Direct correlation of glutathione and ascorbate and their dependence on age and season in human lymphocytes[1–3]

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ABSTRACT

Background: Endogenous reactive oxygen species appear to contribute to aging and cancer and dietary antioxidants, present in fruit and vegetables, counteract these effects.

Objective: The objective was to examine the association between intracellular glutathione, ascorbate (vitamin C), and α-tocopherol (vitamin E) in human lymphocytes.

Design: The study group consisted of 240 healthy nonsmoking volunteers with an approximately equal number of male and female subjects subdivided into 3 age groups: 18–39, 40–59, and ≥60 y). Glutathione, glutathione disulfide, ascorbate, and α-tocopherol were measured in lymphocytes by HPLC.

Results: The average concentration of antioxidants in lymphocytes was 27 ± 8 nmol/mg protein for glutathione, 21 ± 8 nmol/mg protein for ascorbate, and 0.4 ± 0.2 nmol/mg protein for α-tocopherol. There was a strong positive correlation between glutathione and ascorbate ($r = 0.62$, $P < 0.001$). No correlation was observed for glutathione and ascorbate with α-tocopherol. The concentration of glutathione in lymphocytes was inversely correlated with age ($r = -0.19$, $P < 0.01$), as was that of ascorbate ($r = -0.22$, $P < 0.01$), with 10–20% lower values in elderly than in young and elderly subjects. The concentrations of glutathione in lymphocytes were as much as 25% higher and those of ascorbate were as much as 38% higher during the summer than during the winter. The seasonal variation of ascorbate in lymphocytes was described by a linear function for age and a periodic sine function for season.

Conclusion: Glutathione and ascorbate are directly correlated in human lymphocytes. *Am J Clin Nutr* 2000;71:1194–200.

KEY WORDS Vitamin C, vitamin E, glutathione, lymphocytes, ascorbic acid, antioxidants, micronutrients, aging, elderly, cancer, reactive oxygen species, season

INTRODUCTION

It is well known that fruit and vegetables protect against cancer (1–3). The quartile of the population with the lowest consumption of fruit and vegetables has about double the rate of most cancers compared with the quartile with the highest consumption. This effect may be attributed to a variety of anticarcinogenic ingredients in fruit and vegetables, including dietary fiber, folic acid, vitamin A, vitamin C, vitamin E, carotenoids, and selenium. Most epidemiologic studies of specific antioxidants suggest that these compounds contribute to the overall protection against cancer at several sites (3, 4). On the other hand, there have been conflicting results. For example, the incidence of lung cancer was not affected by vitamin E supplementation, whereas it increased with β-carotene supplementation in a large-scale study of male smokers (5). Although the effects of antioxidant supplementation remain to be established in humans, there is much evidence from tissue culture and animal studies indicating that antioxidants decrease oxidative DNA damage, thereby decreasing mutagenesis and carcinogenesis (6, 7). Furthermore, reactive oxygen species damage cellular components, and this damage is associated in part with various diseases, including cancer and atherosclerosis (6, 8).

Glutathione and vitamin C (ascorbate) are the 2 main aqueous-phase antioxidants within cells. Of these, ascorbate is taken up solely through the diet in humans and concentrations can vary significantly without any apparently harmful side effects. Ascorbate is required for the synthesis of collagen and severe deficiency results in scurvy. On the other hand, glutathione is not directly taken up by cells but it is synthesized intracellularly. This compound fulfills numerous functions by assisting in the synthesis of protein and DNA, the maintenance of intracellular thiol groups, the enzymatic reduction of dehydroascorbate, the transport of amino acids into cells, and the elimination of toxic compounds (9). Furthermore, glutathione is required in the elimination of hydrogen peroxide and organic peroxides by glutathione peroxidase. The ratio of glutathione to glutathione disulfide is an indication of the intracellular redox status of cells, which is implicated in proliferation and vital responses such as apoptosis (10, 11). Although much is known about the individual functions of glutathione and ascorbate, not much work has been devoted to the interaction of these antioxidants in cells. The work

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of Meister (12) supports the idea that glutathione and ascorbate mutually spare each other. In particular, high doses of vitamin C were shown to rescue glutathione-deficient animals, which cannot synthesize ascorbate. Conversely, glutathione-enhancing agents like glutathione monoethylester were shown to delay the onset of scurvy. The reasons for these effects are not clear and whether they can be extrapolated to humans is not known.

