

# Nutrients and HIV: Part Three – N-Acetylcysteine, Alpha-Lipoic Acid, L-Glutamine, and L-Carnitine

Lyn Patrick, ND

## Abstract

The role of antioxidants in preventing apoptosis and viral activation in HIV is well documented. N-acetylcysteine, glutathione, and alpha-lipoic acid have been shown to interrupt the process of viral activation and CD4 cell death. L-glutamine has been shown to improve glutathione levels and significantly increase lean body mass in HIV infection. The literature on the use of L-carnitine and acetyl-L-carnitine in treating mitochondrial toxicity, both in muscle and nerve pathologies is relevant in nutritional treatment of HIV, given the mitochondrial toxicity of nucleoside analog reverse transcriptase inhibitor therapy. The current use of highly-active antiviral therapies, their toxicity, and significant failure rates have created the need for a more conservative reassessment of HIV treatment. The adjunctive use of nutrient therapy in the treatment of HIV is reviewed here.

(*Altern Med Rev* 2000;5(4):290-305)

## The Importance of Redox Homeostasis in HIV

HIV infection and the progression to AIDS involves a long period of latent infection characterized by low levels of viral replication that slowly increase to the point of immunosuppression.<sup>1</sup> This progression is accelerated if the latent (non-reproducing) provirus in the nuclei of the lymphocyte is activated.<sup>2</sup> Oxidative stress induces both viral activation of HIV and DNA damage, leading to immunosuppression.<sup>3-5</sup> It is now generally accepted that a central pathologic feature of HIV disease involves oxidative stress, leading to programmed cell death (apoptosis) and depletion of CD4 cells.<sup>6,7</sup> It has been hypothesized by Montagnier and others<sup>8,9</sup> that the majority of T-helper (CD4+) cell loss (the cell most susceptible to fatal injury by HIV) actually occurs by apoptosis and not by direct HIV infection. This phenomenon has been seen in *in vitro* culture and in peripheral blood lymphocytes from HIV-infected patients.<sup>10</sup>

Evidence of increased oxidation reactions,<sup>11</sup> depletion of the glutathione-based antioxidant defense system,<sup>12</sup> and increased levels of oxygen radicals have been demonstrated in the blood and tissues of HIV-infected individuals.<sup>6</sup> Elevated levels of hydroperoxides,<sup>13</sup> malondialdehyde,<sup>14</sup> and deficiencies of the critical antioxidant enzymes manganese superoxide dismutase, glutathione peroxidase, thioredoxin, and catalase have been demonstrated in plasma, lung lining, erythrocytes, and lymphocytes in HIV-infected individuals.<sup>4,7,13</sup> Nutrient malabsorption, glutathione and selenium depletion, and reduction of total thiol (cysteine) levels have all been observed to be associated with the pathology of free radical overload that leads to the cellular apoptosis of T lymphocytes.<sup>15</sup>

---

Lyn Patrick, ND – Associate Editor, *Alternative Medicine Review*; Private Practice, Tucson, AZ.  
Correspondence address: 540 W Prince, Ste A, Tucson, AZ 85705.

## Glutathione: Antioxidant and Antiviral

Glutathione, the most abundant cellular thiol, provides the major antioxidant defense mechanism in all mammalian cells by neutralizing toxic peroxides.<sup>16</sup> It also helps to maintain levels of ascorbate and tocopherol by acting as a reducing agent.<sup>17</sup> Glutathione is necessary for maintaining immune mediated T-cell activation and phagocytosis, in addition to cellular and antibody mediated cytotoxicity,<sup>18</sup> and a normal balance between the T-helper cell 1 (IL-2, IL-12, gamma-interferon) and the T-helper cell 2 (IL-6, IL-4, tumor necrosis factor-alpha, IL-10, IL-1) cytokine response profile.<sup>19</sup> Glutathione conjugation is also the primary mechanism of eliminating electrophilic xenobiotics (some of which are carcinogens) in the liver.<sup>20</sup>

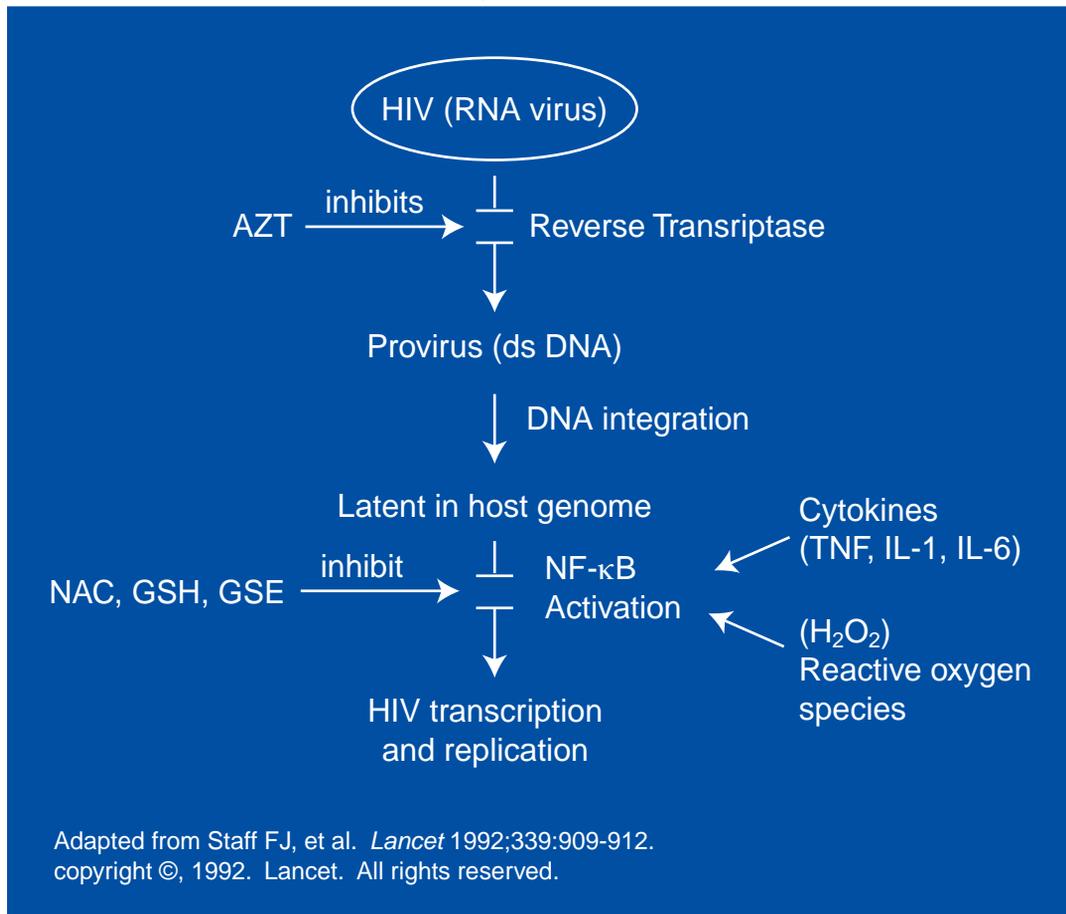
Glutathione deficiency has been theorized to be the cause of the increased sensitivity HIV-infected individuals have to high doses of acetaminophen and sulphamethoxazole, a medication used in the prevention of *Pneumocystis pneumonia*. The metabolic fate of these medications, the hepatic glutathione-S-transferase/mercapturic acid pathway, is less efficient in individuals with glutathione deficiency.<sup>21-24</sup> Plasma glutathione levels in HIV-infected individuals, even in the asymptomatic state, have been found to be depressed as early as three weeks post-infection.<sup>10</sup> Glutathione levels in lung epithelial fluid have been found to be depressed as much as 60 percent when compared to HIV-negative controls.<sup>25</sup> Intracellular glutathione levels in both infected CD4 and CD8 lymphocyte subsets are also significantly depressed; levels from 62-69 percent of normal have been found in the CD4 and CD8 lymphocytes of HIV and AIDS patients.<sup>12</sup> These figures become relevant in light of studies that show glutathione reduction of 10-40 percent is capable of completely inhibiting T-cell activation *in vitro*.<sup>26</sup> While not all studies have found depressed glutathione

levels in plasma or lymphocytes of infected individuals,<sup>27,28</sup> levels of reduced glutathione do appear to be disturbed in HIV.<sup>29</sup> Research assessing ratios of reduced-to-oxidized glutathione in HIV-positive patients found significantly increased levels of oxidized glutathione and subsequently lower levels of reduced glutathione when compared to HIV-negative controls.<sup>30</sup> These disturbances were greater in patients with more advanced disease, the ratios being higher than have been found in human lymphocytes in any other disease state.<sup>30</sup> Low serum thiol levels (precursors to glutathione) have been shown in HIV-infected, injecting drug users (IDU) to be associated with an increased risk of mortality: IDU with low serum thiol levels are 5.65 times more likely to experience an accelerated time-to-death.<sup>5</sup>

