Glutathione, glutathione peroxidase, and selenium status in HIV-positive and HIV-negative adolescents and young adults[1–3]

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ABSTRACT

Background: Antioxidant nutrient deficiencies may hasten the progression of HIV disease by impairing antioxidant defenses.

Objective: The objective of the study was to determine whether HIV infection is associated with poor selenium status and low antioxidant protection by glutathione and glutathione peroxidase (GPX).

Design: In a cross-sectional study of 365 HIV-positive and HIV-negative adolescents and young adults, we examined the relation of plasma selenium, whole-blood glutathione, and whole-blood GPX to HIV status, disease severity, immune activation, and oxidative damage.

Results: Selenium deficiency (plasma selenium <0.070 μg/mL) was not seen in any subjects, and plasma selenium in 244 HIV-positive subjects (0.120 ± 0.0013 μg/mL) did not differ significantly ($P = 0.071$) from that in 121 HIV-negative subjects (0.125 ± 0.0020 μg/mL) . However, multiple regression analysis after adjustment for covariates showed a significant ($P = 0.002$) negative association between HIV-associated immune activation (plasma neopterin) and plasma selenium concentrations. GPX activity was highest in HIV-positive subjects taking antiretroviral therapy (median: 14.2; 25th, 75th percentiles: 11.1, 18.7 U/mL; $n = 130$), intermediate in HIV-positive subjects not taking antiretroviral therapy (11.8; 9.4, 15.1 U/mL; $n = 114$), and lowest in HIV-negative subjects (10.6; 8.6, 12.7 U/mL; $n = 121$; $P < 0.05$ for all comparisons). GPX was also positively associated with malondialdehyde, a marker of oxidative damage.

Conclusions: Subjects had adequate selenium status, although HIV-related immune activation was associated with lower plasma selenium concentrations. GPX activity appears to have been induced by the oxidative stress associated with HIV infection and use of antiretroviral therapy. Thus, young, well-nourished subjects can mount a compensatory antioxidant response to HIV infection. *Am J Clin Nutr* 2007;85:173–81.

KEY WORDS Glutathione, glutathione peroxidase, selenium, oxidative stress, HIV

INTRODUCTION

HIV infection increases oxidative stress, and greater oxidative stress may result in oxidative damage. The level of oxidative damage will be influenced by the extent of oxidative stress and the activity of the body's antioxidant defenses. These defenses include dietary and nondietary antioxidants and antioxidant enzymes. The activity of antioxidant enzymes may be influenced by the intake of nutrients required for enzyme activity. One example is the enzyme glutathione peroxidase (GPX), which requires selenium for activity (1). The expression of such enzymes may be increased by oxidative stress, as appears to occur with catalase (2) and superoxide dismutase (3, 4) during HIV infection.

Several studies have found low plasma selenium concentrations in subjects with HIV infection (5–7). These low concentrations may reflect true selenium deficiency or depression of selenium concentrations as a result of immune activation (8, 9). Such depression may not result in functional deficiency if tissues have sufficient concentrations to meet requirements. However, low plasma selenium during HIV infection has been associated with low GPX activity (5, 6), and selenium supplementation has been shown to increase GPX activity (4) and decrease the need for hospitalization (10, 11), which indicates that functional deficiency can occur during HIV infection.

Most studies of oxidative damage and antioxidant protection in HIV infection have involved adults or young children. We have examined nutrient intakes (12, 13) and the relation of immune activation to oxidative damage (14) in subjects aged 13–23 y who were participating in the Reaching for Excellence in Adolescent Health (REACH) Study. In the REACH Study subjects, oxidative damage was not associated with HIV infection but was positively associated with the use of antiretroviral therapy (ART) and with markers of immune activation. In the current study, we

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[2] Supported by grants no. R01 AI46183 and no. P60 MD00222 from the National Institutes of Health; Project 5306-51530-006-00D from the US Department of Agriculture Agricultural Research Service; and grant no. U01-HD32830 from the National Institute of Child Health and Human Development, the National Institutes on Drug Abuse, National Institute of Allergy and Infectious Diseases, and the National Institute of Mental Health (all: to the REACH Study).

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Received April 30, 2006.

Accepted for publication August 17, 2006.

examined plasma selenium, whole-blood glutathione, and whole-blood GPX concentrations in these subjects.

SUBJECTS AND METHODS

Study population

The REACH Study was a prospective, observational, cohort study of the progression of HIV infection in adolescents conducted at 15 US clinical sites (15, 16). A standardized protocol for the different sites was developed through the Adolescent Medicine HIV/AIDS Research Network. Between March 1996 and November 1999, 325 adolescents aged 12–18 y who had acquired HIV infection through sexual activity or intravenous drug use were recruited. In addition, 171 HIV-negative adolescents were recruited from the same sites with the use of selection criteria that made the HIV-negative and HIV-positive groups comparable with regard to risk-behavior profiles and demographic characteristics (including age, sex, race, and ethnicity). This report describes the analysis of data from a supplemental cross-sectional study focusing on measures of immune activation, antioxidant protection, and oxidative damage that was conducted from January through October 2000. One site did not participate because of logistical difficulties. Of the 436 participants active in the 14 REACH Study network sites at the time of this study, 391 agreed to participate (264 HIV-positive and 127 HIV-negative subjects), and complete data for selenium, glutathione, and GPX were available for 365 of these subjects, who were included in the present analysis.

