

Effects of vitamin C supplementation in human volunteers with a range of cholesterol levels on biomarkers of oxygen radical-generated damage*

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Abstract: Twenty-four men and 24 women, all nonsmoking, and maintaining normal dietary habits were assigned to 3 groups of 16. Each group comprising 4 males with “low” cholesterol levels (<6 mmol/L) matched for age and build with 4 males with “high” cholesterol levels (>6 mmol/L) and 8 similarly matched females. A three-treatment, three-treatment period, cross-over design was adopted. The three treatments were placebo, 60 mg vitamin C/day (the recommended daily allowance) and 6 g vitamin C/day for 14 days with 6 weeks between treatments. Blood samples were taken at the end of each treatment period. Vitamin C supplementation significantly increased plasma vitamin C concentrations and total antioxidant capacity, but did not affect cholesterol status or plasma *ras* p21 protein levels. There was a nonsignificant dose-related decrease in plasma lipid peroxidation breakdown products. DNA damage, measured in lymphocytes by the Comet assay and chromosome aberration test, was not increased after vitamin C supplementation. Sensitivity to hydrogen peroxide (in the Comet assay) was also unaffected, but sensitivity to chromosome aberration induced by bleomycin was increased by supplementation. A significant gender difference was found in plasma vitamin C levels, antioxidant capacity, and number of chromosome aberrations. Results were independent of low and high cholesterol status.

INTRODUCTION

Reactive oxygen species are thought to play a major role in the etiology of a wide variety of diseases including atherosclerosis [1], respiratory tract disorders [2], neurodegenerative disease [3], inflammatory bowel disease [4], cancer [5,6] and in aging in general [5,7,8].

The damaging species are produced not only by environmental agents such as redox-active chemicals and ionizing radiation, but also by natural metabolic processes (see reviews in refs. 9,10). Enzymes such as lipoxygenases, dehydrogenases, cyclooxygenases, and peroxidases and the mitochondrial electron transport system are constant intracellular sources of superoxide radicals, formed by the reduction of oxygen, coupled to substrate oxidation. Extracellular superoxide is produced by phagocytic cells during their reaction to foreign particles, presumably as part of a microbiocidal mechanism. Superoxide, although relatively unreactive, dismutates rapidly to hydrogen peroxide which reacts with reduced metal ions to yield the extremely reactive hydroxyl radical. Thus, constant exposure of human tissues to potentially damaging oxidants is unavoidable.

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Free radicals exert their primary effects by reacting with macromolecules including DNA, lipids, proteins, and carbohydrates. Reactive oxygen species produced by enzymes and phagocytes *in vitro* are able to cause chromosome damage and mutations in mammalian cells [11,12]. Ames [5] has shown that oxidation is a major cause of DNA damage in man and has calculated that the number of oxidative hits on DNA may be more than 10^3 per cell per day. Abstraction of hydrogen atoms from unsaturated bonds in lipids is a major reaction of free radicals and results in lipid peroxidation, a chain reaction ultimately giving rise to toxic products such as 4-hydroxyalkenals and malondialdehyde [13]. Reactions with proteins can result in amino acid modification, cross-linking, inactivation, and fragmentation [14], and damage to carbohydrates can cause the breakdown of polymers such as hyaluronic acid, important in the function of joints [15].

Human tissues are protected from oxidative damage by a variety of mechanisms [16], including small molecular weight antioxidants (e.g., vitamins C and E), enzymes that destroy the reactive species (e.g., catalase) and repair enzymes (e.g., DNA glycosylases). Among the simpler antioxidants, vitamin C is one of the most well known and possibly the most important. Epidemiological evidence suggests that antioxidant vitamins at sufficient concentrations inhibit heart disease [17–19] and cancer [20]. Ames *et al.* [21] have calculated that eating insufficient fruit and vegetables doubles the risk of most cancers and increases the risk of heart disease. Since humans cannot synthesize vitamin C, their main source is dietary fruit and vegetables [22]. However, there is considerable uncertainty about the optimal level of intake. It is widely believed, among the general population, that the greater the amount consumed, the greater the beneficial effect in terms of preventing oxidative damage and the diseases thought to result from it. This concept has been challenged by Diplock [23].