In the present work, the association between antioxidant concentrations was examined in lymphocytes by systematically measuring the concentrations of glutathione, glutathione disulfide, ascorbate, and α-tocopherol in samples of freshly isolated lymphocytes from 240 healthy subjects.

SUBJECTS AND METHODS

Subjects and blood samples

A trained nurse interviewed ≈1500 potential candidates over the telephone during the course of the study. The main criterion for acceptance into our study was that the candidates were in good health, with no indication of heart disease, cancer, or chronic disorders such as diabetes, arthritis, hepatitis, or HIV infection, as determined by a detailed medical questionnaire. In addition, candidates were accepted only if they did not smoke or take prescription medication or daily vitamin supplements. Subjects were recruited continuously (5 subjects/wk) from a list of eligible candidates to obtain an approximately equal number of men and women in 3 age groups: young (18–39 y), middle-aged (40–59 y), and elderly (≥60 y). Blood samples (100 mL) were collected in the morning from fasting individuals by venipuncture into five 20-mL tubes containing sodium citrate as anticoagulant. The experimental protocol was approved by the ethics committee at the Sherbrooke University Institute of Geriatrics, and all subjects gave their written consent before the study began.

Laboratory water and buffers

Water was extensively purified, first by double distillation through a glass still (Fi-stream II; Barnstead Thermolyne, Dubuque, IA) and then by passage through a water-purification system equipped with ultrapure and high-purity Low Toc cartridges (Easypure RF; Barnstead Thermolyne). The final resistivity of this water was no less than 18.3 MΩ · cm. Buffers were made from chemicals with the highest available purity from either Sigma Chemical Co (St Louis) or Aldrich Chemical Co (Milwaukee). Phosphate-buffered saline solution (PBS; 150 mmol/L, pH 7.4) was treated with 1 g Chelex 100 resin/L (Bio-Rad, Richmond, CA) to remove trace metal ions and was filtered before use.

Separation of lymphocytes

The protocol for the isolation of lymphocytes from blood was adapted from an established method (13). First, the blood (100 mL) was divided equally into three 50-mL polypropylene tubes (Falcon; Becton Dickinson, Franklin Lakes, NJ) and the tubes were centrifuged at 300 × *g* for 15 min at room temperature to separate the plasma. The plasma was removed and defibrinated by adding calcium chloride to a final concentration of 33 mmol/L and was used later for the removal of monocytes. Subsequently, 10–15 mL PBS was added to the blood-containing tubes to complete the volume to 30 mL, followed by 15 mL of a 2% dextran solution. The erythrocytes were allowed to settle out for 30 min at 37 °C. The resulting leukocyte suspension was layered onto Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden), with a specific gravity of 1.077, and centrifuged at 750 × *g* for 20 min at room temperature. The mononuclear cell layer was collected and the cells were washed twice with RPMI media (Gibco BRL, Grand Island, NY) at 300 × g for 15 min at room temperature. The cells (lymphocytes and monocytes) were then incubated at 37 °C in RPMI media with 10% fetal bovine serum (ICN, Costa Mesa, CA) under an atmosphere of 5% CO_2 for 50 min by using Falcon tissue culture flasks coated with defibrinated plasma. The monocytes attached to the surface of the flask, allowing the lymphocytes to be washed off. The resulting lymphocytes (≈100 × 10^6 cells) were examined under a microscope and were >90% viable as determined by trypan blue exclusion. There was no visual trace of red blood cells in the final lymphocyte preparation.