## Mechanisms of Apoptosis

The response to antigenic material and the presence of cytokines, hydroxyl radicals or other viruses can trigger the activation of nuclear factor kappaB (NF-kB).<sup>2</sup> A gene-transcription regulating factor present in lymphocytes, macrophages, and monocytes, NF-kB activates genes in the nuclear material of these cells, resulting in production of cytokines and major histocompatibility (MHC) agents.<sup>31</sup> NF-kB also binds to HIV proviral gene material in the nucleus of HIV-infected cells and activates HIV replication.<sup>32</sup> Viral replication, in turn, increases cellular levels of cytokines, like tumor necrosis factor-alpha (TNF $\alpha$ ), that promote the production of free radicals and the activation of NFkB, initiating a vicious cycle of viral replication and free radical production.<sup>23</sup> The presence or absence of reducing thiols (sulfhydryl groups that form the basis for antioxidant enzymes, glutathione, etc.) appears to directly affect this cycle. In cell cultures where glutathione levels or thiol levels have been depleted, the TNF $\alpha$ -stimulated activation of HIV is enhanced.<sup>26</sup> In cell cultures where N-acetylcysteine (NAC)

**Figure 1: Synergistic Effects of Thiol-Enhancing Substances and Anti-retroviral Drugs on HIV Infections.**



(an efficient thiol source and glutathione precursor) is added, the activation of NF-κB is blocked (Figure 1).<sup>26</sup> Glutathione has also been shown to directly inhibit the activity of reverse transcriptase (a major enzyme necessary for HIV replication) by 80-90 percent in cell cultures.<sup>33</sup>

### Glutathione Restoration

The attempt to find antioxidants or glutathione “pro-drugs” that recreate normal glutathione states and block production of TNF-α and NF-κB has led to the creation of antioxidant strategies for preventing CD4 cell decline.<sup>34</sup> Data showing that cysteine, N-acetylcysteine (NAC), reduced glutathione, and ascorbic acid all suppress NF-κB activity and HIV activation in cell lines<sup>33</sup> has resulted in the production of drugs like L-2-oxothiazolidine-4-carboxylic acid (OTC)

or “Procysteine.” While OTC has been shown to increase lymphocyte glutathione levels in healthy subjects, it is not itself an antioxidant and has not been shown to be as effective as N-acetylcysteine.<sup>35</sup> When OTC was compared to NAC, NAC was more effective at decreasing cytokine-induced HIV replication in different cell lines and replenishing intracellular glutathione levels.<sup>35</sup>

Glutathione, administered orally, has been demonstrated to be absorbed by rat intestine, kidney and lung epithelium,<sup>36</sup> but intact glutathione cannot be absorbed by T-cells, unless it is given as a glutathione monoester.<sup>37</sup> Glutathione monoesters, however, have been associated with significant toxicity.<sup>38</sup> Studies with oral dosing of glutathione in healthy human volunteers have shown no increase in cysteine, glutathione, or glutamate levels after a 3-gram dose,<sup>39</sup> leading

the authors to conclude: "It is not possible to increase glutathione to a clinically beneficial extent by the oral administration of a single dose of 3 grams of glutathione." Using the rate limiting amino acid L-cysteine to increase glutathione production may be inadvisable since it appears to autooxidize rapidly in the bloodstream and increase free radical load.<sup>40</sup>

### **N-Acetylcysteine as a Glutathione Regenerator**

N-acetylcysteine has been used successfully to treat hepatic and renal failure caused by glutathione depletion secondary to acetaminophen overdose.<sup>41</sup> It has an extensive history as a mucolytic and has been used in pulmonary diseases including emphysema, tuberculosis, chronic asthma, fibrosing alveolitis, and primary amyloidosis of the lung.<sup>42</sup> The mechanism of action in these respiratory conditions includes the restoration of reduced and total glutathione levels in lung cell fluid.<sup>43</sup> N-acetylcysteine has been demonstrated to have heavy metal chelating capacities for toxic metals, as well as for copper, zinc, and boron.<sup>44</sup> Several studies support evidence that NAC increases glutathione levels *in vivo* and *in vitro*;<sup>45-48</sup> there is also evidence NAC may boost cellular immunity directly.<sup>49</sup>

### **N-Acetylcysteine in HIV/AIDS**

CD4+ and CD8+ T-cells from HIV-infected individuals have an impaired ability to proliferate; they are also unresponsive to recall antigens and unable to secrete normal amounts of interleukin-2, exhibiting properties similar to a state of anergy.<sup>50</sup> This altered response appears to be the reason for the rebound of viral loads to pre-medication levels following discontinuation of triple anti-viral therapies even after one year of continuous and successful treatment.<sup>51</sup> NAC appears to be able to help restore CD4 cell function: in a study of 11 asymptomatic HIV-infected individuals with CD4+ counts of over 300/mL,

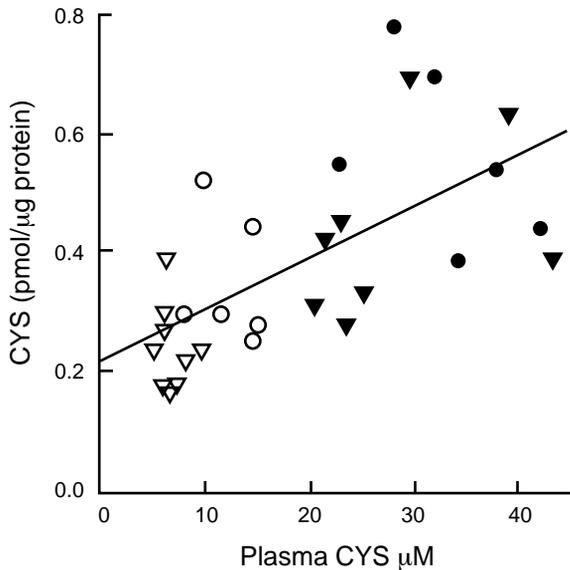
N-acetylcysteine (at 5, 10 and 20 mM) restored normal CD4+ proliferative responses in 8 of 11 patient blood samples.<sup>50</sup>

N-acetylcysteine appears to be beneficial in HIV as a result of its ability to restore normal glutathione levels in lymphocytes and thereby reduce free radical production.<sup>52</sup> NAC also, however, acts directly as an antioxidant.<sup>52</sup> Preincubation with 15 mM concentration of NAC was able to partially protect lymphocytes from asymptomatic HIV-infected patients after exposure to menadione, an oxidizing agent. This study also found a significant relationship between CD4+ counts, plasma peroxidation, and the ability of NAC to preserve the structural characteristics of the lymphocytes. The patients with lower CD4+ counts also had higher levels of lipid peroxidation products and were less protected by the same amount of NAC. As a direct free radical scavenger, NAC reduces hypochlorous acid produced by neutrophils in order to kill target cells.<sup>53</sup> Because NAC has a direct antioxidant action in lymphocytes, concern has been expressed that NAC supplementation would inhibit the natural mechanism of cytotoxicity by neutrophils. Cell studies<sup>54</sup> have shown NAC actually enhances intracellular killing of bacteria by protecting neutrophils and macrophages from free radical damage generated during phagocytosis. Cell studies with HIV-infected monocytes and neutrophils have shown similar results: NAC (at 1 and 5 mM concentrations), enhanced their cytotoxicity.<sup>53</sup> Multiple cell studies have shown NAC acts as an antiviral in HIV-infected cell lines; both by direct inhibition of TNF- $\alpha$ <sup>52,55-57</sup> and direct inhibition of viral transcription.<sup>55</sup>

### **N-Acetylcysteine in Clinical Trials**

The basic question underlying NAC clinical trials is whether the repletion of available thiol groups will normalize lymphocyte glutathione levels, minimize cytokine-induced viral proliferation, and stop the CD4+ cell depletion associated with

**Figure 2: Concentrations of Extra- and Intracellular Cysteine**



Correlation ( $p < 0.01$ ) between the extracellular and the intracellular concentration of cysteine (CYS) in peripheral blood mononuclear cells isolated before ( $\circ, \nabla$ ) and 2h after ( $\bullet, \blacktriangledown$ ) ingestion of 30 mg/kg N-acetylcysteine from HIV-infected patients ( $\circ, \bullet$ ) and healthy patients ( $\nabla, \blacktriangledown$ ). Taken from de Quay B, et al. *AIDS* 1992; 6:815-819.<sup>28</sup> copyright ©, 1992. AIDS. All rights reserved.

glutathione deficiency.<sup>15</sup> Clinical trials to date contain only partial answers to these crucial questions.