The present study was undertaken to address the question of whether any benefit can be demonstrated from the intake of large quantities of vitamin C by a population of healthy, well-nourished individuals. A number of biomarkers were used to investigate the effect of vitamin C supplementation on antioxidant status and oxidant-induced damage. Using a three-treatment, three-treatment period, cross-over design, 48 volunteers were treated for 14 days with a placebo, with vitamin C at 60 mg/day and with 6 g vitamin C/day. The lower dose of 60 mg/day is the recommended daily allowance (RDA) stipulated in the EC Nutrition Labelling Directive (90/496/EEC) and is similar to the reference nutrient intake value [24]. Duthie *et al.* [25,26] have studied the effects of vitamin E administered for 14 days at 100 times the RDA for this vitamin and found no adverse effects. A similar strategy was adopted in the present study, resulting in the use of a high dose of 6 g vitamin C/day.

Blood samples were taken at the end of each treatment period of the study. The effect of vitamin C supplementation was assessed using assays for total antioxidant capacity of plasma, lipid peroxidation breakdown products in plasma, DNA damage in lymphocytes with and without challenge by hydrogen peroxide (using the Comet assay), chromosome breakage in lymphocytes with and without challenge with bleomycin, and *ras* p21 protein levels in plasma.

Cholesterol status was considered to be an important factor in the design of the study since a correlation has been established between serum cholesterol level and risk of coronary heart disease, as well as the severity of atherosclerosis [27–29]. Correlations between cholesterol status and plasma vitamin C level have been extensively studied as a possible link between vitamin C intake and reduced risk of heart disease [30]. Volunteers were therefore selected to cover as wide a range of cholesterol levels as possible, without including people on medication or specific diets to reduce cholesterol levels. A classification of low cholesterol (<6 mmol/L) or high cholesterol (>6 mmol/L) was used in the analysis of the results of the study to determine whether cholesterol status had any influence on antioxidant status and oxidative damage or on the response to vitamin C supplementation.

Smokers were excluded from the study because they are thought to have an inadequate intake of vitamin C [21,31] in relation to their exposure to very high levels of free radicals in cigarette smoke [32]. The effects of vitamin C supplementation in smokers was therefore considered a subject for specific study at a later date.

MATERIALS AND METHODS

Study design

From a panel of over 100 nonsmoking volunteers, 48 were selected to have as wide a range of serum cholesterol levels as possible. These individuals were allocated to 3 groups of 16, each consisting of 4 males with low cholesterol levels (defined as <6 mmol/L), matched for age and build with 4 males with high cholesterol levels (>6 mmol/L) and 8 females matched in the same way. The ages of the volunteers ranged from 20 to 68 years with a mean of 45.7 years. None of the volunteers was taking medication to control cholesterol levels, and they were allowed to maintain their normal dietary habits so as not to compromise their cholesterol status. All procedures were performed to the standards of Good Clinical Practice.

A three-treatment, three-treatment period, cross-over design was adopted (shown in Table 1) to take account of temporal differences in response which have been demonstrated for certain parameters [33,34]. In each treatment period, volunteers were given either a placebo, 60 mg vitamin C/day or 6 g vitamin C/day for 14 days. A period of 6 weeks was allowed between the legs of the study. Blood samples were taken at the end of the treatment period, approximately 8 h after the last dose.

Table 1 Design of the main vitamin C supplementation study

Treatment	Placebo	60 mg/day	6 g/day
Week 1 and 2	A Blood sample 1	B	C
Week 3–8	WASHOUT PERIOD		
Week 9 and 10	B Blood sample 2	C	A
Week 11–16	WASHOUT PERIOD		
Week 17 and 18	C Blood sample 3	A	B

A,B,C represent groups of 16 volunteers; 4 pairs of males and 4 pairs of females, each pair matched for age and build but maintaining greatest possible difference in blood cholesterol level.

Prior to the main study a preliminary study was conducted to test the suitability of the parameters to be examined and the methods to be used [35].

