R. Sokal, F.J. Rohlf, W.H. Freeman and Company, 1981.

Elkins, E.R., 1979, Nutrient content of raw and canned green beans, peaches and sweet potatoes, Food Technology, Feb , 66-70.

Engle, A., Muscat, J.E., Harris, R.E., 1991, Nutritional risk factors and ovarian cancer, Nutrition and Cancer, 15(3-4):239-247.

Fehily, A.M., 1983, Epidemiology for nutritionists: 4: Survey methods, Human Nutrition: Applied Nutrition, 37A:419-425.

Fontham, E.T.H., Pickle, L.W., Haenszel, W., Correa, P., Lin, Y., Ralk, R.T., 1988, Dietary vitamins A and C and lung cancer risk in Louisiana, Cancer, 62:2267-2273.

Garewal, H.S., Ampel, N.M., Watson, P.R., Prabhala, R.H., Dols, C.L., 1992, A preliminary trial of ß-carotene in subjects infected with the human immunodeficiency virus, Journal of Nutrition, 122(3): 728-732.

Garewal, H.S., Meyskens, F.L., Killen, D., Reeves, D., Kiersch, T., Elletson, H., Strosberg, A., Steinbronn, K., 1990, Response of oral leukoplakia to ß-carotene, Journal of Clinical Oncology, 8:1715-1720.

Garewal, H.S., Potential role of ß-carotene in prevention of oral cancer, American Journal of Clinical Nutrition, 53:294s-297s.

Gibson, R.S., 1987, Sources of error and variability in dietary assessment methods: A review, Journal of the Canadian Dietetic Association, 48(3): 150-155.

Goodman, D.S., 1984, Overview of current knowledge of metabolism of vitamin A and carotenoids, Journal of the National Cancer Institute, 73:1375-1379.

Greenberg, E.R., Baron, J.A., Stukel, T.A., Stevens, M.M., Mandel, J.S., Spencer, S.K., Elias, P.M., Lowe, N., Nierenberg, D.W., Bayrd, G., Kwan, T., and the Skin Cancer Prevention Study Group, 1990, A clinical trial of ß-carotene to prevent basel-cell and squamous cell cancers of the skin, New England Journal of Medicine, 323: 789-795.

Greenwald, P., 1992, Colon cancer overview, Cancer, 70:1206-1215,

Gross, J., 1991, "Pigments in Vegetables: Chlorophylls and Carotenoids", Van Nostrand, Reinhold, New York.

Harris, R.W.C., Key, T.J.A., Silcoks, P.B., Bull, D., Wald, N.J., 1991, A case-control study of dietary carotenes in men with lung cancer and in men with other epithelial cancers, Nutrition and Cancer, 15: 63-68.

Haveland-Smith, R.B., 1981, Evaluation of the genotoxicity of some natural food colours using bacterial assays, !981, Mutation Research, 91:285-290.

Henderson, C.T., Mobarhan, S., Bowen, P., Stacewicz-Sapuntzakis, M., Langenberg, P., Kiani, R., Lucchesi, D., Sugerman, S., 1989, Normal serum response to oral ß-carotene, Journal of the American College of Nutrition, 8(6): 624-635.

Hennekens, C.H., Mayrent, S.L., Willett, W., 1986, Vitamin A, carotenoids and retinoids, Cancer, 58: 1837-1841.

Herbeth, B., Spyckerelle, Y., Deschamps, J-P., 1991, Determinants of plasma retinol, ß-carotene and alpha-tocopherol during adolescence, American Journal of Clinical Nutrition, 54:884-889.

Herrero, R., Potischamn, N., Brinton, L.A., Reeves, W.C., Brenes, M.M., Tenorio, F., de Britton, R.C., Gaiton, E., 1991, A case-control study of nutrient status and invasive cervical cancer I: Dietary Indicators, American Journal of Epidemiology, 134(1):1335-1346.

Heywood, R., Palmer, A.K., Gregson, R.L., Humler, H., 1985, The toxicity of ß-carotene, Toxicology, 36: 91-100.

Hinds, M.W., Kolonel, L.N., Hankin, J.H., Lee, J., 1984, Dietary vitamin A, carotene, vitamin C and risk of lung cancer in Hawaii, American Journal of Epidemiology, 119: 227-237.

Hochstein, P., Atallah, A.S., 1988, The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer, Mutation Research, 202: 363-375.

