NUTRITION and AIDS

Second Edition

Edited by

Ronald R. Watson

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We welcome the contribution of the second edition of *Nutrition and AIDS* by Ronald R. Watson. The first edition was very well received indeed by the relevant scientific community. Science does not stand still, and with the rapid expansion of knowledge in the field, a second, updated and expanded edition was needed. The book summarizes the knowledge of how nutrition cofactors can modify the physiology and immunology of HIV-infected individuals. Once a phenomenon is understood, at least in part, it can be manipulated, the aim of experimental therapeutics.

Ira Wolinsky, Ph.D.
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Series Editor

Preface

In May of 1999, the World Health Organization announced that AIDS was the most deadly infectious disease worldwide. In addition it is now the fourth leading cause of premature death in the U.S.A. A wide variety of retroviruses infects a broad spectrum of animals, including HIV-1 which infects humans. Progression to disease, ARC or AIDS, seems variable in humans. In Africa, where nutritional problems are widespread, progression is accelerated. The reasons for the large differences in the rates of progression to disease in adults after infection are unclear. The hypotheses that various foods, nutrients, and nutrient deficiencies affect this process offer opportunities for lifestyle changes to influence progression to AIDS.

Clearly, immunosuppressive conditions like protein malnutrition or low selenium intake reduce resistance to some pathogens, while high intakes of vitamins stimulate immune functions, increasing resistance to infection. Dietary cofactors offer avenues to attack retroviral diseases and serve as tools to understand the mechanisms of action. As we develop greater knowledge of how nutrients modify the physiology and immunology of HIV-1-infected individuals, we will better understand retroviruses and their roles in immunosuppression.

It is now timely to look at dietary materials, supplements, and foods that may benefit or treat AIDS as well as nutrient deficiencies that may accelerate progression to AIDS and death. As HIV infection progresses to AIDS and death, a significant component is undernutrition. Starvation works as a potent immunosuppressant.

Nutritional supports could thus help maintain health in the HIV-infected patient by repleting lost nutrients, compensating for nutritional damage done by the retrovirus-induced immunodeficiency, and stimulating the remaining immune system and cells for better host defenses. Unconventional dietary therapies are being used by AIDS patients.

The goal of this book is to define recent advances in understanding the nutritional deficiencies of AIDS patients and explore the ways nutritional and dietary changes and herbal medicines benefit or harm them. A large variety of alternative herbal and dietary remedies have been proposed, and some have been tested in animals and people to stimulate immune defenses or compensate for changes induced by HIV infection. Animal models are clearly useful in testing novel remedies. Conversely, a number of drugs and ingested and inhaled substances such as cocaine, alcohol, and other immunosuppressive compounds can adversely impact damaged immune systems.

The overall goal of this book is to provide the most current, concise scientific appraisal of the efficacy of nutrients, foods, and herbal (alternative) medicines in preventing or treating AIDS and its symptoms and improving the quality of life.

The Editor

Ronald R. Watson, Ph.D., initiated and directed the Specialized Alcohol Research Center at the University of Arizona College of Medicine for 6 years. The main role of this National Institute of Alcohol Abuse and Alcoholism (NIAAA) grant was to understand the role of ethanol-induced immunosuppression on progression to AIDS in animals. Dr. Watson has edited 50 books, including 3 on cofactors and drug abuse in AIDS, and 2 on nutrition in the aged.

Dr. Watson attended the University of Idaho and graduated from Brigham Young University in Provo, Utah with a degree in chemistry in 1966. He completed his Ph.D. program in biochemistry at Michigan State University in 1971. His postdoctoral schooling in nutrition and microbiology was completed at the Harvard School of Public Health and included a 2-year postdoctoral research experience in immunology. He was an assistant professor of immunology and did research at the University of Mississippi Medical Center in Jackson from 1973 to 1974. He was an assistant professor of microbiology and immunology at the Indiana University Medical School from 1974 to 1978 and an associate professor at Purdue University in the Department of Food and Nutrition from 1978 to 1982. In 1982, he joined the faculty at the University of Arizona in the Department of Family and Community Medicine. He is also a research professor in the University of Arizona's newly formed College of Public Health. He has published 450 research papers and review chapters.

Dr. Watson is a member of several national and international nutrition, immunology, cancer, and research societies. He is also currently the principal investigator on two NIH grants studying the role of alcohol and nutrients in modulating heart disease in a model of AIDS.

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Contents

CHAPTER 1

Wasting and AIDS in the Era of Highly Active Antiretroviral Therapy

Lawrence A. Cone

CONTENTS

INTRODUCTION

AIDS is nearly always complicated by unintentional weight loss, and when the loss exceeds 10% of baseline weight is termed wasting.[1] Wasting is noted in 20 to 30% of patients diagnosed with AIDS and in approximately 25% of patients who have AIDS during the last six months of their lives.[2] Weight loss is also correlated with increased morbidity and mortality.[3] Loss of lean body mass and the presence of other nutritional parameters indicative of malnutrition are associated with death due to AIDS.[4,5] A weight loss as small as 5% adversely affects both survival and the development of opportunistic infections,[6] even in the absence of prior or concurrent AIDS-defining complications.

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Weight loss in patients with HIV infections can be periodic, corresponding to episodes of secondary infection or gastrointestinal disease.[7] It may result from a decrease in energy intake due to anorexia or secondary to drug treatment, upper gastrointestinal disease, or malabsorption of nutrients.[8] Moreover, intermediary metabolism can be changed so that sensitivity to insulin is increased, oxidation of carbohydrates is suppressed, plasma triglycerides are increased, and protein turnover is accelerated.[9-11] Resting energy expenditure is often increased with advanced HIV disease and is suspected to contribute to weight loss.[9,12]

Graham et al.[13] have suggested that the importance of diarrhea as a cause of weight loss in HIV infection may have been overestimated and that fever, thrush, and a $CD4^+$ cell count of <100 cells/μl were the best predictors of weight loss.

More recent studies have shown that levels of HIV replication appear to be causally related to the magnitude of weight loss in some patients with wasting.[14] Resting energy expenditure is correlated with tumor necrosis factor alpha or cachectin in peripheral blood mononuclear cells.[15] There is a significant positive correlation between plasma HIV RNA and the resting energy expenditure after adjustment for lean body mass.[16]

It is widely accepted that reductions in HIV viral load through highly active antiretroviral therapy (HAART) can reverse weight loss. Whether antiretroviral resistance to HAART predicts for wasting is the subject of this communication.

METHODS

Subjects

Seven male patients with AIDS expired at the Eisenhower Medical Center (EMC) in 1999 while under my treatment. Four were defined as having wasting based upon a >10% loss of weight compared to baseline. Two of the remaining three did not lose weight prior to death and one lost less than 10% of his baseline weight.

Assays

HIV-1 RNA Quantitation Utilizing bDNA

HIV-1 RNA was quantitated in plasma by bDNA signal amplification-based hybridation (Quantiplex HIV-RNA assay kit, Chiron) according to the manufacturer's instructions of Specialty Laboratories Inc., Santa Monica, CA.

Genotypic Studies

An assay for the CC-CKR5 delta 32 mutation spanning nucleotides 794-825 was performed along with the HIV-1 genotyping for nucleoside and non-nucleoside reverse transcriptase and protease inhibitor resistance by PCR/DNA sequencing by Specialty Laboratories Inc., Santa Monica, CA. The following 17 drugs or combinations were tested: zidovudine, didanosine, zalcitabine, stavudine, lamivudine, the

zidovudine/lamivudine combination, abacavir, nevirapine, delaviradine, loviride, atevirdine, efavirenz, saquinavir, ritonavir, nelfinavir, indinavir, and amprenavir. Adefovir was administered to all patients but was not tested. Indeterminate results were grouped together with resistant strains.

Flow Cytometry

CD4 cells were enumerated by standard flow cytometry using a flow cytometer (EPICS 752, Coulter) and commercially available monoclonal antibodies (Ortho Diagnostics, Raritan, NJ).

RESULTS

Seven patients in this report expired at EMC during 1999 while under my medical care. Four were diagnosed as having wasting based upon a >10% weight loss from baseline. Their mean age was 30 years at the time when HIV-1 infection was diagnosed and they survived a mean of 9 years. The three patients who had no weight loss or lost <10% from baseline had a mean age of 36 years and survived a mean of seven years following HIV-1 diagnosis. These and other demographic data are summarized in Table 1.

The mean viral load in the wasting group was 51,000/ml, and the four patients averaged 29 $CD4^+$ cells/μl. The non-wasting group mean viral load was 110,000 while the $CD4^+$ cell count averaged 51/μl. Because of the small number of patients, these results do not appear significant. Genotyping of the HIV-1 strains isolated from the wasting patients showed nearly universal resistance to all but one or two antiretroviral drugs of the 17 tested. Conversely, two patients without wasting revealed antiretroviral resistance to one and nine of 17 drugs tested.

CC-CKR5 delta 32 mutation was found in a single patient. He survived 14 years after diagnosis of HIV infection, in contrast to $6^1/_2$ years for the remaining patients who tested negative for the mutation. These data are summarized in Table 2.

DISCUSSION

The development of wasting in HIV-infected patients has been extensively studied and relates to a negative energy balance. While wasting often results from inadequate caloric intake, resting energy expenditure is usually increased in persons with advancing HIV disease.[9] Increasing resting energy expenditure has been shown to correlate with both a rising HIV-1 viral load and elevated tumor necrosis factor alpha concentrations.[15,16]

In the small study described in this presentation, such a correlation between HIV-1 viral load and $CD4^+$ cell count and wasting could not be demonstrated. On the other hand, patients with wasting manifested much higher frequencies of genotypic resistance to all currently available antiretroviral drugs. This could imply that an elevated viral load consisting of resistant strains of HIV-1 generate more

Table 1 Summary of Clinical Data of Seven AIDS Patients Who Expired in 1999

Case No.	Age at Dx (Years)	Ethnicity	Associated Diseases	Opportunistic Infections	Neoplasia	Baseline Weight (kg)	Weight Loss (kg)	Years Survived with AIDS
1	29	Caucasian	Hypertension, asthma	OC, MAC CMV	KS	95	22	14
2	26	Hispanic	None	EC, CNS toxoplasmosis	Lymphoma	69	13	9
3	32	Hispanic	None	PCP	KS	84	16	5
4	31	Black	None	EC, PCP, MAC	None	70	16	4
5	39	Caucasian	Chronic hepatitis B, hepatic cirrhosis	E. coli Sepsis	None	76	0	9
6	39	Caucasian	Sinusitis	Cerebral mucormycosis	KS	76	6	7
7	31	Caucasian	Crohn's disease	OC, PCP, PML	KS	78	0	6

Note: PCP = pneumocystis carinii pneumonia; MAC = *Mycobacterium avium* complex; CMV = cytomegalovirus infection; OC = oral candidiasis; EC = esophageal candidiasis; PML = progressive multifocal leukoencephalopathy; KS = Kaposi's sarcoma.

Table 2 Presence of CC-CKR5 Delta 32 Mutation in Seven AIDS Patients Who Expired in 1999

Case No.	CC-CKR5 Mutation	Viral Load (μl)	$CD4^+$ Cells (mm^3)	Drug Resistance by Genotyping/Total Tested
1	+	41,800	37	16/17
2	–	77,100	42	16/17
3	–	69,500	15	16/17
4	–	17,000	3	15/17
5	–	39,200	138	1/17
6	–	301,000	19	9/17
7	–	200	7	9/17

proinflammatory cytokines, such as TNF-alpha, interleukin-6 (Il-6), and interleukin-1 (IL-1) than genotypically sensitive strains. This results in an increase in resting energy expenditure. In addition, systemic increases in TNF-alpha and IL-1 inhibit the appetite center in the ventromedial hypothalamus.[17] It is also interesting to note that poor gastric emptying was noted radiographically in two patients with wasting, yet upper endoscopy failed to disclose any disease visually or by biopsy. It is noteworthy that TNF- alpha has been shown to inhibit gastric emptying in rats.[18]

An infectious, metabolic, or neoplastic cause for weight loss was not conclusively demonstrated in any of the four patients with wasting. Biopsies of the upper and lower gastrointestinal tracts, and blood cultures for bacteria, acid fast bacilli, and fungi were negative, except in one patient with *Mycobacterium avium* infection who was receiving antimicrobial therapy with ethambutol, azithromycin, and intravenous amikacin. Also, in two patients with wasting, opportunistic infections occurred preterminally. One infection was caused by *Aspergillus* and resulted in extensive lung infection. A microbiologically undiagnosed brain abscess in one patient was revealed by head computerized tomography. In another patient with wasting and unexplained cardiomyopathy, heart muscle biopsy and electron microscopy revealed questionable elements suggestive of microsporidiosis.

Although it was not possible to detect definable infection or neoplasm in any of the patients with wasting, it is essential to exclude an underlying disease other than HIV-1 infection as the cause of wasting.

We have previously reported on the roles of intestinal microsporidiosis and intestinal infection by *Mycobacterium avium intracellulare*[19,20] as causes of malabsorption and wasting in patients with AIDS. I am confident that these etiologies were satisfactorily excluded in the four patients discussed in this report. Additionally, serum carotene levels in two patients with wasting were only minimally depressed.

It will now be necessary to study TNF-alpha, IL-6, and IL-1 blood levels in HIV-infected patients with and without wasting, to determine whether there is a correlation with viral genotypic resistance to antiretroviral drugs.

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CHAPTER 2

Supplementation and Undernutrition Affect Survival in Murine AIDS

Jeongmin Lee and Ronald R. Watson

CONTENTS

INTRODUCTION

Human immunodeficiency virus (HIV) infection is the hallmark of profound immune dysfunction that allows opportunistic infections in acquired immunodeficiency syndrome (AIDS) patients. HIV infection is estimated to affect 15 million people worldwide and continues to spread at an unabated pace. Since over 90% of new cases of HIV infection occur in developing countries, social behaviors and nutritional status became major issues to consider in issues related to progression of the infection to AIDS.[1]

It is true that most studies of the relation between micronutrient deficiency and progression to AIDS have been conducted with human subjects, and they provided data leading to understanding the pathogenesis of HIV. In most studies, serum was used to determine the levels of nutrients. However, serum levels are sometimes considered to have limitations dependent on severity of illness and sample size of study. For some micronutrients, serum levels may not be the most sensitive indicators of status. On the other hand, because no RDA levels have been established for HIV-

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infected individuals, there is some disagreement as to which biochemical cut-off point defines deficiency.

Thus, we still face many unanswered questions regarding nutrition and HIV infection, for example, whether low serum micronutrient levels are primary or secondary effects of HIV, and whether micronutrient intake might actually be a cofactor in the development of AIDS. The animal model may prove effective for studying survival and the relation between nutritional status and HIV infection in humans. Thus, the purpose of this review is to provide insights into the association between micronutrient deficiencies and progression to AIDS, using the murine model of human AIDS.

MICRONUTRIENT DEFICIENCY AND IMMUNE DYSFUNCTION IN MURINE AIDS

Evidence from animal models indicates that altered nutritional status may influence the course of murine AIDS progression and survival.[2] Although micronutrient status is not likely to be the most important etiological determinant, it may alter immune function to facilitate disease progression, influence viral expression, and have a significant impact on survival. Since some micronutrients play essential roles in maintaining normal immune function, micronutrient deficiencies may exacerbate host immunity in retrovirus-infected mice.

Low serum vitamin B-6 positively correlated with lymphocyte response to mitogen.[3,4] An early study by Stoerk on the effects of vitamin B-6 deficiency on immune function found that the thymus glands of vitamin B-6 deficient rats were essentially depleted of lymphocytes and consisted almost entirely of epithelial cells and stroma.[5] Lymphocyte maturation in the thymus was also affected. Willis-Carr reported that thymic epithelial cells of rats fed a vitamin B-6-deficient diet for two weeks were unable to induce maturation of lymphoid precursors.[6] More recent studies suggest that functional subpopulations of lymphocytes may be differentially and selectively affected by vitamin B-6 deficiency.[7]

Using five-week-old female C57BL/6 mice fed low pyridoxine diets, primary and secondary splenic and peritoneal T-cell-mediated cytotoxicities were reduced. However, phagocytosis of sheep red blood cells (SRBC) by macrophages and native and interferon-induced natural killer (NK) cell activities were not affected by dietary levels of vitamin B-6. The other important finding was that increasing the dietary level of vitamin B-6 to as much as seven times the requirement did not further alter immunocompetence, suggesting that megadoses of the vitamin do not produce benefits beyond those observed with moderate supplementation.

Vitamin A deficiency increases susceptibility to disease and, in animal studies, impairs both humoral and cell-mediated immunity.[4] Vitamin A also plays a central role in the growth and function of T and B cells and antibody response.[8]

Some evidence exists that vitamin E protects T cells, B cells, and other immune effector cells against oxidative stress.[9] Since oxidative stress may be a potent inducer of viral activation and DNA damage in virus-infected cells, producing

one of the long-term consequences of HIV infection, immunosuppression, reduced levels of potent antioxidants such as vitamin E may be correlated with the replication of murine retrovirus.[10] Rats that consumed diets deficient in vitamin E and received injections of endotoxin showed more anorexia and had higher IL-6 levels than animals consuming adequate amounts of vitamin E.[11]

Our previous study with female C57BL/6 mice suggested that the increased free radicals produced during murine retrovirus infection cause the increased utilization of antioxidants including membrane vitamin E, resulting in increased tissue lipid peroxidation.[12] Cytokine-induced oxidative stress is also an important factor in enhancing replication of murine retrovirus.[13] In addition, we have previously shown that vitamin E supplementation at dietary intakes of 15 to 450 times greater than the level in the control diet partially normalized immune dysfunction. However, this effect decreased as the infection progressed to murine AIDS.

In the murine model, coenzyme Q_{10} has recently been found to be involved in transplasma membrane electron transport involved in the control of cell growth, and was also implicated as an antioxidant protecting membranes in which it resides.[14] Serum β-carotene concentration is known to be deficient in HIV-infected persons with or without malnutrition. The most likely mechanism for the β-carotene deficiency may be related to impairment of free radical elimination and failure to protect cellular membrane against lipid peroxidation.[15] Deficiency of selenium is associated with glutathione peroxidation activity, cardiomyopathy, immune dysfunction including impaired phagocytic function, and decreased CD4 T-cells.[16,17] In addition, zinc deficiency led to reduced cytotoxic lymphocyte numbers, decreased responses to mitogens, decreased NK cell activity, reduced production of interleukin-2 (IL-2) by T cells, and reduced T cell-dependent antibody production.[18,19]

MECHANISMS OF PREMATURE DEATH IN MURINE AIDS

The recent study in our laboratory suggested that reduced micronutrient intake alone can severely influence survival of mice. Murine retrovirus infected mice consuming reduced micronutrients died earlier than uninfected mice that consumed reduced micronutrients. Reduced micronutrient intake for 12 weeks reduced T and B cell proliferation beyond the immune suppression induced by LP-BM5 murine retrovirus infection. Inhibition of mitogenesis was more severe in T cells than B cells. Production of Th1 cytokines was decreased after consumption of diets with reduced levels of micronutrients, while the secretion of Th2 cytokines was significantly increased. Hepatic vitamin E levels were substantially lowered, resulting in a significant increase of hepatic lipid peroxidation.

The premise that micronutrient deficiencies play a role in progression to murine AIDS is based on two possible mechanisms: immune dysregulation and excessive free radical production. Thurnham reported that the main effect of micronutrient deficiencies is the reduction in cell mass that indirectly affects immune cell function, particularly when T cell numbers are reduced.[4] Decreased cell division was expected due to reduced release of IL-2, a major T cell growth factor. It is thought that reduced

micronutrient intake during progression to murine AIDS involves cytokine dysfunction, since micronutrient deficiencies can modify cytokine production or receptor expression, and cause a shift from balanced Th1 and Th2 cell secretion of cytokines to increased Th2 and decreased Th1 cell cytokine production.[20,21]

In human HIV$^+$/AIDS patients and mice with murine AIDS, T cell proliferation and IL-2 production decline, while IL-4, IL-5, and IL-10 production increases.[22,23] Blocking Th2 cell activation and its excessive cytokine production should retard development of murine AIDS. When IL-4-deficient mice (IL-4 gene knockout) with suppressed Th2 cytokine production were infected with LP-BM5 retrovirus, the usual lethality and the development of T cell abnormalities were delayed.[24] Administration of anti-IL-4 monoclonal antibody in LP-BM5 retrovirus-infected mice also maintains balance of Th1 and Th2 responses, preventing retrovirus-induced suppression of immune responses.[25] Severely reduced micronutrient intake independently altered cytokine secretion, a possible cofactor in perpetuating the cytokine imbalance and increasing the rate of murine AIDS progression.

As the other possible mechanism of premature death, excessive free radical induction may be a result of retrovirus infection and micronutrient deficient diet. For instance, as evidenced by the reduced production of IL-2 during low selenium and zinc intake, most cytokine alteration may be due to deficiency of antioxidant nutrients. Pro-inflammatory cytokines (IL-1, IL-6, and TNF-α) and reactive oxygen species (ROS) are mutually stimulatory.[18,26] The stimulation of cytokine production by ROS involves activation of nuclear factor kappa B (NF-κB) which induces retrovirus replication. Attack by ROS results in the detachment of the inhibitory component from the NF-κB complex and leads to transcription of genes for synthesis of pro-inflammatory cytokines.

In conclusion, low survivability in mice with reduced micronutrient intake might be caused by immune dysfunction and increased oxidative stress exacerbated by murine retrovirus and its reduced tissue antioxidants.

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CHAPTER 3

Antioxidants in Human AIDS

Jeongmin Lee and Ronald R. Watson

CONTENTS

INTRODUCTION

Human immunodeficiency virus (HIV)-1 infection results in the progressive impairment of immune response leading to the development of the acquired immunodeficiency syndrome (AIDS). Among the mechanisms contributing to this progression, oxidative stress induced by the production of reactive oxygen species (ROS) during activation of polymorphonuclear leukocytes and macrophages may play a critical role.[1]

The infection due to HIV-1 is clinically characterized by a long latency period before the onset of AIDS. One of the essential steps in the development of AIDS is the activation of the latent provirus. It has been suggested that the activation of provirus is associated with the excessive production of ROS.[2] In fact, prospective clinical trials have documented an excessive production of ROS in the HIV-infected population, regardless of the extent of their immunosuppression, based on measurements of lipid peroxidation indices in plasma and expired breath.[3,4]

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Excessive ROS production not appropriately compensated by cellular antioxidants can play an pivotal role in pathogenesis of HIV infection through various mechanisms: (1) increased production of proinflammatory cytokines,[5] (2) activation of NF-κB transcription factor,[5] (3) increase of viral burden due to increased HIV replication,[6] and (4) T lymphocyte depletion due to triggering apoptosis.[7] Virus-infected cells display very low levels of antioxidant defenses such as superoxide dismutase Mn, vitamin E, selenium, and glutathione (GSH). Moreover, patients undergoing HIV infection display high serum levels of xanthin oxidase (XOD) and lipid peroxidation,[8] both markers for oxidative stress situations. Furthermore, opportunistic infection from *Mycoplasma* can participate in AIDS progression by induction of oxidative stress mechanisms.[9] Therefore, the roles of oxidative stress due to excessive ROS and antioxidant supplementation in HIV pathogenesis have received considerable attention in recent years.

The purpose of this review is to discuss recent developments related to oxidative stress and antioxidants, and to suggest the possibility of treatment of human AIDS.

ANTIOXIDANT DEFENSE IN HIV INFECTION

HIV-1 activation from the latent state can be stimulated with ROS. Activation is due, at least in part, to the stimulation of oxygen-responsive transcription factors including NF-κB and AP-1, which can activate HIV-1 gene expression from regulatory elements located in the viral long terminal repeat (LTR).[1,10] Conversely, activation can be inhibited by vitamins, trace elements, and enzymes with antioxidant properties. These include vitamin C, vitamin E, glutathione, glutathione ester, NAC, catalase, and glutathione peroxidase.

Of these antioxidants, α-tocopherol is the most potent and abundant lipophilic antioxidant *in vivo* as well as an immunoenhancer.[11] The circulating vitamin E is considerably decreased in HIV-seropositive patients after progression to AIDS stages.[4] Vitamin E is a main antioxidant of cell membranes and plasma lipoproteins, and a deficiency of vitamin E is related to the increase of lipid peroxidation. However, paradoxically, lipid peroxidation is more significant in asymptomatic HIV subjects than it is in AIDS patients.[12] In early HIV-1 infection, elevation of plasma IgE levels precedes the decline of $CD4^+$ T lymphocyte counts and is influenced by vitamin E status.[13] Other antioxidant species, such as ascorbic acid, carotenoids, and selenium, were found to be depleted in HIV-infected patients.[14] Altered levels and/or activities of antioxidant defense enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, as well as increased production of lipid peroxidation products, such as malondialdehyde and 4-hydroxy-2-nonenal (4-HNE), have also been observed in HIV-infected patients.[12] Vitamin C is the major water-soluble antioxidant and acts as the first defense against ROS in whole blood and plasma.[15] In addition, a cooperative interaction exists between the two vitamins. Vitamin C is important in regenerating vitamin E during the antioxidant defense process.[16]

Several studies have indicated that HIV-infected cells have altered antioxidant defenses. Acutely and chronically infected human T cells exhibited reduced thiol levels as well as decreases in the antioxidant enzymes, thioredoxin, superoxide

dismutase, and catalase. Moreover, cysteine, a component amino acid of glutathione, is depleted in plasma of both asymptomatic and symptomatic HIV-infected patients.[17] More recently, of particular interest, is the role of the cytosolic form of glutathione peroxidase, GSHPx-1, in influencing the course of an HIV infection.[18] GSHPx-1 is a selenium-dependent enzyme capable of detoxifying both hydrogen and lipid peroxides, and represents a major cellular defense against ROS. GSHPx-1 levels have shown to be reduced in symptomatic HIV-1-infected individuals and in chronically infected cell lines. Levels of GSHPx-1 may be relevant to the apoptotic responses of T cells to viral infection. T cells demonstrating an HIV associated deficiency in GSHPx-1 activity were more susceptible to apoptosis induced by exposure to lipid hydroperoxides.[19] In addition, elevated GSHPx-1 activity was shown to functionally mimic bcl-2 expression with regard to the inhibition of apoptosis following interleukin withdrawal from human T cells.[20] Especially noteworthy in this regard is the recent report that poxvirus DNA includes a gene that is 74% identical to the human GSHPx-1 gene, including an in-frame TGA triplet that corresponds to the selenocysteine-encoding codon of GSHPx-1.[21] The genomes of several mammalian viruses have been shown to contain genes that function in the inhibition of the apoptotic responses of host cells. The presence of a poxvirus glutathione peroxidase gene suggests that this viral protein may also function as an anti-apoptotic adaptation.

N-acetylcysteine (NAC), an important thiol group supplier, has been shown *in vitro* to inhibit apoptosis induced by pro-oxidant cytokine tumor necrosis factor α (TNF-α), to decrease the number of viral particles in HIV-chronically infected U937 cells,[22] and to revert the impaired proliferative activity of purified $CD4^+$ lymphocytes from HIV-infected patients; NAC acts *in vivo* to counteract the immunological consequences of cysteine and glutathione deficiency, has favorable effects on $CD4^+$ lymphocyte survival when administered to HIV-infected individuals, and reduces the decline in $CD4^+$ lymphocytes when administered to HIV-positive patients.[17] It has been postulated that an increase in the plasma index of peroxidation in HIV-positive patients, probably owing to an excess of circulating oxidized low-density lipoproteins, may affect several immune functions including natural killer cell activity.[23] In contrast, a number of *in vitro* and *in vivo* data suggest that the antioxidant supply could significantly improve immune response and survival. Thus, administration of the GSH prodrug NAC may be potentially advantageous in HIV-infected patients.[24] However, NAC efficacy is limited by its poor bioavailability and its use is still disputed.

Losses of essential coenzyme Q_{10}, β-carotene, and selenium, are associated with immune dysfunction caused by HIV.[25] Coenzyme Q_{10} has recently been found to be involved in transplasma membrane electron transport involved in the control of cell growth, and also been implicated as an antioxidant, protecting membranes in which it resides.[26] Serum β-carotene concentration is known to be deficient in HIV-infected persons with or without malnutrition. The most likely mechanism for the β-carotene deficiency may be related to impairment of free radical elimination and failure to protect cellular membrane against lipid peroxidation.[26] Deficiency of selenium is associated with glutathione peroxidation activity, cardiomyopathy, immune dysfunction including impaired phagocytic function, and decreased $CD4^+$ T cells.[27] Thus, mineral deficiencies could accentuate immune dysfunction in AIDS.

CYTOKINE ALTERATION IN HIV INFECTION

A number of studies have shown that a decrease in antioxidant defenses or enhanced oxidant concentration in tissues increases a number of aspects of the inflammatory response due to unbalanced production of cytokines. Most cytokine alteration may be due to deficiency of antioxidant nutrients and/or less uptake of exogenous antioxidants. Pro-inflammatory cytokines (IL-1, IL-6, and TNF-α) and ROS are mutually stimulatory.[28] The stimulation of cytokine production by ROS involves activation of nuclear factor kappa B (NF-κB) which induces retrovirus replication. For example, attack by ROS results in the detachment of the inhibitory component from the NF-κB complex. The activated transcription factor migrates to the nucleus. At the molecular level, the transcription of HIV genes is directed by sequences called LTR, located at the extremities of the viral genome.[29] These elements present binding sites for viral (i.e., Tat) and cellular transcription factors (i.e., NF-κB). Interestingly, NF-κB can be strongly activated by ROS and pro-inflammatory cytokines (i.e., TNF-α) to induce the expression and replication of HIV which results in transcription of genes for synthesis of pro-inflammatory cytokines. The process is prevented by treatment with antioxidants such as vitamin E derivatives, NAC, and alpha-lipoic acid.[29]

$CD4^+$ CELL DEPLETION AND ANTIOXIDANTS

The most widely available marker of immune system destruction in the HIV-positive patient is a reduction in the number of circulating $CD4^+$ cells.[30] The $CD4^+$ cell count provides a measure of the degree of immune system damage already sustained by the patient and estimates the potential to respond effectively to pathogens.[31] Therefore, this laboratory marker is an excellent predictor of pending risk of HIV-associated opportunistic infection and guides the prophylactic use of antimicrobial medications to prevent the appearance of these infections.[32] As measured by flow cytometry, normal $CD4^+$ counts range from 600 to 1600 cells/mm^3 of blood, with a median count of approximately 1000 cells/mm^3. Initial immune suppression, indicated by a $CD4^+$ count of fewer than 500 cells/mm^3, signals the first appearance of systemic and oral opportunistic infections. Life-threatening infections increase in number and severity with further declines in cell count. With appropriate treatment, severe immune suppression (<200 cells/mm^3) can be reversed in most cases.[33]

The depletion of uninfected $CD4^+$ lymphocytes via apoptosis is thought to be an important contributor to the development of AIDS.[7] Markers of programmed cell death are rarely observed in productively infected cells. Conversely, HIV-1 mRNA products are rarely detected in apoptotic cells. Therefore, other factors of cellular or viral origin must be responsible for this depletion. In fact, synergistic interactions between viral proteins such as gp120 and Tat and cytokines such as IL-1 and TNF-α rather than intracellular virus multiplication are implicated.[34,35] Recently, it has been demonstrated that the HIV-1 Tat protein may prime T cells to undergo apoptosis by inducing a state of enhanced oxidative stress within sensitive cells.[36] This alteration results from diminished levels of cellular antioxidants. Furthermore, addition of purified Tat to normal uninfected peripheral lymphocytes induces apoptosis of the

T cell subset.[37] Therefore, HIV-1 Tat may be responsible for the decreased levels of reduced GSH seen in HIV-1-infected individuals and may ultimately be responsible for the depletion of $CD4^+$ T cells observed during AIDS progression.

Selective early loss of $CD4^+$ and $CD8^+$ cells with high GSH content correlates with $CD4^+$ cell depletion.[38] Glutathione has been suggested to attenuate HIV replication by the selective inhibition of envelope glycoproteins.[39] Consequently, a significant correlation between the progression of the disease, i.e., the decrease in $CD4^+$ lymphocyte number, and oxidative stress susceptibility was found. Furthermore, the thiol supplier NAC was generally capable of counteracting this susceptibility.[19] Lymphocyte function is affected by the intracellular effects of antioxidants, which probably act by interfering with tyrosine phosphorylation mediated by $p56^{lck}$ in response to engagement of the T cell receptor through inactivation of CD45, which critically depends on the supply of sulfhydryl compounds.[19] Thus, antioxidant treatment of lymphocytes from HIV-positive subjects may result in the recovery of phosphatase activity and lymphocyte proliferative responses.

VIRAL LOAD IN HIV INFECTION AND ANTIOXIDANT SUPPLEMENTATION

Explosive data now demonstrate that a single measurement of the level of HIV RNA in a plasma sample is highly predictive of the subsequent clinical course in an individual patient. Quantitative measures of HIV-1 RNA have been used extensively in the study of HIV-1 viral dynamics, individual prognosis, and as a surrogate marker for clinical end points in studies of new treatment regimens.[40] Studies on the dynamics of HIV-1 replication using quantitative HIV-1 RNA viral load assays have demonstrated that the virus, far from being latent during the long clinically asymptomatic phase of the disease, is, in fact, highly active.[41,42] As many as 100 million virus particles are produced each day by HIV-1-infected host cells and, although the immune system may limit the infection, HIV is never eradicated from the host. The lymph nodes appear to be the most active sites for HIV-1 replication,[43] and it is clear that the level of HIV-1 replication in infected individuals is very important to the rate of progression to AIDS and death. This relationship between baseline HIV RNA viral load in plama and risk of progression to AIDS is statistically significant.[44] Patients with viral loads of <4530 copies/ml progress to AIDS at significantly slower rates than patients with viral loads between 4531 and 13,020 copies/ml. The biggest difference in progression to AIDS is between those with viral loads of <4530 copies and those with viral loads of >36,270 copies/ml. This observation of the risk of high viral load leads to the belief that the most effective way to treat HIV-1 was to hit the virus as hard as possible for as long as possible.[45] As a result, it has become common practice to regularly monitor HIV-1 RNA viral load in order to assess the desirability of combination therapy and the effectiveness of treatment.

Zidovudine or AZT (azidothymidine) is an antiviral drug that stops virus replication by interfering with the viral reverse transcriptase and inhibiting DNA synthesis. Interestingly, antioxidants such as N-acetyl cysteine (NAC) and dithiocarbamate are useful agents in AIDS treatment, enhancing the therapeutic effects of

zidovudine.[46] The mechanism of action may be related to the ability of NAC to restore GSH levels more effectively than administration of zidovudine alone. Patients undergoing long-term treatment with AZT develop destructive mitochondrial myopathies with ragged red fibers, a typical pathology strongly related to oxidative stress.[49] For this reason, the coadministration of antioxidants such as NAC and vitamin E significantly reduces the cytotoxic effects of zidovudine.

CONCLUSION

The widespread use of highly active antiretroviral treatment (HAART) should encourage physicians to consider the many facets of the damage inflicted by HIV, which affects lymphocyte integrity in terms of both the cytoskeleton and the redox state. The effects of increased oxidative stress must be considered in order to understand the functional defects observed in HIV disease progression, leading to increased susceptibility to apoptosis and subsequent loss of $CD4^+$ cells. An early treatment associating HAART with antioxidants may therefore be even more beneficial to patients in the long run.

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CHAPTER 4

Trace Elements, Free Radicals, and HIV Progression

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CONTENTS

INTRODUCTION

Three years ago, a review was published on the acquired knowledge of the relationship between the status of trace elements, oxidative stress in the HIV infection, and their eventual implications for the progress of the disease.[1] In effect, a

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number of documents have revealed the presence of anomalies in trace element status in HIV-infected subjects. A decrease of the plasmatic concentration of selenium was brought into evidence in practically all the studies of adult cases as well as in cases of children; the decrease accentuates itself as the disease progresses. The decrease of zinc is often present, but it generally remains moderate; it is more frequent in disease treated with AZT. This moderate drop can have an effect on the immune function. Copper anomalies in blood are rarer. These modifications of the status of trace elements, like those of vitamins (vitamins A and E and carotenoids), increase oxidative stress and influence viral replication and the latency period of the HIV infection.

Since this review, tritherapy has been instituted, at least in developed countries. It has changed the evolution of the disease so that severe malnutrition linked with malabsorption and the wasting syndrome, which were frequently encountered, are no longer the main signs of the infection. Thus, although nutritional factors are certainly not essential determinants in the disease evolution, they can have a significant impact on disease expression, morbidity, and mortality. Adequate nutritional intake by those infected with the disease can contribute to their well-being, improve their immune defenses, and lessen oxidative stress. Furthermore, identification and correction of micronutrient deficiency remain of great importance because the effectiveness of tritherapy diminishes in a growing number of HIV-infected people, about 90% of infected people have no access to the therapy, and the price of possible supplementation is low. Moreover, substantial advances have been made in the biological understanding of the HIV infection, and trace elements and oxidative stress play important roles.

Trace Element Status in the HIV Infection

Several general factors such as other micronutrients can contribute to the deficiency of trace elements in the HIV infection. The supplies can be diminished through loss of appetite and dysphagia. Several studies report that HIV^+ subjects do not consume the quantities of trace elements recommended by the RDA.[2] Malabsorption and diarrhea also contribute to the inadequate status of trace elements. Among HIV^+ adults with moderate deficiencies living in industrialized countries, pregnant women and children are the groups at risk, and the deficiencies are more frequent and more pronounced in infected people in developing countries.

Selenium

Our previous article[1] stated that selenium in blood decreases through the course of the HIV infection. More recent documents confirm these earlier studies. The serum concentration of selenium is lowered in the early stage of the infection, before clinical signs appear and nutritional deficiencies are noted. The decrease accentuates itself through the course of the infection progression. A correlation exists between the serum concentrations of selenium and the number of $CD4^+$ cells, and a negative correlation exists between selenium and β_2 microglobulin. The decrease of selenium is more important when there are opportunist infections.[3,4] Selenium deficiency is

strongly associated with decreaseed survival; it is a predictive element of the seriousness of the disease. That is not true for other micronutrients such as vitamins A, B_{12}, B_6, and zinc[5] which also diminish through the course of the infection. Identitical results have also been found in HIV^+ children.[6]

Among measured parameters (albumin, iron, zinc, selenium), only selenium deficiency is associated with a high risk of mortality, in cases of children (RR = 5.96). The risk is even greater in adults (RR =10.6)[5-7] and independently of the number of $CD4^+$ cells. In children, an increase of selenium deficiency prevalence was found in relation to the disease progression with 2 to 4% in asymptomatic subjects, 33% in stage II, and 75% in stage IV of AIDS.[6] Se is not modified uniquely in plasma. Chariot et al.[8] observed that it is diminished in the muscles, particularly in HIV^+ subjects with muscular symptoms. The decrease of Se can be at the origin of the dilated cardiomyopathy, as sometimes observed in those infected with HIV.[9,10] Correlations between selenium deficiency and disease severity are found in other viral diseases like hepatitis B and cocksackie virus P3.[11] It should nevertheless be noted that not all of the studies found the Se deficiency.[12] Several grounds have been advanced to explain this Se deficiency and its implications on the severity of the disease. At first it was thought that selenium deficiency, like deficiencies of other nutrients, was related to malabsorption, caloric and protein malnutrition, and the wasting syndrome. This does not explain selenium deficiency predominance.

Otherwise, it is known that Se formed as selenoproteins assumes different functions. Eleven selenoproteins have been identified. The four glutathion peroxidases (Gpx), thioredoxine reductase, and selenoprotein P play key roles as antioxidants and in detoxification of radical oxygen species (ROS).[13-15] More recently, it has been shown that the viruses can code for selenoproteins. Taylor et al.[16] were the first to put forward this hypothesis, confirmed by other workers.[17,18] They have identified in the pox virus *Molluscus contagiosius* a functional GPx presenting a 76% analogy with mammal GPx. HIV can equally code for a plasmatic GPx homologue. This molecule, although truncated, contains the enzyme catalytic site.[19]

Through the course of the HIV infection, there is a decrease in the synthesis of cellular selenoproteins of the host and an increase in viral selenoproteins.[20] This could explain why the effects of the infection from HIV were exacerbated in subjects deficient in selenium. The interest of the virus to code a GPx is to assure its defense against free ROS released by immune cells or to increase the viability of viral particles when they are in extracellular compartments or at the time of their attachment to the host cells. Glutathione concentrations are weak in plasma. This GPx could function with glutaredoxine or thioredoxine.[21] A viral GPx could also have a repressive effect on viral replication which is increased through the ROS (H_2O_2). This would allow the virus to maintain a low profile when the immune system is active.[21]

Zinc

Zinc plays an important role in the HIV infection. Its concentration can influence the transcriptions of numerous genes, in particular, genes implicated in the immune system. In this way, zinc participates in the regulation of the growth, development, and functions of neutrophils, macrophages, natural killer cells, and T and B lymphocytes.[22]

Zinc deficiency can change immune functions from predominantly cellular Th1 responses to humoral Th2 responses. HIV multiplies preferably in Th0 and Th2 cells and not all in Th1 cells,[23] and it modulates the HIV virus replication. HIV protease, an enzyme which cuts serially linked virus elements so that they can combine to form new infectious viral particles, remains inactive as long as sufficient zinc ions are bound to it. This enzyme is activated as the availability of Zn or copper together with cysteine in and around host cells is low enough.[24]

In vitro, Zn is equally an inhibitor of the reverse transcriptase of the HIV virus.[25] The nucleocapside proteins, particularly NCP7, depend on the Zn bound to them in order to maintain their form, stability, and functionality.[26-27] Zn also plays an important role in the brain; its deficiency could play a role in AIDS dementia complex. Zinc is present in the synaptic vesicules in a group of glutaminic neurones and it modulates the response of inhibitor or excitor receptors, particularly of NMDA (N-methyl-d-aspartic acid) and GABA (γ-aminobutyric acid).[28] In cerebral tissue, as in other tissues, Zn constitutes a part of the defense system against oxidative stress because it binds to thiol groups and prevents their oxidation. It induces metallothionein (MT) synthesis and metallothioneins are also good ROS scavengers. MTIII offers protection against neuropathology in the brain.[29] It is a constituent of Cu-Zn-SOD and it is a powerful inhibitor of nitric oxide synthase (NOS).

Deficiencies of plasmatic zinc have often been found in HIV$^+$ patients; they are generally more moderate than those of selenium. It should be noted that plasmatic zinc is not always the best reflection of zinc status. For example, in the study of Baum et al.,[30] the average rate of plasmatic zinc is set at the lowest limit of normal values. In a group of 125 subjects, 57% of women and 45% of men have concentrations lower than 0.75 mg/l with a relative risk of mortality at 2.29. There is a significant inverse correlation between HIV-RNA concentration and Zn or CD4 number and Zn.[31] However, the zinc deficiency was not found in all studies. Henderson et al. [12] found rates in normal range in 38 cases of HIV$^+$ children, which included children presenting growth deficiencies.

Zinc is a key element in statural growth and cerebral development. In another study conducted on 24 children, the ratio of zinc was found to be lower than 0.75 mg/l in 11 children. No association exists between zinc deficiency and an increase of mortality.[6] A recent study[32] reports the prevalence of deficiencies of micronutrients (vitamins and trace elements) in HIV$^+$ patients.

As with other nutrients, zinc deficiency can be caused by a lack of supply. It effectively seems that the supplies are lower than RDA recommendations and are weaker in women than in men.[30] However, several other reasons can be considered. The intense immune and inflammatory responses to the HIV infection can diminish the plasmatic ratio of zinc.[33] The response can pass through the intermediary of the secretion of TNFα and IL1, and deplete plasmatic zinc. Zn deficiency can thus be linked to its usefulness for the replication of the HIV virus.

Copper

In combination with cysteine, copper ions are also able to keep HIV-1 protease in activated form.[24] The plasmatic concentrations are often increased in the presence

of HIV infection, as noted throughout the literatture.[1] Published works confirm these results. Copper is increased in the population of pregnant HIV+ women in Zimbabwe.[35] Identical results were obtained from a group of HIV+ adults. Copper increases at the onset of infection, then remains constant through the course of the disease progression. There is no correlation between serum copper and the other nutritional parameters or the number of CD4 cells.[36]

Iron

Iron is not, strictly speaking, a trace element, but recently published works have discussed the modifications of its metabolism through the course of the HIV infection. During infection, malabsorption of iron, folates, and vitamin B_{12} and anaemia can often be observed.[37,38] Anomalies of iron metabolism are superimposed on those described for inflammation.[39] Decreases of serum iron, transferrin, and hemoglobin have been noted, as has an increase of ferritin.[40,41] This last compound could be an independent marker of infection progression.[42]

These anomalies are well established in patients in the advanced stage of the infection. Are they the results of opportunistic infections? It seems that they are consequences of the HIV infection because they occur from the onset of the disease.[43] Complexes could form between the p24 protein of the virus and hemoglobin, leading to a decrease of hemoglobin.[44] The decrease of iron serum accompanies the increase of iron linked to ferritin and hemosiderin in numerous tissues: bone marrow, white cerebral substance, skeletal muscles, and liver.[45] However, other authors find that bone marrow biopsies and serum studies showed that over a third of symptomatic HIV patients had iron deficiencies.[46]

Several mechanisms have been proposed to explain this iron excess:

1. The inflammatory response, which prolongs during the disease, increases the production of cytokines IL1, IL6, and TNFα, which induce the synthesis of ferritin through the macrophages. Ferritin sets iron. This mechanism sequesters the iron in these cells, adding their return toward plasma. It is a protective mechanism of healthy cells against the excess of iron.[45,47,48]
2. Transfusions in order to treat the anaemia.
3. The therapeutic role of the AZT interferes with the biosynthesis of heme and stimulates the absorption of iron.[49]
4. Cigarette use leads to iron excess in alveolar macrophages.[50]

This excess of iron can have a series of consequences. It increases sensitivity to infections by decreasing the effectiveness of part of the immune system[40] and by increasing the multiplication of microorganisms for which iron is an indispensable element.[51,52] Cytomegalovirus infections are more frequent in HIV+ patients.[51] Patients with *Pneumocystis carinii* pneumonia have elevated nontransferrin-bound iron in the bronchoalveolar lavage fluid.[53] The iron loads of smokers' alveolar macrophages can also explain the increased HIV replication in such cells *in vitro*.[54,55] Furthermore, HIV negatively modulates the expression of transferrin receptors (CD71) on lymphoid cell membranes which seems to be parallel to the

cytopathogenicity of the virus.[56] Therefore, the metabolism of iron plays an important role on disease progression and morbidity and the accumulation of iron seems associated with a shorter survival.[57]

Oxidative Stress

The roles of ROS and oxidative stress in general in HIV pathogenicity have received considerable attention in recent years. Some modifications of the balance of oxidants/antioxidants and an increase of oxidative stress have been observed since the debut of the HIV infection, even when patients are asymptomatic, and the imbalance enhances in symptomatic subjects. This increase of oxidative stress is multifactorial: it is due to the diminution of antioxidant nutrients and the increase of the formation of free radicals by the virus or by some of its proteins. The increase of free radicals leads to increased viral replication and an increase of host cell apoptosis, particularly of CD4 cells. Some of the mechanisms have already been explained in our synopsis of recent publications.[1]

Decrease of Defenses

Antioxidant vitamins are equally diminished. The global decrease of the defense system against oxidative stress is illustrated by a study by Malorni et al.[58] When peripheral mononuclear blood cells (PBMCs) from healthy blood donors or from HIV^+ patients are subjected to oxidative stress from menadione, which induces the formation of $O_2^{-}\cdot$, there is a correlation between the sensitivity of PBMC oxidation and the progression of the disease.

N-acetylcysteine (NAC) partially protects the integrity of the PBMCs through the course of the oxidative stress. Selenium, diminished almost constantly in HIV^+ subjects, plays a vital role in the fight against free radicals. Formed as selenocysteine, it is the active site of GPx. GPx detoxifies H_2O_2 and lipid peroxides using reduced glutathione (GSH). This reaction prevents the formation of an extremely toxic hydroxyl radical. Through the course of the reaction, GSH is transformed into GS-SG and regenerated, thanks to NADPH and glutathione reductase. Thus, adequate concentrations of Se and GSH are necessary in order for the system to function.

This is well exhibited in a study by Sandstrom et al.[59] that showed cells over-expressing GPx accelerated viral replication and associated cytopathogenetic effects. A progessive depletion of GSH or plasmatic thiols has been observed through the course of the HIV infection.[60] This decrease is directly correlated with the number of CD4 cells and inversely correlated with the viral load.[61] The erythrocytes and T lymphocytes are equally depleted.[62] There is a poor survival rate for HIV-infected individuals with lower GSH levels. Survival rate improved when GSH was replenished and maintained.[63]

Glutathione has been suggested to attenuate HIV replication by the selective inhibition of envelope glycoproteins.[64] This decrease occurs rapidly at the beginning of the infection. The mechanisms of the decrease are not clearly understood. Since the plasma cysteine precursor of glutathione is also diminished, it could be a limiting factor in the synthesis of GSH. In effect, NAC supplementation increases the rate

of synthesis of GSH concentration in erythrocytes,[65] but the presence of the Tat viral protein could explain the decrease of intracellular GSH.[66] The HIV is able to synthetize selenoproteins with GPx activity and these proteins are capable of taking action on suppressed GSH cells. This can have important therapeutic implications, particularly on therapies which concern supplementation.