Materials

The lower dose of vitamin C was administered in the form of 2 × 30 mg Sanatogen Chewable Vitamin C tablets taken once each day. The high dose was administered as 6 × 1000 mg Redoxon effervescent tablets dissolved in water; 2 tablets were taken approximately every 8 h. The placebo consisted of Horlicks tablets. These materials were purchased from a local retailer.

ABTS (2,2'-azinobis-{3-ethylbenzothiazoline-6-sulphonic acid}) was purchased from Aldrich Chemical Co., Ltd. LPO-586 kits were obtained from Bioxytech SA, France. Bleomycin was obtained from Lundbeck Ltd., Milton Keynes and had an activity of 1.85 IU/mg. All other chemicals were purchased from Sigma Chemical Co., Ltd.

Modified Eagle's Medium (EMEM), antibiotics and glutamine were obtained from Life Technologies Ltd., Paisley, Scotland. Fetal calf serum was from Harlan Sera-Lab Ltd., Crawley Down, Sussex.

Assays and biomarkers

The following assays were conducted on each sample of peripheral blood obtained by venepuncture.

Serum cholesterol

A standard enzymatic clinical chemistry method was used.

Plasma vitamin C

Vitamin C (L-ascorbic acid) was determined by acidic reversed phase HPLC with UV detection at 244 nm, using a method based on that of Liau *et al.* [36]. Plasma samples were denatured with 10% perchloric acid containing 1% metaphosphoric acid and D-isoascorbic acid as internal standard.

Total antioxidant capacity

A modification of the method of Miller *et al.* [37] was employed. Formation of the ABTS radical cation was initiated by addition of 75 mM H₂O₂ to a mixture of 150 mM ABTS and 2.5 mM metmyoglobin and monitored using its absorbance at 734 nm. Addition of plasma (10 mL/mL) prior to initiation of the reaction caused a delay in formation of ABTS radical cation. The "lag time" was used as a measure of total antioxidant capacity since it has been shown to be particularly appropriate for detecting the antioxidant activity of vitamin C [38].

Lipid peroxidation breakdown products

The LPO-586 method from Bioxytech SA, France was employed. In this assay, malondialdehyde and 4-hydroxyalkenals in plasma react with a chromogenic reagent and are estimated by measuring absorbency at 586 nm.

Comet assay

Lymphocytes were separated from a sample of whole blood using Lymphoprep and subjected to electrophoresis as described in Anderson *et al.* [39]. One sample of cells was examined without treatment and another after 30 min exposure to 10 mL H₂O₂. After electrophoresis, DNA was stained with ethidium bromide and the migration estimated by image analysis. A number of parameters was measured, but the results finally expressed as tail moments.

Chromosome aberration test

Each sample of whole blood was used to establish 8 cultures consisting of 0.8 mL blood and 9.2 mL Modified Eagle's Medium supplemented with 15% fetal calf serum. Pairs of cultures were treated with bleomycin at concentrations of 11, 33 and 66 mg/mL. Two cultures were left untreated. Bleomycin exposure was terminated after 3 h by resuspending the cells in fresh medium. Phytohaemagglutinin was then added to all cultures, to stimulate cell division, which were incubated for 48 h. Metaphase was arrested by addition of demecolcine for 2 h, and chromosome slides prepared using standard procedures [34]. Chromosome aberrations were scored in approximately 100 metaphases from each culture, i.e., 200 metaphases were scored per individual per study leg where possible. The results of a preliminary study [35] determined that only the bleomycin dose of 33 mg/mL should be scored initially for chromosome aberrations.

Ras p21 protein estimation

Ras p21 protein was estimated as described previously [40]. Briefly, plasma proteins were separated by polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane by Western blotting, and the blots developed with appropriate primary and secondary antibodies followed by enhanced chemiluminescence. Exposure to light-sensitive film resulted in bands at 21 kD which could then be measured by light densitometry. The protein level (optical density) was represented as the area of the

peaks on the densitometry scans. Based on the suggestion of Perera *et al.* [41], an increased expression of this protein is defined as a peak area greater than the mean for normal controls (the placebo group) plus 2 standard deviations of the mean.

Statistical analysis

For continuous variables, the data were tested for normality and homogeneity. If either of these assumptions was violated, transformations were performed.

