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Antioxidant Micronutrient Intake and Oxidative Stress in Persons with Human Immunodeficiency Virus Infection

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Abstract

An imbalance of the oxidant - antioxidant equilibrium has been associated with disease progression in HIV-seropositive individuals. *In vitro* and *in vivo* studies have demonstrated the efficacy of antioxidant supplementation in reducing the concentrations of oxidative stress markers. The objectives of the present cross-sectional study were to evaluate the dietary intake and nutritional supplementation practices of 24 HIV-seropositive persons in Montréal and explore the relationship between dietary intakes of antioxidant micronutrients (ascorbic acid, vitamin E, β-carotene, zinc, selenium), oxidative stress (plasma malondialdehyde [MDA], leukocyte glutathione [GSH]) and immunological indices (absolute CD4+ counts, polymorphonuclear leukocytes [PMN]). Unexpectedly, and paradoxically, a tendency to higher MDA concentrations in subjects with higher CD4+ counts was observed ($r = 0.39$, $p < 0.10$). Moreover, supplementation with vitamin E was associated with significantly higher MDA concentrations ($p < 0.05$). Ascorbic acid intake, even at levels in excess of the tissue saturation (200 mg / d) did not significantly lower MDA concentrations. Neither GSH concentrations nor CD4+ counts were significantly different between supplement users and non-users. In general, the small sample size of this study may have been in part responsible for the failure to detect statistical significance in some associations, however, trends were noted. These included the observation that vitamin supplement users were more likely to have a history of a clinical event associated with HIV infection (opportunistic infection, neoplasm, unintentional weight loss), have a longer duration of HIV-seropositivity and have significantly higher MDA concentrations ($p < 0.05$). In this study, it appears that antioxidants, and vitamin E in particular, were ineffective in reducing the elevated levels of reactive oxygen species (ROS) associated with HIV infection.

Sommaire

Un déséquilibre entre oxydant-antioxydant a été associé avec une progression de la maladie chez les individus porteur du VIH. Des études *in vitro* et *in vivo* ont démontrées l'efficacité des suppléments d'antioxydants pour réduire la concentration des marqueurs de stress d'oxydation. Les objectifs de la présente étude transversale étaient d'évaluer les pratiques de la consommation alimentaire et de la prise de suppléments nutritionnels chez 24 personnes séropsitives à Montréal, et d'explorer les relations entre la consommation alimentaire de micronutriments antioxydants (acide ascorbique, vitamin E, β-carotène, zinc et sélénium) le stress causé par l'oxydation (malondialdéhyde [MDA] sanguin, glutathion de leucocytes [GSH]) et les indices immunologiques [compte absolu de CD4+, leucocytes polymorphonucléaires (PMN)]. D'une manière imprévue et paradoxalement, les concentrations de MDA étaient plus élevées chez les sujets ayant un compte élevées absolu de CD4+ cellules x 10^6 / L ($r = 0.39$, $p < 0.10$). De plus, la consommation de suppléments de vitamin E était associée avec une concentration plus élevée statistiquement significative de MDA ($p < 0.05$). La consommation d'acide ascorbique, même à des niveaux excédant la saturation tissulaire (200 mg / j), n'a pas fait dimuner la concentration de MDA de façon significative. Les concentrations de GSH et de comptes de CD4+ n'étaient pas différentes de façon significative entre les sujets utilisateurs et non-utilisateurs de suppléments. En géneral, la petitesse de l'échantillon utillisé pour cette étude pourrait avoir été, en partie, responsable de l'échec du depistage d'un effet significatif; par contre, des tendances ont été remarquées. Celles-ci contenaient certaines observations, entre autres, selon lesquelles les utilisateurs de suppléments vitaminiques seraient plus enclin à vivre des événements cliniques reliés à l'infection par le SIDA (infection opportuniste, néoplasme, perte de poids involontaire), à avoir une période plus longue de séropositivité et à avoir des concentrations de MDA plus élevées de façon significative ($p < 0.05$). Dans cette étude, il semblerait que les antioxydants, paticulièrement la vitamine E, n'ont pas été capable de diminuer les niveaux élevés des spécimens d'oxygène réactive (ROS) associés avec l'infection du VIH.

Acknowledgments

Wherefore it appears to me necessary
to every physician to be skilled in nature, and strive to know,
if he would wish to perform his duties,
what man is in relation to the articles of food and drink,
and to his other occupations,
and what are the effects of each of them to every one.

Hippocrates

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Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ARC	AIDS Related Complex
ATP	Adenosine Triphosphate
b.i.d.	Twice Daily
BHT	Butylated Hydroxytoluene
BMI	Body Mass Index
CD	Conjugated Dienes
CDC	Centers for Disease Control
CD4+	Cluster of Differentiation Four
CI	Confidence Interval
DHA	Dehydroascorbate
DNA	Deoxyribonucleic Acid
DTNB	5, 5' - Dithiobis - 2 - Nitrobenzoic Acid
EDTA	Ethylene Diamine Tetraacetic Acid
GPx	Glutathione Peroxidase
GSH	Glutathione
GSSG	Glutathionine Disulfide
HCl	Hydrochloride
HIV	Human Immunodeficiency Virus
HPLC	High-Performance Liquid Chromatography
HPO	Hydroperoxides
H_2O	Water
H_2O_2	Hydrogen Peroxide
IkB	Inhibitor Kappa Beta
IU	International Units
kcal	Kilocaloric
LTR	Long Terminal Repeat
LPO	Lipid Peroxide
MDA	Malondialdehyde
NAC	N-acetylcysteine
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCI-USDA	National Cancer Institute - United States Department of Agriculture
NCP	Nucleocapsid Protein
NFκB	Nuclear Factor Kappa B
NHANES I	First National Health and Nutrition Examination Survey
NHANES II	Second National Health and Nutrition Examination Survey
NHIS	National Health Interview Survey
NK	Natural Killer

OR	Odds Ratio
PBMN	Peripheral Blood Mononuclear
PBS	Phosphate Buffered Saline
PCD	Programmed Cell Death
PHA	Phytohaemgglutinin
PMA	Phorbal Myristate Acetate
PMN	Polymorphonuclear Leukocyte
PUFA	Polyunsaturated Fatty Acids
q.i.d.	Four Times Daily
RBC	Red Blood Cell
RDA	Recommended Daily Allowance
RE	Retinol Equivalent
REE	Resting Energy Expenditure
RH	Relative Hazard
RNA	Ribonucleic Acid
RNI	Recommended Nutrient Intake
RPM	Revolutions Per Minute
ROS	Reactive Oxygen Species
SD	Standard Deviation
SE (M)	Standard Error (of the Mean)
SSA	5' Sulfosalicylic Acid
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TCA	Trichloroacetic Acid
TEA	Tetraethylammonium
t.i.d.	Three Times Daily
TNF-α	Tumor Necrosis Factor-Alpha
χ^2	Chi-Squared

1. Introduction

1.1 Natural Course of HIV Infection

Acquired immunodeficiency syndrome (AIDS) is a disease characterized by profound immunosuppression accompanied by diverse clinical conditions associated with a chronic immunosuppressed state, including opportunistic infections, malignancies and central nervous system degeneration. The etiological agent of AIDS is the human immunodeficiency virus (HIV). It is classified as a member of the lentivirus family of retroviruses based on its genomic sequence homologies, morphology and life cycle. A unique feature of lentiviruses is their ability to produce short-term cytopathic effects or long-term latent infection in cells, however, HIV infection in humans is slowly progressive and ultimately fatal.

The primary target of HIV is the cluster of differentiation four (CD4+) subset of T lymphocytes. This subset of T cells is defined phenotypically by the presence of the CD4 molecule on the cell surface. Monocytes also express the CD4 receptor and any cell expressing this molecule is capable of infection by HIV. HIV replicates extensively within monocytes but with relatively low cytopathicity. Consequently, these cells may act as reservoirs of infection and play a role in the process of extensive viral dissemination throughout the body (Fauci and Lane, 1994; Meltzer et al., 1990). Despite macrophage resistance to HIV-induced cytolysis, they exhibit functional impairments most likely related to abnormal activation of these cells *in vivo* by cytokines (Abbas et al., 1994; Fauci and Lane, 1994; Pantaleo et al., 1993). Cytokines are soluble polypeptide growth factors which regulate normal functioning of immunocompetent cells. They are responsible for essential roles in the communication network that links inducer and effector cells during immune and inflammatory responses (Wang and Watson, 1994).

The natural course of HIV infection follows a common pattern of development, although the length of clinical latency and the specific clinical symptoms experienced varies between individuals (Figure 1). After exposure to HIV there is an initial period between two to 12 weeks in duration where the level of HIV antibodies increases and the individual seroconverts to a HIV antibody-positive status. Within this period, approximately 20 - 70% of individuals experience an acute HIV syndrome. This mononucleosis-like condition is associated a high level of viremia, fevers, headaches, sore throat, generalized

lymphadenopathy and skin rashes persisting up to 14 days (Abbas et al., 1994; Levasseur and Lecorps, 1993; Pantaleo et al., 1993). There follows a HIV-specific immune response with a reduction in extracellular viral load and the individual returns to a clinically asymptomatic stage. This clinically latent period has the greatest variability in duration, lasting months or several years with a median duration of 10 years, prior to the diagnosis of illness indicative of AIDS (Appendix 2) (Centers for Disease Control, 1987; Centers for Disease Control, 1993). During this time, there is a quantitative and qualitative deficiency of CD4+ cells and eventually the level falls beneath a critical level [less than 200 cells per microlitre (μL)] which is associated with a high risk of developing a variety of opportunistic diseases, particularly infections and certain neoplasms (Abbas et al., 1994; Fauci and Lane, 1994; Levasseur and Lecorps, 1993; Pantaleo et al., 1993). Oxidative stress has been implicated as a contributor to disease progression by acting as a stimulatory factor in the induction of viral replication (Legrand-Poels et al., 1990; Schreck et al., 1991) and as a mediator of apoptosis or programmed cell death (Buttke and Sandstrom, 1994; Sandstrom et al., 1993; Sandstrom et al., 1994).

1.2 The Role of Oxidative Stress in HIV Pathology

Oxidative stress is a collective term describing the pathological consequences of an imbalance between the systems producing reactive oxidative species (ROS) and the antioxidative defense systems. This imbalance may occur when an absolute or relative excess of free radicals exceeds the capacities of the antioxidant defense system to neutralize these molecules, thereby initiating free radical-generating chain reactions (Halliwell, 1989). Endogenous and exogenous sources of free radicals may both contribute to an imbalance in the oxidant–antioxidant equilibrium (Figure 2).

Due to their high reactivity, ROS will readily react with cellular macromolecules causing damage directly or indirectly through the initiation of free radical-generating chain reactions (Buttke and Sandstrom, 1994; Halliwell, 1989). The major cellular and extracellular targets for ROS damage are protein and free amino acids, nucleic acid constituents, the polyunsaturated fatty acid (PUFA) components of lipids and lipoproteins, carbohydrates and connective tissue macromolecules. The damage and functional consequences are unique to each target component and the relative importance of the damage depends on the degree of oxidative stress inflicted, by which mechanism it is imposed, the length of time exposed to oxidative stress and finally, the nature of the system stressed (Table 1) (Rice-Evans et al., 1991; Halliwell, 1987; Halliwell, 1993).

1.2.1 Viral Activation

Upon cellular infection with HIV, the viral reproductive cycle is activated. Viral ribonucleic acid (RNA) is transcribed by reverse transcriptase to produce viral deoxyribonucleic acid (DNA). The viral DNA is then integrated within the host cell genome. This permanently integrated form of DNA, known as provirus, is capable of remaining transcriptionally inactive for months or years with limited production of new viral proteins or virions (Abbas et al., 1994; Fauci and Lane, 1994). The rate of provirus transcription and replication is highly variable as anatomically different sites support different rates of proviral replication with the lymph nodes being a particularly active site. There are also interindividual differences in viral replication rates as some individuals exhibit elevated rates very early in the disease. The causes of the individual and anatomical differences have not yet been explained (Fauci and Lane, 1994).

Initiation of proviral gene transcription depends upon the interaction of a number of cellular and viral factors, including T-cell activation and immunoregulatory cytokine stimulation (Figure 2) (Fauci et al., 1991; Fauci and Lane, 1994; Poli and Fauci, 1992). Various T-cell activation signals will initiate HIV transcription: for example, coinfectious viruses, cytokines and physical agents, all of which share the ability to cause oxidative stress (Laurence, 1990; Revillard et al., 1990; Rosenberg and Fauci, 1989; Rosenberg and Fauci, 1990; Rosenberg and Fauci, 1990; Stanley et al., 1990). In two *in vitro* studies, the addition of ROS have been shown to directly increase HIV replication. Legrand-Poels et al. (1990) demonstrated oxidative stress from exposure to hydrogen peroxide (H_2O_2) in a promonocytic cell line (U1) elevated reverse transcriptase activities and transactivation of the viral long terminal repeat (LTR) at 24 h post stress. Schreck et al. (1991) confirmed these results in Jurkat T-cells stressed with 150 μM H_2O_2.

The molecular mechanism by which oxidative stress induces viral replication is unknown. However, evidence implicates a redox-sensitive step in the signal transduction leading to nuclear factor kappa B (NFκB) activation and HIV transcription. NFκB is a promoter factor of viral transcription whose action is controlled by inhibitor kappa B (IκB). When stimulated by oxidative stimuli, NFκB dissociates from IκB allowing viral DNA transcription and production of progeny virus (Schreck et al., 1991).

Tumor necrosis factor–alpha (TNF–α), a cytokine produced primarily by monocyte, macrophage and T lymphocytes normally plays a role in monocyte and macrophage-mediated killing of tumor cells. However, TNF also induces the expression of

HIV in the chronically infected promonocytic line (U1) (Poli et al., 1990) and a chronically infected T-cell clone (ACH-2) (Folks et al., 1989; Poli et al., 1990) as well as primary human macrophages (Mellors et al., 1991). The mechanism by which TNF–α exerts its action may be through the utilization of oxidative stress as a second messenger, thus contributing to the dissociation of the NFκB - IκB complex (Schreck et al., 1991). The suggestion that TNF–α may play a role in viral activation was supported in a recent cross-sectional study by Zangerle et al. (1994). They reported increased serum concentrations of soluble TNF–α receptors were associated with immune activation in 61 HIV-positive individuals at all stages of infection. Immune activation was measured by significant increases in urinary neopterin and serum β_2-microglobulin. Elevated levels of these immunological markers have been validated as predictors of disease progression in HIV infection (Fahey et al., 1990).

1.2.2 Programmed Cell Death

Oxidative stress leading to programmed cell death (PCD) or apoptosis may be an etiological factor in the CD4+ cell depletion which typifies HIV infection (Greenspan and Aruoma, 1994; Laurent-Crawford et al., 1993). It has been suggested that at certain times or conditions, for example during T-cell activation, a cell may arrive at a critical juncture with two possibilities; cellular proliferation or apoptosis (Gougeon and Montagnier, 1993). Apoptosis is a morphologically distinct form of cell death characterized by compaction and marginalization of nuclear chromatin into dense masses, and by condensation of cytoplasm with cell surface blebbing (Buttke and Sandstrom, 1994; Larrick and Wright, 1990; Schwartz and Osborne, 1993). The ability of the cell to maintain an oxidant–antioxidant balance may determine which of these possibilities is favoured (Buttke and Sandstrom, 1994).

Studies *in vitro* have demonstrated that exposure to various ROS or the depletion of antioxidants, such as glutathione peroxidase (GPx), will result in apoptosis (Buttke and Sandstrom, 1994; Sandstrom et al., 1993; Sandstrom et al., 1994). In an oxidative environment, TNF–α alone, or combined with additional metabolic or immunological factors, will promote apoptosis.

1.3 In Vivo Evidence for Oxidative Stress in HIV Infection

The direct measurement of ROS in biological systems is difficult. Consequently, the determination of oxidative stress *in vivo* is frequently assessed by measuring the degradation products of free radical injury in body fluids and tissue. Currently, the techniques which are most developed are those which measure lipid peroxidation byproducts. The primary degradation products of lipid peroxidation are lipid hydroperoxides. These products have the ability to decompose into secondary byproducts such as malondialdehyde (MDA), thiobarbituric reactive substances (TBARS) and conjugated dienes (CD). The free radical byproducts of oxidative injury to proteins or DNA can only be measured with techniques which are not easily accessible, such as mass spectrometry. As a result, the effects of ROS damage in normal or pathological states are commonly assessed by measures of lipid peroxidation. In addition to the reaction byproducts of free radical damage, oxidative stress may also be evaluated by measuring the status of the antioxidant defenses. However, the degree of specificity, reliability and reproducibility for all of these laboratory estimates of oxidative stress have been challenged (Halliwell, 1989; Pryor, 1991).

Interest in the contribution of free radical to disease causation and disease progression has recently increased. Free radical -initiated or -mediated reactions have been implicated at some point in the pathogenesis or pathology of over 50 diseases (Greenspan, 1993; Halliwell, 1987), including atherosclerosis, carcinogenesis and aging. At the present time, a small number of studies have explored the potential role of oxidative stress in HIV pathology.

1.3.1 Lipid Peroxidation Products

There has been five studies measuring the level of circulating lipid peroxidation breakdown products in subjects with HIV; they are summarized in Table 2. Initially, Sönnerborg et al. (1988) compared oxidative stress parameters in 30 HIV-seropositive subjects at various stages of disease progression to a seronegative control group. They observed a significantly higher mean plasma concentrations of MDA in the HIV-seropositive group (0.62 μmol) when compared to the control group (0.46 μmol) but they did not detect any difference in TBARS. Coutellier et al. (1992) and Malvy et al. (1994) both confirmed the significantly higher MDA in disease stages II - IV as defined by the Centers for Disease Control [Appendix 2. Centers for Disease Control surveillance case definition for AIDS (1993)] compared to controls (Table 2). Other investigators have

detected significantly higher TBARS and lipid hydroperoxides (HPO) in CDC II versus seronegative controls (Table 2) (Revillard et al., 1992; Sappey et al., 1994).

In all studies, oxidative stress concentrations were significantly higher in CDC II versus controls indicating early increases in lipid peroxidation; however, CDC IV did not consistently show significant differences compared to controls. However, neither MDA nor TBARS concentrations were correlated with the degree of immunodeficiency as measured by CD4+ lymphocytes. Consequently, it remains to be determined if elevated oxidative stress concentrations are a consequence of the stage of HIV infection and therefore acting as a marker of disease progression or if oxidative stress precedes disease progression and thus, is contributory to HIV pathology.

Recent preliminary results, available only in a non-refereed abstract, examined the effects of supplemental ß-carotene (250 mg / d) and selenium (100 mg / d) on plasma and red blood cell (RBC) MDA concentrations (Peuchant et al., 1995). Although the actual concentrations of MDA were not published, significantly higher concentrations of baseline MDA were reported in the HIV-seropositive groups compared to seronegative controls. A significant reduction in plasma and RBC MDA concentrations in the selenium supplemented group ($p < 0.04$; $p < 0.003$, respectively) and a non-significant trend towards decreased MDA in the ß-carotene group was reported. These preliminary results need to be interpreted with caution due to the small sample size ($n = 15$ controls, 15 selenium, 10 ß-carotene) and the fact that neither supplementation compliance nor randomized assignment to intervention groups were described. If dietary intake was simultaneously evaluated it would be possible to determine the potential effect of other antioxidants on the outcome measured. Different dietary intakes of the other antioxidant micronutrients, for example, high intakes of ascorbate, α-tocopherol and zinc may partially account for their results showing a beneficial association between the intervention and outcome measurement, in this case a reduced lipid peroxidation product, MDA. It is well established that the antioxidant defense system does not function in isolation, but rather as a complex system of synergistic reactions between different antioxidants (Figure 3).

1.3.2 Antioxidant Micronutrient and Glutathione Status

As a result of the cytotoxic damage which ROS are capable of inflicting, a defense or protective system has evolved naturally. In healthy individuals a tight control of free radical production and antioxidant defenses is maintained. This is crucial to the very survival of the cell. The protective and repair functions are mediated by both enzymatic and non-enzymatic antioxidants (Table 3). Specifically, an antioxidant may be defined as any

substance which prevents the transfer of electrons to and from molecular oxygen and organic molecules, stabilizes organic free radicals and / or terminates organic free radical reactions (Bray and Bettger, 1990). The enzymatic antioxidants are comprised of three superoxide dismutases, glutathione peroxidase (GPx) and catalase. The non-enzymatic antioxidants are vitamins E (α-tocopherol) and C, ß-carotene and glutathione (GSH, the tripeptide γ-glutamyl-cysteinyl-glycine). Finally, the extracellular antioxidants include lactoferrin, ceruloplasmin, albumin, haptoglobin, hemopexin, uric acid and transferrin (Diplock, 1994; Halliwell, 1989).

In addition to the individual function each antioxidant micronutrient possess as described in Table 3, they also interact synergistically with each other (Figure 3). Animal studies and *in vivo* evidence suggest a GSH-sparing role for ascorbate. Initially, this was supported by an animal study from Mårtensson and Meister (1991). On the other hand, an experiment by Henning et al. (1991) in eight healthy subjects consuming an ascorbate deficient diet (5 - 20 mg / d) for nine weeks demonstrated low plasma GSH, both plasma and leukocyte ascorbic acid. However, DNA scission levels were not affected. These deficiencies were restored upon cross-over to a diet with either 60 or 250 mg / d ascorbate for four weeks. A study to determine the effects of supplemental ascorbic acid on RBC GSH by Johnston et al. (1993) demonstrated 500 mg / d of supplemental ascorbic acid in nine healthy subjects consuming vitamin C-restricted diets was sufficient to maintain reduced RBC GSH and improve the overall antioxidant capacity of the blood. There appeared to be a threshold effect to the effects of vitamin C as there were no significant differences in mean plasma ascorbic acid or red blood cell GSH with 2000 versus 500 mg / d supplementary ascorbic acid. Since 500 mg / d ascorbic acid is over eight times the recommended nutrient intake (RNI) for the average man (Health and Welfare, 1990), a minimum level required to achieve GSH-sparing needs to be reported.

Not only has ascorbate been shown to spare GSH, but GSH will spare ascorbic acid. Mårtensson et al. (1993) demonstrated that a standardized ascorbate-deficient diet supplemented with GSH monoethyl ester (1.25 nmol / kg body weight t.i.d.) fed to male Hartley guinea pigs was capable of sparing ascorbate. This sparing effect may have been mediated through an increase in the reduction of dehydroascorbate (DHA), which otherwise would have been degraded and irreversibly lost, or the sparing of ascorbic acid may be due GSH acting as an antioxidant.

Collectively, results from this group of studies suggest GSH and ascorbate are essential in physiological functioning and regeneration of the other. Metabolic redundancy

and overlap of antioxidant functions for these antioxidants are apparent. The clinical implications of this synergistic reaction remain unclear as concentrations of GSH and other antioxidant micronutrients have been observed to be low in the HIV-seropositive population. Table 4 provides a global summary of several studies estimating the nutritional status of the antioxidant micronutrients and GSH at different stages of HIV disease progression.