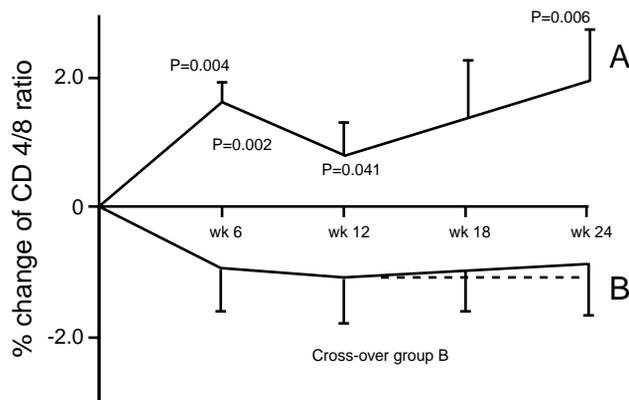
DeQuay et al<sup>28</sup> found significant reductions in cysteine and glutathione in all of nine HIV-infected subjects. Low baseline levels of cysteine in blood and in CD4, CD8 T lymphocytes, B-cells, and monocytes were returned to normal after a single oral dose of N-acetylcysteine (30 mg/kg body weight) (Figure 2). Glutathione levels were elevated four hours later in five of the nine subjects. The remaining four, who had the lowest baseline glutathione levels, each had less than 100/mm<sup>3</sup> CD4+ cells and did not exhibit increased glutathione levels with NAC treatment. The authors commented that glutathione production in CD4 and CD8 cells was slow, and felt a longer period of administration would be necessary to adequately assess glutathione production. The study included an HIV-positive patient whose lymphocyte glutathione levels doubled after seven days of 600 mg NAC three

times daily.

However, NAC clinical studies have not been consistent. In one trial examining 45 HIV-positive men and women, taking 800 mg daily for four months, NAC was successful in normalizing plasma cysteine levels and significantly reducing TNF- $\alpha$ , but there were no changes in glutathione levels.<sup>58</sup> Although none of the individuals was on an antiretroviral regimen, the median CD4+ count remained the same after four months while the control group had a significant CD4+ cell decline ( $p < 0.005$ ). The dosage was considerably lower in this trial (800 mg vs 30 mg/kg), which may account for the inability of NAC to affect glutathione repletion.

Leonore Herzenberg and her group at Stanford<sup>59</sup> studied glutathione repletion in 27 HIV-positive men who were taking 3200-8000 mg NAC daily (median 4400 mg). This high dose was chosen because it was below a previously determined maximum-tolerated dose, and it was based on a prior erroneous study that indicated NAC had low bioavailability. At baseline, the average glutathione levels in uninfected controls were 28-percent higher than the HIV-positive group with CD4+ counts over 200. The NAC treatment group had a significant elevation in whole blood glutathione after eight weeks while the control group remained unchanged. The average increase in the NAC group was 113 percent, an increase that brought the treatment group close to the baseline of uninfected controls (Figure 3). The trial was continued for two years, even though the treatment group was only given NAC for 8-32 weeks (median 24). After two years, the NAC group (25 subjects) had a significantly greater chance of surviving than the group who never took NAC ( $p = 0.002$ ). The most beneficial information derived from this study, however, was the data relating glutathione levels and survival in those who had not taken NAC. In those with CD4+ counts less than 200, 85

**Figure 3: Percent Changes in CD4/CD8 Ratios During 24 Weeks of Treatment with NAC and Selenium**



Taken from Look, MP et al. *Eur J Clin Invest* 1998; 28: 389-397.<sup>61</sup> copyright ©, 1996. European Journal of Clinical Investigation. All rights reserved

percent of the 28 subjects in the high lymphocyte glutathione group were still alive after 2.5 years. Only 18 percent of the 69 subjects in the low glutathione group survived. Although the number of subjects on NAC treatment was too small to attach significance to treatment outcomes, there was a very significant relationship of glutathione levels to survival.

Oliver<sup>60</sup> gave 15 HIV-positive individuals 600-1200 mg NAC daily for over six months. Peripheral blood lymphocyte apoptosis profiles were done at the onset and after six months of treatment. All of the HIV-infected subjects had evidence of significant apoptosis at baseline. The NAC subjects, however, had significantly less evidence of cell death compared to baseline, HIV-infected controls, and HIV-negative controls after six months.

A study combining the effects of NAC (600 mg three times daily) and selenium in the form of sodium selenite (500 mcg daily) was designed to answer questions about the effects of these antioxidants on glutathione production, lymphocyte subsets, and viral

load.<sup>61</sup> Twenty-four HIV-positive, anti-retroviral-naïve men and women (CD4+ counts 200-500/mm<sup>3</sup>) were randomized into two groups, one treated for 24 weeks and the other treated for the last 12 weeks of the study. A control group consisted of 25 healthy HIV-negative men. Baseline serum selenium and plasma glutathione (reduced and oxidized) levels were significantly reduced in the HIV-positive group. After six weeks of treatment, serum selenium concentrations increased by 53 percent and, although they dropped a little, remained at 45 percent above baseline for the duration of the treatment. Glutathione levels did not change in either group, but they were only measured at weeks 6 and 12. CD4+ percent (measured as a percentage of total lymphocytes) increased significantly

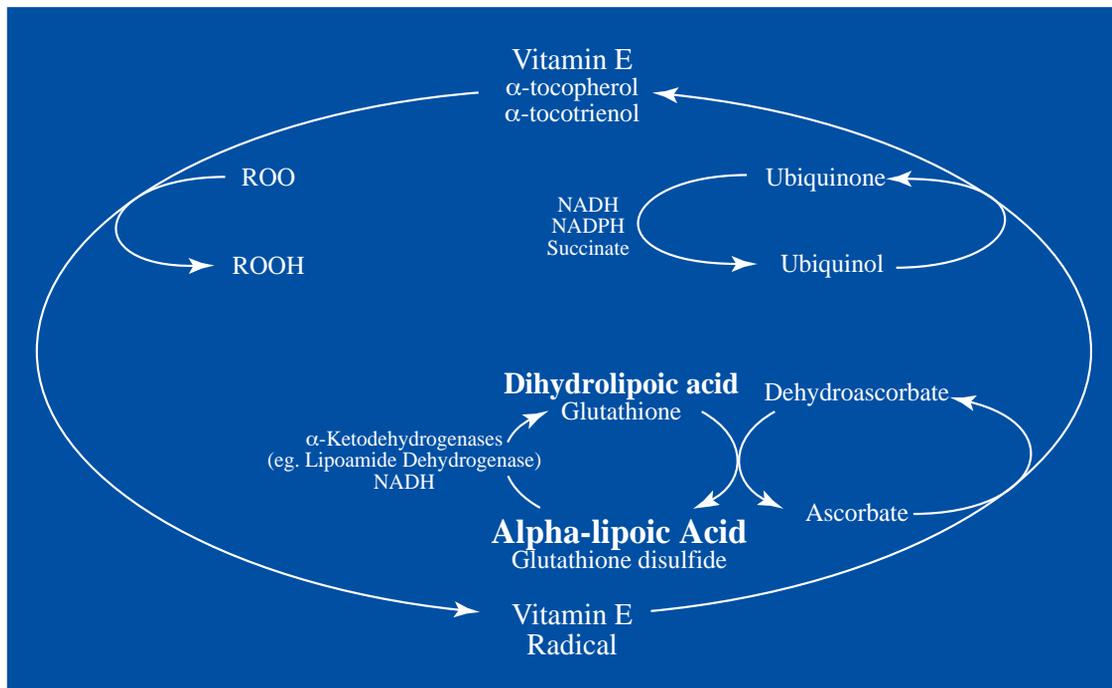
in the first group at week 6 and week 24. Suppressor cell (CD8+) levels fell significantly within six weeks, (closer to control levels) and remained there for 24 weeks. Although the reduction of CD8+ cells is difficult to interpret, the increase in CD4+ percentage was significant; a falling CD4+ percent has been shown to be an indicator of faster progression to AIDS.<sup>62</sup> It is important to note that viral load was unaffected by this treatment.

