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ORIGINAL ARTICLE

Vitamin C pharmacokinetics of plain and slow release formulations in smokers

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Summary *Background & aims*: Combination of the antioxidants ascorbic acid in slow release formulation and α-tocopherol can retard the progression of atherosclerosis. In order to determine if differences in formulation could explain some of the different results in the intervention trials we determined selected pharmacokinetics for two different formulations of ascorbic acid together with α-tocopherol.

Methods: Single-blinded, randomised, placebo-controlled intervention study with 48 healthy men, aged 20–65 years, smoking ⩾5 cigarettes/day. Subjects received 250 mg plain release ascorbic acid and 91 mg plain release d-α-tocopheryl acetate, 250 mg slow release ascorbic acid and 91 mg plain release d-α-tocopheryl acetate or placebo twice daily for 4 weeks. A series of blood samples were collected after administration of the first dose and repeated after 4 weeks of supplementation.

Results: The fluctuation of ascorbic acid plasma concentrations decreased significantly ($P = 0.003$) after 4 weeks supplementation in the slow versus the plain release group.

Conclusions: This study shows that there were pharmacokinetic differences between plain and slow release formulations of ascorbic acid. However, these effects are small and unlikely to be of significant clinical importance.

Introduction

Vitamin C, or ascorbic acid, cannot be synthesised by the human body.[1] Man therefore depends on dietary intake to maintain vital biological functions dependent on ascorbic acid. While it is well accepted that ascorbic acid intake in doses of about 20–90 mg per day are sufficient to prevent scurvy,[2,3] considerable controversy exists about the effects of so-called mega doses, i.e. ''gram'' doses, on the major diseases in the western society: Cancer and atherosclerosis. A few small focused trials[4–6] and larger preventive trials[7] of up to a few years duration have shown benefits from antioxidant supplementation, e.g. reduction of the atherosclerotic progression. These positive findings from intervention studies are at variance with larger and longer preventive trials where it was not

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possible to find positive effects of ascorbic acid in combination with vitamin E and β-carotene.[8–15]

Recently, the antioxidant supplementation in atherosclerosis prevention (ASAP) study showed a beneficial effect on atherosclerotic progression of ascorbic acid and α-tocopherol in combination using a slow release formulation of ascorbic acid.[4,5] The significant effect as shown by a reduced progression of atherosclerosis was only seen for the smoking male and no effect was found in female smokers as well as non-smokers of both genders. Furthermore the ASAP study did not show any effect with ascorbic acid or α-tocopherol individually. The rationale for using a slow formulation of ascorbic acid was to reduce fluctuations in the plasma concentrations of ascorbic acid. Earlier studies had indicated that this was achieved after a single dose.[16] However, more detailed pharmacokinetic studies were not conducted and little is known about the basic pharmacokinetics of ascorbic acid in plain versus slow release formulation. The bioavailability of ascorbic acid in non-smoking men has been found to be dose-dependent and doses exceeding 500 mg are largely excreted or not absorbed.[17] Another pharmacokinetic study did not find any significant effect of smoking on the bioavailability, maximum plasma concentration or elimination for erythorbic acid, a stereo isomer of ascorbic acid.[18]

To further investigate the mechanism that retarded the progression of atherosclerosis in the ASAP study, we have studied the possible pharmacokinetic differences between plain and slow release ascorbic acid in smoking men and expanded these studies to the steady state situation following 4 weeks of supplementation.

Materials and methods

Subjects

Healthy men 20–65 years of age and smoking five or more cigarettes per day were recruited from the Copenhagen area by newspaper advertisements. Exclusion criteria were uncontrolled hypertension (diastolic blood pressure >105 mmHg), abnormal weight (body mass index $<20\,kg\,m^{-2}$ or $>30\,kg\,m^{-2}$), insulin dependent type 1 diabetes, simultaneous participation in other clinical trials, any condition limiting mobility, severe disease shortening life expectancy or other disease or condition worsening the adherence to the measurements or treatment. Although no restrictions regarding ethnicity were made, the study population was exclusively Caucasian. A written informed consent was obtained from all subjects. The Independent Ethic Committee of the City of Copenhagen approved the study protocol and the study was conducted according to the Declaration of Helsinki II and the guidelines of GCP with the exception of external monitoring, as it was a single site, small investigator initiated study. Sixty-six men completed an in-house screening and 50 of these men completed the first trial visit at the Department of Clinical Pharmacology, Copenhagen University Hospital in accordance with the study protocol. Four weeks later 48 subjects completed the second visit. Two volunteers dropped out because of lack of time from newly acquired jobs.