To assess whether decreases in antioxidants and increases in oxidative stress are related to disease progression, Favier et al. (1994) conducted a six month longitudinal study in asymptomatic subjects CDC II and CDC IV. From preliminary results, Favier et al. (1994) concluded there is a progressive decline in antioxidant levels (retinol, carotenoids, zinc, selenium and vitamin E) related to HIV progression, with the exception of selenium level which was normal at CDC II (Table 4). Of these antioxidants, the most dramatic decrease was serum carotenoids. At CDC II, concentrations were only 50% of controls [mean serum carotene ± SD (standard deviation) = 0.94 ± 0.46 vs. 1.76 ± 0.40 µmol / L, respectively]. Despite the overall higher levels of antioxidants at CDC II, Favier as a co-author, observed significantly higher concentrations of lipid byproducts at CDC II compared to CDC IV in the same population (Table 4) (Sappey et al., 1994). Regardless of the principal mechanism, oxidative stress appears to be evident early in the course of infection and precedes the severe micronutrient deficits resulting from wasting which occur more frequently in the later stages of disease progression. This may indicate oxidative stress is a factor in HIV pathology and not simply a consequence of secondary malnutrition, although further investigation is needed to fully elucidate this temporal relationship.

The validity of reports indicating the nutrient status of HIV-seropositive persons must be considered for each nutrient. For example, the association between dietary intake and serum concentrations of ascorbic acid may be altered in subjects with acute stress, elevated temperatures, chronic inflammation, acute and chronic infections and cigarette smoking (Sauberlich et al., 1974). These factors are present in HIV infection and will affect the interpretation of serum ascorbic acid measurements. Although leukocyte measurements may provide a more reliable index of slowly changing tissue stores (Loh, 1972; Omaye et al., 1979; Turnball et al., 1981), they are not commonly assessed.

1.4 Pathophysiological Consequences of Antioxidant Depletion

The etiology of the depletion of antioxidant defenses may be due to increased antioxidant expenditure and / or inadequate replenishment of tissue stores. For example, GSH, once oxidized to glutathionine disulfide (GSSG) is normally regenerated to the reduced form via the GSH reductase pathway. However, irreversible loss of GSH occurs under conditions of intense oxidative stress. The GSH reductase pathway becomes overwhelmed or incapable of coping with the increased concentrations of GSSG. Consequently, the accumulation of toxic GSSG is exported from the cell and thus, it is lost to the reductase pathway (Deneke and Fanburg, 1989; Reed, 1990). This loss is irreversible as GSSG can not be reduced extracellularly or taken up by cells (Uhlig and Wendel, 1992)

Ex novo synthesis of GSH can replace lost GSH but it is dependent on the availability and transmembrane transport of cysteine, the rate-limiting substrate in GSH biosynthesis, into the cell (Sakamoto et al., 1983). However, de Quay et al. (1992) reported significantly lower mean (SD) plasma and mononuclear cell levels of cysteine (plasma = 6.9 ± 1.4 versus 12.0 ± 3.1 μmol / L, $p < 0.001$; mononuclear cells = 0.24 ± 0.07 versus 0.35 ± 0.10 nmol / mg protein, $p < 0.03$) in nine HIV-seropositive subjects compared to a seronegative control group. The decreased levels of cysteine in peripheral blood mononuclear (PBMN) cells were related to stage of HIV infection (controls = 0.35 ± 0.09; early stage = 0.28 ± 0.07; late stage = 0.19 ± 0.04 nmol / mg protein). They speculated that inadequate extracellular cysteine, and the resulting low levels of intracellular cysteine, was a likely cause of intracellular GSH deficiency. Similar observations were reported by Eck et al. (1989). In this study, the decreased plasma cysteine was associated with a significant decrease in intracellular GSH in both PBMN cells and monocytes. Glutamate and cystine compete for the same active transport sites and Eck et al. (1989) demonstrated a high extracellular concentration of glutamate exacerbated the intracellular cysteine / GSH deficiency in peritoneal macrophages.

Diminished GSH and other antioxidants may be a consequence of long-term oxidative stress from many sources including stimulated pro-inflammatory cytokines, such as TNF-α, exposure to viral or opportunistic pathogens and certain drugs (Schreck et al., 1991; Jariwalla and Harakeh, 1994). Human T cells can be separated into cells with high or low concentrations of GSH. Staal et al. (1992) hypothesized the induction of inflammatory cytokines causes selective depletion of GSH in those CD4+ and CD8+ cells

with high intracellular concentrations of GSH. They observed changes early in HIV infection as asymptomatic subjects with normal CD4+ cell counts had a significant decrease in the number of T-cells with high GSH concentrations in both CD4+ and CD8+ phenotypes.

Some of the pathological consequences of HIV infection have been hypothesized to be caused by a glutathione deficiency. Intracellular GSH has been shown to be critical for T-cell functions such as IL-2 dependent activation of T-cells (Meister, 1988; Staal et al., 1992), formation of CD8+ cytotoxic cell blasts (Dröge et al., 1986; Hamilos and Wedner, 1985), activity of cytotoxic and natural killer cells (Dröge et al., 1986) and mixed lymphocyte reactions (Meister, 1988). Levy et al. (1992) demonstrated an impairment in T-cell colony-forming cells harvested from both HIV-seropositive and HIV-negative controls subsequently subjected to artificial thiol depletion, particularly GSH, by buthionine sulfoximine (BSO), cyclohexene-1-one (CHX) and copper phenanthroline (CuP). HIV-infected cells exhibited an increased vulnerability to experimental depletion, perhaps due to decreased initial intracellular thiol concentration. Staal et al. (1993) observed a similar regulatory effect on T-lymphocyte function and viral transcription and replication *in vitro* with intracellular GSH. Using highly purified T-cells from PBMN cells donated by healthy volunteers, Suthanthiran et al. (1990) suggested that GSH can directly modulate proliferation of these T-cells, perhaps at the level of DNA synthesis since GSH is required as a reducing equivalent for glutaredoxin during DNA-synthesis (Holmgren, 1990). GSH deficiency has been observed early in HIV infection prior to CD4+ cell depletion (Staal et al., 1992) and perhaps some of the immunological consequences which occur early in HIV infection may be explained by this GSH deficiency.

There is evidence that GSH deficiency may contribute to the progression of HIV infection. Depleted intracellular glutathione enhances the activity of NFκB, which, in turn, permits the initiation of a viral transcriptional sequence (Kalebic et al., 1991). The depletion of antioxidant defenses also diminishes the ability of cells to withstand oxidative stress and electrophilic aggression leading to progressive deterioration and eventually programmed cell death (PCD) (Buttke and Sandstrom, 1994; Greenspan and Aruoma, 1994; Ruffman et al., 1991). In summary, the hypothesized effects of oxidative stress in HIV are 1) impaired T-cell colony formation, 2) increased activation of NFκB leading to viral activation and 3) apoptosis induction.

During HIV infection, ascorbic acid may fail to provide sufficient defense against free radicals for several reasons. Firstly, depletion of this antioxidant may be directly

related to its primary role as an electron-carrier similar to other non-enzymatic radical scavengers (Cathcart, 1991). Secondly, in the presence of GSH-deficiency, ascorbic acid will compensate and act as an essential antioxidant, consequently, as the concentration of GSH decreases, the demand for ascorbic acid increases (Cathcart, 1991; Martensson et al., 1991). Thirdly, in a normal situation the oxidized form of ascorbic acid, dehydroascorbate (DHA), is reduced by GSH. However, when DHA is unable to be regenerated due to GSH deficiency, it is degraded and ascorbic acid is irreversibly lost. Consequently, this extra burden in an environment of increased oxidative stress may overwhelm the capacities of ascorbic acid, resulting in oxidative stress damage (Jariwalla and Harakeh, 1994). Finally, as the requirements for vitamin C, and other antioxidants are yet to be defined throughout HIV infection, the recommended and actual dietary intake may be inadequate to meet the demand. This may have indirect implications on GSH status considering the synergistic interactions between these nutrients (Figure 3).

Relatively few studies have measured plasma ascorbic acid levels in HIV-seropositive subjects (Table 4). These have demonstrated normal plasma levels of ascorbic acid at all disease stages. Frei et al. (1989) has shown that ascorbate is the only plasma antioxidant which is capable of completely protecting plasma lipids against detectable peroxidative damage inflicted by extracellular aqueous peroxyl radicals. Secondly, ascorbic acid is involved in the production and normal functioning of mononuclear and polymorphonuclear (PMN) leukocytes, thereby affecting cell-mediated immunity. Many independent studies have demonstrated the important role of ascorbate in the regulation and function of the immune system including protection from anaphylactic shock, enhancement of delayed hypersensitivity and graft rejection, enhanced phagocytic motility and function, increased T cell response to mitogens and increased production of interferon, in addition to antibacterial and antiviral effects (Jariwalla and Harakeh, 1994).

1.5 Antioxidant Intervention Studies

The ubiquitous nature of free radical-mediated processes which have been implicated in the pathology of numerous conditions has led many investigators to explore the feasibility and potential of antioxidant intervention clinical situations (Harats et al., 1990; Wartanowicz et al., 1984). Intervention trials in healthy individuals at risk of an immunocompromised state and increased infection-related morbidity, such as the elderly, have shown a role for antioxidant micronutrients in the immune response (Boxer et al., 1976; Joffe et al., 1983; Kennes et al., 1983; Penn et al., 1991; Shilotri and Bhat, 1977).

1.5.1 Effects of Antioxidants on Immune Response

At this time, the intervention studies which have examined the effects of antioxidant supplementation specifically on the immune system are not conclusive. Weak study design including inadequate sample size and lack of double blinded placebo-controlled trials limit any conclusions. Consequently, the results reported may be due to the effect of inappropriately controlled confounders or the true effect may be underestimated due to inadequate power to demonstrate an association.

In a double-blind placebo control trial (n = 10 control, 10 intervention), healthy elderly adults [mean (SD) age = 81 (7) years] at risk for decreased immune competence were supplemented with intramuscular injections of ascorbic acid (500 mg / d) (Kennes et al., 1983). In the elderly, the observed diminished immune competence is principally due to impaired proliferative responses of human blood lymphocytes to T mitogen stimulation, most likely as a result of an intracellular defect with imprecise origins (Adler and Nagel, 1981; Doggett et al., 1981; Goodwin et al., 1982; Makinodan and Kay, 1981). After a period of one month, the intervention group was compared to baseline data and the control group. The results indicate an enhanced cell-mediated immune response as indicated by *in vitro* increase of the proliferative response of blood lymphocytes to mitogens and the *in vivo* tuberculin skin hypersensitivity test. Although improvement in cell-mediated immunocompetence was demonstrated with intramuscular ascorbic acid injections, a more detailed study to determine optimal oral doses is needed. The bioavailability of oral doses of ascorbate may be affected by absorption kinetics, especially in the presence of the maldigestive and malabsorptive disorders common in HIV infection (Watson, 1994).

A study by Boxer et al. (1977) described beneficial effects in immune response as demonstrated by improved stimulation of neutrophil chemotaxis with pharmacological doses (200 mg / d) of ascorbic acid in children with Chediak-Higashi disease, a congenital disorder characterized by drastically altered phagocytic function. Of course, as this study was conducted in a small sample of children with pre-existing conditions affecting their immune system unparalleled to HIV infection, the generalizability or extrapolation ability to HIV infection is limited. Nonetheless, a positive immune enhancing effect was demonstrated.

However, not all reports have observed beneficial immunological enhancement (Shilotri and Bhat, 1977). In this cross-over intervention trial conducted in five healthy subjects, the effects of high dose ascorbic acid supplementation on the bactericidal activity of leukocytes (100 mg / d b.i.d. for 15 d then 200 mg / d q.i.d. for 14 d) were examined. Results indicated no significant differences in bactericidal activity with the lower dose

ascorbic acid, but there was significantly impaired activity with the higher dose supplementation. This effect was reversible and returned to normal levels after withdrawal of supplementation for four weeks. Although the authors did not provide a conclusive explanation for the impaired bactericidal activity, perhaps it was due to a maladaptive shift of the oxidative / antioxidative balance, towards the latter. Leukocytes depend on free radicals to destroy invading pathogens (Curnutte and Babior, 1987) and excessive ascorbic acid and its free-radical quenching capability may have interfered with this process (Halliwell, 1987).

1.5.2 Effects on Oxidative Stress

ROS and antioxidants exist in a tightly controlled balance in the normal, healthy individual. An imbalance favoring the oxidant state is a consequence of a relative increase in ROS production or decrease in antioxidant defenses. A study by Wartanowicz et al. (1984) investigated the relationship between antioxidant supplementation and lipid peroxidation in the elderly, as free radicals have been implicated in the degenerative process of aging. One hundred subjects, mostly women between 60 - 100 years old, were divided into three treatment groups receiving either vitamin E (100 mg, b.i.d.), vitamin C (200 mg, b.i.d.) or both for a duration of one year. Their control group consisted of 20 women who were not supplementing with any vitamins. They observed statistically significant differences ($p < 0.001$) in mean concentrations of TBARS in all supplementation groups at 12 months compared to baseline concentrations [TBARS nmol / mL at baseline vs. 12 months, mean $\pm$ standard deviation (SD): vitamin E group = 3.05 ± 0.37 vs. 2.26 ± 0.55; vitamin C group = 2.73 ± 0.45 vs. 2.37 ± 0.35; both C and E group = 3.05 ± 0.55 vs. 2.30 ± 0.31]. These differences became more significant as tissue saturation increased and an apparent synergistic effect between vitamin C and E was observed. Their control group showed no significant differences in lipid peroxide levels (TBARS nmol / L at baseline vs. 12 months, mean $\pm$ SD: 3.26 ± 0.43 vs. 2.94 ± 0.52). A clinical perspective was not provided as clinical endpoints were not assessed, nor was there any indication of subjective improvement in the status of these subjects.

Parallel studies in HIV-seropositive individuals which assess the safety and efficacy of antioxidant micronutrient intervention on both immunological and oxidative stress parameters are lacking.

1.6 Antioxidant Intervention in HIV Infection

Prior to the implementation of intervention trials in HIV-seropositive subjects, consideration to the following is necessary. Given a nutritional deficiency, it needs to be

determined to what extent correction of this deficiency should be made. What are the biochemical and clinical endpoints which should be set as goals for repletion therapy? The aberrations which influence the nutritional status of an individual can be functions of the disease itself, the associated infections and their concurrent treatments and / or the individual's dietary intake (Canadian Dietetic Association, 1994; Winick, 1988). Some causes of deficiency may be more responsive than others to repletion therapy.

There are four levels of nutrient supply, each associated with clinical and immunological conditions. A scale of four stages has been described by Schmidt (1991): 1) deficient supply, 2) sufficient supply, 3) optimal supply, and 4) overoptimal supply. For example, zinc deficiency results in characteristic immunological abnormalities and increased susceptibility to bacterial infection (Cunningham-Rundles et al., 1990; Schmidt, 1991). These abnormalities include decreased numbers of circulating T-cells, atrophy of lymphatic tissues, leukopenia, reduced antibody responses, reduced production and release of cytokines, impaired cytotoxicity of immune cells and other alterations. On the other hand, at the overoptimal level zinc has immunotoxic consequences including, blocking of membrane receptors, inhibition of cytoskeletal function, inhibition of calcium transport and changes in membrane fluidity (Schmidt, 1991). Functional tests to assess the clinical implications are needed to more precisely define the parameters of sufficient and optimal supply and the two detrimental stages of deficiency and oversupply (Schmidt, 1991).

This seemingly paradoxical nature of antioxidant nutrient status, with both beneficial and detrimental effects depending on nutrient supply, combined with the synergistic antioxidant effects which occur between nutrients (Figure 3) increases the complexity of this problem. For many nutrients there appears to be a large margin of safety, usually many times the normal daily intake, before toxic effects are observed from excessive nutrient intakes (Elias, 1993). However, this information is based on observations in healthy individuals who are neither immunocompromised nor malnourished.

The presence of either malnutrition or immunosuppression in an individual may narrow the margin of safety in both directions. Figure 4 illustrates for different nutrients there may be different ranges of optimal intake which in turn, influence the degree of pathology of HIV infection. For example, zinc may follow the (D) curve because of the apparent detrimental effects in HIV for both deficiencies and excesses. In a study with three groups of 54 HIV-seropositive subjects, low levels of serum zinc were related to progression to AIDS (Graham et al., 1991). A low serum zinc level predicted progression to AIDS [Odds Ratio (OR) = 0.30 / 20 μg / dL increase; 95% Confidence Interval (CI) =

0.14 - 0.66], independent of CD4+ lymphocyte levels, age, and calorie–adjusted intakes of zinc. In this study mean dietary intakes of zinc were not significantly different between progressors and non-progressors (mean ± SD, 24.1 ± 22.9; 20.9 ± 25.4 mg / d, $p > 0.30$, respectively) and did not predict progression to AIDS (OR = 1.03, CI = 0.85, 1.25; $p = 0.73$). However, in a study by Tang et al. (1993) excess dietary zinc intake (> 20.2 mg / d) predicted progression to AIDS [for highest vs. lowest quartiles, Relative Hazard (RH) = 2.06; 95% CI = 1.16 - 3.64]. Moderately elevated zinc intake (> 14.2 mg / d) remained predictive of progression to AIDS, even in their multinutrient model (RH = 1.85; CI = 1.03, 3.31). Both studies utilized similar dietary intake measurement methodology.

A theory which may explain the findings from Tang et al. (1993) originates from an *in vitro* study by South et al. (1990). All retroviruses encode a *gag* gene product which plays a key role in viral packaging and assembly. Prior to viral budding, *gag* proteins are processed by a retroviral protease, thereby allowing the processing of several major structural proteins, including nucleocapsid protein (NCP). NCP apparently acts as a RNA stabilizer in the viral core through non-specific NCP-RNA interactions (Karpel et al., 1987). Using HIV-1 (MN strain) cells grown from H9 cells, South et al. (1990) observed two sites which may function physiologically as zinc-binding domains in NCP. Consequently, it appears the availability of intracellular zinc is necessary for HIV-1 nucleocapsid protein assembly. In other studies it has been observed in mutant *gag* proteins with defective retroviral-type zinc finger arrays an inability to package genomic RNA (Aldovini and Young, 1990; Gorelick et al., 1990). South et al. (1990) has suggested that zinc may be associated with the NCP in virus particles, in addition to functioning in retroviral gene recognition at the *gag* protein level. These findings suggest excessive zinc may negatively influence HIV pathology.

Referring to Figure 4, ß-carotene, vitamin E and vitamin C may be more similar to curve (B) because of their apparent low level of immunotoxicity and hypothesized beneficial effect in HIV pathology at high doses. To support this hypothesis, vitamin C intake at the highest quartile (> 715 mg / d) has been significantly associated with a protective effect against progression to AIDS (RH = 0.55; 95% CI = 0.34, 0.91) (Tang et al., 1993). However, there may be a threshold level of intake where higher doses provide little advantage in reducing HIV pathology like curve (C) illustrates. There needs to be further investigations to more precisely define and confirm these relationships throughout the course of HIV infection.

At this point, the sufficient or optimal nutrient intake levels for HIV infected persons throughout all stages of disease progression have not yet been established. The level of nutrient intake suggested by the RNI (Health and Welfare Canada, 1990) may be sufficient for the general healthy population, but inadequate for the HIV-infected person who may have higher requirements due to the stress of chronic infection and metabolic aberrations associated with HIV infection (Watson, 1994). Evidence from three separate studies are suggestive of the need to further test the hypothesis that ascorbic acid intake, from food and supplements, may be inadequate to meet increased metabolic demands. In the first study by Sharkey et al. (1992), there were no significant differences between the weighed seven day food records of 28 HIV-seropositive subjects at various stages of infection versus eight seronegative subjects. No differences were reported to be significant for the nutrients measured, including ascorbic acid (means $\pm$ SEM; HIV+ = 102 $\pm$ 12 and HIV- = 115 $\pm$ 31 mg /d ascorbic acid). The second study by Abrams et al. (1993) also reported adequate nutritional intake in a prospective cohort of 296 recently diagnosed HIV-seropositive men. They evaluated nutritional intake data from a self–administered nutrition questionnaire in relation to the 1989 Recommended Daily Allowances (RDA) (Food and Nutrition Board, 1989). Intake for all nutrients was above the RDA, except zinc, which was 88% of the RDA. However, in both studies biochemical assessment of nutritional status was not performed in conjunction with these dietary intake data.

On the other hand, plasma ascorbic acid was reported to be deficient in 27% of subjects in a cross-sectional survey of 30 HIV-seropositive subjects at various stages of disease (Bogden et al., 1990). This estimate of ascorbate deficiency was based on the total group combined and was not separated into disease stages [six asymptomatic, 17 AIDS-related complex (ARC) (Appendix 3), seven AIDS]. This presents difficulty in interpreting the significance of this estimate as eight subjects reported a mean weight loss of 8.5 kg in the previous six months, while all other subjects had lost less than 1.4 kg or had stable weights for the same period. Twenty-seven percent deficiency represents eight subjects from this combined group and it would have been helpful to report the dietary intake of subjects and if those with weight loss were also hypoascorbemic. This hypothesis warrants testing in future studies which examine the two nutritional assessment methods within the same population.

1.6.1 *In Vitro* Intervention

In vitro evidence for the anti-HIV activities of ascorbic acid has stimulated particular interest in the potential therapeutic role against HIV disease progression. In two studies by

Harakeh et al. (1990 and 1991) the action of ascorbic acid (150 μg / mL) on HIV-1-infected cells (H9 and H9/HTLV-III_B) was investigated. In the first study (Harakeh et al., 1990), extracellular reverse transcriptase activity and p24 antigen level were reduced by 99 and 90%, respectively. However, removal of ascorbic acid from the cellular medium caused the resumption of viral replication. As the continuous presence of ascorbic acid was necessary to maintain the antiviral activity *in vitro*, this could prove a challenge for the pharmacological delivery of non-toxic doses of ascorbic acid *in vivo*. In the second study (Harekeh et al., 1991), non-cytotoxic concentrations of ascorbic acid, calcium–ascorbate, GSH and N–acetylcysteine (NAC) were compared for anti-HIV activity. Again, the action of ascorbic acid required the continual presence of the antioxidant. The combination of ascorbic acid and NAC showed synergy and the greatest inhibition of viral activity. Further investigation of combination therapies *in vivo* is required to confirm and interpret the clinical challenges and significance of this type of therapy.

In cytokine-stimulated cells, ascorbate blocked the transcriptional activity of a reporter gene linked to HIV LTR (Staal et al., 1993). This inhibitory effect was ascribed to inhibition of the activation of the transcription factor NFκB. However, experiments examining the mechanistic action of ascorbate have provided a clue that HIV inhibition is at the level of post-translational impairment of enzyme activity in infected unstimulated cells (Harakeh et al., 1994). Measurement of HIV RNA molecules synthesized in chronically infected cells (HXB) in the presence of ascorbate (0, 150, 200 μg / mL) demonstrated that nucleic acids were not the main target of ascorbate inhibition. In addition, ascorbate (0, 100, 150 μg / mL) did not interfere with the synthesis and processing of intracellular HIV proteins in HXB cells. However, a dose-dependent inhibition of ß-galactoside in 293.27.2 cells (containing an integrated copy of bacterial *lac* Z fused to HIV LTR) resulted in an 89% reduction in enzymatic activity, relative to controls, in the presence of maximum non-cytotoxic ascorbate levels (200 μg / mL). This supports the interpretation that impairment may be related to the selective modification of susceptible sites in the secondary / tertiary protein structure that influence catalytic activity. At this time, further studies utilizing site directed analysis of model proteins are necessary to elucidate the precise mechanism of ascorbate inhibition.