Hong, W.K., Lippman, S.M., Itri, L.M., Karp, D.D., Lee, J.S., Byers, R.M., Schantz, S.P., Kramer, A.M., Lotan, R., Peters, L.J., Dimery, I.W., Brown, B.W., Goepfert, H., 1990, Prevention of second primary tumours with isotretinoin in squamous-cell carcinoma of the head and neck, New England Journal of Medicine, 323: 795- 801.

Ito, Y., Shima, Y., Ochiai, J., Otani, M., Sasaki, R., Suzuki, S., Hamajima, N., Ogawa, H., Aoki, K., 1991, Effects of the consumption of cigarettes, alcohol and foods on serum concentrations of carotenoids, retinol, and tocopherols in healthy inhabitants living in a rural area of Hokkaido, (Abstract only) Japanese Journal of Hygiene, 46(4):874-882.

Johnson, E.J., Russell, R.M., 1992, Distribution of orally ingested ß-carotene among lipoproteins in healthy men, American Journal of Clinical Nutrition, 56:128-135.

Kaplan, L.A., Lau, J.M., Stein, E.A., 1990, Carotenoid composition, concentrations and relationships in various human organs, Clinical Physiology and Chemistry, 8:1-10.

Kaplan, L.A., Stein, E.A., Kaplan, J.M., 1987, Specificity of loading of lipoproteins with carotenoids, (Abstract), Clinical Chemistry, 33:935.

Kaplan, L.A., Stein, E.A., Willett, W.C., Stampfer, M.J., Stryker, W.S., 1987, Reference ranges of retinol, tocopherols, lycopene and alpha- and beta-carotene in plasma by simultaneous high performance liquid chromatographic analysis, Clinical Physiology and Biochemistry. 5:297-304.

Kaugars, G.E., Brandt, R.B., Chan, W., Carcaise-Edinboro, P., 1991, Evaluation of risk factors in smokeless tobacco-associated oral lesions, Oral Surgery, Oral Medicine and Oral Pathology, 72:326-331.

Kelloff, G.J., Boone, C.W., Malone, W.F., Steele, V.E., 1992, Chemoprevention clinical trials, Mutation Research, 267(2), 291-295.

Khachik, F., Beecher, G.R., Whittaker, N.F., 1986, Separation, identification and quantification of the major carotenoid pigments and chlorophyll constituents in extracts of several green vegetables by liquid chromatography, Journal of Agriculture and Food Chemistry, 34:603-616.

Kneckt, P., Aromaa, A., Maatela, J., Alfthan, G., Aaran, R.K., Nikkari, T., Hakama, M., Hakulinen, T., Teppo, L., 1991, Serum micronutrients and risks of cancers of low incidence in Finland, American Journal of Epidemiology, 134(4):356-361.

Kneckt, P., Aromaa,A., Maatela, J., Aaron, R., Nikkari, T., Hakama, M., Hakulinen, T., Peto, R., Teppo, L., 1990, Serum vitamin A and subsequent risk of cancer: cancer incidence follow-up of the Finnish Mobile Clinic Health Examination Survey, American Journal of Epidemiology, 132(5): 857-870.

Kraemer, K.H., DiGiovanna, J.J., Moshell, A.N., Tarone, R.E., Peck, G.L., 1988, Prevention of skin cancer in xeroderma pigmentosum with the use of oral isotretinoin, New England Journal of Medicine, 318(25): 1633-1637.

Krinsky, N.I., 1989, Antioxidant function of carotenoids, Free Radicals Biology and Medicine, 7:617-635.

Krinsky, N.I., 1990, Caroteniods in Medicine, in "Carotenoids: Chemistry and Biology", Editor, Krinsky, N.I., Plenum Press New York.

Krinsky, N.I., Cornwall, D.G., Oncley, J.L., 1958, The transport of vitamin A and carotenoids in human plasma, Archives of Biochemistry and Biophysics, 73:233-246.

Le Marchand, L., Yoshizawa, C.N., Kolonel, L.N., Hankin, J.H., Goodman, M.T., 1989, Vegetable consumption and lung cancer risk: A population-based case-control study in Hawaii, Journal of the National Cancer Institute, 81: 1158-1164.

Lee, R., Mathews-Roth, M.M., Pathak, M.A., Parrish, J.A., 1975, The detection of carotenoid pigments in human skin, Journal of Investigative Dermatology, 63: 175-177.