Design

The study was a single-blinded (study participants only), single-site, randomised, placebo-controlled intervention study with three treatment groups. The supplementation comprised tablets of (A) 250 mg plain release ascorbic acid and 91 mg plain release d-α-tocopheryl (as acetate corresponding to 100 mg), (B) 250 mg slow release ascorbic acid and 91 mg plain release d-α-tocopheryl (CellaVie®) or (C) identical placebo tablets. Two tablets were consumed daily, one with the morning meal and one with the evening meal. The trial period was 4 weeks, where steady state for ascorbic acid[2,19–21] and α-tocopherol is achieved.[22,23] The supplements were generously donated by Ferrosan A/S (Denmark) and contained by analysis 267 mg ascorbic acid and 104 mg d-α-tocopheryl acetate. The doses of the supplements were identical to the doses used in the ASAP study.[4,5] Subjects were advised to stop supplement of vitamins and natural remedies containing antioxidants as soon as a written informed consent was obtained at the screening visit. Compliance with trial treatment was calculated by counting any remaining tablets at the second trial visit.

Pharmacokinetic trial

Twenty subjects from each supplement group were invited to stay for a 12 h pharmacokinetic study to test the absorption and distribution of ascorbic acid and α-tocopherol. The first blood sample (baseline) was drawn after overnight fasting. After administration of the supplement, twelve blood samples were drawn over the next 12 h with 45 min interval between the first nine samples and prolonged time interval for the last drawn blood samples. A diet with negligible ascorbic acid content was provided

during the pharmacokinetic sampling period. The pharmacokinetic study was repeated after a 4-week supplementation period with plain or slow release formulation. Nineteen subjects completed the second trial visit from each supplement group. To determine a possible seasonal variation, a placebo group consisting of ten subjects was included. We have previously shown that plasma ascorbic acid does not fluctuate among the placebo group[18] and consequently no pharmacokinetic evaluation was conducted for this group.

Measurements of antioxidants

Blood samples were drawn from an indwelling venflon catheter in a forearm vein into pre-cooled evacuated tubes containing 5 ml anticoagulant Li-heparin (Beckton Dickinson). The vacutainer was immediately centrifuged for two minutes at $2000 \times g$ (4°C). All standards were from Fluka (Milwaukee, IL).

Aliquot (500 μl) of plasma was immediately stabilized with an equal amount cold (10%) *meta*-phosphoric acid containing 2 mM EDTA. The precipitate was removed by centrifugation (1 min at $16{,}000 \times g$). The supernatant was stored at −80°C for less than one month until analysis. It has previously been shown that ascorbic acid is stable under these conditions.[24] Total plasma ascorbic acid (i.e. ascorbic acid + dehydroascorbic acid) was separated by reverse-phase ion-pairing high-performance liquid chromatography (HPLC) and quantified by coulometric detection as described elsewhere.[25] Four observations were missing and were replaced after the following principle: if the first observation was missing; it was replaced by the last observation. Other missing observations were replaced by the preceding observation.