Increase of the Production of Radical Oxygen Species (ROS)

Chronic infection increases the production of ROS through immune cells.[67] Iron accumulation in tissues can be a source of ROS. In effect, iron (Fe^{++}) plays an essential role in the formation of a particularly toxic $OH^{-}\cdot$, formed from H_2O_2. Normally, iron linked to proteins is sequestered and does not play a part in the formation of ROS; however, this is not true in cases of excess. This can be illustrated through the phenotype relation of haptoglobin and the progression of the HIV infection. Haptoglobin and hemoglobin form a complex which is rapidly eliminated from plasma. Hence, haptoglobin possesses an antioxidative role in eliminating iron from Hb.

Three phenotypes exist (Hp 1.1; Hp 1.2; Hp 2.2). Hp 2.2 less effectively links Hb and, therefore, has a weaker antioxidating role. HIV^{+} patients with an Hp 2.2 phenotype have a better survival rate than patients with other phenotypes.[68]

The Tat protein is a transcriptional factor of the HIV. Tat links itself to a region of the 5′ untranslated sequences of all RNA viruses (a region called the transactivation response element). Thus, Tat activates viral replication. It also cooperates with the cellular transcriptional factor Sp1. Therefore, it modifies the expression of cellular genes and, in particular, it represses the expression of the mitochrondrial Mn superoxide dismutase (Mn-SOD); it does not act on the Cu-Zn-SOD.[69,70] SODs are enzymes which degrade superoxide anions. The inhibition of this enzyme increases oxidative cellular stress. The Tat protein, as has already been indicated, diminishes intracellular concentration of GSH.[71] The presence of iron and $OH\cdot$ seems necessary to promote the effects of Tat.[72] The Tat protein is toxic for neurons *in vitro*; it induces the activation of caspases, progressive elevation of intracellular and intramitochondrial Ca^{++}, and generation of ROS leading to neuronal death.[73]

The HIV gp120 protein amplifies *in vitro* the activity of tumor necrosis factor α (TNFα); it regulates mRNA for this cytokine[74] and activation of the transcription NFκB factor in inducing intratissular formation of H_2O_2 and a decrease of the GSH/GS-SG ratio. This gp120 effect is inhibited by antioxidants such as butylated hydroxyanisole.[75] The viral gp160 protein also very rapidly induces the production of H_2O_2.[76]

NO (nitrogen monoxide) can play an important role in the constitution of cerebral lesions observed in about 30% of AIDS dementia patients.[77] Interleukin-1 liberated from cerebral tissue of AIDS dementia patients triggers the activation of the inductible NO (iNOS) synthase which permits the synthesis of NO. NO alone is not very toxic but has the capacity to react with $O_2^{-}\cdot$ to form peroxinitrite which is a powerful oxidant and neurotoxic.[78] In particular, NO can oxidate tyrosine residues from proteins and therefore affect the phosphorylations/dephosphorylations implicated in the transduction of neuromediator signals.[79]

In the brains of newborn rats, gp120 positively regulates iNOS[74] and NO contributes to the cytotoxicity of the protein. The gp120 protein also has a property that makes the cerebral macrophages infected by HIV secrete excitor amino acids and other neurotoxins. There is then an activation of the NMDA channel and an influx of Ca^{++} leading to cerebral lesions. Some antagonists of the NMDA receptor could be therapeutic platforms worth exploring.[80] Furthermore, a regulatory loop exists between the homeostasis of iron and the metabolism of NO.[81] NOS is regulated by iron through the transcriptional level.[82] Ferritin induces the expression of iNOS and therefore the production of NO, which carries a post-transcriptional regulation of ferritin and of the transferrin receptor. However, it should be noted that not all the authors have found increases of iNOS in the brains of deceased AIDS dementia patients.[83]

AZT (zidovudine) can also be at the origin of an increase of ROS production and peroxinitrite formation, and they can play roles in the onset of AZT-induced cardiomyopathy.[84]

Consequences of Oxidative Stress Increase

Oxidative stress or deficiencies of particular trace elements (Se, Zn) can exert effects on immune responses, for example, weakening of the membranes of immune cells. Moreover, the envelope proteins of the virus, by linking themselves to cellular membranes directly, cause injuries to neutrophils and diminish their production of $O_2^{-}\cdot$.[85] Other consequences include lymphopenia in the central and peripheral lymphoid tissues, depression of T and B lymphocyte functions such as delayed hypersensitivity and cytotoxic activity, decrease of PHA induced mitogen response, and decrease of natural killer cell activity. Chemotactic responses of neutrophils are impaired.[22,86]

Oxidative stress plays an important role in viral replication. Infection by HIV stimulates the secretion of pro-inflammatory cytokines, one of which is TNFα. TNFα stimulates ROS synthesis, which in turn activates the transcriptional NFκB factor. This cytosolic factor includes three subunits: P50, P65, and IKBα, which is an inhibitor. Through the action of H_2O_2, IKBα is phosphorylated and destroyed. The NFκB factor is translocated toward the nucleus and that permits the expression of genes possessing long term repeats (LTRs), such as the HIV genes.[87]

Furthermore, NFκB regulates the expression of numerous cytokine genes implicated in the immune system, and it can form activation loops. In this way, TNFα stimulates the synthesis of IL8 in HIV^{+} macrophages. IL8 increases the respiratory bursts in neutrophils and granulocytes by increasing oxidative stress.[88] The TNFα action must be much more complex. When primary macrophages are pretreated with TNFα *in vitro*, the virus entrance into these macrophages is inhibited. In this case, the action of the TNFα passes through the receptor of the TNFα RII (75 kDa).

The potentially beneficial role of TNFα in HIV infection could be limited *in vivo* by the presence of soluble RII receptors found in elevated quantities in the blood of HIV^{+} patients.[89] TNFα inhibitor role is not found for all cells, in particular lymphocytes of peripheral blood.[90]

The changes in the redox potential of cells induce apoptosis, which can contribute to the decline of CD4 cells.[86] The Tat protein is implicated in this mechanism, which

has been shown by several authors; while lymphocytes are cocultivated with cells overexpressing Tat, apoptosis is multiplied three times.[91] This increase is inhibited by N-acetylcysteine pre-treatment.[92] Tat is liberated in the extracellular compartments where it behaves like a toxin. Thus, Tat can cause modifications in noninfected cells by inhibiting the proliferation of antigen stimulated T cells.[76]

During the acute stage of the infection, bcl_2 (protein antiapoptotic) is diminished in infected CD4 cells.[93]

As already indicated, several HIV proteins have neurotoxic activities.[94] Tat induces the apoptosis of cortical neurons via a mechanism that interferes with TNFα and NMDA receptors.[95] The gp120 protein induces the apoptosis of cortical and hippocampal neurons by increasing intracellular Ca^{2+} and NO.[96] The inhibition of the NOS activity abolishes the cytotoxicity of gp120.

Therefore, oxidative stress diminishes the defenses of the host (eliminating immune defenses and increasing apoptosis, particularly of CD4 cells) and favors the replication of the virus.

SUPPLEMENTATION

HIV infection is always accompanied by a decrease of plasmatic Se, Zn, and Fe, and, except for Fe, by an increase of the tissular load. A decrease of antioxidants such as GSH and cysteine, a decrease of Mn-SOD activity, and an increase of oxidative stress have also been found. This suggests that supplementation, particularly with antioxidative elements, could diminish viral replication and therefore the viral charge and thus retard the progression of the disease and diminish morbidity and mortality. Before relaying findings on the use of supplementations in HIV^+ patients, it is necessary to first report some of the *in vitro* results in order to justify the *in vivo* tests.

Tests Conducted on Cells in Culture

Se, Zn, and Antioxidants

These substances were tested alone or in combination on cells in cultures. Tests were also performed on animals. *In vitro*, Se inhibits viral cytotoxic effects and reactivation of HIV-1 by hydrogen peroxide and protects against activation of HIV-1 by TNFα. It has been shown to promote GPx and Cu-Zn-SOD activity and to increase intracellular GSH concentration. Peripheral blood mononuclear cells (PMBCs) from HIV^+ patients, pretreated with NAC before being subjected to oxidative stress, were partially protected. A direct relation was found between susceptibility of PMBCs to oxidative stress and the decrease in numbers of CD4 cells in patients at different stages of the disease.[58] Treatment of T cells or PMBCs with NAC has been shown to decrease transcription of NFκB-driven gene constructs containing the HIV-LTR and inhibit intracellular replication of HIV-1.[97,98] 2-Oxothiazolidine-4(R)-carboxylic acid (OTC) derivatives are also used as GSH replenishing drugs. *In vitro,* these drugs exhibit anti-HIV-1 effects and little toxicity at high doses.[99]

Zinc supplementation of HIV-infected cells synergizes proliferation of lymphocytes stimulated by PHA, increases [^{3}H]-thymidine incorporation, and decreases the percentage of apoptotic cells.[100] Zn group metal compounds show anti-HIV activity. They inhibit transcription of HIV-RNA at concentrations at which they do not affect the growth of HIV producing cells.[101] Cupric and ferric ions and bathocuproine disulfonic acid are also *in vitro* inhibitors of HIV replication.[102,103]

When mice infected with murine AIDS, similar to the human HIV virus, are supplemented for four weeks with 0.1 mg/kg of Na_2SeO_3, there is an inhibition of splenomegaly and of the elevation of immunoglobins. Lipid peroxides are diminished and the GPx plasmatic activity is increased. The supplementation does not have an effect on the SOD activity.[104]

During HIV infection, a decrease of plasmatic iron and an increase of tissular iron have been noted. Thus, these are chelators of iron which have been tested. Desferrioxamine (DFX) has been shown *in vitro* to inhibit HIV replication in several cell lines.[105,106] These results have not been found by all authors.[107]

More recent studies show the inhibitor effects of DFX on viral replication. DFX, in addition to cultures of U1 promonocytoid cells and ACH2 lymphotic cells (cells infected with HIV), diminishes the reverse transcriptase activity before it is stimulated by H_2O_2. At the utilized dose (5 μM), it does not have a cytotoxic effect. This antiretroviral action is confirmed in mononuclear cells of peripheral blood.[108] The chelators of iron (DFX, hydroxypyridinone, and biomimetic siderophore SF1) also inhibit *in vitro* the growth of several but not all of the opportunistic pathogenes found in the HIV infection.[45]

DFX inhibits the activation of NFκB modulated by the Tat protein; if iron is added to Jukat cells treated by DFX, the Tat action is restored. Conversely, DFX does not have an effect on the activity of NFκB stimulated by TNFα.[78]

In order to diminish the toxicity of excess iron, it is recommended that one anticipate the liberation of iron from the transferrin in the acid compartments of the cell (endosome and lysosome). Weak bases such as chloroquine and hydroxychloroquine that can locally elevate pH have been used in cellular cultures, resulting in a decrease of gp120 protein synthesis and a decrease of virion number.[48]

All the tests conducted *in vitro* show diminution of oxidative stress and the presence of trace elements in adequate quantities serve as protectors against viral replication. The supplementation tests in men were based on solid scientific arguments. Supplementation tests are generally conducted on a small number of subjects, in populations that have different risks of trace element deficiency, during limited periods of time. Additionally, the authors essentially report the effects of supplementation on biological parameters, not on clinical improvements nor on the long term progression of the disease.

Se-Zn Antioxidant Supplementation

Dousset et al.[1] compiled the results of numerous tests. More recently, Semba and Tang[32] and Patrick[109] summarized some studies focusing primarily on the supplementation of vitamins, but also on trace elements. In this section, only the tests that are not cited in these works are mentioned.

Fifty-five HIV+ subjects having CD4 numbers under 400/mm^3 received over a period of one year either 250 µg a day of selenonomethionine (100 µg Se) or 30 mg a day of β carotene. In the Se group, the number of CD4 cells does not vary, but the concentration of β_2 microglobulin diminishes and oxidative stress markers improve.[110-111]

In one study, 24 HIV+ subjects who did not receive antiviral treatment were given 180 mg a day of NAC and 500 µg a day of Se for 12 or 24 weeks. The supplementation did not affect the viral charge. There was an increase in the CD4/CD8 ratio, and no modification of GPx activity and GSH.[112] Another study was conducted in Zambia to test the hypothesis that the clinical response to albenzole might be improved by oral micronutrient supplementation for AIDS patients with diarrhea wasting syndrome. Sixty-six patients were randomized to albendazole plus vitamin A (10,500 U), vitamin C (300 mg), Se (150 µg), and Zn (200 mg) for two weeks. This short term supplementation did not reduce the periods of diarrhea or reduce the mortality.[113]

Some supplementations have been carried out in order to test particular hypotheses. HIV+ patients are at higher risk of atherosclerosis and endothelium dysfunction which could be related to the loss of antioxidants. One test was conducted on 15 HIV+ subjects who received 100 µg Se a day, 11 HIV+ subjects who received 30 mg of β carotene twice a day, and 15 HIV+ control patients. Control patients showed a stronger increase of Von Willebrand factor and soluble thrombomodulin which implicates damage to the endothelium over the year of the study and protection of endothelium by antioxidant agents.[114]

In HIV+ patients with dilated cardiomyopathies the plasma Se concentration was lowered. Three of these patients received 200 µg of Se a day. After three months, two of the patients showed clinical improvement and improved echocardiographs; the state of the third was not modified.[10]

Iron Deficiency Treatment

Therapy prevents an excess of iron. Although a deficiency may occur with a lowered concentration of serum iron, iron supplies are not recommended unless a real deficiency can be identified. The deficiency should not be treated with transfusions, but rather with erythropoietin. It is also necessary to eliminate concentrations of susceptible substances such as ethanol to increase the absorption of iron. It is equally necessary to pay attention to the supply of vitamin C;[45] this vitamin is an antioxidant. It suppresses reverse transcriptase activity and viral replication. But it increases the intestinal absorption of iron and it can have a pro-oxidating effect. Iron is stored in the Fe^{3+} form linked to ferritin. Vitamin C passes through the pores of ferritin where it converts Fe^{3+} into Fe^{2+} which can be released from ferritin and generated ROS.[115] Tobacco should also be avoided; it triggers iron excess, particularly in the alveolar macrophages.

It is not advisable to give iron to HIV+ subjects, as illustrated by the Salmon-Ceron et al. study.[116] These authors, in order to prevent opportunist infections, treated subjects with dapsone. The formula chosen contained 100 mg of dapsone and 60 mg of iron in tablet form; the subjects could take a half tablet a day. Mortality was

more elevated in treated subjects than in control subjects. Iron chelators also were tested. One test with DFX was conducted on 49 thalassemic HIV+ subjects; nine years after the seroconversion, 10% of the subjects treated with more than 40 mg/kg of DFX entered stage IV of the disease, versus 39% of the subjects who received doses under 40 mg/kg.

One test was conducted on four patients who took hydroxychloroquine (800 mg per day for eight weeks); the quantity of HIV-RNA diminished significantly in cases of treated subjects.[117]

In conclusion, considerable progress has been made in understanding the pathology and metabolism of the HIV. It has now been well established that oxidative stress stimulates the replication of the virus and induces apoptosis, particularly in immunocompetent cells. It has been demonstrated that zinc and selenium deficiences contribute to the decrease of immune defenses and to the increase of oxidative stress in HIV+ subjects. Therefore it would be tempting to make supplementations of antioxidant agents: Se, Zn, GSH, or NAC. However, it is now known that the virus uses Se in order to synthesize a GPx-like product. Zinc plays an equally important role in the stability and function of viral proteins. Its concentration plays a role in the activity of HIV protease. In reality, adequate supplies of trace elements represent an extremely complex problem. These micronutrients can interact; excesses of micronutrients can have harmful effects.[118] Hence, it is necessary that they are made in a bioavailable form, and diffuse and reach the right target cells at the right moments and for an adequate amount of time. All of these factors do not explain the sometimes deceiving results after supplementation.

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CHAPTER 5

Use of Herbs and Non-Nutritive Supplements in HIV-Positive and AIDS Patients

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CONTENTS

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INTRODUCTION

The spread of HIV infection affects men, women, and children of every age and ethnic group. The average person with AIDS is under considerable stress, as he or she is confronted with a debilitating disease, financial strain, stigmatization, and death.[1] The feeling of control rendered by greater participation in self-care is a source of empowerment to patients who seek nontraditional treatments. Some patients turn to nontraditional methods of healing or to healers who believe that there is always hope. This is the point at which an individual might experiment with different "cures" in an effort to alleviate symptoms of illness. In this chapter, we look at some of the more popular nontraditional methods of healing such as herbs and non-nutritive supplements, and the prevalence of these supplements in the HIV-infected population.

NONTRADITIONAL NUTRITIONAL THERAPIES (NNTs)

Treatments such as acupuncture, meditation, yoga, massage, vitamins/minerals, and herbal remedies have been referred to as complementary and alternative medicine, or CAM. CAM has been defined as a group of practices that do not form part of the dominant system for managing health and disease.[2] CAM offers views of disease and treatment that may not be compatible with Western medicine.[3]

From a sociological standpoint, "unconventional" therapies are medical practices that do not conform to the standards of the medical community.[4] "Alternative" therapy implies the unproven treatment is as good or better than a standard, medically accepted treatment. No sufficient scientific evidence supports claims for alternative therapies if appropriate clinical studies have not been performed or published to support their benefits.[5] In this chapter, alternative nutritional therapies will be referred to as nontraditional nutritional therapies (NNT). In the context of clinical medicine, nontraditional (nonbiomedical) therapy implies interventions against diseases that are not part of Western (biomedical) medical tradition.[1] NNT is any self-supplementation of unproven nutritional therapies which are not part of Western medical tradition. Examples include vitamin/mineral megadosing (more than 10 times the RDA), herbal therapies, and the use of non-nutritive supplements such as DHEA and melatonin. Nontraditional and traditional approaches to HIV/AIDS treatment need not be mutually exclusive. They share two fundamental objectives: (1) compassion for the patient, and (2) interest in improving the patient's clinical status.[1] The descriptions of some nontraditional nutritional therapies are provided in this chapter.

PREVALENCE OF NNT USE

The popularity and use of unproven or nontraditional therapies among patients with chronic illnesses, including HIV infection, have increased significantly. Unlike a decade ago, HIV is now more often considered a chronic disease, not an imminently terminal disease.

People with cancer, arthritis, and other chronic illnesses have long been inclined to seek help outside traditional medicine. In one study, nontraditional therapy use was shown to be more prevalent for AIDS patients than for those with cancer or other illnesses.[6] A motivating factor to experiment with nontraditional therapies lies with the fact that traditional medical therapy is not completely curative. HIV/AIDS patients have a hunger for information on natural remedies, supplements, and other treatment methods that the traditional medical establishment does not provide. Research on AIDS and HIV-related illnesses has focused primarily on anti-viral drugs, which produce mixed results and unpleasant side effects.[7]

To fill the gap left by clinical medicine, some patients have become activists on behalf of nontraditional therapies. Lack of faith in medicine has emerged throughout history as people sought quick and simple cures. Quacks were quick to respond with convincing natural remedies.[8] In some ethnic groups, holistic treatments such as herbs, massage therapy, vitamins, and acupuncture are still preferred over Western medicines due to uncertainty of effectiveness or side effects of some drugs.[9]

In 1990, Americans made approximately 427 million visits to providers of nontraditional therapy. That estimate increased to 629 million in 1997, thereby exceeding the total number of visits to all U.S. primary care physicians. Expenditures associated with the use of nontraditional therapy in 1990 amounted to approximately $13.7 billion, $10.3 billion of which was paid out of pocket.[10] Expenditures for alternative medical professional services in 1997 were estimated at $21.2 billion, with at least $12.2 billion paid out of pocket. This figure exceeds the out of pocket expenditures for all hospitalizations in the United States.[11]

Physicians are divided in their opinions about these nontraditional therapies. Some believe them to be quackery, while others are willing to make distinctions and acknowledge some of them. Some physicians even refer patients for alternative treatment, particularly when all else has failed.[12] The American Medical Association has taken the position that although most nontraditional therapies are not proven effective and some are fraudulent they should still be evaluated.[13] Physicians need to educate themselves and their patients about the efficacy and adverse interactions of herbs and the limitations of our present knowledge of them.[14]

CAM practices provided to HIV-infected individuals, provider experience with HIV disease, patient characteristics, and provider perceptions of treatment effectiveness were studied through a survey mailed to 117 providers who offer CAM therapies. This survey showed that providers treat patients in all stages of HIV disease with a variety of CAM practices, have an average of 6.5 years of HIV disease treatment experience, and average 105 patients in treatment per provider. A total of 115 different CAM therapies with an average of 12 treatments per provider were disclosed. Ninety percent of providers claimed their CAM therapies were "somewhat to very effective" at all disease stages, indicating effectiveness for symptom management (96%), quality of life (98%), increasing or maintaining $CD4^+$ lymphocyte levels (66%), slowing progression to AIDS (69%), and extending survival (73%).[15]

Pelletier et al. studied the status of managed care and insurance coverage of CAM and the integration of these therapies offered by hospitals through 18 insurers. The researchers found that for 12 insurers, market demand was the primary motivation

for covering CAM, and the majority of insurers offered some coverage for nutrition counseling, biofeedback, acupuncture, psychotherapy, chiropractic, and osteopathy. Hospitals are limited in offering CAM therapies secondary to the availability of licensed practitioners in those areas. Among the most common obstacles to incorporating CAM into mainstream health care were lack of research on efficacy, economics, ignorance, provider competition/division, and lack of standards of practice.[16]

INTERNET AVAILABILITY OF NONTRADITIONAL/ALTERNATIVE TREATMENT INFORMATION

Media and product advertising are filled with outrageous and inflated claims of efficacy for nontraditional remedies. Unsubstantiated claims instill a false sense of hope where none is warranted. This false hope may lead users of the products to ignore precautions that are necessary to help protect against harm, or abandon traditional therapies and rely only on nontraditional products.[17] Many HIV seropositive individuals seek information that will help them to maintain, build, or restore their immune status. A majority of HIV-infected individuals receive treatment information that may or may not be reliable from peers, printed material, and most recently in the privacy of their homes through Internet access to the World Wide Web.

A Web scan of micronutrient supplementation advice for the HIV-positive population reveals countless micronutrient recommendations. HIV-infected individuals who seek micronutrient recommendations may not know that before guidelines can be universally utilized scientific evidence must prove their safety, effectiveness, and reliability, and evidence must be peer reviewed.[18] Of the micronutrient recommendations found on the Web, few base their recommendations on solid scientific advice, and they may provide information for the purpose of generating sales. As an example, the text of one on-line site's recommendations came from a group of activists in Philadelphia:

> The following vitamin recommendations, like the whole field, have been only meagerly researched. Although they are not a part of the standard of care recommendations, these appear in the 1996 January edition of ACTUP Philadelphia's HIV Adult Standard of Care, co-authored by Jonathan Lax & Kiyoshi Kuromiya. The recommendations are as follows: Multivitamin (Standard Adult Dose); Vit C (2-5 gm as ascorbate); Beta-Carotene (25,000-30,000 IU) [raises T cell count?]; B vitamins (extra B vitamin tablet daily); B-12 vitamin (sublingual, IM injection. A nice energy boost); Vit E (400-800mg).[19]

Internet health fraud has been the target of a comprehensive law enforcement and consumer education campaign conducted by the Federal Trade Commission as "Operation Cure All." This campaign uses the Internet both as a law enforcement tool to stop bogus claims for products and treatments touted as cures for various diseases, and as a communication tool to provide consumers with quality health information. The focus of the new campaign is "quality not quackery." As of December 1998, nearly 22.3 million adults in this country sought health and medical information online, making the health and medical content on the Internet the sixth

most commonly accessed type of information. Twenty-nine percent of all Americans looked to the World Wide Web for medical information and almost 70% of them do so before visiting a physician's office.[20] Cancer-related searches are the most prevalent medical searches, followed by inquiries about heart disease.

HERBAL SUPPLEMENTS

Of all the foods used as medicines or to maintain health, none received more attention as a group than herbs. The herbs popularly known as medicines contain plant materials combined with chemically defined active substances, including chemically defined, isolated constituents of plants.[21] Despite advances in our understanding of medicinal and toxic properties of many herbs, the consumer is confronted with misconceptions regarding the benefits and dangers of herbs.[22] It is assumed that because herbal remedies are natural, they are safer than drugs made from chemicals. Herbal supplements, like their pharmaceutical counterparts, can have adverse side effects.

Known or potential drug–herb interactions exist and should be screened. Immunostimulants (e.g., *Echinacea* and zinc) should not be taken with immunosuppressants such as corticosteroids and cyclosporine. Tannic acids present in some herbs (e.g., St. John's Wort and Saw Palmetto) and teas may inhibit the absorption of iron.[23] Karela and ginseng may affect blood glucose levels and should not be used by patients with diabetes mellitus. Ginseng may cause headache, tremulousness, and manic episodes in patients treated with phenelzine sulfate. Ginseng should also not be used with estrogens or corticosteroids because of possible additive effects. Nonsteroidal anti-inflammatory drugs may negate the usefulness of feverfew in the treatment of migraine headaches. Feverfew, garlic, gingko, ginger, and ginseng may alter bleeding time and should not be used concomitantly with warfarin sodium. Evening primrose oil and borage should not be used with anticonvulsants because they may lower the seizure threshold.[23]

Herbal remedies do not have to meet the same FDA standards of safety and quality that synthetic drugs do. Manufacturers of herbal and other supplements can make certain health claims, proven or not, as long as they include disclaimers stating their products have not been evaluated by the FDA. Consumers do not realize that this disclaimer does not imply safety when they purchase products. Possible interaction of an herbal product with medications is important information that is not included on the package.

To maximize profits, the health food industry must minimize government regulation.[24] A 1994 Congressional act reclassified vitamins, minerals, and herbs as "dietary supplements," reducing the FDA's control over their regulation. According to the Dietary Supplement Health and Education Act of 1994, a dietary supplement is defined as "a product intended to supplement the diet that contains at least one or more of the following ingredients: a vitamin, a mineral, an herb or other botanical substance; an amino acid. Also, a dietary substance for use to supplement the diet by increasing the total dietary intake; or a concentrate, metabolite, constituent, extract, or combination of any of the previously mentioned ingredients" falls in that

category.[21] The ingredients in dietary supplements are not subject to review by the FDA, which gives the manufacturer more freedom in how they are sold.

Many herbal and non-nutritive supplements come with unregulated dosage recommendations written on the labels. Under FDA regulations, "labeling" is not limited to what is on a product container. It also includes claims made by any written or graphic matter that explains a product's use and is physically or contextually connected with its sale. Thus, promotional material used to sell a product or to explain its use can be construed as labeling whether it is used before or after a sale.[24] The concern for the safety of prescribed non-nutritive supplements and herbs is evident from an editorial in the February 2000 issue of the *American Journal of Clinical Nutrition.* The editorial board of that journal is encouraging investigators to research the biological and clinical efficacy and safety of these substances.[25]

HISTORY OF MEDICINAL USE OF HERBS

The medicinal use of herbs is deeply rooted in human history, religion, folklore, and even magic.[26,27] Herbs have been incorporated into the healing practices of practically all human cultures. People have recognized the potential health-promoting effects of plants since early recorded history. A popular concept about herbs is that they exist on earth as our rightful medicines. Interest in herbal remedies and their association with medicinal quackery and natural cures stem from a close association between medicine and botany throughout history. Some herbs are precursors of modern pharmacology. The heart medication digitalis is made from foxglove; morphine and codeine are derived from opium poppies; and vincristine (a drug to treat leukemia) comes from the Madagascar periwinkle.[22]

A system of medicine based on plants, minerals, and animal products was employed in ancient Egypt, Iran, and China. The importance of diet as the foundation of the healing process was a fundamental part of medicinal treatment in fifth century B.C. Greece. During the period of prominence of Arabian and Persian medicine (850–1000 A.D.), the search for plants and herbs to cure diseases led to the development of modern forms of drug delivery such as tinctures, syrups, and ointments. By the Renaissance of the fifteenth century, botany was viewed as the only natural and applied science relevant to medicine.[28]

The use of herbal preparations persisted throughout the ages because it induced a physiological and/or pharmacological response. Lydia E. Pinkham's "Vegetable Compound" used for the treatment of female problems and weakness was popular because it contained iron and herbs with estrogen-like compounds.[28]

In the next section, we will outline some of the more popular herbs and other non-nutritive supplements often used by HIV-positive and AIDS patients, and review some of the research available on the popularity and effectiveness of these supplements.

Garlic

Garlic remains the most popular herbal panacea. It was traditionally used for medicinal purposes, and has been reported as a popular herb used by HIV-positive

individuals. Garlic use can be traced back thousands of years and is mentioned in folklore and traditional herbal beliefs. It is purported to help a variety of symptoms and diseases including hay fever, arthritis, sleep disorders, sinus problems, lung ailments, tuberculosis, athlete's foot, digestive problems, heart disease, cancer, and "cleansing" the blood.[29,30] Since the early 1970s, experimental and epidemiological studies have investigated garlic's influence on risk factors associated with heart disease. In clinical studies, garlic was observed to inhibit lipid synthesis, reverse cholesterol-induced atherosclerosis, dissolve blood clots, and inhibit clot formation. Feeding garlic to patients with coronary heart disease decreased serum cholesterol, triglycerides, LDL, and VLDL, increased HDL levels, and produced a transient increase in fibrinolytic activity.[28]

Evidence suggests that garlic may serve as a biological response modifier by augmenting macrophage and T lymphocyte function.[31] Tang et al. studied the effects and mechanisms of garlic related to preventing oral pre-cancer in rats subjected to a chemical carcinogen. In that study, garlic effectively prevented oral pre-cancer by stimulating the activation of natural killer cells, the function of T lymphocytes, and the level of IL-2.[32]

Cohen et al. investigated the effects of organosulfur compounds on hepatic phase II carcinogen detoxification enzymes. Their results contradicted previous animal model studies in which garlic had a preventative effect against cancer. The S-allylcysteine form of garlic that was used did not exert an inhibitory effect on any index of tumor development, including incidence, latency, multiplicity, or volume, compared with untreated controls.[30]

Many of the effects of garlic are attributed to its sulfur-containing compounds. Organosulfur compounds of garlic inhibit growth of animal tumors and modulate the activities of diverse chemical carcinogens.[30] Garlic bulbs have a sulfur-containing amino acid derivative, alliin (S-allyl-L-cysteine sulfoxide). When the bulbs are ground, alliin is converted to allicin (diallyldisulfide S-oxide). Allicin, one of the active components of freshly crushed garlic, exhibits a variety of antimicrobial activities.[33] In its pure form allicin inhibits:

1. Antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant strains of *E. coli*
2. Antifungal activity, particularly against *Candida albicans*
3. Antiparasitic activity acting on some intestinal parasites such as *Entamoeba histolytica* and *Giardia lamblia*
4. Antiviral activity[33]

The *in vitro* effect of the garlic derivative alliin on mitogen-induced peripheral blood mononuclear cell (PBMC) proliferation and cytokine production was examined by Salman et al. They concluded that alliin *in vitro* exerts an immunomodulatory effect on certain functions of the peripheral blood cells.[34] Diallyl sulfide (DAS), a flavor component of garlic which is used as a food additive, exerts chemopreventive effects at several organ sites by possibly inhibiting carcinogen activation via cytochrome P450-mediated oxidative metabolism.[35]

Consumer Tips

Many garlic products available today contain garlic oil of unknown composition, rendered tasteless and odorless. Since most of the active components of garlic are odoriferous, the usefulness of garlic oil products should be questioned. It is possible that not all the components of garlic affecting blood lipids and blood clotting are present in the volatile oils.[29] Also, the sulfur-containing compounds in garlic are unstable when subjected to heat and are largely destroyed during the cooking process.

The garlic content of proprietary health food capsules may be insufficient to affect platelet aggregation.[28] Garlic is not without side effects, and careful evaluation of the doses needed to elicit an effect on blood lipids must be considered. Documented side effects of garlic overdose include contact dermatitis, nausea and vomiting, diarrhea, weight loss, anorexia, flatulence, and a garlicky body odor.[28]

Milk Thistle or Silymarin

During the middle ages, milk thistle seed was believed to have both nutritional and medicinal properties. It was used to treat conditions such as liver disease and stomach, spleen, gallbladder, and female disorders.[36] A member of the *Asteraceae* or *Compositae* family, milk thistle is also known by several other names: *Silybum marianum, L. gartneri*, *Carduus marianus* L., Mariana thistle, and Our Lady's thistle, to name a few.[29] The active ingredient of milk thistle has been identified as silymarin, which consists of many flavonoligninans.[29,36,37] Today silymarin is known as a liver tonic that has the ability to stimulate protein synthesis to accelerate the regeneration and production of liver cells.[36,38] Researchers report that the herb is used clinically in Europe and Asia as an antihepatotoxic agent.[38,39]

Several anecdotal reports emphasize the herb's usefulness in people with HIV/AIDS. Proponents of the herb claim that it helps reverse the inflammatory effect that HIV has on liver tissue by destroying oxidants, thus protecting the liver from stress and the prolonged use of pharmaceutical drugs.[40] Buyers' clubs on the Internet advertise silymarin in their popular General Liver Protection /Detoxification Protocol.[41]

Cyanobacteria or Blue-Green Algae

A recently developed tetrazolium-based microculture assay was used to screen extracts of cyanobacteria (blue-green algae) for inibition of the cytopathic effects of the human immunodefiency virus (HIV-1). A number of extracts were found to be remarkably active against the AIDS virus. A new class of HIV-1 inhibitory compounds, the sulfonic acid-containing glycolipids, was discovered through the use of the microculture assay to guide the fractionation and purification process. The pure compounds were found to be active against HIV-1 in cultured human lymphoblastoid CEM, MT-2, LDV-7, and C3-44 cell lines in the tetrazolium assay as well as in p24 viral protein and syncytium formation assays.[42]

Echinacea

Echinacea has been considered one of the most usable plants in medical treatment for many years.[43] It has been used for the prevention of various diseases and disorders

and is one of the most commonly used herbal remedies for the immune system. Therefore it is a popular herb among those with HIV/AIDS. Two species of Echinacea are listed in the *European Pharmacopoea: Echinacea angustifolia* and *Echinacea purpurea.* These species differ in morphology and chemical composition. Chemical and biological properties of Echinacea have been studied for about 80 years.[43] Most chemical analyses have been done with *Echinacea angustifolia,* whereas biological activity was tested with *Echinacea purpurea.* Most of the reports, which declared the stimulating biological activity of Echinacea, could not resist critical opinion, so the frequency of medical application of this herb is primarily due to delivered practical knowledge. Schumacher et al. studied the effects of the water-soluble extract of *Echinacea angustifolia* on immune function in mice. Echinacoside is one of the low-molecular compounds and proprietaries contained in this plant. Influence on the unspecified cellular immunity of the mice after intraperitoneal, intravenous, or peroral application was investigated. Under various conditions no effects on the immune system could be found using the carbon clearance test.[43]

Echinacea is also referred to as *E. pallida,* Purple Kansas coneflower, *Rudbeckia purpurea,* echinacin, elhincea, and eahincea.[44,45] The herb is reported to accelerate the healing of wounds, and is believed to produce immune effects when taken internally. Coeugniet et al. tested extracts of *Echinacea purpurea* for their non-specific action on cell-mediated immunity. *In vitro*, these extracts have stimulating effects on the production of lymphokines by lymphocytes.[46] A toxic effect on cells was produced only with very high, clinically irrelevant concentrations. Clinical application of these extracts can produce a stimulation of cell-mediated immunity (one therapeutic administration followed by a free interval of one week) or produce a depressive action (daily administrations of higher doses). These results were confirmed by lymphokine production and assay, 3H-thymidine incorporation, and a skin test with recall antigens.[46]

Clinical studies report on the wound healing effects of Echinacea for a variety of skin conditions.[47] Polysaccharides isolated from large scale plant cell cultures of *Echinacea purpurea* have been shown to activate human and murine phagocytes.[48] Some of these effects are believed to be an increase in the number of WBC and spleen cells, activation of the capacity for phagocytosis by human granulocytes, increase in body temperature, reproduction of T-helper cells, and the production of cytokines.[45]

Elsasser-Beile et al. studied the levels of cytokines in stimulated whole blood cells derived from 23 tumor patients undergoing a 4-week oral treatment with a an extract from *Echinacea angustfolia, Eupatorium perfoliatum,* and *Thuja occidentalis* (Echinacea complex). All patients had curative surgery for localized malignant tumors, and blood was analyzed before treatment and after 2 and 4 weeks of therapy. In the blood cell cultures of all patients, a wide range of cytokine levels was found. After therapy with Echinacea complex, no significant alteration in the production of cytokines was found, and the leukocyte populations remained constant. The researchers concluded that at the dosage of Echinacea given, the therapy with Echinacea complex had no detectable effect on tumor patients' lymphocyte activity as measured by their cytokine production.[49]

See et al. evaluated extracts of *Echinacea purpurea* and Panax ginseng for their capacity to stimulate cellular immune function by PBMC in normal individuals and patients with chronic fatigue syndrome or AIDS. They found that both Echinacea and ginseng, at concentrations greater than or equal to 10 μg/kg, significantly enhanced natural killer cell functions of all groups. Similarly, the addition of either herb significantly increased antibody-dependent cellular cytotoxicity of PBMC in all subject groups. Thus, See et al. concluded that the extracts of *Echinacea purpurea* and *Panax quinquefolia* (ginseng) enhanced cellular immune function of PBMC both in normal individuals and in those with depressed cellular immunity.[50]

NON-NUTRITIVE SUPPLEMENTS

Dehydroepiandrosterone (DHEA)

DHEA, an adrenal steroid hormone produced in abundance by humans and most other warm-blooded animals, is uniquely sulfated (DHEAS) prior to export into the plasma, and exhibits an age-related decline in production.[51] Therefore, many individuals and groups advocate DHEA as an anti-aging product. Some HIV-infected individuals believe that DHEA can inhibit HIV replication and boost immune function.[43] It is popularly believed that taking the steroid internally can help replenish the body's diminishing supply of the hormone.

No major physiological functions have been ascribed to the activity of DHEA, although it serves as an intermediary in reproductive steroid synthesis. Studies on the effects of glucocorticoids (GCS) on the immune system led to the question whether DHEA effects activated lymphocytes to produce interleukin-2. Daynes et al. concluded that DHEA, presumably through receptor-mediated mechanisms similar to those of other types of steroid hormones, may represent a natural and important regulator of interleukin-2 production in normal and pathologic conditions.[51]

A significant protective effect of DHEA was demonstrated in studies of system coxsackievirus B4 and herpes simplex type 2 encephalitis in mice. Histopathological analysis, leukocyte counts, and numbers of spleen antibody forming cells in the coxsackievirus B4 model suggest that DHEA functions by maintaining or potentiating the immune competence of mice otherwise depressed by viral infection. While the molecular basis for DHEA's effect on the immune system is not known, studies suggest that it may counteract the stress-related immunosuppressive effects of glucocorticoids stimulated by viral infection.[52] It is suggested that the utility of DHEA in the therapeutic modulation of acute and chronic viral infections, including HIV infection, deserves further intensive study.

This section covers just a few of the many herbal and non-nutritive supplements some patients who have HIV disease may turn to with the hope of boosting the immune system and improving their quality of life. Use of these supplements can offer hope to the people who use them. In this next section we will explore the therapeutic value of hope, and how hope, or the lack of it, influences a diseased person's outlook on life.

THERAPEUTIC VALUE OF HOPE

The level of hope a sick person has can fluctuate, based not only on his or her personal view, but on others' views of his or her progress. When a person is notified that he or she is HIV positive, the first reactions are often terror and confusion.[53] Early in the course of coping with an HIV diagnosis, suicidal tendencies and behavior may arise as the person begins to envision a future with AIDS. The initial reaction often leads to a process of coping with HIV disease by redefining the meaning of HIV, enhancing his or her sense of control over life, prompting a renewed effort at self-help and seeking help, and forming a new commitment to life and a reappraisal of personal goals. Allowing newly diagnosed HIV-positive patients to discuss suicide may allow them to move forward toward acceptance and commitment to life.[53]

Hope is the expectation that something positive will happen, and it is reinforced or altered by other people's behavior.[54] Patients must acquire a sense of what is and is not possible. Healthy persons can learn to accept the limits of hope; people who are ill and are dependent on others for help may not see or accept these limits.[54]

Since hope relates to the future, it must include alternatives so that one can adapt to unexpected situations.[55] Expectations of most patients, their families, and health care professionals are that the patient will be restored to as normal a condition as possible. One assumption about hope is that it needs to be realistic to be beneficial. Thus, a patient must consider some questions. Does the potential exist for the problem to be relieved? Are there ways to bring about the desired change? Is my caregiver able to provide the means to restore health?[56]

Spirituality is an important and often neglected aspect of pain.[57] The spiritual domain involves meaning, hope, love, and relatedness. Understanding spiritual aspects of pain requires health care providers to be aware of their own spirits in order to relate to a patient in pain. They should offer spiritual assessment and interventions such as presence, attentive listening, acceptance, and judicious self-disclosure, for promoting comfort and diminishing pain.[57] Coleman et al. found that existential well-being (a spiritual indicator of meaning and purpose) was significantly related to the HIV patient's psychological well-being and level of hope. In addition, HIV symptoms were found to be significant predictors of psychological well-being.[58]

Health care providers should recognize their patients' spiritual beliefs and incorporate them into discussions about terminal care. To describe the role of spiritual beliefs in end-of-life decisions, Kaldjian et al. surveyed 90 HIV-positive patients. Forty-four percent of patients felt guilty about their HIV infections, 32% expressed fear of death, and 26% felt the disease was a form of punishment. Prior discussions about resuscitation status were less likely in those who perceived HIV as a punishment and more likely in those who believed in God's forgiveness. Living wills were more common for those who prayed daily. Fear of death was more likely in those who saw HIV as a punishment or felt guilty about having HIV, and less likely in those who read the Bible or attended church regularly. Outcome measures did not vary significantly according to sex, race, HIV risk factors, or educational level. The researchers concluded that religious practices and belief in a forgiving God appeared to facilitate discussions about end-of-life decisions. These discussions were impeded by a patient's interpretation of HIV infection as punishment.[59]

While new discoveries about HIV disease and antiretroviral therapies hold the promise of improved survival, ambiguity about the durability of treatment response and ultimate survival contributes to the level of uncertainty with which a patient must cope. Brashers et al. examined the revival experiences of HIV-positive men and women who once were reconciled to their deaths from HIV/AIDS but, as a result of dramatic treatment responses, now believe they may survive. This dynamic is popularly known as the Lazarus syndrome. Participants described the uncertainty accompanying renewed health and a return to the joys and problems of continued life. They further described physical renewal as an unexpected new stressor forcing them to renegotiate feelings of hope and future orientation, social roles and identities, interpersonal relations, and the quality of their lives.[60]

Hope is not easily obtained by the HIV patient. With diminished hope, HIV patients seek advice on practices that they feel will not only help them, but offer a sense of control over their lives that now seem out of control. Spiritual awareness or belief in a higher power helps some patients cope with their illness; death is not seen as the end, but as the promise of a better place where suffering does not exist. Health care workers should be aware that, like spiritual faith, some patients turn to herbal supplements and other nontraditional practices to provide hope in coping with the disease.

NONTRADITIONAL THERAPY USE

Studies based on samples in limited geographic areas suggest that the use of nontraditional therapies is widespread.[61,62] Complementary and alternative medicine use is common in the general population. Fewer studies are available on the prevalence, cost, and patterns of alternative therapy use among people with HIV infection. The data that are available on nontraditional nutritional therapy use by that specific disease population are summarized in this section.

Eisenburg et al. conducted a random national telephone survey of 1539 adults over a period of three months in 1990. Respondents were asked to report any serious medical conditions, their use of conventional medical services, and use of unconventional therapies. Thirty-four percent of the respondents reported using at least one unconventional therapy in the previous year and one third of these respondents visited providers for these unconventional therapies. They made an average of 19 visits to such providers during the preceding year, at an average cost per visit of $27.60.

Frequency of unconventional therapy use varied among sociodemographic groups. A high rate of use of unconventional therapies was reported by non-black persons from 25 to 49 years of age who had some higher education and higher incomes. The majority of participants used unconventional therapy for chronic, as opposed to life-threatening, medical conditions. For serious medical conditions, most (83%) sought treatment from physicians, and 72% of the respondents who used unconventional therapy did not inform their physicians that they had done so. Nontraditional therapy use was significantly more common among persons with some college education than among persons with higher levels of education.[11]

It might be expected that persons with higher levels of education would be more discriminating when making decisions regarding "miracle" claims made by a health supplement manufacturer or seller. One explanation for this difference might be that with more education comes a greater desire to be in control of health and well-being. An individual can also access existing information about these products through the Internet and libraries. The supplement industry is in the meantime taking advantage of such patients by advertising their claims of miracle cures.

Eisenburg and colleagues conducted a comparable survey in 1997, utilizing 2055 HIV-positive participants. Prevalence, cost, and disclosure of alternative therapy use to physicians were analyzed for comparison against 1990 results. Eisenburg determined that use of at least one of 16 alternative therapies during the previous year increased from 33.8% in 1990 to 42.1% in 1997. The greatest increase was noted for herbs, massage, megavitamins, self-help groups, folk remedies, energy healing, and homeopathy therapies. The probability that users would visit an alternative medical provider increased from 36.3% to 46.3%. There was no significant change in disclosure rates between 1990 and 1997; 39.8% of alternative therapies were disclosed to physicians in 1990 versus 38.5% in 1997.[12]

In a cross-sectional study, 657 HIV/AIDS drug treatment program participants were evaluated for associations between complementary therapy use and participant characteristics. Survey data were gathered between September 1995 and June 1996 on the use of complementary therapies (dietary, medicinal, tactile, relaxation) and the motivations behind the use of these therapies. The researchers concluded that 39% (256) of the participants used complementary therapies, 30% (195) used dietary supplements (not including herbs), and 22% (141) used herbal and other medicinal therapies. The use of complementary therapies overall, in conjunction with HIV/AIDS medications, appeared to be most prevalent in young, university-educated individuals, and in those experiencing the great physical pain that can accompany HIV disease.[63]

Anderson et al. studied 184 HIV-positive individuals for specific alternative therapeutic modalities sought, and the efficacy of both conventional and alternative therapies. Forty percent of patients reported use of alternative or complementary therapies. Recourse to alternative therapy was significantly associated with risk-group affiliation, duration of seropositivity, and gender. The decision to use alternative therapy was not significantly related to age, race, education, religion, or severity of symptoms. Of the respondents using alternative therapies, only 10% expected the unconventional therapies to cure their HIV infections, while 36% expected the therapies to delay the onset of AIDS symptoms.[64]

Lyle et al. studied the prevalence of supplement use and chronic disease in 2152 middle-aged to older adults between 1988 and 1990. They determined that supplement use was more prevalent among women versus men; persons with more than 12 years of education versus those with less education; those with low versus high body mass index; persons with active versus sedentary lifestyles; and those who had never smoked versus current smokers. Intakes of most micronutrients, dairy products, foods high in vitamins C and E, and certain carotenoids were significantly higher

in supplement users than non-users. Relationships between food intake and supplement use differed by gender and type and level of supplement intake.[65]

Smith et al. compared sociodemographic and illness-related factors associated with the use of vitamins, nonprescription medications, herbs, and recreational substances among HIV-infected individuals. Data were derived from 7887 interviews conducted as part of the AIDS Cost and Services Utilization Survey. After adjusting for perceived health status, T cell count, and stage of disease, the results of the survey indicated that blacks were less likely to use nonprescription drugs, vitamins, and herbs, as compared with non-Hispanic whites. College educated individuals were more likely to use vitamins and herbs. Variables such as insurance status and income were associated with use of recreational drugs, while need-for-care variables were associated with use of nonprescription drugs and vitamins.[66]

In a cohort of 127 HIV-positive individuals, the influence of individual income on frequency of utilization of alternative therapies was studied. The sample was divided into two cohorts: those who received Medicaid and those who did not. The number of cases of AIDS in both cohorts was equal. Weekly spending for alternative therapies was significantly higher in the non-Medicaid population. Mean weekly spending averaged $22.24 in the non-Medicaid cohort, and averaged $3.34 in the Medicaid cohort for alternative nutrition therapies. Marv et al. concluded that income positively influences the utilization of alternative therapies by HIV-infected persons. The strong association between income and nontraditional nutrition therapy (NNT) use when demographic and clinical factors were controlled warranted further investigation. If NNTs are helpful to HIV-infected individuals, low socioeconomic status is a factor that could inhibit accessibility to these therapies.[67]

In order to determine the short-term safety and efficacy of a Chinese medicinal herb preparation in treating symptoms of HIV infection, Burack et al. conducted a 12-week randomized, double blind, placebo-controlled clinical trial of 30 adults with symptomatic HIV infection.[68] Participants who had no previous AIDS-defining diagnosis and $CD4^+$ counts of 200 to 499/mm^3 received 28 tablets each day of either a standardized oral preparation of 31 Chinese herbs or a cellulose placebo. All subjects reported taking 94% of prescribed tablets. Improvements in life satisfaction and symptoms were reported among subjects receiving herbal therapy. There were trends toward greater improvements among herb-treated subjects on all symptom subscales except skin disorders. However, believing that one was receiving herbs was strongly associated with reporting that the treatment had helped, but not with changes in life satisfaction or symptoms. The researchers concluded that the question whether Chinese herbs are effective in the management of symptomatic HIV infection may only be adequately addressed by larger trials of longer duration.[68]

Fairfield et al. conducted a telephone survey and medical chart review of 180 HIV-infected patients. Data were collected on the prevalence of alternative therapy use, out-of-pocket expenditures, and associated outcomes of alternative therapy use. The researchers determined that 122 (67.8%) of the patients used herbs, vitamins, or dietary supplements, 81 (45%) visited alternative medical providers, and 43 (23.9%) reported using marijuana for medicinal purposes within the past year. Mean yearly out-of-pocket expenditures for alternative therapies totaled $938 for all

therapies. Patients who saw alternative medical providers made a median of 12 visits per year versus seven visits to primary care physicians. College education and fatigue were associated with alternative medicine provider visits, while memory loss and fatigue were associated with supplement use.[69]

Standish et al. are studying, through the Bastyr University AIDS Research Center, 1500 HIV-positive men and women and the outcomes of those who use both alternative and conventional medicine versus those who use conventional medicine alone. The occurrence of AIDS-related opportunistic infections and neoplasms in HIV-positive patients who are using specific alternative therapies will then be compared with the incidence in those who use conventional medical treatment.[70]

NONTRADITIONAL NUTRITION THERAPY USE IN LOW SOCIOECONOMIC GROUPS

There is a growing concern that HIV-infected individuals may not receive adequate health care due to cultural and/or socioeconomic barriers. Those living in poverty in any ethnic background, including European Americans, face the same problems accessing health care as those classified as racial or ethnic minorities.[10] A number of economic factors contribute to the increase in progression of HIV infection to AIDS. These factors include lack of medical insurance, fear of medical care, limited transportation to a health clinic, and, in some rural areas, inadequate health care facilities.