Le Marchand, L., Hankin, J.H., Kolonel, L.N., Wilkens, L.R., 1991, Vegetable and fruit consumption in relation to prostate cancer risk in Hawaii: a reevaluation of the effect of dietary ß-carotene, American Journal of Epidemiology, 133(3):215-219.

LeMarchand, L., Wilkens, L.R., Hankin, J.H., Haley, N.J., 1991, Dietary patterns of female non-smokers with and without exposure to environmental tobacco smoke, Cancer Causes and Control, 2(1):11-16.

Malaker, K., Anderson, B.J., Beecroft, W.A., Hodson, D.I., 1991, Management of oral mucosal dysplasia with ß-carotene and retinoic acid: a pilot cross over study, Cancer Detection and Prevention, 15(5): 335-340.

Malone, W.F., 1991, Studies evaluating antioxidants and ß-carotene as chemopreventives, American Journal of Clinical Nutrition, 53: 305S-313S.

Marubini, E., Decarli, A., Costa, A., Mazzoleni, C., Barberi, A., Capitelli, E., Carlucci, M., Cavallo, F., Monferroni, N., Pasotrino, U., Salvini, S., 1988, The relationship of dietary intake and serum levels of retinol and ß-carotene with breast cancer, Cancer, 61:173-180.

Mathews-Roth, M.M., 1988, Lack of genotoxicity with ß-carotene, Toxicology Letters, 41:185-191.

Mathews-Roth, M.M., 1990, Plasma concentrations of carotenoids after large doses of ß-carotene, American Journal of Clinical Nutrition, 52: 500-501.

Mathews-Roth, M.M., Abraham, A.A., Gabuzda, T.G., 1976, ß-carotene content of certain organs from two patients receiving high doses of ß-carotene, Clinical Chemistry, 22(6): 924-926.

Menkes, M.S., Comstock, G.W., Vuilleumer, J.P., Helsing, K.J., Rider, A.A., Brookmeyer, R., 1986, Serum ß-carotene, vitamins A and E, selenium, and the risk of lung cancer, New England Journal of Medicine, 315:1250-1254.

Mettlin, C., Graham, S., 1979, Dietary factors in the human bladder cancer, American Journal of Epidemiology, 110(3):255-263.

Mettlin, C., Selenskas, S., Natarajan, N., Huben, R., 1989, ß-carotene and animal fats and their relationship to prostate cancer risk, Cancer, 64: 605-612.

Micozzi, M.S., Brown, E.D., Edwards, B.K., Bieri, J.G., Khachik, F., Beecher, G.R., Smith, J.C. Jr., 1992, Plasma carotenoid response to chronic intake of selected foods and ß-carotene, American Journal of Clinical Nutrition, 55(6):1120-1125.

Mobarhan, S., Bowen, P., Anderson, B., Evans, M., Stacewicz-Sapuntzakis, M., Sugerman, S., Simms, P., Lucchesi, D., Friedman, H., 1990, Effects of ß-carotene repletion on ß-carotene absorption, lipid peroxidation and neutrophil superoxide formation in young men, Nutrition and Cancer, 14:195-206.

Moon, R.C., 1989, Comparative aspects of carotenoids and retinoids as chemopreventive agents for cancer, Journal of Nutrition, 119:127-134.

Negri, E., La Vecchia, C., Franceschi, S., Decarli, A., Bruzzi, P., 1992, Attributable risks for esophageal cancer in northern Italy, European Journal of Cancer, 28A(6-7): 1167-1171.

Nierenberg, D.W., Stukel, T.A., Baron, J.A., Dain, B.J., Greenberg, E.R., 1991, Determinants of increase in plasma concentration of ß-carotene after chronic oral administration, American Journal of Clinical Nutrition, 53(6): 1443-1449.

Nierenberg, D.W., Stukel, T.A., Baron, J.A., Dain, B.J., Greenberg, E.R., and the Skin Cancer Prevention Group, 1989, Determinants of plasma levels of ß-carotene and retinol, American Journal of Epidemiology, 130(3), 511-521.

Nierenberg, D.W., Nann, S.L., 1992, A method for determining concentrations of retinol, tocopherol and five carotenoids in human plasma and tissue samples, American Journal of Clinical Nutrition, 56: 417-426.

