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The Effects of Iron and Vitamin C Co-supplementation on Oxidative Damage to DNA in Healthy Volunteers

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The effects of co-supplementing healthy volunteers with iron (14 mg/day ferrous sulphate) and vitamin C (either 60 mg/day or 260 mg/day as ascorbic acid) on levels of oxidative DNA damage in white blood cells were studied. The subjects were divided into two groups: one group of 20 volunteers with a higher mean initial level of plasma vitamin C (71.9 ± 14.0 μmol/l) and a second group of 18 volunteers with a lower mean level (50.4 ± 25.8 μmol/l). In the first group there was a significant rise in several oxidative DNA base damage products and in total oxidative DNA damage in DNA extracted from white blood cells, but not in 8-hydroxyguanine, after 6 weeks of supplementation. However, after 12 weeks levels returned approximately to normal. In the group with the lower initial level of plasma ascorbate, presupplemental levels of oxidative DNA damage were higher and decreased on supplementation with iron and ascorbate. Since oxidative DNA damage has been suggested as a risk factor for the development of cancer, the implications of increased levels in well-nourished subjects after iron/ascobate supplementation are disturbing in view of the frequent use of dietary supplements containing both iron salts and ascorbate. © 1998 Academic Press

Oxidative damage has been linked to the development of a range of pathologies such as cancers [1, 2] and to the accumulation of problems associated with aging [3]. In particular, it has been suggested that oxidative DNA damage is a major contributor to age-related development of the common cancers, and that increased oxidative damage contributes to the increased risk of cancer seen in many chronic inflammatory diseases [3, 4]. Epidemiological and other studies have led to the attractive proposition that the intake of vitamin C may provide, in part, protection against some cancers [5] and DNA damage in human sperm [6]. However, the general use of vitamin C supplements as being beneficial to health has been disputed [7] and the results of some of these studies involving vitamin C are equivocal [8]. One of the possible complications arising from the ingestion of supplemental doses of vitamin C is the possibility that the vitamin itself would lead to the exacerbation of free radical damage rather than its amelioration [7], particularly if co-supplemented with iron. It can be demonstrated *in vitro* that vitamin C interacts with iron to exert pro-oxidant effects [9], although there is no clear support for the concept of pro-oxidant interactions between ascorbate and iron *in vivo* [9, 10]. It has also been suggested that vitamin C may also increase the bioavailability of iron [11]. Other authors have stated that elevated ascorbic acid has no adverse effects in the normal population [12], or in specific clinical conditions such as "iron overload" in neonates [13] or adults [9]. In this study we have investigated the effects on oxidative DNA damage of vitamin C and iron co-supplementation in non-iron deficient, non-iron overloaded healthy human subjects.

MATERIALS AND METHODS

Study protocol. Ethical permission was obtained from the Lewisham and North Southwark Committee on Ethical Practice. Forty healthy volunteers (20 men, 20 women) aged between 21 and 45 years (30.3 ± 6.28 y; mean age ± S.D.) were recruited. There were no significant differences between the groups with respect to age and body mass index (overall 23.9 ± 3.86 kg/m^2). Exclusion criteria included smoking, high alcohol intake (males >21 units/wk or female >14 units/wk), consumption of vitamin and mineral supplements other than those used in the study, pregnancy, oral contraceptives or consumption of drugs such as antihistamines, antibiotics and anti-inflammatories including aspirin.

The study was carried out in two parts with an initial total of 40 healthy subjects (Fig. 1). In the first study group we examined subjects with an initial mean plasma vitamin C concentration of 71.9

