

# Oxidative stress and plasma antioxidant micronutrients in humans with HIV infection<sup>1-3</sup>

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**ABSTRACT** Increased lipid peroxidation induced by reactive oxygen species may play a role in the stimulation of HIV replication. In this study we compared lipid peroxidation indexes and plasma antioxidant micronutrients between 49 nonsmoking HIV-positive patients with no active opportunistic infection (25 asymptomatic and 24 with AIDS) and 15 age-matched seronegative control subjects. Breath-alkane output, plasma lipid peroxides, antioxidant vitamins, and trace elements were measured. Vitamin C ( $40.7 \pm 3.02$  compared with  $75.7 \pm 4.3$   $\mu\text{mol/L}$ ,  $P < 0.005$ ),  $\alpha$ -tocopherol ( $22.52 \pm 1.18$  compared with  $26.61 \pm 2.60$   $\mu\text{mol/L}$ ,  $P < 0.05$ ),  $\beta$ -carotene ( $0.23 \pm 0.04$  compared with  $0.38 \pm 0.04$   $\mu\text{mol/L}$ ,  $P < 0.05$ ), and selenium ( $0.37 \pm 0.05$  compared with  $0.85 \pm 0.09$   $\mu\text{mol/L}$ ,  $P < 0.005$ ) concentrations were significantly lower in the HIV-positive patients. Lipid peroxides ( $50.7 \pm 8.2$  compared with  $4.5 \pm 0.8$   $\mu\text{mol/L}$ ,  $P < 0.005$ ), breath pentane ( $9.05 \pm 1.23$  compared with  $6.06 \pm 0.56$   $\text{pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.05$ ), and ethane output ( $28.1 \pm 3.41$  compared with  $11.42 \pm 0.55$   $\text{pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.05$ ) were significantly higher in the HIV-positive patients. These results showed an increase in oxidative stress and a weakened antioxidant defense system in HIV-positive patients. Whether supplementation of antioxidant vitamins will reduce this oxidative stress is still unknown. *Am J Clin Nutr* 1998;67:143–7.

**KEY WORDS** Lipid peroxidation, breath alkane, antioxidants, HIV infection, AIDS, oxidative stress, humans

## INTRODUCTION

HIV infection induces a wide array of immunologic alterations resulting in the progressive development of opportunistic infections and malignancy, which results in AIDS. Of the mechanisms contributing to this progression, oxidative stress induced by the production of reactive oxygen species (ROS) may play a critical role in the stimulation of HIV replication and the development of immunodeficiency (1, 2).

Excessive production of ROS such as superoxide anion, hydroxyl radical, and hydrogen peroxide may be related to an increased activation of polymorphonuclear leukocytes during infections or influenced by the prooxidant effect of tumor necrosis factor  $\alpha$  produced by activated macrophages during the course of HIV infection (3). ROS can attack double bonds in polyunsaturated fatty acids, inducing lipid peroxidation (4), which may result in more oxidative cellular damage (5, 6). Thus, measurement of lipid

peroxidation is a means of determining oxidative stress. Such damage may be prevented or moderated by a normal antioxidant defense system that scavenges the ROS. This antioxidant system depends first on the integrity of an enzymatic system that requires adequate intake of trace minerals such as selenium, copper, zinc, and manganese, and second on adequate concentrations of vitamin E, A, and C and  $\beta$ -carotene in the cytoplasm and lipid membrane of the cells. Previous studies showed that humans infected with HIV may have deficiencies in some of these trace minerals and vitamins, such as selenium (7) and vitamin A (8).

The purpose of the present study was to measure the plasma concentrations of various antioxidants (vitamins A, C, and E and  $\beta$ -carotene; other carotenoids; selenium; and zinc) and some lipid peroxidation indexes in an HIV-positive population, either asymptomatic or with AIDS, and to compare these results with those in age-matched seronegative control subjects.