### Other Uses for NAC

NAC has also been used successfully in one case of AIDS-related porphyria cutanea tarda.<sup>63</sup> The patient was unresponsive to treatment (hydroxy-chloroquine 100 mg daily) and was subsequently given NAC 600 mg three times daily. His symptoms improved four weeks later and completely resolved in three months.

NAC has been found to improve response to interferon in hepatitis C patients previously unresponsive to four months of alpha-interferon. Fourteen patients who had not responded to a previous 4-month course of interferon were given a combination of

**Figure 4.**  $\alpha$ -Lipoic Acid and Antioxidant Regeneration



interferon and oral NAC, 600 mg. three times daily for 5 to 6 months. Ten patients who had no prior treatment were given NAC alone. Serum ALT returned to normal in 41 percent of the patients who were given combination therapy while those on NAC alone had no improvement.<sup>64</sup>

The evidence for *in vivo* reduction of viral growth and proliferation by NAC treatment is lacking. Because the cellular concentrations that can be obtained by large oral doses are approximately three orders of magnitude lower than those demonstrated *in vitro* (10 mM-20 mM), and even intravenous doses (10,500 mg over four hours) did not raise blood levels higher than 0.8 mM, NAC may not have direct antiviral effects *in vivo*.<sup>28</sup> Although NAC has not been found to lower viral loads, the evidence for normalization of lymphocyte glutathione levels<sup>28,59</sup> and reduction of oxidation products does suggest a possible use in prolonging survival in HIV-infected individuals.

### Antioxidant Effects of Alpha-Lipoic Acid

Alpha-lipoic acid is a dithiol antioxidant found primarily in the mitochondria where it acts as a coenzyme in the  $\alpha$ -keto-acid dehydrogenase complex.<sup>65</sup> Because it is a low weight molecular substance and is bioavailable from the diet, lipoic acid is absorbed from the gut and readily passes through the blood-brain barrier.<sup>66</sup> Active as an antioxidant in both lipid and non-lipid components, it can act in membranes of the brain and nervous tissue, lymphoid tissue, and in most other cell types.<sup>67</sup> Both as alpha-lipoic acid and in its reduced form dihydrolipoate, it is a potent antioxidant that can scavenge a wide variety of reactive oxygen species, including superoxide and peroxy radicals formed during lipid peroxidation.<sup>66</sup> Lipoic acid is able to regenerate ascorbate and tocopherol and to raise glutathione levels (Figure 4).<sup>67</sup>

Lipoic acid has recently been the subject of research in HIV as a result of its ability to raise glutathione levels significantly (30-70%) both *in vivo* and *in vitro*.<sup>68</sup> Alpha-lipoic acid also interrupts HIV replication by

completely blocking the activation of NF- $\kappa$ B in cell cultures at a concentration of 2 mM.<sup>69</sup> Lipoic acid has direct action as an HIV-1 replication inhibitor at concentrations of 70 mg/L and has a synergistic antiviral effect when combined with zidovudine (AZT) at a concentration of 7mg/mL.<sup>70</sup> The authors of this study conclude that since a 7 mg/mL concentration is reachable with oral therapy and since alpha-lipoic acid works to reduce levels of reverse transcriptase in a manner that is synergistic with AZT, it should be the subject of further research in HIV treatment.

A clinical trial in 11 AIDS-diagnosed patients taking 450 mg lipoic acid daily for 14 days resulted in increases in plasma ascorbate, total glutathione, total plasma thiols, CD4 and CD4/CD8 ratios, and decreases in lipid peroxide levels. The increases in CD4 cells in those patients who responded to the lipoic acid (6 out of 10) were significant: an average increase of  $141 \pm 121$  CD4 cells.<sup>71</sup>

### Alpha-lipoic Acid and HIV Therapeutics

Lipoic acid has been used in multiple trials to successfully treat diabetic neuropathy<sup>66</sup> and has been shown to limit excessive free radical-induced damage in the central nervous system. Although no studies have been published to date on the effect of alpha-lipoic acid in HIV-related peripheral neuropathy, a small placebo-controlled trial comparing alpha-lipoic acid to deprenyl was conducted to assess its effect in HIV dementia.<sup>72</sup> The study involved 36 patients with diagnosed HIV-related cognitive damage taking either lipoic acid, deprenyl, the combination, or placebo, and was designed as a tolerability trial, not an efficacy trial. Patients were given 600 mg alpha-lipoic acid twice daily, deprenyl 2.5 mg three times weekly, or both in combination for ten weeks. The alpha-lipoic acid was well tolerated with no apparent side-effects. Although the study did not show any beneficial effect in cognitive function or daily functioning, the

authors commented the trial may have been too short. The subjects were all intravenous drug users and were using injectable drugs during the study. Alpha-lipoic acid may also have usefulness in decreasing risk of kidney stones, a side-effect of the protease-inhibitor, antiviral drug indinavir (Crixivan).<sup>73</sup>

### Glutamine

L-glutamine is found in the body in higher quantities and concentrations than any other free amino acid; along with taurine it is the most abundant amino acid in skeletal muscle.<sup>74</sup> Although it can be manufactured in all cells, the majority is manufactured in skeletal muscle and transported to intestinal cells, kidney, and lymphocytes, particularly under conditions of physiologic stress. When the stress is prolonged, production in skeletal muscle may not meet the demand in organ and lymphoid tissue, and a deficiency can occur.<sup>75</sup> Lymphocytes, macrophages, and enterocytes of the small intestine are dependent on glutamine as their primary source of fuel.<sup>76</sup> Numerous studies have shown benefit when parenteral glutamine was given to patients undergoing surgery, chemotherapy, or irradiation; glutamine was effective both in improving immunocompetence and preventing small bowel mucosal damage.<sup>77-79</sup> Glutamine also minimizes enterocolitis secondary to chemotherapy in animal models.<sup>80</sup>

HIV infection appears to induce glutamine deficiency, possibly as a result of the rapid turnover of immune cells that occurs in the acute and chronic stages of the infection.<sup>81</sup> Glutamine supplies appear to be depleted even in asymptomatic individuals; supplementation with 20 grams of glutamine daily for a month failed to normalize glutamine levels in a cohort of symptomatic HIV-positive patients.<sup>82</sup>

Loss of lean body mass (also known as “wasting”) is common in the AIDS stage of HIV infection, and glutamine loss from skeletal muscle may further accelerate in situations

**Table 1:** Protocol of Nutrients: Total Daily Intake

L-glutamine .....	10 grams qid
Ascorbic Acid .....	200 mg qid
Alpha-tocopherol .....	125 IU qid
Beta carotene .....	6750 IU qid
Selenium .....	70 mcg qid
N-acetylcysteine .....	600 mg qid

From: Shabert J, et al. *Nutrition* 1999; 15:860-864.<sup>83</sup>

where diarrhea, fever, anorexia, malabsorption, and opportunistic infections occur.<sup>81</sup> Total parenteral nutrition or medications given to stimulate appetite in HIV infection promote weight gain that is predominately adipose tissue or body fluid; they are not successful at promoting gain of lean body mass.<sup>81</sup>