Analysis of glutathione and ascorbate

The intracellular antioxidants glutathione and ascorbate were measured according to the method of Rose and Bode (14). Ten million cells were washed 3 times with ice-cold PBS, suspended in 200 mmol phosphoric acid/L containing 0.1 mmol EDTA/L, subjected to 3 freeze-thaw cycles (4 °C to –80 °C), and centrifuged at 12 000 × *g* for 10 min at 4 °C. Samples were stored at –80 °C until analyzed (within 3 mo). Glutathione and ascorbate were separated by HPLC with an Inertsil 5-(m octadecylsilyl column (150 × 4.6 mm internal diameter; CSC, Montreal) with 200 mmol phosphate/L (pH 3.0) as the mobile phase. The HPLC system consisted of an M6000 pump (Waters, Milford, MA) and an L-ECD-6A amperometric electrochemical detector (Shimadzu, Kyoto, Japan) set at 1.1 V against silver chloride as the reference electrode. The protein concentration in cell lysates was determined with the Bradford assay (Bio-Rad), which was automated by using an AS 3000 autosampler (Hitachi, Tokyo) and a U 3000 spectrophotometer (Hitachi). All standards were stored at –80 °C and aliquots were used for the duration of the study.

Analysis of glutathione disulfide

A method based on HPLC coupled to a specific postcolumn reaction with *o*-phthalaldehyde and fluorescence detection (15) was developed to measure glutathione disulfide in lymphocytes. Briefly, samples of 10 million cells were processed in the same way as were samples for the glutathione analysis, except that 1 mmol *N*-ethyl maleimide/L was added to the cell suspension before cell lysis by freeze-thawing. HPLC analysis was carried out by using a Hypersil 5-(m octadecylsilyl column (150 × 4.6 mm internal diameter; CSC) with a mobile phase consisting of 50 mmol phosphate/L (pH 3.0). To derivatize with *o*-phthalaldehyde, the HPLC eluant was mixed with 200 mmol phosphate/L (pH 12) containing 373 μmol *o*-phthalaldehyde/L flowing through a 2-m reaction loop held at 60 °C. The primary eluant and the postcolumn reaction medium were delivered at a flow rate of 1 mL/min. The final eluant was monitored by a fluorescence detector (model RF-551; Shimadzu) with an excitation wavelength of 340 nm and an emission wavelength of 425 nm. Under these conditions, glutathione disulfide in cell lysates eluted at ≈12 min without any contamination from other reactive compounds.

Analysis of α-tocopherol

The method used to analyze α-tocopherol in lymphocytes was adapted from standard methods (16, 17). Ten million cells were resuspended in 0.1 mol sodium dodecyl sulfate/L (200 μL) plus 10 μL butylated hydroxytoluene (10 g/L), as a lipophilic antioxidant,

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and the sample was stored at −80 °C until analyzed. During thawing, 200 μL water was added to the sample and the suspension was refrozen and rethawed once. To extract α-tocopherol from the sample, 400 μL ethanol containing 100 μmol tocopherol acetate/L as internal standard was added, and then, after being vortexed briefly, 400 μL hexane was added. The mixture was then vigorously vortexed for 5 min at room temperature. The polar and organic phases were separated by centrifugation at 1000 × *g* for 5 min and an aliquot (200 μL) of the top (organic) layer was withdrawn and dried under vacuum. α-Tocopherol was analyzed by HPLC with use of a Sephasil peptide 5-μm octadecylsilyl column (150 × 4.6 mm internal diameter; Pharmacia Biotech) with a mobile phase of methanol:absolute ethanol:2-propanol (72.1:19.7:8.2, by vol). The same HPLC system described above was used to analyze aqueous-phase antioxidants, except that the potential of the electrochemical detector was set to 0.70 V against silver chloride as the reference electrode to optimize the detection of α-tocopherol.

Statistical analysis

Least-squares linear regressions were performed on a personal computer with use of either EXCEL 97 (Microsoft Corp, Redmond, WA) or SPSS (version 8.0; SPSS Inc, Chicago) software. The probability (*P*) that the correlation coefficient (*r*) was zero was determined by using a two-tailed *t* test of the null hypothesis. Probabilities < 0.05 were considered significant. Grouped data are expressed as means ± SDs. Differences in grouped data were determined by using an unpaired *t* test.