1.6.2 *In Vivo* Intervention

Unfortunately, the very limited *in vivo* evidence for ascorbic acid supplementation in HIV-infected subjects must be interpreted with caution due to poor study design

(Cathcart, 1984). Based on a report from a group of 90 AIDS subjects self-supplementing with high doses of ascorbic acid (unreported amounts) and an additional non-randomized group without controls, (12 subjects with AIDS, six of whom were given an unreported amount of intravenous ascorbic acid for a short time), Cathcart recommended very high doses of ascorbic acid in HIV-infection. The recommended dosages of between 40 - 100 g / d in the absence of opportunistic infections and greater than 100 g / d are based on a theory that the dosage required to achieve beneficial effects is directly related to the severity or toxicity of the disease state. Ascorbate acid levels are titrated according to individual bowel tolerance with optimal dosage levels just below the level which causes diarrhea. The criteria for efficacy of this treatment was not well-defined and utilized reports of subjective improvement only. The safety is unknown as side-effects were neither reported nor measured. In any case, CD4+ cell counts remained suppressed.

1.6.3 Prevalence of Self-Supplementation

A significant number of reports have investigated the prevalence of self-supplementation of antioxidant micronutrients in the HIV-seropositive population. Studies investigating this practice have reported various levels of intake, depending on the nutrient considered (Abrams et al., 1993; Beach et al., 1988; Drolet, 1993; Mantero-Atienza et al., 1991; Parisien, 1993; Rakower and Galvin, 1989). To provide perspective for the prevalence of supplementation in the HIV-seropositive population, a number of recent surveys have been completed in the general American population. The Second National Health and Nutrition Examination Survey (NHANES II) determined that 23% of the total sample consumed supplements with vitamin C (Dickinson et al., 1994). In the 1986 National Health Interview Survey (NHIS) (Moss et al., 1986), observed 85% of supplement users consumed a product with vitamin C. This was represented by a median of 120 mg / d in women, 150 mg / d in men and a 90th percentile of one g / d. In the 1987 NHIS, Subar and Block (1990) reported the majority (75%) of supplement users were consuming a multivitamin and the median intake of vitamin C from supplements was 60 mg / d and the 90th percentile intake was just greater than 600 mg / d.

The HIV-specific data clearly show a higher prevalence and dosages of ascorbic acid self-supplementation than the general population. The Baltimore/Washington, DC Multicenter AIDS Cohort study interviewed 281 participants and observed over 75% consumed greater than 250% of the RDA for ascorbate (Tang et al., 1993). This high intake was due primarily to vitamin C supplement consumption. Beach et al. (1988) reported in 25 male subjects diagnosed with HIV, 85% had made major post-diagnosis

dietary modifications and 57% had begun supplementing with high dose vitamins / minerals. Of these subjects, the vast majority were consuming greater than 10 times the RDA for ascorbic acid. In a more recent study by the same author (Mantero–Atienza et. al., 1991), 75 subjects were interviewed and 80% changed their diet at the time of, or subsequent to, diagnosis of HIV infection. Sixty-eight percent of subjects were consuming nutritional supplements at the time of diagnosis, however, of these subjects, 59% reported increasing the dosage after a HIV-seropositive diagnosis. The mean ascorbate intake from supplementation alone was 873 mg / d (standard deviation = 620 mg). In another study by Martin et al. (1991), a voluntary survey of 76 HIV-seropositive persons indicated a mean consumption at the megadose level of 905 mg / d representing a mean level 15 times the RDA (range 0 - 6,000 mg / d, 10th and 100th percentile = 50 and 20,000 mg / d, respectively). In the prospective cohort with 296 subjects by Abrams et al. (1993), the median intake of vitamin C from food alone was 300% of the RDA, and from food and supplements combined the intake was approximately 875% of the RDA. As all studies recruited volunteers a volunteer bias may have influenced the reported results.

A combination of disease-related, therapy-related, societal, patients and physician characteristics influence the type of treatment chosen in many diseases. In the case of HIV infection, many of these favour the choice of unproven nutritional therapies (Figure 5) (Dwyer et al., 1988). According to Congressional hearings, a projected estimate of $1 billion was to be spent on unproven or fraudulent AIDS therapies in 1987 (Segal, 1987). As AIDS is an incurable, fatal disease surrounded by fear and ignorance, HIV-infected persons are particularly vulnerable to the claims of unproven nutritional therapies. It appears there is a considerable amount of nutritional ignorance which may contribute to the choice of unproven therapies. Few of the supplement users in the surveys by Mantero-Atienza et al. (1991) and Beach et al. (1988) were aware of the RDA nor the possible toxic consequences of high dose self-supplementation. This is supported by a more recent survey by Meyer (1994) of 164 clients of two non-profit AIDS organizations who voluntarily participated. Thirty nine percent of the participants felt the more vitamins consumed the more they would benefit. Perhaps some of this ignorance stems from the observation that more people receive their information from friends and the media (17 and 28%, respectively) than qualified professionals who are bound by a professional code of ethics to provide reliable advice, such as dietitians and doctors (10 and 24%, respectively) (Martin et al., 1991). The remaining 20% obtained their information from parents, self or other sources. Other surveys have consistently identified media and friends as more

frequent sources of nutrition information than health care professionals (Bandy et al., 1993; Mantero-Atienza et al., 1991)

The amount of Canadian data estimating the extent of nutritional self-supplementation is limited. Referring to Figure 5, many of the variables which influence treatment choice will be similar between Canadian and American HIV-seropositive individuals. However, there are important differences in the delivery of health care between these countries which may influence the decision-making process. The Canadian health care system, theoretically providing equal access to all persons regardless of economic or employment status, may reduce the reliance on alternative therapies. On the other hand, similar literature and products are accessible to the Canadian public which strongly promote the usage of high-dose nutritional supplementation. To assess the Canadian-specific prevalence of self-supplementation, there is a need for a comprehensive national survey, with consideration of potential regional and cultural differences unique to Canada.

A study by Verhoef et al., (1990) estimated the prevalence of alternative therapy usage among outpatients in a gastroenterology clinic in Calgary, Alberta. Of the 395 subjects interviewed, 36 (9%) had used alternative therapies. Unfortunately, the precise proportion using nutritional therapies cannot be ascertained as the data for various alternative treatments were grouped together. In addition these were not HIV-positive subjects and as Figure 5 suggests, disease- and treatment-specific characteristics will influence choice of therapy. According to the influence of the variables in Figure 5, HIV would be expected to have a stronger influence favouring the choice of alternative nutritional therapies than was observed in the Verhoef et al. (1990) study.

Two M. Sc. thesis projects from the University of Montreal have investigated the nutritional status of HIV-seropositive subjects. The first project (Drolet, 1993) examined nutritional status in 117 subjects between CDC stages III - IV. A 24 h dietary recall and seven day food record estimated the extent of supplement usage. In this sample, 76% of subjects supplemented on a regular basis, and 38.5% supplemented with 2-5 different supplementation products. The most popular supplements were multivitamin with minerals (56.4%) and natural supplements (41%) including ginseng, lecithin, wheat germ and garlic capsules. In comparison to the RNI standards, 67% of subjects were consuming greater than five times the RNI and 27% were consuming greater than 10 times the RNI. Once again, the principle source of information was friends / relatives or self-medication (11 and 43%, respectively) versus physicians or dietitians (30 and five percent, respectively). The

second project (Parisien, 1993) investigated a smaller sample of subjects (n = 37) in CDC II - IV. Twenty-nine percent of the subjects consumed pharmacological doses of ascorbic acid (> 10 x RNI). Although the two Canadian studies did not demonstrate supplementation prevalence levels as high as the American data, additional studies are needed to confirm or dispute their observations.

1.7 Deficiency and Excess: A Precarious Balance

All pharmacological agents have side-effects (Compendium of Pharmaceuticals and Specialties, 1995). When nutrients are consumed at pharmacological levels, 10 times greater than the RNI (Food and Nutrition Board, 1989), it is unlikely side-effects are absent. Potential side-effects of high-dose supplementation with vitamin C, although speculative at this time, need to be ascertained before an informed decision with regards to the risk-benefit ratio is possible. There is evidence that ascorbate will operate as a prooxidant in the presence of transition metals (Cu^{2+} and Fe^{3+}), promoting the formation of ROS, thereby damaging cellular macromolecules (Stadtman, 1991). Under normal physiological conditions, trace elements exist almost entirely sequestered in protein complexes (ceruloplasmin, albumin, transferrin, ferritin, lactoferrin and hemoglobin). Although Stadtman et al. (1991) suggests there is currently little evidence that ascorbate acts as a prooxidant under normal physiological conditions, perhaps this is not the case in HIV infection. Tissue damage results in the release of free metal ions from sequestered sites and release of heme proteins (Halliwell et al., 1989). There are factors promoting tissue damage which may be especially relevant in HIV infection, primarily chronic infection, trauma and radiation therapy. Interestingly, serum copper (Beach et al., 1992) and iron (Beck et al., 1990) have been shown to be significantly higher in HIV-seropositive subjects versus controls and elevated plasma copper was associated with advancing disease stage (Bodgen et al., 1990). Furthermore, a high serum copper concentration predicted progression to AIDS in a study by Graham et al. (1991). The clinical relationship, if any, between these transition metals and ascorbic acids role as an antioxidant or prooxidant is unknown.

Intervention trials using antioxidant therapy should include the monitoring of oxidative stress, antioxidant levels and immunological markers. The beneficial effects from low levels of ROS are the desired outcome, but deleterious effects from excessive antioxidant protection may occur if this balance is shifted too far towards the antioxidant state. An intervention study requires significant commitment in terms of research participation, personnel and finances. Before a trial of this magnitude is undertaken, it is

prudent to clearly assess the presence and strength of an association between antioxidant micronutrient intake and oxidative stress parameters in HIV-seropositive individuals. The current cross-sectional study will allow this type of association to be characterized.

2. Hypotheses and Objectives

2.1 Hypotheses

2.1.1 Primary Hypothesis

Ascorbic acid from food and / or supplements at levels greater than tissue saturation (≥ 200 mg / d) is associated with lower plasma MDA and higher leukocyte GSH concentrations in HIV-seropositive individuals.

2.1.2 Secondary Hypothesis

Dietary intakes of antioxidant micronutrients β-carotene, vitamin E, zinc and selenium are associated with lower plasma MDA and higher leukocyte GSH concentrations in HIV-seropositive supplement users compared to non-users.

2.2 Objectives

2.2.1 General Objective

The present descriptive study will describe and characterize the dietary intake of HIV-seropositive men and women. These nutritional variables will be related to oxidative stress (plasma MDA and leukocyte GSH) and immunological [absolute CD4+ cell and polymorphonuclear (PMN) leukocytes] parameters to provide a basis for future analytical research in antioxidant supplementation therapy.

2.2.2 Primary Objectives

1. To characterize the dietary intake of ascorbic acid.
2. To explore the relationship between nutritional intake of ascorbic acid, oxidative stress measurements (plasma MDA and leukocyte GSH) and indicators of immunodeficiency, absolute CD4+ and PMN leukocyte counts.

2.2.3 Secondary Objectives

1. To describe the dietary intake of the antioxidant micronutrients, β-carotene, vitamin E, zinc and selenium, according to the Recommended Nutrient Intakes for Canadians.
2. To describe prevalence, type and dosage of nutritional supplementation in HIV-seropositive persons.

3. Methods

3.1 Study Design

In selecting the most appropriate study design, consideration was given to the current state of knowledge and feasibility of the research project, for this reason the cross-sectional design was chosen. This design emphasizes a reporting of characteristics of person, place or time for the outcome of interest. It can identify potential associations between risk or protection factors and outcomes, as well as elucidate patterns among particular populations. In the present study, this refers to the risk or protective exposure of antioxidant nutrient supplementation, and the outcome of interest, levels of oxidative stress. As exposure and outcome are measured simultaneously, cross-sectional studies are most useful for assessing and characterizing the presence of an association rather than testing a hypothesis or determining the temporal relationship between the exposure and outcome (Sherry, 1992).

3.1.1 Study Population

The target population of this study was all HIV-seropositive adults. The population sampled was HIV-seropositive men and women of the legal age of consent in Quebec. Prospective subjects were assessed for eligibility by independent screening physicians at the Montréal Chest Hospital Immunodeficiency Unit. Completion of a screening questionnaire (Appendix 4) was used to assess the following inclusion / exclusion criteria:

- Karnofsky score ≥ 80. The Karnofsky Performance Scale was used to estimate the level of performance as a crude estimate of health (Karnofsky et al., 1948). This scale emphasizes the physical aspects of performance rather than social and psychological aspects. Subjects were assigned a notational percentage score (100 = normal; 0 = dead) for each component of the scale by the participating physician. These scores were then summed to produce the overall score (Bowling, 1991).

- Absence of medical conditions unrelated to HIV infection which independently increase oxidative stress levels. Atherosclerosis, heart disease, diabetes, hemophilia, neoplastic disease or inflammatory disorders were criteria for exclusion.

- Absence of clinical symptoms of medical conditions related to HIV infection which independently increase oxidative stress levels for at least six weeks prior to study entry. Oral candidiasis, fever, diarrhea (defined as three or more unformed stools per day) and weight loss (defined as loss of greater than 10% of usual or pre-HIV infection body weight within previous three months) were also criteria for exclusion.

- Oral comprehension of French or English.

3.1.2 Recruitment

3.1.2.1 Subject Sources

Recruitment was continuous from the last week of October 1994 through April 1995, inclusive. Subject recruitment involved advertising strategies designed to reach a broad audience of prospective subjects. Announcements were placed on bulletin boards of physicians' offices and clinics known to provide care to HIV infected persons in the Montréal region. Community support groups providing support for HIV-seropositive persons also served as places of advertisement. These included groups offering service to French- or English-speaking clients (AIDS Community Care Montréal, Comitè des Personnes atteintes de VIH, Centre d'Action SIDA Montréal-Femmes) indigenous persons (Native Friendship Centre) or persons of Haitian origin (GAP-SIDA) and a specialized food bank with distribution to HIV-seropositive persons (AIDS Montréal Direct Assistance Foundation). Monthly newsletter advertisements were placed in community group newsletters and in the McGill AIDS Centre Newsletter. Radio and newspaper advertisements were made at local universities and colleges (McGill, University of Montréal, Concordia, Dawson College). Finally, word-of-mouth recruitment from subjects who had completed the study was encouraged.

3.1.2.2 Study Incentives

To encourage participation, a nutrition counseling session was offered by a registered dietitian and took place subsequent to the collection of study data. It was designed to allow the subject to freely enquire about individual nutritional questions or concerns. This session was strictly for the benefit of the subject and was not related in any way to the collection of data.

3.2 Data Collection

Data collection consisted of a one and a half hour interview and blood test. All nutritional and demographical data were collected by two research dietitians trained in interviewing techniques. All sample preparation was performed by the same research laboratory technician. Each of the two oxidative stress measurements were analyzed in batch fashion by one research laboratory technician.

3.2.1 Demographical-Medical Information

Questionnaires were designed to obtain a general subject profile (Appendix 5 and 6). Basic demographical variables included gender and age. Anthropometric measurements consisted of self-reported height, current weight and usual or pre-HIV infection weight. Medical information was collected on medication usage, date of initial HIV seropositivity and the presence of AIDS-indicator neoplasms and the three most recent AIDS-indicator opportunistic infections. Finally, smoking status was determined and subjects were classified as either a smoker, non-smoker or former smoker, including date of smoking cessation.

3.2.2 Dietary Assessment

The dietary assessment was comprised of two components. The first part was a nutritional supplement frequency questionnaire (Appendix 7) specifically designed for the purposes of this study. This questionnaire consisted of a series of questions designed to elicit quantitative information about individual nutritional supplementation patterns including types / brand-names of nutrients supplemented, dosages and duration.

The second part of the nutrition interview was a diet history (Appendix 8). This research tool is useful in assessing usual or average dietary intake of individuals and was based on the first diet history conceived by Burke (1947). The first section of the questionnaire was a 24 hour recall of actual intake and collection of general information on the overall eating pattern throughout the previous 24 hours. The second section includes lists of foods and groups with similar nutrient values, their frequency of consumption and usual portion sizes (Gibson, 1990). The diet history and standardized food models used to estimate portion sizes were specifically designed by Santé Québec for use in the Québec population (Santé Québec, 1994). Weighted average daily nutrient content was determined using the data entry software CANDI (CANDI: Version 4.0. Health Canada: Ottawa. March 1994). This computer system is comprised of a nutrient database based on food consumption in the Canadian population. It contains standardized recipes for common

combination food dishes, and an internal recipe program allows the creation of particular food items not listed in the nutrient database.

3.2.3 Oxidative Stress Assessment

Twenty of 22 blood samples were taken on the same day as the nutritional interview, two of 22 blood samples were taken within one or two days after the nutritional interview. Samples were obtained from the antecubital vein and collected into evacuated (Vacutainer) tubes. Blood was immediately sent by courier to the Jewish General Hospital for preparation and storage at -80°C for a mean of 11 weeks (range = 0 - 26 weeks) until assayed.

3.2.3.1 Leukocyte Glutathione

Sample Preparation: All sample preparation was performed at the Jewish General Hospital (Dr. M. Wainberg, McGill AIDS Centre, Jewish General Hospital, Montréal). Samples for lymphocyte GSH analyses were collected in heparinized (Vacutainer) tubes. Pellets were obtained by centrifuging at 800 x g [2000 revolutions per minute (RPM)] (Sorvall RT 6000D Centrifuge) for 10 minutes (min) at room temperature. Using a disposable wide bore graduated pipette, the supernatant and buffy coat were removed. The supernatant was diluted with 1:1 volume of phosphate buffered saline (PBS) (pH 7.4) at 37°C. The supernate and PBS were then layered on top of Ficoll (Pharmacia #17084002) to a ratio of 1 Ficoll : 3 sample. This mixture was centrifuged at 400 x g (1500 RPM) for 40 min at 25°C. The lymphocyte layer was resuspended in 10 mL of PBS at 4°C. Centrifugation at 400 x g (2000 RPM) for 10 min at 4°C followed. The pellet was resuspended in 10 mL PBS 1X (pH 7.4) and a cell count using a Hematocytometer was performed according to established procedure. At this point, the cells were suspended to a concentration of 1 x 10^6 cells / tube and centrifuged at 400 x g (2000 RPM) for 10 min at 4°C. The pellet was then resuspended with 970 µL ice cold water (H_2O) and vortexed for one min. Finally, 30 µL SSA (5' sulfosalicylic acid F. Wt. 254.2 Sigma #S-0640) were added to each tube and this mixture was incubated for 15 min on ice. The sample was subsequently centrifuged at 5000 x g for 5 - 10 min at 4°C. The supernatant was removed and frozen at -80°C until GSH determination.

GSH Assay: All GSH analyses were performed at the Montreal Children's Hospital (Dr. S. Baruchel, Montréal Children's Hospital Research Institute, Montréal).

The GSH assay was carried out by the DTNB-GSSG reductase recycling assay (Anderson, 1985). This method combines the usefulness of the colorimetric reaction of DTNB (5,5'-dithiobis-2-nitrobenzoic acid) with the specificity of GSH-reductase and offers high sensitivity for GSH. One hundred µL of sample were added to 700 µL of the working buffer containing 0.3 mM nicotinamide adenine dinucleotide phosphate (NADPH) (pH 7.4) after diluting in stock buffer [0.143 M sodium phosphate containing 6.3 mM ethylene diamine tetraacetic acid (EDTA) (pH 7.4)] and 100 µL of tetraethylammonium (TEA) (1 M pH 8.0). One hundred µL of DTNB solution (6 mM in stock buffer) were added with mixing and the assay was initiated by the addition of 10 µL of non-diluted GSH reductase (120 U / mg) diluted stock buffer. The rate of TNB formation was then followed spectrophotometrically at 412 nm for 2 minutes and compared to a standard curve of known quantities of GSH. Values were calculated as nanomoles of GSH and expressed in nmol GSH / 10^7 cells.

3.2.3.2 Plasma Malondialdehyde

All MDA analyses were performed at Ste-Justine Hospital (Dr. G. Lepage, Pediatric Research Centre, Ste-Justine Hospital, Montreal). Samples for MDA analysis were collected into 0.1% EDTA (Vacutainer) tubes. All glassware and stirring bars were acid washed in hydrochloride (HCl) 1N. To 100 µL of butylated hydroxytoluene (BHT) [0.05% methanol (CH_3OH)], 200 µL plasma, 500 µL of trichloroacetic acid (TCA) (12.5% H_2O) and a stirring bar were added and this mixture was heated for 30 min at 100°C. After heating, the mixture was transferred to cooled Eppendorf conic tubes and centrifuged for 10 min (13 000 RPM; 4°C). The filtered supernatant [Acrodisc LC13 PVDF, 13 mm x 0.2 µm high-performance liquid chromatography (HPLC)-certified filter] was combined with 1 mL thiobarbituric acid (TBA) solution (1 g TBA in 100 mL H_2O). The MDA-TBA complex was heated at 100°C for 60 min. Thereafter, the tubes were cooled and 200 µL of HCl 5N and 1 mL of butanol were added and centrifuged for 10 min (2 900 RPM; 20°C). The top solvent was mixed with 500 µL *n*-butanol and centrifuged. Pooled butanol was completely evaporated at 37°C under nitrogen. Finally, the residue was dissolved with 500 µL H_2O and vortexed. Twenty µL of the sample were injected into the HPLC (Hypersil

ODS C18 100 x 4.6 mm, 5 µm) and the peak of the MDA-TBA complex determined by HPLC (Hewlett-Packard 1090). The gradient was previously described in Lepage et al. (1991). Total plasma MDA was expressed as nmol / L.

3.2.4 Immunological Assessment

Lymphocyte subset enumeration was determined according to standard procedure of the Special Hematology (Cell Marker) Laboratory of the Royal Victoria Hospital. Two 12 x 75 Eppendorf microtubes with either MsIgG2b-ECD/MsIgG1-RD1/MsIgG1-FITC or CD3-ED/T4-RD1/T8-FITC were labeled. Ten µL of the appropriate antibody were added to each tube, then 100 µL of well mixed EDTA whole blood. These samples were subsequently vortexed gently and incubated for 10 min at room temperature. Sample processing followed on Multi-Q-Prep (Coulter) and the samples were run on flow cytometer (Coulter XL-MCL Flow Cytometer) according to established protocol. Absolute CD4+ counts were expressed as number of cells x 10^6 / L and PMN as number of cells x 10^9 / L.

3.2.5 Statistical Analyses

Nutritional, oxidative stress, immunological and clinical / demographical differences between supplement users and non-users were evaluated using parametric methods, with the exception of one nonparametric correlational analysis. All micronutrient total intakes represent the sum of the daily average intake from food and, if consumed, micronutrient supplements. However, data for selenium and vitamin E are based on supplementation intakes only. This is based on two reasons, the first is the nutrient database files used in this study contain incomplete nutrient composition analyses for these particular micronutrients and the second is the inherent variability due in part to regional differences in the selenium content of Canadian soil and seasonal variability which may significantly influence the reported value of these nutrients in a cross-sectional study (Arthur, 1972; Bauernfeind, 1980; Thompson et al., 1975).

- The Student's *t*-test was used to detect differences between two sample means in the following data sets:

    1. Supplement users and non-users were compared to detect differences in the mean total intake of each of the five antioxidant micronutrients, energy, and protein.

2. Differences between the sample mean plasma MDA, leukocyte GSH and CD4+ concentrations were compared in the following dichotomized groups: total ascorbic acid > or < 200 mg / d; supplemental vitamin E intake > or < 30 IU; users and non-users of supplemental ascorbic acid; and users and non-users of supplemental vitamin E.

3. Mean levels of plasma MDA, leukocyte GSH, CD4+ counts and total ascorbate intake in individuals with CD4+ counts > of < 200 x 10^6 cells / L.