All participants provided written informed consent. The study was approved by the human subjects review boards at the University of California, Davis; Iowa State University; the University of Alabama at Birmingham; and each clinic site.

Data collected by the REACH Study

REACH data were collected by using face-to-face interviews, interactive computer interviews, medical record abstractions, and physical and laboratory examinations. HIV-positive subjects were seen every 3 mo, and HIV-negative subjects were seen every 6 mo. A detailed description of the REACH Study protocol was published elsewhere (15, 16).

Anthropometric measurements

Participants were gowned and were weighed at each visit by using digital scales accurate to 0.1 kg. Heights were measured by using calibrated stadiometers installed at each study site. Body mass index (BMI; in kg/m^2) was calculated for each participant.

HIV and immune activation variables

Laboratory tests were performed at local clinic sites according to the REACH Study protocol, as described elsewhere (15–19). Activated $CD8^+$ T cells were measured as described previously (20) by using CD38 and HLA-DR as markers of activation. Absolute $CD4^+$ T-cell counts for HIV-positive participants were stratified according to Centers for Disease Control and Prevention criteria for HIV/AIDS classification: $\geq$500, 200–499, and $<$200 cells/mm^{-3}. Clinical progression was ranked (ie, early and asymptomatic, intermediate and symptomatic, or late and with AIDS-indicator illnesses) by using Centers for Disease Control and Prevention guidelines (21). Quantitative HIV-1 RNA virus load in plasma was measured in a centralized laboratory on frozen specimens by using either nucleic acid sequence–based amplification or NucliSens assays (Organon Teknika, Durham, NC) as described elsewhere (19). ART was coded as a dichotomous variable (receiving or not receiving therapy), and descriptive data on ART use and compliance were previously reported (14).

Variables collected for the current study

Blood collection and processing

Nonfasting blood was collected at a regularly scheduled REACH Study visit. Site-to-site variation in biochemical variables was minimized by providing all sites with the same blood collection and processing tubes from a central source and by processing and analyzing all samples collected for this study at the same time in a central laboratory, as described previously (14).

Immune activation and oxidative damage variables

C-reactive protein (CRP), ceruloplasmin, and neopterin were measured by using commercial assays as described previously (14). Total plasma malondialdehyde was measured by using an HPLC determination of the thiobarbituric acid–malondialdehyde adduct (22). Plasma protein carbonyls were measured by using an enzyme-linked immunosorbent assay (23).

Glutathione

For analysis of total glutathione in whole blood, 100 μL whole blood was removed to a tube containing 0.5 mL freshly prepared 10% metaphosphoric acid (wt:vol) prepared in 0.54 mmol EDTA/L (metaphosphoric acid–EDTA solution) to precipitate protein and stabilize glutathione in the acidified supernatant. The tube was vigorously shaken by vortex for 5 s and then allowed to sit for 10–15 min at room temperature. The tube was then centrifuged at 4 °C for 10 min at 1500 $\times$ *g*. Supernatant fluid (0.5 mL) was removed and frozen in a 1.5-mL cryovial. This processing was done at each clinic site within 4 h of blood collection. Total whole-blood glutathione was measured from this sample by using electrochemical detection after HPLC separation as described (24). Results were expressed per milliliter of whole blood.

Glutathione peroxidase

GPX activity was measured in frozen whole blood by using a Hitachi 902 Autoanalyzer (Hitachi, Brisbane, CA) with reagents from Randox (San Diego, CA). Results were expressed per milliliter of whole blood.

Hemoglobin

Hemoglobin concentrations were measured by using a modified azidmethemoglobin reaction with a HemoCue B-Hemoglobin photometer (HemoCue Inc, Mission Viejo, CA) and the manufacturer's reagents. The same tube of frozen blood that was used for GPX measurement was used for hemoglobin.

Selenium and selenium supplements

Plasma selenium was measured by using HPLC detection of the selenium fluorophore formed by reaction with 2,3-diaminonaphthalene after sample digestion in a mixture of nitric and perchloric acids at a ratio of 5 to 2 (25). The use of dietary

selenium supplementation was assessed by using a food-frequency questionnaire at the same visit as the blood collection, as previously described (13).

Statistical analysis

Selenium reference values

Median serum selenium concentrations for males and females aged 14–17 y (0.121 and 0.119 μg/mL, respectively) and for males and females aged 18–30 y (0.123 and 0.120 μg/mL, respectively) from the third National Health and Nutrition Examination Survey (NHANES III) were used to determine whether concentrations in these REACH Study subjects were below the appropriate median (26). Because pregnant subjects were excluded from the analysis of the NHANES III data, pregnant REACH subjects were excluded from this analysis of serum selenium.