α-Tocopherol and γ-tocopherol were measured as described previously.[26] The conditions for the reversed-phase HPLC assay with electrochemical detection were specifically optimised for the separation of α- and γ-tocopherol.[27] The concentrations reported for γ-tocopherol also include those for β-tocopherol, which does not separate from the former under reverse-phase conditions but represents less than 10% of the two forms in blood plasma.[28]

Statistical methods

Pharmacokinetic parameters were determined by using WinNonlin software version 2.1 (Scientific Consulting, Inc.). A non-compartmental model type was used in combination with a linear/log trapezoidal calculation method. The area under the plasma concentration curve (AUC) was calculated from zero to the last observation. Data were further analysed by using Statistica version 6 (StatSoft, Tulsa, OK). Differences between plain release, slow release and placebo groups were analysed with one-way ANOVA. *t*-Test for independent samples by groups was used to examine the difference between the plain and slow release formulations while effects of supplementations tested by using the *t*-test for dependent samples by groups. A two-tailed P value <0.05 was considered statistically significant.

Results

Ascorbic acid

The study start characteristics of the population are summarized in Table 1. There were no significant differences in the subjects' characteristics between the groups, except for lower compliance (tablet count) in the placebo group ($P<0.001$). When comparing compliance for the plain and slow release groups, no significant differences were observed.

Changes in ascorbic acid plasma concentrations are illustrated in Fig. 1 and Table 2. At study start, no significant differences in plasma ascorbic acid concentrations were observed between groups. After ingestion of a plain release tablet plasma ascorbic acid concentration increased 66% ($P<0.001$) to a maximum concentration of 74.2 ± 24.3 μmol/l at 4.0 h (T_{max}). T_{max} for the slow release group was 5.0 h and the plasma concentration increased 38% ($P<0.001$) to reach maximum concentration (C_{max}) corresponding to 70.2 ± 18.3 μmol/l ($P<0.05$). No significant difference was observed in the span (maximum concentration subtracted the minimum concentration) between the groups. Furthermore, there was no difference in AUC between the groups.

After 4 weeks of supplementation, plasma ascorbic acid C_{max} reached 76.9 ± 17.3 and 82.4 ± 14.6 μmol/l for plain and slow release group, respectively. The plasma concentration in the placebo group decreased to 39.0 ± 17.9 μmol/l. There was a significant difference ($P<0.001$) in the trough ascorbic acid concentration between the groups receiving supplements and placebo, while no difference was found between the plain and slow release groups. At the second visit, the ascorbic acid concentration increased more moderately; 26% ($P<0.001$) (96.9 ± 18.4 μmol/l at T_{max}

Table 1 Characteristics of subjects. Characteristics of the subjects for the plain release, slow release and the placebo groups.

Characteristic	Plain release ($n = 19$)		Slow release ($n = 19$)		Placebo ($n = 10$)	
	Mean	SD	Mean	SD	Mean	SD
Age (yr)	39	12.7	40	12.8	36	10.6
Height (m)	1.79	0.066	1.79	0.072	1.80	0.096
Weight (kg)	76	9.9	75	10.6	79	16.1
Body mass index (kg m^2)	24	2.6	24	3.1	24	2.8
SBP (mmHg)	137	12.9	135	20.1	139	11.1
DBP (mmHg)	83	10.8	83	12.1	90	10.6
Smoking (cigarettes/d)	18	7.3	17	9.7	18	6.9
Pack year (yr, smoking 1 pack/d)	18	14.1	15	13.3	18	16.3
Alcohol (drinks/wk)	11	13.5	12	10.8	17	7.8
Compliance (tablet)	89.5***	12.3	93.8***	10.8	60.8***	9.4
Wash out period (d)	30	13.7	33	15.6	30	6.4
Trial period (d)	27	1.7	28	1.5	28	0.52