To assess the vitamin, mineral, herbal, and non-nutritive supplement use in low socioeconomic HIV-infected individuals, 110 men and women were surveyed using a questionnaire format. Information regarding traditional (biomedical) and nontraditional (nonbiomedical) nutrition beliefs, attitudes, and practices was entered on a 3-part questionnaire. Part 1 consisted of statements relating to sources of nutrition information during HIV infection, current use of anti-viral medications, traditional supplement use (prescribed multivitamins), oral liquid supplement use (Boost, Advera), and past herbal supplement use (if applicable) during HIV infection. Part 2 requested information on 37 listed vitamin/mineral, herb, and non-nutritive supplements, their use, cost, and frequency of use during HIV infection. Part 3 asked for demographic information including gender, age, ethnic/racial background, education, employment, average monthly income, and number of years diagnosed HIV positive. A list of patient characteristics is shown in Table 1.

As shown in Table 1, the patient population at the AIDS clinic was compared to the study sample population. There was a difference in the percentage of individuals in the sample population who were considered “other” in racial/ethnic background. Seven percent of the sample population who answered the questionnaire was of Hispanic origin, and 1% of the study population was of Native American descent. Compared to the racial/ethnic population at the clinic of 1%, only 8% of the sample population was considered “other.” This may be an indication of how the population at the AIDS clinic is expanding in ethnic diversity. It could also indicate a possible increase in prevalence of HIV infection in Hispanic and other populations.

The data presented here extend previous observations[11,66] in that nontraditional nutrition therapy (NNT) use was more prevalent in white (non-Hispanic) HIV-positive

Table 1 Comparison of Patient Characteristics in an AIDS Clinic Population[a]

Variable	Total Population	Sample	Number of NNT Users (%)[b]	
Gender	69% Male	65% Male		
	31% Female	35% Female		
Race/Ethnic	67% Black	64% Black	Nonwhite	Yes 18 (22.8%)
	1% Other[c]	8% Other		No 61 (77.2%)
	32% White	28% White	White	Yes 14 (45.2%)
				No 17 (54.8%)
Age[d]	49%	47%		

[a] Clinic population approximately 1107; sample group = 110.
[b] Chi-square = 5.40; P = 0.02.
[c] Includes Hispanic and Native American individuals.
[d] Largest percentage of the population fell into the age group of 30–39 years.

individuals than in nonwhite HIV positive-individuals. Some studies have shown prevalence rates of use of herbal preparations in different ethnic populations to be lower in whites and Hispanics.[71]

In our study, income and educational level did not show a significant effect on NNT use. Results were similar to a study by Hung and colleagues.[71] This is contradictory to some previous studies[11,63,65,66] in which income and education were factors contributing to NNT use.

NNT users were more likely (chi-square = 4.45, P = 0.03) to believe that vitamin/mineral supplements are beneficial in fighting HIV infection as shown in Table 2.

Two additional factors relating to NNT use were investigated. The participants were asked whether they believed a particular vitamin, mineral, or herbal supplement was beneficial in fighting HIV disease, and whether would they spend their regular food money buying these supplements. A *t*-test revealed that the positive responses were significantly ($P = 0.0008$) related to NNT use. Vitamin C and selenium were the most prevalent vitamins and minerals used by our population group. Garlic was the herb most frequently used, and DHEA was the most popular non-nutritive supplement as shown by Table 3.

Table 2 Belief in Nutrition Supplements and NNT Use during HIV Infection[a]

	NNT Use	No NNT Use	Supplement Use (% of sample population)[b]
Belief	27	51	78 (73%)
No belief	4	25	29 (27%)

[a] n = 107.
[b] Phrased as "vitamin and mineral supplements are helpful in fighting HIV infection" on the questionnaire.

Table 3 Reported Frequency Use of Vitamins, Minerals, Herbs, and Other Supplements

Supplement	Frequency of Use[a]	
	Number of Subjects Reporting Use	Average Number of Tablets/Capsules Taken per Week
Vitamins		
Vitamin C	15 (46.9%)	7–14
Other multivitamin	9 (28.1%)	7
Vitamin E	5 (15.6%)	7–14
Vitamin A	4 (12.5%)	7
Vitamin B6	3 (9.4%)	7
Vitamin B12	3 (9.4%)	7
Minerals		
Selenium	4 (12.5%)	7
Iron	3 (9.4%)	7–21
Zinc	1 (3.1%)	7
Calcium	1 (3.1%)	14
Magnesium	1 (3.1%)	7
Herbs		
Garlic	8 (25%)	7–14
Ginseng	5 (15.6%)	3–14
Echinacea	5 (15.6%)	7
Bee pollen	2 (6.25%)	1–7
Aloe	2 (6.25%)	3–14
Chamomile	2 (6.25%)	3–7
Licorice root	1 (3.1%)	2
Dandelion	1 (3.1%)	2
Ginger	1 (3.1%)	2
Alfalfa	1 (3.1%)	1
Arrowroot	1 (3.1%)	1
Sea salt	1 (3.1%)	7
Shark cartilage	1 (3.1%)	3
Other Supplements		
DHEA	3 (9.4%)	7
L-Carnitine	1 (3.1%)	7
Chromium picolinate	1 (3.1%)	1
Melatonin	1 (3.1%)	2
Creatine phosphate	1 (3.1%)	14

[a] Percentage frequency based on 32 NNT study participants.

CONCLUSIONS/APPLICATIONS FOR HEALTH PROFESSIONALS

The acquired immune deficiency syndrome (AIDS) presents a challenge for dietitians and health care professionals alike. The American Dietetic Association believes that the more health professionals know about dietary supplements, including the benefits and adverse affects, the more able they are to communicate such information effectively to consumers.[72] Dietitians play an integral role in disseminating accurate and appropriate nutrition information within the community and among health care professionals. Qualified health professionals are unlikely to promote the benefits of supplements when the evidence is inconclusive, non-existent, or inaccessible. A more effective system for distributing/disseminating information to health professionals and consumers about the status of research on dietary supplements is needed.[72]

The question must be asked: Do HIV/AIDS patients feel comfortable about divulging information regarding supplement use to their health care professionals? If a patient does not feel confident about how such a coping method will be perceived, then chances are such information will not be provided to the health care worker. These patients sometimes have to overcome several social hurdles before reaching the point of discussing supplement use. These hurdles might include admitting sexual preference, admitting drug use, and ultimately admitting the reality of HIV disease. If keeping the use of supplements hidden will prevent more confrontation, then the patient will do exactly that.

Health care professionals should be especially aware of the need for building rapport with this special patient population. Because the patient needs to feel a certain amount of control over the disease, the dietitian must create an atmosphere in which the HIV-positive patient can feel safe in sharing information regarding the use of nontraditional nutrition therapies.

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CHAPTER 6

AIDS and Food Safety

Ralph Meer and Scottie Misner

CONTENTS

INTRODUCTION

The latest "official" figures on the impact of food-borne illness in the U.S. estimate that 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths are attributed to food and water-borne pathogens each year.[1] Norwalk and Norwalk-like viruses, *Salmonella*, and *Campylobacter* account for 93% of the illnesses while *Salmonella*, *Listeria,* and *Toxoplasma* account for 75% of the deaths attributed to known pathogens. Individuals with weakened immune systems, especially those with human immunodeficiency virus (HIV), belong to the group of sensitive populations who are at greater risk of morbidity and mortality associated with food and water-borne disease.[2-4] For example, one study found that the incidence of salmonellosis was 20-fold higher in men with AIDS compared to men without AIDS, and the incidence of *Salmonella* bacteremia was 45% in the former compared to 9% in the latter.[5] The destruction of helper T cell lymphocytes characteristic of HIV contributes

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to deficits in both cell-mediated and humoral immunity, resulting in an increased susceptibility to opportunistic infections and certain cancers.[6]

Common gastrointestinal illnesses in AIDS patients include giardiasis, amebiasis, cryptosporidiosis, microsporidiosis, salmonellosis, campylobacteriosis, shigellosis, hepatitis A, and cytomegalovirus enteritis.[7,8] Secondary infections, including those transmitted in food and water, can be difficult to treat, are more likely to be chronic in nature, and contribute significantly to the morbidity and mortality of AIDS patients. The prevention of food and water-borne infections can be achieved by implementing proper control measures to prevent, eliminate, or reduce food-borne hazards. Food-borne hazards can be biological (bacteria, parasites, fungi, viruses), chemical (pesticides, antibiotic residues, heavy metals), or physical (metal, glass, plastic). Biological hazards are of primary concern to those with compromised immune systems and are also responsible for the majority of food-borne illnesses associated with known cause.[9]

FOOD-BORNE DISEASE

A variety of microbial pathogens can be transmitted by food and water.[10-14] Tables 1 and 2 list selected pathogens associated with food and/or water-borne outbreaks. Generally speaking, the two types of food-borne disease are infections and intoxications. Food-borne infection results from eating food that contains a pathogenic microorganism (i.e., the agent is ingested and multiplies in the gut). Food-borne intoxication is typically caused by eating food that contains a toxin (i.e., the agent has produced a toxin in the food prior to consumption). Microbes responsible for food-borne infections include *Salmonella*, *E. coli*, *Listeria*, and *Cryptosporidium,* while those responsible for food-borne intoxications include *Staphylococcus aureus* and classical *Clostridium botulinum*. Food may play an active role in disease transmission by supporting growth of an etiological agent, or play a passive role where it does not support growth, but serves only as the vehicle of transmission. Many nonpathogenic microbes present in foods are of minimal significance to healthy individuals but have the potential to become opportunistic pathogens in immunocompromised patients.[15-18]

Pathogens present in raw animal foods or acquired as a result of cross-contamination during processing or preparation are responsible for more cases of food-borne illness than those resulting from contamination of foods by persons with infectious or communicable diseases. However, certain pathogens are more likely than others to be transmitted by infected persons. The Centers for Disease Control (CDC) publishes an annual list of infectious and communicable diseases that can be transmitted through food handling.[23] The latest list is summarized in Table 3. The presence of diarrhea, vomiting, open skin sores, boils, fever, dark urine, and jaundice may indicate infection from a pathogen that can be transmitted by food. The spread of pathogens can occur before symptoms develop, after they subside, or in persons who remain asymptomatic. Therefore, food handler hygiene, particularly proper hand washing, is paramount. Anyone preparing food or drink should first wash his

Table 1 Selected Bacterial Pathogens Associated with Food and/or Water-Borne Illness[19-22]

Organism	Onset/Symptoms/Duration	Associated Vehicle
Bacillus cereus (emetic)	1/2 to 6 hr. Nausea, vomiting, and occasional diarrhea (may resemble *S. aureus* intoxication). Up to 24 hr.	Rice products, starch foods (potato, pasta, and cheese products), sauces, puddings, soups, casseroles, pastries, and salads.
Bacillus cereus (diarrheal)	6 to 15 hr. Abdominal pain, nausea, and watery diarrhea. 24 hr.	Meats, milk, vegetables, and fish.
Campylobacter jejuni	2 to 5 days. Diarrhea, abdominal pain, nausea, fever, muscle ache, and malaise. 2 to 5 days.	Raw poultry, meat, and unpasteurized milk.
Clostridium perfringens	8 to 20 hr. Abdominal pain, nausea, watery diarrhea. 24 hr.	"Cafeteria germ." Improper cooling of cooked meat, and dishes containing meat and/or beans.
Escherichia coli O157:H7	3 to 8 days. Diarrhea (watery, may become bloody) severe abdominal cramps and pain, vomiting, mild fever, and may cause kidney failure in some people. 2 to 9 days.	Raw and undercooked ground beef, imported cheeses, unpasteurized milk and apple cider/juice, dry salami, lettuce, sprouts, and nonchlorinated water.
Listeria monocytogenes	Few days to 6 weeks. Nausea, diarrhea, headache, fever, chills, backache, meningitis, encephalitis, septicemia, spontaneous abortion or stillbirth. Days to weeks.	Raw or inadequately pasteurized milk and dairy products, packaged luncheon meats, and hot dogs.
Salmonella sp.	6 to 48 hr. Nausea, abdominal pain, diarrhea, sometimes vomiting, fever, chills, and headache. 1 to 2 days.	Unpasteurized milk, sprouts, fruit and fruit juice, raw or undercooked poultry, meat, and eggs.
Shigella sp.	12 to 50 hr. Diarrhea (may be bloody), abdominal pain, fever, nausea, cramps, vomiting, chills, fatigue, dehydration. 4 to 7 days.	Salads (potato, tuna, shrimp, chicken, and macaroni), lettuce, and raw vegetables.
Staphylococcus aureus	1 to 7 hr. Nausea, vomiting, abdominal cramps, and diarrhea. 8 to 22 hr.	Ham and other meats, reheated foods, high starch and/or protein salads, custard, and cream filled pastries.
Vibrio sp.	4 to 96 hr. Diarrhea, abdominal cramps, fever, chills, nausea, and septicemia. Days to weeks.	Raw shellfish and crustaceans.
Yersinia enterocolitica	1 to 11 days. Fever and severe abdominal pain (mimics appendicitis), sometimes diarrhea, vomiting, and headache. Days to weeks.	Meats (beef, pork, lamb), fish, oysters, raw milk, tofu, and nonchlorinated water.

Table 2 Selected Viral and Parasitic Pathogens Associated with Food and/or Water-Borne Illness[19-22]

Organism	Onset/Symptoms/Duration	Associated Vehicle
Hepatitis A	10 to 50 days. Fatigue, abdominal pain, jaundice, loss of appetite, nausea, and diarrhea. 1 to 2 weeks (severe cases can last several months).	Person to person contact, water, ice, salads, cold cuts and sandwiches, fresh fruits, juices, and vegetables.
Norwalk and Norwalk-like viruses	1 to 2 days. Nausea, vomiting, non-bloody diarrhea, and abdominal cramps. 1 to 3 days.	Water, food (particularly shellfish and salads), aerosols, and person to person contact.
Rotavirus	1 to 3 days. Vomiting, diarrhea, abdominal pain, and mild fever (more common in young children than adults). 4 to 8 days.	Water, ice, raw, and ready-to-eat (salads, fruits, sandwiches, hors d'oeuvres) foods.
Crytosporidium	1 to 12 days. Severe, watery diarrhea, may have no symptoms. 4 days to 3 weeks (relapses spanning 1 to 2 months are common).	Water, salads and raw vegetables, milk, and apple cider.
Cyclospora	Days to weeks. Watery diarrhea, loss of appetite, weight loss, bloating, gas, abdominal cramps, nausea, vomiting, muscle aches, fever, and fatigue. Days to weeks.	Water, marine fish, raw milk, and produce.
Entamoeba histolytica	1 to 4 weeks. No symptoms to vague gastrointestinal distress and diarrhea with blood and mucus. Potentially severe ulceration of the GI tract. May last several weeks.	Water, food, and person to person contact.
Giardia duodenalis (lamblia)	3 to 25 days. Fatigue, nausea, intestinal gas, weakness, weight loss, and abdominal pain. 1 to 2 weeks or more.	Water, ice, and salads.
Isospora belli	Several days to weeks. Watery diarrhea, abdominal pain, and low grade fever. Up to 1 month.	Water, food, and person to person contact.
Microsporidia	Several days to weeks. Watery diarrhea with intermittent normal stools. Several days to weeks.	Water and food suspected.
Toxoplasma gondii	5 to 20 days. Infections can be asymptomatic, enlarged lymph nodes (head and neck), severe headache, muscle pain, and rash. Days to weeks (relapses are common).	Raw or undercooked meat (pork, lamb, venison, and ground beef). Commonly transmitted via cat feces.

Table 3 Diseases Transmitted through Food Supplies by Food Handlers[23]

Pathogens Often Transmitted Through Food by Food Handlers	Pathogens Occasionally Transmitted Through Food by Food Handlers[a]
Caliciviruses (Norwalk and Norwalk-like viruses)	*Campylobacter jejuni*
Hepatitis A virus	*Cryptosporidium parvum*
Salmonella typhi	*Entamoeba histolytica*
Shigella sp.	Enterohemorrhagic *E. coli*
Staphylococcus aureus	Enterotoxigenic *E. coli*
Streptococcus pyogenes	*Giardia lamblia*
	Nontyphoidial *Salmonella*
	Rotavirus
	Taenia solium
	Vibrio cholerae
	Yersinia enterocolitica

[a] Usually transmitted by contamination at the source, in food processing, or by nonfood-borne routes.

or her hands thoroughly with warm water and soap, especially after using the toilet or changing diapers, handling raw animal foods, cleaning spills from raw animal foods, handling trash, or touching animals including pets.

From 1988 through 1992, the most commonly reported food preparation practice that contributed to food-borne illness was improper holding temperature. The second most common practice was poor personal hygiene of food handlers.[9] Traditionally, temperature control, i.e., adequate pasteurization and cooling/holding temperatures, served as the focus of control measures for preventing food-borne illness. However, over the past decade, the incidence of food-borne illness resulting from the consumption of ready-to-eat foods, e.g., luncheon meats and hotdogs, cereal, fresh produce, and juices, has increased.[24-34]

FOOD SAFETY COUNSELING

Since a significant number of food-borne illnesses result from improper food handling, persons with HIV/AIDS and their caretakers can protect themselves by following basic food safety guidelines. Complications associated with enteric infections (anorexia, maldigestion, malabsorption, diarrhea, weight loss, etc.) can exacerbate the already tenuous nutritional status typical of HIV/AIDS patients.[7,35] It has become a standard of care to incorporate information on food safety measures and their importance into counseling received by persons with HIV/AIDS as part of an overall strategy for defensive living.[22,36-40] A survey of 77 HIV-positive individuals conducted by Heathcock et al.[41] found they lacked knowledge about food storage, recommendations to avoid certain high risk foods, and infections transmitted by water. In addition, only 25% reported receiving information on food safety. This indicates a need to improve delivery of food safety information to these patients.

A number of food safety measures focus on potentially hazardous foods which, as defined by the FDA and USPHS,[42] are natural or synthetic foods that require temperature control to prevent (1) the rapid and progressive growth of infectious or

toxigenic microorganisms, (2) the growth and toxin production of *Clostridium botulinum,* and (3) the growth of *Salmonella enteritidis* in raw shelled eggs. Potentially hazardous foods include: foods of animal origin that are raw or heat-treated; foods of plant origin that are heat-treated (e.g., potatoes, beans, rice, etc.); or consist of raw seed sprouts, cut melons, and unprocessed garlic-in-oil preparations. Potentially hazardous foods do not include air-cooled hard-boiled eggs with intact shells, foods in unopened hermetically sealed containers (cans or jars) processed to achieve and maintain commercial sterility under conditions of nonrefrigerated storage and distribution, or foods that contain one or more barriers to prevent the growth of microorganisms cited earlier in this paragraph.

Common food safety measures are listed in Table 4. Also covered in Table 4 are increased risks of food and water-borne diseases associated with foreign travel, particularly to underdeveloped countries, and the potential for animals as sources of enteric pathogens.[22,43-48]

STERILE AND LOW MICROBIAL DIETS

Sterile and low microbial diets, as reviewed by Moe,[52] have been recommended and are used to reduce the exposure of immunocompromised persons to pathogens and opportunistic infections that can be transmitted by food and water. However, few studies have evaluated their efficacy in these patients. A review by Aker and Cheney[53] on the incidence of infection, morbidity, mortality, and response to treatment in immunosuppressed cancer patients receiving sterile, low microbial, or regular diets concluded that benefits of these diet modifications had not been established. It is challenging to provide a sterile diet (i.e., zero microbes in foods or on food contact surfaces such as utensils, cups, plates, and meal trays) in a clinical setting, let alone at home. A low or reduced microbial diet is more realistically attained; however, the extent of microbial reductions in these diets can vary.[52] An example of a low microbial diet is listed in Table 5. Despite the lack of evidence indicating clear benefits from using an altered or reduced microbial diet, it would seem prudent that HIV-positive patients and especially those with AIDS institute control measures to reduce exposure to infectious organisms transmitted by food and water.

ENTERAL FORMULAS

Nutritional status is frequently compromised in HIV/AIDS patients. Therefore, the use of various liquid or enteral formulas taken as oral supplements or delivered via tube feeding is common. Enteral formulas are considered commercially sterile as received from the manufacturers; however, they have many opportunities, from opening to administration, to become contaminated. The nutritional compositions of these formulas provide exceptional media for bacterial growth. A number of organisms including *Bacillus cereus*, β-hemolytic streptococci, *Enterobacter*, *E. coli*, *Klebsiella*, *Moraxella*, *Proteus*, *Pseudomonas*, *Salmonella enteritidis*, *Serratia, Staphylococcus aureus*, *Staphylococcus epidermis*, and yeasts have been isolated from

Table 4 Measures to Reduce Exposure to Enteric Pathogens and Prevent Food and Water-Borne Illness[40,49-51]

Control	Measures
Hygiene	Wash hands with soap and hot water followed by rinsing and drying: before handling food; after using the rest room or changing diapers; after handling raw animal foods; after handling trash or taking out the garbage; after coughing, sneezing, or touching hair or face; after touching and cleaning up after animals/pets.
Cleaning	Food preparation utensils, dishes, pots, pans, cutting boards, and other food contact surfaces should be cleaned with hot water and soap after each use. Avoid cross-contamination between raw animal foods and cooked or ready-to-eat foods. Above items can be sanitized using a dilute bleach [½ tsp (5.25% household bleach) in one liter of water = 130 ppm] solution. Items need to be adequately cleaned before sanitizers will work effectively. A 1:10 dilution of household bleach should be used to disinfect surfaces exposed to large amounts of blood or body secretions.
High Risk Foods	Avoid raw or undercooked animal foods (beef, pork, poultry, eggs, fish, seafood, milk, dairy products) or items that contain these ingredients. Avoid direct or indirect cross-contamination of these foods or their juices by ready-to-eat foods. Avoid unpasteurized fruit and vegetable juices. Avoid uncooked seed sprouts. Do not consume soft cheeses (brie, cambert, feta, Mexican style, etc.). Due to the prevalence of *Listeria* in hot dogs and luncheon meat, reheat these foods until steaming hot. Thoroughly wash produce in a container (3:1 water to produce) followed by rinsing. Produce should be scrubbed, rubbed, and/or agitated during washing depending on its stability. Heavily soiled produce may require a double wash. Consider peeling produce prior to consumption if applicable. It is important to wash produce even if you do not plan to eat the peel since contamination may occur during cutting or peeling.
Cooking	Cooking raw animal foods or dishes containing them to an endpoint temperature of 165°F will provide an appropriate margin of safety for most foods. Molluscan shellfish (oysters, clams, etc.) should be cooked to 180 to 190°F since some viruses are more resistant to cooking than bacteria. Use a needle tip instant-read thermometer to measure endpoint temperatures. Color is not a reliable indicator of doneness. Due to less uniform heating, microwave cooking requires special precautions. Follow the “stand time” instructions or stir food to ensure proper temperature is reached throughout. Reheat leftover food to 165°F.
Holding	Keep hot foods hot (≥140°F) and cold foods cold (≤41°F). Potentially hazardous foods held in the temperature danger zone (41 to 140°F) for more than 4 hours should be discarded.
Shopping	Read labels; make sure all milk and dairy products are pasteurized. Use foods before use-by or purchase-by date. Avoid foods in damaged packaging. Shop for perishables last. Place raw meat, poultry, fish, and seafood in plastic bags. After shopping put all cold and frozen foods away as soon as possible.
Eating Out	Avoid high risk foods and ingredients as you would at home including raw or lightly steamed fish and seafood (e.g., oysters, clams). Order foods well done.
Water	Because you cannot be absolutely sure tap water is 100% safe, the prudent measure is to boil it for 1 minute. Avoid swimming in streams, rivers, lakes, reservoirs, etc.
Travel	Traveling can mean increased risk of water- and food-borne illness. Alert your healthcare provider about your travel plans and potential need to follow special measures. In developing countries, do not eat raw produce unless you can peel it. Eat cooked foods while they are hot. Consume bottled or canned processed beverages only; otherwise boil all water for 1 minute before drinking it. Use ice made from boiled water only.

Table 4 Measures to Reduce Exposure to Enteric Pathogens and Prevent Food and Water-Borne Illness[40,49-51] *(continued)*

Control	Measures
Pets	Pets can be sources of microbes that can cause secondary infections (e.g., cats and toxoplasmosis, reptiles and salmonellosis). Avoid animals less than 6 months of age especially those with diarrhea. Wash hands thoroughly after handling pets. Use disposable gloves when handling animal feces or material containing feces. Avoid feeding pets raw animal foods. Keep cats indoors so they cannot hunt.

Resources:

http://www.cdc.gov/nchstp/hiv_aids/pubs/brochure.htm
http://www.cdc.gov/health/diseases.htm
http://www.cdc.gov/travel/hivtrav.htm
http://www.cdcnpin.org
http://www.hivatis.org/resoinfo.html
http://www.fsis.usda.gov/OA/pubs/aids.htm
http://www.fsis.usda.gov/thermy/index.htm

If you cannot access the above web pages directly, try searching the main web pages: Centers for Disease Control (www.cdc.gov); Food Safety Inspection Service, USDA (www.fsis.usda.gov); CDC's National Prevention Information Network (www.cdcnpin.org) — formerly the National AIDS Information Clearing House — or the HIV/AIDS Treatment Information Service (www.hivatis.org).

contaminated enteral formulas.[54-59] In addition, a number of studies have indicated that enteral nutrition solutions can be sources of nosocomial infections.[60-66]

Factors identified with microbial contamination of enteral solutions include formula preparation (mixed by hand versus ready to feed), length of hang time, and use of open versus closed feeding systems.[55,63,65,67-70] Recommendations to preclude or minimize the chances of microbial contamination of enteral nutrition formulas include following the manufacturer's instructions; use of commercially sterile products only; aseptic handling of formulas during mixing, including wearing sterile or new non-sterile gloves; limiting hand times to eight hours, although frequent refilling of containers can be a source of contamination[71]; use of closed systems; use of sterile water to reconstitute or dilute formulas; blast freezing; addition of potassium sorbate to powder formulations; and regular bacteriological surveillance of enteral formulas and their delivery.[52,72-75] Patients taking enteral formulas as oral supplements should not let open cans sit at room temperature for more than 4 hours. If they cannot consume all the formula in a container, they should portion out the amounts they will use, cover the open container, and place it in the refrigerator. Refrigerated open cans should be discarded after 24 hours.

FOOD SERVICE ISSUES

Balancing the rights of an HIV-positive worker with the concerns and fears of fellow workers and patrons has been described as "Catch 22."[76] The routine testing of food service workers for the presence of HIV is not recommended as a measure to avoid the transmission of the disease from workers to customers.[77] Employment

Table 5 An Example of a Low Microbial Diet[52]

Food Group	Permitted	Not permitted
Beverages	Instant coffee; instant tea; canned fruit drinks; fruit-flavored powdered drink mix; carbonated beverage; pasteurized beer; bottled seltzer water; sterile water and ice.	Non-pasteurized beer; wine; bottled, distilled water.
Milk and dairy products	Ultra-heat treated milk; instant hot cocoa mix; commercially sterilized milk shake products; canned milk; half-and-half creamer; American cheese; cream cheese in individual packets; processed/pasteurized cheese spread and cheese food spread; canned puddings.	Whipped cream; nondairy whipped topping; pasteurized milk; yogurt; cheese other than American; buttermilk; ice cream (all varieties); sherbet; cottage cheese; sour cream; powdered instant breakfast drinks; homemade and commercially prepared refrigerated puddings.
Fruit and fruit juices	Canned fruit; canned and bottled fruit juices; baked apples.	Fresh fruits and juices; raisins and other dried fruit.
Vegetables and vegetable juices	Canned vegetables and vegetable juices; canned bean salad; well cooked frozen vegetables; baked fresh squash.	Fresh vegetables and juices; onion rings.
Potato and potato substitutes	Cooked white or sweet potatoes; yams; french fries; hash browns; instant mashed potatoes; rice; pasta; noodles cooked in sterile water; chow mein noodles.	Raw potatoes; au gratin potatoes; rice, pasta, potatoes cooked in non-sterile water; potato and macaroni salad.
Breads and cereals	All breads; English muffins; bagels (except onion); hamburger and hot dog buns; dinner rolls; tortillas; hot and cold cereals except as noted; pancakes, waffles, french toast; blueberry and plain muffins, crackers.	All raisin and nut-containing cereals and breads; cinnamon rolls; donuts; onion bagels.
Meat and meat substitutes, mixed entrees	All hot, well-cooked red meats, poultry, and fish; canned meats, poultry, fish, and shellfish; well-cooked hot dogs; well-cooked eggs; spaghetti sauce; frozen commercial mixed entrees, heated thoroughly; smooth peanut butter; canned beans/legumes; jarred baby food.	Deli meats; processed luncheon meats; raw eggs; dried meats (jerky); rare and medium cooked meats, poultry, seafood; lasagna; pizza.
Soups	All hot canned and dehydrated packaged soups, broth, and bouillon.	Home-made soups; commercially refrigerated and frozen soups; cold soups.
Fats and oils	Margarine; vegetable oil; fat for deep fat frying; shortening; mayonnaise; tartar sauce from individual packets; canned gravy and sauces.	Butter, homemade gravy, hollandaise sauce; tartar sauce, and mayonnaise from multi-serving containers.
Condiments and spices	Individually packaged mustard, ketchup, taco sauce, lemon juice, salad dressings, jams, jelly, cranberry sauce, honey and syrup; sugars; salt; canned chocolate syrup; dill pickles; canned black olives; seasonings, spices, and pepper added before cooking.	Condiments from multi-serving containers; green olives; sweet pickle relish; seasonings, spices, and pepper added after cooking.

Table 5 An Example of a Low Microbial Diet[52] *(continued)*

Food Group	Permitted	Not permitted
Desserts and snacks	Pound and angel food cakes; commercial gingersnaps, frosting-filled sandwich cookies, shortbread, vanilla wafers; corn and tortilla chips; crackers; popcorn; canned dips; cupcakes and individually packaged fruit pies; popsicles on sticks; Jello; home-made custard; hard candies, jelly beans, gum drops, orange slices; gummy bears, lemon drops, marshmallows, peanut butter cups, plain chocolate disc candies; chewing gum.	All other cakes; all other cookies; cracker jacks; pies; nuts, all varieties; potato chips; pretzels; ice cream bars; candy made with nuts or dried fruit; candy bars.
Nutritional supplements	Glucose polymers; powdered supplements reconstituted with sterile water; canned supplements.	

Note: Acceptability criteria: $<10^3$ CFU/mL of coagulase-negative staphylococci or *S. viridans* and $<10^4$ CFU/mL of *Bacillus* sp., diphtheroids, or *Micrococcus* sp.

of an HIV-infected individual is not a violation of the Occupational Health and Safety Act because HIV disease is not transmitted by casual contact and does not pose a risk in the work place.[78] The Hatch Amendment to the Americans with Disabilities Act of 1990 requires identification of diseases that can be spread by food handlers by the Department of Health and Human Services. Since HIV infections are not believed to be transmitted by food handlers, HIV-infected persons cannot be removed from food handling positions.[78]

It is recommended that all food service workers abide by the recommended standards and practices of good personal hygiene and food sanitation.[22] All food service workers should avoid hand injuries while preparing food. If injury should occur, aesthetic and health considerations require discarding any food or beverage contaminated with blood.[77] As with other employees, HIV-infected persons do not need to be restricted from work unless they have been diagnosed with a food-borne illness or exhibit symptoms of gastrointestinal illness.

Disposable utensils, plates, and trays are not required for patients infected with HIV because serving items have not been implicated in the transmission of HIV infection.[77,79] However, to reduce fears of food service personnel, educational programs that include food service care for persons with such infections should be provided.[80] Food service establishments need appropriate food safety measures to control food hazards throughout the food product flow while maintaining routine standard sanitation procedures for cleaning, sanitizing, facility maintenance, pest control, and employee hygiene.

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CHAPTER 7

Thiols to Treat AIDS

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CONTENTS

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INTRODUCTION

Infection with human immunodeficiency virus type 1 (hereafter referred to as HIV) results in a progressive impairment of immune function and leads to opportunistic infections and malignancies of the acquired immunodeficiency syndrome (AIDS) if untreated. The fundamental immunologic abnormality is a progressive impairment of the numbers and functions of $CD4^+$ lymphocytes. Because the $CD4^+$ lymphocytes are important immune regulatory cells, various immune functions are affected. During recent years, several reports have suggested that impaired antioxidant defense plays a role in the immunopathogenesis of HIV infection.[1-5]

REACTIVE OXYGEN SPECIES AND ANTIOXIDANTS

As an essential part of human metabolism, oxygen is required to transform different substrates for the release of energy, oxidize endogenous compounds, and detoxify xenobiotics. In these processes, most of the oxygen acts as a terminal 4-electron acceptor and is completely reduced to water. However, a small amount of oxygen is normally only partially reduced, yielding various reactive oxygen species (ROS). ROS include free radicals, i.e., molecular species containing one or more unpaired electrons, such as the hydroxyl radical and superoxide anion, and species that are not, but may readily be, converted to oxygen radicals, e.g., hydrogen peroxide, in the presence of, for example, iron or copper ions.[6,7] Reactions of free radicals with other biological molecules tend to proceed as chain reactions; one radical begets another and so on,[6-8] leading to oxidative damage of proteins, carbohydrates, lipids, and DNA.

The steady state formation of ROS is normally balanced by a similar rate of consumption by antioxidant reactions that are enzymatic and/or nonenzymatic.[7,9,10] Oxidative stress results from imbalance of this ROS-antioxidant equilibrium in favor of the ROS.[10] Important scavenger enzymes are superoxide dismutase that catalyzes the conversion of superoxide anion to hydrogen peroxide, catalase that promotes the conversion of hydrogen peroxide to water and oxygen, and glutathione peroxidase that reduces intracellular oxidants, such as hydrogen peroxide, by the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG).[6,7,9,10]

Additional important factors are compartmentalization of hydrogen peroxide-generating enzymes in peroxisomes, and chelation of free iron or copper ions to transferrin, ferritin, lactoferrin, albumin, or ceruloplasmin, thereby preventing these metal ions from participating in ROS generation.[6-8,11,12]

In addition to the primary defenses (scavenger enzymes and metal-ion sequestration), secondary defenses are also present. Lipid-soluble α-tocopherol, the most effective antioxidant component of vitamin E, and water-soluble ascorbic acid (vitamin C) may function as chain-breaking antioxidants, creating new ROS which are poorly reactive and can be reconverted to the antioxidant compound.[6,7,11,12]

GLUTATHIONE: THE MAJOR INTRACELLULAR ANTIOXIDANT

Glutathione is a cysteine containing tripeptide (γ-glutamyl-cysteinyl-glycine) found in eukaryotic cells at millimolar concentrations and is regarded as the major

intracellular redox buffering principle.[6,7] Glutathione is the substrate of selenium-dependent glutathione peroxidase catalyzing detoxification of hydrogen peroxide and other oxidants, while glutathione reductase catalyzes the regeneration of reduced glutathione from oxidized glutathione. Another antioxidant enzyme, glutathione transferase, inactivates reactive electrophilic species[7] and participates in the metabolism of such endogenous compounds as steroids and leukotrienes.[6,7] Glutathione also has antioxidant properties.[13] Finally, GSH provides reducing power for the maintenance of other antioxidants, e.g., ascorbic acid (vitamin C), vitamin E, and β-carotene.[6,7]

Ascorbic acid is a powerful antioxidant that reacts with superoxide, peroxide, and hydroxyl radicals, causing the formation of dehydroascorbic acid. Dehydroascorbic acid is converted back to the reduced form, ascorbic acid, by GSH.[14] Ascorbic acid and glutathione act together as antioxidants, and evidence suggests that GSH can spare ascorbic acid and vice versa.[15-17]

Vitamin E, the major lipophilic antioxidant protecting cell membranes against lipid peroxidation, is coupled to the hydrophilic antioxidants glutathione and ascorbic acid.[19]

The relative levels of oxidized and reduced glutathione are normally regulated by a series of enzymes which include the glutathione peroxidase and glutathione reductase.[7,19] Normally, almost all (>99%) intracellular glutathione is in the reduced form.[19] However, GSSG can accumulate under certain circumstances such as rapid GSSG production, reduced glutathione reductase activity, or impaired transport of GSSG out of the cell.[19] The synthesis of GSH is regulated by feedback inhibition from its end product, GSH. When GSH is consumed and feedback inhibition is lost, the availability of cysteine as a precursor can become the limiting factor.[20]

THIOL STATUS IN PATIENTS WITH HIV INFECTIONS

During the last years, several reports have suggested that impaired antioxidant defense plays a role in the immunopathogenesis of HIV infection. With regard to glutathione homeostasis, several reports have demonstrated abnormalities *in vivo* during HIV infection, although the results are somewhat conflicting. Decreased levels of reduced glutathione in HIV-infected patients have been found in plasma,[1] in lung epithelial lining fluid,[1] in PBMC,[2] and in $CD4^+$ and $CD8^+$ lymphocytes.[3,4] On the other hand, in two studies, levels of reduced glutathione in PBMC from HIV-seropositive patients were not different from levels found in healthy controls.[21,22] Furthermore, we have demonstrated elsewhere that increased levels of oxidized glutathione and a decreased ratio of reduced to total glutathione, rather than decreased levels of reduced glutathione, are the major glutathione disturbances in $CD4^+$ lymphocytes from HIV-infected patients, particularly in patients with advanced clinical and immunological disease.[5]

It has been claimed that depletion of reduced glutathione in HIV infection is caused by decreased availability of precursor amino acids, particularly cysteine.[2,21,23] However, in one study, depletion of reduced glutathione in serum was accompanied by normal cysteine levels.[24] Furthermore, except for a slight decrease in oxidized

cysteine, studies in our group did not demonstrate any abnormalities in plasma cysteine levels in HIV-infected individuals.[5] As an alternative explanation for the deranged thiol status in HIV-infected patients, Dröge et al. have proposed that elevated circulating concentrations of glutamate in HIV infection may inhibit the uptake of oxidized cysteine into monocytes and macrophages which then will make less reduced cysteine available to lymphocytes for synthesis of reduced glutathione.[25,26] However, we and others[5,27] could not confirm these findings.

Thus, several lines of evidence suggest the existence of important glutathione abnormalities in HIV-infected individuals. However, several controversies also exist concerning thiol status during HIV infection, as discussed in detail elsewhere.[28]

Patients with HIV infections seem to have normal levels of homocysteine.[24,29] However, we have shown that HIV-infected patients have significantly elevated levels of reduced homocysteine.[29] This might possibly contribute to the production of ROS as the sulfhydryl group of homocysteine is believed to act catalytically with cupric or ferric ions to generate hydrogen peroxide and various homocysteinyl radicals.[30-32]

Also, the HIV-infected patients in our study had reduced methionine concentration in plasma. This is in accordance with previous reports.[2,27] As methionine is an essential amino acid, malabsorption may lead to methionine deficiency. However, none of the patients in this study had clinical signs or symptoms suggesting malabsorption.[29] Thus, the reason for reduced methionine levels is unclear.

A significant but modest decrease in the total cysteinylglycine plasma concentration was found in the HIV patients, compared to controls. As cysteinylglycine is a degradation product of glutathione,[19] the decreased concentration of cysteinylglycine might reflect low glutathione turnover. In fact, plasma glutathione tended to be lower in this group of HIV patients, compared to controls.[5] The concentration of reduced cysteinylglycine tended to be slightly elevated in the patient group, resulting in an increased reduced/total ratio. As with homocysteine, elevated levels of reduced cysteinylglycine might contribute to ROS generation due to the presence of a free sulfhydryl group.[32]

INCREASED LEVEL OF OXIDIZED GLUTATHIONE — AN IMPORTANT INDICATOR OF INCREASED OXIDATIVE STRESS DURING HIV INFECTION

Increased levels of oxidized glutathione and decreased ratio of reduced to total glutathione seem to be better parameters of increased oxidative stress than decreased levels of reduced glutathione.[19,33-37] It appears that the capacity of the glutathione redox cycle, rather than intracellular levels of reduced glutathione, may determine resistance to oxidative stress, at least in some cell types.[19,33,34]

While most studies analyzing intracellular glutathione status in PBMC or lymphocyte subpopulations in HIV-infected individuals have only measured levels of reduced glutathione, we have previously determined the glutathione redox balance in lymphocyte subpopulations and monocytes during HIV infection by measuring intracellular levels of both reduced and total glutathione.[5] We found a marked increase in oxidized glutathione and a considerable decrease in ratio of reduced to total glutathione as the major glutathione redox disturbances in $CD4^+$ lymphocytes

from HIV-infected individuals. In contrast to resting mammalian cells, where only a small fraction of total glutathione exists in the oxidized form,[19,38] we found that $CD4^+$ lymphocytes from the majority of patients with symptomatic HIV infection had ratios of reduced to total glutathione below 0.5. The increase in oxidized glutathione and the decrease in ratio of reduced to total glutathione, but not the moderate decrease in levels of reduced glutathione in $CD4^+$ lymphocytes, were significantly correlated with advanced clinical and immunological disease.[5] Among the $CD4^+$ lymphocytes, the most pronounced glutathione abnormalities were found in the "naive" ($CD45RA^+$) subpopulation.[39] These changes reflecting increased oxidative stress may represent important immunopathogenic factors in HIV infection.

Several factors may influence the intracellular levels of oxidized glutathione during oxidative stress, including increased generation, the activities of glutathione reductase and glutathione peroxidase, and the ability to export oxidized glutathione from cells.[19,38,40] Furthermore, a recent study suggests that the regulatory HIV protein Tat may decrease glutathione synthesis, probably through increased sensitivity of biosynthetic enzymes to feedback inhibition by GSH.[41]

Several studies have focused on the cooperation between glutathione and the thioredoxin system.[42,43] Indeed, thioredoxin appears to be of importance for maintenance of glutathione in a reduced state and vice versa.[38,44] Furthermore, it seems that some of the effects seen after increasing the intracellular ratio of reduced to total glutathione may at least in part be mediated by raised thioredoxin levels (e.g., inhibition of apoptosis).[42,45] Masutani et al. found that thioredoxin high producer cells were selectively lost in lymph nodes from AIDS patients.[46] In a study by Nakamura et al., elevated plasma thioredoxin levels were found in HIV-infected patients, especially in those with advanced disease.[47] Thus, the redox disturbances of the glutathione and the thioredoxin system seen in HIV-infected patients may both contribute to the markedly disturbed intracellular redox balance.

ENHANCED OXIDATIVE AND INFLAMMATORY STRESS — A VICIOUS CIRCLE OPERATING IN HIV INFECTION

Several lines of evidence suggest that cytokine dysregulation, especially increased TNFα activation, may contribute to the oxidative stress in patients with HIV infection. TNFα stimulation may decrease levels of reduced glutathione by causing enhanced ROS production which in turn leads to consumption of this glutathione species.[48-50] TNFα stimulation both *in vivo* and *in vitro* has also been demonstrated to increase oxidized glutathione levels and to impair the activity of the glutathione reductase system.[48,51-53] Moreover, it seems that antioxidants such as *N*-acetylcysteine (NAC) and glutathione impair TNFα production from PBMC.[54,55] Furthermore, glutathione redox disturbances as found in $CD4^+$ lymphocytes from HIV-infected individuals may possibly increase the inflammatory cellular response to TNFα stimulation,[56] and proper glutathione redox status is of major importance in protecting against the toxic effects of TNFα.[51,57]

Finally, increased TNFα activation may in turn increase the sensitivity of cells to ROS exposure.[48] Thus, TNFα activation with enhanced ROS generation and

disturbed glutathione homeostasis may represent a vicious circle leading to increased levels of oxidative stress with important clinical, immunological, and virological consequences in HIV infection.

IMMUNOLOGICAL CONSEQUENCES OF GLUTATHIONE REDOX DISTURBANCES IN HIV INFECTION

Disturbed intracellular glutathione metabolism in lymphocytes may result in immunological dysfunction either as a direct effect of decreased levels of reduced glutathione,[58,59] as a direct effect of increased levels of oxidized glutathione,[60,61] or indirectly through intracellular redox disturbances and oxidative stress.[40,62] As enhanced ROS production may induce glutathione redox disturbances, it is difficult to separate the effects of increased ROS generation from disturbed glutathione homeostasis.

Several immunological functions related to HIV infection are dependent on adequate intracellular glutathione redox balance, e.g., lymphocyte activation by mitogens, natural killer cell activation, and T cell mediated cytotoxicity.[58,63,64] Furthermore, a shift from a Th1 (IL-2) to a Th2 (IL-4 and IL-10) cytokine profile, possibly in combination with an activated TNF system, has been suggested to be of importance in the immunopathogenesis of HIV infection.[65-67] Interestingly, studies have found that thiol supplementation or reducing, in contrast to oxidizing conditions, may suppress Th2 cytokine production.[68,69]

Decreased T cell proliferation or anergy upon antigen stimulation is an important immunological feature of HIV infection.[70] This hypoproliferation may manifest long before any decline in the absolute numbers of $CD4^+$ lymphocytes is observed.[71,72] Disturbed intracellular glutathione homeostasis may be of importance for this defect in T cell proliferation. A decrease in intracellular levels of reduced glutathione by as little as 10 to 30% almost completely abrogated the intracellular calcium flux and the proliferative response when T cells were stimulated through the T cell receptor (TCR)/CD3 complex.[56] Also, IL-2 production, which is of major importance for an adequate lymphocyte proliferative response, seems to be impaired by decreased intracellular levels of reduced glutathione,[73,74] although the results are somewhat conflicting.[75,76] Indeed, we have demonstrated a significant correlation beween intracellular redox disturbances in $CD4^+$ lymphocytes and both impaired lymphocyte proliferation and decreased IL-2 production in HIV-infected patients.[5] Furthermore, studies of Cayota et al. have demonstrated that restoration of glutathione redox balance by antioxidant supplementation was able to revert the impaired proliferative activity of $CD4^+$ lymphocytes from HIV-infected patients upon CD3 stimulation.[77]

GLUTATHIONE AND APOPTOSIS IN HIV INFECTION

The markedly disturbed intracellular glutathione homeostasis, particularly when combined with increased TNFα activity, may be of major importance for the induction

of lymphocyte apoptosis in HIV infection.[78,79] Interestingly, mitochondrial changes associated with early stages of apoptosis were demonstrated in circulating T lymphocytes from HIV-infected individuals.[80] It has also been shown that thiol redox status may directly affect mitochondrial function, thus serving as an important regulator of apoptosis.[81] Furthermore, the antioxidant *N*-acetylcysteine (NAC) has been found to inhibit apoptosis in HIV-infected cells.[82]

VIROLOGICAL CONSEQUENCES OF GLUTATHIONE REDOX DISTURBANCES IN HIV INFECTION

Evidence indicates that an abnormally activated immune system is needed to maintain a high level of virus replication.[83,84] TNFα and ROS are both potent stimulators of HIV replication through activation of NF-κB which is essential for the expression of genes controlled by the LTR of HIV.[49,85,86] It has been suggested that increased oxidized glutathione or decreased reduced-to-total glutathione ratio may directly enhance HIV replication through this mechanism.[87,88]

Furthermore, NAC, glutathione, and glutathione esters have been demonstrated to inhibit HIV replication *in vitro,* both in acutely and chronically infected cell lines and in lymphocytes and monocyte/macrophages from HIV-seronegative donors.[49,89-95] It seems that the antiviral effect of glutathione is mediated by inhibition of NFκB activation.[49,89,93] However, Bergami et al.[90] have reported that cystamine, which may enhance intracellular levels of reduced glutathione, inhibits HIV replication in human lymphocytes and macrophages by interfering with the orderly assembly of HIV virions.[96] Furthermore, oltipraz (4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione), which also may increase intracellular levels of reduced glutathione, has been found to be a potent inhibitor of HIV reverse transcriptase.[97,98] Thus, one cannot totally exclude the possibility that the antiviral effects of glutathione and its esters may also be more direct.