### Glutamine and Clinical Trials

A double-blind, placebo-controlled trial examining the effect of glutamine in HIV-infected patients was conducted on 21 HIV-positive men and women (CD4 count 1-364/mm<sup>3</sup>).<sup>83</sup> The subjects had all experienced at least five-percent unintentional weight loss since the onset of their diagnoses and were excluded if they had infectious diarrhea. Only two subjects were taking testosterone and 18 were on antiretrovirals. All patients received nutritional counseling and had bioimpedance analysis done at 0, 1, 2, and 3 months to determine body cell mass and fat mass. The patients were given 40 grams glutamine daily with an antioxidant combination that included N-acetylcysteine. For complete protocol see Table 1. The results of the study showed a significant effect of glutamine on lean body mass gain over the three-month period: the supplemented subjects gained an average of 1.8 kg lean body mass (body cell mass) over 12 weeks. The control group initially gained mass

but could not sustain it, and at three months had only a net gain of 0.4 kg lean body mass. The results for the supplemented group were similar to effects gained with recombinant growth hormone (rhGH), currently the most effective FDA-approved treatment for wasting in HIV, at approximately 1/30 the cost (\$31.00 vs. \$1,000.00). The cost-savings is substantial. To gain 1 kg lean body mass costs approximately \$9200 using rhGH; according to the results of the above study, the same amount of lean body mass could be gained with L-glutamine for \$220.<sup>84</sup>

### L-Carnitine

L-carnitine is a non-essential amino acid that regulates cell membrane transport of fatty acids into the mitochondria, subsequently allowing them to be broken down to acetyl coA via  $\beta$ -oxidation.<sup>84</sup> This is particularly important in muscle, where fatty acids and pyruvate are the main energy substrates. Carnitine is found in the body in the free and ester forms with acyl groups of different chain lengths.<sup>85</sup> L-carnitine is found in high concentrations in leukocytes and peripheral blood mononuclear cells where it acts to support lymphocyte proliferation.<sup>86</sup>

L-carnitine appears to be deficient in certain cohorts of HIV-infected individuals: L-carnitine deficiencies have been found in 72 percent of a group of 29 AIDS patients on AZT.<sup>87</sup> HIV-positive patients are at risk for carnitine deficiency as a result of malabsorption, kidney abnormalities that lead to increased renal excretion, specific antibiotics and antiviral medications, and the loss of adipose tissue that increases fatty acid availability.<sup>86</sup> Even in HIV-positive patients with normal serum carnitine levels, peripheral blood mononuclear cell levels of carnitine were significantly lower than healthy normals<sup>88</sup> which may be related to apoptosis rates in apoptosis sensitivity in CD4 and CD8 cells.

Multiple studies both *in vivo* and *in vitro*, have shown decreases in apoptosis of

**Table 2:** The Effects of L-Carnitine on CD4, CD8, and HIV RNA

Variable	Time points	NO.	Mean	St. Dev.	Min	Median	Max
CD4	T0	11	305.2	139.4	61	331	558
	T1	11	317.7	136.3	62	311	504
	T2	11	322.0	142.3	100	337	522
	T3	11	377.5	154.5	100	390	580
	T4	11	406.3	167.9	101	476	670
CD8	T0	11	860.7	353.7	352	844	1,460
	T1	11	926.3	392.9	419	918	1,745
	T2	11	920.3	425.7	525	737	1,865
	T3	11	947.5	405.4	401	922	1,577
	T4	11	1,025.1	700.0	395	947	2,887
HIV RNA (log <sub>10</sub> )	T0	11	3.67	0.64	2.60	3.84	4.52
	T1	NA					
	T2	10	3.82	0.72	2.65	4.13	4.52
	T3	NA					
	T4	11	3.89	0.54	2.90	4.07	4.51

T0, baseline; T1=day 15; T2=day 30; T3=day 90; T4=day 150.  
 Abbreviations: NA=not available; St. Dev.=standard deviation.  
 Taken from Moretti S, et al. *Blood* 1998; 91:3817-3824.<sup>89</sup>  
 copyright ©, 1998. Blood. All rights reserved.

CD4 and CD8 cells with the addition of L-carnitine and acetyl-L-carnitine.<sup>89,90</sup> The probable mechanisms for this reduction in CD4 and CD8 cell death involve the reduction of ceramide (a mediator of apoptosis) that increases HIV-1 replication, and increases of insulin-like growth factor 1 (IGF-1).<sup>90</sup>

### Clinical Studies with Carnitine/ Acetyl-L-carnitine

Eleven asymptomatic HIV-infected individuals who were not receiving any antiviral treatment, received daily infusion of six grams L-carnitine for four months.<sup>89</sup> Prior to the study they all had been experiencing steady CD4 count declines for 12 months. At day 150, mean CD4 counts were significantly elevated

and a positive trend was observed for increases in CD8 counts (Table 2). Two subjects had a doubling of their CD4 count, two had a more than 50-percent increase, three had approximately 30-percent increases, and three had no significant increase over baseline. The researchers also saw a significant drop in apoptotic cell death in both CD4 and CD8 cells (p<0.001 for both values). Ceramide levels were significantly higher than in HIV-negative individuals, and dropped significantly over the course of the trial. HIV-1 viremia increased slightly over the 150-day period. There was no toxicity related to the L-carnitine therapy.

A follow-up trial by the same group<sup>90</sup> with 11 HIV-positive asymptomatic individuals evaluated three grams of oral

acetyl-L-carnitine daily for five months. Again, significant reductions were seen in CD4 apoptosis and ceramide levels. No significant changes, however, were seen in absolute CD4 or CD8 cell counts or levels of viremia. The treatment did increase serum IGF-1 levels and had no impact on serum growth hormone levels. It is interesting to note that the baseline IGF-1 and growth hormone levels in the HIV-positive individuals were not lower than healthy age-matched normals. There were no side-effects or toxicity reported in this trial, and the authors noted that all subjects reported an improved sense of well-being by the second or third week of therapy.

A randomized trial<sup>91</sup> of six grams oral L-carnitine daily in 20 male AIDS patients on AZT for 14 days resulted in significant decreases in TNF- $\alpha$  and in serum triglycerides. Hypertriglyceridemia occurs in HIV as a result of increased cytokine production and is also a recognized side-effect of protease-inhibitor therapy. Whether L-carnitine will reduce the hypertriglyceridemia and hypercholesterolemia secondary to protease-inhibitor therapy remains to be seen.

### **L-Carnitine, Mitochondrial Myopathy, and Neuropathy**

L-carnitine is a recognized treatment for mitochondrial myopathy and encephalomyopathy, a group of neurological disorders characterized by progressive neurological and muscular weakness and degeneration.<sup>92</sup> AZT and other reverse transcriptase inhibitors have been associated with mitochondrial toxicity. This is most evident in the research exploring mitochondrial myopathy after long-term therapy with AZT as a single antiviral agent.<sup>93</sup> AZT affects muscle mitochondria by inhibiting the  $\gamma$ -DNA polymerase enzyme, resulting in depletion of muscle mitochondrial DNA, and creating defects in the cytochrome system of the respiratory chain.<sup>94</sup> This results in a functional “uncoupling” of

the oxidation and phosphorylation of mitochondrial energy production, and an inability of the mitochondria to use fatty acids as energy, instead being stored as lipid in the muscle tissue. Low levels of carnitine are also found in the muscle tissue of AZT patients, not correlative of the duration of treatment or cumulative dosage of the drug.<sup>93</sup> A small trial of six patients on AZT and lamivudine found that carnitine depletion was occurring through increased renal excretion. Supplementation with 800 mg oral L-carnitine, 3000 mg magnesium chloride, 800 mg L-arginine, and 240 mg glycine daily was able to increase carnitine levels. No symptom-related data was available.<sup>95</sup>

Other antivirals classified as nucleoside reverse transcriptase inhibitors (ddI, ddC, d4T) also impair mitochondrial DNA production. This is believed to be the mechanism through which they produce axonal peripheral neuropathy, a common side-effect of these medications.<sup>96</sup> In a comparison study, patients on these medications with peripheral neuropathy were found to have significant acetyl-L-carnitine deficiencies when matched to patients on the same medications who did not develop neuropathy.<sup>96</sup> The authors stated that acetyl-L-carnitine is critical for peripheral nerve function and deficiencies may be contributing to the neurotoxicity of these medications. L-carnitine and acetyl-L-carnitine have been found to be protective for this type of mitochondrial damage *in vitro*,<sup>97</sup> and a clinical trial with acetyl-L-carnitine and peripheral neuropathy in HIV-negative patients has shown benefit.<sup>98</sup> In this study significant improvements in mobility, subjective and objective symptom rating, and performance were seen after one-gram intramuscular injections were given daily for 15 days.