***$P < 0.001$ by one-way ANOVA test for plain release vs. slow release vs. placebo.

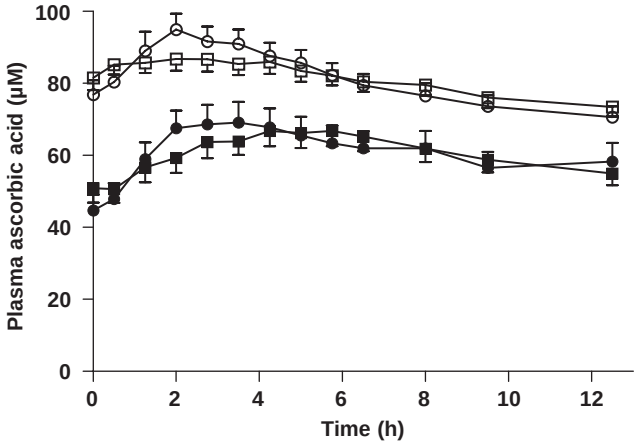

Figure 1 Plasma ascorbic acid concentration. Changes in plasma ascorbic acid concentration after intake of one tablet of plain release ascorbic acid (250 mg) and plain release α-tocopherol (91 mg) (plain release) or slow release ascorbic acid (250 mg) and plain release α-tocopherol (91 mg) (slow release) at study start (closed circles and squares, respectively) and after 4 weeks of supplementation (open circles and squares, respectively). Error bars are SEM.

2.1 h; plain release) and 11% ($P < 0.001$) (91.6 $\pm$ 13.6 μmol/l at T_{max} of 2.4 h; slow release), respectively. The C_{max} was higher in the plain compared to the slow release group and a significant difference for the span between the two groups was observed ($P < 0.005$).

Effect of supplementation

Four weeks of supplementation resulted in significantly increased plasma concentrations of ascorbic acid for both plain and slow release formulations ($P < 0.001$; Table 2). The ascorbic acid concentration decreased 25% in the placebo group ($P < 0.001$). A decrease in T_{max} of about 30% could be observed in both the plain and slow release groups ($P < 0.01$). Moreover, AUC increased 66% and 42% in the plain and slow release groups, respectively, after 4 weeks of supplementation ($P < 0.001$). The maximum ascorbic acid plasma concentration (C_{max}) also increased significantly in both supplemented groups ($P < 0.01$). There was no significant change in the span from study start to after the 4 weeks supplementation period for the two active treatment groups.

Table 2 Plasma concentrations at study start and after 4 weeks. Plasma concentration of ascorbic acid, α-tocopherol and γ-tocopherol for the plain release, slow release and the placebo group.

	Plain release ($n = 19$)		Slow release ($n = 19$)		Placebo ($n = 10$)		P (t-test for independent samples)	P (one-way ANOVA)
	Conc. (μM)	SD	Conc. (μM)	SD	Conc. (μM)	SD	Plain vs. slow	Plain vs. slow vs. placebo
Study start								
Ascorbic acid	44.7	18.6	50.9	17.7	52.8	16.1	0.303	0.419
α-Tocopherol	31.1	10.5	30.2	10.3	31.3	7.2	0.797	0.945
γ-Tocopherol	1.91	0.95	1.63	0.66	1.61	0.60	0.295	0.462
α-/γ-Tocopherol	18.3	7.0	21.2	8.4	21.9	9.3	0.282	0.435
Ascorbic acid/α-tocopherol	1.60	0.85	1.83	0.78	1.78	0.69	0.380	0.636
4-weeks								
Ascorbic acid	76.9***	17.3	82.4***	14.6	39.0*	17.9	0.295	0.000
α-Tocopherol	38.3**	8.5	36.1**	9.3	23.8***	6.0	0.435	0.000
γ-Tocopherol	0.56***	0.37	0.31***	0.26	0.90***	0.36	0.018	0.000
α-/γ-Tocopherol	104.7***	82.7	210.0***	160.8	30.2*	11.9	0.013	0.000

*$P<0.05$; **$P<0.01$; ***$P<0.001$ by t-test for dependent samples for study start vs. 4-weeks.

Tocopherol

When analysing the plasma α-tocopherol concentration for the first ten subjects (six subjects receiving plain release and four receiving slow release), no absorption, distribution or elimination could be observed within the 12 h of blood sampling from either visits (data not shown). Therefore, it was decided to analyse only the baseline samples of all subjects.