Comparison among groups

Comparison of selenium, glutathione, and GPX concentrations among groups was performed by using Student's *t* tests, one-factor analysis of variance (ANOVA), and 2-factor ANOVA. For these analyses, subjects were grouped by HIV status, stage of HIV disease as indicated by $CD4^+$ T-cell count, use of ART, and sex. These analyses were performed by using SIGMASTAT for WINDOWS software (version 3.01; Jandel Scientific, San Rafael, CA). Unless otherwise indicated, data are presented as means ± SEs, and $P < 0.05$ was considered to be significant.

Regression analysis

One objective of the regression analyses was to identify predictors of the antioxidant variables (ie, selenium, glutathione, and GPX) from among variables representing personal characteristics, HIV status, and immune activation. A second objective was to identify predictors of oxidative damage from among the antioxidant variables and the variables representing personal characteristics, HIV status, and immune activation.

The regression analyses included 5 types of variables: *1*) demographic and health status (ie, age, sex, pregnancy status, BMI, race, ethnicity, and smoking); *2*) HIV status and disease severity (ie, HIV status, $CD4^+$ T-cell count, plasma virus load, and use of ART); *3*) immune activation (ie, activated $CD8^+$ T cells, a proxy for HIV-specific cytotoxic T-cells; neutrophils; plasma neopterin, a product of activated macrophages; and 2 acute phase proteins, CRP and ceruloplasmin); *4*) antioxidant (ie, plasma selenium, whole-blood glutathione, and whole-blood GPX); and *5*) oxidative damage (ie, plasma protein carbonyls and plasma malondialdehyde). Multiple linear regression analysis was used to determine whether HIV status was significantly associated with the antioxidant variables when HIV status was included in a prediction equation with all demographic, health status, and behavior variables. In addition, backward stepwise multiple regression analysis was used to identify variables from the first 3 groups that predicted concentrations of glutathione, GPX, and selenium. Interaction of these variables with HIV status and sex was assessed by using interaction terms. Sex and HIV status were forced into the final equations.

This same approach was used to predict malondialdehyde and protein carbonyl concentrations by using variables from the first 4 sets of variables. Because significant variations were seen between the different study sites when markers of oxidative damage were analyzed (14), dummy (ie, 0 and 1) variables representing the different study sites were included in all models, although coefficients were not reported. To confirm that the variables selected in this manner were not unduly influenced by a priori assumptions (eg, inclusion of study site, sex, and HIV status in most models), both forward and backward stepwise analyses were performed to identify predictive variables for glutathione, GPX, and selenium from among all variables (from the 5 types mentioned above) without forced inclusion of any variables. These analyses identified the same significant predictive variables as did the analyses with forced inclusion of study site, sex, and HIV status.

RESULTS

Population characteristics

Subjects in this study ranged in age from 13 to 23 y, and they primarily were female and African American (**Table 1**). Twenty percent were Hispanic. On average, HIV-positive subjects were ≈7 mo older than HIV-negative subjects. In addition, 9.8% (17/174) of the female HIV-positive and 12.1% (11/91) of the female HIV-negative subjects were pregnant at the time of the study visit ($P = 0.71$, chi-square test). Demographic characteristics have been compared in a previous publication (14).

Glutathione peroxidase

GPX activity was significantly higher in HIV-positive than in HIV-negative subjects (**Figure 1**). The mean for HIV-positive subjects—13.8 ± 0.3 U/mL ($n = 244$)—was 28% higher than that for HIV-negative subjects—10.8 ± 0.3 U/mL ($n = 121$; $P < 0.001$). No difference was seen by sex. GPX activity was not associated with severity of HIV disease when subjects were grouped by CD4 T-cell count (data not shown). A positive association of HIV status with GPX activity was also seen when multiple regression analysis was used to adjust for demographic and health status variables ($R^2 = 0.154$; $n = 364$; standardized coefficient for HIV: 0.262 ± 0.052; $P < 0.001$). The results of the analysis were similar when hemoglobin concentration was included in the regression analysis ($R^2 = 0.259$; $n = 364$; standardized coefficient for HIV: 0.235 ± 0.049; $P < 0.001$). Hemoglobin was included to adjust for between-subject differences in red blood cell mass because red blood cells are a principal source of GPX activity in whole blood.

Stepwise regression analysis did not find significant associations between immune activation variables and GPX activity (**Table 2**). However, the use of ART was positively associated with GPX activity when all subjects were analyzed together or when HIV-positive subjects were analyzed separately (Table 2). Inclusion of ART use in the equation decreased the contribution of the HIV term, but HIV remained a significant positive predictor of GPX activity ($P = 0.017$; Table 2). Both reverse transcriptase and protease inhibitors were associated with higher GPX concentrations (data not shown). When GPX activity was compared by HIV status and ART use, HIV-negative subjects had the lowest median, HIV-positive subjects not taking ART had an intermediate median (11% greater than that of HIV-negative subjects), and HIV-positive subjects taking ART had the highest median (21% higher than that of the HIV-positive subjects not taking ART). The medians of the 3 groups differed significantly

TABLE 1
Demographic variables of HIV-positive and HIV-negative subjects from the REACH Study[1]

