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Vitamin C prevents hyperbaric oxygen-induced growth retardation and lipid peroxidation and attenuates the oxidation-induced up-regulation of glutathione in guinea pigs

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Abstract

Hyperbaric oxygen therapy is used to treat various clinical conditions, but it also causes oxidative damage. The objectives of this study are to determine if increased vitamin C intake can prevent hyperbaric oxygen-induced damage and to determine interactions among vitamin C, glutathione and vitamin E in response to oxidative stress. The growth rates of unexposed guinea pigs fed 1.25 mg vitamin C/day were indistinguishable from that of guinea pigs fed 50 mg vitamin C/day. In contrast, hyperbaric oxygen exposure resulted in growth retardation in guinea pigs fed 1.25 mg vitamin C/day, but it had little effect on the growth rates of guinea pigs fed 50 mg vitamin C/day. Increased vitamin C intake also prevented hyperbaric oxygen-induced lipid peroxidation in the liver. In guinea pigs not exposed to hyperbaric oxygen, levels of vitamin C in tissues were closely related to vitamin C intake, but tissue levels of glutathione and vitamin E were not related to vitamin C intake. However, interactions between vitamin C and glutathione were observed upon chronic hyperbaric oxygen exposure. Chronic hyperbaric oxygen exposure resulted in >2-fold increases in the levels of glutathione in liver and lung of guinea pigs fed 1.25 mg vitamin C/day. In comparison, the oxidation-induced increases in glutathione were significantly attenuated in guinea pigs fed 50 mg vitamin C/day. These data show that increased intake of vitamin C can prevent or alleviate the hyperbaric oxygen-induced damage. The interactions between vitamin C and glutathione upon hyperbaric oxygen exposure indicate that there is a homeostatic regulation of antioxidant capacity in guinea pig tissues.

Keywords: Ascorbate; Glutathione; Lipid peroxidation; Hyperbaric oxygen; Vitamin E

1. Introduction

Hyperbaric oxygen (HBO) therapy is a useful method for treatment of various clinical conditions such as wound healing, carbon monoxide poisoning, and acute necrotizing infections [1,2], but it can also cause oxidative damage [3,4]. Long term HBO exposure has been found to cause cataracts in humans [5] and experimental animals [6,7]. Antioxidants and antioxidant enzymes are the primary defenses against oxidative damage. An objective of the study is to determine if supplementation with antioxidants can prevent or alleviate HBO-induced damage, such as growth retardation and cataract formation. Vitamin C was chosen because it is one of the most effective biological antioxidants and its levels in tissues can be altered by changes in dietary intake. It has been shown that vitamin C supplementation can reduce risks for various diseases which are associated with oxidative stress, such as cancer [8,9], cataract [10–14], and arteriosclerosis [15–19].

Antioxidants and antioxidant enzymes in an organism may operate within the context of an integrated system. Components of the system may work in concert or synergistically to scavenge reactive oxygen species and to prevent oxidative damage. In addition to vitamin C, important biological antioxidants include glutathione, vitamin E and some flavonoids. It has been demonstrated that vitamin C is coupled with the recycling of vitamin E and glutathione [20–22]. Interactions between vitamin C, vitamin E and glutathione were observed under deficient conditions in numerous studies [23–26]. However, results regarding interactions between vitamin C, vitamin E and glutathione under non-deficient conditions are controversial. A few human studies showed that supplementation with vitamin C was associated with higher lipid-normalized vitamin E lev-

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els [27,28] and increased vitamin E intake was associated with higher vitamin C levels [28]. In other human studies, the effect of vitamin C intake on vitamin E levels was not observed [29]. In rats, excess vitamin C intake lowered tissue antioxidant potential during marginal vitamin E intake [30]. In guinea pigs, no significant effects of vitamin C intake on tissue vitamin E concentrations, or vise versa, were found [31,32]. Our recent data showed that increasing vitamin C levels beyond the minimal requirement has no significant effect on levels of glutathione and vitamin E in vitamin C-requiring ODS rats [33]. To determine if oxidative stress affects the interactions among these antioxidants under non-deficient conditions, we determined the effect of vitamin C intake on levels of glutathione and vitamin E in both unexposed and HBO-exposed guinea pigs. The results showed that increased vitamin C intake can prevent or alleviate HBO-associated adverse effects. The results also showed that increasing vitamin C levels beyond the minimal requirement had no significant effect on levels of glutathione and vitamin E in unexposed guinea pigs. However, high vitamin C intake attenuated the HBO-induced up-regulation of glutathione in liver and lung of guinea pigs.