RESULTS

Antioxidant profile in lymphocytes

The concentrations of 2 aqueous-phase antioxidants, glutathione and ascorbate, and one lipophilic phase antioxidant, α-tocopherol, together with the oxidation product of glutathione, glutathione disulfide, were measured in lymphocytes obtained from 240 healthy men and women aged 18–86 y. The concentrations are reported as the mean for the 3 age groups (**Table 1**). The values were similar to those in the literature for human lymphocytes [22 nmol glutathione/mg protein (19), 0.4 nmol α-tocopherol/mg protein (20), and 3 mmol ascorbate/L (21)]. Analysis of the data by least-squares linear regression showed a strong correlation between intracellular glutathione and ascorbate in human lymphocytes (**Figure 1**). In addition, the concentrations of glutathione and ascorbate (**Figure 2** and **Figure 3,** respectively) were dependent on season and age. There was no significant correlation of α-tocopherol with ascorbate ($r = 0.066$, $P = 0.30$) or glutathione ($r = 0.048$, $P = 0.45$). Lastly, the ratio of glutathione to glutathione disulfide remained nearly constant in all lymphocyte samples; it was not correlated with either ascorbate ($r = 0.042$, $P = 0.51$) or age ($r = 0.01$, $P = 0.87$), nor was there any change in the ratio with sex. Also note that the ratios of glutathione to glutathione disulfide were > 100 in all samples. The low glutathione disulfide concentrations suggest that exposure of lymphocytes to oxidative conditions was minimal during their separation from blood (22).

Correlation of glutathione and ascorbate

Individual glutathione values ranged from 14 to 55 nmol/mg protein and those for ascorbate ranged from 8 to 40 nmol/mg protein. The correlation between intracellular glutathione and ascorbate was highly significant. In addition, the correlation coefficients for glutathione and ascorbate were similar for each subgroup: $r = 0.63$, 0.68, and 0.70 for women and $r = 0.39$, 0.65, and 0.65 for men in the young, middle, and elderly age groups, respectively. These values indicate that the correlation of glutathione with ascorbate in lymphocytes does not depend on sex or age. There was no obvious reason, however, for the greater scatter and weaker correlation in young men. Lastly, a correlation was observed between glutathione disulfide and ascorbate ($r = 0.18$, $P < 0.01$; data not shown); however, no significant correlation was observed between ascorbate and the ratio of glutathione to glutathione disulfide ($r = 0.042$, $P = 0.51$).

Effect of age and season

On the basis of least-squares linear regression analyses, the concentrations of glutathione and ascorbate were significantly lower with increasing age; the rates of loss were 0.084 and 0.074 $\text{nmol} \cdot \text{mg protein}^{-1} \cdot \text{y}^{-1}$, respectively (Figure 2). For those between the ages of 30 and 70 y, glutathione was 15% lower and ascorbate was 17% lower in the older subjects. Similarly, we observed differences in the average concentrations of glutathione and ascorbate when the data were divided according to the 3 age groups (Table 1). There was no significant interaction of sex and age and no significant difference in the change of antioxidants with age for men and women. Finally, there was no significant correlation between α-tocopherol and age ($r = 0.085$, $P = 0.19$) or season.

In addition to the effect of age, analysis of our data over a 16-mo period showed variations in the concentrations of antioxidants with season. To examine this effect, we initially fit a sine function to the data:

$$y = A \cdot \sin(\omega t + \phi) + B \quad (1)$$

TABLE 1
Antioxidant concentrations by age[1]