4. Differences in the mean plasma MDA, leukocyte GSH and duration of seropositivity between users and non-users of any nutritional supplement, including the non-antioxidant micronutrients, were assessed. The same parameters were compared when users were restricted to those supplementing with vitamin C or E.

5. The demographic variables gender and language (French-speaking or English-speaking), were each dichotomized and differences between mean levels of energy, protein, total ascorbate, plasma MDA, leukocyte GSH, and CD4+ counts were assessed. Smoking status (smoker or non-smoker) was compared in only the three later indicies.

- The Student's paired *t*-test was used to detect differences between usual or pre-illness weight compared to current weight.

- For analyses with greater than two sample means, analysis of variance (ANOVA) was employed. Data were stratified on the basis of mean total ascorbate intake (< 100, 100 - 200, >100 mg / d) and assessed for differences between the mean plasma MDA, leukocyte GSH and CD4+ counts. A second stratification based on CD4+ counts (< 100, 100-200, > 200 x 10^6 cells / L) was assessed for differences between mean plasma MDA concentrations, however, due to the small sample size and non-normality of these strata, Spearman's rank correlation was subsequently used to assess possible associations. This correlation was tested for significance using the Student's *t*-test.

- The potential interactive and synergistic effect of vitamin C and E was explored using analyses of covariance (ANCOVA). The mean nutrient intakes of vitamin C or E (> or < 10 x RNI) were adjusted for the possible interactive effect of either vitamin C or E and mean concentrations of each of plasma MDA, leukocyte GSH and absolute CD4+ counts were evaluated. The same covariates were utilized in a second analysis stratified on the basis of supplementation with either C or E.

- Proportions were compared using the chi-squared (χ^2) test. These included the proportion of each of supplement users and non-users who had experienced a clinical event associated with HIV infection followed by analysis of the proportion who had CD4+ counts > or < 200 x 10^6 cells / L.

- Linear correlation analyses, using Pearson's product moment correlation, were used to quantify the degree to which two continuous dependent variables were related. Prior to correlational calculations, all data were plotted in scatter diagrams to visually inspect for the presence of non-linear associations. Associations were assessed in the following correlational analyses sets:

    1. Each of the five antioxidant micronutrients [total intake (food plus supplements): vitamin C, zinc, β-carotene: food only: vitamin C, zinc, β-carotene; supplement only: vitamin C, E, β-carotene, zinc, selenium], protein, energy, the oxidative stress measurements (plasma MDA, leukocyte GSH) and the immunological marker (absolute CD4+ cell count) were correlated with each of plasma MDA, leukocyte GSH and absolute CD4+ counts.

    2. Users and non-users of supplements (each of vitamin C, β-carotene, zinc and any nutritional supplement) were correlated with concentrations of each of plasma MDA, leukocyte GSH and absolute CD4+ counts.

    3. The mean total intakes of vitamin C or E were stratified on the basis of > or < 10 x RNI and intakes within each strata were correlated with each of plasma MDA, leukocyte GSH and CD4+ counts.

    4. The demographical observations body mass index (BMI) and age were each correlated with plasma MDA, leukocyte GSH, CD4+ and the dietary indices total ascorbate, energy and protein.

    5. Smoking status (smoker or non-smoker) was correlated with plasma MDA, leukocyte GSH and CD4+ counts.

    6. Neutrophils were correlated with dietary intake (total: vitamin C, zinc, β-carotene; supplemental: vitamin E, selenium). CD4+ counts and oxidative stress markers (plasma MDA and leukocyte GSH).

All analyses, except covariate analyses, were performed using Microsoft Excel (Microrsoft, Cambridge, Massachusetts: Version 5.0, 1994). Covariate analyses were performed using Statistical Analysis System (SAS) (SAS Institute, Cary, North Carolina: Version 5.18, 1988). The minimum level of significance required to reject the null hypothesis was 0.05 and tests of significance were one-tailed.

3.2.6 Ethics

All aspects involving research design, conduct, reporting and interpretation were made according to the Guidelines on Research Involving Human Subjects (Medical Research Council of Canada, 1987). The experimental protocol was submitted to independent Research Ethics Boards at the Royal Victoria Hospital / Montréal Chest Hospital and Macdonald Campus of McGill University. Individual freedom of choice to participate was informed and voluntary and subjects were made aware of their right to decline or withdraw from the study at any time, without prejudice. The invitation to participate was proffered by independent health care professionals, community groups or advertisements and not by the investigator. Communication of the final decision to participate was made only after prospective subjects study-specific questions were answered by the research investigator. Written informed consent was obtained and subjects were provided with a permanent record of consent and an information form. Confidentiality was maintained by implementing the following measures: the only form with personally identifying information, the record of consent, was stored in a locked cabinet at the Montréal Chest Hospital and all other reports and analyses included coded (anonymous) data only.

4. Results

4.1 Subject profiles and clinical details

Twenty four subjects were recruited over a period of seven months and the characteristics of this sample population are summarized in Table 5. The group was primarily male (79%) and French-speaking (54%). The mean (SE) age was 41 (1.8) years. Regular cigarette smokers comprised 46% of this group.

At the time of study participation, the medical history and physical exam revealed all subjects to be in generally good health without clinical evidence of active HIV-related conditions. This was supported by a high mean Karnofsky score 93 (1.8), a crude indicator of overall health. Furthermore, the majority (91%) of BMI scores were greater than 20 kg / m^2, which is a value associated with decreased health risks within a healthy population (Health and Welfare Canada, 1988b). The remaining nine percent of subjects had BMI scores of 19.5 kg / m^2, just below this lower cutoff value. The mean (SE) self-reported usual or pre-illness weight was 67.6 (1.8) kg while the current weight was 66.7 (1.9) kg, representing a non-significant ($p = 0.21$) weight loss of 0.9 (1.1) kg since the time of HIV seropositivity (Table 5).

Correlational analyses for the demographical variables demonstrated uniformly weak and non-significant relationships between age or BMI and oxidative stress (GSH, MDA), immunological (CD4+) and dietary (ascorbic acid, energy, protein) parameters (Table 6). Language, as a crude proxy for culturally-influenced differences in dietary consumption between French-speaking and English-speaking subjects, was not associated with mean (SE) daily intakes of ascorbate [441 (194) versus 737 (248) mg; $p = 0.18$], energy [3111 (319) versus 3441 (251) kcal; $p = 0.21$] and protein [122 (10.9) versus 139 (14.4) g; $p = 0.18$] in French and English-speaking subjects, respectively. Gender had a significant influence on mean (SE) daily intakes of energy [3408 (241) versus 2710 (287) kcal; $p = 0.05$) and protein [135 (10.8) versus 110 (6.2) g; $p = 0.03$] as would be expected, but the mean total intake of ascorbic acid [454 (135) versus 1045 (503) mg; $p = 0.15$) was not related. The high mean ascorbic acid intake in females was affected by 2 / 5 (60%) subjects consuming greater than 10 times the RNI while in men only 2 / 17 (12%) consumed doses at this level. CD4+ counts [221 (57) versus 270 (104) cells x 10^6 / L; $p = 0.35$] or the oxidative stress measurements GSH [17.7 (1.7) versus 20.9 (7.5) nmol / x

10^7 cells: p = 0.35] and MDA [585 (115) versus 1058 (313) nmol / L: p = 0.11] were not significantly different in males and females, respectively.

4.2 Dietary intake

Nutrient intakes are summarized in Table 7. The general quality of the diet of supplement users and non-users was compared using the dietary markers of energy and protein. The Student's *t*-test revealed no significant differences (Table 7). The antioxidant supplement users consumed a mean (SE) of 132.0 (11.1) g protein versus 125.7 (15.2) g in the non-users group. Energy intake was 3315 (238) kcal / d in the users and 3174 (399) kcal / d in the non-users. The most commonly supplemented antioxidants were ascorbic acid and vitamin E (50%) followed by β-carotene and selenium (41%) and zinc (39%). To determine the influence of supplementation on total dietary intake, this sample was divided into antioxidant supplement users and non-users (Table 7). For two of 24 subjects who were supplementing with antioxidants, the precise intake could not be quantified. This was due to inadequate product information, consequently these subjects were excluded for relevant analyses. Significant differences between users and non-users were observed for the mean (SE) total intake of ascorbic acid [956.6 (269.7) versus 221.7 (37.8) mg: $p \leq 0.01$) and zinc [36.7 (4.0) versus 16.1 (1.8) mg: $p < 0.001$]. Table 8 lists the types of nutritional supplements consumed by each subject and illustrates the majority (67%; 16 / 24) of subjects were consuming some form of nutritional supplement.

The contribution of dietary supplementation is expressed as percentage of the 1990 Recommended Nutrient Intakes (RNI) for Canadians, male and female aged 25 - 74 years, in Figure 6 (Health and Welfare Canada, 1990). It is apparent the RNIs for these antioxidant micronutrients were exceeded by both the mean intake from diet alone and with supplementation combined. For descriptive purposes only rather than to suggest adequacy of the RNIs for the HIV-seropositive population, the percent of supplement users consuming greater than or equal to 10 times the RNI was calculated (Table 7). Only users of supplemental ascorbic acid (36%; 4 / 11) or vitamin E (45%; 5 / 11) exceeded levels greater than 10 times the RNI whereas two subjects (15%; 2 / 13) exceeded this level for β-carotene from food sources alone. The consumption of high levels of dietary β-carotene alone is feasible as this nutrient occurs naturally in certain foods at high concentrations, whereas for other micronutrients, the doses required to achieve this level are typically only available through dietary supplements. Moreover, the reported β-carotene intake was

checked for credibility and accuracy by the research dietitian. Inclusion or exclusion of these two high β-carotene consumers did not significantly change the group mean intake, therefore, they remained within the supplement non-users group for subsequent analyses.

Since vitamin C and E are known to have synergistic effects (Bendich, 1990), they were analyzed as covariates. Using ANCOVA, the mean intake of vitamin C at levels greater than and less than 10 times the RNI was stratified and the mean plasma MDA, leukocyte GSH and absolute CD4+ counts between the two strata were compared, controlling for vitamin E intake. The same parameters were assessed with vitamin E intake stratified at levels greater than or less than 100 IU, controlling for vitamin C intake. A second covariate analysis was performed on the basis of whether a subject was a supplement user of vitamin C and mean MDA, GSH and CD4+ levels were compared, controlling for vitamin E intake. The same analysis was repeated on the basis of whether a subject was a user of vitamin E, controlling for vitamin C intake. These analyses also showed no significant differences between groups in this sample.

To explore associations between supplement usage and internal or external cues indicative of disease progression, the proportion of supplement users who had previously experienced a clinical event associated with HIV infection was compared to non-users. In this regard, a clinical event was defined as a documented history of HIV-related illnesses or self-reported unintentional weight loss. Supplement users included subjects who consumed any form of nutritional supplement (antioxidants and / or natural supplements like ginseng, cyanobacteria, coenzyme Q, etc.). For descriptive purposes only, rather than to suggest the validity of these indices as markers of disease progression, external cues were defined as those indices typically known to the subject, for example, CD4+ levels, clinical events and duration of HIV seropositivity. On the other hand, internal cues were included those typically unknown to the subject, for example, oxidative stress levels.

In standard practice, CD4+ counts are frequently assessed and the patient is informed of their status. Counts below 200 x 10^6 / L are indicative of AIDS according to the most recent definition of AIDS in the United States (Centers for Disease Control, 1993) and these persons are at a high risk of developing HIV-related complications (Fauci and Lane, 1994). The prevalence of supplement use was observed in 42% of those with CD4+ counts less than 200 and 56% in those with CD4+ counts greater than 200 cells x 10^6 / L. These proportions were not significantly different (χ^2 = 0.37; p = > 0.25). Dichotomization between supplement use and non-use revealed a tendency towards higher mean (SE) absolute CD4+ counts in users analyzed by the Student's *t*-test [274 (66) versus

162 (67) x 10^6 / L p = 0.12, respectively]. When this dichotomization was restricted to users of vitamin C or E only, CD4+ counts did not indicate differences exist in this sample [mean (SE) = 267.0 (71) versus 184.1 (63) x 10^6 / L; p = 0.20], respectively. Of those persons who were supplement users, 75% (6 / 8) had experienced a clinical event, whereas in the non-users group only 36% (4 / 12) had experienced a clinical event (χ^2 = 2.8; p = < 0.10). Duration of HIV seropositivity revealed a trend indicating supplement users were HIV-seropositive for a longer period than non-users but this did not achieve significance [mean (SE) = 42.6 (9.0) versus 32.8 (8.2) months; p = 0.21), respectively.

The internal cues did not indicate a consistent pattern. Interestingly, the mean (SE) MDA concentration of supplement users was significantly higher than non-users [882 (191) versus 498 (115) nmol / L; p = 0.05). On the other hand, mean (SE) GSH levels showed no significant differences between supplement users and non-users, respectively [17.5 (2.5) versus 19.6 (4.1) nmol / x 10^7 cells; p = 0.33). Restriction of the supplement users group to only consumers of vitamin C or E demonstrated significantly higher MDA but not different GSH concentrations in users compared to non-users [mean (SE) = MDA 913.1 (209) versus 493.0 (92) nmol / L; p = 0.05; GSH 17.5 (2.5) versus 19.6 (3.5) nmol / x 10^7 cells; p = 0.33].

To explore further the finding of significantly higher MDA concentrations in supplement users, supplementation usage was separated into those persons consuming either vitamin C or E (Table 9). The effect of supplementation with ascorbic acid indicated a non-significant inverse association with MDA concentrations while users of vitamin E had significantly higher plasma MDA concentrations. A similar pattern was evident when mean daily intakes of ascorbic acid from food and if applicable, supplements, were dichotomized into levels greater than or equal to and less than the tissue saturation level for ascorbic acid (200 mg / d) (Friedman et al., 1940) and above or below the median vitamin E intake (30 IU). As the upper tissue saturation point for ascorbic acid has been reported to have a range between 100 - 200 mg / d (Friedman et al., 1940) data for mean MDA, GSH and CD4+ concentrations were stratified on the basis of less than 100, 100 - 200 and greater than 200 mg / d mean ascorbic acid intake. ANOVA analyses revealed no significant differences between groups for any of the parameters. It should be noted that the stratum with mean intake less than 100 mg / d ascorbate acid had only one or two subjects, therefore the first dichotomy is more appropriate for this particular analyses. Consistent trends were not apparent for either leukocyte GSH or absolute CD4+ counts (Table 9).

4.3 Oxidative stress and immunological determinations

4.3.1 Comparisons

Table 10 shows the sample means for oxidative stress parameters (plasma MDA, leukocyte GSH), CD4+ counts and total ascorbate intake. The means of these four indicies were compared on the basis of the dichotomy of CD4+ counts (greater than or less than CD4+ cells x 10^6 / L) and smoking status (smoker or non-smoker). Differences between groups did not achieve statistical significance, however a tendency towards higher MDA concentrations was observed in the group with CD4+ counts higher than 200 x 10^6 / L as is illustrated in Figure 7. As this finding was unexpected and could be due to chance alone since statistical significance was not detected, additional analyses were performed. The Spearman's rank correlation detected a positive association between plasma MDA and CD4+ counts approaching significance ($r = 0.39$, $p < 0.10$) while the ANOVA did not reveal a similar trend (groups = > 200, 100-200, < 100 cells x 10^6 / L, $p = 0.33$).

Finally, as elevated oxidative stress concentrations have been associated with smoking (Halliwell, 1989), differences in MDA, GSH, CD4+ and daily ascorbic acid intakes were compared in smokers and non-smokers (Table 10). In concurrence with the findings of others, mean (SE) plasma MDA was significantly higher in smokers compared to non-smokers [884.4 (193.8) versus 481.4 (93.3), respectively].

4.3.2 Correlations

The presence and strength of significant associations between dietary intake and oxidative stress and immunological determinations were assessed by linear correlational analyses (Table 11). To assess the possibility that food or supplements alone could have a stronger association with oxidative stress or immunological measurements than the total combined intake, all mean intakes from food only, supplement only or the total intake (food and supplement combined) were correlated with each of MDA, GSH and CD4+ counts (Table 11). As significant correlations were not evident, total nutrient intake was utilized in analyses where applicable.

Table 11 suggests oxidative stress (MDA, GSH) and the immunological parameter, CD4+, were not associated. The relationship between another immune cell, polymorphonuclear (PMN) leukocytes, was also explored. These cells utilize free radicals during phagocytosis (Halliwell, 1989) and could be in part responsible for the tendency to higher MDA concentrations in subjects who were less immunocompromised as indicated by higher CD4+ counts. In this sample, the mean (SE) PMN level was 1.91 (0.13) x 10^9 /

L and within the normal range. Correlational analyses revealed PMN cells were not associated with plasma MDA, leukocyte GSH, CD4+ counts or the antioxidant nutrients.

In Table 13, mean total nutrient intakes were stratified on the basis of use of each of vitamin C, zinc and β–carotene supplements. A second stratification used mean energy and protein intakes based of the usage of any antioxidant supplement (vitamin C or E or zinc or β–carotene or selenium). Lastly, mean total intakes of each of vitamin C and E were stratified on the basis of consumption of vitamin C or E at levels greater than or equal to 10 times the RNI. All strata were correlated with each of MDA, GSH and CD4+. Generally, these analyses revealed weak correlations which were not statistically significant, with the exception of MDA and vitamin E intake less than 10 x RNI ($r = 0.92$; $p < 0.05$). However, it is likely this could be significant by chance alone considering the large number of correlational analyses performed.

Since the Student's *t*-test showed significantly higher MDA concentrations in smokers, MDA, GSH, CD4+ and total intake of ascorbic acid were correlated with MDA, GSH and CD4+ counts in both smokers and non-smokers (Table 14). Only MDA and GSH concentrations in smokers were positively associated. As oxidative stress has been shown to be associated with smoking, the lack of significance may, once more, reflect the problem of the small sample size in achieving statistical significance.

5. Discussion

This is the first reported study to explore the relationship between dietary intakes of five antioxidant micronutrients and oxidative stress levels in HIV-seropositive men and women. Furthermore, as a descriptive study, it augments the limited knowledge currently available regarding the dietary intake and nutritional supplementation practices of Canadian persons living with HIV.

The multifactorial mechanisms which initiate and promote HIV disease progression are complex and remain to be fully understood. In this regard, a particularly intriguing observation from the present study warrants further investigation. Plasma MDA concentrations were found to have a tendency towards higher concentrations in those individuals with higher absolute CD4+ counts (Figure 7). Evidence of oxidative stress early in HIV infection has important implications since oxidative stress has been linked to viral replication and apoptosis of CD4+ cells which may contribute to the profound immunodeficiency characteristic of HIV infection (Fauci et al., 1991; Pantaleo et al., 1993). Moreover, this observation suggests that oxidative stress likely precedes the secondary opportunistic infections, neoplasms and severe malnutrition characteristic of AIDS. Like the current study, other investigators assessing additional oxidative stress markers, including plasma MDA (Sonnerborg et al. 1988), plasma TBARS and plasma lipid hydroperoxides (Sappey et al. 1994) observed a tendency to higher concentrations in stage II versus IV subjects (Table 2). On the other hand, Revillard et al. (1992) found plasma MDA to be significantly higher in the HIV-seropositive stage IV versus II subjects, however, methodological differences may be in part responsible for this discrepancy. Revillard et al. (1992) employed the fluorimetric assay to estimate plasma MDA versus the HPLC methods used by Sonnerborg et al. (1988), Sappey et al. (1994) and the present study. According to Bird and Draper (1984), the fluorometric method is not widely used because other components that fluoresce may be present and fluorescent derivatives having the same excitation and emission maxima are formed by compounds other than MDA, thereby reducing the specificity of this method compared to HPLC.

In addition, studies have consistently demonstrated oxidative stress concentrations of HIV-seropositive subjects, irrespective of disease stage, exceed those of seronegative healthy controls (Coutellier et al., 1992; Favier et al., 1994; Malvy et al., 1994; Sappey et

al., 1994; Revillard et al., 1992; Roederer et al., 1993; Sonnerborg et al., 1988) (Table 2). The sample mean (SE) plasma MDA concentration of the current study was at a comparable level (Table 10) to that observed by Sonnerborg et al. (1988) (613 (28) nmol / L).

Although classification according to disease stage indicates early elevation of oxidative stress, markers of disease progression and immune activation have not shown a strong correlation. Neither MDA nor GSH were found to be significantly correlated with CD4+ levels in the current study (Table 11), on the other hand a trend to greater MDA concentrations with higher CD4+ was demonstrated in Figure 7. Similarly, correlation between leukocyte GSH or plasma MDA and absolute CD4+ counts (Malvy et al., 1994; Roederer et al., 1993; Sonnerborg et al., 1988), serum B2-microglobulin, IgG and IgA concentrations (Malvy et al., 1994) has not been demonstrated. In this regard, the absence of evidence for a strong correlation may provide a clue indicating these immune markers are not the appropriate correlates and there are other, as yet, unidentified factors which are more closely be related to oxidative stress concentrations at the different disease stages. On the other hand, it may indicate the association between oxidative stress and immunosuppression is weak, and would remain weak even with a larger sample, thereby diminishing the predictive ability of these variables.

One possible explanation for elevated oxidative stress is related the role of transition metal ions in the initiation and promotion of lipid peroxidation via the Fenton reaction (Halliwell, 1989). Hydrogen peroxide will react with copper (Cu^+) or iron (Fe^{2+}) to produce hydroxyl radical ($OH^\bullet$), the most highly reactive oxygen free radical. This may have particular relevance in HIV infection as Beck et al.(1990) observed 47% [range: mean: SD = 0.68 - 19.1; 5.51; 5.8 µg / mL) and 22% (range: mean: SD = 0.72 - 38.5; 6.51; 7.4 µg / mL) of 59 HIV-seropositive subjects in all stages of disease progression with elevated serum copper and iron concentrations, respectively. In a prospective cohort, Graham et al. (1991) reported significantly higher serum copper in HIV-seropositive progressors compared to non-progressors. However, the homeostasis of transition metals is complex, particularly in disease states. Moreover, there is not any evidence linking dietary intake of these micronutrients and serum levels in HIV infection as a possible etiology of the elevated levels.

High oxidative stress levels could also be due in part to the normal immunological process of phagocytosis. Activated phagocytic cells produce $O_2^\bullet$ in order to destroy invading pathogens (Curnutte and Babior, 1987) and neutrophils or polymorphonuclear

leukocytes (PMN) also utilize the enzyme myeloperoxidase to produce H_2O_2 from the dismutation of $O_2^{\cdot}$. Hydrogen peroxide is then used to oxidize chloride ions into hypochlorous acid (HOCl), a powerful antibacterial agent (Weiss, 1989). Since a trademark of early HIV infection is a hyperactivated immune system (Abbas et al., 1994), this additional source of endogenous free radicals could contribute to the early elevated oxidative stress concentrations which have been observed. Accordingly, a positive correlation between PMN and MDA would be expected but this was not evident in the current study (Table 12). However, Bandres et al. (1993) observed increased phagocytosis and ROS generation by PMN leukocytes of asymptomatic HIV-seropositive men (stage I, without previous history of bacterial or fungal infections) compared to seronegative controls. They suggested the enhanced production of ROS may reflect a systemic release of agents which induce functional maturation or activation of circulating leukocytes or it may be an attempt for the HIV virus to create the most favorable environment for the viral replication that actively continues in lymphoid tissue during the early stage of infection. In an *in vitro* study by Allen et al. (1972), the continued viability and consequently, the excessive production of superoxide anion was negatively affected in hyperstimulated PMN. PMN functional activity was examined in HIV-infected subjects with more advanced stages of disease progression (LAS, ARC, AIDS - Centers for Disease Control, 1982) (Gabrilovich et al., 1994). A reduction in activity was observed in all groups compared to a seronegative control group, with a tendency towards increased suppression in AIDS subjects. This may suggest a mechanism which could explain in part the diminished oxidative stress concentrations in later disease, however, it remains speculative and further investigation is required.