CORRECTION OF THIOL REDOX STATUS — POSSIBLE THERAPEUTIC APPROACHES IN HIV-INFECTED PATIENTS

The cornerstone in treatment of HIV-infected patients is so-called highly active antiretroviral therapy (HAART) with combinations of different classes of reverse transcriptase inhibitors and protease inhibitors.[99,100] Although the results of HAART have been impressive, there are also several problems related to this treatment, such as the emergence of drug resistant HIV variants, long term side effects, difficulties with strict adherence to treatment regimens, and lack of immunological reconstitution.[99-103] These difficulties have led to a renewed interest in other treatment modalities, including immunomodulating agents. One possible immunomodulatory strategy is a treatment that restores the dysregulated thiol redox balance in patients with HIV infections. In particular, modulation of the intracellular glutathione redox status in $CD4^+$ lymphocytes is promising.

CYSTEINE PRODRUGS

N-acetyl-L-cysteine (NAC) is a direct ROS scavenger and is metabolized *in vivo* to cysteine which restores intracellular GSH levels.[104,105] NAC presently is used as an antidote for paracetamol (acetaminophen) overdose[106] and in the treatment of various respiratory diseases.[107,108] It can be given orally, even if the bioavailability is less than satisfactory, and is well tolerated.[109] NAC effectively inhibits TNF-α-induced activation of the HIV long terminal repeat, leading to reduced HIV replication in infected cells.[89,93] This effect of NAC is due to a specific blocking of the induction of NF-κB.[49,110] However, a recent study suggests that NAC does not inhibit all NF-κB activation pathways.[111] Also, NAC has been shown to enhance antibody-dependent cellular cytotoxicity of cells from patients with HIV infection.[112] When studied *in vitro,* the impaired proliferative capacity of $CD4^+$ lymphocytes from HIV-infected patients was restored by NAC.[77] In cultured T cells from HIV-seronegative donors, NAC enhanced both the proliferative capacity and IL-2 production.[73] Furthermore, NAC has been shown to inhibit apoptosis of T lymphocytes and monocytoid cells,[113,114] and also in HIV-infected monocytoid cells.[82,115] However, this anti-apoptotic effect of NAC may not be mediated by an increase in intracellular GSH.[116]

The effect of NAC administration to patients with HIV infection has been adressed in other studies. De Quay et al.[21] noted a moderate increase in glutathione levels in PBMCs from eight HIV-infected patients in response to a single dose of NAC. However, the glutathione redox balance in $CD4^+$ lymphocytes was not assessed in this study. In a study over 14 weeks, Walker et al. treated HIV patients with four different dosage levels of NAC and did not observe serious toxicities.[117] In another study, six patients were given NAC for 2 weeks, but no increase in glutathione level in PBMCs or plasma was noted.[118] In a double-blind placebo-controlled trial, Akerlund et al. treated 45 HIV-seropositive patients with $CD4^+$ lymphocyte counts $> 200 \times 10^6$/l for 4 months with orally administered NAC (800 mg daily) or placebo and found that the decline of the $CD4^+$ lymphocyte count was less steep in the NAC group compared to the placebo group.[119] Hertzenberg et al. administered NAC for 8 weeks to 27 HIV-seropositive patients and found a significant increase in whole blood GSH as well as increased survival, compared to placebo.[120] Finally, Look et al. administered NAC and sodium selenite for 24 weeks to asymptomatic HIV-infected patients and found increased CD4/CD8 ratios, a trend toward an increase in the percentage of $CD4^+$ lymphocytes, but no alteration in viral load as assessed by HIV-RNA quantification in plasma.

In a pilot study,* we examined virological and immunological effects of antioxidant combination treatment for 6 days with high doses of NAC and vitamin C in eight patients with HIV infections. In the five patients with the most advanced immunodeficiency ($CD4^+$ lymphocyte counts $< 200 \times 10^6$/l), a significant rise in $CD4^+$ lymphocyte count, reduction in HIV RNA plasma level of 0.8 log, enhanced lymphocyte proliferation, and an increased level of intracellular glutathione in $CD4^+$ lymphocytes were found.

* Submitted for publication.

There may be several reasons for the lack of GSH response to NAC treatment in most studies. First, as mentioned above, the bioavailability of NAC is low by oral treatment.[109] Also, the control point in glutathione synthesis, γ-glutamylcysteine synthetase, is subject to feedback inhibition by GSH, and this may inhibit the effect of supplementation with NAC or other cysteine prodrugs.[19]

Another cysteine prodrug, *L*-2-oxothiazolidine-4-carboxylic acid (OTC; also called procysteine), has been shown to increase GSH levels in lymphocytes when given to healthy volunteers.[122] OTC enters the cells independently of the cystine transport pathway and is converted to cysteine by oxoprolinase inside the cell.[107] While NAC has the dual ability to replenish GSH and scavenge oxidants, OTC is strictly a glutathione precursor. When NAC and OTC were compared with respect to inhibition of cytokine-induced HIV replication in various cell lines, it was observed that NAC was far more effective than OTC.[94] Also, NAC fully replenished intracellular GSH, while OTC did not.[94] In a phase I/II trial of intravenous OTC for six weeks in 24 asymptomatic HIV-infected patients, no change in $CD4^+$ lymphocyte count or HIV viral load was observed, although in the subgroup of patients receiving the highest OTC dose, whole blood glutathione was significantly higher at the end of the study, compared to baseline levels.[123]

The thiol compound, cystamine, has been shown to inhibit HIV replication both in lymphocytes and macrophages *in vitro*.[90,124] Cystamine can efficiently increase GSH in cells.[96] In addition, cystamine inhibits HIV replication by interference with two steps of the viral life cycle, namely, inhibition of proviral DNA formation and assembly of HIV virions, causing the production of defective viral particles.[90] Interestingly, cystamine also inhibited lipopolysaccharide (LPS) induced TNFα production in macrophages.[124] However, cystamine has not been used in humans; its structurally related form, cysteamine, has low toxicity in man.[125] In one study, cysteamine was found to be a potent inhibitor of HIV replication *in vitro* at concentrations similar to those obtained by oral administration for the treatment of cystinosis, an inherited disorder.[126] Thus, this agent might have promise for further clinical studies in HIV-infected patients.

Promising *in vitro* studies of the GSH prodrug γ-glutamylcysteine ethyl ester (γ-GCE) have shown that this compound inhibits replication of HIV, even at relatively low drug concentrations.[127]

TREATMENT WITH GLUTATHIONE

In one study, intravenous infusion of GSH was given to eight HIV-infected patients. The plasma levels of both cysteine and GSH increased, but the intracelluar GSH concentration in mononuclear cells did not increase.[128] The authors suggest that GSH levels remained low due to decreased systemic synthesis of GSH.[128] However, as we have noted above, glutathione levels may differ among different cell types. Measurements of intracellular glutathione in mononuclear cells may thus merely reflect the net effect of increased or decreased proportions of particular cell types and are therefore difficult to interpret.

MODULATION OF GLUTATHIONE PEROXIDASE

The activity of the selenium-dependent glutathione peroxidases is important for intracellular protection against oxidative damage. Patients with HIV infections have an impaired selenium status,[129-131] and this may well affect glutathione peroxidase activity. In fact, selenium supplementation has been shown to increase glutathione peroxidase activity in HIV-infected T lymphocytes.[132] Thus, selenium supplementation in patients with HIV infections may be of importance and may also have a synergistic effect in combination with GSH replenishment, e.g., with NAC. However, in one study where selenium and NAC were co-administered to HIV-infected patients, only modest changes in lymphocyte subpopulations and no changes in glutathione levels or viral load were noted.[121]

MODULATION OF GLUTATHIONE REDUCTASE

The elevated intracellular level of oxidized glutathione in $CD4^+$ lymphocytes from HIV-infected patients may well be due to impaired activity of glutathione reductase. Flavin adenosine dinucleotide (FAD) is synthesized from riboflavin and is required as a cofactor for glutathione reductase. Thus, one might speculate that reduced uptake of riboflavin due to malabsorption could lead to reduced activity of glutathione reductase. However, no data are available from HIV patients regarding this action.

Also, glutathione reductase is dependent on NADPH, and it is important that pro-inflammatory cytokines such as TNFα deplete intracellular NADPH levels[51] and thus may inhibit the reductase. The effects of TNFα are further potentiated by the HIV regulatory protein Tat.[133] Furthermore, Tat caused decreases in the intracellular glutathione levels in different T cell lines.[133]

Thus, anti-TNFα treatment may well enhance the activity of glutathione reductase in HIV-infected patients. Highly active antiretroviral therapy (HAART) has been shown to reduce the activation of the TNF system.[134,135] Furthermore, several clinical trials with anti-TNF compounds thalidomide[136,137] and pentoxifylline[138-140] have been performed, but glutathione status has not been evaluated in these trials. It is thus important that the intracellular glutathione redox balance is evaluated in future trials where anti-TNFα treatment is given.

OTHER ANTIOXIDANTS WITH EFFECTS ON THE GLUTATHIONE REDOX BALANCE

In vitro studies with alpha-lipoic acid and vitamin E derivatives have shown that they inhibit TNFα-induced NF-κB activation in T cell lines, and it has been suggested that these compounds may be possible candidates for clinical trials in HIV-infected patients.[141,142]

Ascorbic acid inhibits HIV replication *in vitro*.[91,143] Furthermore, ascorbic acid has a synergistic effect in combination with NAC with regard to reduction of HIV

replication.[91] However, the antiviral effect of ascorbic acid seems not to be mediated by suppression of NF-κB activation.[144] Interestingly, our pilot study of combination treatment with NAC and vitamin C showed that viral load decreased, while CD4 lymphocyte numbers, lymphocyte proliferation, and lymphocyte glutathione levels increased.

CONCLUSION

Two lines of evidence suggest that patients with HIV infections may benefit from antioxidant treatment. First is the impaired glutathione status in their $CD4^+$ lymphocytes. Second, *in vitro* data indicate that oxidative stress leads to impairment of important lymphocyte functions and causes increased HIV replication in infected cells. Whether treatment with antioxidants will prove to be useful in the treatment of patients with HIV infection awaits the results of larger future clinical trials, where such agents must be given in combination with state-of-the-art HAART regimens.

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CHAPTER 8

Drugs of Abuse Modulate Immune and Nutritional Status in AIDS

David Solkoff and Ronald R. Watson

CONTENTS

ALCOHOL USE IN THE UNITED STATES

In the Unites States, alcohol (EtOH) contributes to approximately 100,000 deaths annually, making it the third leading cause of preventable death, after tobacco and diet/activity patterns.[1] According to the most recently available (1998) statistics[2] 5.9% of the U.S. population (12.4 million Americans) are heavy drinkers, with heavy drinking defined as five or more drinks on the same occasion on at least 5 different days in the past month. Almost three times as many men as women are problem

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drinkers, and prevalence is highest for both sexes in the 18- to 29-year-old age group.[3] Fetal EtOH syndrome, which can occur from drinking during pregnancy, is the leading known environmental cause of mental retardation in the Western World.[4]

From 1985 to 1992, the economic costs of alcoholism and EtOH related problems in the U.S. rose 42% to $148 billion. Two thirds of these costs were related to lost productivity due to EtOH related illness or premature death. Most of the remaining costs covered health care expenditures to treat the medical consequences of EtOH consumption, as well as the property and administrative costs of EtOH related motor vehicle accidents, and the various costs of EtOH related crime. Based on the most recently available projected inflation and population growth estimates, the predicted costs for 1995 totaled $166.5 billion.[5] Nearly one fourth of all persons admitted to general hospitals have alcohol problems or are undiagnosed alcoholics being treated for the consequences of their drinking. On average, untreated alcoholics incur general healthcare costs at least 100% higher than costs for nonalcoholics.[4]

Chronic alcohol use leads to abnormalities of humoral[6-12] and cellular immunity,[13-17] including malfunction of suppressor,[14,18-20] helper,[21] and cytotoxic lymphocyte activities.[22-27] It has also been determined that lymphocytes precultured *in vitro* with varying concentrations of ethanol (EtOH) suffered reductions in lymphokine-activated killer (LAK) cell activity, natural killer (NK) cell activity, and antibody-dependent cellular cytotoxic (ADCC) activity,[28] even when the lymphocytes were derived from healthy, nonalcoholic donors.

IMPACT OF HIV ON THE IMMUNE SYSTEM

Acquired immune deficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV). More than 688,000 cases of AIDS have been reported in the U.S. since 1981, and the number of new HIV infections in the year 2000 is estimated to be 40,000. Half of all newly infected individuals are under 25 years of age, and minority populations are disproportionately affected. Internationally, AIDS has become a major epidemic and it is estimated that in the year 2000 40 million people will be infected worldwide.[29]

HIV selectively and persistently infects monocytes, macrophages, and lymphocytes such as T cells, and is particularly virulent toward the $CD4^+$ class of helper T cells.[30-34] It wages a long battle with these rapidly replicating T cells, but eventually the rate of cell destruction exceeds the rate of replication. AIDS also causes a deterioration of the ability of B cells to proliferate in response to antigen stimulation. By killing or impairing cells of the immune system, HIV progressively destroys the body's ability to fight infections and certain cancers. Infected people become susceptible to life threatening diseases called opportunistic infections (OIs), which are primarily caused by microbes that usually do not cause illness in healthy people.

OIs are caused by a wide range of microorganisms, including protozoa, viruses, fungi, and bacteria. Many of these organisms are pervasive in the environment but do not cause disease until host immunity declines. Their occurrence often marks the progression from early HIV infection to advanced HIV disease (AIDS). Examples of OIs and the organs they attack include toxoplasmosis and cryptococcal meningitis

(brain); cytomegalovirus (eyes); thrush/*Candida albicans* (mouth); and *Pneumocystis carinii* pneumonia, tuberculosis, and histoplasmosis (lungs).[35] The gut is subject to OIs such as cytomegalovirus, cryptosporidosis, and *Mycobacterium avium* complex, while the skin may be infected with Herpes simplex virus, shingles, and Kaposi's sarcoma. The genitals of HIV/AIDS patients are also subject to OIs such as genital herpes, human papillomavirus, and vaginal candidiasis. OIs can be very severe and cause significant morbidity and death. However, effective prophylaxis and treatment for OIs can significantly reduce their incidence and severity, especially when used in conjunction with highly active antiretroviral therapy (HAART).[28]

HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

Over the last 4 to 5 years, HAART therapy using a combined drug "cocktail" has helped change AIDS from an automatic death sentence to what is now often a chronic but manageable disease. Powerful HAART therapy helped the U.S. AIDS death rate drop by approximately 60% from 1995 to 1997, the most recent period for which figures are available.[36] In addition to the drug therapy, other factors have played roles in reducing U.S. HIV related deaths, including increased access to care, growing expertise and experience in caring for HIV infected people, and the decrease in new HIV infections in the late 1980s due to prevention efforts. In 1997, for the first time since 1990, AIDS fell out of the top ten causes of death in the U.S., dropping from 8th to 14th place. According to recent data, U.S. patients infected with HIV had 71,000 fewer hospitalizations in 1997 than in 1995, a 30% decline. Patients who were hospitalized had shorter stays, resulting in almost 900,000 fewer total days of hospital care in 1997 compared to 1995.[36] By 1998, about 16,000 Americans who would have died during the previous year had AIDS mortality continued at its former rate were still alive.

In addition to asymptomatic patients who are willing to accept therapy and adhere to the prescribed regimen, HAART has been recommended for patients with less than 500 $CD4^+$ T cells/mm^3 or plasma HIV RNA levels exceeding 10,000 copies/ml (bDNA assay) or 20,000 copies/ml (RT-PCR assay). Results of clinical trials have shown that maximum suppression of viral load (a measure of new virus produced in the body) may be best achieved with a potent protease inhibitor (PI) in combination with two nucleoside analogue reverse transcriptase inhibitors (NRTI's).[37] Both classes of drugs employ the strategy of inhibiting crucial enzymes required for successful viral replication.

The NRTI group of antiretroviral drugs inhibits an enzymatic pathway during the early stages of virus replication, and includes zidovudine (Retrovir, AZT), lamivudine (Epivir, 3TC), didanosine (Videx, ddI), stavudine (Zerit, d4T), zalcitabine (HIVID, ddC), abacavir (Ziagen, ABC), and Combivir, which is a mixture of AZT and 3TC that allows patients to reduce the number of pills needed, which can be more than 20 a day for certain drug combinations. PIs interrupt another enzymatic pathway during a later stage of the viral life cycle. Examples include indinavir (Crixivan), nelfinavir (Viracept), ritonavir (Norvir), saquinavir (Fortovase), and amprenivir (Agenerase).

Recently approved non-nucleoside analogue reverse transcriptase inhibitors (NNRTIs) such as nevirapine (Viramune), delavirdine (Rescriptor), and efavirenz (Sustiva) are also in use. Results of therapy are evaluated primarily via plasma HIV RNA levels. These are expected to show a one log (tenfold) decrease at eight weeks, and no detectable virus (<500 copies/ml) at 4 to 6 months after initiation of treatment.

Another encouraging development in the U.S. battle against AIDS has been the major reduction in perinatal transmission that occurs before birth, during birth, or after birth as a result of breast feeding. American investigators have found that a specific regimen of AZT given to the mother during pregnancy and to the newborn after birth can reduce the incidence of maternal transmission by 66%.[29] It must be noted, however, that due to prohibitive treatment costs and complicated logistics, 1,800 HIV infected infants worldwide are still being born every day.[29]

HAART therapy is not an AIDS cure. Although HIV may not be detectable in the blood following successful HAART treatment, it is believed that the virus is still present, hiding in organs such as the lymph nodes, brain, testes, and retina. Therefore, it is strongly suspected that patients successfully undergoing HAART treatment can still spread the virus, even though they have greatly diminished viral loads. HAART treatment regimens do not work for all patients. In some individuals, substantial levels of circulating virus persist despite use of the newer drug combinations. HAART therapy is also very expensive (costing more than $12,000 per year for the drugs alone) and the prohibitive price tag makes HAART therapy largely unavailable in the developing world, where 95% of the 5.6 million new HIV infections (15,000/per day) occurred in 1999.

Those who can afford HAART therapy need to strictly adhere to complicated treatment regimens or the result can be the emergence of HIV strains that are resistant to treatment. Several studies have also shown that viral load can quickly rebound to high levels if patients discontinue part or all of their HAART therapy.[38,39] HAART therapy may also prompt the onset of diabetes or a worsening of existing diabetes and hyperglycemia.[40,41] It has also been recently reported that hepatitis C virus (HCV) RNA levels were increased at 48 and 96 weeks after initiation of HAART therapy when compared against baseline values in a study cohort of 21 hemophilic men coinfected with HIV and HCV.[42] The disturbing finding that PIs used in HAART therapy may transiently increase HCV viral load is significant because the prevalence of HCV and HIV coinfection ranges from 30% to 50% depending on the population studied.[43] Persons infected with HIV who are also intravenous (IV) drug abusers are at particular risk of becoming coinfected with HCV.[44] HAART treatment can also have adverse interactions with other medications. In particular problems can arise by taking PIs in conjunction with drugs commonly used to treat tuberculosis.[45,46]

Some persons receiving HAART have experienced a type of weight redistribution. Face and limbs become thin, while stomach, neck, or breasts appear to have enlarged.[39,40] Research is presently being conducted to determine whether protease inhibitors cause permanent changes in fat metabolism, and whether the increase in serum cholesterol that often accompanies weight redistribution increases the risk for cardiovascular complications such as strokes or heart attacks.[47]

Failure of HAART therapy (i.e., plasma HIV RNA levels exceeding 500 copies/ml) at 4 to 6 months has been known to occur, and may be due to non-adherence

to the prescribing regimen, inadequate potency of the drugs, use of sub-optimal doses, resistance, or other factors that are still poorly understood. It is recommended that patients whose therapy fails should be given at least two new agents that are not likely to show cross-resistance with drugs given previously. Unfortunately, rational changes in therapy may be especially difficult to achieve for patients in whom the preferred therapy has failed, due to present limitations in available alternative antiretroviral regimens that have documented efficacy.[37]

Progression from HIV to AIDS may occur anywhere from 3 to 10 years following seroconversion. This variation in length of time required for patients to develop AIDS after they have been exposed to or infected with HIV has led investigators to suspect that, in addition to the etiologic agent HIV-1, cofactors that affect disease progression also exist.[47] It has therefore been postulated that repeated exposure to HIV, malnutrition, coincident infections, and use of recreational drugs with the potential for abuse (EtOH, cocaine, opiates), alone or in combination, may raise the vulnerability of the host to HIV infection and/or subsequent development of AIDS. EtOH ingestion has actually been shown to increase retrovirus replication via NF-κB activation, which has been associated with increased viral burden and inhibition of $CD8^+$ T lymphocyte function.[48]

ALCOHOL USE AND PROGRESSION OF AIDS

Human studies examining the modulation of immune status in AIDS due to EtOH have been limited. One previous effort examined the *in vitro* effects of EtOH on proliferative responses of lymphocytes from healthy donors and AIDS patients to a recombinant fusion peptide, env-gag, corresponding to portions of the gp41 envelope (env) and internal core (gag) proteins of HIV.[6] The same study also examined the effects of EtOH on NK cell activities of lymphocytes from healthy donors and patients with AIDS. Peripheral blood mononuclear cells (PBMCs) from both normal donors and AIDS patients produced significant levels of lymphocyte proliferative responses to the HIV env-gag peptide. These responses, however, were significantly higher among patients with AIDS, thus showing the specificity of the response. Results showed that EtOH at a low level (0.1%) produced significant ($p < 0.05$) suppression of the env-gag-induced proliferation of lymphocytes from AIDS patients. The env-gag-induced proliferative responses of lymphocytes from normal subjects were significantly ($p < 0.05$) suppressed only when cultures contained high EtOH levels (0.2% and 0.3%).

With respect to the natural killer cell activities of lymphocytes, experimental results revealed that when EtOH at concentrations of 0.2% and 0.3% was added to cultures containing both lymphocytes from AIDS patients and NK target cells, there was a significant ($p < 0.05$) reduction in NK activities when compared with infected controls. This suppressive effect was noted at all effector to target (E:T) cell ratios tested. No such reduction in the NK activity of lymphocytes from uninfected donors was noted when these cultures were subjected to EtOH concentrations of 0.1%, 0.2%, or 0.3%.

Several factors account for the limited use of human experimental models in this area of research.[49] These include confounding variables such as genetic differences

and nutritional variations among the subjects. Studies involving human subjects are often economically prohibitive, and ethical concerns as well as necessary safety restrictions frequently render the human model impractical. For those reasons, the use of animal models has become essential in elucidating the roles of drugs such as EtOH in modulating immune and nutritional status in AIDS.

The lentivirus known as murine leukemia retrovirus (LP-BM5)causes murine AIDS and has become the preferred small animal model of human AIDS.[50] It offers multiple advantages over the human model, including low cost and high reproducibility of results, while at the same time possessing many functional similarities to human AIDS.[51] LP-BM5 preferentially infects B cells and does not destroy $CD4^+$ T cells.[52] Actually, the presence of functional T cells is required for LP-BM5 mice to develop lymphoproliferation and B cell abnormalities.[53] Although HIV infects and destroys $CD4^+$ T cells, disease symptomology is similar. Both human and murine AIDS are characterized by lymphadenopathy, splenomegaly, hypergammaglobulinemia, defects in both T and B cell function, impaired cytokine release, loss of tumor suppression capabilities, and late stage onset of opportunistic infections.[51,54]

The existence of multicellular organisms requires the development of biochemical intercellular messengers that could coordinate physiological and cellular activities, such as cytokines and hormones. Cytokines are low molecular weight regulatory proteins that may be produced by nearly every nucleated cell type in the organism. Such cell types would include lymphocytes, monocytes/macrophages, epithelial cells, and inflammatory cells. Included in the cytokine family are interleukines (IL), monokines, chemokines, growth factors, interferons (IFN), and colony stimulating factors (CSF). Individual cytokines are able to stimulate the production of others, generating an interactive immune system "communications network" that may affect other cell regulators such as hormones.

Endocrine hormones, usually produced by specialized glands or cells, are found in the systemic circulation, and their basic function is to maintain systemic homeostasis. Cytokines, however, generally act over short distances and carry autocrine or paracrine intercellular signals in localized tissues, while only occasionally entering the circulation in order to initiate systemic reactions. Cytokines are of vital importance in the regulation of immunity, inflammation, tissue remodeling, and embryonic development.[50] Understanding the cytokine network and its molecular mechanisms in disease states may have important clinical implications. Since cytokines are soluble mediators of immunity and inflammation, their efficacy continues to be evaluated as a potential therapeutic modality for the treatment of malignancies, chronic viral and parasitic infections, and rare congenital immune deficiencies. It is suspected, however, that excessive uncontrolled production of cytokines may be pathogenic.

The T helper (Th) subsets of T cells produce and secrete cytokines.[55,58] Th1 cells produce interferon-gamma (IFN-γ) and interleukin-2 (IL-2), which promote cell mediated effector responses and delayed hypersensitivity responses.[57] Th2 clones produce IL-4, IL-5, IL-6, and IL-10, which promote humoral responses.[58] IL-6 facilitates the differentiation of B cells in mature immunoglobulin secreting cells,[59] and is a regulator of protein synthesis in human hepatocytes.[60] *In vivo,* B cells and macrophages from HIV^+/AIDS patients produced high levels of IL-6[61] as did lipopolysaccharide (LPS) stimulated splenocytes and peritoneal macrophages from

LP-BM5 retrovirus infected mice.[62] Abnormally elevated IL-6 production is believed to promote the hypergammaglobulinemia and global B cell dysfunction observed in HIV and LP-BM5 infections.[59] Abnormally elevated levels of IL-6 have also been found to contribute to the stimulation of HIV viral replication in monocytes, macrophages, and T cells.[63,64]

Th1 and Th2 responses are reciprocally cross-regulated by IFN-γ, which inhibits Th2 cells,[56] and by IL-10, which inhibits Th1 cells,[55,65] It is suspected that following retroviral infection, progression to AIDS is facilitated by aberrant cytokine production that occurs via a switch from a Th1 to a Th2 response.[65] Indeed, previous studies indicate that in both human HIV+/AIDS and murine AIDS there is a decline in T cell proliferation and IL-2 production, and an increase in IL-4, IL-5, IL-6, IL-10, and immunoglobulin production.[62,66,67]

Some investigators have tried to validate experimentally the hypothesis that the increase in Th2 cytokine production may be wholly or partly responsible for the fatal nature of the illness. For example, IL-4 deficient (IL-4 gene "knockout") mice are known to be defective in Th2 cytokine responses. When such mice were infected with LP-BM5, the typical lethality outcome did not occur, and the development of some T cell abnormalities associated with murine AIDS was delayed.[68] In a similar experiment, administration of anti IL-4 monoclonal antibody to retrovirally infected mice normalized the previously described imbalance of Th1 and Th2 responses, while at the same time prevented retrovirus-induced suppression of immune responses, and ameliorated splenomegaly and hypergammaglobulinemia.[69]

Another important molecular component of the immune system is tumor necrosis factor alpha (TNF-α). Secreted by appropriately stimulated monocytes and macrophages, TNF-α is involved in immunomodulation and the inflammatory response, and has been shown to possess anti-tumor activity.[70] As was the case with IL-6, B cells and macrophages from HIV+/AIDS patients produced high levels of TNF-α,[61] as did lipopolysaccharide (LPS) stimulated splenocytes and peritoneal macrophages from LP-BM5 retrovirus infected mice.[62] Previous study has suggested that elevated levels of TNF-α play a role in the "wasting" phenomenon observed in both human and murine AIDS, as well as promoting lipid metabolism problems such as the induction of hypertriglyceridemia.[71,72] Like IL-6, abnormally elevated levels of TNF-α have also been found to contribute to the promotion of HIV viral replication in monocytes, macrophages, and T cells.[63,64]

To determine whether EtOH use synergizes with retrovirus infection to exacerbate cytokine dysregulation, several studies using LP-BM5 mice have been conducted. In one such effort[73] mice were infected with LP-BM5 and fed 5% (v/v) EtOH. After 12 weeks, spleens were harvested for cell culture, wherein mitogen-stimulated splenocytes released cytokines into the supernatant. These cytokines were detected by sandwich type enzyme linked immunosorbent assay (sandwich ELISA). Results showed that the levels of Th2 cytokines released by concanavalin-A (CON-A) stimulated splenocytes (IL-5 and IL-6), which were already elevated due to murine AIDS, were increased even further by EtOH consumption. Therefore, it has been suggested that chronic EtOH abuse may increase the severity of AIDS related symptoms such as hypergammaglobulinemia and global B cell dysfunction, since IL-5 and IL-6 are known to be B cell growth factors. Also found was further evidence indicating that

chronic EtOH use synergizes with LP-BM5 *in vitro* to further abnormally elevate the disease related rise in IL-6 and TNF-α elicited from LPS-stimulated splenocytes. Results from this part of the study showed that EtOH consumption during murine AIDS was found to have significantly ($p < 0.05$) increased IL-6 and TNF-α production to a level higher than the abnormal elevation caused by the disease.

Another study sought to determine whether healthy mice fed EtOH pre-infection would be more susceptible to post-infection cytokine dysfunction when compared with mice that did not receive EtOH.[74] One group of mice was fed 5% (v/v) EtOH for ten weeks, and the other was not. After ten weeks, both groups were infected with LP-BM5. At nine weeks post-infection, spleens were harvested for cell culture and cytokine analysis. Cytokines were detected by use of sandwich ELISA, and it was found that CON-A stimulated splenocytes released lowered amounts of the Th1 cytokine IL-2. This suppression was even more pronounced in mice fed EtOH pre-infection. Data also revealed that levels of Th2 cytokines such as IL-5, IL-6, and IL-10 rose in both groups but, again, significantly ($p < 0.05$) more in mice fed EtOH pre-infection. In another portion of the same experiment, results showed that increased release of IL-6 and TNF-α produced by LPS-stimulated splenocytes during murine AIDS was further increased in EtOH-fed mice at six and nine weeks post-infection. These results suggest that chronic EtOH abuse prior to retrovirus infection aggravates progression of immune dysfunction, thus supporting the hypothesis that alcoholics, upon infection with HIV, are predisposed to accelerated immune dysfunction and that chronic EtOH abuse can function as a cofactor in increasing the severity of the disease.

It remains to be determined how chronic EtOH consumption further suppresses the retrovirus associated decrease in the release of splenic Th1 cytokines, while it further stimulates the infection related increase of splenic Th2 cytokine levels. Perhaps the effects of EtOH on cytokine release result from complex interactions in the poorly understood cytokine-glucocorticoid hormone network. From previous research[75] it is known that EtOH abuse produces a marked upregulation of hypothalamic-pituitary-adrenal (HPA) axis activity. In response to chronic alcohol use, central nervous system (CNS) signals reach the brain based HPA system and stimulate an upregulation in the secretion of corticotropin releasing hormone (CRH) and possibly vasopressin from cells in the median eminence region of the hypothalamus.[76,77] They then stimulate an upregulation in the release of adrenocorticotropic hormone (ACTH) from the pituitary gland located at the base of the brain. The increased amounts of ACTH then stimulate the cortex (outer layer) of the adrenal glands, resulting in a large upregulation in the production and secretion of glucocorticoid hormones such as cortisol.[82] Large doses of cortisol, whether produced *in vivo* as a result of chronic EtOH consumption or administered pharmacologically as an anti-inflammatory agent, have previously been shown to elicit strong suppressive effects on the immune process.[78] It should be noted, however, that small physiologic doses of cortisol are thought to be required for the proper development and maintenance of normal immunity.[79]

In addition to splenocytes, the parent classification for immune cells such as lymphocytes that develop and mature in the spleen, thymocytes are immune cells

that develop and mature in the thymus. This primary lymphoid organ supplies other organs such as the lymph nodes and the spleen with mature lymphocytes such as functionally competent T cells. Also supplied with mature lymphocytes by the thymus are the linings of the intestinal, respiratory, genital, and urinary tracts.[80] Soon after birth, the thymus begins secreting the hormone thymosin, which is targeted to certain members of the resident lymphocyte population. Their response is to mature into B cells, which are lymphocytes that later specialize in forming antibodies. Over time the human thymus changes. Most prominent in children, its growth peaks at puberty. It then begins to atrophy, becoming almost unrecognizable in older people.[81] In order for the thymus to convert bone marrow produced pro-thymocytes into functionally competent T cells, several cellular proliferation and differentiation events which are mediated by cytokines must occur.[82-85]

Chronic EtOH use has previously been shown to diminish $CD4^+$ and $CD8^+$ thymocyte counts, thymocyte weight, and thymocyte mitogenesis,[16,86,87] and as previously stated, chronic EtOH consumption prior to retrovirus infection altered cytokine production by splenocytes during murine AIDS. In order to determine whether chronic EtOH use prior to retrovirus infection would also alter cytokine production by thymocytes during murine AIDS, an experiment was conducted whereby mice chronically fed EtOH (or a control diet) for ten weeks were then infected with LP-BM5. Upon conclusion of the experiment, thymus glands were harvested for analysis of thymocyte proliferation and cytokine production, specifically IL-2, IL-4, IL-6, and IFN-γ. With respect to thymocyte count and proliferation, data showed that thymocyte numbers were slightly decreased by LP-BM5 compared to uninfected normal mice. EtOH consumption prior to retrovirus infection significantly ($p < 0.05$) further reduced thymocyte counts when compared to controls at six and nine weeks post-infection. Thymocyte proliferation was decreased by LP-BM5, and it was found to be significantly ($p < 0.05$) further reduced by pre-infection EtOH use when compared to controls six weeks after infection. Further results from this experiment showed the disease related decrease in IL-2 production by thymocytes was significantly ($p < 0.05$) further exacerbated by chronic pre-infection EtOH use.

In contrast to previously mentioned findings describing the retrovirus related increase in splenic IL-6, data from this experiment indicate a reduction in thymic IL-6 levels in murine AIDS, while thymic IL-4 levels rose with retroviral infection. This study further revealed that pre infection chronic EtOH use significantly ($p < 0.05$) further reduced thymic IL-6 production compared to controls at six weeks post-infection while at the same time significantly further elevated thymic IL-4 levels.

From this study it is reasonable to speculate that chronic EtOH use prior to retrovirus infection contributes to the further aggravation of infection related disturbances in production of cytokines which play vital roles in the normal differentiation, development, and maturation pathways of thymus derived T cell subpopulations. Chronic EtOH use is believed to further disrupt these pathways by inducing the production of corticosteroids,[88] which can result in thymocyte apoptosis. EtOH is also thought to facilitate the spread and replication of retrovirus infection by increasing oxidative stress and retarding activation of the immune response.[89]

Susceptibility to Cryptosporidiosis

An additional problem for retrovirally infected hosts is their increased susceptibility to the OI known as cryptosporidiosis, which is caused by *Cryptosporidium parvum* infection.[90] *C. parvum* is a single cell protozoan parasite that lives in the intestines of animals and people.[91] In otherwise healthy subjects, cryptosporidiosis is characterized primarily by watery diarrhea, and possibly abdominal cramps, nausea, low grade fever, dehydration, and weight loss. Cryptosporidiosis has long been a veterinary health problem, predominantly in farm animals such as young calves. First recognized as a cause of human disease in 1976, it was rarely reported in humans until 1982, when the number of detected cases began to rise rapidly along with the AIDS epidemic and the development of methods that allowed for identification of parasites in stool samples.

The dormant, inactive form of *C. parvum*, called an oocyst, is excreted in the feces of infected humans and animals. Healthy individuals who contract *C. parvum* are usually ill with cryptosporidiosis for several days, but rarely longer than two weeks. Some seem to recover, then get worse. Some may not even get sick. Infected sufferers may shed oocysts in their stools for months, even after they no longer appear to be ill.

Cryptosporidiosis may cause complications for pregnant women; and for those with illnesses such as alcoholism and diabetes, the effects of prolonged diarrhea and dehydration can be dangerous. Cryptosporidiosis is most severe and long lasting in immunocompromised individuals such as cancer patients on chemotherapy, transplant patients, those taking medications that suppress the immune system, and those infected with HIV/AIDS. Cryptosporidiosis can be life threatening for the immunocompromised, and several AIDS patients died as a result of drinking water tainted during the 1993 *C. parvum* outbreak in the Milwaukee, Wisconsin Municipal Drinking Water System. Past studies estimate that 16% to 50% of HIV/AIDS patients contract cryptosporidiosis.[92,93]

Research has shown that normal cytokine production is required to elicit intestinal immune responses necessary for successful host defense against cryptosporidiosis and other infections.[94] However, in retrovirally infected subjects and chronic EtOH users, accumulated damage to the intestines and immune system can reduce resistance to various pathogens, including *C. parvum*. As a consequence, production of cytokines that exert direct cytotoxic effects against invading parasites and viruses such as TNF-α and IFN-γ[94] may be disrupted, due to a reduction in intestinal $CD4^+$, $CD8^+$, and IgA^+ immune cell counts,[95-98] thus causing dysregulation in the production of other needed cytokines.

To study the immunomodulating effects of LP-BM5 infection and chronic ethanol ingestion on resistance of mice to *C. parvum* infection, female C57BL/6 mice were assigned to one of four treatment groups: control, EtOH (5%v/v) diet, retrovirus infected, and EtOH (5%v/v) plus retrovirus. Sixteen weeks after retrovirus infection, the mice randomly chosen to receive alcohol diets began ingestion, and then at 20 weeks after retroviral infection all mice were infected with 2×10^5 *C. parvum* oocysts via stomach tube.[90]

Ten days later, spleens and 2 cm portions of the terminal ileum were harvested from each mouse for splenocyte culture/cytokine analysis and quantification of *C. parvum* oocysts in the intestine and feces. Results showed that retrovirus related reductions in splenic IL-2 and IFN-γ were not further exacerbated by EtOH. Neither were retrovirus related increases in TNF-α further increased by EtOH. Persistent oocyst shedding into the feces was observed for all murine AIDS mice and significantly ($p < 0.05$) more oocysts were found in the feces of murine AIDS mice that had been fed alcohol before parasite challenge; 69.4% of the retrovirally infected mice fed EtOH died from cryptosporidiosis as compared with a mortality of 42.9% for the murine AIDS mice that consumed EtOH free diets.

Susceptibility to Pneumonia

Pneumonia is another OI that HIV/AIDS patients are at high risk of contracting. The most common etiologic agent is the bacteria known as *Streptococcus pneumoniae*.[99] Besides pneumococcal disease, symptoms of *S. pneumoniae* infection include bacteremia, meningitis, otitis media, sinusitis, and peritonitis. As a consequence of meningitis, neurologic sequelae and/or learning disabilities can occur, and recurrent otitis media can cause serious hearing impairment.[100] Strains of drug resistant *S. pneumoniae* have become common in the United States. Many penicillin resistant pneumococci are also resistant to other antimicrobial drugs, such as β-lactam agents, erythromycin, and trimethoprim-sulfamethoxazole. The widespread overuse of antibiotics is frequently mentioned as a cofactor in the development of anti-pneumococcal drug resistance.

Ninety serotypes of *S. pneumoniae* exist, and 89% have been incorporated into the 23-valent polysaccharide vaccine first licensed in 1983 to help prevent pneumococcal disease. As to the efficacy of vaccinating HIV/AIDS patients against *S. pneumoniae* infection, recent studies indicate this is a wise course of action. One such study[101] concluded that since HIV-1 infection in Kenyan women is associated with decreased levels of natural antibodies to selected pneumococcal capsular serotypes, the vaccine could be immunogenic in such patients who are at high risk of invasive pneumococcal disease. Another study[102] also highlighted the need for early immunization of HIV-infected persons with currently available polysaccharide vaccines.

In order to investigate the possibility that chronic EtOH use reduces survivability of murine AIDS mice challenged with *S. pneumoniae,* a study[103] found the percentage of survival after bacterial challenge of murine AIDS mice fed the EtOH diet was significantly ($p < 0.05$) suppressed by dietary EtOH, when compared with retrovirally infected non-EtOH controls.

Vitamin E Supplementation

The search for treatments that can offer prevention and/or protection against EtOH exacerbated retroviral immune dysfunction is presently in its infancy. Dietary supplementation with d-α tocopherol (vitamin E) has been shown in both human and animal models to be associated with increases in lymphoproliferative response

to mitogens, T helper activity, antibody synthesis, neutrophil antimicrobial activity, lymphoblastic cell viability, and macrophage phagocytic activity.[104-106] Vitamin E supplementation has also been shown to enhance the overall immune response by stimulating splenocytes to secrete cytokines.[107,108] High doses of vitamin E have not been shown to be toxic,[109-112] and have improved host resistance to pathogens.[113,114]

Vitamin E is also a powerful antioxidant.[115,116] This is significant because approximately 5% of the O_2 required by an aerobic organism for the generation of adenosine triphosphate (ATP) energy is converted to highly unstable reactive oxygen species (ROS), which are also known as free radicals.[117] ROS carry out some necessary and beneficial functions.[118-121] Certain ROS are a necessity for the phagocytic functions of cells, and there is evidence that free radicals may sometimes function as intracellular signaling molecules.[121-124] ROS, however, are also known to play roles in the underlying bases of many disease states.[125] One means of ROS production is the reaction whereby superoxide anion (O_2^-) in the presence of H_2O_2 or transition metals such as Fe is converted into the extremely powerful oxidant known as the hydroxyl radical (·OH). This radical, in concert with iron-oxygen complexe,s is capable of initiating lipid peroxidation (LPO), which is the free radical catalyzed lipid damage that occurs when ROS such as ·OH attack biological membranes that contain polyunsaturated fatty acids, resulting in their oxidative degradation.[126]

When EtOH is oxidatively metabolized, it is initially converted to acetaldehyde by alcohol dehydrogenase enzyme catalysis and the microsomal EtOH oxidizing system, resulting in the production of ROS. Acetaldehyde is then further metabolized by aldehyde oxidase and xanthine oxidase, with the generation of additional ROS and the induction of LPO.[127-129] Although oxidative stress on murine AIDS mice that chronically consumed EtOH promoted disease states such as esophageal tumor growth, dietary vitamin E supplementation resulted in a significant ($p < 0.05$) inhibition of such growth.[130]

Vitamin E has also been shown to decrease the EtOH compounded burdens of free radicals on immune cells[115,116] by reacting with and quenching ROS byproducts of EtOH metabolism, thus inhibiting cellular LPO by terminating ROS autocatalysis and branching chain reactions.[131,132] The constant requirement for high levels of antioxidants that can quench the excess production of ROS generated by EtOH metabolism often results in vitamin E deficiency.[107] This deficiency, usually accompanied by an increase in tissue LPO, can be further exacerbated by retroviral infection, as indicated by the results of several studies in animals and humans that have suggested links between LP-BM5 or HIV infection and vitamin E deficiency.[133-136] Along with EtOH related oxidative stress and retroviral infection come cofactors that also contribute to tissue vitamin E deficiency. Among these are inadequate and/or imbalanced intake of nutrients, maldigestion, malabsorption, impaired metabolism, decreased tissue uptake and storage, increased loss of nutrients through the urine, and illness related increases in nutritional requirements.[136,137]

Since hormone production is reduced by retrovirus infection and chronic EtOH use, it was hypothesized that supplementation might lessen compound immune dysfunction in subjects who are both chronic EtOH users and retrovirally infected and retard tissue vitamin E loss and prevent an increase in LPO.

DHEAS Supplementation

To test this hypothesis, dehydroepiandrosterone sulfate (DHEAS) treatment was investigated[138] since it had already been shown to possess immune enhancing properties during murine AIDS[139] and because it is suspected that EtOH and DHEAS act via a common pathway.[140] DHEAS is the storage form of immunologically active dehydroepiandrosterone (DHEA), a weakly androgenic and estrogenic steroid that is synthesized primarily by the adrenal cortex and derived from androsterone.

Study data showed that *in vitro* proliferation of splenocytes stimulated by CON-A and LPS was significantly ($p < 0.05$) decreased by LP-BM5. EtOH supplementation for 16 weeks further inhibited proliferation of T and B cells in murine AIDS mice when compared with both infected and uninfected controls. DHEAS treatment was found to significantly ($p < 0.05$) increase proliferation of T and B cells in infected mice when compared with untreated murine AIDS mice. It was also found that EtOH-consuming infected mice provided with DHEAS supplementation significantly maintained T and B cell proliferation near levels found in EtOH-free infected mice. With respect to cytokine production by splenocytes, *in vitro* production of the Th1 cytokine IL-2 by CON-A stimulated splenocytes was significantly ($p < 0.05$) inhibited in murine AIDS mice compared with uninfected controls. When compared against EtOH free murine AIDS mice, it was found that IL-2 release was further inhibited from splenocytes harvested from murine AIDS mice that consumed the EtOH diet.

Additional data revealed that DHEAS supplementation partially restored the retrovirus induced decrease in IL-2 levels and also overcame the compound lowering of IL-2 noted in mice that had murine AIDS and were chronic EtOH consumers. With respect to the *in vitro* LPS-stimulated production of Th2 cell cytokines IL-4 and IL-6, results showed a significant ($p < 0.05$) increase in murine AIDS mice. Chronic EtOH supplementation further exacerbated this increase when compared against EtOH free controls. DHEAS supplementation of EtOH consuming murine AIDS mice modulated detectable secretions of IL-4 and IL-6 to levels below those noted for the EtOH free murine AIDS mice.

Further results showed that DHEAS supplementation significantly ($p < 0.05$) retarded additional hepatic vitamin E loss in EtOH-consuming murine AIDS mice compared against EtOH-free infected controls. Also noted was the finding that DHEAS supplementation of EtOH-consuming murine AIDS mice caused hepatic LPO to remain at approximately the same incidence as that measured in infected EtOH-free controls.

EXACERBATION OF IMMUNE DYSFUNCTION BY COCAINE

Another drug of abuse that can cause modulation of immune status during retrovirus infection is cocaine. Cocaine is a powerfully addictive stimulant that directly affects the brain. Coca leaf, the source of cocaine has been chewed for thousands of years, and the pure chemical cocaine hydrochloride has been an abused substance for more than 100 years.[141] Pure cocaine was first extracted from the leaves of the *Erythroxylon* coca bush in the mid 19th century. Cocaine is usually produced

into one of two forms. The first is the hydrochloride salt or powdered form which dissolves in water. When abused, it can be taken intravenously (IV) or intranasally. The second form is the "freebase." The freebase form has not been neutralized by an acid in order to make the hydrochloride salt. Freebase cocaine can be smoked.

Another notorious variant of cocaine is known as "crack." Crack is the street name given to the product of a process that converts powdered cocaine hydrochloride back into a smokable freebase form. In 1998, an estimated 1.8 million Americans were current cocaine abusers. The figure includes approximately 604,000 crack smokers. This statistic reflects a large reduction in American consumption of cocaine since 1985, when it was estimated that 5.7 million Americans actively used it. Adults aged 18-25 are the most frequent users of cocaine and, overall, men use cocaine more frequently than women. In 1996, the latest year for which data are available, there were approximately 152,000 cocaine related hospital emergency room visits.[141]

Enormous medical complications are associated with cocaine abuse.[143] Some of the most frequent complications are cardiovascular effects, including accelerated heartbeat, heart attacks, and chaotic heart rhythm known as ventricular fibrillation. Accelerated breathing, increased body temperature, and high blood pressure are other common side effects of cocaine abuse. Blurred vision, muscle spasms, convulsions, and coma may also occur. A user may also suffer respiratory effects such as chest pain or outright respiratory failure and neurological complications such as strokes, seizures, and headaches. Gastrointestinal problems may also arise and include abdominal pain and nausea. Persons who abuse cocaine intranasally frequently experience nosebleeds, hoarseness, problems with swallowing, loss of sense of smell, and a general irritation of the nasal septum which may lead to a chronically inflamed runny nose. Since cocaine has a tendency to decrease food intake, many cocaine abusers lose their appetites and can experience significant weight loss and malnourishment.[143]

Cocaine abusers are at increased risk of developing hepatitis B(HBV), HCV, and HIV/AIDS. Cocaine and other drugs of abuse have become the leading risk factors for new cases of AIDS.[43] Drug abuse can further the spread of HIV through sharing of contaminated drug use paraphernalia, such as contaminated syringes in the case of those using the IV route of administration. Research has also shown that drug abuse can interfere with proper judgment about risk taking behavior. Drug abuse by both sexes can lead to ignoring precautions about having sex and bartering of sex for drugs.[43]

Results of experiments designed to investigate the effects of cocaine exposure on immune responses in human and animal studies have been generally inconclusive. A variety of studies have generated conflicting conclusions, often as a consequence of differences among *in vitro* human models, animal models, and the actual conditions experienced by persons acutely abusing the drug.[142]

One study reported divergent effects of cocaine on cytokine production by lymphocytes and monocytes/macrophages.[143] In another study, the effects of cocaine infusion (40 mg) on IFN-γ and IL-10 secretion were examined in 15 cocaine dependent subjects.[144] Baseline IFN-γ levels were lower and IL-10 levels higher in addicted subjects compared to cocaine free controls. Pre- and post-cocaine infusion peripheral blood mononuclear cells (PBMC) were stimulated with phytohemagglutinin (PHA)

and then cultured for 48 hours. Supernatant cytokines were then measured by ELISA. Cocaine infusion increased IFN-γ secretions and decreased IL-10 secretions when compared against controls. Cocaine infusion also increased white blood cell counts, lymphocyte counts, and $CD4^+$ and $CD8^+$ counts. In another part of the same study, when PBMC from addicted subjects were treated *in vitro* with cocaine, suppressions of IFN-γ and IL-10 were noted. Therefore, data from this experiment suggest that acute intravenous *in vivo* cocaine administration enhances Th1 type immune responses while while inhibiting Th2 type responses.