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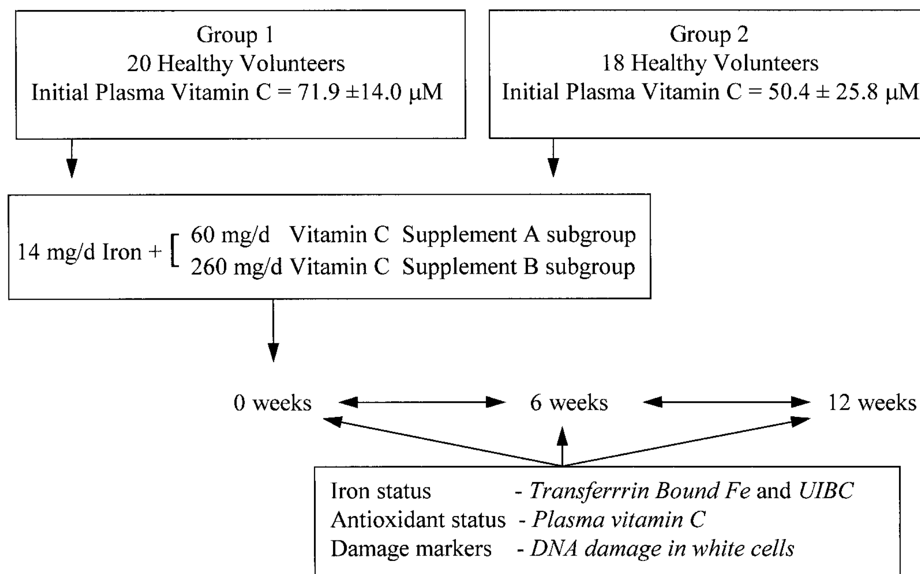

FIG. 1. The Study Protocol. Iron supplied as ferrous sulphate and vitamin C as ascorbic acid. UIBC, unsaturated iron binding capacity.

±14.0 μmol/l (mean ± SD; n=20) indicative of a good dietary intake of vitamin C [14]. This was significantly higher than in the second study group (50.4 ±25.8 μmol/l; n=18). In each study, subjects were randomly assigned to one of two supplementation groups (5 males and 5 females/group) and all the subjects received proprietary brand (Boots Company, Nottingham, UK) vitamin C and iron supplements containing 14 mg of iron (as ferrous sulphate) and 60 mg vitamin C (as ascorbic acid) (Supplement A subgroup) or with an additional 200 mg vitamin C (total vitamin C, 260 mg) (Supplement B subgroup) per day for 12 weeks. Blood samples were taken on 3 occasions, before supplementation started, and after 6 weeks and 12 weeks supplementation. The subjects fasted for 12 h immediately before the blood samples were taken. The subjects were also required to complete a general health questionnaire and an 8-day food diary, which was assessed using the COMP-EAT dietary analysis computer programme [15]. Compliance was determined by supplying a documented excess of these supplements and counting the number of capsules returned by the subjects at the 6 wk and 12 wk visit, and was equally high in all groups (overall 99.5%). All the subjects completed the first study, but two subjects (one from each group) withdrew from the second study.

Whole blood was collected by venepuncture and divided into aliquots for the subsequent analyses to be performed. For transferrin bound iron levels and iron binding capacity blood serum was analysed and for ascorbic acid determination plasma samples were separated.

All chemicals were of the highest quality available from Sigma Chemical Co. (Poole, Dorset, UK), BDH Chemical Co. (Gillingham, Dorset, UK), and /or Aldrich Chemical Co. (Milwaukee, WI).

DNA damage. Standards of 8-hydroxyadenine, FAPY guanine and thymine glycol (cis) were synthesised, purified [16], and purity assessed by mass spectrometry. 2-hydroxyadenine, 5-hydroxycytosine, and 5-hydroxymethylhydantoin were gifts from Dr Miral Dizdaroglu (National Institute of Standards and Technology, Gaithersburg, MD).

DNA isolation from whole blood was carried out by a modification of the method of John et al. [17]. Analysis of DNA base modification by gas chromatography-mass spectrometry (GC-MS) was by a modification of the method of Spencer et al. [18]. Briefly, the DNA samples were dialysed in distilled water for 18-20 h using regenerated cellulose tubular membrane (Cellusep T_1) with a nominal relative molecular mass cut-off of 3500. The amount of DNA recovered was determined spectrophotometrically at 260 nm (A_{260} of 1.0 = 50 μg DNA/ml), aliquoted into 100 μg DNA samples, and 25 μl of 20 μM internal standard (azathymine and diaminopurine) was added. The samples were then lyophilised overnight, followed by hydrolysis in evacuated and sealed tubes with 500 μl 60% w/v formic acid for 45 min at 150 °C. Samples and standards were then lyophilised again overnight and derivatised in poly (tetrafluoroethylene)-capped hypovials in which air was displaced with N_2. 15 μl acetonitrile (methylcyanide silylation grade) and ethanethiol, premixed at a ratio of 3:1 respectively, and 60 μl N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylchorosilane (TMCS) was injected into each sample. The samples were mixed well and incubated at room temperature for 2 h. This protocol has been shown to prevent artifactual oxidation of DNA bases during the derivatization procedure [19].