## SUBJECTS AND METHODS

### Subjects

Forty-nine HIV-positive patients [25 asymptomatic: Centers for Disease Control and Prevention (CDC) class A1 or A2; and 24 with AIDS: CDC class A3, B3, and C3] with a mean age of 39 y (range: 25–64 y) were recruited from the Immunodeficiency Clinic at The Toronto Hospital. All subjects underwent an initial screening that included a detailed history (medical, smoking, diet, and alcohol and supplemental vitamin intakes) and anthropometric (weight and height) and biochemical (complete blood count, glucose, creatinine, urea, and liver enzymes) measurements. Patients were eligible if they had no acute opportunistic infection. Exclusion criteria were as follows: smoking, initiation of antioxidant vitamin therapy before the study, hyperlipidemia, diabetes, kidney or liver dysfunction, intractable diarrhea (more than six liquid stools per day), vomiting, or evidence of gastrointestinal bleeding. Subjects were then placed

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<sup>2</sup> Supported by a grant from the Canadian Foundation for AIDS Research (CANFAR).

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Received February 4, 1997.

Accepted for publication July 22, 1997.

on a controlled diet that provided a polyunsaturated to saturated fatty acid ratio of 0.3:1 for 2 wk before measurements were taken. Informed consent was obtained from all participants and the study protocol was approved by the Toronto Hospital Committee for Research on Human Subjects.

### Plasma samples

Two weeks after the screening period, biochemical measurements and breath collection were performed. Blood was drawn from subjects for analysis of plasma carotenes, plasma  $\alpha$ - and  $\gamma$ -tocopherol, vitamin C, lipid peroxides, selenium, and zinc.

Blood was collected into EDTA-containing tubes for the determination of carotenes, tocopherols, vitamin C, and lipid peroxides. Blood was collected into trace element-free tubes for analysis of zinc and selenium. The samples were put on ice and centrifuged promptly at 2400 rpm for 10 min at 4°C. The plasma was removed and frozen until analyzed. Plasma for vitamin C assays was stabilized immediately with 100 g HPO<sub>3</sub>/L (2.0 mL plasma plus 2.0 mL HPO<sub>3</sub>).

### Analyses and measurements

#### Breath-alkane output

Breath analysis was performed as described previously (9). Briefly, subjects were first required to breathe hydrocarbon-free air for 4 min to wash contaminating hydrocarbons from their lungs. Subsequently, expired air was collected for 2 min and analyzed by gas chromatography (Shimadzu 6-AM GC; Shimadzu Seisgkusho Ltd, Kyoto, Japan). Fifty milliliters of air was passed through a stainless steel loop packed with alumina and cooled to -95°C to adsorb the injected sample. The loop was then heated to desorb the gas thermally. Pentane and ethane were analyzed on a Porasil D column (Chromatographic Specialties Inc, Brockville, Canada) by using a calibration curve derived from known concentrations of the gases. Concentrations of breath pentane and ethane were expressed in pmol · kg<sup>-1</sup> · min<sup>-1</sup>.

#### Lipid peroxide measurements

Plasma lipid peroxides were measured with a kit from Kamiya Biochemicals LPO (Thousand Oaks, CA). In this procedure, hemoglobin catalyzes the reaction of hydroperoxides with a methylene blue derivative, forming an equimolar concentration of methylene blue. Lipid peroxides are quantitated by calorimetry at 675 nm, and methylene blue formation is measured.

#### Vitamin and trace element measurements

Retinol and other carotenoids, including  $\beta$ -carotene, were analyzed by HPLC according to the method of Sapuntzakis et al (10). In this method, a reversed-phase C<sub>18</sub> column was used with an isocratic solvent system (methanol:acetonitrile:tetrahydrofuran, 50:45:5, by vol) after hexane extraction with 200  $\mu$ L serum.  $\alpha$ - and  $\gamma$ -Tocopherol were analyzed with isocratic reversed-phase HPLC and fluorescence spectrophotometry at 294 nm according to the method of Nata et al (11).