A clear relationship between the immunological status, as estimated by absolute CD4+ counts, and antioxidant micronutrient intake was not evident in the current study (Table 11). Impaired leukocyte functioning in HIV infection has been observed (Fauci et al., 1991; Pantaleo et al., 1993) and these functional impairments may exist long before enumerative impairments are detected (Vedhara et al., 1995). For example, Bendich (1993) suggested the protective functioning of immunocompetent cells may be impaired by cellular membrane lipid peroxidation. Lipid peroxidation alters membrane fluidity which has been directly related to a decreased ability of lymphocytes to respond to challenges to the immune system defenses. Consequently, dietary intake may have a greater effect on immune function rather than the absolute cell counts and assessment of functionality could provide a better representation of the effects of differing antioxidant intakes.

Limited number of studies have assessed immune function or cell counts. A cross-over trial using β–carotene (180 mg / d x 28 days) did not demonstrate a significant effect on the absolute CD4+ count, however, the total white blood cell count, % change in CD4+ count and % change in CD4+ / CD8+ ratios were significantly improved (Coodley et al., 1993). Garewal et al. (1992) provided β–carotene (60 mg / d) to 11 subjects for 4 months and observed little change in total T cell numbers or CD4+ / CD8+ ratios, however, the number of natural killer (NK) cells was significantly increased. In clinical trials of healthy HIV-seronegative subjects, improvements in cell-mediated immunity in subjects supplemented with vitamin C, A and / or E have been reported. Both leukocyte functional tests (Kennes et al., 1983) and absolute CD4+ counts were enhanced, most significantly with the combination of vitamin C, E and A (Penn et al., 1991).

In vitro and *in vivo* studies have clearly demonstrated free radical-mediated peroxidation is inhibited by supplemental antioxidant intake. Supplementation with vitamin E (200 mg / d) and / or C (400 mg / d) for one year was shown to suppress lipid peroxide concentrations in healthy elderly individuals (Wartanowicz et al., 1984). These levels correlated with the degree of tissue saturation and the effect was strongest with the combination of vitamin E and C, followed by vitamin E, then C alone. Likewise, Peuchant et al. (1995) demonstrated a significant reduction in red blood cell and plasma MDA in HIV-seropositive subjects (CDC IV) with selenium supplementation (100 mg / d) and a trend to MDA reduction with β–carotene (250 mg / d). However, caution must be excised when interpreting these findings as the actual levels of MDA were not published and plasma MDA may be independently associated advanced disease. In addition, the selenium dosage significantly exceeds the RNI of approximately 70 µg / d and adverse effects from selenium toxicity are suspected, although this was not reported.

Antioxidant supplementation has also proven efficacious in decreasing indices of lipid peroxidation associated with smoking. Smoking increases oxidative stress levels directly from the ROS present in cigarette smoke and indirectly by local pulmonary activation of leukocytes which produce ROS during the inflammatory response. Even chronic cigarette smoke exposure, likely including second-hand smoke, has the effect of lowering lymphocyte functions, proliferation and antibody production. Increased risk of infections, decreased NK activity and increased precancerous lesions have been observed in chronic smokers (Halliwell, 1987). A study in healthy smokers (Brown et al., 1994) demonstrated a significant reduction in plasma TBARS, LPO and conjugated dienes levels

in smokers after vitamin E supplementation (280 mg / d *dl*-α–tocopherol acetate x 10 weeks). In a second study, plasma TBARS were again significantly reduced in healthy smokers after supplementation with vitamin C (500 mg / d x 2 or 4 weeks) or E (200 mg / d *dl*-α–tocopherol acetate x 4 weeks) (Harats et al., 1990).

Like Brown et al. (1994) and Harat et al. (1990), the results of the present study demonstrate smokers had significantly higher levels of plasma MDA than non-smokers (Table 10). On the other hand, leukocyte GSH was not significantly different between smokers and nonsmokers. In the current study, blood was sampled from the antecubital vein and perhaps measurements of pulmonary GSH concentrations, the primary exposure site of cigarette smoke, would have shown a significant decrease. Buhl (1994) has reported a significantly reduced GSH in the lung epithelial lining fluid and plasma of HIV-seropositive (CDC II) subjects versus seronegative controls. The data did reveal a significant positive correlation between GSH and MDA ($r = 0.78$, $p < 0.05$) in smokers (Table 14.) This association could be statistically significant due to the large number of correlations performed as the scatter plot of the data does not enable conclusions to be made at this time (Figure 8).

Taken together, these studies provide strong evidence for the efficacy of antioxidants in reducing oxidative stress concentrations. Paradoxically, in the current study users of vitamin E supplements had significantly higher MDA levels which increased when the dietary intake of vitamin E exceeded 30 IU / d (Table 9). Ascorbic acid supplementation users did not have significantly different MDA concentrations compared to non-users and there was little difference when ascorbic acid intake exceeded the tissue saturation level (200 mg / d). As the functions of micronutrients may change as the dosage increases, particularly beyond 10 x RNI, dosages in excess of these levels may acquire pharmacological properties. However, in the current study the interpretation of the results from correlational analyses may be restricted due to the limited data available as a consequence of sample size (Table 13).

One possible explanation for this paradoxical effect observed is related to the hypothesized state of the oxidant - antioxidant balance in HIV infection. In this case, an imbalance may be shifted towards the oxidant state in excess compensatory capacities of the antioxidant defenses. A relative excess concentration of oxidants, whether from increased production or decreased inactivation by antioxidants will further propagate free radical-generating reactions (Figure 2). Evidence for the later was not supported by the nutrient analyses in this study as the dietary intake of the five antioxidants exceeded the

RNIs. However, studies assessing the biochemical status of antioxidants have shown evidence of deficiencies, even early in HIV infection (Table 4). Although complete agreement between studies is not consistent, a general trend towards decreasing levels of antioxidants as HIV progresses is evident. High oxidative stress concentrations early in the infection could account for the initial decrease in antioxidant concentration and as HIV progresses, the influence of nutritional factors such as anorexia, malabsorption and drug-nutrient interactions which become more prevalent as the disease progresses may contribute to the antioxidant decline (Winick, 1989).

Nutritional analyses and assessment of demographical variables did not suggest the current sample was nutritionally compromised at the time of study participation. Comparison to Canadian studies revealed samples with similar age, height and BMI (Hogg et al., 1995; Sharkey et al., 1992). In the current study, neither age nor BMI were significantly correlated with oxidative stress concentrations (MDA, GSH), CD4+ counts or dietary intakes of ascorbate, energy or protein (Table 6). Other studies have demonstrated an independent relationship between BMI or weight loss and CD4+ levels in the prediction of disease progression (Chlebowski et al., 1995; Suttman et al., 1995). Contrarily, Sharkey et al. (1992) found CD4+ levels to be correlated with energy and weight loss, particularly at CD4+ levels below 100 cells x 10^6 / L. This finding was confirmed in a later study by Risser et al. (1995). However, BMI or weight loss measurements are not sensitive to quantitative changes in body composition of HIV-seropositive individuals. Although body composition was not directly measured in the present study, subjects with weight loss greater than 10 % of usual body weight in the previous three months were excluded from participation.

In contrast to BMI and age, the mean current weight was approximately 6.4 kg (9%) lower than those reported by Sharkey et al. (1992) [mean (SE) = 73.2 kg (0.4) in HIV-seropositive subjects CDC II-IV free from enteric pathogens] and Hogg et al. (1995) [median (SE) = 77 kg (0.3) in HIV-seropositive, AIDS-free subjects, according to the current definition of AIDS in Canada (Centers for Disease Control, 1987)], however, direct comparison with these studies is impossible as their samples were exclusively male.

Weight may be considered a crude proxy for the general nutritional status of individuals and low weights may identify a greater risk of nutritional deficiency. In HIV infection, unintentional weight loss has been associated with malabsorption, metabolic changes and anorexia (Winick, 1989). Even though a mean weight loss of 0.9 kg since the time of HIV seropositivity was observed in this sample, an increased risk of nutritional deficiency due to weight loss is unlikely for three reasons. First, BMI correlates with

many health-related indices such as mortality risk (Waaler, 1984). This parameter takes into account height and weight and provides a standardized value for men and women. The major drawback to using BMI in a HIV-seropositive group is this measurement is based on data from a healthy population and does not account for differences in body composition. Nonetheless, in the present sample, which included 21% women, the average BMI was within the ideal range associated with the lowest risk of illness for most people (Health and Welfare Canada, 1988b) and was comparable to the BMI levels of HIV-seropositive subjects as reported by Hogg et al. (1995).

Secondly, individual weight histories revealed a non-significant weight loss since the time of HIV-seropositivity (Table 5). When determining individual weight histories, the reference parameter chosen, for example usual (pre-illness) weight versus % ideal body weight, is important for assessing weight changes over a long period. This is especially relevant for a population which may differ in a number of ways compared to the standard reference population. Estimating weight changes according to pre-illness weight, as was the case in the current study, allows comparison of weight changes relative to the individual. Ideal body weight is defined as the percentage of current weight compared to life insurance industry statistics, however, these tables provide values related to height and frame size and are based on longevity rates from healthy individuals and are therefore inadequate for Canadian HIV-seropositive persons. Firstly, they are based on the predictive longevity of healthy persons measured in their early twenties and followed to their death. Secondly, the tables predominantly reflect data from American upper middle class Caucasian groups (Pi-Sunyer, 1988) which may limit the comparability in ethnically diverse HIV-seropositive samples. This is supported by evidence from a large American cohort indicating HIV-seronegative homosexual males may have below average weights (Hoover et al., 1992). Average weights in 1984 and 1990 of HIV-seronegative and HIV-seropositive homosexual men were compared. They observed a significant increase in weights (3.7 kg) in the seronegative group between this time period. A similar trend was observed in the HIV-seropositive men between 1984 to late 1988, but there was a significantly smaller increase in this group after 1989, most likely around the time of seroconversion.

Thirdly, insufficient energy intakes, a common cause for weight loss, is unlikely in this sample. Common symptoms of inadequate energy intake include fatigue and general apathy. Although activity levels were not directly measured in this study, a very high mean Karnofsky score was observed. This implies an excellent quality of life pertaining to the physical dimensions of daily living. The strongest evidence that insufficient energy intakes

were not contributing to the lower observed weights is the mean (SE) energy intake of 3262 kcal (206). This represents an average intake of 48 kcal / kg of usual weight, which is well above the 30 kcal / kg suggested as adequate to maintain usual weight in HIV-seropositive persons [predominantly male (94%); asymptomatic or with minimal symptoms] (Chlebowski et al., 1995). Their estimate was based on a seven day food record of energy intake, however, this method may have underestimated their "true" energy intake and consequently, underestimated the required level to maintain weight.

The reported intakes of both energy and protein were clearly higher compared to the other Canadian studies. The most likely explanation originates from differences between the dietary intake methodologies that were employed in each study. In the first study (Hogg et al., 1995), only one self-implemented 24 hour recall was used to estimate dietary intake of 139 HIV-seropositive males. The mean (SE) energy intake reported was 2 239 kcal (73) and protein was 98 g (4). As the success of the 24 hour recall is highly dependent on the subject's motivation, memory and ability to provide accurate portion size estimates (Acheson et al., 1990), it is most commonly implemented by a trained interviewer. In addition, a single 24 hour recall can be affected by intraindividual variation in nutrient intakes. Furthermore, this method assesses recent intake and is not appropriate for estimating usual nutrient intakes of individuals (Hankin, 1992). Sharkey et al. (1992) utilized seven day weighed food records to estimate the dietary intake of 28 HIV-seropositive male volunteers [mean (SEM) for energy (kcal) = 2725 (143); protein (g) = 97 (7)]. This method requires motivated and literate volunteers who may not be representative of the general study population. It is particularly affected by atypical reporting periods, for example during times of illness. Apparently, this was the case as 25 % of their sample did not complete food records.

In the current study, the diet history, in conjunction with food models, was implemented by trained research dietitians to estimate dietary intake (Appendix 8). This tool is useful for estimating the usual intake of individuals over a long period of time, although precision is somewhat reduced as a consequence. The diet history and food models developed by Santé Québec (Santé Québec, 1994) were specifically designed to assess the eating patterns of persons in Québec. In addition, the supplement questionnaire (Appendix 7) was designed for this study. However, the diet history, like the previously described methodologies, is affected by respondent and recorder errors, interviewer and reviewer errors and nutrient database errors (Witschi, 1990). Nonetheless, Hankin (1992) suggested diet histories are likely more representative of the "true intake" even though they

provide estimates greater than those of 24 hour recalls or food records. This may explain in part the comparatively higher dietary intakes observed in this sample.

The dietary intake methodology differences most likely accounted for the apparent discrepancies in macronutrient intakes. However, in light of the large difference in reported caloric intakes, consideration of the possible influence of hypermetabolism in this sample is warranted. Preliminary data on HIV and resting energy expenditure (REE) has been contradictory and there have not been studies concurrently assessing oxidative stress concentrations. Hommes et al. (1991) reported an early increase in the REE by 8% in CDC II subjects versus a seronegative control group ($p < 0.05$). When subjects with all stages of disease were assessed concurrently, the intermediate stages of infection exhibited the highest REE (Slusarczyk, 1994; Suttman et al., 1991) but this was contradicted by Grunfeld et al. (1992) (compared to seronegative controls: HIV+ 11%, AIDS 25%, AIDS with secondary infection 29%). On the other hand, Kotler et al. (1990) observed subjects with clinically stable AIDS were hypometabolic compared to both seronegative controls and with the predicted metabolic rate based on the Harris-Benedict equation. It has been suggested that during the initial stages of the syndrome viral replication may cause an increase in REE. Lean muscle mass is an important influencing factor on the REE and as the disease progresses lean muscle mass is lost with a decline in REE (Slusarczyk, 1994). Interestingly, the early increase in REE, and comparatively lower level in clinically stable AIDS subjects, parallels the pattern of plasma MDA as suggested by the results of the current study. As energy metabolism increases, so does the endogenous production of ROS via the uncoupling of the electron transport chain during the oxidative phosphorylation reaction (Stryer, 1988). Unfortunately, in the current study, the degree to which elevated energy metabolism may have influenced either oxidative stress levels on caloric intake is impossible to assess.

In accordance with the macronutrient intake comparisons, large differences exist between the observations of the current study and the limited published data available describing antioxidant intakes of HIV-seropositive Canadians. Compared to the ascorbate intake reported by Sharkey et al. (1992) [mean (SE) = 102 (2.3) mg], the present study estimated much higher total ascorbic intake (Table 7). On the other hand, American studies from Mantero-Atienza et al. (1991) and Abrams et al. (1994), observed mean (SE) ascorbic acid intakes closer to this study [ascorbate (mg) = 873 (71); 1219 (86) (supplement users only), respectively]. Again mean (SE) dietary intakes of vitamin E and zinc were similar in this study (Table 7) as reported by Mantero-Atienza et al. (1991) [vitamin E = 224 (37) mg; zinc = 36.4 (3.2) mg]; however, lower selenium levels [23.5 (9.5) µg] were reported in

the American study. Since selenium intake in both the current and the Mantero-Atienza et al. (1991) study were based solely on supplement intake, recent changes in multivitamin formulations to include higher concentrations of antioxidant vitamins and minerals may account for this difference. Interestingly, very similar dietary intake methodology (diet history by trained nutritionist and a request to bring supplements to the interview with follow-up telephone calls for missing supplement data) were used in the current study and the study by Mantero-Atienza et al. (1991).

There are several possible explanations which may account for the differences between the dietary intake of ascorbic acid in the Canadian studies. These include seasonal variations (Vanderslice, 1991), individual variations (Gallagher et al., 1992) and nutrient database differences (Barr et al., 1994), however, considering the large discrepancy, it is possible that the lower intake is due to misclassification by an error of omission. Block et al. (1994) suggested misclassification by omission has the most serious implications for vitamin C or E. This conclusion was based on information collected from the NHANES I (1971-1975) (Block et al., 1988), NHIS (1987) (Subar, Block, 1990) and NHANES II (1976-1980) (Murphy et al., 1990). At that time, supplements did not normally contain added antioxidants and it is likely that similar caution should now apply for the other antioxidant micronutrients. The NCI-USDA (National Cancer Institute- United States Department of Agriculture) study suggested plasma concentrations of vitamin E may not increase notably until single-nutrient supplements are consumed (often 10 - 20 times higher than multivitamin supplements) (Sinha et al., 1993). Obviously, misclassification by omission of antioxidant micronutrient supplementation increases the difficulty in achieving significant and consistent results, especially those studies which explore the relationship between dietary intake and disease outcomes.

Analyses of the results reveal the majority of subjects in this sample were using some form of nutritional supplement (Table 8) and many subjects were supplementing with ascorbic acid, vitamin E, β-carotene, selenium and / or zinc (Table 7). Dichotomization on the basis of specific supplement usage showed significantly higher mean intakes of vitamin C and zinc in users compared to non-users (Table 7). Similarly, Abrams et al. (1994) observed significant differences in ascorbic acid, zinc and vitamin E intake between supplement users and non-users. However, caution should be used when generalizing supplementation prevalence from the current study since subjects were recruited voluntarily and there is a strong possibility of volunteer bias resulting in over- or under-estimation of the true prevalence. Subjects who were interested in nutrition may have been more likely to participate, on the other hand, those who were supplementing with unorthodox nutrient

doses and nutritional products may have been reluctant to participate due to fear of judgment or the desire to maintain anonymity.

As comprehensive data on the antioxidant supplementation practices of HIV-seropositive persons is quite limited at this time, extrapolation, with caution, may provide an indicator of expected prevalence. In a healthy population, 23 - 35% reported daily supplementation with multivitamins and this percentage varies according to the demographic group (Block et al., 1988; Kim et al., 1993; Koplan et al., 1986; Subar et al., 1990). For example, white Americans were more likely than black Americans to supplement with single nutrient sources. In 1992, nine percent of the population took more than one supplement and three percent took more than two (Slesinski et al., 1995). Consumer reports from vitamin retailers indicate a 19% increase in retail sales of all supplements between 1987 and 1992 (Council for Responsible Nutrition, 1993) which is consistent with data from the NHIS reports, suggesting an overall 17% increase in the number of persons consuming daily supplements (Subar and Block, 1990). HIV-specific data suggest 50.8 and 61.9 % of AIDS-progressors and non-progressors, respectively, consumed a daily multivitamin in a prospective cohort (Abrams et al., 1993). These data were not quantified with regards to the usage of single nutrients which tend to have higher dosages. However, it is likely that a number of subjects were taking considerable dosages as inferred by the large mean differences and standard deviations of nutrient intakes when separated into intakes from food and supplements, particularly for vitamin C and E. High - dose supplementation prevalence has been reported as high as 57% (Beach et al., 1988). Together these studies suggest nutritional supplementation in both the HIV-seropositive population and the normal healthy population is not only likely, but must be quantified in order to accurately assess and characterize dietary intakes of antioxidant micronutrients.

The results from this study indicate of those persons who were consuming vitamin E or C supplements, 36 and 45%, respectively, were consuming doses in excess of 10 times the RNI (Table 7). Observations from two Master's theses by Drolet (1992) and Parisien (1992), showed lower contributions from high-dose supplementation (vitamin C > 10 x RNI = 8, 21%; vitamin E > 10 x RNI = 7, 19%, respectively) in Montreal-area HIV-seropositive subjects, stages II-IV. However, interpretation of the clinical significance of intakes compared to the RNIs must be cautioned in the HIV-seropositive population. The RNI for Canadians are standards established for a healthy population. In the case of vitamin C, it is noteworthy that the American recommended daily allowance (RDA) for ascorbic acid is based solely on its function in collagen synthesis (Levine, 1986) and not on its antioxidant activity (Frei et al., 1989). Upon assessing the efficacy of the role of

ascorbate as an antioxidant, Frei et al. (1989) suggested the RDA should be increased from the current 60 mg / d to approximately 150 mg / d to maximize the total body pool and provide antioxidative benefits to human health. In any case, even this estimate does not consider the added oxidative stress burden of chronic infection with HIV and HIV-related illnesses (Baruchel and Wainberg, 1992; Favier et al., 1994; Greenspan, 1993; Sonnerborg et al., 1988). Consequently, the value required to maintain nutritional adequacy and an oxidant-antioxidant balance may be much higher for vitamin C and the other antioxidants and this may explain the apparent inefficacy of the antioxidants to reduce oxidative stress levels in the current study.

In addition to the prevalence of micronutrient supplementation, clinical variables associated with supplement usage were considered. Some studies have shown a relationship between the use of unproven therapy, including high-dose micronutrient supplementation, and diminishing CD4+ cell counts (Senterfitt et al., 1990), increasing severity of illness (Cohen et al., 1990) or longer duration of HIV seropositivity (Anderson et al., 1993). In the current study, supplement users did show differences in CD4+ counts or duration of HIV seropositivity but tended to be more likely to have experienced a clinical event associated with HIV infection. From these proxy indicies of immunodeficiency, conclusions about supplement use and disease progression are difficult to make. Firstly, duration of seropositivity is only an estimate of the length of time since seroconversion. Some cases present shortly after seroconversion while others present at the time of clinical symptoms indicative of AIDS. Secondly, the temporal sequence between initiation of supplementation and the development of clinical events can not be determined with the cross-sectional study design. Perhaps supplement users began supplementing after the appearance of symptoms which warranted consultation with a health professional recommending this practice. On the other hand, in a study by Mantero-Atienza et al. (1991), 80% of reported making positive changes to their diets soon after diagnosis, including 59% who reported increasing their micronutrient intake. Moreover, Sutherland and Verhoef (1995) found the severity of symptoms was not significantly associated with the use of alternative medicine, primarily being herbalism and nutritional therapies.

MDA and GSH, both measurements typically unknown to subjects, did not appear consistently associated with supplement usage. MDA was significantly higher in users while GSH showed little difference. A possibility exists that those with higher MDA concentrations were experiencing a greater feeling of malaise which subsequently prompted initiation of supplementation. However, this study suggests MDA may be higher in individuals at an earlier disease stage, thus more likely asymptomatic. As there are not

reports in the literature linking these variables, the explanation is speculative and needs to be confirmed by additional investigation.

6. Primary Findings of This Study

- In accordance with previous studies, malondialdehyde (MDA) concentrations were elevated and this was evident early in the disease. Possible explanations from the literature which support this finding include the production of reactive oxygen species (ROS) during the immune response of polymorphonuclear leukocytes, the catalysis of the Fenton reaction by transition metals, cytokine dysregulation with production of ROS as second messengers, ROS produced during increased oxidative phosphorylation as a consequence of an elevated energy metabolic rate and a relative insufficiency of antioxidants to maintain an oxidant - antioxidant equilibrium.

- Dietary antioxidant intake at the levels consumed by sample did not appear efficacious in reducing oxidative stress concentrations. Smoking, a known exogenous source of ROS, was associated with higher malondialdehyde concentrations.

- Nutritional, demographical and clinical variables indicate this sample was otherwise healthy at the time of study participation. The mean dietary intakes met and exceeded the Recommended Nutrient Intakes for Canadians from diet alone and with supplementation.

- Energy intake was comparatively high for a population of this weight. Dietary intake methodology or an elevated energy metabolic rate may account for these differences.