The derivatised samples were analysed by a Hewlett-Packard 5971A mass selective detector interfaced with a Hewlett-Packard 5890II gas chromatograph. The injection port and the GC-MS interface were kept at 250 °C and 290 °C respectively. Separations were carried out on a fused silica capillary column (12m x 0.2 mm i.d.) coated with cross-linked 5% phenylmethylsiloxane (film thickness 0.33 μm) (Hewlett-Packard Ltd., Stockport, Cheshire, UK).

Helium was the carrier gas with a flow rate of 0.93 ml/min. Derivatised samples (2 μl) were injected into the GC injection port using a split ratio of 8:1. Column temperature was increased from 125 to 175°C at 8°C/min after 2 min at 125°C, then from 175 °C to 220°C at 30°C/min and held at 220 °C for 1 min, and finally from 220 °C to 290 °C at 40 C/min and held at 290 °C for 2 min. Selected ion monitoring was performed using the electron-ionisation mode at 70 eV with the ion source maintained at 190 °C. Quantitation of modified bases was achieved as described in references 19, 20.

Antioxidant status and transferrin bound iron. For ascorbic acid analysis plasma samples were treated with 0.3 M trichloroacetic acid (TCA; 1 ml plasma: 4 ml TCA), centrifuged at 1,500g and the supernatant stored at −70 °C. Total ascorbic acid levels were deter-

mined fluorimetrically using a modification of the method of Deutsch and Weeks [21]. In brief, the TCA treated samples were oxidised with iodine and a complex formed with orthophenylenediamine (OPD). The resulting fluorescence was read on a Shimadzu RF-1501 fluorimeter (excitation wavelength 348 nm; emission wavelength 423 nm). The concentration of ascorbic acid in the incubation mixtures was determined by comparison with standards.

To determine the transferrin-bound iron, ferric iron from serum samples was dissociated from the transferrin and reduced using ascorbic acid. The ferrous iron was then complexed with ferene chromogen (Randox Serum Iron/UIBC Assay Kit, Randox Laboratories Ltd., UK) and the chromophore formed was quantified spectrophotometrically at 595 nm by comparison with standards.

Results are expressed as mean ± SD. Statistical analysis was performed using Microsoft Excel. All tests were two-tailed and P values <0.05 for t in differences between two values were considered to be significantly different.

RESULTS

Dietary analysis. Analysis of the nutritional intake (without supplementation) of the subjects showed that over both studies the presupplemental daily amounts of dietary iron ingested varied by a factor of six from a minimum of 6 mg to a maximum of 36 mg. Vitamin C intake ranged between 42 and 290 mg/d in the first group and between 29 and 161 mg/d in the second , so over both studies the presupplemental daily amounts of dietary vitamin C varied by a factor of ten from 29 mg/d to 290 mg/d, and intake was positively correlated with plasma levels ($r = 0.42$; $p < 0.05$; $n = 38$)

Oxidative DNA base damage. GC/MS was used to measure a wide range of DNA base damage products, allowing a quantitative assessment of oxidative damage to all four DNA bases. In the first study with subjects who had a higher initial level of plasma vitamin C, in both subgroups of low and high vitamin C co-supplementation (Table 1), total base damage was initially lower than that seen in subjects in the second study. Further, total base damage was not significantly different at the start of the study and after 12 weeks supplementation. However, in both subgroups a rise in damage was observed at 6 weeks, due largely to increases in 5-OH methylhydantoin, 5-OH hydantoin and FAPY guanine (Table 1). By contrast, levels of 8-OH guanine, the most commonly-used parameter of oxidative DNA damage [20,22], did not rise significantly at 6 weeks (although there was a trend to a rise) and fell at 12 weeks.