Variable	HIV-positive subjects	HIV-negative subjects	P^2
Female [% (*n*/total)]	73 (179/244)	77 (93/121)	0.55
Age (y)	20.0 ± 0.10 (244)[3]	19.4 ± 0.13 (121)	< 0.001
Age range (y)	13.8–23.2	14.8–22.9	—
Black [% (*n*/total)]	73 (179/244)	66 (80/121)	0.19
Hispanic [% (*n*/total)]	19 (46/244)	21 (26/121)	0.65
Smokers [% (*n*/total)]	42 (103/243)	35 (42/121)	0.20
BMI (kg/m^2)			
Females	29.8 ± 0.6 (179)	29.1 ± 0.8 (93)	0.31[4]
Males	23.6 ± 1.0 (65)	25.4 ± 1.5 (28)	
Neopterin ($\log_{10}$ μg/L)	0.832 ± 0.0157 (232)	0.632 ± 0.0210 (116)	< 0.001
Using antiretroviral therapy [% (n/total)]	53 (130/244)	—	—
Hemoglobin (g/dL)			
Females	14.7 (13.1, 17.1; 179)[5]	13.6 (12.8, 15.8; 93)	0.016[6]
Males	16.5 (15.1, 17.9; 65)	17.3 (16.2, 18.8; 28)	0.054[6]

[1] $n = 365$.
[2] Student's *t* test or chi-square test.
[3] $\bar{x}$ ± SE; *n* in parentheses (all such values).
[4] BMI comparisons were made by 2-way ANOVA using 1/BMI to normalize the distribution and provide equal variance (BMI differed by sex, $P < 0.001$; P for interaction = 0.084); comparison using only nonpregnant females gave essentially identical results.
[5] Median; 25th, 75th percentiles and *n* in parentheses (all such values).
[6] Hemoglobin data were not normally distributed and differed by sex ($P < 0.001$) using Wilcoxon's rank-sum test. Differences by HIV status were determined independently for each sex by using the same test.

(**Figure 2**). Plasma virus load was not associated with GPX activity (data not shown).

Selenium

Because selenium is required for GPX activity, we assessed selenium status in the REACH Study subjects by using plasma selenium concentrations. Providing selenium to deficient subjects increases GPX activity, but the increase diminishes to insignificant levels when subjects have initial serum selenium concentrations between 0.070 and 0.090 μg/mL (27). Subjects with concentrations <0.070 μg/mL are considered deficient. This cutoff is based on the plasma concentration of selenium needed to maximize erythrocyte GPX activity (1). None of the 365 subjects in the current study had a plasma selenium concentration <0.070 μg/mL. Nor did plasma selenium show any association with GPX activity when it was analyzed by bivariate ($R^2 = 0.006$, $P = 0.15$; $n = 365$) or multiple regression analysis (Table 2).

To compare the selenium status of REACH Study subjects with that of the overall US population, we determined the percentage of REACH Study subjects who had selenium concentrations below age- and sex-appropriate median according to NHANES III data, as described in Subjects and Methods. Overall, 55% (201 of 365) of subjects had concentrations below the median, a proportion that is not significantly different from the expected 50% ($P = 0.18$). The percentage of subjects below the median did not differ by sex [male: 50.5% (47/93); female: 56.6% (154/272); $P = 0.37$] or HIV status [HIV-positive: 56.6% (138/244); HIV-negative: 52.1% (63/121); $P = 0.48$].

The mean plasma selenium concentration for the 93 males, 0.126 ± 0.0021 μg/mL, was 6% higher than that for the 272 females, 0.119 ± 0.0012 μg/mL ($P = 0.002$, 2-factor ANOVA comparing sex and HIV status) (Figure 1). The mean for the 244 HIV-positive subjects, 0.120 ± 0.0013 μg/mL, was 4% lower than that for the 121 HIV-negative subjects, 0.125 ± 0.0020 μg/mL, a difference that was of marginal significance ($P = 0.071$). No significant sex × HIV status interaction was seen ($P = 0.62$).

When multiple regression analysis was used to adjust for demographic and health status variables, HIV status was found to be a significant negative predictor of plasma selenium ($R^2 = 0.168$; $n = 357$; standardized coefficient for HIV: −0.107 ± 0.052; $P = 0.039$). When stepwise analysis was used to identify significant predictors of plasma selenium, plasma neopterin, a marker of macrophage activation that is elevated by HIV infection (28), was found to be a significant negative predictor (Table 2). Inclusion of the neopterin term rendered the HIV term nonsignificant. Plasma virus load was not associated with plasma selenium (data not shown).

Glutathione

Whole-blood glutathione concentrations did not differ by sex or HIV status (Figure 1). Nor was HIV status a significant predictor of glutathione concentration when multiple regression analysis was used to adjust for demographic and health status variables with or without adjustment for hemoglobin (data not shown). When stepwise analysis was used to identify significant predictive variables, only hemoglobin was identified (Table 2). No significant association was seen between glutathione concentration and severity of disease (data not shown).