The mean study start concentrations of α-tocopherol and γ-tocopherol were similar in all groups. After 4 weeks of supplementation, α-tocopherol concentration increased 32% (plain release) and 25% (slow release) ($P<0.001$). During the same period the concentration in the placebo group decreased 24% ($P<0.001$). In contrast, the γ-tocopherol concentration decreased 69% after 4 weeks of supplementation with plain release tablets, 81% for subjects receiving slow release tablets and 42% for controls. The γ-tocopherol concentration was significant different for the plain release versus slow release group ($P<0.01$). After 4 weeks of supplementation, a several-fold increase in the α-/γ-tocopherol plasma ratio was found in both supplemented groups, while a smaller increase was observed in the placebo group.

Plasma ratios for ascorbic acid and α-tocopherol were also examined (data not shown). Neither at study start nor after 4 weeks of supplementation a significant difference was observed for the plain and slow release groups versus placebo. There was no difference between the two supplemented groups.

Discussion

The possible beneficial effects of antioxidant supplementation continue to be subject to much debate. Most preventive trials and a meta-analysis of randomised trials have not found a benefit of antioxidant supplementation,[8–15] while a few smaller and more focused trials investigating people with pre-existing vascular disease such as the ASAP study[4,5] have found significant beneficial effects. In most preventive trials where ascorbic acid was used as supplement, ascorbic acid was formulated as plain release. In contrast, the ASAP study used ascorbic acid in a slow release formulation. Thus, we wanted to investigate if the above discrepancy could relate to pharmacokinetic differences from different formulations of ascorbic acid.

In the present study, we examined the pharmacokinetics of the vitamin supplement used in the ASAP study (slow release formulation) and compared them with those of a similar plain release supplement and placebo. A larger span in plasma ascorbic acid concentration for the plain versus slow release group was expected.

After 4 weeks of supplementation with the slow release formulation, fluctuations in plasma ascorbic acid were below those found in the plain

Table 3 Pharmacokinetic parameters at study start and after 4 weeks. Pharmacokinetics at study start and after 4 weeks of supplementation with plain or slow release tablet.

	Plain release ($n = 19$)			Slow release ($n = 19$)			P (t-test for independent samples)
	Mean	SD	n	Mean	SD	n	Plain vs. slow
Study start							
T_{max} (h)	4.0	2.7	19	5.0	2.5	18	0.234
AUC (h μmol/l)	761	255	19	755	200	19	0.939
C_{max} (μmol/l)	74.2	24.3	19	70.2	18.3	19	0.570
$T1/2$ (h)	24.4	9.0	17	61.8	128.2	19	0.239
Span (μmol/l)	30.2	11.0	19	25.0	9.7	18	0.133
4-weeks							
T_{max} (h)	2.1*	0.6	19	2.4*	1.6	16	0.525
AUC (h μmol/l)	1012***	193	19	1010***	147	19	0.966
C_{max} (μmol/l)	96.9**	18.4	19	91.6***	13.6	19	0.320
$T1/2$ (h)	29.4	19.8	19	34.2	18.4	17	0.650
Span (μmol/l)	28.3	9.4	19	21.4	6.4	19	0.003

*$P<0.05$; **$P<0.01$; ***$P<0.001$ by t-test for dependent samples for study start vs. 4-weeks.

release group. This finding is in agreement with an earlier smaller study.[16] However, we found no difference in T_{max} for the plain release group versus the slow release group after intake of the first tablet. After 4 weeks of supplementation a steady state was reached[23,29] and T_{max} decreased approximately 30% in both supplemented groups compared to study start. Such findings have been reported earlier,[16,29] but no previous reported mechanism for the T_{max} decrease has been suggested. During the 12 h pharmacokinetic studies, the AUC and C_{max} values of ascorbic acid did not differ significantly between the two supplemented groups, neither at study start nor after 4 weeks of supplementation (Table 3).