Plasma iron and antioxidant status. There were no significant differences in transferrin-bound iron (Fig. 2A) or percent saturation of transferrin (Table 1) with either level of supplementation in this first study (high initial plasma ascorbate), nor were there any significant differences in the total iron binding capacity from the initial levels of 51.9 ± 6.2 μmol/l (Supplement A subgroup) and 54.0±9.9 μmol/l (Supplement B subgroup). Supplementation with iron and either level of vitamin C did not produce any significant changes in

TABLE 1

Co-supplementation of Healthy Human Volunteers with a High Initial Plasma Vitamin C Concentration with Iron and Vitamin C: Effects on DNA Base Damage

DNA base damage product	Supplement A (nmol base/mgDNA)			Supplement B (nmol base/mgDNA)		
	0 wk	6 wk	12 wk	0 wk	6 wk	12 wk
5-Cl uracil	0.06 ± 0.020	0.01 ± 0.010*	0.005 ± 0.0033*	0.06 ± 0.027	0.02 ± 0.011*	0.01 ± 0.011*
5-OH uracil	0.11 ± 0.039	0.05 ± 0.016*	0.04 ± 0.011*	0.11 ± 0.024	0.05 ± 0.008*	0.04 ± 0.013*
5-OH Me uracil	0.03 ± 0.014	0.02 ± 0.008	0.05 ± 0.012*	0.04 ± 0.012	0.02 ± 0.009*	0.05 ± 0.011
5-OH Me hydantoin	0.22 ± 0.087	1.0 ± 0.43*	0.13 ± 0.054*	0.23 ± 0.084	1.1 ± 0.31*	0.16 ± 0.13
5-OH hydantoin	0.12 ± 0.044	0.51 ± 0.21*	0.09 ± 0.044	0.12 ± 0.044	0.59 ± 0.24*	0.08 ± 0.025
5-OH cytosine	0.10 ± 0.019	0.05 ± 0.014*	0.15 ± 0.054*	0.09 ± 0.013	0.05 ± 0.026*	0.12 ± 0.034*
Thymine glycol (cis)	0.35 ± 0.22	0.25 ± 0.10	0.87 ± 0.45*	0.30 ± 0.15	0.30 ± 0.087	0.73 ± 0.20*
Thymine glycol (trans)	0.006 ± 0.0048	0.08 ± 0.028*	0.15 ± 0.099*	0.008 ± 0.006	0.06 ± 0.041*	0.10 ± 0.073*
FAPY adenine	0.54 ± 0.68	0.68 ± 0.35	1.1 ± 0.29	0.69 ± 0.72	0.95 ± 0.49	0.97 ± 0.20
8-OH adenine	0.33 ± 0.17	0.17 ± 0.079	0.08 ± 0.049*	0.40 ± 0.27	0.18 ± 0.072	0.07 ± 0.031*
2-OH adenine	0.15 ± 0.077	0.20 ± 0.066	0.14 ± 0.11	0.10 ± 0.060	0.21 ± 0.064*	0.11 ± 0.024
FAPY guanine	0.33 ± 0.22	1.8 ± 0.98*	0.24 ± 0.13	0.32 ± 0.23	1.6 ± 0.50*	0.21 ± 0.048
8-OH guanine	0.24 ± 0.087	0.31 ± 0.081	0.12 ± 0.034*	0.23 ± 0.11	0.34 ± 0.12	0.15 ± 0.067
Total base damage	2.6 ± 0.77	5.2 ± 1.9*	3.1 ± 0.87	2.7 ± 0.98	5.6 ± 1.2*	2.8 ± 0.39
‡Plasma vit. C μmol/l	73.7 ± 15.1	64.7 ± 15.9*	78.7 ± 17.7	70.5 ± 13.4	72.4 ± 12.4	79.5 ± 10.9
‡%Sat. transferrin	32.7 ± 9.8	37.9 ± 12.2	30.8 ± 10.6	33.9 ± 14.	38.2 ± 9.9	31.9 ± 8.4

Note. Mean values ± SD shown; n=10 in both Groups. * statistically significantly different from 0 wk, P<0.05 paired t test. Vit. C, vitamin. %Sat., %saturation; TIBC, total iron binding capacity. Supplementation: iron 14 mg/d together with either 60mg/d vitamin C (Supplement A subgroup) or 260 mg/d vitamin C (Supplement B subgroup). GC/MS analysis was carried out as in Refs. 19, 20.