Age group	Mean age	Ascorbate	Glutathione	GSH:GSSG	Tocopherol
	y	*nmol/mg protein*	*nmol/mg protein*		*nmol/mg protein*
Young, 18–40 y ($n = 93$)	27	21.7 ± 7.2[2]	28.1 ± 8.5	124 ± 50	0.44 ± 0.26
Middle-aged, 40–59 y ($n = 61$)	48	20.3 ± 6.8	27.5 ± 8.4	139 ± 67	0.38 ± 0.25
Elderly, ≥60 y ($n = 86$)	72	18.8 ± 7.4[3]	23.9 ± 9.5[3]	141 ± 81	0.50 ± 0.25

[1] An average protein concentration of 0.025 mg/10^6 cells can be used to convert to nmol/10^6 cells and an estimated volume of 200 nL/10^6 cells (18) converts to mol/L. For example, 21.7 nmol/mg ≈ 0.54 nmol/10^6 cells ≈ 2.7 mmol/L. GSH, glutathione; GSSG, glutathione disulfide.

[2] $\bar{x}$ ± SD.

[3] Significantly different from the young group, $P < 0.01$.

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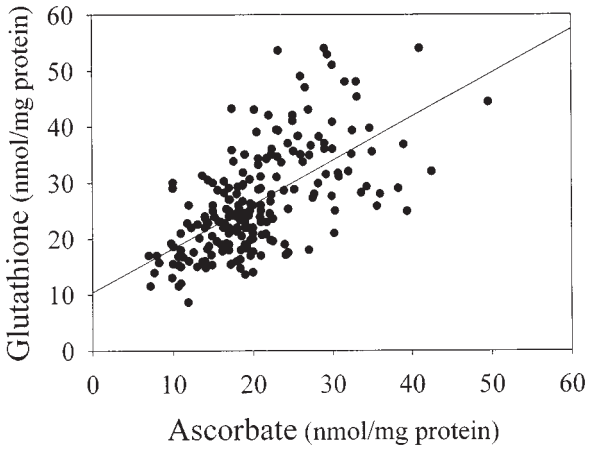

FIGURE 1. Correlation of ascorbate and glutathione concentrations in human lymphocytes. The following linear function was fitted to the data: $y = 10.4 + 0.78x$ ($r = 0.62$, $P < 0.001$; $n = 240$) where x and y are the intracellular concentrations of ascorbate and glutathione, respectively, in human lymphocytes. The compounds were normalized to total protein as measured by the Bradford assay.

where y is the concentration of glutathione or ascorbate, t is the date of blood donation, A and B are constants, and ω and ϕ represent the periodicity and constant of the sine function, respectively. A least-squares fit of this function to the data was significant for both glutathione and ascorbate ($P < 0.05$). Furthermore, the calculated value of ω corresponds to a periodicity of 376 d, confirming that the effect of season occurs on an annual cycle. The concentration of glutathione was found to fluctuate by as much as 25% between the low values observed in January compared with the high values observed in June; the variation in the concentration of ascorbate was 38%.

The variation of intracellular ascorbate with season was stronger for men and women in the young age group (18–34 y) than in either the middle-aged or the elderly group (Figure 3). To describe this effect, we constructed a mathematical model that takes into account the interaction of season and age:

$$\text{Asc} = (C + D \cdot \text{age}) \cdot \sin(\omega t + \phi) + (E + F \cdot \text{age}) \qquad (2)$$

where $(C + D \cdot \text{age})$ represents the age-dependent change in amplitude of the periodic function $[\sin(\omega t + \phi)]$ and $(E + F \cdot \text{age})$ represents the age-dependent change in baseline. A least-squares Levenberg-Marquardt analysis of the data with use of the above function yielded the following parameters ($P < 0.05$): $C = -8.02 \pm 1.96$ nmol/mg protein, $D = 0.096 \pm 0.039$ nmol · mg protein$^{-1} \cdot y^{-1}$, $E = 24.2 \pm 1.2$ nmol/mg protein, $F = -0.074 \pm 0.024$ nmol · mg protein$^{-1} \cdot y^{-1}$, $\omega = 0.017 \pm 0.003$/d, and $\phi = 1.94 \pm 0.54$ (Figure 3). The values for modulation of the amplitude $(C + D \cdot \text{age})$ indicate that the effect of season diminishes at a rate of 1.2%/y. Although this model is significant, the low correlation of determination (0.15) suggests that season and age account for a small percentage (15%) of the variation observed in the concentration of ascorbate in lymphocytes.