2. Methods and materials

2.1. Animals

Male Hartley white guinea pigs (1 month old) were obtained from Charles River Breeding Laboratories (Wilmington, MA). Animals were individually housed in stainless steel suspended rodent cages and were given free access to Certified Guinea Pig Chow (PMI, Richmond, IN) and water for 2 weeks. The guinea pigs were then fed a vitamin C-free diet (AIN 93M powdered diet) and orally dosed with vitamin C daily for four months. The high vitamin C (HC) group was supplied 50 mg vitamin C/day and the low vitamin C (LC) group received 1.25 mg vitamin C/day. The choice of the two levels of vitamin C was based on prior experiments which showed that 1.25 mg/day vitamin C is sufficient to prevent scurvy and 50 mg/day is enough to saturate the plasma vitamin C levels [34–36]. The absolute amount of vitamin C, rather than per unit of body weight was chosen for convenience. Although the amount of vitamin C per unit body weight decreases with increased body weight, 1.25 mg/day vitamin C was still above the minimal requirement of 1 mg/kg body weight [35,36] by the end of the experiment. The animals were maintained in an environmentally controlled atmosphere (23°C, 45% relative humidity with 15 air changes of 100% fresh hepa-filtered air per hour, and a 12/12 hr light/dark cycle) in facilities accredited by American Association for the Accreditation of Laboratory Animal Care. This study was reviewed and approved by the Animal Care and Use Committee. All animals were closely monitored daily for clinical signs of disease. Body weights were measured monthly. After 4 months of vitamin C supplementation, 9 guinea pigs from the HC group and 9 guinea pigs from the LC group were exsanguinated under CO_2 narcosis and tissues were harvested and were flash-frozen immediately in liquid nitrogen. The frozen tissues were stored at −80°C until use.

2.2. Exposure to HBO

Guinea pigs were obtained and treated the same as described above. After 2 weeks of acclimation with high and low vitamin C intake, the guinea pigs were divided into two subgroups, 5–6 guinea pigs in each subgroup. One subgroup was exposed to hyperbaric oxygen twice a week, 2 hr each time. The other subgroup served as the control. For exposure to HBO, animals were placed in a high-pressure chamber. Air in the chamber was purged with oxygen until the oxygen concentration in the chamber reached 95%, which was monitored with an oxygen sensor in the chamber. Then the pressure in the chamber was increased to 2.5 atmospheric pressures and the oxygen concentration in the chamber was maintained at ~95% with a flow rate of 10 L/min. During HBO exposure, all the animals were given free access to water and food and were closely monitored for any sign of oxygen toxicity. If any sign of oxygen toxicity, such as convulsion, was observed, the animals were removed from the chamber. After each exposure, the pressure in the hyperbaric chamber was decreased to atmospheric pressure in a period of 45–60 min and the animals were returned to their cages. The eyes of these animals were examined once a month using slit-lamp microscope to determine if there were signs of lens opacity. After 72 exposures (during a 7 month period), the animals were exsanguinated under CO_2 narcosis. Organs were harvested and were flash-frozen immediately in liquid nitrogen. The frozen tissues were stored in tightly closed vials at −80°C until use.

2.3. Blood handling

Blood samples were drawn via cardiac puncture under CO_2 narcosis into EDTA coated Vacutainer® glass tubes, shielded from light, and kept on ice until processed. The whole blood was separated into plasma and erythrocytes by centrifugation at 1000 × g for 15 min. Plasma intended for vitamin C analysis was deproteinized by vortexing equal volumes of 0.5 mol/L perchloric acid (PCA, Aldrich, Milwaukee, WI), centrifuged, and aliquots of the clear supernatant were stored at −80°C until analysis.