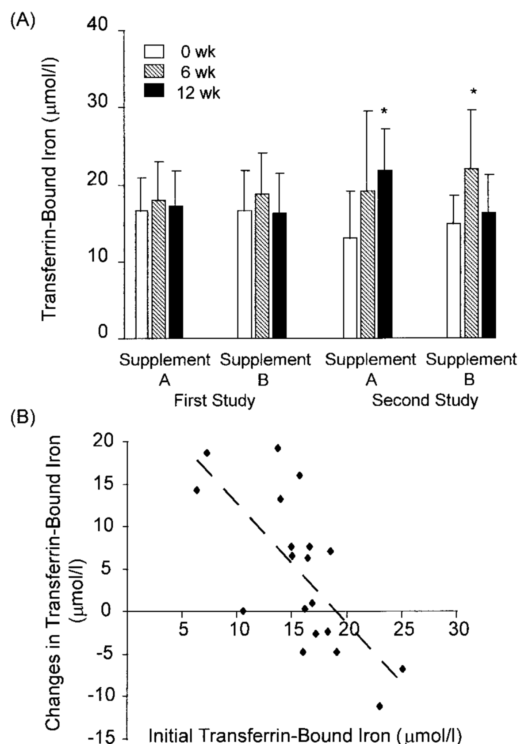

FIG. 2. The effects of co-supplementing with iron (14 mg/d) and either 60 mg/d vitamin C (Supplement A subgroup) or 260 mg/d of vitamin C (Supplement B subgroup) for 12 wk on (A), the transferrin bound iron, and (B), the relationship between the initial presupplemental transferrin bound iron concentrations and the change in transferrin-bound iron after 6 wk supplementation with 260 mg/d vitamin C + iron. First Study initial plasma vitamin C concentration = 71.9 ± 14.0 μmol/l; Second Study = 50.4 ± 25.8 μmol/l. Mean values ± SD are shown in (A); n=10 in each supplement subgroup of First Study Group and n = 9 in each Supplement subgroup of Second Study Group; *statistically significantly different *vs* initial (presupplemental) level, $P < 0.05$ paired t test. In (B) n = 19 pooled data Supplement B subgroup both studies; r = −0.706, $P < 0.05$; linear regression intercept = 17.4 μmol/l; slope −1.4.

the initial total haemoglobin levels of 14.2 ± 3.2 g/dl (Supplement A subgroup) and 14.8 ± 1.4 g/dl (Supplement B subgroup).

Further, in the first study consisting of subjects having a higher initial plasma level of vitamin C, following supplementation with iron and 60 mg/d vitamin C (Supplement A subgroup) the plasma concentration slightly decreased, returning to basal levels at 12 wk, whereas the Supplement B subgroup (260 mg/d) showed no significant changes in vitamin C plasma concentration (Table 1).

A second study was also conducted with subjects who had a lower initial level of plasma vitamin C (Fig. 1; Table 2). A higher initial level of base damage was observed which decreased on supplementation. However, full statistical analysis of this DNA data was not possible because it was necessary to pool the samples to extract sufficient DNA for analysis. Further, in the subgroup taking the lower supplemental amount of vitamin C (Supplement A), the mean concentration of transferrin-bound iron increased to a level that was significantly different from that of the initial value ($P < 0.05$) after 12 wk (Fig. 2A). On the other hand, subjects from the subgroup having the higher intake of vitamin C (Supplement B), showed a significant increase ($P < 0.05$) in transferrin-bound iron after 6 wk which subsequently decreased towards initial concentrations after 12 wk (Fig. 2A). The values for the saturation of transferrin (Table 2) showed a similar trend to those of the transferrin-bound iron (Fig. 2A). Supplement A subgroup showed increased saturation of transferrin after 6 wk and after 12 wk, while the initial level of Supplement B subgroup was increased at 6 wk returning to basal levels after 12 wk (Table 2).