Oxidative damage

Stepwise regression analysis was used to determine whether plasma selenium, whole-blood glutathione, and whole-blood GPX were associated with plasma protein carbonyl and malondialdehyde concentrations. None of these variables was significantly associated with plasma protein carbonyls, but whole-blood GPX was positively associated with malondialdehyde (**Table 3**). When the use of ART was forced into the equation, its effect was not significant and did not alter the positive association of GPX with malondialdehyde (data not shown).

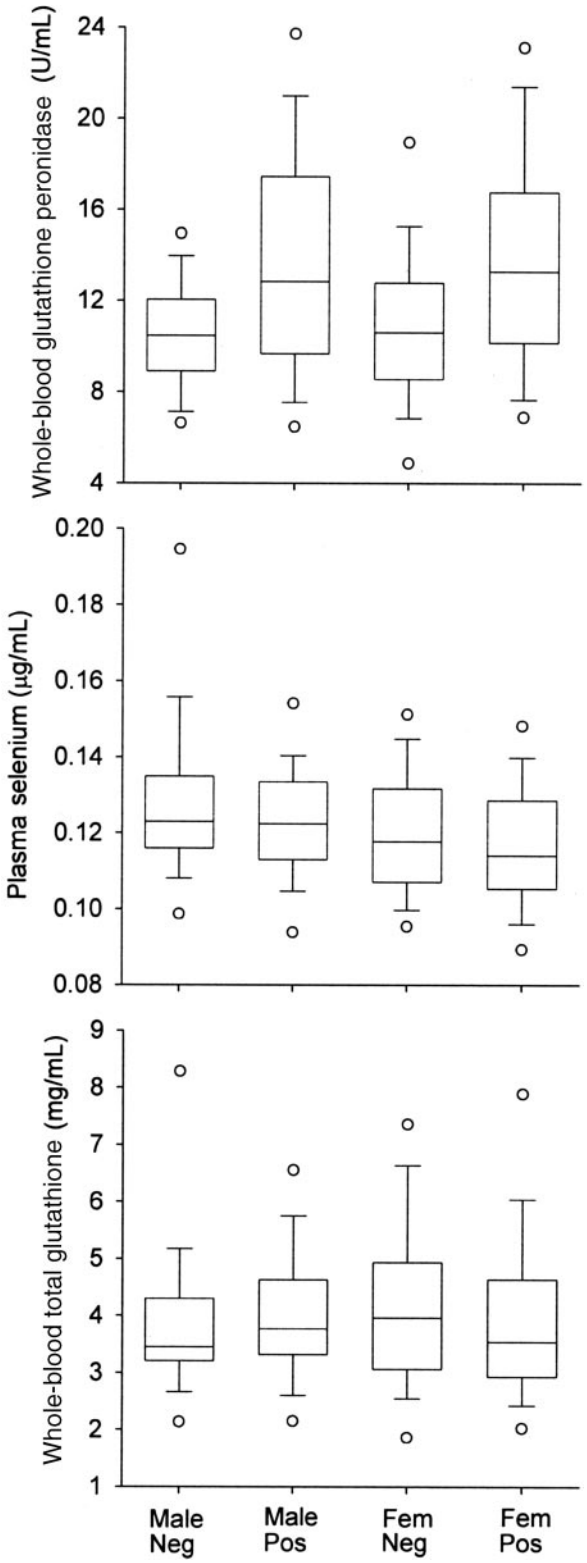

FIGURE 1. Whole-blood glutathione peroxidase, total glutathione, and plasma selenium concentrations in study subjects grouped by sex and HIV status. Box plots show 5th (open circle at bottom), 10th (lower error bar), 25th (bottom of box), 50th (solid line inside box), 75th (top of box), 90th (top error bar), and 95th (open circle at top) percentiles. $n = 28$ for HIV-negative males (Male Neg), 65 for HIV-positive males (Male Pos), 93 for HIV-negative females (Fem Neg), and 179 for HIV-positive females (Fem Pos). Two-factor ANOVA showed that mean $\log_{10}$ glutathione peroxidase concentrations differed significantly by HIV status ($P < 0.001$) but not by sex ($P = 0.78$; P for interaction = 0.67). Mean selenium concentrations differed significantly by sex ($P = 0.002$) and trended toward significance by HIV status ($P = 0.071$; P for interaction = 0.62). Mean $\log_{10}$ glutathione concentrations did not differ significantly by sex ($P = 0.87$) or HIV status ($P = 0.88$; P for interaction = 0.39).

DISCUSSION

The REACH Study cohort is unique among HIV study populations because its members are young, primarily female, and mostly African American or Hispanic. In addition, a high percentage of subjects are overweight or obese. Unlike in many previous studies, no association of HIV infection with oxidative damage was found in the REACH Study cohort. However, oxidative damage was observed in REACH Study subjects, particularly in association with smoking, marijuana use, immune activation, and use of ART (14). In addition, the higher levels of GPX activity found in the current study in association with both HIV infection and ART use suggest that oxidative stress in REACH Study subjects elicited an adaptive response of increasing antioxidant protection. This response may have minimized oxidative damage in HIV-positive members of the cohort, thus accounting for the lack of association of oxidative damage with HIV infection in these subjects.