The data were subjected to an analysis of variance testing for treatment, leg, cholesterol, and gender effects. Pair-wise comparisons between groups were made using a least significant difference test (LSD). A level of probability of less than 0.05 was taken as indicating statistical significance.

RESULTS

Serum cholesterol

Volunteers were selected initially on the basis of a pre-study measurement to represent a wide range of serum cholesterol values. Figure 1 shows how the serum cholesterol of these subjects varied during the study. In some cases there was wide variation, but in general there was reasonable consistency in the classification of low or high. There was, as might be expected, a significant difference between the means for the low and high groups, but no significant difference between males and females. During the study, there was no evidence of an effect of vitamin C supplementation on serum cholesterol levels.

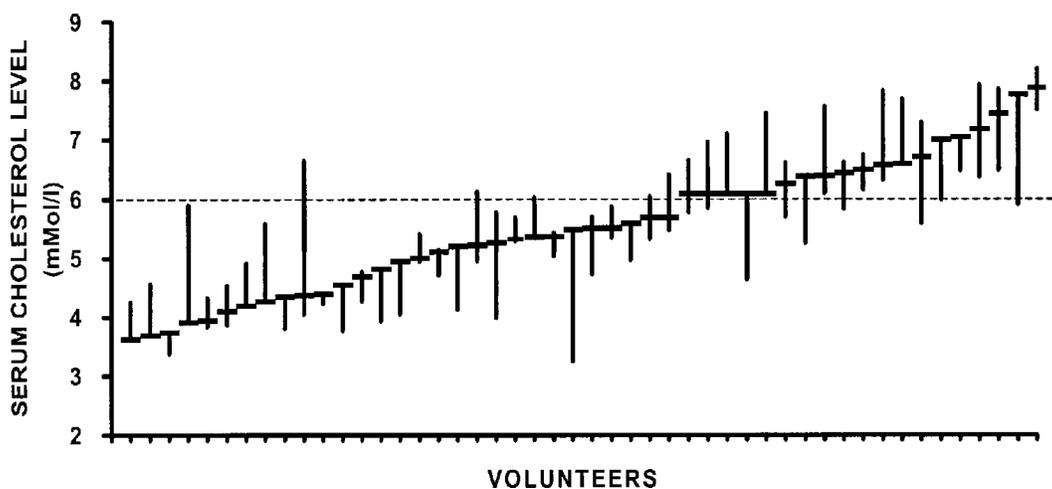


Fig. 1 Serum cholesterol levels of volunteers in the main study. Serum cholesterol was measured before the study and at the end of each of the three treatment periods. For each volunteer, the value obtained prior to the study is indicated by a short horizontal line, and the highest and lowest of the four measurements are linked by a vertical line, thus indicating the range of values obtained. For clarity, the data are arranged in order of increasing pre-study cholesterol level. The value of 6.0 mmol/L was used to classify volunteers as having high or low cholesterol levels during selection for the study.

Plasma vitamin C

For the 48 individuals, both levels of vitamin C supplementation caused a statistically significant increase in mean plasma vitamin C level (placebo 11.19 ± 0.66 mg/mL; low vitamin C 13.84 ± 0.54 mg/mL).

high vitamin C 20.12 ± 0.91 mg/mL). The value for the higher supplementation dose was also statistically significantly higher than that for the lower supplementation level. There was no statistically significant difference between vitamin C levels in volunteers with low and high cholesterol levels, but there was a statistically significant difference between male and female volunteers. Those volunteers who had a relatively low vitamin C level in the placebo leg showed the greatest increase when given vitamin C supplements, but that supplementation had relatively little effect once a certain level had been reached.

Lipid peroxidation

The quantities of lipid peroxidation breakdown products found in plasma varied very little between volunteers and between treatments. No statistically significant differences were found between low and high cholesterol groups, males and females, or the three treatment groups. However, there was a small dose-related reduction in lipid peroxidation breakdown products with vitamin C supplementation.

Total antioxidant capacity

The overall data (Table 2) showed a statistically significant difference between the low and high cholesterol groups, the antioxidant capacity being greater in the high cholesterol group. Male volunteers had a higher mean antioxidant capacity than the females. Vitamin C supplementation significantly increased antioxidant capacity but there was no statistically significant difference between the low and high levels of vitamin C supplementation.