- Nutritional supplementation was practiced by the majority of subjects. The prevalence of high-dose supplementation was comparatively lower than previously published American studies, but higher than rates reported in Montréal. Although many factors may influence this practice, supplement users were more likely to have higher MDA concentrations and a tendency to have experienced a clinical event associated with HIV infection while duration of HIV seropositivity, CD4+ counts nor GSH concentrations appeared to have an influence on supplementation usage.

7. Conclusions

This study was designed to explore the relationship between dietary antioxidant micronutrients and oxidative stress in HIV infection. The results from this study indicate oxidative stress is elevated early in HIV infection, however, antioxidant levels do not appear strongly correlated in this sample. Immunological parameters were not related to oxidative stress concentrations nor dietary intakes, with the a trend to greater MDA levels with higher absolute CD4+ counts. Many of the associations may not have achieved statistical significance due to a small sample size. Furthermore, a more comprehensive number of oxidative stress, immunological and biochemical markers of nutritional status may have contributed to further understanding of the relationships investigated.

Analyses of the dietary intake revealed a high mean intake of energy and protein in an otherwise healthy sample, which may be indicative of increased energy metabolism. For the most part, micronutrient intakes exceeded the Recommended Nutrient Intakes (RNI) and the majority of subjects were consuming some form of nutritional supplement. Nonetheless, the prevalence of high dose supplementation was comparably lower than American estimates. This may be due to demographical, sociocultural or geographical differences influencing supplementation practices.

The findings of this study provide an interesting foundation for further research. Investigation into the etiology of the elevated oxidative stress concentrations, whether from a relative insufficiency of antioxidants, an elevated energy metabolism or other mechanism, may provide clues regarding disease progression. Elucidation of the pathophysiological consequences of oxidative stress in HIV infection would allow therapeutic strategies to be appropriately designed. Although the apparent inefficacy of antioxidant micronutrients to decrease oxidative stress concentrations is puzzling, it is likely they are beneficial. Studies examining the correlation between the dietary intake and tissue concentrations of antioxidants will assist in understanding this paradox and further define the timing and dosages required for maximum effectiveness. Finally, HIV infection and AIDS involve cytokine dysregulation, immune dysfunction, oxidative stress and nutritional aberrations, consequently future research must reflect these dimensions to meet the challenge.

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9. Tables

Table 1. Cytotoxicity of oxidative stress

Target	Damage	Consequences
DNA / RNA	Base modifications, base-free sites, single- and double-strand breaks, crosslinks and enhanced expression of oncogenes	Mutations, translational errors and inhibition of protein synthesis
Protein	Aggregation and cross linking; fragmentation and breakdown; modification of thiol groups	Modification of enzyme activity, ion transport and intracellular calcium homeostasis
Polyunsaturated Lipid	Loss of unsaturated bonds; formation of reactive metabolites	Altered lipid fluidity, membrane permeability and membrane - bound enzyme activity

Source: Adapted from Halliwell (1987) and Rice-Evans et al. (1991).

Table 2. Indicators of lipid peroxidation in HIV infection

Study Indicator	Controls	CDC II*	CDC III	CDC IV	CDC II-IV
Sönnerborg et al., 1988 Plasma malondialdehyde (MDA) (μmol / L)	0.46 ± 0.08[a] n = 20	0.72 ± 0.24[b] n = 6	0.58 ± 0.10[b] n = 19	0.60 ± 0.12[c] n = 5	
Coutellier et al., 1992 Plasma MDA (μmol / L)	0.90 ± 0.30 n = 30				1.48 ± 0.34[d] n = 29
Revillard et al., 1992 Plasma thiobarbituric reactive substances (TBARS) (μmol / L)	2.51 ± 0.15 n = 32	2.93 ± 0.20[e] n = 26		3.32 ± 0.24 n = 35	
Sappey et al., 1994 Plasma TBARS (μmol / L)	2.51 ± 0.31	2.73 ± 0.35[f]		2.68 ± 0.40	
Plasma hydroperoxides (HPO) (μmol / L)	127 ± 14 n = 16	176 ± 58[f] n = 25		155 ± 67 n = 18	
Malvy et al., 1994 Plasma TBARS (μmol / L)	2.31 ± 0.85[g] n = 10	6.31 ± 1.27[g] n = 10			

[a] Mean ± standard deviation.

[b] Difference between controls vs. CDC II or CDC III: $p < 0.001$; Student's *t*-test.

[c] Difference between controls vs. CDC IV: $p < 0.01$: Student's *t*-test.

[d] Difference between controls vs. CDC II: $p < 0.001$; Student's *t*-test.

[e] Difference between controls or CDC IV vs. CDC II: $p < 0.001$; one-way ANOVA.

[f] Difference between controls vs. CDC II: $p < 0.05$; one-way ANOVA.

[g] Difference between controls (HIV-seronegative hemophilic children) vs. HIV-seropositive hemophilic children: $p < 0.001$; Wilcoxon test.

* Centers for Disease Control (1986; 1987) classification system of HIV infection - Appendix 1.

n Sample size.

Table 3. The antioxidant defense system: The antioxidant micronutrients and glutathione

Defense	Mode of Action	Comments
Ascorbic acid	Facilitates the quenching of reactive oxygen species (ROS) by acting as a carrier or source of electrons.	Superior intra- and extracellular hydrophilic antioxidant.
α-tocopherol	Breaks free radical-chain reactions by trapping peroxyl and other reactive radicals and thus, protecting the polyunsaturated fatty acids (PUFAs) in cell membranes and lipoproteins.	Major, possibly only, lipid-soluble chain-breaking antioxidant in human plasma.
ß-carotene	Effective singlet oxygen and free radical-trapping antioxidant.	Other carotenoids may possess antioxidant capabilities.
Selenium glutathione peroxidase (GPx)	Catalytic function of GPx depends on presence of selenium. Catalyzes reduction of H_2O_2 and lipid hydroperoxides.	Primarily interferes at the level of free radical initiation.
Zinc (Zn^{2+}) [Cu/Zn-superoxide dismutase (SOD)]	Zinc most likely does not function in the catalytic process directly, but rather as an enzyme stabilizer. This enzyme catalyzes a reaction which reduces superoxide radicals.	Cytoplasmic antioxidant.
Glutathione (GSH)	Scavenger of hydroxyl radicals and singlet oxygen. Also provides reducing equivalent for GPx.	Major intracellular antioxidant and main defense against H_2O_2.

Sources: Bendich (1990); Diplock (1994); Halliwell (1987); Wang and Watson (1994).

Table 4. Nutritional status of the antioxidant micronutrients and glutathione (GSH) during the evolution of HIV infection

Ascorbic acid status, according to different authors.

Study	Sample size	Subjects	Plasma / serum ascorbate[a] (mg / dL) N = 0.2-2.0	Percent Deficient
Bogden et al., 1990	6	Asymptomatic[b]	0.52 ± 0.16	27
	17	ARC	0.88 ± 0.18	for all stages
	7	AIDS	0.91 ± 0.17	combined
Javier et al., 1990	70	CDC III	0.93 ± 0.07	7
Beach et al., 1992	42	Control	1.10 ± 0.05	0
	100	CDC III	1.02 ± 0.06	7
Coodley et al., 1993	15	CDC IV (W)[c]	0.87 ± 0.16	23
	11	CDC IV (NW)[d]	1.11 ± 0.28	12
	21	CDC III (NW)	1.09 ± 0.11	6

[a] Mean ± standard error (SE).

[b] Disease staging according to the Centers for Disease Control (CDC) classification system of HIV infection. Appendices 1 - 3.

[c] W = involuntary weight loss greater than 10% of baseline body weight.

[d] NW = absence of wasting.

N Normal reference range according to Monsen, 1987.

Table 4. Continued.

Carotenoid status, according to different authors.

Study	Sample size	Subjects	Plasma / serum ß-carotene[a] (μmol / L) N = 0.9 - 4.6	Plasma / serum carotenoids[a] (μmol / L) N = 0.9 - 4.6	Percent Deficient
Bogden et al., 1990	6	Asymptomatic[b]		2.83 ± 0.24	31 for all stages combined
	17	ARC		1.81 ± 0.06	
	7	AIDS		2.04 ± 0.00	
Coodley et al., 1993	30	HIV +	2.74[c] (0.37 - 11.8)		
Coodley et al., 1993	15	CDC IV (W)[d]		0.66 ± 0.17	85
	11	CDC IV (NW)[e]		1.24 ± 0.24	42
	21	CDC III (NW)		1.43 ± 0.13	20
Sappey et al., 1994	16	Control	0.56 ± 0.07	1.76 ± 0.10	
	25	CDC II	0.24 ± 0.03	0.94 ± 0.09	
	18	CDC IV	0.21 ± 0.05	0.42 ± 0.09	
Ullrich et al., 1994	33	Control		1.37[f] (0.88 - 2.92)	0
	116	HIV +		0.48 (0.06 - 1.69)	77

[a] Mean ± standard error (SE).

[b] Disease staging according to the Centers for Disease Control (CDC) classification system of HIV infection. Appendices 1 -3. HIV+ indicates unspecified disease stages.

[c] Mean (range).

[d] W = involuntary weight loss greater than 10% of baseline body weight.

[e] NW = absence of wasting.

[f] Median (range).

N Normal reference range according to Monsen, 1987.

Table 4. Continued.

Vitamin E status, according to different authors.

Study	Sample size	Subjects	Plasma / serum (mg / L) vitamin E[a] N = 5-20	Percent Deficient
Javier et al., 1990	70	CDC III[b]	8.20 ± 0.04	27
Bogden et al., 1990	6	Asymptomatic	9.30 ± 0.10	12
	17	ARC	1.09 ± 0.15	for all stages
	7	AIDS	0.87 ± 0.13	combined
Beach et al., 1992	42	Control	9.80 ± 0.73	0
	100	CDC III	8.90 ± 0.48	19
Coutellier et al., 1992	30	Controls	13.00 ± 0.47	
	29	HIV+	9.60 ± 0.52	
Coodley et al., 1993	15	CDC IV (W)[c]	14.40 ± 1.30	0
	11	CDC IV (NW)[d]	16.80 ± 1.78	0
	21	CDC III (NW)	13.70 ± 0.92	0
Lack et al., 1993	120	Asymptomatic		7
	for all stages combined	ARC	*	11
		AIDS		50
Baum et al., 1995	108	HIV+	8.64 ± 0.46	

[a] Mean ± standard error (SE).

[b] Disease staging according to the Centers for Disease Control (CDC) classification system of HIV infection. Appendices 1 -3. HIV+ indicates unspecified disease stages.

[c] W = involuntary weight loss greater than 10% of baseline body weight.

[d] NW = absence of wasting.

N Normal reference range according to Monsen, 1987.

* Values not published.

Table 4. Continued.

Glutathione (GSH) status, according to different authors.

Study	Sample size	Subjects	Plasma / serum GSH[a] (μmol / L) N = 57 - 80	Lung ELF GSH[a] (μmol / L)	Percent Deficient
Buhl et al., 1989	19	Control	183.9 ± 29.8	270 ± 14	
	14	CDC II[b]	60.5 ± 12.5	170 ± 23	
de Quay et al., 1992	6	Control	181.3 ± 73.7		
	9	HIV +	116.7 ± 0.9		
Coodley et al., 1993	15	CDC IV (W)[c]	68.7 ± 8.3		14
	11	CDC IV (NW)[d]	64.3 ± 17.5		25
	21	CDC III (NW)	64.2 ± 13.1		30

[a] Mean ± standard deviation (SD).

[b] Disease staging according to the Centers for Disease Control (CDC) classification system of HIV infection. Appendices 1 and 2. HIV+ indicates unspecified disease stages.

[c] W = involuntary weight loss greater than 10% of baseline body weight.

[d] NW = absence of wasting.

N Normal reference range according to Coodley et al., 1993.

ELF Epithelial lining fluid.

Table 4. Continued.

Zinc status, according to different authors.

Study	Sample size	Subjects	Plasma / serum zinc[a] (μmol / L) N = 11.5 - 18.5	Percent Deficient
Falutz et al., 1989	23	Heterosexual HIV-	17.3 ± 2.3	
	25	Homosexual HIV-	18.2 ± 2.0	
	29	Homosexual CDC II[b]	16.8 ± 2.8	
	47	CDC III	16.6 ± 3.3	
	24	CDC IV	13.0 ± 2.4	
Beck et al., 1990	26	Healthy	17.9 ± 3.5	0
	59	HIV+	18.1 ± 4.9	2
Bogden et al., 1990	6	Asymptomatic	11.9 ± 2.4	30
	17	ARC	12.5 ± 2.7	for all
	7	AIDS	11.9 ± 4.2	stages combined
Walter et al., 1990	14	Control	9.6 ± 0.3	
	7	Asymptomatic	12.7 ± 1.2	
	16	ARC	11.5 ± 0.8	
	13	AIDS	13.3 ± 1.7	
Conri et al., 1991		Controls	19.1 ± 1.9	
	29	CDC II	10.2 ± 2.6	
	14	CDC III	10.2 ± 3.2	
	42	CDC IV	9.8 ± 3.2	
Graham et al., 1991	54	Control	13.7 ± 2.1	
	54	Stable HIV+	13.5 ± 1.8	
	54	Progressor HIV+	12.6 ± 1.7	
Beach et al., 1992	42	Homosexual HIV-	12.9 ± 2.5	17
	100	Homosexual HIV+	12.6 ± 1.9	26
Coodley et al., 1993	15	CDC IV (W)[c]	17.4 ± 6.4	0
	11	CDC IV (NW)[d]	14.8 ± 4.6	20
	21	CDC III (NW)	17.6 ± 3.8	0
Sappey et al., 1994	16	Control	12.3 ± 2.5	
	25	CDC II	10.5 ± 2.7	
	18	CDC IV	7.8 ± 1.4	
Baum et al., 1995	108	HIV+	13.7 ± 6.3	

[a] Mean ± standard deviation (SD).

[b] Disease staging according to the Centers for Disease Control (CDC) classification system of HIV infection, Appendices 1 -3. HIV+ indicates unspecified disease stages.

[c] W = involuntary weight loss greater than 10% of baseline body weight.

[d] NW = absence of wasting.

N Normal reference range according to Monsen, 1987.

Table 4. Continued.

Selenium status [plasma, serum and erythrocyte selenium, plasma and erythrocyte glutathione peroxidase (GPx)], according to different authors.

Study	Sample size	Subjects	Plasma / serum selenium[a] (μg / L)	Erythro-cyte selenium[a] (μg / L)	Plasma GPx[a] (U / L)	Erythro-cyte GPx[a] (U / g Hb)	Percent Deficient
Zazzo et al., 1988	10	Control	87 ± 12				
	10	AIDS	59 ± 21				
Beck et al., 1990	26	Healthy	76 ± 15				7
	59	HIV+	62 ± 15				17
Javier et al., 1990	70	CDC III	108 ± 18				18
Cirelli et al., 1991	15	Control	103 ± 6				
	7	CDC II	69 ± 9				
	7	CDC III	68 ± 13				
	30	CDC IV	65 ± 17				
Olmsted et al., 1991	28	Control		195 ± 20			
	26	ARC		126 ± 38			
	24	AIDS		123 ± 30			
Coutellier et al., 1992	30	Controls	88 ± 17		334 ± 41	26.0 ± 4.0	
	29	CDC II	84 ± 16		285 ± 46	29.7 ± 9.2	
Sappey et al., 1994	16	Control	72 ± 12		375 ± 78	42.4 ± 11	
	25	CDC II	70 ± 14		310 ± 71	47.0 ± 11	
	18	CDC IV	44 ± 19		238 ± 81	44.2 ± 23	

[a] Mean ± standard deviation (SD).

[b] Disease staging according to the Centers for Disease Control (CDC) classification system of HIV infection, Appendices 1 - 3. HIV+ indicates unspecified disease stages.

Hb Hemoglobin.

Table 5. Characteristics in 24 HIV-seropositive men and women

Characteristic	Mean (SE)	Range	%
Age (years)	41.0 (1.8)	30-61	
Gender			
male			79
female			21
Weight			
usual weight[a] (kg)	67.6 (1.8)	50.0-83.0	
current weight[a] (kg)	66.7 (1.9)	47.5-80.0	
weight change[a,b,c] (kg)	(-) 0.9 (1.1)	(-) 18.3-(+) 9.7	
Body mass index[d] ($kg.m^2$)	22.7 (0.6)	19.5-30.0	
Karnofsky score	93 (1.8)	70 - 100	
Current cigarette smoker			46
Language			
French			54
English			46

[a] Data available for 22 subjects.

[b] Group weight change = Σ (individual $weight_{usual\ (pre\text{-}illness)}$ - individual $weight_{current}$).

[c] Difference between mean usual weight and mean current weight was not significant based on Student's paired *t*-test; p = 0.21.

[d] Data available for 21 subjects.

SE Standard error.

Table 6. Linear correlational analyses: Association between body mass index (BMI) or age and oxidative stress, immunological and nutritional variables in HIV-seropositive men and women

Parameter	BMI (kg / m^2) r (n)	Age (years) r (n)
Plasma malondialdehyde (MDA) (nmol / L)	-0.23 (18)	-0.19 (20)
Leukocyte glutathione (GSH) (nmol / x 10^7 cells)	-0.16 (15)	-0.31 (17)
CD4+ (cells x 10^6 / L)	0.00 (19)	-0.12 (22)
Ascorbic acid (mg)	-0.13 (19)	-0.14 (22)
Energy (kcal)	0.35[a] (21)	-0.10 (24)
Protein (g)	0.34[a] (21)	-0.08 (24)

[a] P =< 0.10.

r Coefficient of correlation.

n Sample size.

Table 7. Comparison of HIV-seropositive antioxidant supplement users and non-users with respect to mean daily intakes of antioxidant micronutrients, energy and protein

Nutrient	All Subjects	Supplement Users	Supplement Non-Users	p value[a]
Ascorbic acid (mg)[b]				
mean (SE)	589.0 (157.1)	956.6 (269.7)	221.7 (37.8)	≤ 0.01
n (% of n)	22 (100)	11(50)	11 (50)	
% > 10 x RNI[c]	19	36	0	
Zinc (mg)[b]				
mean (SE)	23.8 (2.7)	36.7 (4.0)	16.1 (1.8)	< 0.001
n (% of n)	23 (100)	9 (39)	14 (61)	
% > 10 x RNI	0	0	0	
β-carotene (RE)[b]				
mean (SE)	3323.8 (824.6)	2060.7 (337.3)	2934.1 (1275.1)	0.26
n (% of n)	22 (100)	9 (41)	13 (59)	
% > 10 x RNI[d]	9	0	15	
Energy (kcal)[e]				
mean (SE)	3262.0 (206.1)	3393.0 (223.6)	3174.4 (333.3)	0.38
n	24	16	8	
Protein (g)[e]				
mean (SE)	130.0 (8.8)	127.7 (11.0)	125.7 (15.2)	0.37
n	24	16	8	
Selenium (μg)[f]				
mean (SE)	*	68.9 (27.4)	*	*
n (% of n)	22 (100)	9 (41)	13 (59)	
% > 10 x RNI	*	0	*	
Vitamin E (IU)[f]				
mean (SE)	*	193.5 (69.6)	*	*
n (% of n)	22 (100)	11 (50)	11 (50)	
% > 10 x RNI	*	45	*	

[a] Differences between antioxidant supplement users & non-users based on Student's *t*-test.

[b] Value represents combined food and supplement intake.

[c] Source: Health & Welfare Canada, Nutrition Recommendations, 1990.

[d] 1 RE (retinol equivalent) = 10 IU (international unit) β-carotene. RNI (vitamin A) = 1000 (males); 800 (females) RE / d.

[e] Value represents food intake only.

[f] Value represents supplementation intake only.

SE Standard error; n = sample size; RNI = Recommended Nutrient Intake; * = data unavailable.

Table 8. Supplementation profile of 24 HIV-seropositive men and women

Subject	Nutritional Supplement[a] (known)	Nutritional Supplement[b] (unknown)
011	vitamin C, E, β-carotene, zinc, selenium, iron, magnesium, B-complex, super C and rosehips	acidophilus, garlic, lecithin, folic acid-cyanocobalamin, copper, ubiquinone, folic acid,
150	*	*
159	multivitamin / minerals plus antioxidants	ginseng, energy powder
168	*	*
175	multivitamin / minerals plus antioxidants	*
180	*	*
184	vitamin C	*
188	multivitamin / minerals plus antioxidants	*
196	*	*
232	multivitamin / minerals plus antioxidants, vitamin C	garlic, yarrow, power time B, vitamin B, E
325	*	algae
342	*	*
398	*	spirulina (cyanobacteria), yogurt
461	*	*
468	vitamin E, A, B, D-calcium, magnesium	yogurt, garlic, wheat germ oil
486	multivitamin / minerals	*
512	*	*
660	multivitamin / minerals plus antioxidants, vitamin E, β-carotene, selenium	garlic, horsetail extract
704	vitamin C	*
775	*	alfalfa
802	multivitamin / minerals plus antioxidants	*
835	vitamin C, E, B-complex, B-complex-antioxidants, folic acid, zinc	primonol, bioflavinoid, Siberian ginseng, yogurt
877	multivitamin / minerals plus antioxidants	relaxen, Chinese herbal: composition A
963	*	*

[a] Nutritional supplements with known compositions and average daily dosages.

[b] Nutritional supplements with unknown antioxidant compositions, or average daily dosages.

* Subject did not report supplementation usage.

Source: Canadian Pharmaceutical Association: Compendium of Pharmaceuticals and Specialties, 1995; Self-Medication Product Information, 1993.

Table 9. Comparison of dichotomized groups with respect to plasma malondialdehyde (MDA), leukocyte glutathione (GSH) and absolute CD4+ concentrations in HIV-seropositive men and women

Parameter	Ascorbic Acid[a] (mg / d)		Vitamin E[b] (IU)		Ascorbic Acid (mg / d)		Vitamin E (IU)	
	≥ 200	< 200	≥ 30	< 30	User	Non-User	User	Non-User
Malondialdehyde (MDA) (nmol / L)								
mean	667	825	1155	824	621	826	971[c]	483
(SE)	(177)	(211)	(427)	(243)	(136)	(229)	(224)	(84)
n	11	7	4	5	12	8	9	11
Glutathione (GSH) (nmol / x 10^7 cells)								
mean	16.4	22.8	22.1	15.5	20.3	16.4	18.0	18.9
(SE)	(2.1)	(5.1)	(2.6)	(3.8)	(3.2)	(2.5)	(2.7)	(3.2)
n	11	5	3	5	9	8	8	9
CD4+ (x 10^6/ L)								
mean	253	144	197	351	179	288	287	168
(SE)	(73)	(38)	(50)	(120)	(53)	(82)	(74)	(58)
n	14	6	5	7	11	11	12	10

[a] Value represents combined food and, if applicable, supplement intake.

[b] Value represents supplement intake only.

[c] $p < 0.05$ for the differences between groups within dichotomized categories based on the Student's *t*-test.

IU International units.

SE Standard error.

n Sample size.