2.4. Analysis of vitamin C

The vitamin C assay was a modification of a method described by Behren and Madere [37]. Frozen livers were homogenized with 1.2 mol/L perchloric acid (PCA, 10 ml/g). For measurement of reduced vitamin C, the supernatant was directly injected into a reversed-phase high performance liquid chromatography (HPLC) column (Biosil ODS 5S 150 × 4 mm column from Bio-Rad, Richmond, CA). For measurement of

total vitamin C, the dehydroascorbic acid was completely reduced with homocysteine and then injected into the reversed-phase HPLC column. Elution was accomplished with a mobile phase consisting of 40 mmol/L sodium acetate, 1.75 mmol/L n-octaylamine and 0.5 mmol/L EDTA at pH 4.0 using a flow rate of 1.5 ml/min and a run time of 3.0 min. The HPLC instrumentation consisted of a Waters Model 510 pump, a Waters 710B autosampler (Waters Associates, Inc., Milford, MA), and an LC4B electro-chemical detector (Bioanalytical Systems, West Lafayette, IN). Intra-assay variation for this method was 4.2%, and the inter-assay variation was 5.1%.

2.5. Analysis of lipid peroxidation

Lipid peroxidation in guinea pig livers was measured by determining the level of malondialdehyde (MDA) using thiobarbituric acid reaction [38]. Frozen livers were homogenized in 150 mmol/L KCl containing 1 mmol/L butylated hydroxytoluene and 1 mmol/L EDTA (10 ml/g wet tissue). The liver homogenate (0.2 ml) was mixed with 0.2 ml of 280 mmol/L SDS, 1.5 ml 3 mol/L acetic acid, pH 3.5, and 1.5 ml 60 mmol/L thiobarbituric acid solution. The mixture was adjusted to 4 ml with deionized water and then incubated at 95°C for 60 min. After cooling with tap water, 1 ml of deionized water and 5 ml of the mixture of butanol and pyridine (15:1, v/v) were added and the tubes were shaken vigorously. After centrifugation at 500 × g for 10 min, fluorescence in the organic layer was measured (excitation 515 nm; emission 553 nm) using a Perkin-Elmer fluorescence spectrophotometer. A standard curve was prepared using MDA generated by acid hydrolysis of 1,1,3,3-tetramethoxypropane.

2.6. Analysis of glutathione

Levels of reduced and oxidized glutathione in liver and lung were determined using the HPLC as described by Fariss and Reed [39]. Frozen livers and lungs were pulverized with 1.2 mol/L perchloric acid containing 1 mmol/L bathophenanthrolinedislfonic acid (10 ml/g). After centrifugation at 5000 × g for 10 min, the supernatant was first reacted with iodoacetic acid to convert reduced glutathione and other thiols to stable S-carboxymethyl derivatives. Then 1-fluoro-2,4-dinitrobenzene was added to convert the primary amine to dinitrophenyl derivatives. After derivatization, samples were separated with the HP 1100 HPLC system (Wilmington, DE) using an amine column and were monitored at 365 nm. Levels of reduced glutathione and oxidized glutathione were determined by comparing their peak areas with the peak area of the internal standard, γ-Glu-glu.

2.7. Vitamin E analysis

Levels of alpha-tocopherol (vitamin E) in livers were determined by reversed-phase HPLC as described previously [40]. In brief, livers were homogenized in phosphate-buffered saline (PBS) at concentrations of 100 mg/ml. One mL of the homogenate was mixed with 1 ml of 150 mmol/L pyrogallol in ethanol. After addition of 100 μl of 9 mol/L KOH, the mixture was incubated at 70°C for 30 min and then was cooled to room temperature. One mL distilled water and 3 ml hexane containing 2 μg Tocol (internal standard) were added to the mixture. After vigorously shaking, the organic solvent layer was transferred to a new tube and evaporated under a stream of nitrogen. The dried samples were then dissolved in methanol and injected onto a reversed phase column, Bio-Sil ODS-5S 150 × 4 mm (Bio-Rad, Richmond, CA), equipped with a guard column, ODS-5S 30 × 4.6 mm (Bio-Rad). The mobile phase was pure HPLC-grade methanol. The HPLC instrumentation was from Waters Associates Inc. (Milford, MA), and consisted of a Model 510 pump, a 710B WISP autosampler, and a 490 Multiwavelength detector set at 292 nm for α-tocopherol detection.

2.8. Statistical analysis

The body weight growth data were formally evaluated using repeated measures ANOVA. Differences were considered significant when the p value was less than 0.05. Student's t test for independent samples was used to compare levels of antioxidants in tissues between low and high vitamin C intake guinea pigs.