### **Coenzyme Q**

Coenzyme Q10 (CoQ10) is present in all eukaryotic cells and all lipoproteins. Cellularly it acts as an electron carrier in the mitochondrial respiratory chain and as a free

radical scavenger in liposomal membranes.<sup>99</sup> It appears to be as efficient as  $\alpha$ -tocopherol in preventing free peroxy radical production in lipid membranes and prevents oxidation of  $\alpha$ -tocopherol in *in vitro* studies designed to mimic human physiological conditions. Reversal of depressed host defense systems in animals treated with chemotherapy has been achieved with administration of CoQ10<sup>100</sup> and CD4/CD8 ratios have been elevated as a result of increased CD4 counts in HIV-negative subjects on 100 mg CoQ10 daily for 60 days.<sup>101</sup>

CoQ deficiencies have been demonstrated in HIV infection.<sup>100</sup> The deficiencies (measured both in whole blood and intracellular erythrocytes and lymphocytes) in 12 HIV-infected individuals were significantly greater in symptomatic HIV-positive individuals than HIV-negative controls and CoQ10 levels dropped with progressive stages of the infection.<sup>100</sup> CoQ10 at 200 mg daily was given to seven of these HIV-positive individuals. Three had AIDS diagnoses and four were symptomatic for AIDS-related complex (ARC): fever, night sweats, diarrhea, weight loss, and lymphadenopathy.<sup>100</sup> Treatment lengths varied in each case (4-14 months), but in all cases, whole blood CoQ10 levels rose substantially, as high as 4.51 mg/mL in one subject (mean levels in HIV-negative controls were 0.79 and 0.84 mg/mL). Five of the seven patients who were able to be followed improved symptomatically and had no opportunistic infections after 4-7 months.<sup>100</sup> A follow-up paper by the same author evaluated the effect of CoQ10 on two of the same HIV-positive ARC patients treated with CoQ10 for 4-5 years. Both had stabilized with remission of lymphadenopathy and no evidence of opportunistic infection. Information on concomitant antiviral regimens was not available.

Folkers<sup>101,102</sup> found substantial increases in both whole blood levels of CoQ10, serum IgG levels, absolute CD4 counts, and CD4/CD8 ratios in HIV-negative subjects when CoQ10 (200 mg daily) was administered

together with 300 mg vitamin B6 (pyridoxine). CoQ10's role as an antioxidant and immunomodulator makes further research necessary; trials with CoQ10 need to be conducted in much larger populations and in conjunction with other therapies.

## Conclusion

The role of antioxidants as anti-apoptotic and antiviral agents in the treatment of HIV has been supported in the literature in multiple studies.<sup>6,17,21,24,29,33</sup> The important role of ascorbate, tocopherol, lipoic acid, and N-acetylcysteine in maintaining both glutathione levels and a redox state (that stabilizes NF- $\kappa$ B and inhibits activation of latent provirus) needs further documentation in large-scale clinical trials. Until that time, the use of antioxidant therapies in HIV is indicated in doses that correspond to those used in human trials or that can be safely extrapolated from *in vitro* cell cultures with HIV. The evidence for L-glutamine in restoring lost lean body mass in an economically realistic context necessitates its use in wasting syndrome. The use of L-carnitine and acetyl-L-carnitine in protecting neural tissue and mitochondria from damage induced by antiviral therapies is warranted.

## References

1. Rosenberg ZF, Fauci AS. Immunopathogenesis of HIV infection. *FASEB J* 1991;5:2382-2390.
2. Greene WC. The molecular biology of human immunodeficiency virus type 1 infection. *N Engl J Med* 1991;324:308-317.
3. Baruchel S, Wainberg MA. The role of oxidative stress in disease progression in individuals infected by the human immunodeficiency virus. *J Leukoc Biol* 1992;52:111-114.
4. Polyakov VM, Shepelev AP, Kokovkina OE, et al. Superoxide anion production and enzymatic disbalance in peripheral blood cells isolated from HIV-infected children. *Free Radic Biol Med* 1994;16:15.
5. Marmor M, Alcabes P, Titus S, et al. Low serum thiol levels predict shorter times-to-death among HIV-infected injecting drug users. *AIDS* 1997;11:1389-1393.

6. Greenspan HC, Aruoma OI. Oxidative stress and apoptosis in HIV infection: a role for plant-derived metabolites with synergistic antioxidant activity. *Immunol Today* 1994;15:209-213.
7. Pace GW, Leaf CD. The role of oxidative stress in HIV disease. *Free Radic Biol Med* 1995;19:523-528.
8. Gougeon ML, Montagnier L. Apoptosis in AIDS. *Science* 1993;260:1269-1270.
9. Ameisen JC. Programmed cell death and AIDS: from hypothesis to experiment. *Immunol Today* 1992;13:388-391.
10. Buttke TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. *Immunol Today* 1994;15:7-10.
11. Sato N, Iwata S, Nakamura K, et al. Thiol-mediated redox regulation of apoptosis. Possible roles of cellular thiols other than glutathione in T cell apoptosis. *J Immunol* 1995;154:3194-3203.
12. Staal FJ, Roederer M, Israelski DM, et al. Intracellular glutathione levels in T cell subsets decrease in HIV-infected individuals. *AIDS Res Hum Retroviruses* 1992;8:305-311.
13. Sandstrom PA, Tebbey PW, Van Cleave S, Buttke TM. Lipid hydroperoxides induce apoptosis in T cells displaying a HIV-associated glutathione peroxidase deficiency. *J Biol Chem* 1994;269:798-801.
14. Jarstrand C, Akerlund B. Oxygen radical release by neutrophils of HIV-infected patients. *Chem Biol Interact* 1994;91:141-146.
15. Droge W, Eck HP, Mihm S. HIV-induced cysteine deficiency and T-cell dysfunction – a rationale for treatment with N-acetylcysteine. *Immunol Today* 1992;13:211-214.
16. Meister A, Anderson ME. Glutathione. *Annu Rev Biochem* 1983;52:711-760.
17. Meister A. Glutathione-ascorbic acid antioxidant system in animals. *J Biol Chem* 1994;269:9397-9400.
18. Hamilos DL, Wedner HJ. The role of glutathione in lymphocyte activation. I. Comparison of inhibitory effects of buthionine sulfoximine and 2-cyclohexene-1-one by nuclear size transformation. *J Immunol* 1985;135:2740-2747.
19. Muller F, Aukrust P, Svardal AM, et al. The thiols glutathione, cysteine and homocysteine in immunodeficiency virus infection. In: Watson RR, ed. *Nutrition and Foods in AIDS*. Boca Raton, FL: CRC Press; 1998:35-39.
20. Murray RK. Metabolism of xenobiotics. In: Murray RK, Granner DK, Mayes PA, et al, eds. *Harper's Biochemistry*. 24<sup>th</sup> ed. Stamford, CT: Appleton and Lange; 1996.
21. Carr A, Cooper DA, Penny R. Allergic manifestations of human immunodeficiency virus (HIV) infection. *J Clin Immunol* 1991;11:55-64.
22. Shriner K, Goetz MB. Severe hepatotoxicity in a patient receiving both acetaminophen and zidovudine. *Am J Med* 1992;93:94-96.
23. Schreck R, Rieber P, Baeuerle P. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. *EMBO J* 1991;10:2247-2258.
24. Israel N, Gougerot-Pocidal MA, Aillet F, Virelizier JL. Redox status of cells influences constitutive or induced NF-kB translocation and HIV long terminal repeat activity in human T and monocytic cell lines. *J Immunol* 1992;149:3386-3393.
25. Buhl R, Jaffe HA, Holroyd KJ, et al. Systemic glutathione deficiency in symptom free HIV-seropositive individuals. *Lancet* 1989;2:1294-1298.
26. Staal FJ, Roederer M, Herzenberg LA, Herzenberg LA. Intracellular thiols regulate activation of nuclear factor kB and transcription of human immunodeficiency virus. *Proc Natl Acad Sci USA* 1990;87:9943-9947.
27. Pirmohamed M, Williams D, Tingle MD, et al. Intracellular glutathione in the peripheral blood cells of HIV-infected patients: failure to show a deficiency. *AIDS* 1996;10:501-507.
28. de Quay B, Malinverni R, Lauterburg BH. Glutathione depletion in HIV-infected patients: role of cysteine deficiency and effect of oral N-acetylcysteine. *AIDS* 1992;6:815-819.
29. Aukrust P, Svardal AM, Muller F, et al. Markedly disturbed glutathione redox status in CD45RA+CD4+ lymphocytes in human immunodeficiency virus type 1 infection is associated with selective depletion of this lymphocyte subset. *Blood* 1996;88:2626-2633.
30. Aukrust P, Muller F. Glutathione redox disturbances in human immunodeficiency virus infection: immunologic and therapeutic consequences. *Nutrition* 1999;15:165-167.
31. Schreck R, Meier B, Mannel DN, et al. Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells. *J Exp Med* 1992;175:1181-1194.
32. Nabel G, Baltimore D. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature* 1987;326:711-713.
33. Kalebic T, Kinter A, Poli G, et al. Suppression of human immunodeficiency virus expression in chronically infected monocytic cells by glutathione, glutathione ester, and N-acetylcysteine. *Proc Natl Acad Sci USA* 1991;88:986-990.