Glutathione peroxidase

Whole-blood GPX concentrations were higher in HIV-infected REACH Study subjects than in uninfected control subjects. Previous studies made similar observations (4, 29, 30). A logical explanation for these findings is that HIV infection causes increased oxidative stress during erythropoiesis, which, in turn, increases the level of GPX-1 expression, the predominant form of the enzyme found in erythrocytes (1). Such a hypothesis is consistent with the higher concentrations of plasma catalase (2) and erythrocyte superoxide dismutase (3, 4) that have also been seen during HIV infection. Another possibility is that increased GPX activity in whole blood could be due to a putative, HIV-encoded GPX expressed in HIV-infected cells (31). However, no evidence exists for in vivo expression of such an enzyme, and that explanation seems unlikely.

In contrast with our observation of elevated GPX activity during HIV infection, several studies have reported that erythrocyte GPX concentrations are lower in HIV-infected subjects with advanced disease than in uninfected control subjects or in subjects with less advanced HIV disease (5, 6, 32). One possible reason for this difference is that relatively few REACH Study subjects had advanced disease (eg, only 13% had $CD4^+$ T-cell counts $<200/mm^{-3}$), although, even in REACH Study subjects with low CD4 cell counts, no indication was found of a trend toward decreasing GPX concentrations. In addition, the selenium deficiency that appears to have contributed to the lower GPX concentrations seen in more advanced disease in at least one earlier study (6) was not seen in the Reach Study subjects.

Higher GPX activity was associated with use of ART in HIV-positive REACH Study subjects. One other study has reported a similar observation (29). ART use has also been associated with mitochondrial toxicity and cellular oxidative stress in cell culture (33) and with increased oxidative stress (ie, higher plasma F_2 isoprostane concentrations) in patients (34). On the other hand, decreased malondialdehyde concentrations have also been seen after the initiation of ART in patients (35). These different results could be due to differences in the stage of HIV disease and the

TABLE 2
Prediction of plasma selenium, whole-blood glutathione (GSH), and whole-blood glutathione peroxidase (GPX) by multiple regression analysis for all REACH Study subjects[1]

	All subjects (no ART)			All subjects[2]			HIV-positive subjects		
Variables	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
$\log_{10}$ GPX (U/dL)[3]									
HIV-positive	0.240	0.048	< 0.001	0.133	0.055	0.017	-	-	-
Female	0.112	0.054	0.040	0.116	0.053	0.030	0.100	0.069	0.148
BMI	−0.139	0.050	0.006	−0.136	0.049	0.006	−0.105	0.066	0.110
Hispanic	−0.156	0.057	0.007	−0.155	0.056	0.006	−0.126	0.071	0.076
Hemoglobin (g/dL)	0.300	0.052	< 0.001	0.284	0.051	< 0.001	0.247	0.066	< 0.001
Antiretroviral therapy				0.212	0.058	< 0.001	0.232	0.065	< 0.001
$\log_{10}$ Selenium (μg/mL)[4]									
HIV-positive	−0.049	0.054	0.369						
Female	−0.105	0.054	0.051						
Log_{10} neopterin	−0.173	0.055	0.002						
$\log_{10}$ GSH (mg/dL)[5]									
HIV-positive	−0.060	0.051	0.241						
Female	0.045	0.056	0.419						
Hemoglobin (g/dL)	0.238	0.055	< 0.001						

[1] REACH, Reaching for Excellence in Adolescent Health; ART, antiretroviral therapy. Variables were selected by backward stepwise regression analysis, as described in Subjects and Methods. No significant interactions were seen between HIV status and sex, BMI, ethnicity, or hemoglobin. The *F* test (*P*) was used for each coefficient.
[2] ART was included as a variable in this equation for all subjects, even though only HIV-positive subjects may receive ART.
[3] $n = 364$, 364, and 243 and $R^2 = 0.223$, 0.252, and 0.188, for All subjects (no ART), All subjects, and HIV-positive subjects, respectively.
[4] $n = 348$ and $R^2 = 0.181$.
[5] $n = 365$ and $R^2 = 0.110$.

level of oxidative stress at the time of initiation of ART. In the REACH Study subjects, the use of ART was associated with both higher GPX and higher malondialdehyde concentrations, which supports the notion of increased oxidative stress in subjects taking ART. Such observations suggest that monitoring antioxidant enzyme activity may be a useful method of assessing oxidative stress during HIV infection and treatment.