A different investigation used a group of patients who were positive for cocaine or cocaine metabolites to evaluate a variety of functional parameters pertaining to T lymphocytes and other peripheral lymphoid cell populations.[143] When compared to cocaine free subjects, it was found that cocaine users had T cell functional assays which were essentially normal, with the exception of a slight depression in PHA stimulation. It was also found that peripheral blood lymphocytic populations showed normal percentages and numbers of T cell subsets such as $CD4^+$, $CD8^+$, NK, and B cells.

In another study[145] unstimulated PBMC isolated from eight healthy HIV type 1 (HIV-1) seronegative volunteers were exposed to cocaine. Exposure was *in vitro*, at substance concentrations compatible with blood levels seen during clinical abuse observations. Results showed that PBMC cultures treated with cocaine had significantly ($p < 0.05$) increased levels of HIV-1 replication by the 10th day post-infection when compared against untreated controls. A similar study[146] demonstrated that cocaine applied *in vitro* to infected T cells increased HIV replication via a mechanism using tumor growth factor beta (TGF-β).

An *in vitro* human model has recently been used to show that cocaine enhances opening of the blood-brain barrier (BBB) to HIV-1 invasion[147] this suggests that cocaine may play a role in promoting and quickening the onset of AIDS related dementia. A normally functioning *in vivo* BBB in a healthy person regulates the entry of macromolecules and microbial pathogens, and also restricts immigration of monocytes and other leukocytes into the CNS except for a few patrolling cells.[148] It consists partially of the microvascular endothelium and associated astrocyte processes, which are found in close apposition to the abluminal sides of the vascular endothelial cells (EC).[149] Previous study has shown that immune activated HIV-infected monocytes induce adhesion molecules on brain endothelium, and are thus allowed to breach the BBB and enter the brain,[150] partly as a consequence of the ability of TNF-α to open a paracellular route.[151]

Cocaine is believed to enhance HIV-1 neuroinvasion by actions directed at the BBB. In a recent study effects of cocaine on the BBB were investigated using an *in vitro* BBB model consisting of human brain microvascular endothelial cells and fetal astrocytes.[147] Cocaine (10^{-5} *M* and 10^{-6} *M*) was found to increase molecular permeability of the barrier and viral invasion by macrophage-tropic $HIV\text{-}1_{JR\text{-}FL}$ into the brain chamber. Cocaine was also found to increase human monocyte secretions of chemokines such as IL-8, interferon-inducible protein 10, macrophage-inflammatory protein 1α, monocyte-chemoattractant protein 1, and cytokine TNF-α. TNF-α was also found to enhance invasion of the brain compartment by macrophage-tropic, lymphotropic, and bitropic HIV-1 strains. Furthermore, cocaine augmented apoptosis

of brain endothelial cells and monocytes. Examined collectively, these data suggest that HIV-1 neuroinvasion can be enhanced by the paracrine effects of cocaine-induced proinflammatory cytokines and chemokines on the BBB and by direct effects on brain microvascular endothelial cells.

In similar experiments using the same *in vitro* BBB model,[152] it was found that 10^{-5} *M* cocaine enhanced monocyte migration across the barrier by approximately 100%, increased secretion of IL-6 in an LPS-activated brain microvascular endothelial cell/monocyte coculture, and also upregulated expression of endothelial adhesion molecules that facilitate breaching of the BBB. A list of these molecules includes intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), platelet/endothelial cell adhesion molecule 1 (PECAM-1), and endothelial leukocyte adhesion molecule 1 (ELAM-1).

Many animal model studies have been conducted to determine the immunomodulating effects of cocaine. In recently reported research, a daily short term (20 day) cocaine administration protocol was used to determine whether the immune systems of aging mice were more susceptible to the effects of cocaine than those of young mice.[153] Results showed that old mice suffered decreases in the absolute numbers of both splenic and thymic Thy 1^+ cells. Old mice also experienced cocaine induced decreases in absolute numbers of splenic $CD4^+$ cells, splenic $CD8^+$ cells, splenic B cells, splenic cells possessing the IL-2 receptor ($IL\text{-}2R^+$), and splenic MAC 1^+ cells which possess MAC 1 on their surfaces. MAC 1 is a leukocyte membrane adhesive heterodimer glycoprotein that allows phagocytic cells to adhere to endothelial cells and migrate to sites of infection.[154] Old mice also showed lowered numbers of both $CD8^+$ cells and IgA plasma cells produced by antigen stimulation after removal from the intestinal lamina propria. In the young mice, cocaine was found to decrease thymic levels of Thy 1^+ cells and decrease the number of $CD4^+$ cells from the intestinal lamina propria.

Another recently published study[155] investigated the *in vitro* effects of cocaine on the functions of T and B lymphocytes, NK cells, and macrophages in a mouse model. Splenocytes from mature C57BL/6J mice were cultured with cocaine at concentrations that varied from 4 to 64 μg/ml for 24 hours. *In vitro* cocaine exposure was found to cause reduced T cell responses to stimulation by CON-A, PHA, and IL-2. Lowered production of IFN-γ, attenuated killing capacity by NK cells and diminished responses of B cells to LPS stimulation were also noted. Cocaine also disrupted monocyte/macrophage function, resulting in decreased ability to inhibit the growth of tumor cells.

Chronic cocaine treatment in rats significantly increased rolling white blood cell flux and leukocyte-endothelium adhesion.[156] Daily cocaine injection for short periods of time (< 14 days) has also been shown to cause reduced body, spleen, and thymus weights in experimental animals.[157-159] Short term cocaine exposure affects resistance to infection and tumor cell growth in experimental animals, and increases the responsiveness of lymphocytes to both PHA and CON-A while concomitantly suppressing the B lymphocyte response to LPS.[157-159]

Modulation of the immune response in LP-BM5 mice by cocaine has also been evaluated, with one investigation[160] showing that NK cell activity was further increased in murine AIDS mice receiving cocaine when compared to cocaine-free

controls. Two other studies[161,162] revealed that cocaine worsened the LP-BM5 related decrease in the percentages and absolute numbers of T lymphocyte precursor cells in the thymus, such as Thy 1.2^+, $CD4^+$, and $CD8^+$.

One component of immunity is known as the mucosal immune system and is active in areas such as the intestinal lamina propria. This part of the immune system is a definable subunit of humoral and cellular immunity and functions to guard the body's interface with the environment. The intestinal mucosal immune system plays a critical role in protecting the body against pathogenic organisms, especially those responsible for enteric infections associated with diarrheal disease.[163] Immune cells such as $CD4^+$ and IgA^+ play important roles in intestinal mucosal immunity. In the clonal selection theory of the humoral immune response[31] IgA^+ cells are types of B lymphocytes that carry the IgA antibody on their surfaces. When IgA^+ cells encounter appropriate antigens, they can be stimulated by T helper cells to multiply, forming clonal populations. Some of the clones are known as plasma cells, and they produce soluble IgA which is excreted from the cell and can bind to the invading substance. This binding either precipitates the foreign substance or marks it for destruction by macrophages.

In previous research, cocaine was also found to exacerbate the LP-BM5 related decrease in the number of IgA^+ and $CD4^+$ cells in the intestinal lamina propria.[164] In another experiment showing that cocaine exacerbates AIDS related immune dysfunction,[165] chronic cocaine use synergized with LP-BM5 murine AIDS infection in female mice to cause an additional ten-fold increase in the number of fecal *C. parvum* oocysts when compared against cocaine free controls.

HEALTH AND SOCIAL CONSEQUENCES OF HEROIN ADDICTION

Since it is the most abused and most rapidly acting of the opiates, heroin is considered even more addictive than morphine, the parent compound from which it is processed. Morphine is extracted from the seed pods of certain varieties of poppy plants.[166] It is available as an off-white powder or as the black sticky substance known as "black tar heroin." Most street heroin is to an extent prediluted with supposedly inert substances. Even so, evidence collected from recent law enforcement seizures in the U.S. indicates that the strength and purity of what is being consumed heroin have increased. The most recent (1998) estimate of 253,000 U.S. heroin addicts is believed to be very conservative, due to underreporting of the population of heroin abusers.[167] However, the same statistics are also believed to accurately reveal a significant increase in new American heroin users since 1992. A large number of new users are believed to be under the age of 26, and are suspected to have began their heroin use by intranasally sniffing or smoking the drug rather than by injecting it, although all three forms of heroin administration are addictive. It has been reported that first time heroin use in the U.S. by those 12 to 17 years of age rose a disturbing four-fold from 1980 to 1995. Researchers have observed a shift in heroin abuse patterns from injection to sniffing and smoking, due to the greater availability of high purity heroin.[166] Intravenous heroin abuse, however, remains the main reason people seek drug abuse treatment in the most heavily

populated sections of the U.S. In New York and Seattle, heroin abuse ranked a close second behind cocaine.

Perhaps the most deleterious long term effect of heroin (like cocaine) is the addiction itself, which is characterized by compulsive drug seeking and use and neurochemical and molecular changes in the brain.[166] Heroin also produces profound degrees of tolerance and physical dependence, which further reinforces compulsive use and abuse. Like abusers of other addictive drugs, heroin abusers gradually spend greater percentages of their time and energy obtaining and using the drug, until eventually the primary purpose in life becomes obtaining and consuming heroin. As physical dependence develops, the body adapts to the presence of the drug and withdrawal symptoms develop when use is sharply restricted. Withdrawal may occur within a few hours after last use, and has not been reported to be fatal. Symptoms include restlessness, irritability, discontentment, potentially severe muscle and bone pain, insomnia, constipation or diarrhea, vomiting, cold flashes, and involuntary muscle twitches.[166] Major withdrawal symptoms usually peak 24 to 48 hours after the last dose of heroin and subside after approximately one week.

Depending on the route of administration, the medical consequences of chronic intravenous heroin use include scarred and or collapsed veins, bacterial infections of the blood vessels and heart valves, abscesses (boils) and other soft tissue infections, and liver or kidney disease. Lung complications such as pneumonia and tuberculosis may result from the generally poor health condition of an addict as well as from the ability of heroin to depress respiration. Additives used to dilute the heroin prior to sale may have the potential to clog blood vessels leading to the lungs, liver, kidneys, and brain, thus possibly leading to infection or even necrosis of small areas of cells in vital organs. It is also possible that immune reactions to these contaminants may cause arthritis or other rheumatologic conditions. Of particularly important consequence to heroin abusers is the potential for infection with HBV, HCV, and/or HIV from the sharing of contaminated injection equipment. Tragically, the infected drug abusers may then pass these infections to their sexual partners, or perhaps even to their children.

EFFECTS OF INTRAVENOUS MORPHINE USE ON IMMUNOCOMPETENCE

To determine the effects of intravenous morphine on immunocompetence, an investigation was conducted using rhesus monkeys (*Macaca mulata*).[168] Monkeys on daily morphine regimens (3.2 mg/kg) should suppressed PBMC natural killer cell activity, a decrease in the percentages of $CD8^+/CD16^+$ cells when compared against saline treated controls, but an increase in numbers of $CD8^+$ lymphocyte cells. Also found was a reduction in the total percentage of $CD4^+$ lymphocytes and $CD4^+/CD45RA^+$ cells. The $CD4^+/CD29^+$ population increased 17% from that noted in morphine free controls. In addition, cultured PBMC from monkeys receiving the daily morphine regimen had significantly higher ($p < 0.01$) polyclonal immunoglobulin G and polyclonal immunoglobulin M levels when compared against levels seen in untreated controls.

With respect to retrovirally infected animals, further studies showed that murine AIDS and long term morphine administration modified spleen and thymus cell subsets[169] while tending to reduce IFN-γ secretions from spleen cells of murine AIDS mice.[170] Also of note is an experiment utilizing human lymphocytes[171] which found that human lymphocyte proliferative responses to HIV viral proteins was significantly inhibited by morphine in a dose dependent fashion, thus supporting the role of morphine as a cofactor in the pathogenesis of HIV infection.

CONCLUSION

In conclusion, drugs of abuse have been shown to exacerbate immune and nutritional dysfunction initiated by HIV/AIDS by causing disturbances in humoral immunity, cellular immunity, cytokine profiles, vitamin E levels, and, in the case of cocaine, they enhance the ability of the infected immune system to infiltrate the BBB and probably promote the development of AIDS related dementia.

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CHAPTER 9

Cigarette Smoking and AIDS

Herman R. Lucero and Ronald R. Watson

CONTENTS

INTRODUCTION

Smoking causes heart disease, weakens the immune system which promotes pulmonary infections, and accentuates cancer and other health related problems. The human immunodeficiency virus (HIV) also attacks and severely damages the immune system resulting, in most cases, in progression to the development of AIDS. In the United States one person dies every 11 minutes due to complications of the HIV infection. An estimated 5.8 million new HIV infections of adults and children occurred worldwide in 1997. This translates to about 16,000 new infections per day, with 90% of new infections in developing countries.[1]

Since both smoking and the HIV virus damage the immune system and reduce lung function, could the combination result in faster progression to AIDS in HIV-infected individuals who smoke? Do these two agents promote in other ways conditions favoring premature death or disease? Does smoking accentuate the loss of

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quality of life without accelerating premature death? Based on the continuing epidemic of HIV infections, the answer to these questions could have considerable implications for HIV-infected individuals who smoke. While several studies have been done on HIV-infected people who smoke, the results are mixed on whether smoking hastens the progression to AIDS.

AIDS CASE DEFINITION

The current AIDS case definition includes all HIV-infected persons who have fewer than 200 CD4 T lymphocyte counts per microliter of blood, CD4 T lymphocyte to total lymphocyte ratios of less than 14, or HIV-infected persons who get opportunistic diseases.[2] The list of clinical conditions was expanded in 1993 by the Centers for Disease Control and Prevention (CDC) to include the addition of:

1. Pulmonary tuberculosis
2. Recurrent pneumonia
3. Invasive cervical cancer

The revised definition retained the 23 clinical conditions in the AIDS surveillance case definition published by the CDC in 1987. According to the CDC, the most common opportunistic disease diagnosed in men and women with AIDS is *Pneumocystis carinii* pneumonia (PCP). Other common opportunistic diseases are candidiasis, esophagitis, cytomegalovirus (CMV), mycobacteriosis, Kaposi's sarcoma (KS), and toxoplasmosis. This definition is important as individuals with those disease may be more susceptible to accelerated progression due to external parameters.

STUDIES THAT INDICATE SMOKING HAS NO EFFECT

In a fairly extensive study, Galai et al.[3] investigated cigarette smoking as a risk factor in the progression of HIV-1 disease in the Multicenter AIDS Cohort Study of homosexual men. Longitudinal data for T cell subsets, HIV-related clinical symptoms, smoking behavior, and AIDS medication were collected semiannually from 2499 HIV seropositive men for up to nine years. This study found that smoking had no impact on the progression of HIV infection to AIDS (CDC 1987 definition), *Pneumocystis carinii* pneumonia, or death, but may affect incidence of oral thrush. This was true regardless of the amounts smoked or the baseline levels of CD4 lymphocytes.

A large and thus important study by Burns et al.[4] of a prospective cohort of 3221 HIV-1 seropositive men and women enrolled in the Terry Beirn Community Programs for Clinical Research on AIDS found no association between cigarette smoking and the overall risk of HIV-1 disease progression. The study found no difference between current smokers and those who never smoked in the overall risk of opportunistic diseases or death. The study found that smokers were more likely to develop bacterial pneumonia, oral candidiasis, and AIDS dementia. This finding is consistent

with an earlier study these researchers did in 1991, employing 202 homosexual men enrolled in a prospective cohort study of AIDS risk. They also found no detectable difference in the risk of AIDS or *Pneumocystis carinii* pneumonia with respect to smoking.[5]

Similarly, Conley et al.[6] collected data on 516 HIV-infected men from cohorts of homosexual and bisexual men between 1988 and 1992. They focused on 232 HIV-positive men who had well defined dates of seroconversion. Analyses were performed to assess the relationship between cigarette smoking and loss of CD4 T lymphocytes, diagnosis of any AIDS-defining illness, and specific diagnosis of Kaposi's sarcoma, *Pneumocystis carinii* pneumonia, oral candidiasis, hairy cell leukoplakia, and community-acquired pneumonia. They concluded that cigarette smoking was not associated with an increased likelihood or rate of developing Kaposi's sarcoma, *Pneumocystis carinii* pneumonia, or AIDS (CDC 1987 definition), but was associated with developing community-acquired pneumonia, oral candidiasis, and hairy leukoplakia in these HIV-infected men.

Some studies have indicated that smoking by HIV-infected persons has an effect on CD4 and CD8 cell counts. Craib et al.[7] investigated the effects of cigarette smoking on the percentages of CD4 and CD8 cells within a prospective study of homosexual men and compared progression rates to AIDS among seroincident smokers and nonsmokers.[7] They found that percents of CD4 cells were significantly elevated and percents of CD8 cells were significantly lower in seronegative smokers compared to nonsmokers. Craib, however, found no significant association between smoking and progression to AIDS (CDC 1987 definition) or *Pneumocystis carinii* pneumonia. The interaction between the effects of HIV-1 infection and cigarette smoking on leukocyte profiles was also studied in 307 HIV-1 seroconverters in the Multicenter AIDS Cohort Studys for up to 7 years by Park et al.[8] They studied only individuals for whom time to seroconversion was known within ±4 months. They analyzed both absolute cell numbers and proportions of all major leukocyte components in peripheral blood. They found the effects of smoking on CD4 cell numbers were (1) nonspecific, (2) maximal in seronegative individuals, and (3) lost by 3 years after seroconversion. Although the study did not address the question directly, the results suggest cigarette smoking does not accelerate progression to AIDS.

Bertrup et al.[9] also investigated the prognostic effect of time from seroconversion to AIDS. They also analyzed the effects of possible cofactors on the progress to AIDS: age at time of seroconversion, smoking status, number of male partners per year, and CD4 lymphocyte numbers. In their study, 259 Danish and 254 American homosexual men were followed for up to 14 years from 1981 to 1995. Two hundred and one persons seroconverted during the study period and 112 had developed AIDS before the end of the follow up. Bertrup found that CD4 lymphocytes numbers were highly correlated with the risk of developing clinical AIDS. However, the development of AIDS was not affected significantly by age at infection, smoking, or number of male partners. According to this study, a CD4 count between 100 and 200 increased the risk nine times and a CD4 count less than 100 increased the risk 30 times relative to the risk for a person with a CD4 count greater than 800. AIDS was defined as clinically manifested AIDS, thus a diagnosis based solely on a low CD4 count (CDC 1993 definition) was not considered AIDS-defining in their analysis.

STUDIES THAT INDICATE SMOKING ACCELERATES PROGRESSION TO AIDS

A very small study of 84 individuals investigated whether HIV-1 seropositive cigarette smokers progress more rapidly to AIDS than HIV-1 seropositive nonsmokers. Smokers developed *Pneumocystis carinii* pneumonia more rapidly than non-smokers with a median time of 8.2 months compared with 14.5 months for non-smokers.[10] The study concluded that cigarette smoking by HIV-1 seropositive individuals is associated with more rapid development of AIDS, and in particular of *Pneumocystis carinii* pneumonia. The study also found that cigarette smoking has no significant effect on time of progression to AIDS when *Pneumocystis carinii* pneumonia was not a factor.

Palacio et al. assessed whether the presence of specific HIV-related oral lesions was associated with cigarette smoking.[11] This is significant because oral lesions are known to be markers for more rapid progression of HIV disease. They analyzed cross-sectional data (CD4 cell count, smoking history, and oral examination findings) from 1058 HIV-infected male patients. After adjusting for CD4 cell counts, current smokers were significantly more likely to have candidiasis and warts and less likely to have aphthous ulcers than were current nonsmokers. Results suggest a strong association between cigarette smoking and the presence of HIV-related oral lesions.

Royce et al. analyzed the association of smoking and incidence of AIDS in 387 HIV-infected individuals from 1984 to 1992, controlling for baseline CD4 and CD8 cell numbers, thrush, age, and lifetime number of male sexual partners.[12] They found that the relative hazards for AIDS for smokers compared to nonsmokers were significantly elevated. They concluded that smoking may adversely affect HIV progression independently of T cell numbers and thrush.

Zang et al. indicated in a study on antioxidants and AIDS that oxidative stress plays a major role in the progression of HIV infection to AIDS and has been suggested to contribute to the decline in CD4 lymphocytes.[13] Since smoking is known to cause oxidation, the Zang report supports the theory that smoking causes faster progression to AIDS via oxidative damage.

EFFECTS OF SMOKING ON QUALITY OF LIFE

Although many studies found that that smoking did not accentuate the progression from HIV infection to AIDS, smoking was found to cause numerous other infections and diseases which diminish the quality of life. For example, it was found that smoking may cause oral thrush,[3] bacterial pneumonia, oral candidiasis, and AIDS dementia.[4] Smoking was associated with causing community-acquired pneumonia and hairy leukoplakia.[6] It was also found that HIV-infected smokers were more likely to have oral lesions and warts.[10]

DISCUSSION

Based on several of the studies analyzed, it appears that smoking by HIV-infected individuals does not hasten the progression to AIDS. Of particular importance in

substantiating this conclusion are the large and comprehensive studies done by Galai et al.[3] and Park et al.[4] One of the other studies indicates a strong association between oral lesions and smoking but does conclude that smoking accentuates the progression to AIDS.[11] The study by Nieman et al.[10] indicating that smoking hastens progression to AIDS when due to *Pneumocystis carinii* pneumonia was criticized. Galai et al. stated that the study was subject to severe sample bias because it did not control for length of infection.[3] Park et al. also criticized the study as being severely limited because it failed to control for CD4 cell count at study entry, for duration of HIV infection, and for exclusion of data that generated a severe sample bias and made the results from the lifetable analysis meaningless.[14]

SUMMARY

While most of the studies cited indicate smoking by HIV-infected individuals does not hasten the progression to AIDS, it is quite clear that smoking causes numerous other diseases and infections and has a detrimental effect on the quality of life of these individuals. Medical care providers should advise HIV-infected persons of the potential dangers of smoking and encourage them to quit.

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CHAPTER 10

Lipodystrophy: The Most Recent Development in HIV Nutrition Care

Jennifer Muir Bowers

CONTENTS

INTRODUCTION

"Lipodystrophy" or "fat redistribution syndrome" is the latest feature in the ever-changing, challenging, and complicated scenario of HIV nutrition care. Despite its seemingly recent development, some researchers claim that lipodystrophy was present long before protease inhibitor therapy came into use. One study compared the weights, body fat, body cell masses, and waist-to-hip ratios in HIV-infected subjects prior to 1996, to those after 1996 and to healthy HIV-negative controls.[1] It was concluded that HIV-infected subjects studied since 1996 did not demonstrate significantly different amounts of fat when compared to HIV-infected subjects studied prior to 1996. Furthermore, the waist-to-hip ratios in all HIV-infected subjects, regardless of year studied, were higher than in controls ($p < 0.001$). This appears to suggest that HIV-infected subjects have always had lipodystrophic symptoms such

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as truncal adiposity even before the introduction of protease inhibitors. The purpose of this chapter is to introduce the various symptoms of lipodystrophy, the proposed etiologies, and treatments for lipodystrophy that are under current investigation.

DESCRIPTION OF LIPODYSTROPHY

Although clinicians observed lipodystrophy (LD) earlier, descriptive publications regarding lipodystrophy were first printed in early 1998.[2,3] The primary focus was on indinavir as the culprit, due to its recent emergence as the first protease inhibitor. These early publications described lipodystrophy as evidenced by redistribution of adipose tissue. Ten HIV-infected subjects who complained of increased abdominal girth were studied by researchers at the National Institutes of Health.[3] Results from abdominal computed tomography (CT) showed that visceral adipose tissue was significantly increased when compared to HIV-infected patients who were taking indinavir but not experiencing increased abdominal girth. This abdominal distension was labeled "Crix belly," due to its perceived correlation with the protease inhibitor Crixivan (indinavir). Later the description used more frequently was "protease paunch," after other protease inhibitors became commercially available and patients on protease inhibitors other than indinavir experienced truncal adiposity.[4,5]

Another anatomical site for adipose accumulation is on the upper back just below the neck. This enlargement of the dorsocervical area was branded "buffalo hump" and compared to the clinical features seen in Cushing's syndrome.[6] Because of the assumed similarity with Cushing's syndrome, a research group in San Francisco measured plasma cortisol levels, 24-hour urinary free cortisol excretion, and an overnight low-dose dexamethasone suppression test on eight HIV-infected men who displayed buffalo hump.[2] Results demonstrated normal plasma cortisol values and urinary free cortisol excretion, as well as normal suppression of cortisol values following dexamethasone administration. Additionally, no other clinical signs of Cushing's syndrome were observed in these subjects. In the same study, HIV-infected men with buffalo hump had significantly higher proportions of truncal adiposity measured by dual-energy X-ray absorptiometry (DXA) compared to HIV-infected men without lipodystrophy.

This accumulation of visceral adipose tissue is unique in terms of weight gain. Generally, when obese individuals gain adipose weight, it accumulates subcutaneously.[7] Furthermore, the increase in visceral adipose tissue did not always coincide with overall weight gain. Instead, it appeared more like a redistribution of existing adipose tissue.[8] Concurrently, tissue wasting appears at other body sites. The loss of fat in the limbs, buttocks, and face has been described by various researchers.[8-10] Apoptosis (or programmed cell death) has been shown recently to occur in subcutaneous adipocytes taken from the antero-lateral aspects of the right legs of HIV-infected subjects experiencing atrophy of subcutaneous fat.[11]

While specific diagnostic criteria have not been developed or approved yet, some published body composition data will help form these standards in the future. Cross-sectional studies testing the bioelectrical impedance analysis (BIA) of HIV-infected patients displaying lipodystrophy have been performed.[12] This group studied 111

subjects who were prescribed protease inhibitor containing drug regimens. Results demonstrated an increase in intracellular water in lipodystrophic subjects when compared to non-lipodystrophic subjects. However, fat mass changes did not show statistical significance. As expected, whole-body BIA was concluded to be an unreliable method for detecting fat redistribution.

A prospective study of HIV-infected men and women was conducted utilizing DXA scans and whole body magnetic resonance imaging (MRI) to compare body composition to healthy, matched controls.[13] Fat and fat-free masses of the arms, legs, and truncal regions were similar in the HIV-infected subjects and controls. However, MRI displayed lower subcutaneous adipose tissue areas in HIV-infected subjects. Additionally, HIV-infected patients with truncal obesity had higher amounts of visceral adipose tissue and total adipose tissue. This study suggests that MRI is a valuable tool with which to detect increased visceral adipose tissue levels, potentially diagnostic of lipodystrophy.

Altered serum lipid profile is another component in the lipodystrophy presentation. Clinicians attribute hyperlipidemia to specific protease inhibitors such as ritonavir and the combination of ritonavir and saquinavir.[14-16] Hypercholesterolemia and hypertriglyceridemia have been described by various research groups.[9,17] A French research cohort reported hypertriglyceridemia in 79% of lipodystrophic HIV-infected patients, versus only 35% of non-lipodystrophic HIV-infected patients.[17] Statistically significant differences in lipid profiles were found between pre-protease inhibitor and post-protease inhibitor use in a group of HIV-infected males.[18] This study showed a significant difference ($p < 0.01$) in cholesterol, LDL, and glucose, but less significant changes ($p < 0.25$) in triglyceride and HDL values.

Decreased HDL cholesterol levels have also been reported in lipodystrophic HIV-infected patients.[19-21] This decrease appears to be correlated with disease progression. A group of Spanish researchers studied HDL cholesterol levels in HIV-infected patients and compared lipid levels to cellular and humoral immune markers.[21] This study demonstrated a positive correlation between HDL level and $CD4^+$ cell count ($r = 0.45$), as well as negative correlations between HDL level and interferon-α, tumor necrosis factor-α, and β2-microglobulin. As $CD4^+$ cell count declined, HDL cholesterol declined concurrently. This study additionally reported a correlational decline in apolipoprotein-A1 (Apo-A1) levels and $CD4^+$ cell count. Because Apo-A1 appears to be the main structural protein for HDL, this link seems logical.[22] Furthermore, the Spanish study reported gradual elevations of apolipoprotein-B (Apo-B) as HIV infection progressed, evidenced by the decline of $CD4^+$ cell count. Apo-B is the major protein contained in chylomicrons, VLDL, and LDL cholesterol particles. Reductions in HDL cholesterol and elevated Apo-B values have been associated with an increase in cardiovascular disease.[23]

The next logical question seems to be, "Do these lipid alterations predispose HIV-infected patients to cardiovascular disease?" The literature points to an affirmative answer. The cardiology community has shown interest in this question as well.[24] Even before the initiation of protease inhibitor therapy, various cardiac manifestations, including needs for coronary artery bypass graft surgery, were documented in young HIV-infected patients.[25,26] In 1995, asymptomatic atherosclerosis was detected in 36.7% of HIV-infected patients, as diagnosed by echography and

Doppler examination of cervical and lower limb arteries and the abdominal aorta.[27] This compelling study found that patients with plaques had slightly lower $CD4^+$ cell counts (not statistically significant), lower HDL cholesterol levels ($p = 0.03$), and higher triglyceride levels ($p = 0.03$) than control subjects. Upon autopsy, young HIV-infected patients were found to have major atherosclerotic lesions in the coronary vessels with significant obstruction.[28] Importantly, these subjects did not die from cardiovascular complications. It has been theorized that the link of chronic infection, inflammation, and immune response with cardiovascular disease may also apply to the human immunodeficiency virus.[29] Furthermore, the longer life spans of HIV-infected patients may play a role in the development of symptomatic atherosclerosis.[30]

Newly documented cases of atherosclerotic disease in HIV-infected patients emerge more frequently with the presence of lipodystrophy. Two young AIDS patients who developed cardiovascular disease were described by clinicians in Minnesota.[31] A 26-year-old HIV-infected patient was diagnosed with a large occlusive thrombus within the right coronary artery by coronary angiography performed following his complaints of angina. The other case was a 37-year-old HIV-infected patient with angina, diagnosed with occlusion of the left anterior descending artery and severe atherosclerosis of the right coronary artery. Both patients had severely low $CD4^+$ levels (<15 cells/μl) and high HIV RNA levels (>685,000 copies/ml). Several similar cases were described by researchers in New York, Germany, and France in 1998.[32] See Table 1 for a brief representation of these cases.

HIV-infected patients have developed higher incidences of glucose intolerance and frank diabetes mellitus since the onset of protease inhibitor therapy. One study compared oral glucose tolerance test (GTT) results, plasma glucose, proinsulin and insulin in HIV-infected patients with marked facial lipoatrophy.[17] These subjects were compared to non-lipodystrophic HIV-infected subjects. Diabetes or insulin resistance was diagnosed in 79% of the lipodystrophy patients. In contrast, only 20% ($n = 3$) of the non-lipodystrophic subjects presented with diabetes or impaired GTTs. Another group of researchers found basal hyperinsulinemia and abnormal GTTs in their population of lipodystrophic HIV-infected patients.[19] HIV-infected patients on protease inhibitors given intravenous insulin tolerance tests demonstrated decreased insulin sensitivity when compared to protease inhibitor naïve patients.[33] This group of researchers reported peripheral insulin resistance in patients with impaired GTTs ($n = 4$), diabetics ($n = 9$), and even in patients with normal GTT results ($n = 4$). Conversely, protease inhibitor naïve patients had normal insulin sensitivity. Higher fasting insulin levels were found in HIV-infected patients with lipodystrophy when compared to non-lipodystrophic HIV-infected patients.[9] These studies all seem to point to the conclusion that lipodystrophy and impaired glucose tolerance perform in concert with one another.

LIPODYSTROPHY IN HIV-INFECTED WOMEN

Few studies solely dedicated to researching lipodystrophy in female HIV-infected patients have been conducted. It appears that the most common complaint of females

Table 1 Types of Cardiac Disease Found in Nine HIV-Infected Patients

Case #	Age and Gender	CD4 (cells/μl) HIV RNA (copies/ml)	Diagnosed Cardiovascular Disease or Event
		Behrens et al., Hanover Medical School, Germany	
1	60 years Male	204/μl n/a	Anterolateral MI
2	58 years Male	228/μl <400 copies/ml	Large embolic occlusion of right femoral artery Atherosclerosis of carotid arteries
		Vittecoq et al., Hospital Paul Brousse, France	
1	36 years n/a	190/μl 5.5 log/ml	Anteroseptal MI 90% stenosis of LAD coronary artery
2	40 years n/a	210/μl <2.3 log/ml	Transient recurrent ischemic attacks
3	47 years n/a	290/μl 4.6 log/ml	Anteroseptal MI 90% stenosis of LAD coronary artery 70% stenosis of the left circumflex and obtuse marginal arteries
4	36 years n/a	30/μl 5 log/ml	Inferior MI 90% stenosis of the RCA 70% stenosis of the LAD and left circumflex coronary arteries
		Gallet et al., Victor Dupouy Hospital, France	
1	33 years Male	n/a 60,000 copies/ml	Inferoposterior wall MI Subtotal occlusion of RCA Second MI due to re-occlusion of RCA
2	54 years Male	n/a n/a	Angina (Patient declined coronary arteriography)
3	32 years Male	n/a 649 copies/ml	Anterolateral wall MI 90% stenosis of LAD coronary artery

Note: n/a = not available; MI = myocardial infarction; LAD = left anterior descending; RCA = right coronary artery.

Data compiled from *Lancet* 351, 1958–1960, June 27, 1998.

is breast hypertrophy, which was one of the earliest symptoms of lipodystrophy in women.[34] In Rhode Island, 19 HIV-infected women who complained of body habitus changes and used protease inhibitor therapy were studied.[35] Research methods included BIA, physical examination, and serum lipid profiles. Patient interviews revealed specific complaints: 71% with breast hypertrophy, 71% with increased abdominal girth, 47% with weight gain, 47% with peripheral wasting, 29% with gluteal wasting, and 23% with dorsocervical adipose pad or buffalo hump. BIA results confirmed a mean body fat level of 38%. Lipid abnormalities appeared to mimic those in males, with increases in total cholesterol, LDL, and triglycerides, and concurrent decreased HDL values.

Another group of researchers in Massachusetts studied 75 HIV-infected women and 30 healthy controls.[36] Body composition tests with DXA revealed significantly

higher percentages of truncal adiposity in the HIV-infected group when compared to the healthy controls ($p < 0.05$). Additionally, the HIV-infected group was found to have hyperinsulinemia and an increased insulin-to-glucose ratios ($p < 0.001$). Interestingly, these results were found independent of protease inhibitor use. Further studies in female HIV-infected patients are warranted to determine whether hormonal levels, age, nutrient intake or exercise are factors in lipodystrophy development.

LIPODYSTROPHY IN HIV-INFECTED CHILDREN

Even fewer studies or cases have addressed lipodystrophy in pediatric HIV-infected patients. One group of researchers compiled a database of HIV-infected children to determine the incidence and types of lipodystrophy in this populace.[37] Approximately 1.0% of HIV-infected children experienced abnormal body fat distribution, and 64% were females. This population also presented with a mean age of 10.9 years (range, 5 to 17 years); 43% were African American, 29% were Caucasian, and 21% were Hispanic. Abnormal fat distribution occurred in the upper and lower back, abdomen, face, and neck. These children had been prescribed a protease inhibitor for 1–14 months. This is the most descriptive information addressing lipodystrophy in HIV-infected children at this point.

PROPOSED ETIOLOGIC THEORIES

The specific etiology of lipodystrophy syndrome is currently an area of controversy and research. Several researchers have proposed mechanisms behind the development of dyslipidemia and fat redistribution. Due to the fact that lipodystrophy syndrome appeared after the emergence of highly active antiretroviral therapy (HAART), various medications and specific drugs have been implicated as causes. The Food and Drug Administration maintains an ongoing database regarding adverse drug reactions and tracks lipodystrophy symptoms reported.[38] The current stance of FDA is that a link to a specific drug or class of drugs is uncertain.

Early on, protease inhibitors were indirectly blamed for fat redistribution disorders by several researchers.[3,8,9,33,39] A group of British investigators demonstrated patterns of lipodystrophy emergence in patients taking nevirapine-containing HAART regimens.[40] Nevirapine is a non-nucleoside reverse transcriptase inhibitor (NNRTI). The group discovered that 16% of these patients exhibited symptoms of lipodystrophy. The most common symptoms were truncal adiposity (78%) and peripheral fat wasting (67%). Additionally, these researchers reported that all cases of nevirapine-associated lipodystrophy were associated with undetectable viral loads. Other NNRTI medications have not yet specifically been studied.

Stavudine, a nucleoside reverse transcriptase inhibitor (NRTI), was the suggested cause of lipodystrophy by a group of French researchers.[41] In their study, a group of HIV-infected patients prescribed stavudine-containing HAART regimens was compared to a group of HIV-infected patients taking zidovudine-containing HAART regimens and a control group of HAART-naive patients. The stavudine

group demonstrated lower overall body fat percentage ($p < 0.05$) when tested by skinfold thickness measurements, and less subcutaneous fat in the mid-thigh region ($p < 0.05$) when compared with the zidovudine group. Lipid profiles were not statistically different between the groups. These researchers concluded that long-term NRTI therapy, particularly with stavudine, may induce lipodystrophy.

One report from an English group also suggests that nucleoside analogues in combination with NNRTIs lead to lipodystrophy.[42] This group describes nine protease inhibitor naive patients demonstrating weight loss, increased abdominal girth, breast hypertrophy, hypertriglyceridemia, wasting of extremities, and facial adipose wasting. The two most common medication regimens in these subjects were zidovudine plus lamivudine and stavudine plus lamivudine. These results suggest that the amalgamation of nucleoside analogues and NNRTIs may be allied with lipodystrophy.

More evidence that medications cause lipodystrophy symptoms was demonstrated by a case report from France.[43] This report discusses an HIV-negative individual taking a regimen of zidovudine, lamivudine, and indinavir for post-exposure prophylaxis. He had a documented negative HIV ELISA test and began to develop abnormal fat redistribution in the form of central adipose deposition, a "moon face," and loss of fat in the limbs. These symptoms were coupled with a weight loss of 7 kg despite his 3100-kcal/day intake. This case suggests that lipodystrophy is caused by a component (or mixture of components) in HAART as opposed to the HIV virus alone.

Another theory on the etiology of lipodystrophy focuses on lipid metabolism.[44] The HIV-1 protease enzyme has 63% parallel amino acid sequence to regions within cytoplasmic retinoic-acid binding protein type 1 (CRABP-1) and low-density lipoprotein-receptor-related protein (LRP), two proteins that regulate lipid metabolism. This group of Australian researchers hypothesized that the protease inhibitor medications that bind to the HIV-1 protease, subsequently prohibiting HIV-1 viral replication, can also bind to CRABP-1 and LRP, altering lipid metabolism. Interference with CRABP-1 function would inhibit the binding of retinoic acid, resulting in reduced *cis*-9-retinoic acid production, reduced retinoic X receptor activity, and finally, reduced differentiation and increased apoptosis of peripheral adipocytes. Hyperlipidemia ultimately would occur due to reduced triglyceride storage and lipid release into the circulation. Binding of the protease inhibitor drug to LRP may exacerbate hyperlipidemia due to the decreased amount of available LRP and subsequent reduced chylomicron clearance. These researchers also theorized that inhibition of LRP and lipoprotein lipase promotes adipose deposition and insulin resistance. These hypotheses are compelling, but require further research.

As mentioned earlier, symptoms of lipodystrophy have been compared to Cushing's syndrome. Thus, studies have attempted to describe the roles of cortisol and catecholamines in HIV-related lipodystrophy. One study compared the hypothalamic-pituitary-adrenal axes and metabolic measurements of HIV-infected patients with lipodystrophy symptoms to healthy controls and patients with Cushing's syndrome.[45] The HIV-infected subjects showed normal diurnal variation in spontaneous plasma cortisol, and lower cortisol levels at all times of the day or night than the Cushing's syndrome subjects ($p < 0.005$). Urinary free cortisol was significantly lower in the HIV-infected subjects than in the healthy controls or the Cushing's syndrome subjects ($p < 0.001$). Additionally, the HIV-infected subjects had greater

hydroxycorticosteroid excretion than healthy controls ($p < 0.001$), but lower levels than the Cushing's syndrome subjects ($p < 0.01$). Compared to the Cushing's syndrome subjects, the HIV-infected subjects had normal glucocorticoid receptor numbers, similar glucose and insulin values, and greater serum triglycerides. The researchers concluded that HIV-related lipodystrophy is distinct from any known form of hypercortisolism, and therefore cannot be appropriately compared to Cushing's syndrome.

Conversely, a French research group studied body compositions and various endocrine measurements in HIV-infected individuals with altered adipose deposition.[46] Results were compared to those of HIV-infected individuals without symptoms of lipodystrophy, who were matched for age, stage of disease, and HAART use. As expected, HIV-infected subjects with lipodystrophy displayed larger amounts of visceral adipose tissue ($p = 0.002$) when analyzed via CT. Interestingly, these subjects had significantly increased 24-hour urinary output of catecholamines when compared to controls ($p = 0.013$). The authors suggest that the catecholamines induce lipolysis, which in turn increases metabolic rate and may be involved in the peripheral adipose wasting seen in lipodystrophy. As with other etiological theories of lipodystrophy, more research in this area is warranted.

The most recent hypothesis revolves around the mitochondrial toxicity of nucleoside-analogue reverse transcriptase inhibitors (NRTIs).[47] This theory is based on findings that demonstrate that DNA polymerase γ, the only enzyme responsible for mitochondrial DNA replication, is inhibited by NRTIs. This may cause depletion of mitochondrial DNA and mitochondrial DNA-encoded enzymes, resulting in altered mitochondrial function. Many drug-related side effects that occur with NRTI use are also seen in inherited mitochondrial diseases, such as neuropathy, myopathy, and cardiomyopathy. Clinical studies addressing this mitochondrial theory have not yet been conducted.

TREATMENT OF LIPODYSTROPHY

Current therapies to decrease or reverse symptoms of HIV-related lipodystrophy are under rigorous investigation. Numerous individual patients have fought lipodystrophy by simply discontinuing prescribed HAART medications, which is obviously a suboptimal solution. One research group found decreases in cholesterol and triglycerides, and partially reversed fat redistribution symptoms, in subjects switched to HAART regimens in which nelfinavir was the only protease inhibitor prescribed.[48] However, due to viral mutation and resistance, changing medications repeatedly is not the ideal method of treating lipodystrophy.

Diet and exercise serve as the first line of intervention in hyperlipidemias and obesity. Therefore, it seems logical that they would be the first line of treatment in HIV-related lipodystrophy. To date, few published clinical studies elaborate on nutritional intervention exclusively. Published studies that mention dietary intervention have utilized the National Cholesterol Education Program Guidelines (NCEP) as their protocols,[49,50] yet elaboration on this topic does not exist. The published

studies additionally provide subjects with gemfibrozil and/or atorvastatin; therefore, they do not solely concentrate on dietary modification. One study included a diet and exercise group, in which 12 of 20 subjects were deemed treatment failures after an unknown amount of time, and were prescribed lipid-lowering medication.[50] The two studies combining diet, exercise, and lipid-lowering medication have shown reductions in serum cholesterol and triglycerides.[49,50]

One comprehensive investigation into the effects of exercise training on HIV-related fat redistribution was conducted by a cohort in Boston.[51] A group of ten HIV-infected men with increased abdominal girth was involved in a 16-week program of progressive resistance training and aerobic exercise. Training was conducted three times per week, with one session supervised by volunteer trainers. Resistance training lasted for approximately one hour per session and worked all major muscle groups (legs, back, and arms). Aerobic activity consisted of a 20-minute workout on a treadmill or stationary bicycle. Mean compliance to the program was assessed at 77%. Results demonstrated a significant decline in overall body fat ($p < 0.01$) as measured by DXA, with the majority coming from the trunk ($p < 0.03$), despite no change in body weight, body mass index, lean body mass, or bone mineral density. These are promising results since exercise therapy is a cost-effective intervention. Further investigation with longer trials and comparisons of serum lipid and glucose values would be beneficial.

Nandrolone decanoate and other anabolic steroids have been investigated to assess their effects on fat redistribution symptoms and the studies produced varying results.[52,53] Similarly, a study of the effect of recombinant human growth hormone (rhGH) on fat redistribution is ongoing. One study investigated rhGH in the treatment of dorsocervical fat pads and truncal adiposity in two AIDS patients (one male and one female).[54] The researcher reported total resolution of the dorsocervical fat pad and decreased truncal obesity in the female subject after 12 weeks of rhGH therapy. Additionally, the male subject experienced a 50% reduction of the dorsocervical fat pad. A slightly larger study, with ten HIV-infected subjects (seven males and three females), provided rhGH therapy for 12 weeks.[55] Results included decreases in waist-to-hip ratios and increased mid-thigh circumferences. While these data are noteworthy, there is not yet sufficient evidence to support the recommendation for rhGH treatment in all HIV-infected patients with lipodystrophy.

Pharmaceutical treatment for dyslipidemia is another area of current exploration. As noted above, gemfibrozil has shown promise in decreasing triglyceride levels in HIV-infected patients.[49,50,56] One study compared diet and exercise, gemfibrozil, and atorvastatin, and combinations of these variables in HIV-infected subjects.[57] Data revealed that the most significant declines in serum cholesterol and triglycerides occurred in a group taking atorvastatin and gemfibrozil in conjunction with diet and exercise counseling. However, this drug combination is not always feasible due to increased risk of myopathy, as well as increased risk of toxicity when both atorvastatin and protease inhibitors are taken.

Liposuction has been performed on HIV-infected patients with fat redistribution.[58,59] Reports of these procedures are increasing as patients become frustrated with their distorted body images and lack of results or access to other therapies. Whether adipose depositions recur after liposuction has not yet been elucidated.

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Research in the area of HIV-related lipodystrophy will revolve around the etiology, clinical manifestations, and management of the syndrome. Nevertheless, first and foremost, a uniform diagnostic definition of HIV-related lipodystrophy needs to be developed and agreed upon by practitioners. This diagnosis may involve one or several syndromes. Diagnostic criteria will allow for more accurate reports on the incidence and prevalence of HIV-related lipodystrophy. Until this uniform definition and specific criteria are created, patients and clinicians alike may be overutilizing the terminology of "lipodystrophy" and "fat redistribution."

Underlying mechanisms and confounding factors leading to metabolic and body composition alterations need continued investigation. Genetic studies similar to those studying congenital generalized lipodystrophy (CGL) may explain why only certain HIV-infected individuals develop overt symptoms.[60] Exploration of the role of leptin in fatty acid oxidation and energy metabolism in CGL may be applicable to HIV-related lipodystrophy. Leptin was found to reverse insulin resistance and diabetes mellitus in transgenic mice with CGL, but these factors have not yet been investigated in HIV-infected humans.[61] It has been shown that plasma leptin levels are low in AIDS patients, but are not correlated with overall percent body fat or plasma levels of TNFα.[62] The relationship of leptin and lipodystrophy warrants further examination.

The study of how to optimally treat lipodystrophy symptoms must continue, with additional analysis of the risks and benefits of therapies such as anabolic steroids, growth factors, and anti-hyperlipidemic drugs. Furthermore, the evaluation of clinical sequelae of body composition changes, dyslipidemia, and alterations in glucose metabolism will be beneficial to the overall investigation of what comprises optimal care of the HIV-infected patient.

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CHAPTER 11

Vitamins in HIV Infection

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CONTENTS

INTRODUCTION

Altered nutritional states and/or malnutrition are common and often striking findings in patients with HIV infections. As a result, HIV-positive patients, even those who attempt to follow reasonable diets, often present with specific vitamin deficiencies. The pathogenesis of malnutrition and vitamin deficiency in AIDS is multifactorial and includes (at a minimum) decreased food intake, decreased nutrient absorption, and decreased efficiency of utilization, combined with increased nutritional needs and increased tissue metabolism.[1-3]

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Vitamins may have considerable clinical importance in HIV infection. Vitamin deficiencies are known to be related to altered immune, hematologic, and neurologic status in these patients.[1,2] This chapter will review current information about vitamins and their potential roles in HIV infection.