3. Results

3.1. Effects of vitamin C intake on HBO-induced toxicity

To test if increased vitamin C intake can prevent or alleviate HBO-induced damage, guinea pigs fed 1.25 mg vitamin C/day (LC) or 50 mg vitamin C/day (HC) were exposed to HBO twice a week, 2 hr each time. Signs of oxygen toxicity and growth rates were monitored during the 7 months of HBO exposure. All of the guinea pigs not exposed to HBO appeared to be healthy regardless of vitamin C intake and there were no signs of scurvy in these animals. However, two thirds of the HC and all of the LC guinea pigs which were exposed to HBO for more than 6 months experienced seizures, a common sign of oxygen toxicity [4]. After the last (72nd) exposure to HBO, 2 guinea pigs from the LC group and 1 from the HC group died. The exposure to HBO was terminated at that point to avoid further loss of animals. Slit lamp microscope examination of the eyes did not find any lens opacity during the 7 month exposure to HBO. However, increased light scattering in the lens nucleus was observed in all the HBO-exposed guinea pigs, but not in the unexposed animals. There was no detectable difference of light scattering between the HC and the LC groups.

The growth rates of LC guinea pigs not exposed to HBO were comparable to those of HC guinea pigs (Fig. 1). The growth rates of HBO-exposed HC guinea pigs were also indistinguishable from those of unexposed guinea pigs (Fig. 1). In contrast, HBO exposure slowed the growth rates of LC

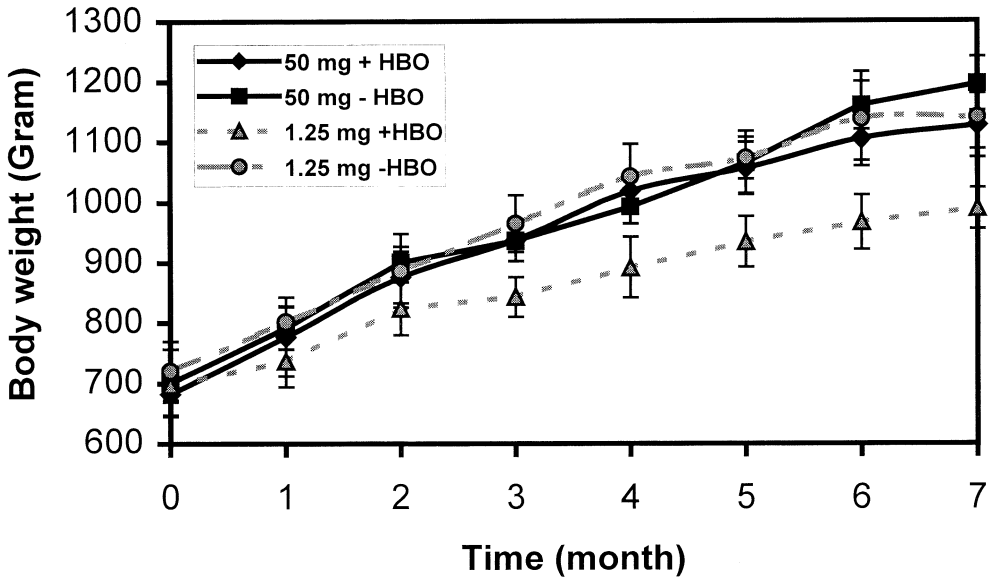

Fig. 1. Effect of Vitamin C and HBO exposure on the growth rates of guinea pigs. Two-month-old male guinea pigs fed vitamin C free diet were supplied 1.25 mg/day or 50 mg/day vitmain C. Guinea pigs supplemented with 1.25 mg/day and 50 mg/day vitamin C were divided into two subgroups. One group was exposed to HBO twice a week, 2 hr each time. The other group served as the control. Body weights of these guinea pigs were determined once a month. Data in this figure are mean ± SD of body weights of each group. There are 5–6 guinea pigs in each group. The growth rates of guinea pigs fed 50 mg/day vitamin C with or without exposure to HBO and the guinea pigs fed 1.25 mg/day vitamin C without exposure to HBO were comparable during the period of this study ($p > 0.05$). The growth rate of guinea pigs supplied 1.25 mg/day vitamin C and exposed to HBO was significantly lower than other groups after three months ($p < 0.05$).

guinea pigs (Fig. 1). These results indicate that 1.25 mg/day vitamin C is sufficient to prevent scurvy and to prevent growth retardation under normal conditions. However, this level is insufficient under conditions of oxidative stress.