In general over both studies, the higher the initial basal values of transferrin-bound iron the lower the extent of any further rise. For example, in the Supplement B subgroup (both studies), there was an inverse relationship (r = −0.706, n = 19, $P < 0.05$) between the initial transferrin bound iron and the change in bound iron after 6 wk (Fig. 2B), and the absence of statistically significant increases in the first study may be related to the higher initial plasma levels The initial transferrin bound iron intercept was 17.4 μmol/l and the slope −1.4. The total iron binding capacity of subjects in the second study did not significantly alter from the initial values of 54.0 ± 10.4 μmol/l (Supplement A subgroup) and 59.2 ± 13.1 μmol/l (Supplement B subgroup). Supplementation with iron and either level of vitamin C did not produce any significant changes in the initial total haemoglobin levels of 15.0 ± 2.2 g/dl (Supplement A subgroup) and 14.2 ± 1.7 g/dl (Supplement B subgroup) from the second study.

In contrast with the first study, in the second study, supplementation with iron and either of the vitamin C concentrations studied increased the plasma ascorbate concentrations in both supplementation subgroups at 6 wk and 12 wk (Table 2). Further, as observed with the transferrin-bound iron, there was an inverse relationship between existing plasma levels and rises in plasma concentration after supplementation. For example, in the case of subjects supplemented with 60 mg/d vitamin C + iron (over both studies), there was a significant correlation between the initial plasma vitamin C concentration and the change in concentration after 6 wk supplementation, r = −0.85 (n = 19, $p < 0.05$). The initial plasma vitamin C intercept was 73.5 μmol/l and the slope −1.02. Further, although the plasma ascorbate levels rose with both levels of vitamin C supplementation in this second study, the total DNA base damage decreased at both 6 and 12 weeks of supplementation (Table 2).

DISCUSSION

The results of these studies suggest that in well-nourished subjects with an above average initial level

TABLE 2

Co-supplementation of Healthy Human Volunteers with a Low Initial Plasma Vitamin C Concentration with Iron and Vitamin C: Effects on DNA Base Damage

DNA base damage product	Supplement A (nmol base/mgDNA)			Supplement B (nmol base/mgDNA)		
	0 wk†	6 wk‡	12 wk‡	0 wk†	6 wk‡	12 wk†
5-Cl uracil	0.35	0.10 ± 0.05	0.09 ± 0.04	0.35	0.12 ± 0.04	0.06
5-OH uracil	0.38	0.12 ± 0.05	0.10 ± 0.04	0.41	0.13 ± 0.05	0.07
5-OH Me uracil	0.06	0.02 ± 0.005	0.01 ± 0.005	0.06	0.02 ± 0.01	0.01
5-OH Me hydantoin	0.18	0.07 ± 0.01	0.08 ± 0.03	0.30	0.13 ± 0.02	0.07
5-OH hydantoin	0.14	0.03 ± 0.21	0.04 ± 0.01	0.17	0.05 ± 0.02	0.08
5-OH cytosine	0.13	0.08 ± 0.03	0.06 ± 0.007	0.20	0.10 ± 0.04	0.07
Thymine glycol (cis)	0.52	0.19 ± 0.03	0.17 ± 0.10	0.36	0.20 ± 0.02	0.24
Thymine glycol (trans)	ND	0.006 ± 0.004	0.004 ± 0.003	ND	0.003 ± 0.002	ND
FAPY adenine	ND	0.18 ± 0.05	0.12 ± 0.03	0.48	0.15 ± 0.01	0.06
8-OH adenine	0.37	0.12 ± 0.01	0.13 ± 0.05	0.47	0.20 ± 0.11	0.14
2-OH adenine	0.14	0.09 ± 0.05	0.06 ± 0.007	0.21	0.07 ± 0.003	0.04
FAPY guanine	1.2	0.43 ± 0.33	0.51 ± 0.03	3.5	0.73 ± 0.34	0.48
8-OH guanine	0.40	0.17 ± 0.03	0.16 ± 0.07	0.52	0.25 ± 0.14	0.15
Total base damage	3.9	1.6 ± 0.14	1.5 ± 0.14	7.0	2.2 ± 0.79	1.5
‡Plasma vit. C μmol/l	53.6 ± 29.9	80.0 ± 10.4*	76.0 ± 5.0*	47.2 ± 22.3	104.4 ± 33.7*	84.2 ± 11.91*
‡%Sat. transferrin	23.6 ± 10.7	31.4 ± 14.4	39.1 ± 9.4*	26.0 ± 8.0	40.5 ± 15.7*	30.2 ± 8.6