34. Kalayjian RC, SKowron G, Emgushov RT, et al. A phase I/II trial of intravenous L-2 oxothiazolidine-4-carboxylic acid (procysteine) in asymptomatic HIV-infected subjects. *J Acquir Immune Defic Syndr* 1994;7:369-374.
35. Raju PA, Herzenberg LA, Herzenberg, LA, Roederer M. Glutathione precursor and antioxidant activities of N-acetylcysteine and oxothiazolidine carboxylate compared in *in vitro* studies of HIV replication. *AIDS Res Hum Retroviruses* 1994;10:961-967.
36. Hagen TM, Wierzbička GT, Sillau AH, et al. Bioavailability of dietary glutathione: effect on plasma concentration. *Am J Physiol* 1990;259:G524-G529.
37. Anderson ME, Powrie F, Puri RN, Meister A. Glutathione monoethyl ester: preparation, uptake by tissue, and conversion to glutathione. *Arch Biochem Biophys* 1985;239:538-548.
38. Kidd P. Glutathione: systemic protectant against oxidative and free radical damage. *Altern Med Rev* 1997;2:155-176.
39. Witschi A, Reddy S, Stofer B, Lauterburg BH. The systemic availability of oral glutathione. *Eur J Clin Pharmacol* 1992;43:667-669.
40. Saez G, Thornalley PJ, Hill HA, et al. The production of free radicals during the autooxidation of cysteine and their effect on isolated rat hepatocytes. *Biochim Biophys Acta* 1982;719:24-31.
41. Smilkstein MJ, Knapp GL, Kulig KW, Rumack BH. Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the national multicenter study, (1976-1985). *N Engl J Med* 1988;319:1557-1562.
42. Azarnoff D, Arnold GJ, Anderson J, et al. Acetylcysteine. In: *Mosby's Gen Rx*. St. Louis, MO: Mosby-Year Book Inc.; 1998.
43. Behr J, Maier K, Degenkolb B, et al. Antioxidative and clinical effects of high-dose N-acetylcysteine in fibrosing alveolitis. Adjunctive therapy to maintenance immunosuppression. *Am J Respir Crit Care Med* 1997;156:1897-1901.
44. Kelly G. Clinical applications of N-acetylcysteine. *Altern Med Rev* 1998;3:114-127.
45. De Flora S, Bennicelli C, Camoirano A, et al. In vivo effects of N-acetylcysteine on glutathione metabolism and on the biotransformation of carcinogenic and/or mutagenic compounds. *Carcinogenesis* 1985;6:1735-1745.
46. Hoffer E, Baum Y, Tabak A, Taitelman U. N-acetylcysteine increases the glutathione content and protects rat alveolar type II cells against paraquat-induced cytotoxicity. *Toxicol Lett* 1996;84:7-12.
47. Corcoran GB, Wong BK. Role of glutathione in prevention of acetaminophen-induced hepatotoxicity by N-acetyl-L-cysteine in vivo: studies with N-acetyl-D-cysteine in mice. *J Pharmacol Exp Ther* 1986;238:54-61.
48. Bernard GR, Wheeler AP, Arons MM, et al. A trial of antioxidants N-acetylcysteine and procysteine in ARDS. The Antioxidant in ARDS Study Group. *Chest* 1997;112:164-172.
49. Kinscherf R, Fischbach T, Mihm S, et al. Effect of glutathione depletion and oral N-acetyl-cysteine treatment on CD4+ and CD8+ cells. *FASEB J* 1994;8:448-451.
50. Cayota A, Vuillier F, Gonzalez G, et al. In vitro antioxidant treatment recovers proliferative responses of anergic CD4+ lymphocytes from human immunodeficiency virus-infected individuals. *Blood* 1996;87(11):4746-4753.
51. Pantaleo G. How immune-based interventions can change HIV therapy. *Nat Med* 1997;3:483-486.
52. Malorni W, Rivabene R, Santini MT, Donelli G. N-acetylcysteine inhibits apoptosis and decreases viral particles in HIV-chronically infected U937 cells. *FEBS Lett* 1993;327:75-78.
53. Roberts RL, Aroda VR, Ank BJ. N-acetylcysteine enhances antibody-dependent cellular cytotoxicity in neutrophils and mononuclear cells from healthy adults and human immunodeficiency virus-infected patients. *J Infect Dis* 1995;172:1492-1502.
54. Oddera S, Silvestri M, Sacco O, et al. N-acetylcysteine enhances in vitro the intracellular killing of *Staphylococcus aureus* by human alveolar macrophages and blood polymorphonuclear leukocytes and partially protects phagocytes from self-killing. *J Lab Clin Med* 1994;124:293-301.
55. Roederer M, Staal FJ, Raju PA, et al. Cytokine-stimulated human immunodeficiency virus replication is inhibited by N-acetyl-L-cysteine. *Proc Natl Acad Sci USA* 1990;87:4884-4888.
56. Peristeris P, Clark BD, Gatti S, et al. N-acetylcysteine and glutathione as inhibitors of tumor necrosis factor production. *Cell Immunol* 1992;140:390-399.
57. Ho WZ, Douglas SD. Glutathione and N-acetylcysteine suppression of human immunodeficiency virus replication in human monocyte/macrophages in vitro. *AIDS Res Hum Retroviruses* 1992;8:1249-1253.