3.2. Vitamin C prevents HBO-induced lipid peroxidation

In guinea pigs not exposed to HBO, levels of MDA in the livers from the LC and HC groups were similar (Table 1). After exposure to HBO, levels of MDA increased significantly in the LC guinea pigs, but not in the HC guinea pigs. These results indicate that 1.25 mg vitamin C/day provides sufficient protection under normal conditions, and therefore, an increase in vitamin C intake has little effect on levels of lipid peroxidation under non-stress conditions. However, under oxidative stress conditions, higher levels of vitamin C are required to prevent lipid oxidation. It was anticipated that HBO-exposure causes more lipid peroxidation to the lungs than to the livers. However, lipid peroxidation information in lungs of these guinea pigs was not available because the organs were harvested without perfusion and red blood cells in the lung interfered with the thiobarbituric acid reaction.

Table 1
Effect of vitamin C on HBO-induced lipid peroxidation in the liver (nmol MDA/g)

Vitamin C intake	Control	HBO exposure
50 mg/day	16 ± 3 (5)	14 ± 4 (4)
1.25 mg/day	16 ± 3 (4)	25 ± 1 (4)*

Guinea pigs fed 1.25 mg or 50 mg vitamin C were exposed to HBO twice a week for 7 months and levels of MDA were determined in livers of these animals. Data in this table are means ± SD. Numbers in the parenthesis are numbers of animals examined.

* Indicates $p < 0.01$ as compared between control and HBO-exposed low ascorbate fed guinea pigs, as well as between low vitamin C intake and high vitamin C intake groups of the HBO-exposed animals.

3.3. Vitamin C levels in plasma and livers are closely related to the levels of vitamin C intake

Consistent with previous reports [34–36,41], levels of vitamin C in tissues were associated with levels of vitamin C intake. The level of reduced vitamin C in the plasma and livers of HC guinea pigs were 900% and 1600%, respectively, of those in LC guinea pigs (Table 2). The levels of oxidized vitamin C (dehydroascorbate) in plasma and livers were also higher in HC guinea pigs as compared to those of LC guinea pigs, but with less magnitude than those observed for the reduced vitamin C (Table 2). Thus, the ratio of reduced/oxidized vitamin C in plasma and liver of HC guinea pigs was higher than that in LC guinea pigs, indicating that high vitamin C intake not only results in higher tissue vitamin C levels, but may also result in an altered redox status.

3.4. High vitamin C intake attenuates the increase of glutathione in liver and lung upon HBO exposure

To determine the interactions among vitamin C, glutathione and vitamin E in response to oxidative stress, the effects of vitamin C intake and HBO exposure on levels of glutathione and vitamin E were determined. The levels of reduced glutathione in the liver were significantly higher in

Table 2
The relationship between vitamin C intake and levels of vitamin C in plasma and liver under non-stress conditions

Vitamin C intake	Reduced vitamin C		Oxidized vitamin C		Reduced/oxidized	
	Plasma, nmol/ml	Liver, nmol/g	Plasma, nmol/ml	Liver nmol/g	Plasma	Liver
50 mg/day	63 ± 16	629 ± 146	8 ± 9	132 ± 90	8.2	4.8
1.25 mg/day	7 ± 3**	38 ± 14**	3 ± 2*	18 ± 8*	2.3	2.1

Guinea pigs fed vitamin C-free diet were supplemented with 1.25 mg or 50 mg vitamin C/day for 4 months. Levels of vitamin C in plasma and liver were determined. Data in this table are means ± SD, n = 9.

* Represents $p < 0.05$ and ** represents $p < 0.001$ when compared between the high vitamin C intake group and the low vitamin C intake group.