VITAMIN B_{12} (COBALAMIN)

Numerous studies have demonstrated that vitamin B_{12} deficiency occurs commonly in HIV-infected patients and may occur at any stage of infection.[1,2,4,32] Herbert and colleagues suggested that serum B_{12} levels may underestimate the degree of deficiency in AIDS. They reported that serum levels of the delivery protein holotranscobalamin II and the amount of B_{12} bound to it were more sensitive markers of early deficiency and suggested that functional B_{12} deficiency may occur in almost half of HIV-infected patients.[11,27,28]

The majority of studies have suggested that B_{12} deficiency results from malabsorption.[8-10,13,14,23] Harriman et al. studied 11 AIDS patients, 3 with low and 8 with normal B_{12} levels. Eight had abnormal Schilling tests even when given both intrinsic factor and pancreatic enzyme supplements. Intestinal biopsies in these patients revealed histopathologic evidence of chronic inflammation with evidence that HIV-1 virus was present in the lamina propria. The investigators concluded that malabsorption, perhaps secondary to a direct inflammatory effect of the HIV-1 virus, may be the principal cause of B_{12} deficiency in HIV infection.[8] Similarly, Remacha reported that all 6 patients studied with B_{12} deficiency and HIV infection had abnormal Schilling tests, showing evidence of ileal malabsorption.[13,14] A number of studies have reported that intrinsic factor secretion is reduced in patients with AIDS in association with decreased gastric acid secretion, suggesting parietal cell dysfunction.[9] Herbert suggested that B_{12} deficiency at the cellular level may be exacerbated by the deficiency of the delivery protein holotranscobalamin II (TC II) that occurs early in B_{12} deficiency, although Hansen et al. reported that low plasma B_{12} was not simply due to low concentrations of B_{12} binding proteins.[11,12] Another potential mechanism of B_{12} deficiency may be an effect of Retrovir.[29,30] Richman et al. reported in a double blind study of Retrovir in HIV infection that recipients had higher frequencies of reduction in B_{12} levels than patients taking placebo.[30] The mechanism of how Retrovir could act to decrease B_{12} levels is unknown.

The extent that vitamin B_{12} deficiency contributes to the anemia found in HIV infection is unclear, although it has been noted.[30-36] Vitamin B_{12} replacement has been shown to lead to decreased anemia in cases of documented B_{12} deficiency in HIV infection.[14] Vitamin B_{12} deficiency in HIV infection has been linked variably to neutropenia.[30-33,35,36,43] Richman et al. in a study of Retrovir noted that lower serum B_{12} levels, even within the normal range, were independently correlated with increased development of neutropenia in patients.[30]

Four large studies have looked at whether prophylactic B_{12} would lessen the hematologic toxicity of Retrovir. These studies failed to show any benefit from prophylactic B_{12} in preventing anemia or increasing tolerance to Retrovir.[37-40]

Vitamin B_{12} deficiency has also been associated with neurologic dysfunction in HIV-infected patients.[15-18,25] Baum et al. administered cognitive function tests to 100 asymptomatic HIV-seropositive males. Patients with clear or marginal B_{12} deficiencies had poorer cognitive functions than patients with normal B_{12} levels. Moreover, those patients whose B_{12} levels normalized with intramuscular (IM) B_{12} therapy also experienced improvement in subsequent cognitive testing. Patients whose B_{12} levels did not normalize with IM B_{12} therapy showed no significant improvements in cognition. The authors concluded that B_{12} deficiency was associated with cognitive impairment and that correction of the deficiency could result in functional improvement.[15,25] These authors went on to study 84 patients in a 4 year longitudinal study. Patients with low B_{12} levels had abnormal cognitive functions that improved when vitamin B_{12} levels became adequate.[26] Similarly, Kiebwurtz et al. reported that HIV-infected patients referred for neurological problems frequently had vitamin B_{12} deficiencies, and vitamin B_{12} treatment resulted in clinical improvement.[16] In contrast, Stern et al. reported that in asymptomatic HIV disease, neurocognitive deficiency did not correlate with B_{12} level.[17]

Other authors have speculated whether HIV-associated condition spinal vacuolar myelopathy was secondary to B_{12} deficiency, noting that the same regions of the spinal cord affected in this condition were those affected by B_{12} deficiency.[16,41,42] To date, this hypothesis has not been proven.

Some researchers have suggested that B_{12} is also related to immune function. Baum et al. reported a longitudinal study of the correlation between vitamin levels and CD4 counts over 18 months in a group of 108 HIV-positive patients.[31] Low baseline B_{12} significantly predicted accelerated HIV progression as determined by CD4 counts. Development of B_{12} deficiency was associated with a decline in CD4 cell counts ($p = 0.038$), while normalization of B_{12} was associated with higher CD4 counts ($p = 0.006$).[31] Tang and associates reported a longitudinal study of 310 HIV-positive patients over 9 years in which the risk of progression to AIDS was 3.4 times as high in patients with low vitamin B_{12} levels as in those with normal levels. Patients with B_{12} levels less than 125 pmol/L had a mean 4 year survival compared to 8 years in patients with normal B_{12} levels.[32]

In summary, vitamin B_{12} deficiency is common in all stages of HIV infection, although its prevalence varies considerably among studies. The major cause of B_{12} deficiency appears to be malabsorption. B_{12} deficiency may contribute to cognitive impairment in HIV infection and its correction may lead to improved cognition. B_{12} deficiency is also a treatable cause of anemia and possibly neutropenia in HIV-infected patients.

VITAMIN B_6 (PYRIDOXINE)

Pyridoxine (vitamin B_6) deficiency has also been reported to be common in patients with HIV infection, with prevalences ranging from 12% to 52% of patients in a variety of studies.[7,20,24,31,32,43] The pathogenesis of pyridoxine deficiency in AIDS is unclear. Mantero-Atienza and colleagues reported that pyridoxine deficiency occurred despite apparently adequate dietary intake, although they also noted that

patients who took at least 20 mg of B_6 daily were able to avoid deficiency.[28] In contrast, Coulston et al. suggested that HIV patients may have inadequate dietary intakes of vitamin B_6. They found that vitamin B_6 intake was below the recommended daily allowance (RDA) in a group of 26 patients with early HIV disease.[44]

Animal and human studies suggest that B_6 deficiency results in impairment of both cell-mediated and humoral immune responses, including impaired interleukin-2 production and lymphocyte proliferation in response to mitogens.[45,46] Mantero-Atienza and colleagues reported that HIV-infected patients with vitamin B_6 deficiency had lower CD4 cell counts and other immune parameters than those without B_6 deficiency.[41] Two other reports suggest that vitamin B_6 deficiency in HIV-infected patients is associated with reduced natural killer cell cytotoxicity and decreased lymphocyte mitogen responsiveness.[31,42] One study of 108 HIV-positive patients suggested that B_6 deficiency occurred commonly (in 30%), was significantly correlated with tension/anxiety and bipolar manic behavior, and that correction of B_6 deficiency resulted in decreased depression.[47]

In conclusion, vitamin B_6 deficiency has been identified commonly in HIV infection, and may result in immunologic and psychiatric impairment. Further studies are needed to confirm the benefits of vitamin B_6 therapy.[43]

THIAMINE

While Beach et al. and Bogden et al. failed to find any cases of thiamine deficiency in their studies of 50 and 30 patients, respectively, Malcolm et al. reported that 2 of 16 patients with AIDS/ARC had low thiamine levels. Butterworth reported that 9 of 39 (23%) of patients with AIDS or ARC had biochemical evidence of thiamine deficiency, using the erythrocyte transketolase activation assay.[19-21,48]

There are three case reports of Wernicke's encephalopathy in AIDS patients and a fourth case of acute encephalitis treated with AZT, dexamethasone, and thiamine with rapid clinical resolution.[49-52] A fifth case of thiamine deficiency presented with lactic acidosis and heart failure which responded to thiamine replacement.[53] These cases have led the authors to postulate that thiamine deficiency may be more common than has been reported. Butterworth et al. argued that thiamine supplementation should be started in all new cases of AIDS or ARC.[21] Since thiamine deficiency in non-HIV-infected patients is usually secondary to malnutrition, it is likely that malnutrition is the etiology in HIV-infected patients as well.

RIBOFLAVIN AND NIACIN

Data on whether riboflavin and niacin deficiencies are clinically important in HIV-infected patients are limited. While two studies failed to find any patients with riboflavin deficiency, a third reported that 27% of 100 asymptomatic HIV-positive males had low levels of riboflavin.[20,24,48] Fouty et al. reported three cases of AIDS patients who developed lactic acidosis on triple drug therapy. Their lactic acidosis

was cured by the administration of riboflavin.[54] Fouty noted that mitochondrial DNA synthesis can be impaired by riboflavin deficiency, which could lead to lactic acidosis and hepatic stenosis.[54] Luzzati reported a similar case of an HIV-positive pregnant woman with lactic acidosis who was cured with riboflavin supplementation.[55] In the only reported study of niacin, Bogden et al. found that 7% of 30 HIV-infected patients at various stages of disease had serum niacin levels below the normal range.[20] This finding was not clearly correlated with stage of disease.

VITAMIN A

Vitamin A deficiency occurs commonly in HIV infection. Multiple studies of HIV-infected patients found evidence of vitamin A deficiency with frequencies ranging from 5% to 29% of patients.[7,19,20,24,32,33,48,56-64] Several studies have reported that decreased vitamin A concentrations were associated with increased mortality and decreased immune function in HIV-infected patients.[65,66] Read et al., however, failed to show increased vitamin A deficiency had any link to morbidity in a study of HIV-positive children in the U.S.[67] Javier reported that vitamin A deficiency in a group of asymptomatic patients correlated with decreased natural killer (NK) cells and blood levels of IgG.[57] Phuapradit et al. reported that vitamin A and beta carotene levels had a positive correlation with CD4 count, CD4 percentage, and CD4/CD8 ratio.[58] A study of HIV-infected mothers in Rwanda reported that low maternal serum retinol levels were correlated with increased fetal, neonatal, and postnatal death.[62] Another Rwanda based study reported an association between decreasing retinol and increasing viral load over time.[63] Coodley et al. reported that serum vitamin A levels were significantly lower in patients with wasting.[7]

Malabsorption secondary to HIV-induced intestinal dysfunction may to be the likely etiology of vitamin A deficiency. Two studies of urinary retinol excretion initially did not find increased urinary excretion among stable clinic patients, but found increased excretion in acutely ill, hospitalized patients.[68,69] Allard et al. reported that oxidative stress was higher in HIV-infected patients and was associated with decreased vitamin levels. Allard suggested that deficiency of antioxident vitamins (A, E, C, and beta carotene) may be due to increased utilization secondary to oxidative stress rather than inadequate dietary intake or malabsorption.[60]

Prior to the HIV epidemic, numerous studies from cell cultures, animals, and humans linked vitamin A to the risk of infection. These studies showed increased rates and severity of infection when vitamin A is deficient.[64,70-74] They also demonstrated that vitamin A repletion or prophylactic administration may reduce the incidence or severity of infection.[70,71] Therefore, vitamin A deficiency could further predispose HIV-infected patients to more frequent or severe infections.

Studies of the links between vitamin A therapy and risks of progression have yielded positive and negative results.[75,76] A prospective double-blind placebo controlled trial of vitamin A supplementation in HIV-infected children with pneumonia in Tanzania showed that vitamin A supplements reduced mortality by 63%. Diarrhea related deaths were reduced by 92%. The authors postulated that vitamin A

deficiency may result in impaired gastrointestinal defenses against gastrointestinal infections.[77] Baum et al. reported that normalization with vitamin A was associated with increased CD4 counts ($p = 0.049$).[35]

Studies have also investigated whether vitamin supplementation affects CD4 count or viral load.[78-80] Coutsoudis reported that vitamin A supplementation did not change the viral load in a cohort of HIV-infected pregnant women.[78] Humphrey and colleagues reported that a single dose of 300,000 IU of vitamin A had no effect on viral load or lymphocyte subsets.[79] Semba et al. found that 200,000 IU vitamin A did not affect viral load or T-cells in a cohort of injection drug users.[80]

Several other studies of vitamin A supplementation in HIV-infected patients were reported. Coutsoudis et al. reported that vitamin A supplementation reduced diarrhea in HIV-infected children.[81] In this South African placebo controlled, randomized trial, HIV-positive children supplemented with vitamin A every two months had risks of morbidity associated with diarrhea that were half those of the placebo-controlled children. Because diarrhea is such an important cause of morbidity and mortality among children in Africa, Coutsoudis et al. argued that vitamin A supplementation may be an inexpensive and beneficial intervention.[64,81]

A number of studies have looked at whether vitamin A supplementation could reduce HIV transmission from mother to child.[59,82-87] Semba et al. reported in two large studies from Malawi that decreased vitamin A levels were independently linked to both increased mother to child transmission and increased infant mortality.[82,83] The same team also found an association between the risk of maternal infant transmission and vitamin A deficiency among a cohort of 133 HIV-infected women in the U.S.[84] In contrast, Burger et al.[85] and Wiratachai[59] reported that vertical transmission was not associated with levels of vitamin A and carotenoids.

Two large prospective placebo controlled trials of vitamin A supplementation in pregnant women in Tanzania and South Africa have been reported.[86,87] Both studies showed that vitamin A supplementation had no overall effect on the risk of HIV transmission from mother to child or on infant mortality.[86,87] However, the South African study reported a decrease in pre-term delivery in vitamin A supplemented women and fewer HIV infections among pre-term infants.[86] A similar trial of vitamin A supplementation in Malawi among HIV-infected pregnant women reported that it resulted in decreased low birth weight deliveries in the vitamin A supplemented women.[88]

Initially, a number of studies suggested that beta carotene, a carotenoid with provitamin A (retinol) activity, might have clinical efficacy in HIV infection. Uncontrolled trials of beta carotene, in doses ranging from 60 to 180 mg a day, produced variable results in terms of increasing numbers of CD4 cells and natural killer cells.[89-91] Coodley et al. conducted a placebo controlled trial of beta carotene (180 mg a day) versus placebo and found that the beta carotene resulted in an increased percent change in CD4/CD8 ratio and total WBC count, and a trend to increased numbers of CD4 cells compared to placebo.[92] However, a subsequent larger study by Coodley et al.[93] and uncontrolled study by Nimmagadda et al.[94] failed to show any beneficial effect on CD4 counts or HIV RNA titers. Ullrich and colleagues reported that carotene deficiency occurred in 77% of patients studied and that mean

beta carotene level correlated with CD4 count.[95] Other authors have also reported frequent carotene deficiency.[19,96]

In summary, the available literature demonstrates that vitamin A deficiency occurs in HIV infection and likely increases with more advanced disease. Limited data also suggest the possibility that vitamin A and/or beta carotene may be useful therapeutic agents in HIV-infected patients.

VITAMIN C

Many studies have reported on vitamin C deficiency in HIV infections with prevalences in 0 to 27% of patients.[7,20,48,57,97,98] Studies that included patients with more advanced diseases tended to find more deficiency, although serum levels did not always correlate with stages of disease.[7,20] Javier reported that vitamin C deficiency correlated with decreased IgM levels.[57] The benefit of vitamin C therapy in HIV infection is not clear. In three studies, Harekeh et al. reported that ascorbic acid reduced HIV reverse transcriptase activity in HIV-infected T lymphocyte cell lines *in vitro* and inhibited syncytia formation and reverse transcriptase activity of extracellular HIV virus.[99-101] Fortis et al. showed that *in vitro* ascorbic acid reduced bacterial adherence to buccal epithelial cells from HIV-infected patients, suggesting that this might result in decreased bacterial infections.[102] Rawal also reported an *in vitro* decrease in HIV caused by ascorbic acid supplementation.[103] Lianou reported that ascorbic acid had an antibacterial effect and improved certain immune parameters in a group of HIV-infected patients.[104] Allard and colleagues conducted a randomized, placebo controlled, double study giving 49 HIV-positive patients a placebo or vitamin C (1000 mg daily) and vitamin E (800 IU daily).[105] In this 3 month study, the vitamin supplemented group had decreased lipid peroxidation with a trend toward a reduction in viral load.[105]

VITAMIN D

Three studies that investigated vitamin D deficiency in HIV-infected patients reported quite different results. Malcolm et al. found normal vitamin D levels in a cohort of 14 patients with AIDS/ARC.[19] In contrast, Coodley et al. reported that 17% of 47 patients at different stages of HIV infection had 25 OH vitamin D deficiency and 10% had 1,25 $(OH)_2$ vitamin D deficiency. In this study, lower vitamin D levels appeared to correlate with decreased CD4 count and possibly with wasting.[7] Haug's group also reported decreases in serum levels of 1,25 $(OH)_2$ vitamin D in HIV patients, correlating with the degree of immunodeficiency, survival, and risk for *Mycobacterium avium* complex.[105-107]

The etiology of vitamin D deficiency is unclear. Haug and colleagues reported that inadequate hydroxylation of 25 hydroxy vitamin D, possibly secondary to elevated tumor necrosis factor, appears to be the cause of the deficiency.[108]

Several *in vitro* studies have suggested that vitamin D may enhance HIV replication.[100,108-112] For example, Locardi noted that vitamin D produced increased

macrophage differentiation and a marked increase in HIV infection.[109] Skolnick and colleagues reported in two studies that $1,25(OH)_2$ vitamin D greatly enhanced HIV-1 replication in monocyte cell cultures and peripheral blood monocytes, suggesting that vitamin D might be one of the most potent enhancers of HIV replication.[111,112] In contrast, Rigby and colleagues reported that vitamin D added to monocyte cell culture reduced productive infection of cells by HIV-1 by 95%.[113] Haug et al. reported that vitamin D supplementation may slightly decrease *Mycobacterium avium* replication in macrophages from HIV-infected patients.[114]

Studies are also contradictory on the effect of vitamin D on immune responsiveness. While Girasole reported that vitamin D stimulated monocyte chemotaxis in sera of AIDS patients, Tobler et al. reported that vitamin D may suppress GM-CSF expression in lymphocytes, thus further attenuating the normal immune responses to infections.[115,116] These *in vitro* studies suggest that vitamin D may have an important, although as yet unclear, influence on the immune response, particularly that of T lymphocytes.

VITAMIN E

Multiple studies have been reported on the prevalence of vitamin E deficiency in HIV infection. Beach et al. and Coodley et al. reported normal vitamin E levels in all patients studied, while others reported that 12% to 50% of HIV patients had vitamin E deficiency.[7,20,35,48,56,95,117,118] This deficiency generally did not correlate with changes in immune parameters including CD4 count.[56,57,117]

The cause of vitamin E deficiency is unclear, although it may relate to the malabsorption associated with worsening HIV infection. Jordao and colleagues demonstrated that a group of AIDS patients had decreased plasma vitamin E levels and increased urinary excretion of vitamin E when compared to healthy controls, suggesting another mechanism for vitamin E deficiency.[119]

Several investigators have hypothesized that vitamin E may favorably modulate the immune response in HIV infection. Odeleye and Watson noted that vitamin E has been shown to increase the CD4/CD8 ratio, lymphocyte count, natural killer cell activity, phagocytosis, and mitogen responsiveness. They reported that supraphysiological levels of vitamin E have been shown to increase immune responsiveness and host resistance to microorganisms.[122] Hollins argued that vitamin E stimulates the helper function of T cells, the mitogenesis of T cells, and perhaps T and B cell cooperation.[120] Kline et al. reported that vitamin E, studied in two animal retrovirus models, reduced retrovirus-induced T suppressor activity, increased interleukin-2 production, and decreased PGE2 production.[121] Wang et al. reported that mice with murine AIDS treated with vitamin E showed improvement of multiple immune parameters including increased interleukin-2, interferon, and natural killer cell activity.[123]

Gogu and colleagues studied the effects of adding vitamin E to the HIV-1 virus in cell culture. They added vitamin E with and without AZT to HIV-1-infected human lymphocytic cell lines.[124] They found that while the addition of vitamin E alone had no significant effect, in combination with AZT it acted synergistically to inhibit the HIV-1 virus. These authors postulated that vitamin E may modulate

glycosylation of viral proteins and act with AZT to create a sequential blockade of HIV replication. They also noted that vitamin E reduced the toxicity of AZT on bone marrow cells in culture and suggested that this might result from a stimulating effect of vitamin E on cellular proliferation or by activating erythropoietin.[124]

Some studies have now been reported on vitamin E *in vivo* in HIV-infected patients. Tang and colleagues compared vitamin E levels to the risk of progression to AIDS over time in a cohort of 311 patients in Baltimore and Washington.[75] They found that patients in the highest quartile of vitamin E levels had 34% decreases in risk of progression compared to the lowest quartile.[75] As previously noted, one placebo controlled study of vitamin E (800 IU qid) plus vitamin C (1000 mg qid) supplementation showed decreased lipid peroxidation and a nonsignificant trend to decreased viral load (mean –0.45 log, 10 copies/ml versus +0.5 log, 10 copies/ml for placebo, $p = 0.1$). Larger studies have not been reported. In contrast, Wiratachai failed to find any link between vitamin E status and the role of vertical transmission.[59]

VITAMIN K

The only available study of vitamin K and HIV infection reported that menaquinone (a bacterial vitamin K) suppressed HIV-1-induced syncytia formation in cell culture, but had no effect on virus production. The authors postulated that if vitamin K could interfere with HIV-induced syncytia formation, it might be useful in containing HIV-1 *in vivo*.[125]

FOLATE

There is marked disagreement about whether folate deficiency is a common clinical problem in HIV infection. Four studies reported that most patients had normal or elevated folate levels and that folate deficiency was rare.[6,7,20,48] In contrast, a number of studies have reported that folate deficiency occurs commonly in HIV-infected patients.[22,23,97] Boudes and colleagues evaluated folate levels in 74 HIV-infected patients and found that 64% of their patients not receiving folate replacement were folate deficient. Further, they found that only those patients receiving folate replacement had elevated serum folate levels. The authors concluded that folate deficiency may be common in HIV infection and that elevated serum levels likely reflect vitamin supplementation.[22] Herbert et al.[23] and Zeitz[126] similarly reported that folate deficiency occurred in 66% and 41% of patients studied, respectively.

Revell reported that HIV-infected patients had significant impairments of absorption of folic acid, regardless of disease stage or degree of gastrointestinal symptoms, suggesting a possible mechanism of folate deficiency.[127] Similarly, other researchers have suggested that the elevated serum folate levels observed in HIV infection may be spurious, and secondary to cellular destruction or increased immunoglobin binding in HIV infection leading to transient elevations of serum levels of folate.[128]

Three reports have suggested that cerebrospinal fluid (CSF) folate deficiency may occur in HIV infection and be a cause of neurologic dysfunction.[129-131]

The literature is also contradictory regarding the benefit of folinic acid in preventing the hematologic toxicity of antifolate drugs such as trimetrexate or trimethoprim in HIV infections. Two studies using trimetrexate to treat *Pneumocystis carinii* pneumonia showed that leucovorin (folinic acid) minimized marrow toxicity and allowed the trimetrexate to be well tolerated.[132-134] Other investigators have argued that prophylactic folinic acid use does not always prevent or reverse the cytopenia commonly observed in AIDS.[135,136] While there is no consensus about the role of folate in HIV infection, its deficiency can be measured easily and potentially corrected if present.

MULTIPLE VITAMIN SUPPLEMENTATION

It is important for clinicians to realize that HIV-infected patients may believe in the utility of vitamin supplementation and take one or more vitamin supplements, often without consultation with their physicians. Three studies have suggested that as many as 50% may be taking vitamin supplements.[137-139] It has been reported that vitamin supplementation is greater among whites and individuals with more education than among HIV-infected blacks and Hispanics.[139] Skurnick et al. reported that HIV-positive patients who took vitamin supplements had consistently less vitamin deficiency regardless of disease stage.[97] Vitamin deficiencies have been reported to be more common in HIV-infected women than men.[35] Vitamin intake below the RDA has been reported to occur both in developing nations and in American injection drug users.[33,34] While vitamin levels often correlate with nutritional intake, Baum et al. reported that deficiencies of vitamins B_6, B_{12}, B_2, A, C, and E may occur despite consumption of vitamins at or above the RDA.[140] Abrams et al.,[141] Allard et al.,[98] and Constans et al.[56] also reported that dietary intakes of most vitamins appeared to exceed the RDA in the HIV patients studied.

Some large studies have also reported that supplementation with different and multiple vitamins was associated with improved survival. Tang et al. found a positive association between higher intakes of vitamin B_1, niacin, and B_6 and improved survival in a cohort of 281 HIV-infected patients in the U.S.[142] Fawzi et al. reported, in a study of 1075 HIV-infected pregnant women in Tanzania, that multivitamin supplementation decreased the risks of adverse pregnancy outcomes and fetal deaths and significantly increased CD4, CD8, and CD3 cell counts.[77]

In an uncontrolled study of the utility of multivitamin supplements, Priestley reported a series of 203 HIV-infected patients taking multivitamin supplements. Supplementation appeared to result in stabilization of CD4 counts and improved survival, compared to survival of patients with similar CD4 counts in other studies.[143] Abrams et al. found that intake of vitamin E was inversely correlated with the development of AIDS in 296 HIV-positive patients followed over six years.[141] A case control study in South Africa reported that vitamin B complex supplementation slowed progression to AIDS.[144]

Complications can arise when HIV-infected patients take nutritional supplements that contain vitamins, without knowing the precise contents of these supplements

or informing their health care providers. A recent study by Piscitelli et al.[145] reported that subjects taking St. John's Wort (*Hypericum perforatum*) had significantly decreased plasma bioavailable indinavir levels (greater than 50% reductions). Because these "complementary medicines" are widely used and users lack information about their pharmacology, pharmcokinetics, and potential drug-drug interactions, multiple vitamin and nutritional supplementation taken by HIV-positive patients (and any other patients treated by physicians or other health care providers) should be thoroughly discussed and evaluated.

SUMMARY

There is considerable and convincing evidence that vitamins (and other micronutrients) are deficient and, not surprisingly, are clinically important to HIV-infected patients. Deficiencies of folate, B_{12}, and thiamine have been linked to neurological impairment. A number of vitamins, including A, B_6, C, and E, may enhance (and their deficiency may impair) immune responses in HIV-infected patients. Baum et al. suggested that patients take levels of certain vitamins in excess of the RDA.[140] Further research is needed to establish which deficiencies should be screened for in HIV-infected patients. In addition, further studies of the benefits of supplementation with particular vitamins, especially in terms of immune function, are needed.

Currently, clinicians may want to consider screening all HIV-infected patients for deficiencies of vitamins A, B_6, B_{12}, and folate by measuring serum levels. This is despite the caveat that serum vitamin levels do not always reliably reflect nutrient status secondary to technical limitations. In patients with advanced AIDS, and particularly those with evidence of malnutrition and wasting, clinicians may consider screening more broadly for vitamin deficiency.[141] While empiric vitamin supplementation conceivably could prevent development of deficiencies and their sequelae, such treatment has not yet been proven to prevent deficiency, and the issue of malabsorption raises questions about oral supplementation. Vitamins, particularly the fat soluble vitamins, can also cause toxicity, particularly when taken in large doses. Nevertheless, supplementation of all HIV-infected patients with daily multivitamins appears to be a prudent step at this time.

Further recommendations must be based on the results of further research before they can be suggested for clinical implementation.

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CHAPTER 12

Nutrition and HIV/AIDS Patients in Japan

Chizuko Maruyama

CONTENTS

INCIDENCE OF HIV AND AIDS IN JAPAN

In Japan, the number of patients with HIV/AIDS has continued to increase since the first case was reported in 1985. As of October 31, 1999, a total of 4,747 HIV carriers have been reported to the Ministry of Health and Welfare.[1] Among them, 1,434 (30.2%), 1,417 males and 17 females, were infected with HIV through use of coagulation factor products. Infection with HIV by other causes was noted for 1,916 Japanese nationals (1,621 males and 295 females). The proportion of HIV carriers in the entire population is 0.0027% (2.7 per 100,000). Among them, 2,165 (1,948 males and 217 females) are AIDS patients and 631 AIDS patients were infected with HIV through use of coagulation factor products.

HIV carriers who are not Japanese nationals account for 29.4% (males, 21.1%; females, 74.5%) of the carriers who were infected by routes other than coagulation factor products. The proportion of foreigners from South Asia and Southeast Asia is highest, followed by Latin America and sub-Saharan African countries. However, the reporting of new foreign patients has decreased in recent years.

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The male to female ratio of Japanese HIV/AIDS patients is 90.7% to 9.3%, while the male to female ratio of AIDS patients is 94.8% to 5.2%. The proportion of males is clearly higher and the number of patients is extremely high in the large cities.

A breakdown of patients by age has only been published. The report excludes carriers who acquired HIV via coagulation factor products but includes foreigners. The relative number of males is high, in the order of ages 30s > 20s > 40s, while the proportion of female patients in their twenties is highest (67%). In recent years, the number of Japanese citizens infected with HIV within Japan has continued to increase, especially among young people. Based on these findings, it is predicted that there will be 15,400 Japanese HIV carriers by 2003, which is double the current figure, and 3,300 AIDS patients, which is more than three times the present number.[2] In order to prevent an increase of HIV carriers, it is necessary to provide education about prevention of this infection to school students and the general public.

As an indicator of the onset of AIDS, *Pneumocystis carinii* pneumonia is the most common (about 40%) in both Japanese and foreign patients, while candidiasis and the HIV debility syndrome account for more than 10% each. The incidence of active tuberculosis is lower in Japanese nationals (7%) than in foreigners (14%).

NUTRIENT INTAKE IN JAPAN

The results of the National Nutrition Surveys provide data on nutrient intake by the Japanese people. The Ministry of Health and Welfare conducts a national nutrition survey every November. In 1997, about 5,000 families including 13,289 subjects (6,243 men and 7,046 women) were selected at random in various districts of Japan and examined to assess their physical condition, nutrient intake, and eating habits.[3] This section deals with the results obtained from subjects in their twenties to forties, because the incidence of HIV/AIDS is higher in these age groups.

The nutrient intake was calculated for energy,* protein, lipid, calcium, iron, and vitamins A, B_1, B_2, and C. Unfortunately, the intake of other minerals and vitamins could not be calculated because of the incomplete food table. Nutrient intake was evaluated in comparison with the average recommended dietary allowances for the subjects. As a result, the energy intake and calcium intake were insufficient for men in their twenties to forties. In women, energy, calcium, and iron intake were insufficient. In particular, comparison of nutrient intake by people living alone with the average nutrient sufficiency rates of the subjects showed that intakes of the nutrients mentioned above were insufficient. Energy and calcium intakes were insufficient in men and the sufficiency rates for energy, calcium, and iron were 90%, 73%, and 69%, respectively. Nutrient intake is obviously inadequate for men and women living alone, compared with intakes of families including two or more members. The same trend has continued in recent years, and it is feared that anemia and decreases in bone mineral density may increase, especially in women. The intakes of other nutrients were generally sufficient (Figure 1).

* Energy is calculated by added values originating from carbohydrates, fats, and protein.

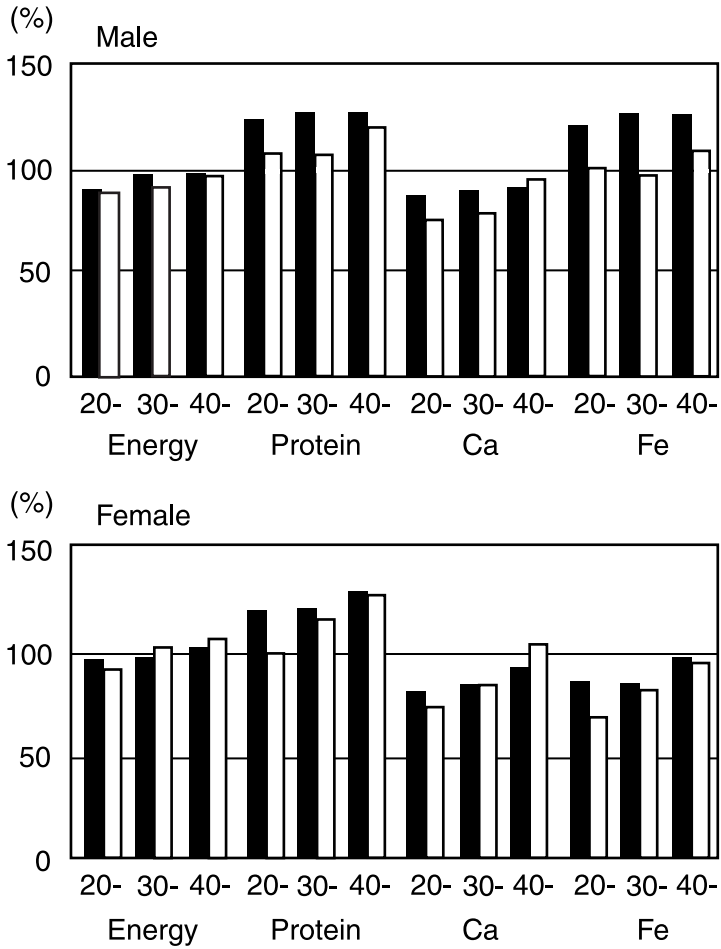

Figure 1 Nutrient sufficiency data based on 1997 Japan Ministry of Health and Welfare Survey. Black columns indicate average values in all subjects. White columns indicate average values in subjects living alone.

The reasons for the low sufficiency rates of the nutrients described will be discussed on the basis of data on eating habits and nutrient intake for each food group obtained from the above survey. The percentage of people not eating breakfast has continued to increase markedly, especially among men (33%) and women (16%) in their twenties and also among men (21%) and women (9%) in their thirties. This trend is one cause of insufficient nutrient intake. Further, the rates of eating evening meals outside the home are respectively 21% and 13% among men and women in their twenties, and 15% and 8% for men and women in their thirties. Rates decline for people in their forties and fifties. In particular, the practices of eating out for lunch or the evening meal and omitting lunch or the evening meal are increasing in large cities. The nutrient sufficiency rates are lower than the recommended dietary allowances with respect to energy, protein, calcium, iron, and various vitamins

among subjects eating out in the evening when compared with subjects eating at home. Thus, the quality of nutrition is lower in persons who eat out often.

Comparison of energy intake among meals in subjects aged 20 to 49 years shows that breakfast, lunch, the evening meal, and snacks account for 15 to 20%, 30 to 35%, 40 to 45%, and 5 to 10% respectively of daily energy intake, on the average. In particular, the energy provided by the evening meal is high. In the 20 to 49 year age group, the time of the evening meal also tends to be irregular. These findings indicate that going without meals, along with the high energy content of the evening meals, has a significant influence on nutrition. Sufficient vegetables are not eaten for the evening meal by 32%, 25%, and 21% of males in their twenties, thirties, and forties, respectively, and by 22%, 11%, and 11% of women in their twenties, thirties, and forties, respectively. Insufficient vegetable intake is higher in these age groups than among subjects aged above 50 years. Furthermore, the intake of oils and fats and meats is high in the 20 to 49 age groups, and their consumption of seaweed, fish, and shellfish is low. The utilization of cooked and processed, frozen, dried, and canned foodstuffs and the consumption of precooked food (prepared dishes and take-away food) have shown marked increases.

It is feared that the above trends may lead to an increase in the intake of cholesterol and saturated fatty acids and an increase in the percentage of total energy provided by fat, as well as a decrease in the intake of antioxidant vitamins, dietary fiber, and minerals such as copper, iron, magnesium, and zinc. It has been indicated that our eating habits should be improved to prevent obesity, hyperlipidemia, hypertension, diabetes mellitus, and arteriosclerosis, because the incidence of these diseases is increasing rapidly among younger Japanese.

TRADITIONAL JAPANESE DIET AND TYPICAL FOODS

The basic traditional Japanese-style menu consists of soup, a main dish, two other dishes, plus a staple food. It is considered desirable for different foodstuffs or methods of cooking to be used for preparing the individual dishes of a meal. Japanese-style meals can be recommended from the standpoint of balance and quality of nutrients. In recent years, Japanese eating habits have become more Americanized and the frequency of eating Japanese-style meals has decreased, especially among young adults. It is feared that this may lead to the onset of various lifestyle-related diseases.

It is recommended that the following foodstuffs and cooking methods be used to prepare a basic Japanese-style meal, consisting of soup, a main dish, two other dishes, plus a staple food.

Staple food: Cereals, such as rice and noodles [e.g., soba (buckwheat noodles) and udon (noodles)], as sources of polysaccharides.

Main dish: Fish and shellfish, meat, chicken and eggs, or soybeans and soybean products as sources of protein. It is considered appropriate to cook the dish by boiling, roasting, steaming, or frying.

Other dishes: Other dishes are provided to supply nutrients which cannot sufficiently be obtained from the staple food and main dish. It is recommended that two

other dishes be offered at every meal. In general, one is a warm dish, such as cooked food or warm vegetables, and the other is a cold dish, such as boiled vegetables, dressed with various sauces, or a salad. Vegetables, potatoes, seaweed, mushrooms, and konjak (a paste made from konjak flour) are the main items used.

Soup: Miso soup or clear soy soup containing vegetables, seaweed, mushrooms, and other ingredients. Fundamentally, soup and cooked foods are prepared with soup stock made from konbu, dried bonito, and small dried sardines, which are seasoned with soy sauce, miso, sake, mirin, sugar, and other seasonings.

With a Japanese-style menu, it is possible to offer low-energy meals and decrease the percentage of total energy provided by fat. If fish and shellfish and soybeans and soybean products are used, it is possible to decrease the proportion of animal foods and the intake of cholesterol and saturated fatty acids, while increasing the intake of polyunsaturated fatty acids. Since various green and yellow vegetables, seaweed, and mushrooms are used to prepare the other dishes and the soup, dietary fiber and various vitamins and minerals will be provided.

However, a high-energy diet may be the result if too much of the staple food and main dish are eaten, too much fried food and oils for frying are consumed, or too much dressing and mayonnaise are added to the salad. Further, the intake of salt may be too high if too much seasoning, such as salt, miso, and soy sauce, is used or if large amounts of pickles and salt-preserved foods (dried fish, fish-paste products, and processed foodstuffs) are eaten.

Among traditional foodstuffs, the following are consumed in larger amounts in Japan than in foreign countries. The physiological effects of these foodstuffs have been recognized.

Soybeans: Soybeans and soybean products, such as tofu (soybean curd), natto, abura-age (deep-fried tofu cutlet), and miso, supply isoflavones including daidzein, genistein, and saponin. Attention has been paid to the female hormone-like effects of isoflavones. Soybean saponin improves liver damage related to oxgen radicals and has anti-inflammatory, sedative, and immunoactivating effects. It has also been reported that soybean saponin has an antiviral effect on HIV *in vitro*.[4] Natto inhibits angiotensin-converting enzyme and its antihypertensive and antitumor effects have been noted *in vitro*.[5] However, since the amino acid score of soybeans and soybean products is 68 to 91, excessive intake of soybean products as a source of protein should be avoided.

Green tea: Green tea contains caffeine, (–)-epigallocatechin gallate, theanine, and vitamin C. Caffeine is expected to relieve fatigue, keep one awake, and relieve stress by stimulating the central nervous system, and also to promote circulation and activate metabolism by its cardiotonic and diuretic effects. It has been reported that about 1 g of (–)-epigallocatechin gallate, the principal component of tannin in green tea, is drunk daily by the Japanese. Attention has been focused on its antioxidant, lipid-lowering, antimicrobial, antiviral, antidotal, antimutagenic, and anticarcinogenic effects. According to the results of epidemiological studies, the risk of stomach cancer was decreased by drinking at least 10 cups of green tea daily[6] and the risk of breast cancer was reduced by drinking at least 5 cups of green tea daily.[7]

Seaweed: Wakame, konnbu (kelp), hijiki (a kind of brown algae), and laver are the most popular edible seaweeds in Japan. Each of these seaweeds contains large

amounts of minerals such as iodine, calcium, potassium, iron, magnesium, phosphorus, and zinc. Further, they are useful as sources of dietary fiber because of their high alginic acid content. We are encouraged to eat seaweed to prevent and treat constipation, obesity, diabetes, and hyperlipidemia. Also, fucoxanthin in hijiki has been reported to show radical-scavenging activity.[8]

Fish: Fish contains n-3 fatty acids, fish protein, and taurine. Various types of fish including horse mackerel, tuna, mackerel, sardine, salmon, hake, Pacific saury, and amberjack are eaten habitually throughout Japan. The anti-arteriosclerotic and anti-inflammatory effects of n-3 fatty acids have been reported all over the world. We previously reported that icosapentanoic fatty acid had a positive influence on the carotid blood flow velocity in subjects eating about 200 g of fish daily.[9] Inhibition of platelet activity and a decrease of serum triglyceride levels were observed in an intervention study in which subjects ate 200 to 400 g of fish (equivalent to 10 g n-3 polyunsaturated fatty acid) daily for 17 days.[10] Habitual consumption of fish is useful for preventing arteriosclerosis. However, it should be remembered that the risk of oxidative damage may increase with increasing intake of fish oil.

DIETS OF HIV/AIDS PATIENTS IN JAPAN

Many sexually infected patients are not rich and some of them must reduce their food costs to pay for medical care. There have been few reports concerning the eating habits and nutritional status of HIV/AIDS patients in Japan. Unfortunately, no studies have investigated nutritional intervention. In 1995, we carried out a diet survey by the 24-hour recall method to compare nutrient intakes of subjects who had CD4-positive cell counts <200 and ≥200. We noted no differences in mean nutrient intakes between the two groups. However, there were wide variations, and intakes of several nutrients were extremely low in some subjects (Table 1). At that time, drug therapy consisted of nucleic acid reverse transcriptase inhibitors. Since treatment was often associated with adverse reactions including nausea, vomiting, diarrhea, and anemia, it was difficult for patients to intake sufficient nutrients. Further, many male patients with AIDS due to sexual transmission lived alone, so they often ate out or ate commercially available prepared dishes and food sold at convenience stores. It was difficult for them to have well-balanced diets.[11]

The dietary problems showed some changes after combination therapy was introduced. According to the results of a survey of six institutions reported at the Japan AIDS Society meeting in 1999, 80% of patients receiving drug therapy took a combination of three agents. Since viral load can certainly be decreased by combination therapy with a nucleic acid reverse transcriptase inhibitor and a protease inhibitor, the progression of the disease is inhibited and the patient's condition is improved. As a result, it becomes possible for patients to eat sufficient amounts of food.

However, dietary restrictions are essential during treatment with protease inhibitors. In order to maintain blood levels, it is necessary to take Indinavir three times daily every eight hours either an hour before a meal or two hours after it. Since we usually eat 3 meals daily, mealtimes tend to be irregular. Further, since it is necessary to drink 1.5 L or more of water to prevent renal calculi, Indinavir therapy has the

Table1 Nutrient Intakes in HIV/AIDS Outpatients

Nutrient	CD4+ Cell Counts	
	<200 cells/μl (19 Patients)	≥200 cells/μl (25 Patients)
Energy (kcal/kg#)[a]	32.9 ± 10.6[b]	32.3 ± 10.4
(min-max)	(10.2–59.1)	(18.8–67.0)
Protein (g/kg#)	1.09 ± 0.42	1.05 ± 0.49
(min-max)	(0.31–2.20)	(0.46–3.11)
Lipid (g/kg#)	0.92 ± 0.38	0.96 ± 0.49
(min-max)	(0.29–1.84)	(0.39–2.54)
Calcium (mg/kg#)	6.9 ± 3.9	7.6 ± 4.8
(min-max)	(2.4–14.2)	(1.7–21.8)
Iron (mg/kg#)	0.14 ± 0.05	0.14 ± 0.07
(min-max)	(0.06–0.25)	(0.06–0.43)
Vitamin A (IU/kg#)	28.8 ± 19.1	58.8 ± 94.5
(min-max)	(7.8–80.4)	(3.6–383.1)
Thiamin (mg/1000 kcal)	0.51 ± 0.14	0.47 ± 0.15
(min-max)	(0.30–0.79)	(0.21–0.72)
Riboflavin (mg/1000 kcal)	0.57 ± 0.20	0.64 ± 0.34
(min-max)	(0.37–1.12)	(0.25–1.76)
Ascorbic acid (mg/kg#)	1.5 ± 0.8	1.4 ± 1.1
(min-max)	(0.2–3.2)	(0.3–6.0)

[a] # = Ideal body weight calculated by a formula of height $(m)^2 \times 22$.
[b] Values are presented as mean ± SD.

disadvantage that sufficient food cannot be eaten at mealtimes. It is considered that the blood level of Nelfinavir, which is taken three times daily every eight hours, is increased by fatty foods, so are patients likely to eat meals and snacks with higher fat content. Any protease inhibitor has the risk of causing hyperglycemia, diabetes, diabetic ketoacidosis, and aggravation of diabetes. Even after these symptoms develop, treatment with protease inhibitors is continued and blood glucose levels are controlled with oral hypoglycemic agents or insulin in many patients. Furthermore, lipodystrophy syndrome may develop.

In order to control these adverse reactions, nutritional guidance is given to counter diabetes and hyperlipidemia. However, improvement of eating habits is often difficult for patients living alone and keeping irregular hours. Many homosexual patients we surveyed tend to sleep by day and work by night, resulting in a potentially unbalanced diet. An additional problem is the possibility of unbalanced nutrient intake due to high alcohol intake. According to the results of our recent survey by the 24-hour recall method, the intake of each food group before and after nutritional guidance was as shown in Table 2 for male HIV/AIDS outpatients without symptoms. At the time of initial nutritional guidance, the consumption of vegetables, fruits, dairy products, and fish was low, and consumption of meat was high. Few patients were utilizing supplements. After nutritional guidance, the consumption of rice was increased, and consumption of meat was decreased. However, these changes were not associated with changes of immunological parameters such as viral load and CD4 cell levels.

Table 2 Food Intake in HIV-Infected Outpatients Before and After the Nutritional Education

	Before	After	Provability
Rice	360 ± 222	485 ± 253	$p < 0.05$
Wheat	174 ± 109	133 ±	
Nuts	2.6 ± 7.3	0.4 ± 1.2	
Potatoes	23 ± 30	42 ± 72	
Sugar	14 ± 21	5 ± 7	
Sweets	21 ± 31	17 ± 49	
Fats and oils	22 ± 12	19 ± 17	
Beans	36 ± 45	35 ± 32	
Fruits	53 ± 82	29 ± 97	
Green vegetables	41 ± 49	40 ± 57	
White vegetables	121 ± 54	103 ± 77	
Mushrooms	1.3 ± 4.8	1.4 ± 4.8	
Algae	1.2 ± 1.3	0.7 ± 1.0	
Beverages	505 ± 574	342 ± 651	
Fish and shellfish	46 ± 65	76 ± 88	
Meat and chicken	115 ± 76	65 ± 42	$p = 0.05$
Eggs	42 ± 57	34 ± 31	
Milk and dairy products	98 ± 121	122 ± 195	

RECOMMENDATIONS

How might diets be modified to benefit Japanese HIV/AIDS patients? No nutritional recommendations can prevent the onset of AIDS in HIV carriers or treat AIDS. Based on the results of studies carried out around the world, I suggest provisional nutrient intake recommendations to maintain nutritional status in HIV carriers (Table 3). In Japan, it is impossible maintain a high intake of vitamins from meals, so the use of vitamin supplements may be essential. Further, it is impossible (because of the incomplete food table) to specify the recommended intakes of various vitamins, minerals, and physiologically active substances.

In order to prevent the onset of AIDS, Japanese-style meals are recommended to achieve a well-balanced intake of nutrients. Patients without complications who do not take anti-HIV drug therapy should consume cow's milk, dairy products, and one egg every day, and should select fish, meat, or soybean products as the protein

Table 3 Nutrient Recommendations to Maintain Nutritional Condition for HIV/AIDS Patients

Energy (kcal/kg standard body weight)	35 ~ 40
Protein (g/kg standard body weight)	1.5 ~ 2.0 (1.5–2.0 times the RDA)
Lipid/total energy (%)	20 ~ 30
Ca (mg)	600
Fe (mg)	12
Vitamin A (IU)	2000 ~ 5000
Vitamin B_1 (mg/1000 kcal)	0.8 ~ 2.0 (2–5 times RDA)
Vitamin B_2 (mg/1000 kcal)	0.8 ~ 2.0 (2–5 times RDA)
Vitamin C (mg)	200 ~ 5000

Table 4 Recommended Volume of Daily Food Consumption

Food	2000 kcal	2600 kcal
Cereal	340	440
Nuts and seeds	3	3
Potatoes	60	80
Sugar and sweets	5	10
Fats and oils	15	25
Soy bean products	60	100
Other pulses	5	5
Fruits	150	150
Green vegetables	100	100
White vegetables	200	200
Sea weeds	5	10
Beverages and seasonings	50	50
Fish and shellfish	50	80
Small fish	5	10
Meats and chicken	50	60
Eggs	40	50
Milk and dairy products	200	300

source for each meal. In order to ensure a sufficient intake of vitamins, minerals, and dietary fiber, a patient should eat green, yellow, and white vegetables, seaweed, mushrooms, and konjak as supplementary dishes. In particular, sufficient amounts of green and yellow vegetables, Japanese tea, and citrus fruits should be eaten to avoid deficiency of antioxidant vitamins. However, eating fruit should be prohibited in patients with hypertriglyceridemia. Vitamin E should be obtained from seeds or nuts, such as sesame seeds, walnuts, and fresh vegetable oils. Lycopene should be obtained from tomatoes or processed tomato products. Drinking vegetable juice is recommended for patients who cannot cook. Oils, especially vegetable oils, should be used for cooking and preparing fried and deep-fried foods and dressings. Lightly salted delicious dishes can be prepared if the amounts of salty seasonings such as salt, miso, and soy sauce are decreased and spices such as ginger, garlic, lemon, and pepper are used. It may be useful to consult the minimum volume and composition of food shown in terms of required nutrient intake for the healthy Japanese population (5th revision issued from the Ministry of Health and Welfare) (Table 4). However, this is not the optimum model for the patients.