This coherence was also found by Nyyssonen et al.,[16] while disagreement has been expressed in the area with respect to pharmacokinetic differences for the slow versus plain release formulation. One study observed higher AUC and C_{max} values after the first dose in film-coated tablets compared with an enteric-coated pellet preparation, while at steady state the AUC and C_{max} values were higher in the enteric-coated pellet preparation.[30] In agreement, other studies found a higher AUC in slow release tablets versus plain release tablets,[29,31] but higher AUC for plain release forms versus slow release forms have also been observed.[32]

As expected, 4 weeks supplementation with ascorbic acid and α-tocopheryl acetate increased the plasma concentrations of ascorbic acid and α-tocopherol significantly. In contrast, decreased ascorbic acid and α-tocopherol plasma concentrations were observed for the placebo group. Due to a general misconception, subjects commonly avoided fruits and vegetables rather than continuing with their normal diet as planned. This phenomenon was presumably equally distributed among all groups. Previous work showed that α- and γ-tocopherol are taken up without preference by the intestine and secreted in the chylomicron particles.[33,34] The chylomicron remnants, which are not transported and transferred to peripheral tissues,[35] are subsequently taken up by the liver. The liver has a preference for incorporating RRR-α-tocopherol into lipoproteins in plasma through the specific α-tocopherol transfer protein,[34,36–38] which decreases the incorporation of γ-tocopherol into lipoproteins and increases the excretion of γ-tocopherol through the liver. In the present study, we also found that the plasma concentration of γ-tocopherol decreased more for subjects receiving the slow release dosage form of ascorbic acid compared with the plain release.

In conclusion, we found significant differences in fluctuation of plasma ascorbic acid when supplemented as a plain or slow release formulation, respectively, in combination with α-tocopherol. However, the authors find it unlikely that the relative small differences observed between these two formulations be of major clinical importance.