HBO exposed-animals than in unexposed animals (Table 3). As compared with unexposed animals, levels of reduced glutathione in livers of the HBO-exposed animals increased 136% in the LC guinea pigs (Table 3), but the increase was only 65% in the HC animals. Although the levels of reduced glutathione in livers from unexposed HC and LC guinea pigs were comparable, the differential changes in glutathione levels in response to HBO exposure resulted in a higher level of reduced glutathione in the HBO-exposed LC guinea pigs than in the HBO-exposed HC guinea pigs ($P < 0.05$). The increase in levels of reduced glutathione upon HBO exposure was also reflected in the GSH/GSSG ratio which increased significantly in LC guinea pigs upon HBO-exposure (Table 3).

To further study the effect of HBO on glutathione metabolism, levels of reduced and oxidized glutathione in the lung were also compared between unexposed and HBO-exposed guinea pigs. Similar to the changes observed in liver glutathione levels, levels of reduced glutathione in lungs were higher in HBO-exposed animals than in unexposed animals (Table 4). The HBO-induced increase in glutathione levels was greater in LC guinea pigs, in which the levels of reduced glutathione increased nearly two-fold (Table 4). These data indicate that levels of reduced glutathione are up-regulated in response to oxidative stress, and that high vitamin C intake attenuates the the HBO-induced up-regulation of glutathione.

HBO exposure also resulted in a 2.7-fold increase in levels of vitamin C in the livers of LC guinea pigs ($p < 0.05$). A similar increase was observed in the HC animals, but the difference was not statistically significant (Table 5). In contrast to glutathione, vitamin E levels in the liver were not affected by vitamin C intake, neither in unexposed, nor in the HBO-exposed guinea pigs (Table 5).

4. Discussion

Data presented here demonstrate that while minimal non-scorbutic levels of vitamin C are sufficient to maintain health under non-stressed conditions, such levels of vitamin C are insufficient to cope with oxidative stress. The data in Fig. 1 and Table 1 show that increased vitamin C intake can prevent HBO-induced growth retardation and lipid peroxidation in guinea pigs. The results indicate that it would be beneficial to take vitamin C at doses that are higher than the minimal requirement, particularly under conditions of oxidative stress. This finding also implies that increased vitamin C intake may prevent at least some of the adverse effects of HBO treatment in humans.

The data also show that increasing vitamin C levels beyond the minimal requirement has little effect on the levels of glutathione and vitamin E in the unstressed guinea pigs. Our data are consistent with previous results obtained in guinea pigs [31,32,41,42], in ODS rats [33], and in human [27]. A recent human study showed that vitamin C supplementation was associated with an increase in lipid-standardized plasma vitamin E, but without alteration in the net plasma vitamin E levels [28]. The increase in lipid-standardized plasma vitamin E most likely was due to a

Table 3
Effects of HBO exposure on levels of glutathione in the liver

Vitamin C intake	HBO	GSH, μmol/g	GSSG, μmol/g	GSH/GSSG
50 mg/day	−	3.12 ± 1.45 (5)	0.36 ± 0.17 (5)	8.6
	+	5.10 ± 1.67 (5)*	0.67 ± 0.28 (5)	7.6
1.25 mg/day	−	3.0 ± 0.99 (4)	0.43 ± 0.08 (4)	6.9
	+	7.1 ± 1.0 (4)**	0.59 ± 0.25 (4)	12

Guinea pigs fed 1.25 mg or 50 mg vitamin C were exposed to HBO twice a week for 7 months and levels of reduced and oxidized glutathione were determined in livers of these animals. Data in this table are means ± SD. Numbers in the parenthesis are numbers of animals examined.

* Indicates $p < 0.05$ and ** indicates $p < 0.01$ between unexposed and HBO-treated groups.

GSH represents reduced glutathione and GSSG represents oxidized glutathione.

Table 4
Effects of HBO exposure on levels of glutathione in the lung

Vitamin C intake	HBO	GSH, μmol/g	GSSG, μmol/g	GSH/GSSG
50 mg/day	−	2.74 ± 0.65 (5)	0.30 ± 0.07 (5)	9
	+	3.63 ± 0.80 (5)	0.28 ± 0.09 (5)	13
1.25 mg/day	−	2.76 ± 0.80 (4)	0.34 ± 0.07 (4)	8
	+	5.02 ± 1.13 (4)**	0.31 ± 0.1 (4)	16

Guinea pigs fed 1.25 mg or 50 mg vitamin C were exposed to HBO twice a week for 7 months and levels of reduced and oxidized glutathione were determined in lungs these animals. Data in this table are means ± SD. Numbers in the parenthesis are numbers of animals examined.