Table 2. Antioxidant capacity (lag time in seconds)

	Overall	Placebo	Low Vitamin C	High Vitamin C
Overall	258.9 ± 7.0 n = 141	241.5 ± 9.5 n = 47	269.9 ± 13.5 n = 48	265.0 ± 12.8 n = 46
Low cholesterol	242.0 ± 7.9 n = 95	229.7 ± 11.0 n = 31	252.4 ± 17.0 n = 32	243.5 ± 11.6 n = 32
High cholesterol	293.7 ± 12.9 n = 46	264.4 ± 17.1 n = 16	305.0 ± 20.1 n = 16	314.3 ± 29.2 n = 14
Males	292.0 ± 11.1 n = 72	268.9 ± 15.2 n = 24	305.8 ± 22.5 n = 24	301.4 ± 19.2 n = 24
Females	224.3 ± 6.2 n = 69	213.0 ± 7.9 n = 23	234.1 ± 11.5 n = 24	225.3 ± 12.2 n = 22

Means ± standard errors, and levels of significance resulting from data that were subjected to an analysis of variance and pair-wise comparisons made using a least significant difference test;

^{ns} = not significantly different ($p > 0.05$); * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Comet assay

The results of Comet assays on lymphocytes without challenge and with hydrogen peroxide challenge respectively showed that there were no statistically significant differences in the amount of DNA damage measured by this assay due to cholesterol status, gender, or treatment.

Chromosome aberrations

Tables 3 and 4 show the results of chromosome aberration tests on lymphocytes without challenge and with bleomycin challenge respectively. Without challenge, there were no statistically significant

Table 3. Chromosome aberrations in lymphocytes without bleomycin challenge: % aberrant metaphases (without bleomycin challenge)

	Overall	Placebo	Low Vitamin C	High Vitamin C
Overall	1.72 ± 0.13 n = 139	1.77 ± 0.29 n = 47	1.61 ± 0.17 n = 47	1.80 ± 0.18 n = 45
Low cholesterol	1.87 ± 0.18 n = 93	1.88 ± 0.43 n = 31	1.77 ± 0.23 n = 31	1.96 ± 0.24 n = 31
High cholesterol	1.43 ± 0.13 n = 46	1.56 ± 0.25 n = 16	1.28 ± 0.20 n = 16	1.44 ± 0.24 n = 14
Males	1.73 ± 0.21 n = 69	1.90 ± 0.56 n = 23	1.68 ± 0.23 n = 23	1.62 ± 0.19 n = 23
Females	1.72 ± 0.15 n = 70	1.65 ± 0.21 n = 24	1.54 ± 0.24 n = 24	1.98 ± 0.32 n = 22

Means ± standard errors, and levels of significance resulting from data that were subjected to an analysis of variance and pair-wise comparisons made using a least significant difference test; ^{ns} = not significantly different (p > 0.05); * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

Table 4. Chromosome aberrations in lymphocytes with 33 µg/mL bleomycin challenge: % aberrant metaphases

	Overall	Placebo	Low Vitamin C	High Vitamin C
Overall	6.25 ± 0.24 n = 139	5.28 ± 0.37 n = 47	6.77 ± 0.44 n = 47	6.70 ± 0.42 n = 45
Low cholesterol	6.19 ± 0.29 n = 93	5.27 ± 0.45 n = 31	6.41 ± 0.50 n = 31	6.88 ± 0.54 n = 31
High cholesterol	6.38 ± 0.43 n = 46	5.29 ± 0.65 n = 16	7.53 ± 0.83 n = 16	6.30 ± 0.61 n = 14
Males	5.80 ± 0.33 n = 69	4.69 ± 0.50 n = 23	6.20 ± 0.50 n = 23	6.51 ± 0.62 n = 23
Females	6.69 ± 0.35 n = 70	5.84 ± 0.52 n = 24	7.35 ± 0.69 n = 24	6.89 ± 0.56 n = 22

Means ± standard errors, and levels of significance resulting from data that were subjected to an analysis of variance and pair-wise comparisons made using a least significant difference test; ^{ns} = not significantly different (p > 0.05); * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

differences in the numbers of metaphases with aberrations attributable to cholesterol status, gender, or treatment. After challenge with bleomycin, however, there was a statistically significant increase in the incidence of chromosome aberrations after vitamin C supplementation. The effect was significant at both supplementation levels, but was slightly less marked at the higher level.