DISCUSSION

The plasma concentration of vitamin C is below the reference range in smokers, alcoholic persons, women taking oral contraceptives, pregnant and lactating women, hospitalized patients, and autonomous as well as institutionalized elderly (4). Interestingly, vitamin C is also lower than the reference range in patients with diabetes (23), hepatitis (24), AIDS (25), and lung cancer (26). This suggests that low concentrations of vitamin C reflect greater oxidative stress. In a parallel study with an overlapping sample set, we showed that glutathione and ascorbate were negatively correlated with oxidative DNA damage (27). Here, we report that glutathione and ascorbate concentrations are strongly and directly correlated in human lymphocytes. This is an important finding that contributes to our understanding of the complex functions and interactions of these antioxidants in vivo.

Several studies with rodents have pointed to an association between glutathione and ascorbate. The concentration of ascorbate in the tissues of newborn rats or guinea pigs that are not able to synthesize ascorbate decreases significantly after treatment with buthionine sulfoximine, an agent that inactivates glutamate–cysteine ligase and reduces tissue glutathione (12). In these animals, the administration of ascorbate increases tissue glutathione, indicating that ascorbate spares glutathione. Conversely, the effects of glutathione esters in ascorbate-deficient guinea pigs

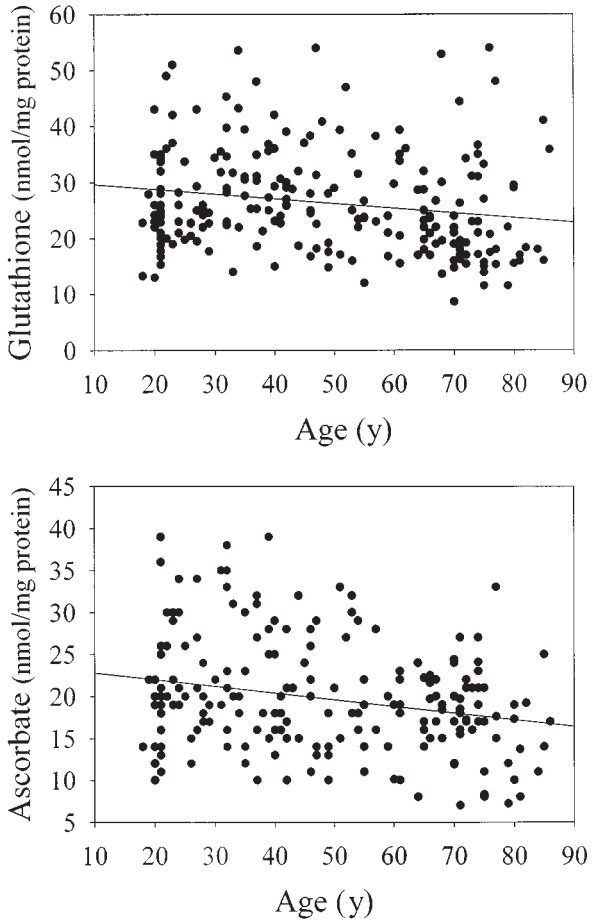

FIGURE 2. Correlation of glutathione and ascorbate concentrations with donor age in human lymphocytes. The following linear function was fitted to the data for glutathione: $y = 30.4 - 0.084x$ ($r = -0.19$, $P < 0.01$; $n = 240$), where y is glutathione and x is donor age. The following linear function was fitted to the data for ascorbate: $y = 23.4 - 0.074x$ ($r = -0.22$, $P < 0.01$; $n = 240$), where y is ascorbate and x is donor age.