NRC., 1986, Nutrient Adequacy: Assessment Using Food Consumption Surveys, in "Subcommitee on criteria for dietary evaluation: co-ordinating committee on Evaluation of Food Consumption Surveys, Food and Nutrition Board, Commission on Life Sciences.

O'Brien, P.J., 1988, Free-radical mediated chemical carcinogenesis, New York Academy of Sciences, 534: 552-564.

Ohno, Y., Yoshida, K., Okada, K., Yamabe, H., Schroeder, F.H., 1988, Dietary ß-carotene and cancer of the prostate: a case control study in Kyoto, Japan, Cancer Research, 48: 1331-1336.

Olsen, J.A., 1989, Provitamin A function of carotenoids: the conversion of ß-carotene into vitamin A, Journal of Nutrition, 119:105-108.

Paganini-Hill, A., Chao, A., Ross R. K., Henderson, B.E., 1987, Vitamin A, ß-carotene and the risk of cancer: a prospective study, Journal of the National Cancer Institute, 79:443-448.

Palan, P.R., Mikhail, M.S., Basu, J., Romney, S.L., 1991, Plasma levels of antioxidant ß-carotene and alpha-tocopherol in uterine cervix dysplasias and cancer, Nutrition and Cancer, 15:13-20.

Parker, R.S., 1988, Carotenoid and tocopherol composition of human adipose tissue, American Journal of Clinical Nutrition, 47: 33-36.

Parker, R.S., 1989, Carotenoids in human blood and tissues, Journal of Nutrition, 119:101-104.

Peters, R.K., Pike, M.C., Garabrant, D., Mack, T.M., 1992, Diet and colon cancer in Los Angeles County, California, Cancer Causes and Control, 3(5):457-473.

Potischman, N., Herrero, R., Brinton, L.A., Reeves, W.C., Stacewicz-Sapuntzakis, M., Jones, C.J., Brenes, M.M., Tenorio, F., de Britton, R.C., Gaiton, E., 1991, A case-control study of nutrient status and invasive cervical cancer, American Journal of Epidemiology, 134(11): 1347-1355.

Prabhala, R.H., Garewal, H.S., Hicks, M.J., Sampliner, R.E., Watson, R.R., 1991, The effects of 13-cis retinoic acid and ß-carotene on cellular immunity in humans, Cancer, 67: 1556-1560.

Prabhala, R.H., Maxey, V., Hicks, M.J., Watson, R.R., 1989, Enhancement of the expression of activation markers on human peripheral blood mononuclear cells by in vitro culture with retinoids and carotenoids, Journal of Leukocyte Biology, 45: 249-254.

Premachandra, B.R., Vasantharajan, V.N., Cama, H.R., 1976, Improvement of the nutritive value of tomatoes, Current Science, 45(2):56-57.

Ramaswamy, P.G., Krishnamoorthy, L., Rao, V.R., Bhargava, M.K., 1990, Vitamins and provitamin A levels in epithelial cancers: A preliminary study, Nutrition and Cancer, 14: 273-276.

Rao, C.N., Rao, B.S.N., 1970, Absorption of dietary carotenes in human subjects, American Journal of Clinical Nutrition, 23(1):105-109.

Richards, G.A., Theron, A.J., Van Rensburg, C.E.J., Van Rensberg, A.J., Ven Der Merwe, C.A., Kuyl, J.M., Anderson, R., 1990, Investigation of the effects of oral administration of vitamin E and ß-carotene on the chemiluminescence responses and the frequency of sister chromatid exchanges in circulating leukocytes from cigarette smokers, American Reviews of Respiratory Diseases, 142: 648-654.

Rock, C.L., Swendseid, M.E., 1992, Plasma ß-carotene response in humans after meals supplemented with dietary pectin, American Journal of Clinical Nutrition, 55: 96-99.

Russell-Briefel, R., Bates, M.W., Kuller, L. H., 1985, The relationship of plasma carotenoids to health and biochemical factors in middle aged men, American Journal of Epidemiology, 122(5):741-749.

Santamaria, L., Bianchi, A., Arnaboldi, A., Ravetto, C., Bianchi, L., Pizzala, R., Andreoni, L., Santagati, G., Bermond, P., 1988, Chemoprevention of indirect and direct chemical carcinogenesis by carotenoids as oxygen radical quenchers, New York Academy of Sciences, 534: 584-596.