Table 10. Comparison of dichotomized groups with respect to plasma malondialdehyde (MDA), leukocyte glutathione (GSH), absolute CD4+ concentrations and mean daily ascorbic acid intake in HIV-seropositive men and women

Parameter	Sample mean	CD4+ ($\times 10^6$ / L)		Smoker	
		> 200	< 200	Yes	No
Malondialdehyde (MDA) (nmol / L)					
mean	703.1	937.6	578.8	884.4[b]	481.4
(SE)	(121.0)	(200.3)	(167.5)	(193.8)	(93.3)
n	20	8	10	9	9
Glutathione (GSH) (nmol / $\times 10^7$ cells)					
mean	18.5	13.7	20.5	18.0	19.2
(SE)	(2.1)	(3.6)	(2.9)	(3.4)	(1.8)
n	17	5	9	7	10
CD4+ ($\times 10^6$/ L)					
mean	232.7	422.3[c]	79.8	215.6	244.8
(SE)	(48.9)	(78.6)	(17.5)	(49.6)	(77.0)
n	22	10	12	9	13
Ascorbate intake[a] (mg)					
mean	589.0	619.9	608.8	496.5	653.1
(SE)	(157.0)	(24.1)	(228.1)	(267.9)	(197.8)
n	22	9	12	9	13

[a] Value represents combined food and, if applicable, supplement intake.

[b] $p < 0.05$; [c] $p < 0.001$ for differences between groups within dichotomized categories based on the Student's *t*-test.

[c] It should be noted this is statistically significant due to the dichotomy criteria established by the investigator and is not meant to imply clinical significance.

SE Standard error.

n Sample size.

Table 11. Linear correlational analyses: Mean daily micronutrient intakes, malondialdehyde (MDA), leukocyte glutathione (GSH) and CD4+ levels versus oxidative stress (MDA, GSH) and immunological (CD4+) parameters in HIV-seropositive men and women

	Plasma MDA (nmol / L) r[a] (n)	Leukocyte GSH (nmol / 10^7 cells) r (n)	Absolute CD4+ (x 10^6 / L) r (n)
Dietary			
Total (supplement & food) intake:			
Ascorbate (mg)	0.33 (18)	0.03 (15)	-0.05 (20)
β-carotene (RE)	-0.19 (18)	-0.35 (16)	-0.23 (21)
Zinc (mg)	0.12 (19)	0.20 (17)	-0.07 (21)
Supplement intake only:			
Ascorbate (mg)	0.46 (8)	0.26 (8)	-0.18 (11)
β-carotene (RE)	-0.46 (7)	0.01 (7)	-0.10 (9)
Zinc (mg)	-0.35 (6)	*	0.32 (9)
Selenium (μg)	-0.52 (7)	-0.11 (6)	0.07 (9)
Vitamin E (IU)	0.17 (9)	0.32 (8)	-0.16 (11)
Food intake only:			
Ascorbate (mg)	0.08 (20)	0.01 (17)	-0.28 (22)
β-carotene (RE)	-0.11 (20)	-0.33 (17)	-0.20 (22)
Zinc (mg)	-0.11 (20)	0.32 (17)	-0.27 (22)
Energy (kcal)	-0.13 (20)	0.17 (17)	-0.31 (22)
Protein (g)	-0.10 (20)	0.28 (17)	-0.30 (22)
Oxidative Stress			
Plasma MDA (nmol / L)	-	0.17 (16)	0.13 (18)
Leukocyte GSH (nmol/10^7 cells)	0.17 (16)	-	-0.48 (15)
Immunological			
Absolute CD4+ (x 10^6 / L)	0.13 (18)	-0.48 (15)	-

[a] All values were not statistically significant at p = 0.05

r Coefficient of correlation.

n Sample size.

RE Retinol equivalents.

* All subjects with GSH measurements supplemented with the same level of zinc, therefore correlational analysis is not applicable.

IU International units.

\- Not applicable.

Table 12. Linear correlational analyses: Polymorphonuclear (PMN) leukocytes versus plasma malondialdehyde (MDA), leukocyte glutathione (GSH), absolute CD4+ counts and the five antioxidant micronutrients (vitamin C, E, β-carotene, zinc, selenium) in HIV-seropositive men and women

Parameter	Polymorphonuclear leukocytes (cells x 10^9 / L) r (n)
Plasma malondialdehyde (MDA) (nmol / L)	0.10 (17)
Leukocyte glutathione (GSH) (nmol / x 10^7 cells)	-0.46 (12)
CD4+ (cells x 10^6 / L)	0.37 (21)
Vitamin E - supplemental (IU)	-0.48 (11)
Selenium - supplemental (µg)	-0.54 (9)
Ascorbic acid - total (mg)	-0.13 (20)
Zinc - total (mg)	0.20 (20)
β-carotene - total (RE)	-0.08 (20)

r Coefficient of correlation.
n Sample size.
RE Retinol equivalents.
IU International units.

Table 13. Linear correlational analyses: Dietary intakes, malondialdehyde (MDA), glutathione (GSH) and CD4+ levels versus oxidative stress (MDA, GSH) and immunological (CD4+) parameters in stratified groups of HIV-seropositive men and women

Strata	Dependent Variables	Plasma MDA (nmol / L) r (n)	Leukocyte GSH (nmol / 10^7 cells) r (n)	Absolute CD4+ (x 10^9 / L) r (n)
Vitamin C	Total ascorbate (mg)			
user		0.42 (8)	0.31 (8)	-0.25 (11)
non-user		-0.24 (10)	-0.29 (8)	-0.28 (10)
β-carotene	Total β-carotene (RE)			
user		-0.01 (9)	0.17 (7)	-0.21 (9)
non-user		-0.29 (13)	-0.48 (9)	-0.32 (12)
Zinc	Total zinc (mg)			
user		-0.59 (6)	0.25 (6)	-0.02 (8)
non-user		0.12 (12)	0.36 (10)	-0.19 (13)
Supplement[a]	Energy (kcal)			
user		-0.28 (12)	0.32 (10)	-0.29 (15)
non-user		0.07 (8)	0.12 (7)	-0.42 (7)
Supplement[a]	Protein (g)			
user		-0.21 (12)	0.38 (10)	-0.29 (15)
non-user		-0.05 (8)	0.27 (8)	-0.32 (7)
Vitamin C	Total ascorbate (mg)			
≥ 10 x RNI		1.00 (2)	-0.18 (2)	1.00 (3)
< 10 x RNI		-0.12 (16)	0.04 (14)	-0.07 (18)
Vitamin E	Supplemental vitamin E (IU)			
≥ 10 x RNI		-0.09 (4)	-0.14 (4)	-0.72 (5)
< 10 x RNI		0.92[b] (5)	0.50 (5)	0.58 (6)

[a] Supplement is defined as the consumption of any antioxidant supplement (vitamin C, E, β-carotene, selenium, zinc).

[b] Value was statistically significant at $p < 0.05$

r Coefficient of correlation.

n Sample size.

RE Retinol equivalents.

IU International units.

Table 14. Linear correlational analyses: Mean plasma MDA, leukocyte GSH, absolute CD4+ and total ascorbic acid intake versus oxidative stress (MDA, GSH) and immunological (CD4+) parameters stratified on the basis of smoking status in HIV-seropositive men and women

Strata	Dependent Variables	Plasma MDA (nmol / L) r (n)	Leukocyte GSH (nmol / 10^7 cells) r (n)	Absolute CD4+ (x 10^6 / L) r (n)
Smoker				
	MDA (nmol / L)	-	0.78[a] (7)	0.41 (9)
	GSH (nmol / 10^7 cells)	0.78[b] (7)	-	0.15 (5)
	CD4+ (x 10^6 / L)	0.41 (9)	0.15 (5)	-
	Total ascorbate (mg)	0.51 (9)	0.33 (6)	-0.04 (8)
Non-Smoker				
	MDA (nmol / L)	-	-0.34 (9)	0.17 (9)
	GSH (nmol / 10^7 cells)	-0.34 (9)	-	-0.54 (10)
	CD4+ (x 10^6 / L)	0.17 (9)	-0.54 (10)	-
	Total ascorbate (mg)	-0.22 (9)	-0.13 (10)	-0.07 (13)

[a] Value was statistically significant at $p < 0.05$.
r Coefficient of correlation.
n Sample size.
- Not applicable.

10. Figures

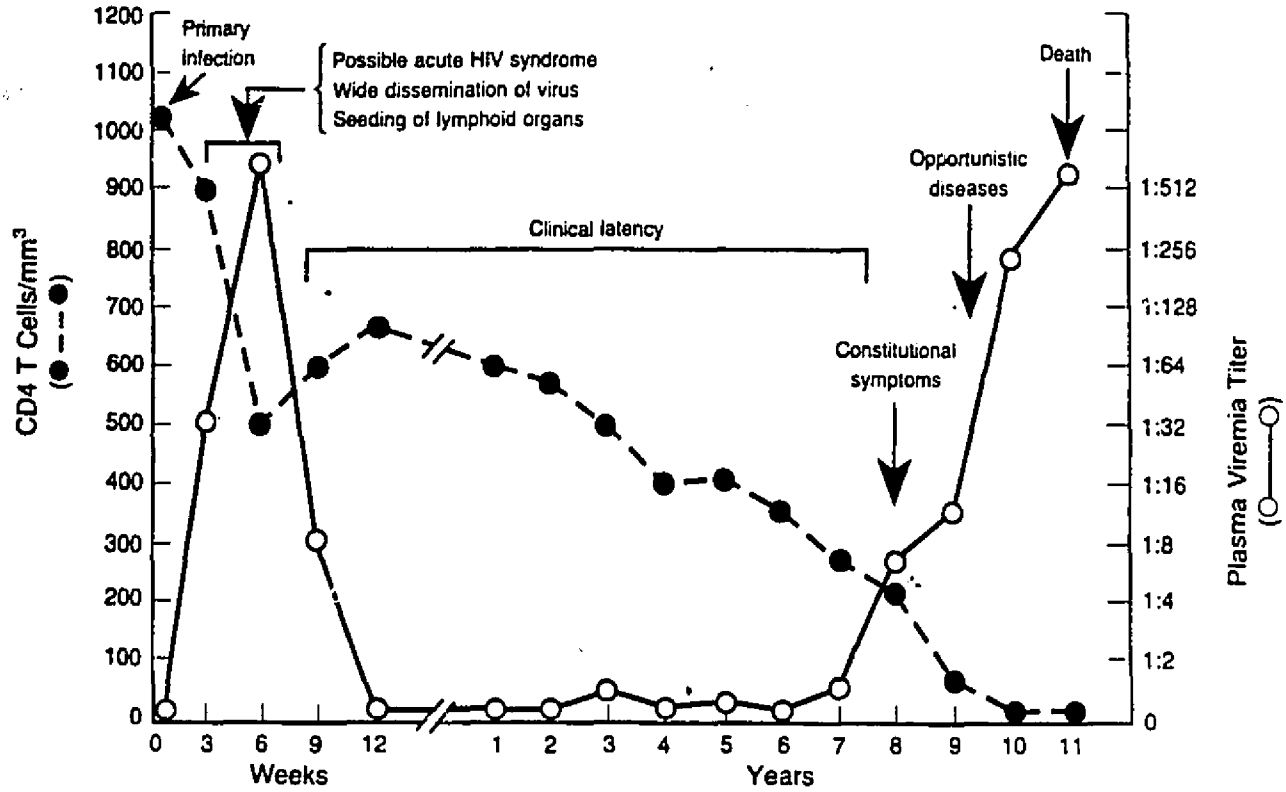

Figure 1. Evolution of the typical course of HIV infection

After about 12 weeks post-infection, blood-borne virus (plasma viremia) is undetectable. However, CD4+ cell levels steadily decline during this clinical latency period, probably because of active viral replication and T-cell infection in lymph nodes. When CD4+ levels fall below a critical level (about 200 / mm^3), there is a high risk of infection.

Source: Abbas et al., 1994; Pantaleo et al., 1993.

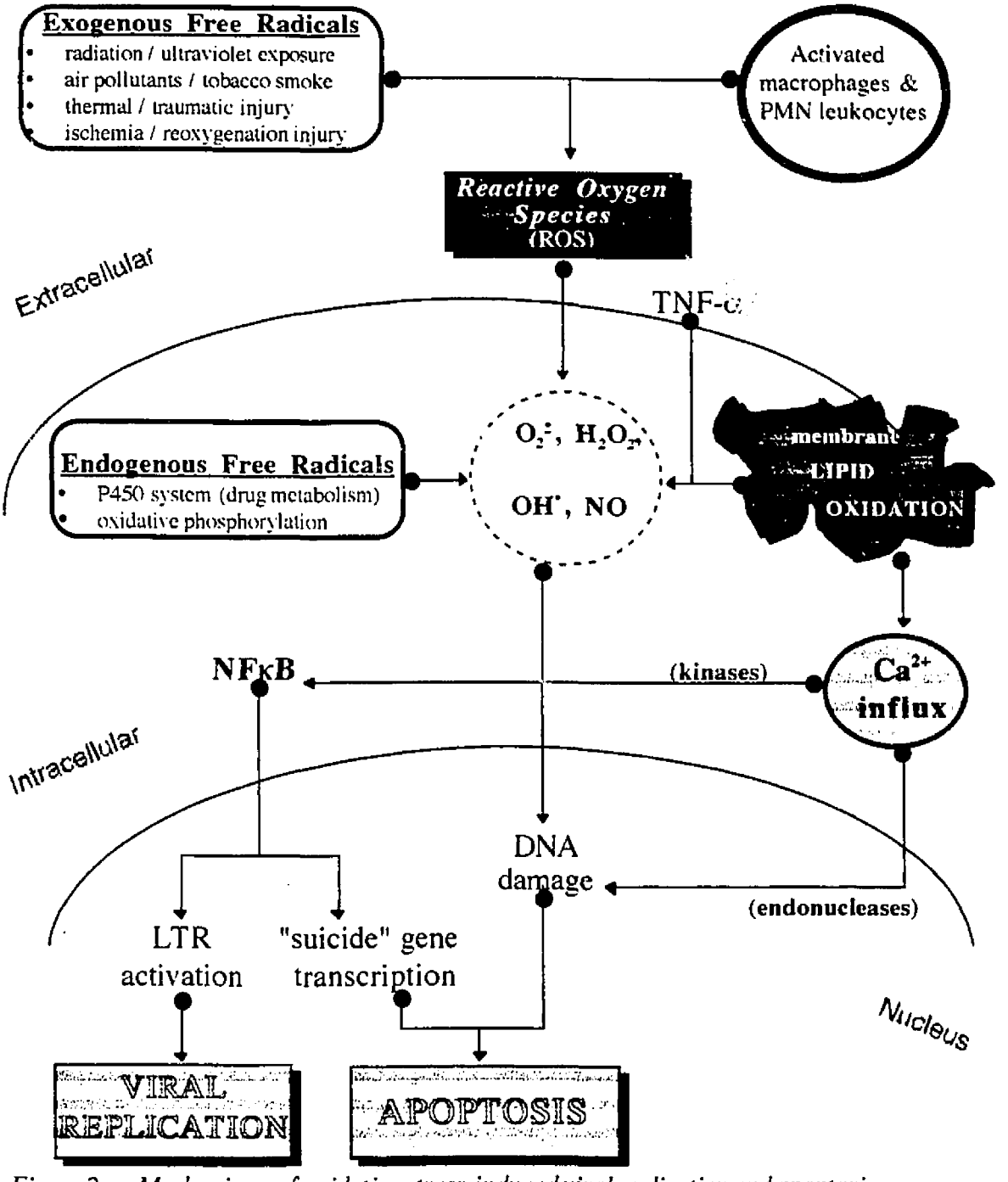

Figure 2. Mechanisms of oxidative stress-induced viral replication and apoptosis

T cells maintain a suitable redox equilibrium by balancing intracellular levels of oxidants and antioxidants. Free radicals and the calcium influx resulting from cellular membrane lipid oxidation may cause the dissociation of NFκB from IκB allowing translocation to the nucleus. Ultimately, these factors are associated with long terminal repeat (LTR) activation leading to HIV replication and programmed cell death or apoptosis.

Source: Adapted from Buttke and Sandstrom, 1994; Larrick and Wright, 1990.

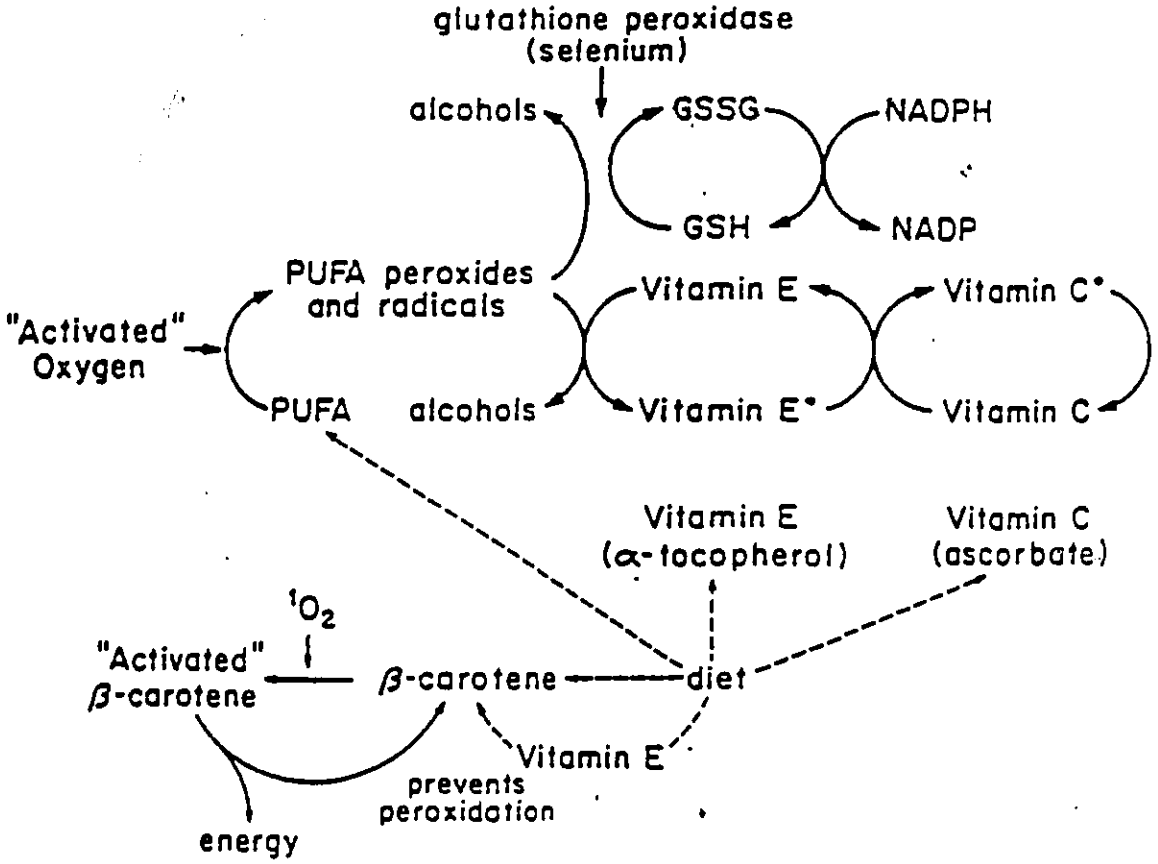

Figure 3. Antioxidant micronutrient interactions

Source: Machlin and Bendich, 1987

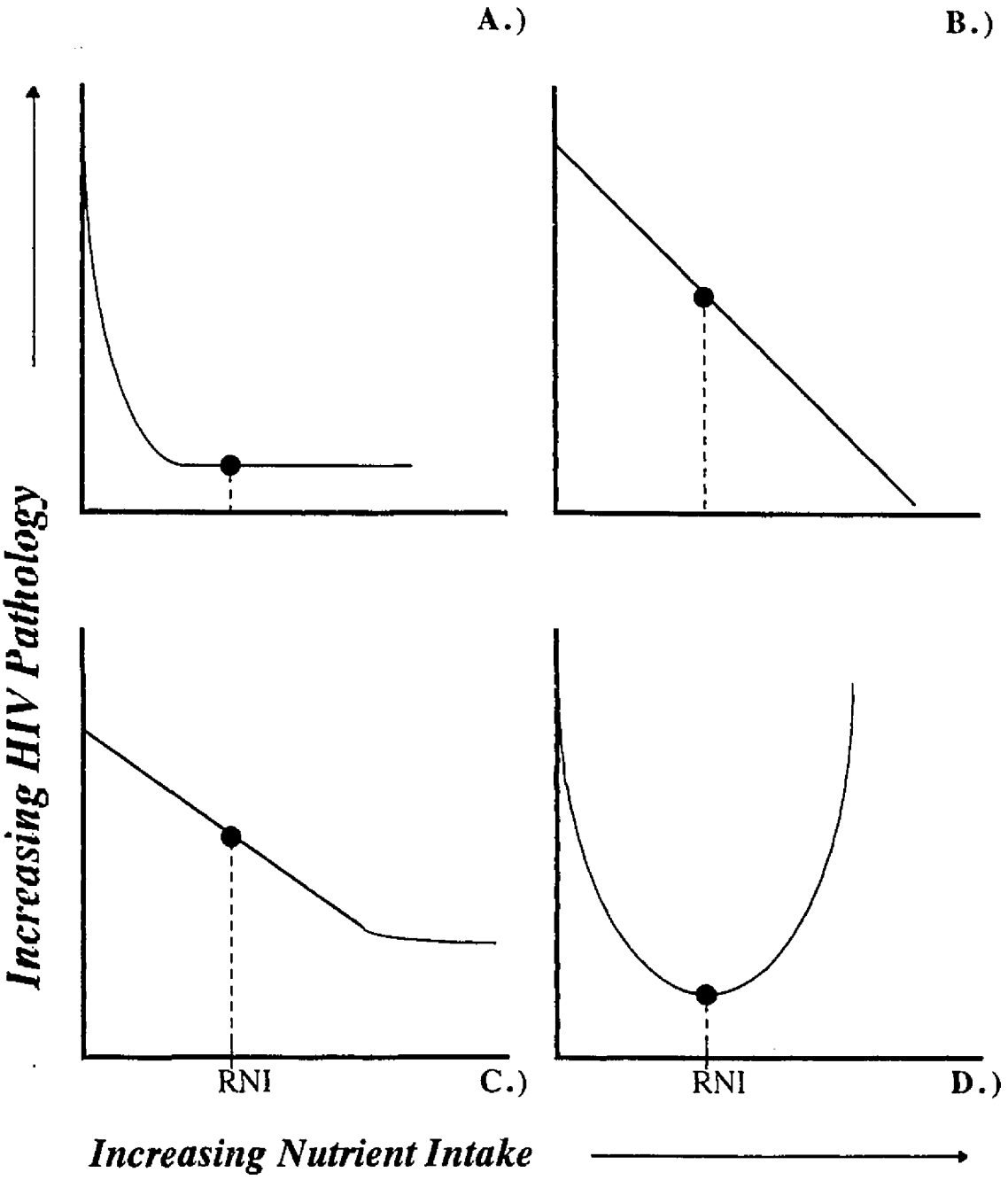

Figure 4. Differential response to micronutrient supply
Hypothesized pathological responses to various nutrient intakes. The micronutrient A provides the maximum protection it can afford against HIV at an intake at, or even slightly below, the Recommended Nutrient Intake (RNI) (Health and Welfare Canada, 1990). For micronutrient B, increased protection is provided at and beyond the RNI. For micronutrient C, however, the protection afforded against HIV pathology continues only to a certain point, therefore demonstrating a threshold effect. Finally, micronutrient D shows benefit at or close to the RNI, but detrimental effects against HIV are shown both at very low or high intakes.
Source: Adapted from Pryor, 1991.