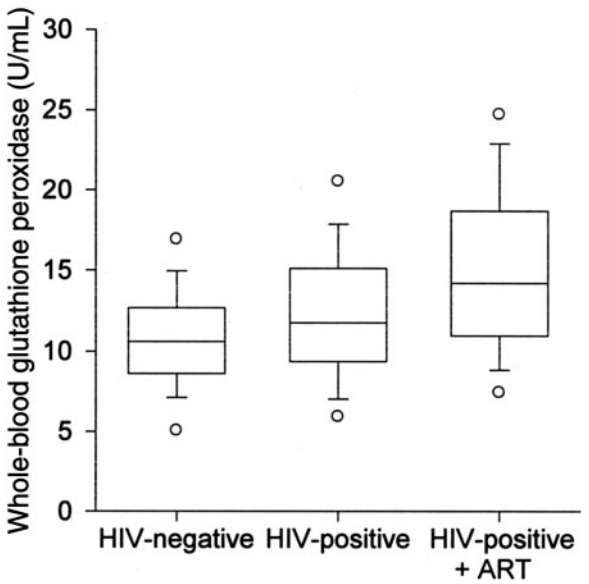

FIGURE 2. Whole-blood glutathione peroxidase concentrations in HIV-negative subjects ($n = 121$) and HIV-positive subjects not taking ($n = 114$) or taking ($n = 130$) antiretroviral therapy (ART). Box plots show 5th (open circle at bottom), 10th (lower error bar), 25th (bottom of box), 50th (solid line inside box), 75th (top of box), 90th (top error bar), and 95th (open circle at top) percentiles. The difference among the 3 groups was significant, $P < 0.001$. Each of the 3 groups differed significantly from each of the other groups, $P < 0.05$ (one-factor ANOVA on ranks using Dunn's method for all pairwise multiple comparisons).

Regression analysis found sex, BMI, and Hispanic ethnicity to be associated with GPX concentrations. In REACH Study subjects, BMI had a negative association with GPX activity. Two previous studies also found such negative associations (36, 37). It is interesting that all 3 of these studies included a significant number of overweight and obese subjects. Whether a similar association would be seen in subjects with lower BMIs is uncertain. Females had slightly higher GPX activity levels than did males by multiple regression analysis, but this association was not seen when males and females were compared directly, unless GPX activity was analyzed in a ratio to hemoglobin (data not shown). Previous studies with subjects in this age range have observed higher GPX concentrations in erythrocytes (38) or plasma (27) from females than from males, but the underlying reason for this sex difference is not clear. To our knowledge, no

TABLE 3
Prediction of plasma $\log_{10}$ malondialdehyde (μmol/L) by multiple regression analysis for all REACH Study subjects[1]

Variables	Coefficient	SE	*P*
Female	−0.164	0.056	0.001
HIV-positive	0.0369	0.051	0.458
Log_{10} GPX (U/g hemoglobin)	0.162	0.055	0.001

[1] $n = 364$. REACH, Reaching for Excellence in Adolescent Health; GPX, glutathione peroxidase. Variables were selected by backward stepwise regression analysis, as described in Subjects and Methods. The *F* test (*P*) was used for each coefficient. $R^2 = 0.223$.

previous study has associated Hispanic ethnicity with GPX activity. This association could be spurious, but the low *P* value (Table 2) suggests that an underlying genetic or environmental factor may account for this observation.

Selenium

According to our analysis of plasma selenium concentrations, the selenium status of both the HIV-negative and HIV-positive REACH Study subjects was adequate. Several observations support this conclusion. First, no subject in this study has a serum selenium concentration <0.070 μg/mL, which is the commonly accepted cutoff for deficiency according to maximal GPX activity (1, 39). Second, 45% of subjects had serum selenium concentrations above the age- and sex-appropriate medians for the US population (26). This proportion was not significantly different from the expected 50%. Third, GPX activity levels were used as a functional marker of selenium status because GPX requires selenium for activity and has been shown to have a positive association with plasma selenium, particularly at low plasma selenium concentrations in populations (eg, that of China) with demonstrable deficiency (27, 39, 40) but also in populations (eg, that of the United Kingdom) in which dietary selenium is considered adequate and plasma selenium concentrations are higher (41). In the latter instance, correlations are not nearly as strong as are those from areas of selenium deficiency, but they nonetheless have been shown, at least in subjects <18 y of age. The absence of such a correlation in the current study suggests that the selenium status of the REACH Study subjects was adequate to maintain GPX concentrations, which supports the conclusion that these subjects were not selenium deficient.

Previous studies found lower plasma selenium concentrations in HIV-positive than in HIV-negative subjects and also found lower concentrations in subjects with more advanced disease than in those with less advanced disease (5–7). In the current study, plasma selenium did not differ significantly by HIV status when the groups were compared directly, although a significant negative association was seen for HIV status by using multiple regression analysis to adjust for demographic and health status variables. In agreement with our finding of a minimal association of HIV with plasma selenium, one study indicates that low selenium concentrations are now less prevalent in HIV-positive subjects than they were before the introduction of highly active ART, even when disease severity is controlled in the analysis (42). Thus use of highly active ART by the REACH Study subjects may have also contributed to the maintenance of adequate plasma selenium concentrations, possibly by improving overall health and appetite.