In general, the above advice also applies to patients on drug therapy.

It is necessary to fast while taking Indinavir. For this reason, patients tend to eat only two meals daily, resulting in a potentially insufficient nutrient intake. It is desirable to eat three or four meals. A patient should eat vegetables, meat, and fish at snack times if he or she cannot consume enough foods in each meal. Drinking more than 1.5 L of water is required to prevent renal calculi.

It is important to intake lipids when Nelfanivir is administered. However, lipids should be limited to 30 g per meal to avoid excessive intake. Since excessive lipid intake may lead to accumulation of fat or induction of hyperlipidemia, blood lipid levels should be checked at regular intervals. It is desirable for lipids to be mainly supplied as vegetable oils. Patients with hypercholestrolemia should avoid eggs and drink low-fat or skim milk.

Patients living alone who cannot avoid eating out or utilizing commercially available take-out and prepared foods should observe the following instructions:

1. Pay attention to nutrient compositions noted on labels.
2. Buy from shops selling lightly salted dishes to avoid excessive intake of salt.
3. Include vegetable dishes or vegetable juice with every meal.
4. Select Japanese-style menus to avoid excessive fat intake.
5. Avoid intake of energy mainly from snacks and do not skip meals.

Since the traditional Japanese-style diet has been shown to be useful for preventing arteriosclerosis and cancer, it is considered that it has the potential to benefit HIV carriers. It is necessary to clarify the relationships of meals, nutrient intake, and nutritional status, and establish a system of nutritional therapy for Japanese patients as soon as possible.

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CHAPTER 13

Nutrition and HIV Infection/AIDS in Sub-Saharan Africa

Cyril O. Enwonwu and Rueben C. Warren

CONTENTS

INTRODUCTION

Africa, the second largest continent in the world, occupies about 25% of the world's land mass, and contains no less than 52 politically independent countries. Sub-Saharan Africa, with an estimated population of more than 500 million people in 1990, and projected to reach a population of 678 million by the year 2000, harbours 45 of the 47 least developed countries in the world.[1] At least 45% of the total

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population of Sub-Saharan Africa is aged under 15 years. Children under 5 years of age constitute about 20% of the population and account for 60% of all deaths.

The HIV-infection/AIDS pandemic ravaging the African continent is now a leading cause of death in 15 to 49 year old groups in several countries,[2] all of which are located in Sub-Saharan Africa. Seven of the eight nations in Africa most affected by AIDS are those with the poorest economies, ranging from US $51 to $350 per capita compared to the African average of $642.[3] As many as 40% of Sub-Saharan Africans live on less than US $1.00 per day. A profound economic decline has characterized the last two decades in Sub-Saharan Africa,[2,4] and in most of its countries, external debt now exceeds the annual gross national product.[4] In Nigeria (a country that accounts for 20% of the population of Sub-Saharan Africa), for example, the average annual growth rate in GNP was 6.9% between 1965 and 1980, but decreased thereafter to an average annual rate of 1.1% following a precipitous fall in living standards below the 1950 level, a trend which continued in the last decade of the 20th century.[4]

Sub-Saharan African countries are characterized by rapidly deteriorating agriculture, mounting pressures of rapid population growth rate, widespread civil unrest, grossly inadequate water supply, severe underfunding of health infrastructures, and a prominent decline in access to good quality primary health care.[2,4] Murray and Lopez[5] have reported that on a global basis, Sub-Saharan Africa has the largest population of total DALYs (disability-adjusted life years) of 21.4%, but only a very small proportion of health expenditures (0.7%). The comparative figures for the economically most developed region are 7.2% and 87.3% respectively.[5] Over the last two decades, malnutrition has remained stable in South America, decreased in Asia and Central America, increased in the African sub-region,[6] and is now very severe in several countries.[7] Food self-sufficiency, a crude index of the prevalence of hunger, was below 100% in 41 Sub-Saharan African countries from 1986 to 1988, and 32 of these countries had dietary energy supplies (DES) below requirements for similar activity levels for all countries in the world.[8]

Escalating widespread protein-energy malnutrition (PEM) in Sub-Saharan Africa, usually complicated by concurrent micronutrient deficiencies and a plethora of endemic communicable infections (e.g., malaria, measles, diarrhea, tuberculosis, acute respiratory infections, cholera, and others), underlies the very high infant and childhood mortality rates observed in the sub-region.[2,9] In Nigeria, the most densely populated Sub-Saharan African country with a population estimate of 115 million, and potentially one of the most economically viable countries in the region, 36% of the children have moderate or severe malnutrition, 43% are stunted, and 9% are wasted.[2,10] It is against this background of widespread poverty, chronic malnutrition, and endemic communicable diseases in Sub-Saharan Africa that this report examines the HIV/AIDS pandemic currently ravaging the sub-region, and the obstacles to AIDS prevention and treatment in the area. This approach is necessitated by reported observations that low socioeconomic status[4,11] and the degree of malnutrition[12,13] are key factors that predict the clinical course and increased mortality from HIV infection, even after controlling for potential confounders like age, disease stage, and access to health care.

ASPECTS OF EPIDEMIOLOGY OF HIV/AIDS IN SUB-SAHARAN AFRICA

Although viewed with skepticism, especially by African biomedical scientists, it is believed particularly in the Western World that HIV originated in Africa via introduction of the virus from a primate population into humans.[14,15] Current estimates put the number of people worldwide living with HIV/AIDS by the end of 1997 at about 31 million.[2,16,17] The rate of spread of the epidemics is slowing in North America and taking off in Asia and Latin America, but Africa remains very hard hit by the disease. Ninety percent of all HIV/AIDS patients in the world reside in developing countries, with over two thirds of the total number living in Sub-Saharan Africa.[2,4,15] Four out of five HIV-positive women and 87% of HIV-positive children in the world are believed to reside in Africa.[16] East and Central Africa, an area accounting for only 15% of the total population of Sub-Saharan Africa, was until 1996 reported to harbour 50 to 65% of the global burden of HIV infections.[15] The reason for that is not clear.

Common alleles (variants at a single locus) or polymorphisms constitute the basis of human diversity, including the ability to cope with hostile environmental challenge.[20] There is some evidence that host genetic factors may influence susceptibility to HIV infection and its subsequent progression to AIDS.[21,23] In Central African countries where HIV-1 infection is very prevalent, the group specific component Gc/fast (Gc/f allele) predominates in the indigenous populations.[22] Studies suggest that progression from HIV infection to AIDS has a strong positive correlation with the Gc/f allele.[20]

HIV/AIDS was first reported in Uganda in 1985, and by 1997, more than 1.8 million of a total population of 19 million people were infected by HIV-1.[18] In 1997, the estimated number of HIV-infected individuals in Kenya was 1.2 million, and this number is projected to exceed 1.7 million by 2001.[19] The published prevalence rates of HIV infection in some Sub-Saharan African countries are Kenya (12%), Malawi (15%), Mozambique (14%), Rwanda (13%), and Zambia (19%).[16] In several Southern African countries, namely South Africa, Zimbabwe, Botswana, and Namibia, the prevalence rates of HIV infection in the adult populations currently exceed 20%.[16]

Sero-prevalence rates of HIV among pregnant women in the African sub-region range from 5 to 35%, with the highest rates reported in the urban centers of Kampala (Uganda), Lusaka (Zambia), Blantyre (Malawi), Kinshasa (Zaire), and Abidjan (Ivory Coast). A prominently high HIV prevalence rate in excess of 80% has been reported for female sex workers in Central Africa.[24] The Republic of South Africa has witnessed a three-fold increase in HIV prevalence from 2.4% in 1992 to 7.5% in 1994, among women attending antenatal clinics.[15]

Very disturbing is the HIV/AIDS situation in Nigeria, a country that accounts for 20% of the total population of Sub-Saharan Africa, where the disease is spreading rapidly among commercial sex workers and their clients.[25] At the end of 1998, the cumulative number of HIV patients in Nigeria was in excess of 3.1 million, rated highest in West Africa, and second after Ethiopia in Sub-Saharan Africa.[26] The

Federal Ministry of Health of the Republic of Nigeria[27] puts the current overall national HIV prevalence rate in the country at about 5.4% with youths 20 to 24 years of age showing rates as high as 10% in some parts of the country. Since 1995, the HIV prevalence rates in the worst affected geopolitical zones of the country have increased by more than 700%.[27] The current projections are that by 2002, Nigeria will have in excess of 5.5 million cumulative HIV/AIDS cases, with at least 1.4 million deaths in adults and children resulting from the disease.[26] Perhaps more ominous is the recent reported observation that new strains of HIV-Type 1, more aggressive than previously known sub-types, and believed to possess increased probability of vertical transmission from mother to fetus, have been isolated in Nigeria and in other West African countries.[28]

DIETARY HABITS OF AFRICANS: BRIEF REVIEW

For most Africans, dietary practices are influenced to a large extent by economic status and the almost exclusive reliance on plant foods. Some ethnic and cultural differences notwithstanding, there are striking similarities in the staple foods and dietary habits of the large majority of Africans who are socioeconomically underprivileged. The popular staples and their protein-calorie ratios (percent) are wheat (11%), millet (12%), rice (8%), maize (10%), taro (7%), yam (6%), plantain (4%), and cassava (3%). The major meals are monotonous, bulky, and often prepared/stored under less than acceptable hygienic conditions. The amounts of food consumed in a day do not provide the recommended dietary allowance (RDA) for energy, protein, and other essential nutrients.[29,30]

The dietary energy supply (DES) per capita per day in Sub-Saharan Africa in 1990 was less than 60% of the supply in North America or Western Europe.[8] Although Sub-Saharan Africa has an extensive array of protein-rich foods (e.g., beans, cowpeas, groundnuts, sesame, soy), foods rich in the micronutrients (green leafy vegetables, banana, mango), and energy-rich foods (palm oil, groundnut oil), most of these are expensive or culturally underestimated by the people.[29,31] Plain unsupplemented cereals and cassava yam porridge feature prominently as foods for children and for the sick in some communities.[29]

Breast feeding is critical for child survival in Sub-Saharan Africa, and is very widely practised during the first 1 to 2 years of life in most communities.[29] Both in the rural areas where most Africans reside and in the overcrowded urban centers, complementary foods are usually introduced when the infant is 3 to 6 months of age. The traditional complementary foods popularly used in Sub-Saharan African communities generally contain low energy density, low protein quality, and low micronutrient contents, and are frequently contaminated with high loads of microorganisms.[29,32] These complementary foods which are often prepared and served under poor hygienic conditions set the stage for repeated episodes of diarrhea frequently encountered in underprivileged African children.[30,33] For most African infants, weaning is usually from breast milk to cereal-based products or gruels which are often microbiologically hazardous.[32,34] These weaning foods are not only

deficient in the micronutrients (vitamins, minerals), but also are low in fat content, thus impairing the absorption of ingested fat-soluble vitamins.

For virtually all Sub-Saharan African countries, the decades of the HIV/AIDS epidemic constitute a period of severe economic crisis, characterized by unsustainable foreign debt burden, national currency devaluation, and a staggering increase in consumer price indices with no real increase in wages for the people.[35,36] In the Republic of Zambia, for example, where urbanization is close to 60%, most people depend on wages to buy foods; and while the consumer price index in the country rose from 125 in 1976 to 1,625 in 1988, a 13-fold increase, real wages actually decreased slightly.[37] In most Sub-Saharan African countries, food production has continued to lag behind population growth rate.[2,5,8,38] In Ethiopia alone, there were no less than 9 million famine victims in 1983, a pattern recently repeated in several other African countries, and often resulting from civil unrest. In 1993, almost 16 million Africans were refugees or displaced people, a situation that aggravates the poor food situation in the region.

MALNUTRITION, INFECTIONS, AND IMMUNE SYSTEM SUPPRESSION

Easily the commonest nutritional problem in Africa is protein-energy malnutrition (PEM), which is generally complicated by concurrent deficiencies of several micronutrients.[1] The large endemic foci of infectious diseases in the world are superimposed on geographical areas of severe hunger and malnutrition in Africa, and the idea that a malnourished host has a greater susceptibility to infections and a relatively worse prognosis than a well-nourished subject is now universally accepted.[9,39] Poor diet and malnutrition impair several parameters of the host's specific and nonspecific defense systems, resulting in increased susceptibility to infections, and that, in turn, intensifies the severity of malnutrition. Several detailed reports[9,40,41] have examined the multifaceted impact of malnutrition on immune function, which includes functional abnormalities of B and T cell lymphocytes, prominent depression in delayed cutaneous hypersensitivity responses to recall and new antigens, impaired phagocytosis, reduction in number of mature fully differentiated T lymphocytes and serum thymic factor activity, marked depression in number of helper $CD4^+$ cells, with less marked alterations in the number of suppressor T cells, resulting in prominently reduced helper/suppressor T cell ratio. Indeed, what is now clear from work in several laboratories is that the immunological dysfunctions in malnourished African children who are serologically negative for HIV infection are reminiscent of the alterations encountered in individuals seropositive for the disease.[41,42]

Marked tissue depletion of the key antioxidant micronutrients (e.g., α-tocopherol, β-carotene, ascorbic acid, retinol, zinc, selenium, and others) as well as depletion of γ-glutamyl-cysteinyl-glycine (GSH) occurs in African children with PEM.[21,23,30,43,44] Selenium deficiency is very common in many Sub-Saharan African countries, particularly in Central Africa.[45] Blood GSH concentration is significantly reduced to almost 50% of control levels in protein-energy deficient African children

who are HIV-1 seronegative.[44] That is not surprising since meats, which are not readily available to impoverished Africans, and certain vegetables are rich sources of GSH and of cysteine, but most foods are, however, low in cysteine.[46]

The roles of micronutrients either as antioxidants or as key components of antioxidant enzymes are well known.[1,43] GSH is normally present in mammalian cells at concentrations of 0.5 to 10 mmol/L, and accounts for more than 90% of cellular non-protein thiols.[47] Apart from its important role as a modulator of T cell activation,[47] this tripeptide participates in the GSH peroxidase-dependent metabolism of hydroperoxides and direct scavenging of reactive oxygen species, and may contribute significantly to the antioxidant network system through regeneration of vitamins C and E from their oxidized inactive forms.[48] Additionally, the antioxidant activities of selenium and vitamin B_6 are GSH-dependent since Se functions as a cofactor of GSH peroxidase, while pyridoxal phosphate facilitates availability of Se for GSH peroxidase activity.[48]

Tissue concentration of cysteine, a constituent of GSH, is prominently reduced in African children with PEM,[49] and there is good evidence that this sulfur amino acid functions in its own right as a cytokine that regulates the functional activities of lymphocytes.[50] The possible causes of GSH deficiency in the malnourished African child include not only dietary insufficiency, but also increased oxidative stress with formation of glutathione disulfide (GSSG), extensive formation of mixed disulfides, and a drain on the body's pool of sulfur amino acids as a consequence of frequent diarrhea. Regeneration of GSH from GSSG requires glutathione disulfide reductase in the presence of NADPH,[48] and the activity of this flavin enzyme is influenced by tissue content of riboflavin which is reduced in PEM.[1,49]

As indicated in a model of the complex interactions of infections, nutritional status, and immune function (Figure 1), impaired gastrointestinal function resulting in episodes of diarrhea is a frequent feature of the malnourished African child, [30,39] although it is not certain whether this is due to undernutrition *per se*, or is elicited by enteric infections very often seen in malnourished hosts. Even in the oral cavity, PEM in the African child promotes a shift in the microbial ecology toward a preponderance of spirochetes and anaerobic organisms[40,51,52] as well as increased carriage of yeasts and subsequent oral candidiasis.[53] Other reported features of malnutrition in the African child that may be relevant to the pathogenesis of HIV infection include altered inflammatory response and cytokine action,[54,55] as well as endocrine function adaptations such as decreased synthesis of insulin, somatomedin-C, estrogen, androgen, and increased circulating concentrations of growth hormone and the glucocorticoids.[49,56] Some relatively recent reviews have examined the potential effects of hypercortisolemia in HIV-infected subjects.[57,58] Glucocorticoid excess in PEM contributes to impairment of various parameters of the host's response to infection,[59] including inhibition of the induction of the Ca^{2+}-independent nitric oxide synthase (iNOS) in macrophages, neutrophils, and other cells.[60]

Finally, it must be noted that sole use of clinical criteria to separate severe malnutrition from pediatric HIV/AIDS in Africa is beset with problems in view of the marked similarities between the clinical features of both conditions. Schuerman and colleagues[61] have reported that the severity of malnutrition and other clinical criteria (e.g., chronic diarrhea, failure to thrive, generalized lymphadenopathy, and

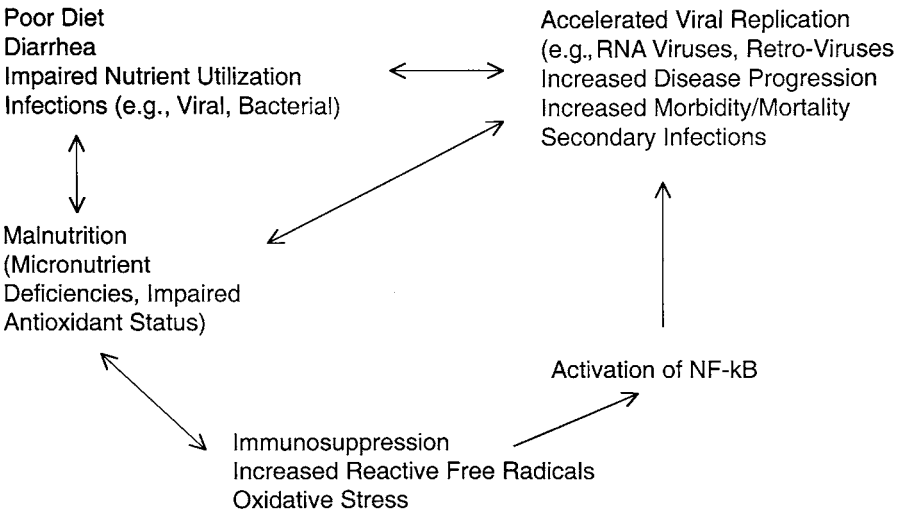

Figure 1 A model of complex interactions of infections, nutritional status, and immune function. This scheme shows the bilateral relationship between malnutrition and impaired immunity, as well as between the former and poor dietary intake and infections. Deficiency of micronutrient antioxidants promotes oxidative stress, and consequently, accelerated HIV replication as well as progression of HIV infection/AIDS.

oropharyngeal candidiasis) was comparable between 30 HIV-positive and 64 HIV-negative children studied in rural Equatorial Africa. Although there is still lack of consensus regarding the role of the pre-existing state of malnutrition on the acquisition of HIV infections, several investigators have advanced the hypothesis that malnutrition is a major underlying cause for the full clinical expression of AIDS in HIV-infected individuals.[42,62,63]

INFLUENCE OF HIV INFECTION ON NUTRITIONAL STATUS

Impact of HIV-infection/AIDS on nutritional status is the subject of several excellent recent reviews.[13,21,23] Both hypometabolism[64] and hypermetabolism[65] have been reported, depending on the duration of HIV seropositivity and the presence or absence of secondary opportunistic infections. HIV/AIDS induces several metabolic disorders including changes in whole-body protein turnover, negative nitrogen balance, hyperlipidemia, altered carbohydrate metabolism, endocrine dysfunction, and body weight loss among others.[1,13,16,57] Causes of malnutrition in HIV disease include insufficient dietary intakes resulting from economic poverty, anorexia, dysphagia, odynophagia, nausea, emesis, diarrhea, malabsorption, and impaired metabolism and storage of nutrients.[1,21,42]

A very prominent feature of HIV infection/AIDS is depleted micronutrient status,[21,23] and this is expected to be more profound in impoverished Sub-Saharan African communities with habitual low dietary intakes of these nutrients.[1,3,8] Even

in affluent Western countries, many studies suggest that despite high dietary and supplemental intakes of micronutrients, HIV-infected individuals display low serum concentrations of retinol, pyridoxine, cyanocobalamin, ascorbate, α-tocopherol, folate, carotenoids, selenium, zinc, and magnesium,[66] an observation confirming earlier reports to the effect that the recommended dietary allowances (RDAs) do not meet the nutritional needs of HIV-infected patients.[1] Other micronutrients whose blood levels are reported to be significantly reduced in HIV infection are copper, 1,25-dihydroxycholecalciferol, and niacin.[21,23] It must be underscored that altered serum levels of several micronutrients in HIV subjects may be partially due to metabolic responses to infections. Abnormally low circulating levels of pro-vitamin A carotenoids may occur in 30 to 80% of HIV-infected individuals.[21] Selenium deficiency, assessed by plasma and red blood cell levels as well as GSH peroxidase activity, is a common finding in HIV patients and is attributed in part to reduced dietary intake and malabsorption.[68] Plasma GSH concentration in asymptomatic HIV-positive individuals can be reduced to as low as 30% of control levels.[69]

PROGRESSION OF HIV DISEASE IN AFRICANS

The clinical course and outcome of HIV-1 infection are very variable, with some cases progressing very rapidly to AIDS within months of HIV-1 seroconversion, while at the other extreme are cases that remain asymptomatic for more than a decade.[70] Studies by Mellors and colleagues[71] indicate that in some developed countries, 20% of cases progress from HIV infection to AIDS within 5 years, while about 12% may remain free from AIDS for 20 years. Rapid progression is the norm in Sub-Saharan Africa. In a study of HIV-1 progression in rural Uganda,[72] the estimated median time for progression to AIDS from enrollment was 4.3 years for the prevalent group, with a cumulative probability of 54% at 5 years. This time is much shorter than for similar cohorts in Europe and North America, although experimental designs such as differences in stage of disease on enrollment, recruitment sites, age of participants, patients' socioeconomic status, support systems, and other factors might limit the value of such comparisons. Although virological and genetic factors may be involved in determining disease progression, some data suggest a role for malnutrition, particularly micronutrient deficiencies.[13,21,23]

Univariate Cox proportional-hazards analysis, performed to examine the effects of baseline variables on long-term survival of HIV-infected subjects, showed that initial nutritional status, characterized by CD4 count, plasma albumin, prealbumin, and C-reactive protein levels, as well as classes of body weight loss, were all significantly associated with survival, and that patients showing low CD4 counts and/or advanced malnutrition have diminished survival rate.[73]

In an earlier study, Melchior and colleagues[74] were able to show that in HIV-infected individuals with severe malnutrition and wasting — consistent features of underprivileged children in Sub-Saharan Africa — provision of adequate nutrition may dramatically influence survival as exemplified by prolongation of survival time from 57 to 211 days following administration of total parenteral nutrition to severely malnourished patients.

Semba and Tang[21] have recently summarized several studies showing that increased risk of rapid progression from HIV infection to AIDS is associated with low serum levels of retinol, vitamin E, cyanocobalamin, zinc, selenium, and other micronutrients. It has also been demonstrated that high intakes of ascorbate, thiamine, and niacin reduce the relative risk of disease progression, while retinol intake has a U-shaped relationship with disease progression rate in that very high and very low intakes are associated with accelerated rate.[75] Equally important is the evidence that HIV-infected subjects with low levels of GSH in their $CD4^+$ lymphocytes have decreased survival.[76]

Abnormalities in cytokine function characterize HIV infection, with impaired production of some like IFN-γ, and overproduction of TNF and others.[1] Mean serum level of TNF-α progressively increases across the clinical spectrum of HIV infection, with the highest levels observed in patients with marked weight loss and extensive opportunistic infections. Whether these changes result from the viral infection per se or are due to the underlying state of malnutrition is not clear.[1] IFN-α, -β, and -γ induce host-cell resistance to a wide range of viral infections, including many animal retroviruses, and are believed to retard HIV replication.[1] Underproduction of IFN should therefore promote the progression of HIV infection.

Both TNF[77] and IL-6 are elevated in HIV infection, and, indeed, AIDS is considered a TNF disease[77] since TNF-α and -β are considered to play roles in the transcriptional enhancement of HIV replication.[77,78] Similarly, IL-6 is reported to activate HIV post-transcriptionally.[79] The stimulation of HIV replication by TNF is linked to induction of NF-κB (nuclear factor kappa B), a DNA-binding protein with binding sites in the viral enhancer. It is a heterodimeric nuclear protein consisting of 50- and 65-kDa subunits. In unstimulated cells, the majority of NF-κB activity is present in the cytosolic fraction as a cryptic form unable to bind to DNA until the p:50 p:65 heterodimer is dissociated from a 37-kDa inhibitory polypeptide, IkB.[80] A protease encoded by the HIV-infected cell can promote activation of NF-κB.[81] Similarly, excessive production of reactive oxygen species relative to the host's antioxidant status — a consistent feature of malnutrition and infections — can activate NF-κB.[82] GSH, whose cellular levels are profoundly depleted in HIV infection[69] and in malnourished African children,[44] has been shown to be effective in blocking the ability of TNF and other mitogens to stimulate HIV-1 replication by inhibiting activation of NF-κB.[83] Impaired cellular antioxidant status resulting from tissue depletion of micronutrients is a prominent finding in African children with malnutrition, and may well play a key role in the reported rapid progression of HIV infection to AIDS in the continent.

MICRONUTRIENT DEFICIENCY, OXIDATIVE STRESS, AND VIRAL REPLICATION

More than three decades ago, Scrimshaw and his colleagues[84] published a trailblazing monograph on the interactions between nutrition and infection, which among other things attempted to explain in scientific terms why impoverished communities in developing countries are very vulnerable to various communicable diseases

including viral infections. The monograph unfortunately focused almost exclusively on the effects of malnutrition on the host, with little attention given to the possibility that for an infecting microorganism such as an RNA virus, the nutritional status of the host can alter the genotype of the pathogen, and therefore its virulence.[45,85] The potentiation of measles virus virulence in malnourished Africans, particularly in vitamin A deficient children,[45,84,86] has traditionally been attributed to diminished host immunity.[87] The measles virus, like the viruses for hepatitis and influenza, as well as for HIV, is a RNA virus. RNA viruses have high rates of mutation in the range of 10^{-3} to 10^{-5} substitutions per copied nucleotide.[88,89]

Recent studies indicate that the benign Coxsackievirus, a RNA enterovirus and member of the Picornavirus family, is genetically altered and converted to a virulent microorganism following replication in a host deficient in the micronutrients/antioxidants vitamin E and/or selenium.[45,90] If the findings with respect to the Coxsackievirus apply to other RNA viruses, this will shed very useful light on the management of viral infections, including HIV infection, in African communities where chronic, multiple, micronutrient deficiencies are the rules rather than the exceptions.[2,7,33] For example, selenium deficiency is very common in several Sub-Saharan African countries, e.g., Zaire and Burundi. Selenium has been suggested to play a role in the speculated mutational events and subsequent horizontal transmission of closely related simian retroviruses to humans in Central Africa.[45,91]

Oxidative Stress and HIV Replication

HIV-infected individuals are generally under chronic oxidative stress.[92] Possible mechanisms for increased oxidant production are suggested to include the stimulatory effects of gp125 and Tat, the viral-transactivating protein secreted from virus infected cells.[92,93] Additionally, certain mycoplasmas produce hydrogen peroxide through activation of the phagocytic cells, and also from T cells through co-infection with the HIV.[93] Activated macrophages and neutrophils generate reactive oxygen species (ROS) whose overproduction relative to the impaired antioxidant defense system[1,94] leads to oxidative stress. Several studies indicate that HIV-1 gene expression is subject to control by intracellular transduction pathways that are redox-regulated, a finding suggestive of an important role for oxidative stress in the pathogenesis of HIV infection/AIDS.[95] This finding is supported by reported observations that HIV-1 gene activation and virus replication in human T cells can be induced by ROS such as H_2O_2,[96] and that HIV-1-infected subjects exhibit abnormally depleted levels of antioxidants such as total acid-soluble thiols, cysteine, GSH, and several other relevant micronutrients in plasma and peripheral blood monocytes.[1,21,23,67,94]

It is highly conceivable that depleted tissue levels of antioxidant micronutrients would be more profound in malnourished African victims of HIV compared with their better nourished counterparts in the rich, developed countries. T cells with high intracellular GSH levels are selectively lost as the HIV infection progresses to AIDS,[94] and plasma concentration of malondialdehyde is elevated, suggesting increased oxidative stress. Chronic oxidative stress is a commonly reported feature of malnourished African children, regardless of their HIV status,[43, 44] and reactive

free radicals are implicated in the pathogenesis of the kwashiorkor syndrome of PEM in children.[43,44,97] As indicated in Figure 1, oxidative stress, through activation of NF-κB, promotes not only viral replication and disease progression, but also the occurrence of secondary opportunistic infections.[85,94] Both TNF-α and ROS are among the known factors that activate NF-κB which then binds to and activates a κB enhancer element in the HIV proviral long terminal repeat, resulting in increased viral gene expression.

VERTICAL TRANSMISSION OF HIV IN AFRICA: INFLUENCE OF MATERNAL MALNUTRITION

Breast feeding, both as a source of high quality, easily digestible nutrients, and as a means of promoting protection from common infections, is widely practiced during the first two years of life in Sub-Saharan African countries.[29,30,98] Breast feeding by HIV-positive mothers is still a controversial issue,[99,100] but the WHO/UNICEF Expert Consultation[101] and others[102,103] have concluded that in situations such as those prevailing in Sub-Saharan Africa, where infectious diseases and malnutrition are the major causes of the extraordinary high infant mortality rate, breast feeding should be encouraged regardless of maternal HIV status. This view receives support from reported observations in milk of a factor that inhibits binding of the HIV epitope-specific MAB to recombinant CD4 receptor molecules as well as the binding of gp120 to CD4, with no difference in titers of inhibitor activity between samples from HIV-seropositive and -seronegative mothers.[100] Dietary requirements of nutrients increase during pregnancy and lactation, a factor contributing to the widespread occurrence in poor African communities of maternal malnutrition which adversely affects the volume of breast milk, as well as its contents of certain nutrients, particularly the micronutrients.[30]

Mother-to-child transmission of HIV accounts for about 500,000 cases each year worldwide, and these occur mainly in Africa.[104] Prior to routine administration of antiretrovirals, rates of mother-to-child HIV transmission ranged from 14 to 25% in developed countries, compared to 25 to 30% in developing countries.[105] Most cases occurred during pregnancy and at parturition, although an additional 5 to 15% of the cases in Africa may become infected during breast feeding.[104]

Observational studies in Sub-Saharan African countries indicate an inverse relationship between maternal serum retinol level in pregnancy and the risk of mother-to-child HIV transmission.[106,107] Studies of HIV-infected women in Kenya show that the risk of vaginal shedding of HIV DNA increases considerably as serum retinol declines below 1.4 μmol/L.[108] Mastitis, an inflammatory process in the breast, affecting no less than 20 to 33% of lactating mothers who breast feed, has been shown in a study in Malawi to promote an increased viral load in breast milk and the risk of mother-to-child transmission of HIV.[104] There is evidence that deficiencies of micronutrients (e.g., selenium, retinol, α-tocopherol, and β-carotene) may promote susceptibility to mastitis which is characterized by increased oxidative stress and is a major problem globally for the dairy industry (see Semba and Neville[104] for extensive review).

CONCLUSION

On a worldwide basis, chronic malnutrition is the biggest cause of immune suppression in humans, and the starving Sub-Saharan Africans, perhaps like immunosuppressed homosexual men, hemophiliacs, and drug addicts, should be considered a special risk group for HIV infection/AIDS. Today, AIDS in the developed rich countries is increasingly viewed as another complex chronic illness with many treatment modalities directed at both the HIV infection and the associated opportunistic infections which constitute major causes of morbidity, mortality, and cost for the patients.[109] Access to expensive, multi-regimen therapies has revolutionized the management of HIV disease in the developed World.[110]

In Sub-Saharan Africa, the HIV/AIDS epidemic is spinning out of control, and the highly active antiretroviral drugs marketed and used in countries in the developed market economies are not generally available. For people with HIV/AIDS in poor African countries, there must be some useful therapy to offer that is better than nothing, something that is consistent with sustainable health interventions in terms of cost, appropriate technology transfer, and minimal requirements for sophisticated monitoring.[21]

Primary micronutrient deficiencies are widespread and severe in Sub-Saharan African countries which constitute the large endemic foci of HIV/AIDS in the continent. Inter-relationships between HIV infection and nutritional status of the victims (before the infection and during disease progression) should be of considerable interest to workers attempting to control the ravages of the viral disease.

The idea that a malnourished individual has a greater susceptibility to RNA viral infections including HIV/AIDS and a relatively worse prognosis than a well nourished subject should be vigorously examined. There are suggestions that impaired nutritional status, particularly micronutrient deficiency, promotes the risk for HIV seroconversion[21,23,104] and the rapid progression of the infection to AIDS.[94,95,104] Food nutrients, particularly the antioxidant micronutrients, should be carefully studied for their potential therapeutic benefits against HIV disease, and specifically for their role in preventing mother-to-child transmission of the virus. In these studies, the micronutrient supplementation protocol should take into account the integrated nature of most components of the antioxidant defense network.[48,112]

Micronutrient supplementation has been reported to have the highest cost-benefit ratio known for any health interventions,[113] and if shown to be effective, it can serve as a readily available public health measure to reduce the scourges of HIV/AIDS in Africa.[23] There are, however, many unanswered fundamental questions regarding malnutrition and HIV/AIDS in Africa which require research attention. These include determining:

1. The interrelationship of residential exposure of poor malnourished Africans to high-grade respiratory and enteric pathogens in unsanitary environments and progressive HIV-induced immunosuppression
2. Specific micronutrient deficiencies that are common in various communities/population groups in different Sub-Saharan African countries

3. Factors other than dietary intake that contribute to the development of micronutrient deficiencies in the various groups
4. The extent to which diarrhea and malabsorption in malnourished subjects impair bioavailability of the micronutrient supplements

Guidelines for aggressive nutrition programs for the management of patients with HIV infection/AIDS in the U.S. are available.[114,115] Wholesale transfer of these guidelines to Sub-Saharan Africa is neither feasible nor recommended. African countries must develop their own national programs, taking into account the types and extents of nutrient deficiencies, the potential nutritional and health complications of endemic diseases peculiar to the various countries, and the available food resources.

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CHAPTER 14

Traditional and Popular Uses of Food as Therapy for HIV/AIDS

Jacquelyn H. Flaskerud

CONTENTS

INTRODUCTION

Foods and herbs have traditionally been used as remedies or therapies to prevent, treat, and cure illness. This tradition continues today with an emphasis on health and a healthy lifestyle that includes diet. The last two decades have seen a growing interest of the American public in nutrition, nutritional supplements, and the use of herbal remedies or natural drug products (as opposed to synthetic drugs) to promote

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health and prevent illness.[1,2] The media have been involved in publicizing the health benefits of foods and nutrient supplements as measures to prevent and reduce heart disease, Alzheimer's disease, various cancers, and HIV/AIDS.[3-5] Diets rich in plant foods such as fruits, vegetables, teas, and herbs have been promoted in popular and clinical literature as having preventive and restorative effects.[6-8] Special diets, botanicals (plant foods and herbs), and nutritional supplements have been used frequently as complementary therapies by people with life-threatening illnesses and poor long-term prognoses, such as people with cancers and HIV/AIDS.[9-11]

An interest in nutrition and nutrient supplements is spurred in people with HIV/AIDS and cancer because of the weight loss and malnutrition commonly associated with the disease and its medical treatments.[1,12] People with HIV/AIDS use nutritional therapies that are associated with antiviral action, immune system enhancement, and infection prevention and treatment properties.[13]

Although many of the current uses of nutrition to promote health are considered modern, some are grounded in traditional beliefs and theories about the uses of food and herbs to promote health and prevent illness. In traditional systems, foods and herbs are classified according to various properties and prescribed for diseases with opposite properties to restore balance. These traditional conceptualizations of food and herbal medicines remain popular and newer foods, herbs, and medicines are constantly incorporated into them.

Logan notes that the cognitive system underlying various classifications remains constant.[14] Consequently, remedies not yet known will, upon introduction, be classified into an existing system. Harwood takes a similar view, noting that traditional theories of illness and treatment are very functional and order a great deal of health behavior, thus reinforcing and validating their use for groups who practice them.[15] Because of traditional classifications of food and herbs as therapies, today's popular uses enjoy credibility and acceptance.

A review of the major traditional theories of illness and ethnomedicine provides a background for the longstanding use of food and herbs as therapies and for understanding nutrition beliefs about HIV/AIDS and its treatment. It also provides a framework for understanding today's popular uses of diet, nutrition, and herbal medicines as alternative therapies for HIV/AIDS. This chapter will focus on both traditional and popular beliefs and practices of Americans related to nutrition, and specifically on beliefs and practices involving use of foods and herbs to prevent and treat AIDS.

TRADITIONAL THEORIES AND NUTRITION

Americans are pluralistic in ethnicity and culture, but many of their beliefs about the uses of food as therapy have common explanations for the relationship between nutrition and health, how specific foods work, and when to use them. Common themes are that foods build body strength, prevent illness, fight illness, and cleanse the body of impurities. The notion of balance in the diet and the concepts of excess and deficiency are prevalent in most traditional beliefs.

Yin/Yang Classifications

Perhaps the oldest of all known food and herb classification systems is derived from the ancient philosophy of Tao and proposes a balance in the universe based on elements of yin and yang. Yin is cold, darkness, earth, repose, and femaleness; and yang is heat, light, heaven, movement, and maleness.[16,17] Yin and yang are often translated into English as cold and hot although this is an inadequate translation. Plant foods, meat, herbs, teas, and tonics are also grouped as yin or yang. Foods have properties such as cool, warm, cold, and hot; and flavors such as sweet, sour, bitter, pungent, and salty.[18] A balance of yin and yang foods in the diet is considered vital to good health. Proper balance promotes harmony and health, and diseases result from imbalances between yin and yang.[13] Yin conditions require yang foods for treatment and vice versa in order to maintain balance within the body. Foods are used as therapy in prevention of disease and for cure and nourishment.[18]

Americans of Asian origin who share traditional food therapy beliefs based on yin and yang include Chinese, Filipinos, Koreans, Japanese, and Southeast Asians.[13,18-22] Food is important for sustaining life and good health, for basic body strength, and for curing and nourishment.[18] Staying healthy is based on the principles of balance and harmony between yin and yang forces in the body, and body restoration and maintenance through the yin and yang properties of food, along with the practice of meditation.[13] Yang foods are used as therapies to strengthen the body and build or fortify the blood when imbalances occur.[16-18]

Shivering, wasting, cancer, and diseases of the lungs are considered Yin conditions and should be treated with yang foods, while salty and cold foods should be avoided.[16,18] Yang foodstuffs include beef, eggs, garlic, ginger root, ginseng, leeks, mushrooms, liquor, peanuts, spicy foods, sour foods, wine, red beans, tomatoes, red peppers, and red foods. Yang conditions require yin foods. Fevers, infections, venereal disease, upset stomach, and constipation are examples of yang conditions and are treated with yin foods. These foods include most fresh fruits, vegetables, greens, fish, gingko, milk, water, winter melon, plant foods, cold foods, and white foods.[16,23] Some foods are considered neutral and may be used to treat either yin or yang conditions.

Hot/Cold Classifications

The Hippocratic theory of humoral medicine guides the traditional use of food as therapy for a wide range of Americans. Humoral science is thought to have originated in the Mediterranean area, spread to the Arabic world, and arrived in the Americas and parts of Asia during Spanish colonialism.[14,24–26] The resemblance between Hippocratic humoral theory and the centuries older Chinese yin and yang philosophy might suggest a center of origin for humoral theory outside the Mediterranean area.[14] Ancient Hindu medicine also refers to humors, and there is some disagreement about whether Indian medicine preceded its Greek counterpart. Americans who had their origins in the Mediterranean, Middle East, some Asian countries, India, and Latin America share similar beliefs about the properties and uses of food and herbs as medicine.[19,21,22,27] Properties of hot and cold are assigned to foods and

herbs and to illnesses, similar to yin and yang principles. The concept of balance is used to prescribe particular foods, depending on the property of the illness.

Cultural groups who incorporate traditional hot-cold classifications into their medical beliefs categorize herbs and foodstuffs as hot or cold, with a number of foods and herbs classified as cool. Some are neutral, and have neither hot nor cold qualities.[15,24,26,28] These properties of food have functional significance and order health behaviors. Health is maintained by diet as long as the equilibrium of hot and cold is not upset. Balance of hot and cold is important to prevent disease and prolong life, as is moderation in ingestion of food and drink. Eating too many hot or cold foods can create a reaction that may be alleviated by eating foods of the opposite property.

Warm foods are believed to be more easily digested than cold foods.[24] A diet consisting of entirely warm foods will not necessarily make a healthy person sick, but a diet consisting of cold foods alone will cause illness in even the healthiest person. Consuming too many cold foods without equivalent amounts of hot foods can cause sickness, as can the reverse. Certain physical problems resulting from the overconsumption of, for instance, hot foods, can be ameliorated by specific cold foods or drink.[26]

Sickness is not caused solely by the overconsumption of one category of food. Illnesses have other causal agents as well. The ultimate importance of foodstuffs and herbs comes from their roles in curing diseases. In general, diseases or symptoms classified as hot are treated with foods, medicines, and herbs that are cold and vice versa. Blood is the primary source of life in the body, of strength and warmth. Any condition that results in loss of blood also results in weakness and an excess of cold. Such a condition is treated by the use of strengthening foods with hot properties, ingesting blood or foods containing blood, and also by ingesting foods, liquor, wine, and spices with darker colors.[14,22,24,26,28,29]

Fever, stomach ache, diarrhea, constipation, nausea, rashes, and pustules are often attributed to impurities in the blood and excess heat. Cleaning the blood of impurities can be accomplished by the use of foods and herbal remedies considered to have cold qualities and cleansing or purgative properties.[15,26,30] Many fresh fruits and vegetables and greens have cold properties. Chills, chronic cough without blood, tuberculosis, and pneumonia without blood are all considered an excess of cold in the body. Treatments would avoid foods, medicinal plants, and modern medicines with cold properties. The practice of giving hot or cold foods in particular kinds of clinical conditions is a balancing technique for treating the condition and not necessarily related to nourishment.[14,24,30] Nourishment is, however, important in recovery from illness. During convalescence, the body is considered weak, and proper foods will strengthen the blood and the body. In addition to aiding recovery, food and drink supply nourishment and strengthen resistance to disease.

Natural/Unnatural Illnesses and Food Therapies

Snow has conceptualized the beliefs of African Americans about maintaining health and the cause and treatment of illnesses as falling into natural and unnatural categories.[31,32] Natural illnesses can be attributed to nature and nature's God. Unnatural illnesses are attributed to forces of evil, witchcraft, and the devil. For purposes

of this discussion, natural illnesses are reviewed because they are associated with food therapy and nutrition.

Natural illnesses are caused by cold, dirt or impurities, improper behavior, and diet.[32] All of these causes are related to blood and its functions, and clearly indicate a need for a moderate and healthy lifestyle that emphasizes balance. Blood is viewed as good or bad, clean or dirty, thick or thin, high or low, and sweet or bitter. These beliefs are found among African Americans, White southerners, and immigrants from Haiti, Jamaica, and the Bahamas.[30,32–35]

Exposure to cold air, cold water, and dampness causes acute illnesses associated with mucous production such as pneumonia, bronchitis, cold, flu, arthritis, and rheumatism. Persons whose blood is thin are more susceptible to cold. The young, old, and menstruating and postpartum women have thin blood. Building up or thickening the blood through food can protect a susceptible person against cold and should be practiced in the winter.[33] Specific foods include liver, pork, beets, and wine. Other protective measures against cold are dressing warmly and avoiding cold air, drafts, dampness, and water.

Dirt, impurities, and germs are causes of illnesses associated with heat and exemplified by conditions such as fever, inflammation, skin eruptions, measles, and venereal disease.[31,32] Impurities are circulated within the body through the blood and expelled through menses, bowel movements, hives and rashes, and the pores. Impurities in the blood are caused by failure to bathe, dirty clothes and living conditions, impeded menses, irregular bowel movements, and sexual excess. The blood can be cleansed with purgatives, sulfur and molasses, greens, and teas.[32,34]

Diet is believed to prevent illness, cause illness, and possess curative properties.[35] Two conditions are associated with diet: (1) high blood, thick blood, sweet blood, or too much blood; and (2) low blood, thin blood, or not enough blood. High blood is caused by too many rich foods or blood builders in the diet. These are red foods: red meats (especially pork and liver), beets, grape juice, red wine, raisins, and black molasses.[32,34] The blood is believed to be thinned by white, colorless, or bitter foods such as lemon, vinegar, epsom salts, garlic, and onions that lower or thin the blood through the pores, bowels, and menses. Persons with low blood are treated with blood builders mentioned earlier. Protective procedures are to build up the blood for winter and thin it out for spring and summer.[33,34] Balance in the diet and monitoring the state of the blood are considered essential to health. These concepts, like those for hot/cold and yin/yang classifications, include notions of illness expressive of cold and heat, of blood as signifying body strength, and of the use of food to treat variations in the blood. The foods used to build and thin the blood are remarkably similar among the three conceptualizations.

Cold/Fever/Germ Classifications and Nutritional Therapies

Several investigators have documented the use of folk conceptualizations of illness and folk remedies among Americans of European origin.[36-40] These beliefs and practices are practiced by lower and middle class and urban and rural subjects.[36,38,39] Many of the remedies endorsed may be classified as dietary substances.[37,39]

The conceptualizations of illness used by white Americans of European origin bear striking resemblances to many of the beliefs of other American ethnic groups and a particular affinity to the beliefs of African Americans.[32,40] Helman[40] describes a folk model of illness that includes hot illnesses generally associated with fever (and with infectious diseases in biomedicine) and cold illnesses associated with chills. Cold illnesses involve the relationships between persons and the natural environment, and hot illnesses involve relationships with other persons in society. Cold illnesses are caused by dampness, rain, cold air, night air, drafts, and climatic changes.[39,40] Such illnesses enter through the skin, ears, top of the head, back of the neck, and feet. They cause colds, flu, rheumatism, bronchitis, pneumonia, cold loose stools, and cold in the kidneys.[37,39,40] Prevention of cold illnesses involves staying warm and strengthening the body with foods and tonics. Hot drinks, rest in a warm bed, and ample warm foods are used to treat cold. Tonics, vitamins, cod liver oil, and whiskey are also used to strengthen the person with a cold illness, but the most important treatment is to feed the person.

Hot illnesses such as fevers, hot diarrhea, boils, and rashes are caused by impurities in the blood and concentrations of dirt, bugs, germs, or viruses. They originate in other people (not in the natural environment), are transported by people, and enter the body through orifices such as the mouth, nose, anus, urethra and vagina.[30,39,40] Hot illnesses are treated by restricting foods and flushing impurities from the system with lots of fluids. Evidence that these hot illnesses are leaving the body are diarrhea, vomiting, sweating, expectorating, urinating, blowing the nose, and skin eruptions and rashes. Remedies used to flush out the body or cleanse the blood are laxatives, vinegar, ginseng, and garlic. Fresh fruit juices in large amounts are the most common fluids recommended for flushing out the system when fever is present.[30,39] Reducing food intake is used concomitantly with forcing fluids for a feverish person.

AIDS AND TRADITIONAL FOOD THERAPIES

Flaskerud and colleagues studied the beliefs about AIDS of low income Anglo, African American, and Latin women in Los Angeles.[38,41,42] Data were gathered through semi-structured focus group interviews. Each ethnic group was interviewed separately by data collectors who shared the ethnicity, language, and gender of the participants. Knowledge of AIDS differed among the participants. Those who had no personal experience with the disease tended to classify it as a hot illness. AIDS was associated with venereal disease, impurities in the blood, fever, rashes, and dirty practices and habits.[43] AIDS was believed to be transmitted from one person to the next through body orifices and pores by direct contact with an infected person or indirectly by contact with objects such as toilet seats, furniture, eating utensils, swimming pools, and spas contaminated with body fluids (especially urine, blood, feces, and saliva). The AIDS virus was believed to be excreted from the body through urine, feces, sweat, menstrual blood, and sexual fluids. To cleanse the body and purify the blood of the virus, Latina women recommended eating fresh fruit, drinking wheat and barley waters, and purging the body with herbs, mineral oils, laxatives, and other purgatives. Ingestion of garlic also was recommended to purify the body

and blood of the AIDS virus and cleanse the stomach and bowels. Additionally, garlic was thought to protect against AIDS infection through mosquito bites. Several herbal remedies available in Mexico included teas made from roots or bark believed to purify the blood of syphilis and AIDS. Finally, treatment with snake blood was recommended to drive out the poisons.