* Indicate $p < 0.05$ and ** Indicates $p < 0.01$ between the unexposed and HBO-exposed groups.

GSH represents reduced glutathione and GSSG represents oxidized glutathione.

Table 5
Effect of HBO exposure on levels of vitamin C and vitamin E in the liver of guinea pigs fed 1.25 and 50 mg/day Vitamin C

Vitamin C intake	HBO	Vitamin C, nmol/g	Vitamin E, nmol/g
50 mg/day	−	488 ± 200 (5)	34 ± 10 (5)
	+	640 ± 188 (5)	35 ± 13 (5)
1.25 mg/day	−	30 ± 16 (4)**	31 ± 10 (4)
	+	81 ± 31 (4)*	38 ± 14 (4)

Guinea pigs fed 1.25 mg or 50 mg vitamin C were exposed to hyperbaric oxygen twice a week for 7 months and levels of vitamin C and vitamin E were determined in livers of these animals. Data in this table are means ± SD. Numbers in the parenthesis are numbers of animals examined.

* Indicate $p < 0.05$ as compared between the control and the HBO-exposed groups of the low vitamin C intake guinea pigs and ** Indicates $p < 0.01$ as compared between high and the low vitamin C intake groups of unexposed guinea pigs.

decrease in plasma cholesterol levels in high vitamin C intake subjects [28,35].

The HBO-induced increase in levels of glutathione may represent an adaptive response to oxidative stress. Similar adaptive induction of antioxidant or endogenous antioxidant enzymes has been observed in various animal models [43–48]. The sources of the increased glutathione remain to be determined. It has been shown that γ-glutamylcysteine synthetase, the rate-limiting enzyme for glutathione synthesis, is up-regulated in response to various stresses [49]. Thus, it is most likely that the increased glutathione level is due to increased endogenous synthesis. The attenuation of HBO-induced increase in the levels of glutathione in HC guinea pigs (Table 3 and 4) may be related to the capability of vitamin C to alleviate the HBO-induced oxidative stress. One indicator for the suppression of oxidative stress by high vitamin C intake is the reduced levels of lipid peroxidation in HBO-exposed guinea pigs (Table 1). Another interpretation for the attenuation of the HBO-induced increases in glutathione levels in HC guinea pigs is the homeostatic regulation of total antioxidant capacity [42,50,51]. It has been reported that treatment of human arterial smooth muscle cells with vitamin C markedly attenuates adaptive increases in endogenous antioxidant gene expression [17]. Homeostatic regulation of endogenous antioxidants was also observed in human subjects and experimental animals. For example, increased vitamin C or vitamin E resulted in a decline in plasma uric acid, a major antioxidant in plasma [28,32,42]. The interaction between vitamin C and glutathione under oxidative stress conditions suggests that vitamin C and glutathione work in concert to cope with oxidative stress. The increased levels of glutathione upon oxidative stress may provide reducing equivalents to compensate for the lower tissue vitamin C levels.

Levels of vitamin C, vitamin E and glutathione reported in this study were within the ranges reported by others [34,36,42,48,52,53]. Decreased ratio of reduced/oxidized vitamin C in low vitamin C intake guinea pigs was also reported [34,41,52]. This ratio, together with ratios of GSH/GSSG and NADPH/NADP or NADH/NAD, may be an indicator of intracellular redox status. The individual variation in vitamin E levels presented here was higher than that reported previously [32,40]. This variation most likely resulted from variation in food intakes, because the guinea pigs were fed ad libitum with a diet which contains 75 mg vitamin E/kg. It was not due to analytical variation, since the variation of repeated measures of the same sample was within 10%.

Taken together, the data clearly indicate that increased vitamin C intake can prevent or alleviate some of the adverse effects associated with HBO exposure. The inverse relationship between vitamin C and glutathione in tissues of HBO-exposed animals supports the idea that there is a homeostatic regulation of total antioxidant capacity. Although the interaction between vitamin C and vitamin E was observed in scorbutic animals [23], the interaction between vitamin C and vitamin E is not detectable in the non-scorbutic guinea pigs and the interaction between vitamin C and glutathione in the non-scorbutic guinea pigs is only observed under conditions of oxidative stress.

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