Samples were analyzed for vitamin C by spectrophotometry (12). In this method, total biologically active vitamin C concentrations were determined spectrophotometrically at 521 nm with 2,4-dinitrophenylhydrazine as the chromogen.

Plasma zinc was analyzed by atomic-absorption spectrophotometry (Varian Techtron model 1200; Varian Associates, Canada Ltd, Malton, Canada) with the method described by Wolman et al (13). Plasma selenium was measured by atomic-absorption spectrophotometry at 196 nm (14). In this method, nickel salt was added as a matrix modifier to prevent volatilization of selenium during ashing.

### Statistical analyses

All group data are expressed as means  $\pm$  SEMs. The HIV-positive group was compared with the seronegative control subjects by using unpaired *t* tests. The minimal level of significance was identified at *P* < 0.05.

## RESULTS

The HIV-positive asymptomatic patients (24 men and 1 woman) had a CD4 cell count >200  $\times$  10<sup>9</sup>/L and the AIDS patients (23 men and 1 woman) had a cell count <200  $\times$  10<sup>9</sup>/L. Most patients were receiving antiretroviral treatment (zidovudine, lamivudine, and saquinavir). Self-reported infection routes were as follows: homosexual behavior (>90%), blood transfusions, intravenous drug use, and heterosexual behavior. The control group was composed of 15 (10 men and 5 women) healthy, seronegative nonsmokers with a mean age of 35 y (range: 21–60 y) recruited from the local population, primarily hospital staff and workers from the local area. Control subjects had no acute or chronic illness and were not taking any medications or nutritional supplements.

There were insignificant differences in age (39  $\pm$  2 y compared with 35  $\pm$  4 y), weight (72.1  $\pm$  2.4 kg compared with 71.3  $\pm$  2.0 kg), and body mass index (in kg/m<sup>2</sup>) (23  $\pm$  1 compared with 24  $\pm$  1) between the HIV-positive patients and control subjects, respectively. HIV-positive patients had significantly lower antioxidant vitamin concentrations (vitamin C,  $\alpha$ -tocopherol, and  $\beta$ -carotene) than control subjects (**Table 1**). Other carotenoids, such as lycopene,  $\beta$ -cryptoxanthin, and lutein/zeaxanthin were also significantly lower in the plasma of the HIV-positive group than in the control subjects (**Table 2**).

Lipid peroxidation determined from breath-alkane output and plasma lipid peroxide concentrations were significantly higher in the HIV-positive group than in control subjects (**Table 3**). There were no significant differences in lipid peroxidation or plasma antioxidant vitamins between HIV-positive asymptomatic subjects and those with AIDS (data not shown).

**TABLE 1**

Plasma antioxidant vitamins in HIV-positive subjects and seronegative control subjects<sup>1</sup>

	HIV-positive patients (n = 49)	Control subjects (n = 15)
$\alpha$ -Tocopherol ( $\mu$ mol/L)	22.52 $\pm$ 1.18	26.61 $\pm$ 2.6 <sup>2</sup>
$\gamma$ -Tocopherol ( $\mu$ mol/L)	2.69 $\pm$ 0.21	7.65 $\pm$ 0.80 <sup>3</sup>
Vitamin C ( $\mu$ mol/L)	40.7 $\pm$ 3.02	75.7 $\pm$ 4.3 <sup>3</sup>
$\beta$ -Carotene ( $\mu$ mol/L)	0.23 $\pm$ 0.04	0.38 $\pm$ 0.04 <sup>2</sup>
Retinol ( $\mu$ mol/L)	2.03 $\pm$ 0.09	2.11 $\pm$ 0.12
Zinc ( $\mu$ mol/L)	15.45 $\pm$ 0.92	14.53 $\pm$ 1.53
Selenium ( $\mu$ mol/L)	0.37 $\pm$ 0.05	0.85 $\pm$ 0.09 <sup>3</sup>

<sup>1</sup> $\bar{x} \pm$  SEM.