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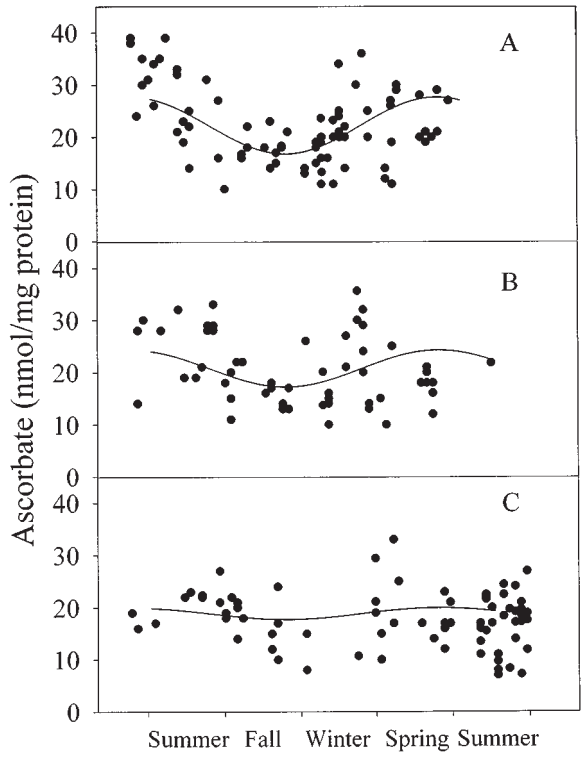

FIGURE 3. Effect of season on the concentration of ascorbate in lymphocytes in the young (A), middle-aged (B), and elderly (C) groups over a 16-mo period. A function was fitted to the data by using least-squares analysis (*see* text for details). The solid line shows the computed change in ascorbate for each group.

suggest that glutathione spares ascorbate (12). Interestingly, a correlation similar to that shown in Figure 1 was observed between total glutathione and ascorbate in rat heart, to which oxidative stress was induced by ischemia reperfusion (28). In another study, the concentration of ascorbate in rat hepatic cells was shown to double after administration of lipoic acid, a compound that is known to increase the concentration of intracellular glutathione (29). However, in a recent study with ascorbate-requiring shionogi rats with osteogenic disorder, the concentrations of glutathione in liver and kidney were unaffected by a 60–70% diet-induced decrease of ascorbate in these tissues (30).

The study of glutathione and ascorbate synergy in humans was limited to the measurement of glutathione in plasma or erythrocytes. For example, Henning et al (31) reported that a reduction in the concentration of vitamin C in plasma to 6 μmol/L led to a significant decrease in the concentration of total plasma glutathione as well as in the ratio of glutathione to glutathione disulfide. Conversely, 2 studies reported significant increases in erythrocyte glutathione after supplementation with vitamin C. A 4-wk regimen of 1 g vitamin C/d was reported to increase erythrocyte glutathione by 28% (32). Similarly, erythrocyte glutathione was shown to increase nearly 50% after supplementation with 0.5 or 2 g vitamin C/d for 1 wk, although there was a large variation in individual responses (33).

The direct correlation between glutathione and ascorbate in lymphocytes may be the result of many factors. First, the correlation may be related to dietary intake, ie, foods rich in ascorbate may also be rich in compounds that enhance the synthesis of glutathione (eg, cystine, cysteine, and methionine). In addition, increases in dietary glutamine enhance glutathione synthesis and preserve glutathione stores in depleted tissues (34). Another factor that may be important in the correlation of glutathione and ascorbate is that these antioxidants mutually spare each other in the reaction with intracellular reactive oxygen species. On the basis of competition kinetics and on the assumption that the regeneration of glutathione or ascorbate is efficient, one would predict that an increase in the concentration of ascorbate would spare glutathione and vice versa. Lastly, glutathione and ascorbate may be associated within cells because glutathione is required in the chemical or enzymatic regeneration of ascorbate from its oxidized state (35–39). In particular, there is evidence that the regeneration of ascorbate involves glutaredoxin, which requires glutathione as an electron donor and was found to be the major dehydroascorbate-reducing activity in neutrophils (37). Alternatively, other enzymes that catalyze the reduction of dehydroascorbate use NADPH as reducing equivalents, including thioredoxin reductase and 3α-hydrosteroid dehydrogenase (38, 39). Indeed, removal of selenium from the diet of rats decreased dehydroascorbate-reducing activity in liver homogenates, suggesting the involvement of selenium-containing thioredoxin reductase (38).