Note. Mean values ± SD shown; † n = 1-3 pooled data; ‡ n=9. * statistically significantly different from 0 wk, $P<0.05$ paired t test. ND, not detected. Vit., vitamin. %Sat., %saturation. Supplementation: iron 14 mg/d together with either 60mg/d vitamin C (Supplement A subgroup) or 260 mg/d vitamin C (Supplement B subgroup). GC/MS analysis was carried out as in Refs. 19, 20.

of plasma ascorbate, supplementation with iron and vitamin C produces a transient rise in white cell oxidative DNA damage which reverts to normal levels with continued supplementation.

The extent of changes in the total ascorbate levels in the plasma over both studies was affected by the initial plasma concentration of the subject, i.e. the lower the initial plasma concentration of vitamin C, the larger the increase. This has been observed in previous human studies with vitamin C supplementation. For example, Calzada et al., [14] reported a level of 81.8±4.2 μmol/l with a supplementation of 250 mg/d for 8 wk, and vitamin C supplementation of 900 mg/d vitamin C for 13 wks has been reported as producing an increase from 66 μM plasma vitamin C to only 84 μM [23], suggesting homeostatic regulation at these concentrations irrespective of the size of the supplement [discussed in 14]. Other studies have shown that the effects of ascorbate on oxidative DNA damage may also depend on initial nutritional status. For example Fraga et al. [6], found that elevated 8-OHdG levels in sperm DNA from scorbutic subjects could be normalized by 60 mg/day of vitamin C, and 250 mg/day gave no further decrease. The 8-OHG data reported here seem consistent with this (see Table 2).

The measurements of oxidative DNA damage using GC/MS, with precautions to avoid any artifactual oxidation of DNA bases [19] also revealed no evidence for any long-term pro-oxidant effect of combined ascorbic acid and iron supplementation on the total levels of oxidized DNA bases. Nevertheless, both supplementation regimens in subjects with high basal vitamin C produced a rise at 6 weeks, due only to increases in levels of certain base products. Levels of 5Cl-U went down; this base is a marker of attack by HOCl or other reactive chlorine species [24], for which ascorbate is an excellent scavenger [25]. Measurements only of 8-OH guanine, frequently used as an index of oxidative DNA damage, would not have revealed this change, an illustration of the value of GC/MS methodology [20]. Changes in various DNA damage products can occur as a result of changes in the redox state of a cell which can affect the pathway followed by different initial products of free radical attack on the DNA bases [e.g., ref. 20]. Our data show a rise in total oxidative DNA damage at week 6, which normalised by week 12. The rise in certain base products at week 6 perhaps leads to adaptations in the cell that protect the DNA, e.g., increases in levels of repair enzymes or better sequestration of iron for example into ferritin, so that it is no longer pro-oxidant [26]. These findings for DNA damage contrast with those for lipid and protein damage reported by Berger et al. [13] showing that in iron overloaded pre-term infants, ascorbate acts as an antioxidant and bleomycin-detectable iron does not cause oxidative damage. Further, other studies using tissue culture techniques found no pro-oxidant effects of iron and ascorbate co-supplementation on fibroblast growth [27]

and using an animal model [28] found no effects on the susceptibility of liver microsomes to peroxidation.

Since oxidative DNA damage may be a risk factor in the development of cancer, and the use of the type of supplements described here is widespread, the transient rise in oxidative DNA damage at week 6 needs further investigation. How crucial is the state of nourishment? Is the effect due to iron, ascorbate or them both in combination? Could these supplements be harmful because of the mutagenicity and potential carcinogenicity of some of these DNA base damage lesions [29-33], or alternatively, could they be advantageous by stimulating a low level pro-oxidant-induced up-regulation of DNA repair systems?

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