Among African American respondents, cleansing the blood and the body were considered methods of ridding the body of the AIDS virus. Food therapies recommended were epsom salts, black draught, castor oil, other laxatives, greens, astringents, and acids such as vinegar and lemon, lemon tea, or lemon stew. Some African American subjects experienced or heard that persons with AIDS had fevers and large amounts of diarrhea. They believed it was a mistake to try to stop the diarrhea because diarrhea represented an attempt by the body to excrete toxins. Otherwise, the toxins in wastes would be reabsorbed by the bloodstream. Keeping the system "cleaned out" was considered a method of staying healthy as well as a possible cure. Fevers accompanying AIDS were treated by purging the blood via laxatives, greens, and acidic foods.

Many Anglo women participants believed AIDS was accompanied by fevers transmitted through concentrations of impurities and germs, and associated with venereal disease. They recommended cleaning the system with lots of fluids, especially fruit juices. Sweating out the germs associated with AIDS could be accomplished by drinking hot teas and herbs.

Prevention of AIDS also was related to nutrition and to the notion of building resistance, body strength, and blood. In addition to foods, moderate rest and exercise, moderation in smoking and alcohol intake, and avoiding chill were recommended to build resistance. Among African American women, foods that were considered to build strength and build up the blood against AIDS were red meat, liver, red wine, grape juice, egg yolks, nutmeg, vitamins, pepper, and chocolate. However, these were not to be eaten as a steady diet because they might cause other illnesses. Rather they were to be eaten when "the system is low" or the "body is not at its highest peak." Persons who were too thin, old, young, pregnant, or menstruating might have to strengthen their resistance to the virus.

Anglo women also believed that a person whose immunity was "run down" or a person who had "low blood deficiencies" would be more susceptible to AIDS. Red meat and vitamins were recommended to build up defenses and the immune system. Latina women recommended maintaining balance in the diet as a method of avoiding AIDS and emphasized the need to purify the body and blood regularly with foods, herbs, and purgatives to prevent AIDS. These methods of keeping the internal body clean were to be combined with measures to keep the external body clean.

Some of the women in each ethnic group had experience with AIDS patients. These women often equated AIDS with pneumonia. If the pneumonia was not accompanied by fever and blood, it was usually considered a cold illness and treated with medicines and foods with hot properties or blood building properties. For these women, food was thought of and used as nourishment to the body, to assist in convalescence and recovery, and strengthen resistance. Warm soups and liquids were used early in recovery from AIDS pneumonia. As persons with AIDS regained strength and were able to tolerate heavier foods after episodes of pneumonia, blood

builders such as meat, eggs, liver, and vitamins were recommended by African American subjects.

Latina women used warm foods during the recovery period after AIDS pneumonia because these are more easily digested and they rebuild the blood and body strength. Many foods considered neutral or warm by the subjects were recommended in small amounts for the beginning of the recovery period: flour tortillas, rice, bread, potatoes, frijoles, and ginger tea. As a person regained strength, meat, eggs, and a little wine were added to the diet to help the body regain health. Vitamins and iron were also considered essential to rebuilding strength.

Anglo women who had experience with AIDS pneumonia recommended warm, bland, and soft foods for the recovery period: chicken soup and other soups, tea, toast or bread, oatmeal or cooked cereal, and soft boiled or scrambled eggs. These foods were considered "easy on the stomach." This description and the foods Anglo women recommended seemed similar to the beliefs of Black and Latina women about the diet during recovery from AIDS pneumonia.

CURRENT POPULAR FOOD CLASSIFICATION

Current popular beliefs and science also link foods, plants, herbs, and teas to health maintenance and restoration. As with traditional classifications, foodstuffs are classified according to their properties. However, the current belief is that these classifications are scientific and therefore more sophisticated than older traditional categorizations. The most recognizable classification used today is the food pyramid based on carbohydrates, proteins, and fats, and consumption of a recommended number of calories from each category. A health promoting diet currently consists of a variety of foods from each of seven food groups displayed as a pyramid: two or three servings of food from each of the protein (15 to 20% of calories) and dairy groups each day (30% or fewer calories from fat), seven to twelve servings from the starch-grain group, two servings of vitamin C-rich fruits and vegetables, one serving of vitamin A-rich fruits and vegetables, and three servings of other fruits and vegetables (50 to 55% of calories).[44,45] An examination of this diet reveals confusing and inconsistent classifications of foods including calories; protein, carbohydrates, and fat; vegetables, fruits, grains, and dairy products; and micronutrients (vitamins A and C). The food pyramid is certainly as confusing as traditional classification systems.

Other categorizations abound and include micronutrients (vitamins, minerals, and trace elements); herbal remedies such as saw palmetto, St. John's wort, and echinacea; and nutrient (food) supplements such as amino acids, enzymes, fatty acids, hormones, and shark cartilage, to name a few.[1,8,13] A new language for classifying key components of foods that may provide health benefits has developed based on their actions. However, the terms used to describe nutritional components and their actions are poorly understood by the general public. Recent categorizations of the nutrient properties of foods have no logical congruence with specific health benefits or the diseases for which they might be helpful for the general public. This was not true of the traditional classifications of foods based on a folk understanding

that can be applied to health practices. Despite the apparent disadvantage to the public in the classifications of nutrients, herbal remedies and the so-called food supplements have become the folk remedies of today. In 1990 nearly $14 billion was spent on alternative remedies.[2]

Many macronutrients and micronutrients have been associated with health promotion and with preventing specific diseases. The media publicize these diet recommendations in the popular press, and the American public is bombarded with information on diet, health and illness, and often conflicting claims.[3–5]

Heart disease provides a good example of the relationships proposed between food and health. It also illustrates the constantly changing recommendations regarding diet and heart disease. In general, a low fat, low salt diet, high in green vegetables, fruits, grains, oat bran, and soy, and low in red meat, coffee, and liquor was recommended.[4] However, recent changes have included recommendations for some fats (omega-3 fatty acids and monounsaturated fats), red wine (resveratrol), onions (flavonoids), and garlic (polyphenols). Salt and coffee are permissible. Nonherbal black, green, and oolong teas are thought to protect the heart against disease. Oat bran which had been thought to have protective effects against heart disease is no longer considered a preventive. The changes keep coming. Soy, which was recommended for protecting against heart disease, has now been suggested to decrease mental abilities, play a role in thyroid abnormalities, and contribute to cancers of the breast and pancreas in people eating large quantities.[5] Cautions have been applied to green tea which contains the antioxidant epigallocatechin-3 gallate because of disappointment with more famous antioxidants, vitamins C, E, and beta carotene, in preventing heart disease and cancer.

A plethora of dietary regimens have been recommended to promote health. Diets low in fat, and/or diets low (or high) in carbohydrates have been recommended to protect against cancers and heart disease. A diet rich in antioxidants from foodstuffs and micronutrients is thought to protect against heart disease and cancers and is recommended for people with AIDS because it promotes immune function.[46,47] Diets high in cruciferous vegetables (and their micronutrients) have been recommended to prevent or treat cancer, AIDS, and hypercholesterolemia.[48] The Mediterranean diet replete with flavonoid-rich foods (e.g., Allium and Brassica vegetables: garlic, onions, and leeks; cabbage, broccoli, cauliflower, turnips, and brussel spouts; and red wine) is associated with decreased incidence of cardiovascular disease. It has been suggested also that because flavonoids may have antiviral and carcinostatic properties, diets high in flavonoids may have a place in the control of retrovirus infections such as AIDS.[49]

AIDS AND POPULAR FOOD THERAPIES

The use of alternative and complementary therapies to treat HIV/AIDS is concentrated in the gay white community and includes, in addition to nutritional remedies, treatments such as acupuncture, massage, and meditation. Popular nutritional remedies consist principally of herbal medicines (plant foods), micronutrients (vitamins, minerals, trace elements), and substances sold as food supplements with

pharmacologic properties (alpha lipoic acid, iscador, N-acetylcysteine, and superoxide dismutase).[1,8,13] Nutritional remedies used by people with HIV/AIDS are thought to produce antiviral action, enhance immune functioning, and treat or prevent opportunistic infections.

Herbal (plant food) remedies have many ties to traditional food therapies. Herbal remedies are used to enhance the immune system, treat infections and contaminants in the blood, and exert antiviral actions in people with HIV/AIDS. Echinacea, astragalus, ginseng, and garlic are herbal remedies long used traditionally for treating fevers and inflammations, removing impurities from the blood, restoring energy, and preventing and treating infection. Their current use for HIV/AIDS describes in scientific terms a similar set of actions. Echinacea is used to treat immune deficiency and as an antiviral; astragalus, ginseng, and shiitake mushrooms are used to increase immune function and restore energy; garlic is used as an antibacterial and antiviral agent.[1,13,48]

Bitter melon and its extract, compound Q, are other herbal medicines used by people with HIV/AIDS and are thought to have antiviral activity.[1,10] Hypericin (from the St. John's wort plant), usually thought of as an antidepressant, is taken by people with HIV because of its antiviral properties. Curcumin, used as a spice, is classified as an antioxidant and is thought to inhibit viral replication. SPV-30, extracted from boxwood evergreen, has been found to have antiviral action and decrease viral load. *Aloe vera,* used for centuries as a skin emollient, when taken internally has antiviral, antibacterial, antifungal, and anti-inflammatory properties.[1,13]

Flower essences are other forms of herbal medicine taken by people with HIV/AIDS. Flower essences have a long tradition of use in people who subscribe to hot/cold traditional classifications of foods.[23] They are typically associated with foods categorized as cool and are common to Hispanic cultures. In current use, they are taken to establish balance and harmony in emotional, physical, and spiritual spheres.[13]

Other categories of nutritional remedies used by most people with HIV/AIDS are the micronutrients (vitamins, minerals, and trace elements). These are often recommended and taken in megadoses by people with HIV.[8,10] Supplementations of B vitamins and folate are taken to compensate for the demands of opportunistic infections and fever, drugs taken for the control of nausea and pain, and the malabsorption of nutrients that accompanies HIV disease, the use of AZT, and drugs taken for infection. Antioxidant vitamin supplementation (vitamins A, C, and E) is used by people with HIV to prevent and treat infections, enhance immune functioning, and increase the antiviral effects of AZT.[8,13]

Supplementation with minerals in people with HIV is usually limited to magnesium, and it is highly recommended for its positive effects on recovery from severe and life threatening infections. Trace elements of significance to people with HIV are zinc and selenium.[8,13] Selenium is an antioxidant that increases immune function. Zinc, usually taken to stimulate the immune system, has been reported recently to weaken immune system function and lower calcium levels in HIV-positive men.[8]

Other forms of supplementation for people with HIV/AIDS are products sold in health food stores as foods or food supplements. All are thought to enhance immune response through their antioxidant effects and antiviral actions. These include superoxide dismutase, N-acetylcysteine, and alpha lipoic acid. Other food supplements sold as immune-boosting dietary supplements and taken by people with HIV are thymus extracts, bovine colostrum, and shark cartilage.[1,8,13]

Complete dietary regimens often followed by people with HIV are macrobiotic diets, anti-yeast diets, and immune power diets. The macrobiotic diet is based on traditional principles of balance and harmony between yin and yang forces and has been constructed specifically to address diseases such as cancer and AIDS, thought to result from long term imbalances. This diet consists of whole grains (50–60%), vegetables (20–30%), legumes and seaweed (5–10%), and soups based on soybean paste (5%).[13] Other dietary regimens include the yeast-free diet thought to prevent opportunistic yeast (fungal) infections, particularly *Candida albicans* (thrush). All foods made with yeast or those that provide a yeast-promoting internal environment are avoided.[50] The Immune Power Diet[51] is another total dietary regimen used by some people with HIV. This diet avoids foods that typically are associated with allergies.

COMPATIBILITY OF FOOD THERAPIES AND AIDS TREATMENT

Popular Dietary Therapies

The use of herbal remedies is not without its dangers. These drugs can be purchased without prescriptions and are considered natural. That does not mean they are benign. The same is true of food supplements. Not only do some of these preparations have serious side effects, they also interact with conventional prescribed medications and with each other. Some supplements exclude the use of others. People with HIV who use complementary food therapies need to be particularly aware of contraindications for certain supplements. Because of the plethora of supplements available, the rapid development of new treatments, and the constantly changing information on contraindications and interactions, it takes a highly motivated, well educated, and self-determined person to use herbal remedies and food supplements safely.

Megadoses of micronutrients can also be problematic. Megadoses can create nutrient-nutrient or nutrient-medication competitive environments, cause toxic effects and neurologic damage, and result in weakening of the immune system.[52] Romeyn[8] reports a particularly troubling situation arising in several studies in which HIV-positive men took high doses of zinc. High zinc intakes were related to faster progression to AIDS. The investigators of these studies suggested that high zinc levels supported the assembly of the new virus.[8] At least some micronutrients taken in excess can weaken immune system function. Again, it takes a disciplined and vigilant person to keep aware of changes in information on micronutrient supplementation. For a variety of reasons, many people with HIV cannot stay informed without help. Health professionals must acquire current data about alternative nutritional therapies on an ongoing basis to effectively assist their clients.

Traditional Dietary Therapies

Some traditional beliefs about the use of food as therapy held by the Anglo, Black, and Latina women interviewed in the studies by Flaskerud and colleagues[38,41,42] are considered compatible with the biomedical treatment of AIDS

indicator diseases (opportunistic infections, neoplasms, and wasting). Other beliefs, however, could be detrimental to treatment and health. Many of the opportunistic infections associated with AIDS are accompanied by fever. The use of increased amounts of fluids in the diet is not incompatible with the treatment of fever. On the other hand, the notions of "flushing out the system" and "sweating it out," especially when herbs, minerals, oils, laxatives, and purgatives are used to accomplish this end, is considered risky. This is particularly true when opportunistic infections are accompanied by diarrhea or when HIV wasting syndrome is the diagnosis. Fluid depletion becomes a real danger to the person with AIDS in those situations. Although diets high in carbohydrates, fat, and lactose can exacerbate gastrointestinal problems, high calorie, high protein diets are still recommended for persons with HIV wasting and diarrhea.[12]

The idea of preventing AIDS by building resistance through the use of foods has an inherent general health complication if the diet becomes too dominated by the so-called blood builders: red meat, egg yolks, liver, chocolate, and other foods. Such a diet might be considered cholesterol rich in current biomedical thought. However, most of the respondents recommended this diet with a view toward moderation in its use. None of them believed the diet should be a steady one. They all cautioned that the diet should be used only when the "system is low." From an AIDS perspective, the "blood building" diet would have to be used cautiously in order to prevent polymicrobial enteric infections which are potent immunosuppressants. To reduce microbes in the diet, meats must be well cooked and prepared foods such as egg salads and cold meats should be avoided.[12] The blood building diet might contribute to such infections if blood products are not cooked well and prepared foods are not properly refrigerated or are held too long.

The use of bland, soft, warm foods for the recovery period from AIDS-related pneumonia might be considered a beneficial use of traditional food therapy. Generally persons with *Pneumocystis carinii* pneumonia (PCP) are allowed to eat whatever they can tolerate if the foods are not contraindicated by their medications.[12] Several current medications in use for PCP produce adverse side effects such as anorexia, vomiting, diarrhea, alteration in taste, and hyperglycemia or hypoglycemia. All these side effects must be considered in developing nourishing and tolerable diets for persons with PCP.

IMPLICATIONS FOR HEALTH PROFESSIONALS

Every health care professional involved with persons who have AIDS should have a working knowledge of lay conceptualizations of illness and the use of food and herbs as therapy. Framing prevention and treatment approaches within lay conceptualizations of health, illness, AIDS, and the therapeutic use of food and herbs may well have a greater chance of success than use of a biomedical approach alone. Harwood[15] makes the point to health care workers that the probability of changing an individual's conception of disease and treatment in a few short encounters is very small. This is especially true when that conceptualization orders a great deal of health

behavior, is supported by the family and social group, and is frequently validated by the prescriptions of the biomedical system. It is far more productive, in a pragmatic sense, to accept and work within the existing system of beliefs and practices than to impose a biomedical regimen that probably will not be followed.

Dietary education efforts by health care workers must include the recognition that various groups of people will integrate information on AIDS into their already existing beliefs and practices about illness, its treatment, and the use of foods and herbs as therapies. It is necessary to understand these beliefs and practices in order to provide relevant, safe, and effective education, prevention, and treatment programs. Dietary education programs should be delivered within the context of a person's beliefs and practices to enhance acceptance and safety. Health professionals may design dietary treatment programs congruent with existing beliefs and practices and should be alert to possible conflicts and dangers in dietary approaches to disease and treatment.

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CHAPTER 15

HIV and Infant Growth

Cara Frankenfeld and Douglas Taren

CONTENTS

INTRODUCTION

The growth patterns of infants and children are the foundations of monitoring systems for determining the health and nutritional status of children. Understanding the various environmental and genetic determinants of growth provides a theoretical and practical basis for developing community-based prevention and treatment programs for undernutrition.

Historically, the roles of dietary intake and infectious diseases have been the primary issues for understanding undernutrition and have led the efforts to determine the intervention points for preventing and treating undernutrition. Classically, the association between nutritional status and infectious diseases has been considered to be a two-way relationship,[1] and produced the dictum that increased incidence of

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infectious diseases, such as diarrheal and respiratory diseases, leads to undernutrition; and undernutrition leads to increased incidence of disease. Many mechanisms have been defined for this relationship including the anorexia and increased metabolic rates associated with infections and the decreased immunity that occurs with undernutrition.

However, with the current epidemic of childhood HIV, many associations have been made between the disease and childhood growth that may not parallel classical thought. Understanding whether an infection, such as HIV, can affect growth in the absence of anorexia, fever, and co-morbidity will expand the understanding of the relationship between nutritional status and infectious diseases. One can ask, do HIV-infected infants have growth patterns similar to non-infected children?

The importance of this information can be directly applied to the estimated 620,000 children infected with HIV in 1999.[2] With these children in mind, this chapter will focus on how HIV is associated with birthweight and with growth during the first year of life. This chapter presents the epidemiological data on the birthweight and growth of HIV-positive and HIV-negative children born to HIV-infected mothers. It also discusses potential reasons for these findings.

BIRTHWEIGHT

Birthweight is an important component of infant morbidity and mortality. Infants with low birthweight (LBW), weighing less than 2500 grams at birth, are at increased risk of death in during infancy, particularly in the neonatal period.[3] This increased risk has been shown to persist after controlling for birth order, maternal HIV and syphilis infections, maternal education, and socioeconomic status (SES).[4] The relationship of birthweight with morbidity is less well characterized.[3] LBW was documented to be associated with intrauterine transmission to infants in New York City.[5] The LBW infants had a four times greater risk of intrauterine transmission, after adjusting for AZT use during pregnancy, maternal CD4 counts, and duration of membrane rupture.[5] This underscores the importance of understanding factors that influence birthweight in populations where maternal HIV infection is present.

There are some important considerations for interpreting studies of birthweight in HIV-infected populations. The outcome of interest is usually whether maternal HIV status or infant HIV status influences birthweight. When considering maternal status, it is important to control for variables known to influence birthweight, such as smoking or concurrent infections. Determining the impact of infant HIV status on birthweight is more difficult. With studies that utilized antibody testing for infants in breastfeeding populations, it was not possible to determine the actual timing of HIV transmission for the infants who became positive. Recently, the importance of distinguishing between intrauterine and intrapartum transmission has been established. Risk factors may differ between these two types of vertical transmission. To make this distinction, an early RNA-polymerase chain reaction (RNA-PCR) test must be administered to the infant. The test should be conducted as soon after birth as possible. It is recommended to test infant blood within 48 hours following birth.[6] Since a rapid increase in sensitivity of PCR occurs in the first week of life, the

further from birth the measurement is taken, the less likely a distinction between intrauterine and intrapartum transmission can be made.[7] Umbilical cord blood is not a reliable assessment of infant HIV status, because of potential contamination with maternal blood.

Inconsistent results have been observed regarding the significance of maternal HIV infection with birth size in developed and developing countries (Table 1). Most studies conducted in developed countries observed no effect of maternal HIV infection on birthweight. Mean birthweights were not different between HIV-positive and HIV-negative women in five studies conducted in developed countries.[8-12] LBW was observed more frequently in HIV-positive women compared with HIV-negative women in one study conducted in New York,[13] but not in two others conducted in Maryland and Scotland.[8,14] Twenty-nine percent of the HIV-positive women in the New York study gave birth to infants with LBW, compared with approximately 9% of HIV-negative women.[13] After adjustment for cigarette and illicit drug use, SES, and prenatal care, the HIV-positive women had a two-fold higher risk (odd ratio: 2.09, 95% confidence interval: 1.54 to 2.69) of delivering LBW infants.

Significant effects of HIV infection on birthweight in pregnant women have been observed more frequently in developing countries. HIV-positive women in the Democratic Republic of Congo, Rwanda, and Haiti were observed to give birth to infants with lower mean birthweights compared with HIV-negative women.[15-17] The differences in mean birthweights between infants born to HIV-negative and HIV-positive women ranged from 119 g[16] to 170 g[15] lower for infants of HIV-positive women. LBW has also been observed to be significantly lower for infants born to HIV-positive women compared with HIV-negative controls.[16-20] HIV-positive women consistently produce a greater incidence of LBW infants compared with HIV-negative women. These differences have ranged from 7.2% to 13.1%, with one study reporting a 28% rate of LBW in HIV-positive women.[16,17,19] The increased risk of giving birth to an infant with LBW persisted after adjustment for variables known to influence birthweight. After adjustment for sociodemographic factors and sexual history variables, the observed odds were 1.5 (95% confidence interval: 1.0 to 2.4) for LBW with maternal HIV infection.[16] After adjustment for SES and concurrent infections, the observed odds ratio for LBW with maternal HIV infection was 5.7 (95% confidence interval: 2.5 to 12.8).[19]

Additionally, a meta-analysis was conducted utilizing 17 prospective cohort studies from developing and developed countries. An overall odds ratio for LBW of 2.04 (95% confidence interval: 1.86 to 2.35) was predicted for HIV-positive women. Not surprisingly, the authors noted significant heterogeneity among the studies.[21] Problems with many of the past studies include failure to control for potential confounders, and inability to adjust for the same confounders. This indicates the need for careful interpretation of results.

Differing results have also been observed for the significance of infant HIV infection upon birthweight. Many earlier studies conducted in developing countries cannot be interpreted for this result. Studies that utilized antibody testing in breastfeeding populations to determine infant status do not allow for distinction of whether infection was present at birth or occurred through postpartum transmission. In non-breastfeeding populations of HIV-positive women, antibody testing can distinguish

Table 1 Summary of Studies Examining Maternal HIV Infection and Birthweight

Authors	Location	Type of Study	Subjects	Outcomes Measured	Variables Controlled	Results
			Developed Countries			
Agostoni, C. et al. (1998)	Milano, Italty	Prospective	92 HIV+ women 65 HIV- women All infants negative	Mean WAZ	No multivariate analysis	Maternal HIV infection not significantly related to WAZ
Alger, L.S. et al. (1993)	Baltimore, Maryland	Prospective	101 HIV+ women 97 HIV- women	Mean BW, LBW, SGA, LGA	No multivariate analysis	No differences for mean BW, SGA or LGA; trend for LBW
Johnstone, F.D. et al. (1996)	Edinburgh, Scotland	Prospective and retrospective	82 HIV+ women 693 HIV- women	Mean BW, WAZ	Drug use, smoking, parity, maternal weight, housing, hemoglobin, age	Mean BW not significantly different, WAZ significantly lower
Johnstone, F.D. et al. (1988)	Edinburgh, Scotland	Prospective	50 HIV+ women 64 HIV- women	LBW	No multivariate analysis	Not significantly different
Markson, L.E. et al. (1996)	New York	Medicaid claims review	772 HIV+ women 2377 control women	LBW	Drug use, race, age, education, tobacco, year, location, Medicaid, prenatal care	Significant odds for LBW
Minkoff, H.L. et al. (1990)	Brooklyn and Bronx, New York	Prospective	91 HIV+ women 126 HIV- women	Mean BW	Drug use, tobacco, age, clinic	No significant difference
Selwyn, P.A. et al. (1989)	New York, New York	Prospective	25 HIV+ women 44 HIV- women	Mean BW, SGA	Prematurity	No significant difference for mean BW or SGA
			Developing Countries			
Bailey, R.C. et al. (1999)	Kinshasa, Democratic Republic of Congo	Prospective	260 HIV+ women 258 HIV- women	Mean BW, WAZ, LBW	Maternal stature	Mean weights of infants born to HIV+ women similar, but less than controls; significant risk of LBW

Bulterys, M. et al. (1994)	Butare, Rwanda	Prospective	318 HIV+ women 309 HIV- women	Mean BW, LBW	For LBW: age, parity, marital status, income, education, cigarette use, syphillis, other STDs	Significant lower mean BW and and higher LBW incidence
Halsey, N.A. et al. (1990)	Port-au-Prince, Haiti	Prospective	199 HIV+ women 1994 HIV- women	Mean BW, LBW	Non-drug using population	Significant lower mean BW and and higher LBW incidence
Leroy, V. et al. (1998)	Kigali, Rwanda	Prospective	384 HIV+ women 381 HIV- women	LBW, IUGR	Gestation; for IUGR: STDs, anamia, malaria, education, age, gravida, ulceration	Significant relative risk for LBW, IUGR not significant
Sutton, M.Y. et al. (1999)	Kinshasa, Democratic Republic of Congo	Nested case-control	215 HIV+ women 206 HIV- women	LBW	Malaria, age, SES, gravida, trichomoniasis, chorioaminoitis	Significant odds for LBW
Taha, T.E.T. et al. (1995)	Blantyre, Malawi	Prospective	694 HIV+ women 691 HIV- women	LBW, IUGR	No multivariate analysis	Significantly higher IUGR and LBW incidence
			Meta-Analysis			
Brocklehurst and French (1998)	N/A	Meta-analysis	31 prospective studies conducted in developed and developing countries	LBW, IUGR	N/A	Odds ratio for LBW: 2.09 (1.86-2.35); for IUGR: 1.70 (1.43-2.02)

Note: HIV+: HIV-infected, HIV-: HIV-negative, BW: birthweight, LBW: low birthweight, SGA: small for gestational age, LGA: large for gestational age, WAZ: weight-for-age z-score, IUGR: intrauterine growth retardation, STDs: sexually transmitted diseases.

whether the infection was present at birth. In five such studies in non-breastfeeding populations, no significant difference was observed for mean birthweight in HIV-positive infants, compared with seroreverters.[22-26] Distinction between intrauterine and intrapartum transmission cannot be made when HIV infection is determined by polymerase chain reaction after one week of age. This distinction is important because intrauterine transmission may be causative for lower birthweight, whereas lower birthweight may be causative for intrapartum transmission.

Two studies that utilized an early PCR test observed different results regarding the association of LBW with presumed intrauterine and intrapartum transmissions.[5,27] The number of subjects and the covariates considered were similar for both studies. Mock et al. studied a cohort of 218 formula-fed infants in Thailand.[27] Twelve infants had presumed intrauterine transmissions and 37 had presumed intrapartum transmissions. Kuhn et al. studied a cohort of 276 infants of HIV-positive women in New York.[5] Twelve infants had presumed intrauterine transmissions and 36 had presumed intrapartum transmissions. Both studies controlled for obstetric factors, maternal immunological parameters at birth, and length of gestation. Mock et al. observed that LBW was associated with presumed intrauterine transmission. The adjusted odds ratio for this outcome was 5.2 (1.3 to 18.9).[27] Kuhn et al. observed that LBW was associated with presumed intrapartum transmission, with an adjusted odds ratio of 4.26 (1.57 to 11.56).[5] They also observed an association of small-for-gestational age and presumed intrauterine transmission. The different results noted may reflection the differing proportions of infants with LBW and presumed intrapartum transmission present in the studies. Only two of the 37 infants with presumed intrapartum transmissions were observed to have LBW in Thailand,[27] but 19 of 36 were observed to have LBW in New York.[5] Further research is needed to elucidate an understanding of the association of birth size and presumed intrauterine and intrapartum transmissions.

GROWTH

Growth is an important predictor of morbidity and mortality for HIV-infected infants. Growth impairment has been observed to be associated with the occurrence of persistent diarrhea[28] and bacterial lung infections in HIV-infected infants.[29] The presence of these illnesses also increased the risk for mortality.[20,28,30] Most studies indicate that impaired weight growth is predictive for survival among HIV-infected children.[31-34] However, the impact of impaired length growth is less well defined. Carey et al. noted that both weight and height velocity provided significant information about the risk of death,[32] but McKinney et al. observed no predictive value of initial height-for-age z-score and height growth rates for survival.[34] These studies suggest that maintaining adequate growth of HIV-infected infants could be important for decreasing morbidity and increasing survival time.

Limited studies are available assessing longitudinal growth of children born to HIV-positive women, particularly in developing countries.[15,31,35,36] Three studies were conducted utilizing antibody testing for HIV determination in children.[31,35,36] In these breastfeeding populations, actual timing of transmission could not be known from

antibody testing. The timing of transmission is an important consideration, because differences in growth may exist prior to and following seroconversion. However, growth before and after seroconversion has yet to be evaluated.

Length

Impairment of linear growth has been frequently observed in children infected with HIV infection (Table 2). Many of the studies conducted in developed countries assessed the difference in linear growth between HIV-positive infants and seroreverted infants. Four of six such studies observed significantly lower linear growth in the infected infants and children.[22,24,26,37] The observed age at which HIV-infected infants began to exhibit lower length-for-age compared to seroreverted infants varied from birth to 15 months. In Europe, HIV-positive infants followed from birth to 48 months of age were noted to be 0.8 cm shorter by 3 months of age.[22] They remained significantly shorter than seroreverted infants throughout the study. From birth to 18 months, mean length-for-age z-scores were observed to be significantly lower in HIV-infected infants.[24] The HIV-infected infants were calculated, after adjustment for gender and maternal characteristics, to be 2.25 cm shorter than seroreverted infants by 18 months of age. HIV-infected children followed from birth to 70 months of age did not exhibit significantly lower height than seroreverted children until after 15 months of age.[26]

In a retrospective analysis of HIV-infected and seroreverted infants, seroreverted infants were observed to be longer from 4 months to 24 months of age.[37] Additionally, 33% of HIV-infected children aged 3 months to 15 years exhibited severe height growth failure in a multicenter United States clinical trial.[32] This height growth failure was independent of examining center, parity, race, sex, gestational age, and caregiver.

Two studies did not observe differences in linear growth between infected and seroreverted infants. These studies utilized smaller sample than the other studies, and that may have limited their power to detect differences in growth. Compared with seroreverters, lower mean length values for HIV-infected infants were noted only at months 3 and 6 in a New York City study in which 64 children were followed for their first 18 months of life.[25] Peters et al. only observed stunting in symptomatic children receiving nutritional support.[38] In contrast to other studies of HIV and growth, very sick children were included.

In developing countries, the presence of HIV infection has been observed to be associated with stunting in cross-sectional and longitudinal analyses. HIV-infected children aged 17 to 41 months in Uganda had approximately 6.5 times greater odds of being stunted compared to HIV-negative children in a cross-sectional analysis.[39] All four longitudinal studies conducted in developing countries observed lower linear growth in HIV-infected infants.[15,31,35,36] Infants followed for 25 months were noted to have lower length-for-age growth curves than both seroreverted infants and infants born to HIV-negative women.[31] This difference in growth persisted after the exclusion of children with early mortality.

Two studies observed lower growth in HIV-infected infants from birth. In Rwanda, infants were followed from birth to 48 months of age.[35] HIV-infected infants in this study generally had significantly lower length-for-age values than infants

Table 2 Summary of Studies Examining Infant HIV Status and Growth

Authors	Location and Infant Ages	Type of Study	Subjects and HIV Testing[a]	Outcomes Measured	Variables Controlled	Results
			Developed Countries			
Agostoni, C. et al. (1998)	Milan, Italy, 1 to 24 months	Prospective	119 seroreverted infants 65 with HIV- mothers, CDC criteria	WAZ, LAZ, WLZ	For LAZ at 18 months: first born, father's income, maternal drug addiction during pregnancy, maternal education	Seroreverted had lower adjusted LAZ at 18 months, no differences in WAZ, unadjusted WLZ lower from 1-3 months for seroreverted
Carey, V.J. et al. (1998)	United States, 3 to 180 months	Prospective	1338 HIV+ children	Height and weight velocity	No multivariate analysis for growth	33% of HIV+ infants exhibted severe height growth failure, and 20% exhibited severe weight growth failure
European Collaberative Study (1995)	Europe, birth to 48 months	Prospective	123 HIV+ children 654 seroreverted children, Ab, Vcult, Ag	Weight, height	Examining center, parity, maternal race, maternal IV drug use, care giver, sex and gestational age	HIV+ infants significantly lighter and shorter at 3 months throughout the study
McKinney, Jr., R.E. et al. (1993)	Durham, North Carolina, 1 to 25.5 months	Retrospcctive	62 HIV+ infants 108 seroreverted infants, Ab, Vcult	WAZ, LAZ, WLZ	No multivariate analysis for growth	HIV+ infants shorter from 4 to 24 months, HIV+ infants lighter throughout (except months 1, 3, 21, 24), no difference in WAZ
Moye, J. et al. (1996)	United States and Puerto Rico, birth to 18 months	Prospective	59 HIV+ infants 123 seroreverted infants, Vcult	Weight, length, WAZ, LAZ, WLZ	Sex, prenatal smoking, alcohol and drugs, maternal education, prenatal CD4+	HIV+ had significantly lower LAZ, WAZ, and WLZ at 18 months

Peters, V.B. et al. (1998)	New York, New York, 1 to 168 months	Retrospective	54 HIV+ children 16 seroreverted children, documented HIV+	WAZ, LAZ, WLZ	No multivariate analysis for growth	HIV+ children receiving nutritional support had significantly lower WAZ, LAZ, WLZ than other children, no differences between other groups
Pollack, H. et al. (1997)	New York, New York, birth to 18 months	Retrospective	22 HIV+ infants 42 seroreverted infants, Ab, Vcult, Ag, PCR	Weight, length	No multivariate analysis for growth	No difference for weight, length only significantly different at 3 and 6 months
Saavedra, J.M. et al. (1995)	Baltimore, Maryland birth to 70 months	Prospective	59 HIV+ children 50 seroreverted children, Ab, Vcult, Ag	WAZ, HAZ	Separation analyses for boys and girls	HAZ significantly lower for HIV+ after 15 months, WAZ significantly lower for HIV+ after 36 months
			Developing Countries			
Bailey, R.C. et al. (1999)	Kinshasa, Democratic Republic of Congo, birth to 20 months	Prospective	69 HIV+ infants 191 seroreverted infants 256 with HIV- mothers, Ab, Vcult, PCR	WAZ, LAZ, WLZ	No multivariate analysis for general growth	HIV+ infants were shorter and lighter by 3 months and remained than other 2 groups, HIV+ had lower WLZ after 12 months than other 2 groups, seroreverted had lower WLZ at 10 months and remained than those with HIV- mothers
Berhane, R. et al. (1997)	Kampala, Uganda, birth to 25 months	Prospective	84 HIV+ infants 251 seroreverted infants 124 with HIV- mothers, Ab	WAZ, LAZ	No multivariate analysis for growth	HIV+ had lower mean weight at 6 months than other 2 groups, HIV+ had lower WAZ and LAZ curves than other 2 groups

Table 2 Summary of Studies Examining Infant HIV Status and Growth *(continued)*

Authors	Location and Infant Ages	Type of Study	Subjects and HIV Testing[a]	Outcomes Measured	Variables Controlled	Results
Lepage, P. et al. (1996)	Kigali, Rwanda, birth to 48 months	Prospective	46 HIV+ children 140 seroreverted children 218 with HIV- mothers, Ab	WAZ, HAZ	No mutlivariate analysis for growth	HAZ and WAZ less in HIV+ infants than those with HIV- mothers for first 36 months, WLZ only lower for HIV+ at 5, 6, 24 and 36 months, no differences between seroreverted and those with HIV- mothers
Semba, R.D. et al. (1997)	Blantyre, Malawi, birth to 24 months	Prospective	328 infants born to HIV+ women, Ab	Weight, length	Infant age, sex, maternal BMI, maternal vitamin A	Weight and length were significantly lower in HIV+ infants than seroreverted infants
Waibale, P. et al. (1999)	Kampala, Uganda, 17 to 41 months	Cross-sectional	50 HIV+ children 193 HIV- children, Ab	HAZ	No multivariate analysis for HAZ	Odds ratio for stunting with HIV+ was 6.62 ($p < 0.0001$)

Note: HIV+: HIV-infected, HIV-: HIV-negative, Ab: antibody testing, Vcult: viral culture, Ag: antigen testing, PCR: polymerase chain reaction, WAZ: weight-for-age z-score, LAZ: length-for-age z-score, WLZ, weight-for-length z-score, HAZ: height-for-age z-score, BMI: body mass index.

[a] Not all HIV testing methods were used for each infant.

from birth to 36 months of age born to HIV-negative mothers. Throughout a 24 month study in Malawi, linear growth was significantly lower after adjustment for vitamin A status in HIV-infected infants compared with seroreverted infants.[36] Length values were not noted to be significantly different at birth in infants studied in the Democratic Republic of Congo.[15] HIV-infected infants in this group were significantly shorter by 3 months of age, and remained shorter than seroreverted infants and infants born to HIV-negative women throughout the 20 month study. HIV infection and associated illnesses were independent predictors of growth impairment in this population. Additionally, the odds of an infant falling below –2 standard deviations of mean reference curves for length for age by 20 months was 2.10 (95% confidence intervals: 1.30 to 3.39), after controlling for maternal stature.[15]

Fewer studies have compared seroreverted children with children born to HIV-negative women. In Italy, a study comparing growth of seroreverted infants and infants born to HIV-negative women observed lower length-for-age z-scores at 18 months of age for seroreverted infants.[40] The effect of being a seroreverter persisted after adjustment for SES, sex, and maternal characteristics. However, growth values of seroreverted infants and infants born to HIV-negative women were observed to be similar in developing countries. Utilizing control groups of infants born to HIV-negative women and comparing growth with seroreverted infants in the Democratic Republic of Congo and Rwanda, no differences in linear growth were observed between these two groups.[15,35]

Weight

Impairment of weight has also been observed in HIV-infected children, and exhibits a similar pattern to differences in length (Table 2). The same four studies that observed differences in length between HIV-infected infants and seroreverted infants also observed differences in weight between these two groups. Three months after birth, HIV-positive infants were observed to be an average of 400 g lighter than seroreverted infants.[22] The HIV-infected infants were significantly lighter at 3 months of age and remained lighter for the 48-month study. Mean weight-for-age z-scores were lower in HIV-infected infants measured from birth to 18 months of age.[24] The HIV-infected infants were calculated to be 710 g lighter compared with seroreverted infants at 18 months, after adjustment for gender and maternal characteristics.[24] Compared to observations that length differed between HIV-infected and seroreverted children after 15 months of age, significant differences in weight were not observed until after 36 months of age.[26] This is an interesting finding because the pattern is contrary to the usual development of malnutrition, in which significant weight impairment is noted before significant length impairment. Weight was generally lower in HIV-infected infants in a retrospective analysis of infants under 25.5 months of age.[37] Additionally, 20% of HIV-infected children in a U.S. multicenter trial exhibited severe weight growth failure independent of socio-demographic variables.[32] The two studies that did not observe significant differences between HIV-infected and seroreverted children utilized smaller samples[25,38] and very sick children.[38]

In developing countries, HIV-infected infants were observed to be significantly lighter in four studies conducted. HIV-infected infants were observed to have

significantly lower weight-for-age growth curves than seroreverted infants and infants born to HIV-negative women in the first 25 months of life.[31] Lower growth curves remained significant after exclusion of infants with early mortality. In Rwanda[35] and Malawi,[36] HIV-infected infants were noted to be lighter compared with controls at birth and remained lighter throughout the studies, which were conducted for 48 and 24 months, respectively. HIV-infected infants in the Democratic Republic of Congo were significantly lighter by 3 months of age compared with seroreverted infants and infants born to HIV-negative women.[15] These infected infants remained significantly lighter throughout the 20-month study. The HIV-positive infants in this sample had an observed odds ratio of 2.84 (95% confidence interval: 1.58 to 5.11) for falling below –2 standard deviations of mean reference curves for weight-for-age after control for maternal stature.

In general, seroreverted infants have not exhibited lower weight growth compared to infants born to HIV-negative women in both developed and developing countries. Differences in weight-for-age z-scores were not observed between seroreverted infants and infants born to HIV-negative women in Italy followed for 2 years after birth.[40] Similarly, differences in weight growth patterns were not observed between seroreverted infants and infants born to HIV-negative women in the Democratic Republic of Congo and Rwanda followed for 20 months and 48 months, respectively.[15,35]

Weight-for-Length

Inconsistent results regarding differences in weight-for-length between HIV-infected and non-infected infants have been observed (Table 2). In the U.S., HIV-infected infants exhibited lower mean weight-for-length z-scores than non-infected infants did during the first 18 months of life.[24] HIV infection was the only independent predictor of weight-for-length at 18 months of age when assessed in multivariate regression with gender, prenatal smoking, prenatal alcohol, prenatal drugs, maternal education, and prenatal $CD4^+$ percentages as covariants in the model. No differences in weight-for-length between infected and non-infected children less than 25.5 months of age were observed in the U.S.[37] HIV-infected children in the Democratic Republic of Congo became significantly wasted after 12 months of age and remained wasted through a 20-month study.[15] Weight-for-length, measured at 3 month intervals in the first year of life and at 6 month intervals thereafter for 48 months, was generally not observed to be lower in HIV-infected infants.[35] For HIV-infected infants, weight-for-length z-scores remained near or above reference medians.[37] This finding may not reflect growth impairment in HIV-infected children if both length and weight are proportionally decreased.

Seroconversion and Growth as Risk Factors for Transmission

Studies that examine the effect of HIV seroconversion after birth on infant growth patterns are limited. Growth impairment after birth has not been examined as a potential risk factor for postnatal HIV transmission. Assessing the impact of seroconversion upon growth and whether impaired growth is a risk factor for transmission would be difficult. The majority of infants who are infected postpartum are infected

within their first few months of age. This limits the number of growth measurements available prior to seroconversion. Determining the exact timing of HIV transmission is also difficult. However, the effect of HIV infection upon immunologic factors and the effect of growth impairment upon immune function indicate that associations may exist between seroconversion and growth.

The effect of HIV on immunologic factors indicates that seroconversion may negatively impact growth. Cytokine production, particularly of interleukin-6 (IL-6), may have osteotrophic effects. In mice, treatment with mouse IL-6 and soluble mouse IL-6 receptor resulted in osteoclast-like multinucleated cell formation.[41] Results of this study suggested that the soluble IL-6 receptor induces osteoclast formation in the presence of IL-6. Elevated levels of IL-6 and the IL-6 receptor were present in cells of adult donors infected with HIV.[42] These observed elevated levels of IL-6, compared with HIV-negative controls, were irrespective of the diagnoses for the subjects as positive without AIDS related complex (ARC) or AIDS, as having ARC, or as having AIDS. Similar metabolic responses to cytokine production may also occur in children with HIV, who exhibit elevated levels of IL-6 compared with controls.[43]

Malnutrition exhibits a negative impact on immune function.[44] Among many factors, reduction in numbers of mature fully differentiated T lymphocytes and a decreased proportion of helper T cells during malnutrition indicate a decrease in immunocompetence. A decreased proportion of helper T cells can result in a reduced CD4 to CD8 cell ratio.[44] Thus, undernutrition and accompanying impaired immunocompetence of an infant may influence postnatal transmission through breastfeeding.

Mechanisms of Impaired Growth for HIV-Infected Infants

The specific etiology for growth impairment in HIV-infected infants has not yet been elucidated, but likely involves a combination of mechanisms. Malnutrition and subsequent growth impairment occur as a result of decreased intake, increased energy demands, or both. Decreased intake may be secondary to decreased oral intake or decreased absorption. Increased demands can occur as a result of altered metabolic or endocrine functions. The presence of HIV infection may put infants at greater risk for such alterations. Abnormalities in absorption, metabolism, and endocrine function are frequently observed in HIV-infected adults with wasting.[45] However, relatively few pediatric studies have been conducted to examine the mechanisms of HIV infection and growth impairment and the primary effect of HIV growth after controlling for co-morbidity.

Decreased oral intake results in subsequent growth impairment. In a cohort of HIV-infected children in the U.S., whose energy intakes met the U.S. Recommended Daily Allowances, their intake predicted better weight outcomes.[46] Although the children had energy intakes meeting recommendations, their growth was below standard, suggesting that suppressed growth resulted despite intakes meeting the recommendations.

Decreased intake can also be secondary to decreases in gastrointestinal absorption. The presence of HIV may increase the risk of infection with enteric pathogens. These pathogens may act directly to decrease absorption by causing diarrhea,

gastrointestinal bleeding, and intestinal dysfunction.[45] Malabsorption may also occur indirectly through the effect of HIV infection of the epithelium, although this has not been documented directly in children. Malabsorption is a frequent finding in children with HIV infection.[47,48] However, it is not likely to be the sole etiology of growth impairment. Growth failure in children has been observed in the absence of clinical symptoms, indicating malabsorption.[47]

Increased demands can occur as a result of altered endocrine or metabolic function. Increases in some cytokines, such as tumor necrosis factor (TNF), can induce a catabolic state in humans. Elevated TNF has been observed in HIV-infected children,[49] but an associating with growth impairment has not been established. Endocrine function was assessed in 14 HIV-infected children, aged 6 months to 10 years.[50] Overt abnormalities in endocrine function were not observed. The children were clinically and biochemically euthyroid, but the authors noted a state of compensated hypothyroidism in a subset of five of the children.[50] A similar but not significant trend of hypothyroidism was also observed in the assessment of nine HIV-infected children.[51] Whether decreased thyroid function impairs growth in HIV-infected children is not known.

It is also important to consider co-morbidity as a factor in growth impairment of HIV-infected infants. Diarrheal disease is associated with increases in growth impairment of children.[52] The etiology for this is likely multifactorial, involving mucosal changes, metabolic responses, and decreased energy consumption. Energy consumption from non-breastmilk foods, but not breastmilk, has been observed to decrease in infants with diarrhea or fever.[53] Diarrhea also increases the transit time of nutrients through the gastrointestinal tract. HIV-infected infants are more susceptible to concurrent infection than uninfected infants. HIV-infected children in Zaire were noted to have significantly more episodes of acute and persistant diarrhea compared with uninfected children.[28] It is difficult to measure the relative contributions of concurrent infections and HIV infection upon infant growth and currently no studies have separated the effects of these two factors on growth.

CONCLUSION

Birthweight and subsequent growth are important factors in infant morbidity and mortality. HIV infection has been documented to negatively influence both birthweight and growth. Results differ regarding whether maternal HIV infection is associated with lower birthweight, particularly between developed and developing countries. A negative association of HIV infection in the mother with birthweight has been observed more frequently in developing countries. Birthweight and the presence of HIV infection in infants has not been widely studied. Recent data illustrate an association between low birthweight and infant HIV infection when controlling for intrauterine or intrapartum transmission. However, whether infant HIV infection is associated solely with intrauterine or intrapartum transmission, or whether it is associated with both types of transmission, has not yet been fully established.

Longitudinal growth, measured as weight and length, is decreased in HIV-infected infants, compared with both infants born to HIV-negative women and

HIV-negative infants born to HIV-positive women. This decrease in growth persists after controlling for other factors known to influence growth. Differences in weight-for-length measurements have not been observed in HIV-infected infants, suggesting proportional decreases in weight and length. Presence of maternal infection does not appear to negatively influence growth of seroreverted infants. Differences in longitudinal growth of seroreverted infants and infants born to HIV-negative women have not been observed.

Although some studies indicate that infants born to HIV-positive women exhibit similarities in birth size, regardless of infant HIV status, the growth patterns of infected infants differ from those of seroreverters. Lower length-for-age and weight-for-age values are commonly observed for HIV-infected infants, but decreased weight-for-length is not as frequently observed. Significant differences in growth between non-infected infants born to HIV-positive mothers and infants born to HIV-negative mothers have not been observed. When seroreverted infants are smaller at birth, they appear to exhibit a catch-up growth that does not occur in HIV-infected infants. After this catch-up growth, seroreverted infants exhibit growth similar to infants born to HIV-negative women.

Whether HIV infection decreases infant growth or impaired growth is a risk factor for infection is not known. However, immunologic changes that occur with HIV infection indicate that seroconversion could increase the risk for decreased growth. Additionally, malnutrition is known to impair immunocompetence, which could influence the risk of postpartum HIV transmission. The mechanisms of growth impairment in HIV-infected infants have not been fully elucidated. Growth impairment appears to have a complex etiology, involving absorption, and endocrine and metabolic factors.

In areas where HIV testing and related medical therapy are accessible, both provide important supplements to adequate prenatal care. Antiretroviral therapy can be beneficial for both reducing vertical HIV transmission and improving birthweight. In other areas, general preventative measures involved in prenatal care, such as maternal weight monitoring and treatment of concurrent infections, are crucial for aiding in reducing the incidence of LBW. Continued growth monitoring of HIV-infected infants is important for early identification of and intervention for infants whose growth is impaired. Since the etiology of growth impairment appears to be complex, treatment should take into consideration all potential factors that may be negatively influencing growth.

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