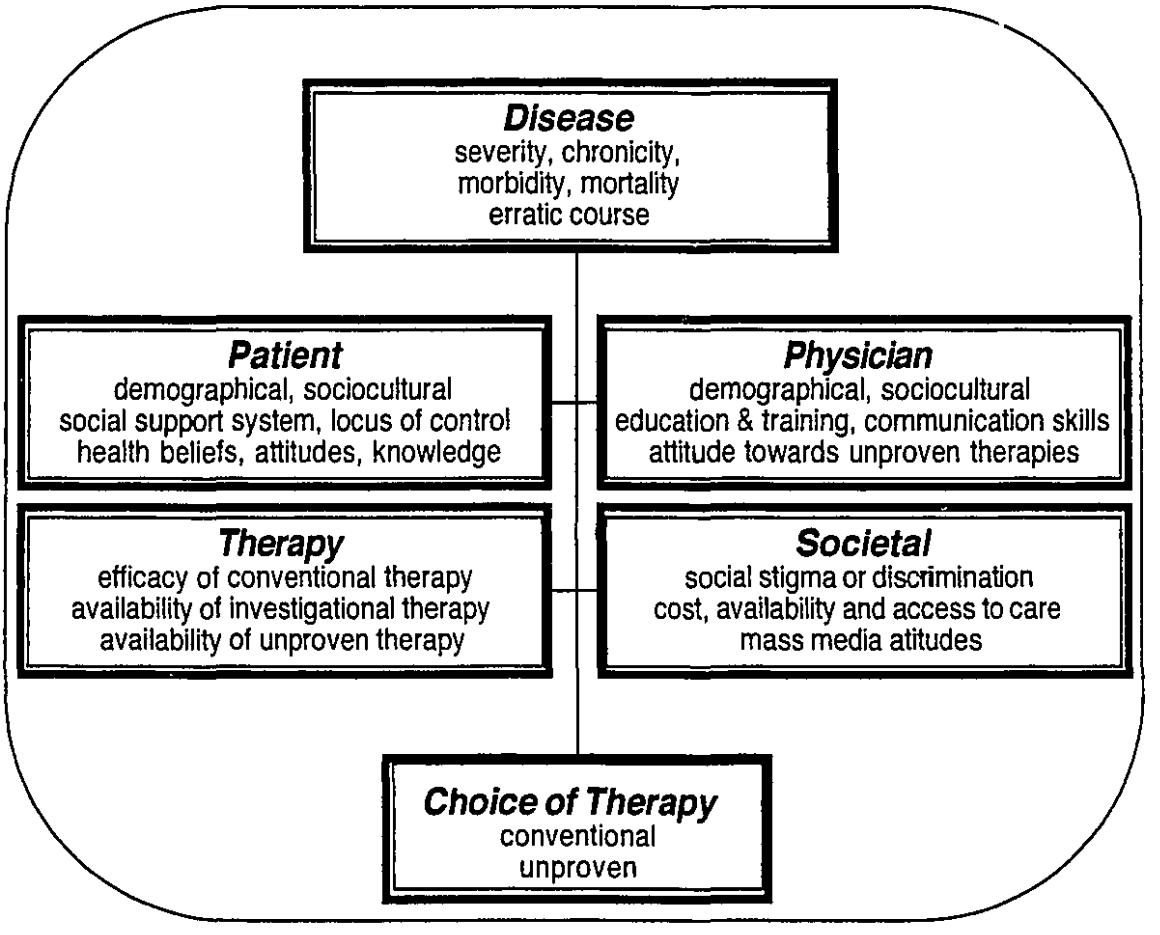

Figure 5. Factors contributing to unproven therapy choice

Unproven therapy is defined as therapy with insufficient evidence to establish efficacy or safety, while conventional therapies have evidence and experience attesting to their safety, efficacy and palliative qualities. The social stigma associated with HIV infection combined with the wide variety and availability of unproven nutritional therapies favours the choice of unproven therapies.

Source: Adapted from Dwyer et al., 1988.

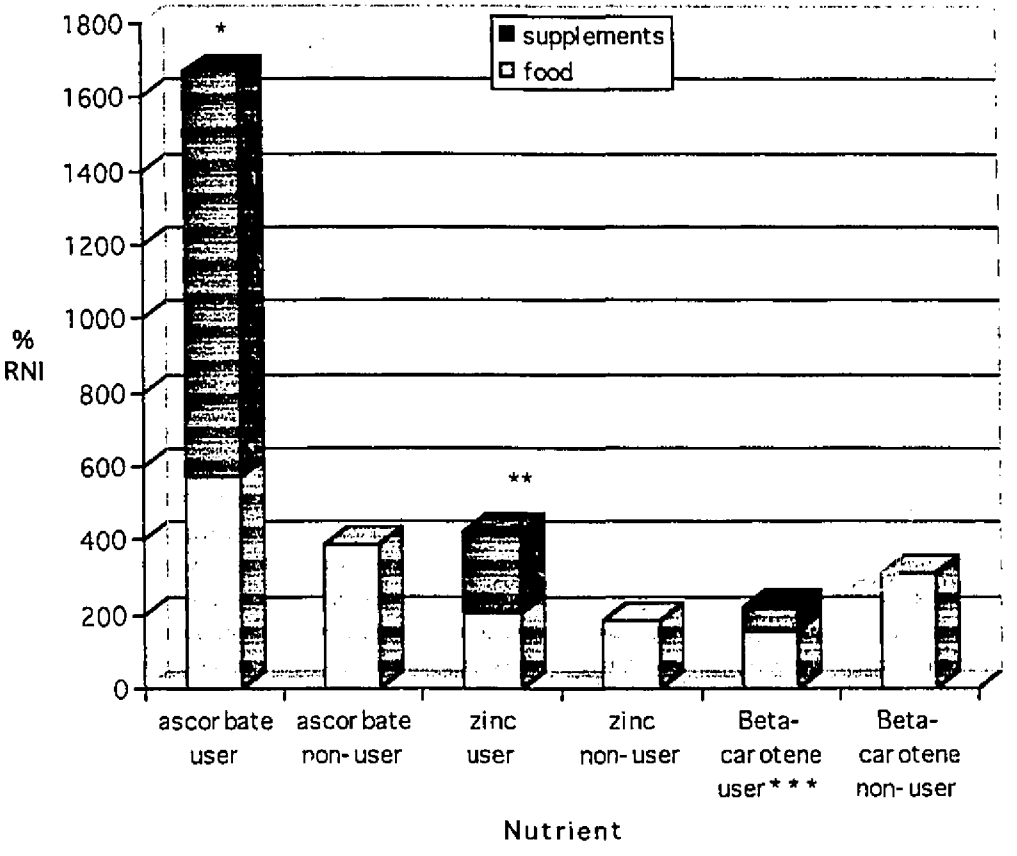

Figure 6. Mean daily intakes of antioxidant nutrients expressed as a percent of 1990 Recommended Nutrient Intakes (RNI) for Canadians in HIV-seropositive supplement users and non-users

* Difference between total mean ascorbate intake of supplement users vs. non-users: $p < 0.01$; Student's *t*-test.

** Difference between total mean zinc intake of supplement users vs. non-users: $p < 0.001$; Student's *t*-test.

*** 1 RE (retinol equivalent) = 10 IU β-carotene. RNI (vitamin A) males = 1000 RE / d; females 800 RE / d.

Source: Health and Welfare Canada, 1990.

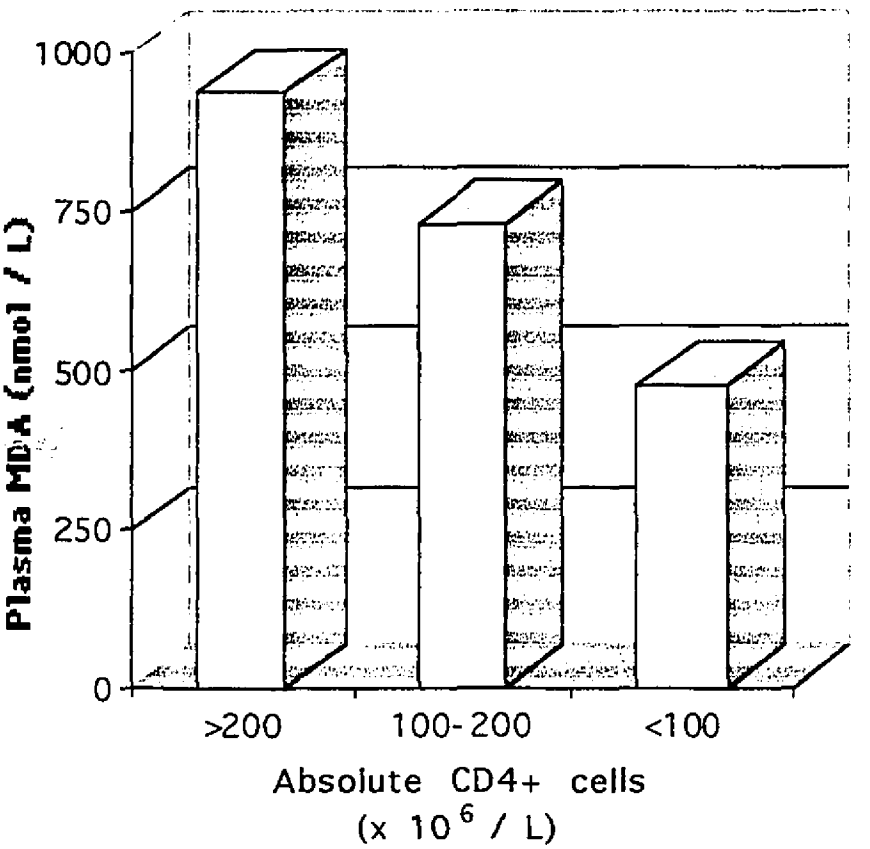

Figure 7. Comparison of mean plasma malondialdehyde (MDA) concentrations with respect to absolute CD4+ cell counts in HIV-seropositive men and women

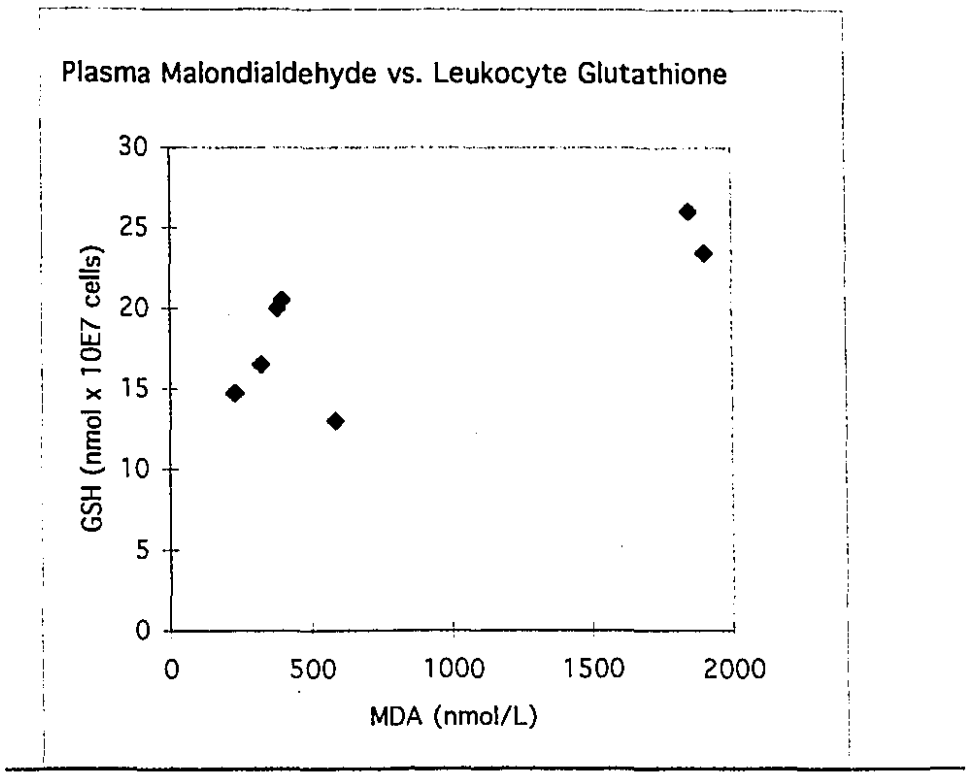

Figure 8. Scatter plot diagram of plasma malondialdehyde (MDA) concentration and absolute CD4+ cell counts in HIV-seropositive men and women

11. Appendices

Appendix 1. Centers for Disease Control classification system of HIV infection

Acute HIV syndrome	Group I
Asymptomatic infection	Group II
Persistent generalized lymphadenopathy	Group III
Other diseases:	Group IV
Constitutional disease	Subgroup A
Neurological disease	Subgroup B
Secondary infectious diseases	Subgroup C
Secondary neoplasms	Subgroup D
Other conditions	Subgroup E

Source: Modified from the Centers for Disease Control, 1986; 1987.

Appendix 2. Centers for Disease Control surveillance case definition for AIDS (1993)

A. Indicator diseases diagnosed definitively in the absence of other causes of immunodeficiency and without laboratory evidence of HIV infection

1. Candidiasis of the esophagus, trachea, bronchi or lungs
2. Cryptococcosis, extrapulmonary
3. Cryptosporidiosis with diarrhea persisting > 1 month
4. Cytomegalovirus disease of any organ excluding liver, spleen and lymph nodes patient > 1 month of age
5. Herpes simplex virus infection causing a mucocutaneous ulcer persisting > 1 month; or bronchitis, pneumonia or esophagitis in a patient > 1 month of age
6. Kaposi's sarcoma in a patient < 60 years of age

Lymphoma of the brain (primary) in a patient < 60 years of age

1. Lymphoid interstitial pneumonia and / or pulmonary lymphoid hyperplasia in a child < 13 years of age
2. *Mycobacterium avium* complex or *M. Kansasii* disease (disseminated)
3. *Pneumocystis carinii* pneumonia
4. Toxoplasmosis of the brain in a patient > 1 month of age

B. Indicator diseases diagnosed definitively regardless of the presence of other causes of immunodeficiency and in the presence of laboratory evidence of HIV infection

1. Any disease listed in section A
2. Bacterial infections (multiple or recurrent) in children < 13 years of age caused by *Haemophilus*, *Streptococcus*, or other pyogenic bacteria
3. Coccidioidomycosis, disseminated
4. HIV encephalopathy
5. Histoplasmosis, disseminated
6. Isosporiasis with diarrhea persisting > 1 month of age
7. Kaposi's sarcoma at any age
8. Non-Hodgkin's lymphoma of B cell or unknown phenotype and having histologic type of small non-cleaved lymphoma or immunoblastic sarcoma
9. Any mycobacterial disease, disseminated, excluding *M. Tuberculosis*
10. *M. Tuberculosis*, extrapulmonary
11. *Salmonella* (nontyphoid) septicemia, recurrent
12. HIV wasting syndrome

C. Indicator diseases diagnosed presumptively in the presence of laboratory evidence of HIV infection

1. Candidiasis of the esophagus
2. Cytomegalovirus retinitis with loss of vision
3. Kaposi's sarcoma
4. Lymphoid interstitial pneumonia and / or pulmonary lymphoid hyperplasia in a child < 13 years of age
5. Mycobacterial disease, disseminated
6. *Pneumocystis carinii* pneumonia
7. Toxoplasmosis of the brain in a patient > 1 month of age

D. Indicator diseases diagnosed definitively in the absence of other causes of immunodeficiency and in the presence of negative results for HIV infection

1. *Pneumocystis carinii* pneumonia
2. Other indicator diseases listed in section A and CD4+ T lymphocyte count < 400 per microlitre

E. The 1993 expanded definition includes:

1. All HIV-infected persons who have < 200 CD4+ T lymphocyte counts per microlitre, or a CD4+ T lymphocyte percentage of total lymphocytes of < 14
2. Pulmonary tuberculosis
3. Recurrent pneumonia
4. Invasive cervical cancer

Source: Centers for Disease Control 1987; 1993

Appendix 3. Criteria for AIDS-related complex (ARC)

The Centers for Disease Control does not provide a precise definition of ARC but rather classifies it as an AIDS like syndrome, noting that it is synonymous with pre AIDS and prodromal AIDS. The medical community has adopted its own definition based on exclusion clauses in the CDC definition of AIDS.

Persons with HIV infection who have "constitutional symptoms" or clinical abnormalities that accompany lymphadenopathy are described as patients with ARC. These include patients with a fever persisting for more than one month, involuntary weight loss greater than 10% of baseline, or diarrhea lasting more than one month in the absence of other conditions that could explain these findings; patients with hairy leukoplakia, multidermatomal herpes zoster, recurrent *Salmonella* bacteremia, nocardiosis, tuberculosis, or oral candidiasis; and patients with chronic lymphoid interstitial pneumonitis. Note that ARC is not synonymous with persistent generalized lymphadenopathy (PGL).

ARC patients present with a spectrum of immunological abnormalities ranging from minimal alterations to severe cellular immunodeficiency comparable to those patients with frank AIDS. An absolute CD4+ count cannot be used as a diagnostic measure since some asymptomatic HIV infected individuals have had low CD4+ cell counts while other patients with constitutional symptoms have had relatively high counts. Many ARC patients are anergic and many exhibit hypergammaglobulinemia, hypersplenism and sometimes a diminution in primary antibody responses. Some ARC patients also have mild anemia, leukopenia, or thrombocytopenia. Patients with PGL or ARC generally progress to full-blown AIDS, usually within a three year period.

Source: AIDS 90 Summary, 1991.

Date: _____

Physician: _____ **Subject Code:** _____

Study: **Oxidative stress and ascorbic acid self-supplementation in HIV infection.**

Medical Checklist:

HIV positive: Y N

Date of initial HIV seropositivity: ___/___/___ (Y/ M/ D)

Karnofsky score: _____

3 most recent AIDS-indicator O.I.:

#1 _____ Date (Y/M/D): _____

#2 _____ _____

#3 _____ _____

Are AIDS-indicator neoplasms present? Y N

If "yes", specify: _____

date of diagnosis: ___/___/___ (Y/ M/ D)

Are any of the following conditions currently present?

oral candidiasis (more than a scant amount) Y N

fevers in last 3 weeks Y N

diarrhea (defined as 3 or more unformed or liquid stools /day) Y N
for how long? _____

atherosclerosis/heart disease Y N

diabetes Y N

active neoplastic disease, (unrelated to AIDS) Y N

Any other condition which is associated with obvious signs of inflammation? (e.g., widespread skin rashes, swollen joints, fever) Y N

Subject Code: _____

Medications:

acetominophen Y N
dose: _____

aspirin Y N
dose: _____

ALL medications used in the previous 6 weeks:

NAME	DOSE	FREQUENCY	DATE STARTED
AZT			
DDI			
DDC			
SEPTRA (or other combinations of sulfonamide-trimethoprin)			
OTHERS:			

Appendix 6. Demographic Questionnaire

Date: _____

Dietitian: _____ **Subject Code:** _____

Study: **Oxidative stress and ascorbic acid self-supplementation in HIV infection.**

Weight history

usual or pre-illness weight _____

current (date) _____

> 10% weight loss over 3 months Y N

height _____

Date of birth _____

Gender _____

Smoking status
smoker, non-smoker, former smoker (date) _____

Date: _____ Subject Code: _____

Dietitian: _____

NUTRITIONAL SUPPLEMENTATION QUESTIONNAIRE

1. In the **PAST 3 WEEKS**, have any MULTIvitamin/mineral supplements been consumed?

   1. No. Proceed to Page 2.

   2. Yes.

Brand Name of:	**Number of pills per day OR week?**	**Number of weeks, months, OR years that you've been taking the supplement?**
Multivitamin/mineral:		
1. _____	_____ / day _____ / week	_____ weeks _____ months _____ years
2. _____	_____ / day _____ / week	_____ weeks _____ months _____ years
3. _____	_____ / day _____ / week	_____ weeks _____ months _____ years
4. _____	_____ / day _____ / week	_____ weeks _____ months _____ years

2. In the **PAST 3 WEEKS**, have any of the following **SINGLE** nutrient supplements been consumed?

1. No

2. Yes.

Type	Brand Name	mg, IU, or mcg per pill/dose?	Number per day or week?	# of wks, mo, or yrs that you've been taking the suppl?
Beta-carotene			____ / day ____ / wk	____ wks ____ mo ____ yrs
Vitamin C			____ / day ____ / wk	____ wks ____ mo ____ yrs
Vitamin E			____ / day ____ / wk	____ wks ____ mo ____ yrs
Zinc			____ / day ____ / wk	____ wks ____ mo ____ yrs
Selenium			____ / day ____ / wk	____ wks ____ mo ____ yrs
Copper			____ / day ____ / wk	____ wks ____ mo ____ yrs
Cysteine			____ / day ____ / wk	____ wks ____ mo ____ yrs
Cod liver oil, Halibut liver oil			____ / day ____ / wk	____ wks ____ mo ____ yrs
Glutathione			____ / day ____ / wk	____ wks ____ mo ____ yrs
Iron			____ / day ____ / wk	____ wks ____ mo ____ yrs

3. In the **PAST 3 WEEKS**, have any **Other** nutritional supplements been consumed?

Type	Brand Name	mg, IU, or mcg per pill/dose?	# per day or week?	# of wks, mo, or yrs that you've been taking the suppl?

Nom: Taille: Poids: Subject Code: _____

Qui prépare les repas

Régime spécial suivi:

Nombre de repas pris à l'extérieur: Mangez-vous seul?

DIETARY HISTORY

24 Hour recall

Morning meal	Noon meal	Evening meal
Collation	Collation	Collation

	Grosseur Portion		Fréquence de consommation		
Groupes alimentaire et Description	Quantité habituelle	Métrique	Par semaine	Par jour	Commentaires
Lait et prod. laitiers Lait % M.G. Milk Drinks					
Fromage % M.G.					
crème glacée/Lait glacée					
Yogourt aux fruits nature					
Pouding/Costard (dessert au lait)					
soupe Crème avec lait ou crème					
viande et substitues abâts foie...					
Sandwich viande charcutrie autres					

Bacon/saussisse					
Poulet avec ou sans peau rôti bouillie frit					
poisson sorte: rôti bouillie frit					
boeuf coupe rôti bouillie avec ou sans gras porc coupe rôti bouillie avec ou sans gras					
pizza					
Oeufs					
hot-dog hamburger					
Légumineuses (haricot, pois)					

	grosseur des portions		Fréquence de consomation		
groupe alimentaire et description	portion habituelle	Metrique	par semaine	par jour	Commentaires
Légumes verts cuit non cuit					
jaune					
Autres					
Pomme de terre purée, bouilli, au four avec ou sans beurre					
pomme de terre frites					
Soupe aux légumes					
Fruits citrins					

Autres					
séchés					
Jus					
pain et petit pains **beurre** **sans beurre**					
Bagels avec ou sans beurre					
Muffins (maison, achetés) beurre sans beurre biscuit (maison, achetés) beurre sans beurre					
Craquelins beurre sans beurre					

Céréales: Sucrées non-sucrées :						
nines et suppléments						
nts diététique						
Riz sans ou avec beurre						
Pâtes sauce tomates sans viande sauce tomate avec viande sauce blanche + fromage avec beurre						
Beurre d'arachide noix						
Bière régulière .05 vin alcool						

	Grosseur des portions		Fréequence de consomation		
Groupes d'aliment et Description	Portion habituelle	Métrique	Par semaine	Par jour	Commentaires
Gras Beurre Margarine sorte:					
Huile sorte: Vinaigrette régulière lègère Mayonnaise régulière légère					
Crème % gras Crème sûre % gras					
Gâteaux Tartes Patisseries					
Beignes					
Shorbet Jello					

Gaufres Crêpes (avec ou sans sirop)					
Confiture Gelée Miel					
Sucre Sirop					
Friandises (bonbons) Chocolat					
Popcorn avec beurre sans beurre Chips Bretzels					
Boisson gaseuse (rég. ou diète)					
Gomme à machée régulière sans sucre					