Many studies have reported changes in antioxidant concentrations with age. Most of these studies were based on measurements of antioxidants in whole blood, red blood cells, serum, or plasma. Although there is a trend toward lower glutathione values in elderly than in young or middle-aged individuals, there have been contradictions. Lang et al (40) reported a 17% lower glutathione concentration in whole blood in the elderly (60–79 y; $n = 60$) than in the young (20–39 y; $n = 40$) ($P < 0.001$). In contrast, Julius et al (41) observed a positive correlation of glutathione with age ($n = 33$; $P < 0.01$). Lastly, Richie et al (42) also observed lower glutathione concentrations in subjects aged >55 y than in younger subjects, but the difference was significant only in women ($n = 231$; $P < 0.01$). Yang et al (43) reported approximately half the concentration of plasma glutathione in elderly than in young subjects. In a similar study, however, there was no significant difference in the concentration of glutathione with age (44). In contrast with studies of glutathione, studies of ascorbate in plasma more consistently point to a decrease in the concentration of this antioxidant with age. For example, Mezzetti et al (45) reported a significantly lower (by 26%) plasma ascorbate concentration in middle-aged (54 ± 16 y; $n = 100$) than in free-living elderly (84 ± 3 y; $n = 91$) subjects; an even greater difference was observed in a comparison with disabled elderly subjects (81 ± 4 y; $n = 62$). Another study showed a 25% lower plasma ascorbate concentration in subjects aged <50 y than in those aged 75–99 y (46). However, no significant effect of age was observed for plasma ascorbate in a group of 219 healthy, age-stratified subjects (47).

Recently, van Lieshout and Peters (19) reported lymphocyte glutathione concentrations that were as much as 50% lower (12.3 nmol/mg protein) in the elderly (60–80 y) than in the young (20–40 y) (21.5 nmol/mg protein), with no significant difference between men and women. In comparison, we observed higher concentrations of glutathione in lymphocytes and smaller differences in glutathione concentrations between different age groups. The decrease in glutathione and ascorbate with age may have been underestimated in our study because the elderly sub-

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jects represented only a fraction of the elderly candidates meeting our stringent criteria for acceptance (86 of ≈1000 elderly candidates). Nevertheless, our study confirms that there is a significantly lower concentration of glutathione in lymphocytes in older than in younger individuals. This may reflect a degeneration in the ability to defend against reactive oxygen species, an increase in the production of these species, or both. Although there is a decrease in the concentration of glutathione with age, the correlation of glutathione with ascorbate remains strong in each age group, suggesting that the underlying factors responsible this correlation do not change with age.

The variation in ascorbate with season may be attributed to a lower consumption of fruit and vegetables during the winter than during the summer, when these commodities are more readily available. Serum or plasma concentrations of vitamin C are strongly correlated with the consumption of fruit and vegetables. Several investigators have reported a fluctuation in plasma vitamin C with season (48–51). In a dramatic example, the plasma concentration of vitamin C reportedly increased by as much as 7-fold in a cohort of pregnant and lactating Gambian women from the rainy season in September and October to the mango season in May and June (51). However, one does not expect to find such a large variation in the concentration of plasma or lymphocyte vitamin C in industrialized countries, where vitamin C–rich foods are available year-round. Therefore, it is unusual that a marked variation in vitamin C in lymphocytes was observed in the present study. Interestingly, the effect was greater in young than in older subjects, suggesting dietary differences between the groups.

In summary, glutathione and ascorbate concentrations are directly correlated in human lymphocytes, dependent to a certain extent on age and season. These results suggest that vitamin C supplementation, which increases the concentration of ascorbate in lymphocytes, will also increase the concentration of glutathione in lymphocytes, a hypothesis that is currently under investigation.

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