<sup>2,3</sup>Significantly different from HIV-positive patients: <sup>2</sup>*P* < 0.05. <sup>3</sup>*P* < 0.005.

**TABLE 2**Other plasma carotenoids in HIV-positive subjects and in seronegative controls subjects<sup>1</sup>

	HIV-positive patients (n = 49)	Control subjects (n = 15)
α-Carotene (μmol/L)	0.061 ± 0.009	0.115 ± 0.035
Lutein ± zeaxanthin (μmol/L)	0.19 ± 0.02	0.35 ± 0.03 <sup>2</sup>
β-Cryptoxanthin (μmol/L)	0.09 ± 0.01	0.16 ± 0.03 <sup>3</sup>
Lycopene (μmol/L)	0.32 ± 0.04	0.64 ± 0.07 <sup>2</sup>

<sup>1</sup> $\bar{x} \pm \text{SEM}$ .<sup>2,3</sup>Significantly different from HIV-positive patients: <sup>2</sup> $P < 0.005$ . <sup>3</sup> $P < 0.05$ .

## DISCUSSION

The results of this study showed that in this group of HIV-positive subjects, oxidative stress was significantly higher than in seronegative control subjects as determined from breath-alkane output and plasma lipid peroxide concentrations. There were no significant differences in lipid peroxidation between asymptomatic patients and those with AIDS. The increase in lipid peroxidation was also associated with lower plasma concentrations of antioxidant micronutrients such as vitamin C, α-tocopherol, β-carotene, and selenium.

Breath-alkane output was also studied by our group in previous studies (15, 16). Breath pentane and ethane evolve from the peroxidation of n-6 and n-3 fatty acids, respectively. These volatile hydrocarbon gases are produced by the β-scission of polyunsaturated fatty acids and are passed from the lungs into the expired air (9). In human studies, measurement of these alkanes in the breath is noninvasive and has been used and validated as a measure of lipid peroxidation (9, 17, 18). Intake of n-3 and n-6 fatty acids can influence the composition of the alkane produced (pentane and ethane) (19). For this reason, the subjects were given instructions about their dietary fat intake 2 wk before the measurements. Smoking (20), liver disease (21), and alcohol consumption (22) can also affect breath-alkane output. The subjects were screened before being enrolled and excluded if these confounders were present.

The finding of increased lipid peroxidation by this method is consistent with the finding of other studies that showed oxidative stress in HIV-positive patients (23–26) as evidenced by plasma lipid peroxide and malondialdehyde concentrations. Increased breath-pentane output was also reported in a small number of HIV-positive patients (25). Another index of lipid peroxidation, malondialdehyde concentrations, was also significantly higher in 26 asymptomatic HIV-positive patients (stage II) than in seronegative control subjects, and even higher in patients with AIDS (24). However, in our study we did not detect a significant

difference between asymptomatic HIV-positive patients and those with AIDS.

The mechanisms underlying the increased oxidative stress in the HIV population remain unclear. In addition to an excessive production of ROS, which may be explained by polymorphonuclear leukocyte activation during infectious conditions or by a prooxidant effect of tumor necrosis factor α produced by activated macrophages (3), a weakened antioxidant defense system may play a role. To our knowledge, our study is the first to document significantly higher oxidative stress and lower concentrations of major plasma antioxidants (ascorbic acid, α-tocopherol, β-carotene, and selenium) in the same HIV-positive subjects than in seronegative control subjects.

Another study by Constans et al (27) also showed that HIV-positive patients had significantly lower plasma vitamin A and selenium concentrations than control subjects and showed that alterations in these antioxidants were correlated with a decrease in polyunsaturated fatty acids, a target of free radicals. Another study (28) reported significantly higher superoxide dismutase and glutathione peroxidase activities in HIV-positive patients than in uninfected control subjects. There was no significant effect of selenium or β-carotene supplementation on superoxide dismutase activity compared with baseline but glutathione peroxidase activity and glutathione status increased. Because it is known that glutathione peroxidase plays a central role in the metabolism of ROS, this study suggests that antioxidant supplements may have an effect on oxidative stress in HIV-positive patients. This could be of great interest because deficiencies in antioxidant vitamins and trace minerals are common in HIV infection, especially in advanced stages of the disease (29–33). In fact, in HIV-positive patients plasma and red blood cell selenium concentrations (29) and glutathione concentrations were found to be low (30, 31). Deficiencies in antioxidant vitamins were also reported for vitamins E (32) and C (33).