Figure 2 summarizes the chromosome aberration data and relates them to the mean plasma vitamin C levels observed.

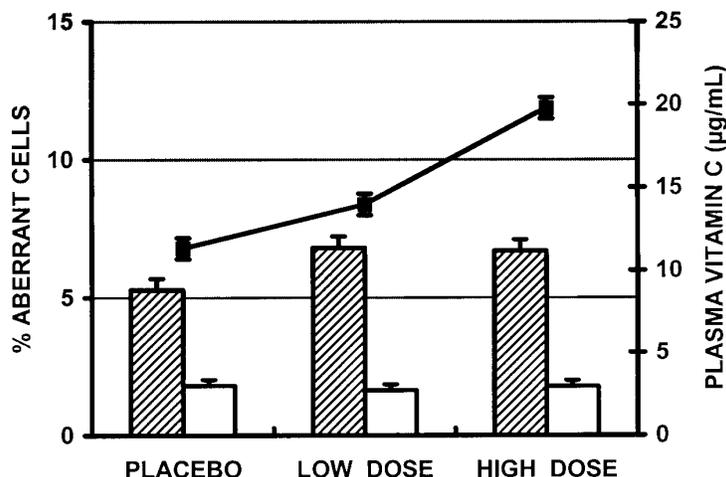


Fig. 2 Incidence of metaphases with chromosome aberrations in lymphocytes and vitamin C plasma levels. Volunteers were given placebo, 60 mg vitamin C/day (Low) or 6 g vitamin C/day (High) for 14 days. Data from all 3 treatment periods are combined. Two sets of cultures were established from a blood sample taken from each volunteer at the end of each treatment period. One set was immediately treated with 33 mg/mL bleomycin for 3 h. The other set was untreated. Chromosome analysis was carried out after 48 h of culture. 200 metaphases were examined for each set of cultures. Each column represents the mean value for all the volunteers who had received the same treatment (16 for a to c; 48 for d). Hatched columns represent data for bleomycin-treated cultures, and unhatched columns represent data for untreated cultures. The mean plasma vitamin C values for each group are linked by a line. The error bars represent one standard error of the mean.

Ras p21 protein levels

The plasma levels of *ras p21* protein were measured in only one treatment period of the trial. An increased expression of this protein is defined as a peak area greater than the mean for normal controls (the placebo group) plus two standard deviations of the mean. Only one sample met this criterion, and that was in the placebo group. Thus, there was no detectable difference in *ras p21* protein levels between the treatment groups.

DISCUSSION

The purpose of this study was to investigate whether vitamin C supplementation has a measurable protective effect against oxidative damage in healthy people consuming their normal diet.

Cholesterol status was considered to be an important factor in the design of the study since a correlation has been established between serum cholesterol level and risk of coronary heart disease as well as the severity of atherosclerosis [27–29]. Volunteers were selected to cover as wide a range of cholesterol levels as possible, without including people on medication or specific diets to reduce cholesterol levels. The study was constructed on the basis of a single assay of cholesterol status but the assays performed during the trial showed that there was a reasonable degree of stability in serum cholesterol for most individuals throughout the duration of the study.

The volunteers in this study had plasma vitamin C concentrations of 4.73–23.54 mg/mL after placebo treatment and none could therefore be regarded as deficient in vitamin C. Supplementation with 60 mg/day of vitamin C increased the mean plasma level from 11.19 to 13.84 mg/mL, a modest increase which was largely due to substantial increases in those individuals with a low value (less than about 10 mg/mL) in the placebo treatment group. Supplementation with 6 g vitamin C/day increased the mean value further to 20.12 mg/mL but this was again a modest increase relative to the 100-fold increase in vitamin C dose. A leveling-off in plasma vitamin C levels with increasing doses of vitamin

supplementation has been reported by Hallfrisch *et al.* [30]. It can be concluded that most of a high-dose vitamin C supplement is either not absorbed [42] or rapidly excreted. Higher plasma levels of 800 to 900 mg/mL after chronic intake of vitamin C have been observed in other individuals [42]. A higher supplementation time might have produced higher plasma levels.