Schmitz, H.H., Poor, C.L., Wellman, R.B., Erdman Jr, J.W., 1991, Concentrations of selected carotenoids and vitamin A in human liver, kidney and lung tissue, Journal of Nutrition, 121:1613-1621.

Shekelle, R.B., Tangney, C.C., Rossof, A.H., Stamler, J., 1992, Serum cholesterol, ß-carotene and the risk of lung cancer, Epidemiology, 3(4): 282-287.

Shibata, A., Pagannini-Hill, A., Ross, R.K., Yu, M.C., Henderson, B.E., 1992, Dietary ß-carotene, cigarette smoking and lung cancer in men, Cancer Causes and Control, 3(3):207-214.

Simic, M.G., 1988, Mechanisms of inhibition of free-radical processes in mutagenesis and carcinogenesis, Mutation Research, 202:377-386.

Simpson, K.L., Chichester, C.O., 1981, Metabolism and nutritional significance of carotenoids, Annual Reviews of Nutrition, 1:351-374.

Singh, V.S., Gaby, S.K., 1991, Premalignant lesion: role of antioxidant vitamins and ß-carotene in risk reduction and prevention of malignant transformation, American Journal of Clinical Nutrition, 53: 386s-390s.

Slattery, M.L., Schuman, K.L., West, D.W., French, T.K., Robison, L.M., 1989, Nutrient intake and ovarian cancer, American Journal of Epidemiology, 130(3):497-502.

Smith, A.H., Waller, K.D.,1991, Serum ß-carotene in persons with cancer and their immediate families, American Journal of Epidemiology, 133(7):661-671.

Sokal, R. R., Rohlf, F.J., 1981, Biometry, The principles and practice of statistics in biological research, 2nd Edition, W.H. Freeman and Company, U.S.A.

Stahl, W., Schwarz, W., Sundquist, A.R., Sies, H., 1992, *cis-trans* Isomers of lycopene and ß-carotene in human serum and tissues, Archives of Biochemistry and Biophysics, 294(1), 173-177.

Stahlein, H.B., Gey, K.F., Eichholzer, M., Ludin, E., Bernasconi, F., Thurneyson, J., Brubacher, G., 1991, Plasma antioxidant vitamins and subsequent cancer mortality in the 12 year follow up of the prospective Basel Study, American Journal of Epidemiology, 133:766-775.

Stahlein, H.B., Gey, K.F., Ludin, E., 1991, ß-carotene and cancer prevention: the Basel Study, American Journal of Clinical Nutrition, 53:265s-269s.

Stahlein, H.B., Rosel, F., Buess, E., Brubacher, G., 1984, Cancer, vitamins and plasma lipids: prospective Basel Study, Journal of the National Cancer Institute, 73:1463-1468.

Stich, H.F., Hornby, A.P., Dunn, B.P., 1985, A pilot ß-carotene intervention trial with Inuits using smokeless tobacco, International Journal of Cancer, 36: 321-327.

Stich, H.F., Hornby, A.P., Dunn, B.P., 1986a, ß-carotene levels in exfoliated mucosa cells of population groups at low and elevated risk for oral cancer, International Journal of Cancer, 37: 389-393.

Stich, H.F., Hornby, A.P., Dunn, B.P., 1986b, Beta-carotene levels in exfoliated human oral mucosa cells following its oral administration, Cancer Letters, 30:133-141.

Stich, H.F., Hornby, A.P., Mathew, B., Sankaranarayanan, R., Nair, M.K., 1988, Response of oral leukoplakias to the administration of vitamin A, Cancer Letters, 40: 93-101.

Stich, H.F., Rosin, M.P., Hornby, A.P., Mathew, B., Sankaranarayanan, R., Nair, M.K., 1988, Remission of oral leukoplakias and micronuclei in

tobacco/betel quid chewers treated with ß-carotene and with ß-carotene plus vitamin A, International Journal of Cancer, 42: 195-199.

Stich, H.F., Rosin, M.P., Vallejera, M.O., 1984, Reduction with vitamin A and ß-carotene administration of proportion of micronucleated buccal mucosal cells in Asian betel nut and tobacco chewers, Lancet, 1: 1204-1206.

Stryker, W.S., Kaplan, L.A., Stein, E.A., Stampfer, M.J., Sober, A., Willett, W.C., 1988, The relationship of diet, cigarette smoking, and alcohol consumption to plasma ß-carotene and alpha-tocopherol levels, American Journal of Epidemiology; 127(2):283-296.