58. Akerlund B, Jarstrand C, Lindeke B, et al. Effect of n-acetylcysteine (NAC) treatment on HIV-1 infection: a double-blind placebo-controlled trial. *Eur J Clin Pharmacol* 1996;50:457-461.
59. Herzenberg LA, De Rosa SC, Dubs JG, et al. Glutathione deficiency is associated with impaired survival in HIV disease. *Proc Natl Acad Sci USA* 1997;94:1967-1972.
60. Olivier R, Dragic T, Lopez O, et al. An anti-oxidant prevents apoptosis and early cell death in lymphocytes from HIV infected individuals. *Int Conf AIDS* 1992 July 9-24;8(2):A65 (abstract no. PoA 2376).
61. Look MP, Rockstroh JK, Rao GS, et al. Sodium selenite and N-acetylcysteine in antiretroviral-naive HIV-1-infected patients: a randomized, controlled pilot study. *Eur J Clin Invest* 1998;28:389-397.
62. Ferbas J, Kaplan AH, Hausner MA, et al. Virus burden in long-term survivors of human immunodeficiency virus (HIV) infection is a determinant of anti-HIV CD8+ lymphocyte activity. *J Infect Dis* 1995;172:329-339.
63. Binet H, Simonart T, Van Vooren JP, et al. Porphyria cutanea tarda in a human immunodeficiency virus-infected patient: treatment with N-acetyl-cysteine. *Int J Dermatol* 1998;37:718-719.
64. Beloqui O, Prieto J, Suarez M, et al. N-acetyl cysteine enhances the response to interferon-alpha in chronic hepatitis C: a pilot study. *J Interferon Res* 1993;13:279-282.
65. Rodwell V. Biosynthesis of the nutritionally nonessential amino acids. In: Murray RK, Granner DK, eds. *Harper's Biochemistry*. 24<sup>th</sup> ed. Stamford, CT: Appleton and Lange; 1996:327-328.
66. Packer L, Witt EH, Tritschler HJ. alpha-Lipoic acid as a biological antioxidant. *Free Radic Biol Med* 1995;19:227-250.
67. Packer L, Tritschler HJ, Wessel K. Neuroprotection by the metabolic antioxidant alpha-lipoic acid. *Free Radic Biol Med* 1997;22:359-378.
68. Han D, Tritschler HJ, Packer L. Alpha-lipoic acid increases intracellular glutathione in a human T-lymphocyte Jurkat cell line. *Biochem Biophys Res Commun* 1995;207:258-264.
69. Packer L, Suzuki YJ. Vitamin E and alpha-lipoate: role in antioxidant recycling and activation of the NF-kappa B transcription factor. *Mol Aspects Med* 1993;14:229-239.
70. Baur A, Harrer T, Peukert M, et al. Alpha-lipoic acid is an effective inhibitor of human immuno-deficiency virus (HIV-1) replication. *Klin Wochenschr* 1991;69:722-724.
71. Fuchs J, Schofer H, Milbradt R, et al. Studies on lipoate effects on blood redox state in human immunodeficiency virus infected patients. *Arzneimittelforschung* 1993;43:1359-1362.
72. Kiebertz K, Schifitto G, McDermott M, et al. A randomized, double-blind, placebo-controlled trial of deprenyl and thioctic acid in human immunodeficiency virus-associated cognitive impairment. Dana Consortium on the Therapy of HIV Dementia and Related Cognitive Disorders. *Neurology* 1998;50:645-651.
73. Jayanthi S, Varalakshmi P. Tissue lipids in experimental calcium oxalate lithiasis and the effect of DL alpha-lipoic acid. *Biochem Int* 1992;26:913-921.
74. Bergstrom J, Furst P, Noree LO, Vinnars E. Intracellular free amino acid concentration in human muscle tissue. *J Appl Physiol* 1974;36:693-697.
75. Souba WW, Smith RJ, Wilmore DW. Effect of glucocorticoids on glutamine metabolism in visceral organs. *Metabolism* 1985;34:450-456.
76. Newsholme EA, Crabtree B, Ardawi MS. Glutamine metabolism in lymphocytes: its biochemical, physiological and clinical importance. *Q J Exper Physiol* 1985;70:473-489.
77. O'Riordain MG, Fearon KC, Ross JA, et al. Glutamine-supplemented total parenteral nutrition enhances T-lymphocyte response in surgical patients undergoing colorectal resection. *Ann Surg* 1994;220:212-221.
78. Ziegler TR, Young LS, Benfell K, et al. Clinical and metabolic efficacy of glutamine-supplemented parenteral nutrition after bone marrow transplantation. A randomized, double-blind, controlled study. *Ann Intern Med* 1992;116:821-828.
79. Souba WW, Smith RJ, Wilmore DW. Glutamine metabolism by the intestinal tract. *JPEN J Parenter Enteral Nutr* 1985;9:608-617.
80. Fox AD, Kripke SA, DePaula J, et al. Effect of a glutamine-supplemented enteral diet on methotrexate-induced enterocolitis. *JPEN J Parenter Enteral Nutr* 1988;12:325-331.
81. Shabert JK, Wilmore DW. Glutamine deficiency as a cause of human immunodeficiency virus wasting. *Med Hypotheses* 1996;46:252-256.

82. Young L, Gatzen C, Wilmore K, et al. Glutamine supplementation fails to increase plasma glutamine levels in asymptomatic HIV+ individuals. *JADA* 1992;92:A88.
83. Shabert J, Winslow C, Lacey JM, Wilmore DW. Glutamine-antioxidant supplementation increases body cell mass in AIDS patients with weight loss: a randomized, double-blind controlled trial. *Nutrition* 1999;15:860-864.
84. Mayes PA. Oxidation of fatty acids: ketogenesis. In: Murray RK, ed. *Harpers Biochemistry*. Stamford, CT: Appleton and Lange; 1996:224-228.
85. Famularo G, De Simone C. A new era for carnitine? *Immunol Today* 1995;16:211-213.
86. Deufel T. Determination of L-carnitine in biological fluids and tissues. *J Clin Chem Clin Biochem* 1990;28:307-311.
87. De Simone C, Tzantzoglou S, Jirillo E, et al. Carnitine deficiency in AIDS patients. *AIDS* 1992;6:203-205.
88. De Simone C, Famularo G, Tzantzoglou S, et al. Carnitine depletion in peripheral blood mononuclear cells from patients with AIDS: effect of oral L-carnitine. *AIDS* 1994;8:655-660.
89. Moretti S, Alesse E, Di Marzio L, et al. Effect of L-carnitine on human immunodeficiency virus-1 infection-associated apoptosis: a pilot study. *Blood* 1998;91:3817-3824.
90. Di Marzio L, Moretti S, D'Alo S, et al. Acetyl-L-carnitine administration increases insulin-like growth factor 1 levels in asymptomatic HIV-1-infected subjects: correlation with its suppressive effect on lymphocyte apoptosis and ceramide generation. *Clin Immunol* 1999;92:103-110.
91. De Simone C, Tzantzoglou S, Famularo G, et al. High-dose L-carnitine improves immunologic and metabolic parameters in AIDS patients. *Immunopharmacol Immunotoxicol* 1993;15:1-12.
92. Campos Y, Huertas R, Lorenzo G, et al. Plasma carnitine insufficiency and effectiveness of L-carnitine therapy in patients with mitochondrial myopathy. *Muscle Nerve* 1993;16:150-153.
93. Dalakas MC, Leon-Monzon ME, Bernardini I, et al. Zidovudine-induced mitochondrial myopathy is associated with muscle carnitine deficiency and lipid storage. *Ann Neurol* 1994;35:482-487.
94. Arnaudo E, Dalakas M, Shanske S, et al. Depletion of muscle mitochondrial DNA in AIDS patients with zidovudine-induced myopathy. *Lancet* 1991;337:508-510.
95. Davis HJ, Miene LJ, van der Westhuizen N., et al. L-carnitine and magnesium as a supportive supplement with antiviral drugs. *Int Conf AIDS* 1998;12:851 (abstract no. 42384).
96. Famularo G, Moretti S, Marcellini S, et al. Acetyl-carnitine deficiency in AIDS patients with neurotoxicity on treatment with antiretroviral nucleoside analogues. *AIDS* 1997;11:185-190.
97. Virmani MA, Biselli R, Spadoni A, et al. Protective actions of L-carnitine and acetyl-L-carnitine on the neurotoxicity evoked by mitochondrial uncoupling or inhibitors. *Pharmacol Res* 1995;32:383-389.
98. Onofri M, Fulgente T, Melchionda D, et al. L-acetylcarnitine as a new therapeutic approach for peripheral neuropathies with pain. *Int J Clin Pharmacol Res* 1995;15:9-15.
99. Frei B, Kim MC, Ames BN. Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proc Natl Acad Sci USA* 1990;87:4879-4883.
100. Folkers K, Langsjoen P, Nara Y, et al. Biochemical deficiencies of coenzyme Q10 in HIV-infection and exploratory treatment. *Biochem Biophys Res Commun* 1988;153:888-896.
101. Folkers K, Hanioka T, Xia LJ, et al. Coenzyme Q10 increases T4/T8 ratios of lymphocytes in ordinary subjects and relevance to patients having the AIDS related complex. *Biochem Biophys Res Commun* 1991;176:786-791.
102. Folkers K, Morita M, McRee J. The activities of coenzyme Q10 and vitamin B6 for immune responses. *Biochem Biophys Res Commun* 1993;193:88-92.