This antioxidant deficiency in HIV-positive populations is probably due to increased utilization of antioxidant micronutrients because of increased oxidative stress rather than to inadequate dietary intake (34) or malabsorption (35). However, although most of the HIV-positive individuals in the study by Baum et al (36) generally consumed dietary amounts that were equal to or greater than the recommended dietary allowance (RDA) (eg, for vitamins B-6, B-12, and A), other micronutrients such as vitamin E and zinc generally were consumed in amounts less than the RDA. Another study also showed that reported intakes of various micronutrients from food exceed 100% of the RDA, except for energy, zinc, thiamine, and vitamin E (37). Our own preliminary data from 3-d food records collected from the population studied indicated that dietary macro- and micronutrient intakes were equivalent to the RDA at the time of the study


**TABLE 3**Lipid peroxidation indexes in HIV-positive subjects and seronegative control subjects<sup>1</sup>

	HIV-positive patients (n = 49)	Control subjects (n = 15)
Breath-pentane output (pmol·kg <sup>-1</sup> ·min <sup>-1</sup> )	9.05 ± 1.23	6.06 ± 0.56 <sup>2</sup>
Breath-ethane output (pmol·kg <sup>-1</sup> ·min <sup>-1</sup> )	28.1 ± 3.41	11.42 ± 0.55 <sup>2</sup>
Plasma lipid peroxide (μmol/L)	50.7 ± 8.2	4.5 ± 0.8 <sup>3</sup>

<sup>1</sup> $\bar{x} \pm \text{SEM}$ .<sup>2,3</sup>Significantly different from HIV-positive patients: <sup>2</sup> $P < 0.05$ . <sup>3</sup> $P < 0.005$ .

(data not shown). Because our HIV population had a normal nutritional status, on the basis of body mass index, we speculate that malabsorption was not significant. Thus, it is possible that the reduced plasma antioxidant concentrations observed in our population were due to an increased consumption of these micronutrients secondary to chronic oxidative stress from infection. A weakened antioxidant defense system, in turn, could lead to further enhancement in lipid peroxidation.

This increase in oxidative stress documented in our HIV-positive population may have some clinical significance because there is experimental evidence implicating oxidative stress in the stimulation of HIV replication. *In vitro* experiments (2) have shown that ROS such as hydrogen peroxide can specifically activate the nuclear factor  $\kappa$ B to induce the expression and replication of HIV-1 in a human T cell line, and addition of antioxidant vitamins blocked activation of nuclear factor  $\kappa$ B and inhibited HIV replication (38–40). Although observational studies suggest that an increased intake of some antioxidants may delay progression to AIDS (37, 41), eg, >750 mg vitamin C/d, >130 mg vitamin E/d, and >7243 RE (retinol equivalents) carotenoids/d (37), no clinical trials have investigated the effect of antioxidant supplementation on oxidative stress and viral load.

In conclusion, this study showed that lipid peroxidation, measured by breath-alkane output and lipid peroxide concentrations, was significantly higher in HIV-positive patients than in seronegative control subjects, and plasma concentrations of various antioxidant vitamins and selenium were significantly lower. These results along with the findings reported in the literature suggest that a weakened antioxidant defense system may play a significant role in the increased oxidative stress found in this population, whether due to increased consumption or reduced intakes of antioxidant micronutrients. However, it remains to be determined whether antioxidant supplementation will have any effect, not only on oxidative stress but also on viral replication and disease progression. 

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