Sugerman, S.D., Mobarhan, S., Bowen, P.E., Stacewicz-Sapuntzakis, M., Langenberg, P., Henderson, C., Kiani, R., Friedman, H., Lucchesi, D., 1991, Serum time curve characteristics of a fixed dose of ß-carotene in young and old men, Journal of the American College of Nutrition, 10(4):297-307.

Swanson, C.C., Mao, B.L., Lubin, J.H., Yao, S.X., Wang, J.Z., Cai, S.K., Hou, Y., Luo, Q.S., Blot, W.J., 1992, Dietary determinants of lung cancer risk: results from a case-control study in Yunnan Province, China, International Journal of Cancer, 50(6):876-880.

Tangney, C.C., Shekelle, R.B., Raynor, W., Gale, M., Betz, E.P., 1987, Intra- and inter-individual variation in measurements of ß-carotene, retinol, and tocopherols in diet and plasma, American Journal of Clinical Nutrition, 45:764-769.

Tannock, I.F., Hill, R.P., 1987, The Basic Science of Oncology, Pergammon Press, New York.

Toma, S., Benso, S., Albanese, E., Palumbo, R., Cantoni, E., Nicolo, G., Mangiante, P., 1992, Treatment of oral leukoplakia with ß-carotene, Oncology, 77-81, 1992.

Valsecchi, M.G., 1992, Modeling the relative risk of esophageal cancer in a case-control study, Journal of Clinical Epidemiology, 45(4):347-355.

Van T'Veer, Pieter, Kolb, C.M., Verhoh, P., Kok, F.J., Schouten, E.G., Hermus, R.J.J., Sturmans, F., 1990, Dietary fiber, ß-carotene and breast cancer: results from a case-control study, International Journal of Cancer, 45(5):825-828.

Van Eenwyk, J., Davis, F.G., Bowen, P.E., 1991, Dietary and serum carotenoids and cervical intraepithelial neoplasia, International Journal of Cancer, 48(1):34-38.

Watson, R.R., Prabhala, R.H., Plezia, P.M., Alberts, D.S., 1991, Effect of ß-carotene on lymphocyte subpopulations in elderly humans: evidence for a dose-response relationship, American Journal of Clinical Nutrition, 53:90-94.

Weisburger, J.H., 1991, Nutritional approach to cancer prevention with emphasis on vitamins, antioxidants and carotenoids, American Journal of Clinical Nutrition, 53: 226s-237s.

West, D.W., Slattery, M.L., Robison, L.M., French, T.K., Mahoney, A.W., 1991, Adult dietary intake and prostate cancer risk in Utah: a case-control study with special emphasis on aggressive tumours, Cancer Causes and Control, 2(2): 85-94.

White, W.S., Kim, C., Kalkwarf, H.J., Bustos, P., Roe, D.A., 1988, Ultraviolet light-induced reductions in plasma carotenoid levels, American Journal of Clinical Nutrition, 47: 879-883.

Winn, D.M., Zeigler, R.G., Pickle, L.W., Gridley, G., Blot, W.J., Hoover, R.N., 1984, Diet in the etiology of oral and pharyngeal cancer among women from the southern United States, Cancer Research, 44:1216-1222.

Zaridze, D., Lifanova, Y., Maximovitch, D., Day, N.E., Duffy, S.W., 1991, Diet, alcohol consumption and reproductive factors in a case control study of breast cancer in Moscow, International Journal of Cancer, 48(4): 493-501.

Zeigler, R.G., 1989, A review of epidemiologic evidence that carotenoids reduce the risk of cancer, Journal of Nutrition, 119:116-122.

Zeigler, R.G., Brinton, L.A., Hamman, R.F., Lehman, H.F., Levine, R.S., Mallin, K., Norman, S.A., Rosenthal, J.F., Trumble, A.C., Hoover, R.N., 1990, Diet and the risk of invasive cervical cancer among white women in the United States, American Journal of Epidemiology, 132(3):432-444.

Zeigler, R.G., Subar, A.F., Craft, N.E., Ursin, G., Patterson, B.H., Graubard, B.I., 1992, Does ß-carotene explain why reduced cancer risk is associated with vegetable and fruit intake?, Cancer Research, 52:2060s-2066s.