Vitamin C supplementation produced a dose-related but statistically nonsignificant reduction of the level of lipid peroxide breakdown products in plasma, suggesting that within the range of concentrations seen in this study, vitamin C had some small effect in controlling plasma lipid peroxidation.

The total antioxidant capacity of plasma was increased significantly by vitamin C supplementation. However, the increases were small (as were the increases in plasma levels of the vitamin itself), and there was no increase between the 60 mg/day and the 6 g/day supplementation levels. Miller *et al.* [37] cite calculations from their own results and those of others, using different methods, showing that vitamin C contributes only about 10% of the total antioxidant capacity of most human plasma samples.

Previous studies in this laboratory [39] have shown that, *in vitro*, vitamin C can protect human lymphocytes against DNA damage induced by oxidative agents such as hydrogen peroxide, although at high concentrations it can exacerbate such damage. In this present study, the results of Comet assays on lymphocytes showed no evidence of an effect of vitamin C supplementation on either background levels of DNA damage or on DNA damage induced by hydrogen peroxide.

The chromosome aberration tests showed no evidence of a change in the background frequency of aberrations in lymphocytes after vitamin C supplementation. The background frequency of aberrations is low but the results presented are based on examination of between 8,000 and 8,500 metaphases for each treatment group, allowing conclusions to be drawn with some confidence. The induction of chromosome aberrations by bleomycin was significantly increased, however, after vitamin C supplementation, suggesting that the vitamin even at moderate levels of intake might exacerbate DNA damage induced by certain oxidative agents. The significance of the increase in sensitivity to bleomycin is unclear, but may suggest that increased vitamin C intake predisposes cells to genetic damage by agents that generate oxygen radicals.

The mechanism by which bleomycin damages DNA has been studied extensively [43] and shown to involve the formation of a complex between bleomycin, DNA, and a reduced metal ion, usually ferrous. An increased concentration of ferrous ion in the blood samples from vitamin C-treated volunteers could have increased the effect of bleomycin. Retrospectively, some serum samples were examined using a commercial colorimetric assay for serum iron and iron-binding capacity (Sigma assay). No systematic difference between unsupplemented and vitamin C-treated volunteers was found. However, release of ferrous ions from ferritin might have occurred intracellularly, causing no net change in plasma. The effect of vitamin C on iron status and iron-catalyzed oxidative reactions needs more extensive investigation.

There was no effect on *ras* p21 proteins. These proteins were measured because it has been shown that in workers exposed to various hazardous wastes [44,45] there were increases in *ras* oncoprotein levels. Such increases have been used as biomarkers of exposure to supposed genotoxic agents. By analogy, increased *ras* oncoproteins have been shown to accompany chromosome damage [46], and oxidative stress is known to cause chromosome damage, so it was thought that an increase in *ras* p21 proteins might be a biomarker for oxidative stress.

There was no evidence that cholesterol status affected any of the parameters measured in this study except for the total antioxidant capacity of plasma that was significantly increased in the high cholesterol subjects. There were shown to be significant differences between males and females for plasma vitamin C levels, the antioxidant capacity of the plasma and for chromosome aberrations after bleomycin challenge. Gender differences have been shown on previous occasions for other endpoints such as chromosome damage, sister chromatid exchanges, proliferative rate index, and mitogen-induced blastogenesis [33,47,48].

The results of this study provide no evidence for any of the biomarkers studied of any benefit to be gained from vitamin C supplementation if an adequate diet is consumed. The volunteers used in the

study were all healthy and able to provide themselves with a good diet. The observed plasma vitamin C levels were all within the normal range. Some effect of supplementation on plasma concentration was seen in individuals with relatively low starting levels but for most, there was very little effect. It is possible, however, that tissue levels were increased more than plasma levels. The higher supplementation dose was 100 times the RDA, but had no measurably greater effect than the low dose, apart from raising plasma concentration by a small amount.

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