The negative association between HIV status and plasma selenium concentrations that was seen by regression analysis in the REACH Study subjects may be due to inflammation-induced changes in selenium distribution. Immune activation, as indicated by elevated neopterin concentrations, was associated with lower plasma selenium concentrations in the REACH Study subjects. Neopterin is a marker of macrophage activation and is elevated in persons with HIV and other infections (28, 43). The anti-HIV response may thus be associated with reductions in plasma selenium. This hypothesis is supported by our additional observation that, when neopterin was removed from the stepwise regression procedure used to select significant predictors of selenium, the concentration of activated CD8 T cells, a marker of the anti-HIV cytoxic T-cell response (44), was found to have a significant negative association with plasma selenium (data not shown). Previous work also pointed to an association of immune activation with lower plasma selenium. For example, minor surgery, which induces the acute phase response, transiently decreases selenium but simultaneously increases CRP (9). The decrease in selenium was primarily attributed to decreased plasma selenoprotein P, which accounted for half of the plasma selenium concentration. Others have also reported a negative association between CRP and selenium (45). In addition, one animal study suggested that the reduction in plasma selenium during the acute phase response is due to transient redistribution to other tissues. Administration of bacterial lipopolysaccharide to rats transiently reduced plasma (and liver) selenium concentrations but increased muscle, lung, spleen and heart selenium concentrations (8). Thus, redistribution may account for the negative correlation between neopterin and selenium seen in the current study and one previous HIV study (6). Because such immune activation persists during HIV infection, the resulting depression of plasma selenium may also be chronic, rather than transient. The consequences of such chronic depression of plasma selenium are not clear; in the current study, the apparent depression was modest, because no subjects had selenium concentrations indicative of deficiency.

Low plasma selenium concentrations have been linked to a poor prognosis for HIV-positive subjects (7, 46). This association could be causal, in that poor selenium status may diminish immune function and increase virus replication, or it could be spurious, in that low plasma selenium may simply be a marker for increased immune activation in more advanced disease. It is interesting to note that increasing neopterin concentrations are linked both to low plasma selenium concentrations, as seen in the current study, and to poor prognosis during HIV infection (28). This linkage of neopterin to poor prognosis presumably reflects the underlying activation of macrophages by increasing amounts of HIV replication. However, selenium supplementation during HIV infection has been shown to reduce the rate of hospital admission (10, 11), which suggests that a functional selenium deficiency does exist for some populations with HIV infection and low plasma selenium concentrations. Female sex was also associated with lower plasma selenium concentrations in the REACH Study subjects, an observation that has been made previously (26, 47, 48) and that perhaps reflects greater selenium intake in males.

Glutathione

Whole-blood glutathione concentrations did not differ by HIV status, nor were significant predictors of glutathione concentrations found among the immune activation variables in this study. Low whole-blood glutathione concentrations were previously reported during HIV infection (4, 49–51), although the finding was not universal (52, 53). Again, differences may have been seen in previous studies because of the presence of more-advanced HIV disease than was seen in the REACH Study subjects.

Oxidative damage

Whereas oxidative damage was not associated with HIV infection in the REACH Study subjects, as previously shown (14), the current analysis indicates that GPX concentrations were positively associated with plasma malondialdehyde concentrations.

This association may seem counterintuitive because GPX decreases peroxide concentrations that can lead to the production of the lipid peroxidation product malondialdehyde. However, if chronic oxidative stress leads to increased concentrations of GPX in nascent erythrocytes, then such a relation may be produced. The current study supports this hypothesis, because GPX concentrations were also positively associated with use of ART in the REACH Study subjects, and ART is associated with elevated malondialdehyde concentrations (14). Thus, an adaptive response to oxidative stress may be mounted in the REACH Study subjects by an increase in the GPX concentrations in erythrocytes. A similar association has been seen between malondialdehyde and GPX in diabetic rats with oxidative damage and nondiabetic rats with less oxidative damage (54).

Conclusions

The current analysis indicates that REACH Study subjects had adequate selenium status, although immune activation was associated with lower plasma selenium concentrations. Antioxidant defenses, as measured by GPX activity, were elevated in HIV-positive subjects and are further elevated in subjects using ART. This elevation also suggests that underlying selenium status was sufficient to allow for this apparent increase and that a protective antioxidant response was mounted in the young, relatively well-nourished, and healthy REACH Study subjects during HIV infection. The current data also suggest that ART may have unanticipated effects on antioxidant defenses that should be further explored. Monitoring changes in antioxidant enzymes may be useful in such studies.

We thank all of the investigators and staff members of the Adolescent Medicine HIV/AIDS Research Network (1994–2001) and the participants for their valuable contributions. We also thank Wayne Chris Hawkes for performing the selenium analysis and contributing to the analysis of the data. We acknowledge Alan Buckpitt and members of his laboratory for assistance with the glutathione analysis; Alina Wettstein for performing the neopterin, glutathione, and glutathione peroxidase assays; Zeynep Alkan for performing the selenium assays; Manuel Tengonciang for performing the hemoglobin assays; Leslie Woodhouse for supervising the laboratory work; and Ginny Gildengorin and Bruce Mackey for assistance with regression analysis.

CBS, GSM, and CMW participated in the design and implementation of the study and in the analysis of the data. LAK and SDD participated in the implementation and analysis. CBS wrote the draft of the manuscript, and all of the authors reviewed the draft and approved the final version of the manuscript. None of the authors had a personal or financial conflict of interest.

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