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References

1. Nishikimi M, Yagi K. Molecular basis for the deficiency in humans of gulonolactone oxidase, a key enzyme for ascorbic acid biosynthesis. *Am J Clin Nutr* 1991;**54**:1203S–8S.
2. Hornig D. Metabolism and requirements of ascorbic acid in man. *S Afr Med J* 1981;**60**:818–23.
3. Hodges RE, Baker EM, Hood J, Sauberlich HE, March SC. Experimental scurvy in man. *Am J Clin Nutr* 1969;**22**: 535–48.
4. Salonen JT, Nyyssonen K, Salonen R, et al. Antioxidant supplementation in atherosclerosis prevention (ASAP) study: a randomized trial of the effect of vitamins E and C on 3-year progression of carotid atherosclerosis. *J Intern Med* 2000;**248**:377–86.
5. Salonen RM, Nyyssonen K, Kaikkonen J, et al. Six-year effect of combined vitamin C and E supplementation on atherosclerotic progression: the antioxidant supplementation in atherosclerosis prevention (ASAP) study. *Circulation* 2003;**107**:947–53.
6. Fang JC, Kinlay S, Beltrame J, et al. Effect of Vitamins C and E on progression of transplant-associated arteriosclerosis: a randomised trial. *Lancet* 2002;**359**:1108–13.
7. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ. randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 1996;**347**:781–6.
8. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet* 2003;**361**:2017–23.
9. Heart Protection Study Collaborative Group. MRC/BHF heart protection study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002; **360**: 23–33.
10. Blot WJ, Li JY, Taylor PR, et al. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst* 1993;**85**:1483–92.
11. The Alpha-tocopherol and Beta-carotene Cancer Prevention Group. The effect of vitamin E, Beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-tocopherol, Beta carotene Cancer Prevention Study Group. *N Engl J Med* 1994; **330**: 1029–35.
12. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P. Vitamin E supplementation and cardiovascular events in high-risk patients. The heart outcomes prevention evaluation study investigators. *N Engl J Med* 2000;**342**:154–60.
13. GISSI-prevenzione Investigators. Dietary supplementation with N-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano Per Lo Studio Della Sopravvivenza Nell'Infarto Miocardico. *Lancet* 1999; **354**: 447–55.
14. de Gaetano G. Low-dose aspirin and vitamin E in people at cardiovascular risk: a randomised trial in general practice. Collaborative Group of the Primary Prevention Project. *Lancet* 2001;**357**:89–95.
15. Waters DD, Alderman EL, Hsia J, et al. Effects of hormone replacement therapy and antioxidant vitamin supplements on coronary atherosclerosis in postmenopausal women: a randomized controlled trial. *J Am Med Assoc* 2002;**288**:2432–40.
16. Nyyssonen K, Poulsen HE, Hayn M, et al. Effect of supplementation of smoking men with plain or slow release ascorbic acid on lipoprotein oxidation. *Eur J Clin Nutr* 1997;**51**:154–63.
17. Levine M, Conry-Cantilena C, Wang Y, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc Natl Acad Sci USA* 1996;**93**:3704–9.
18. Lykkesfeldt J, Bolbjerg ML, Poulsen HE. Effect of smoking on erythorbic acid pharmacokinetics. *Br J Nutr* 2003;**89**: 667–71.
19. Mangels AR, Block G, Frey CM, et al. The bioavailability to humans of ascorbic acid from oranges, orange juice and cooked broccoli is similar to that of synthetic ascorbic acid. *J Nutr* 1993;**123**:1054–61.
20. Jacob RA, Omaye ST, Skala JH, Leggott PJ, Rothman DL, Murray PA. Experimental vitamin C depletion and supplementation in young men. Nutrient interactions and dental health effects. *Ann N Y Acad Sci* 1987;**498**:333–46.
21. Jacob RA, Pianalto FS, Agee RE. Cellular ascorbate depletion in healthy men. *J Nutr* 1992;**122**:1111–8.
22. Traber MG, Winklhofer-Roob BM, Roob JM, et al. Vitamin E kinetics in smokers and nonsmokers. *Free Radical Biol Med* 2001;**31**:1368–74.
23. Baker H, Handelman GJ, Short S, et al. Comparison of plasma alpha and gamma tocopherol levels following chronic oral administration of either all-rac-alpha-tocopheryl acetate or RRR-alpha-tocopheryl acetate in normal adult male subjects. *Am J Clin Nutr* 1986;**43**:382–7.
24. Lykkesfeldt J, Loft S, Poulsen HE. Determination of ascorbic acid and dehydroascorbic acid in plasma by high-performance liquid chromatography with coulometric detection—are they reliable biomarkers of oxidative stress? *Anal Biochem* 1995;**229**:329–35.
25. Lykkesfeldt J. Measurement of ascorbic acid and dehydroascorbic acid in biological samples. In: Maines M, Costa LG, Hodson E, Reed DJ, Sipes IG, editors. *Current protocols in toxicology.* New York: Wiley; 2002. p. 7.6.1.
26. Sattler W, Mohr D, Stocker R. Rapid isolation of lipoproteins and assessment of their peroxidation by high-performance liquid chromatography postcolumn chemiluminescence. *Methods Enzymol* 1994;**233**:469–89.
27. Christen S, Woodall AA, Shigenaga MK, Southwell-Keely PT, Duncan MW, Ames BN. Gamma-tocopherol traps mutagenic electrophiles such as NO(X) and complements Alpha-tocopherol: physiological implications. *Proc Natl Acad Sci USA* 1997;**94**:3217–22.
28. Handelman GJ, Machlin LJ, Fitch K, Weiter JJ, Dratz EA. Oral alpha-tocopherol supplements decrease plasma gamma-tocopherol levels in humans. *J Nutr* 1985;**115**:807–13.
29. Zetler G, Seidel G, Siegers C-P, Iven H. Pharmacokinetics of ascorbic acid in man. *Eur J Clin Pharmacol* 1976;**10**: 273–82.
30. Vidgren M, Kumpusalo E, Silvasti M, Mykkanen M, Parviainen M. Absorption of ascorbic acid from a film-coated tablet and from a new enteric-coated pellet preparation in subjects with inadequate plasma levels of ascorbic acid. *Arzneimittelforschung* 1992;**42**:143–6.
31. Siegers CP, Seidel G, Iven H. Absorption of vitamin C from a delayed-action preparation. *Med Welt* 1975;**26**:206–9.
32. Yung S, Mayersohn M, Robinson JB. Ascorbic acid absorption in humans: a comparison among several dosage forms. *J Pharm Sci* 1982;**71**:282–5.

33. Kayden HJ, Traber MG. Absorption, lipoprotein transport, and regulation of plasma concentrations of vitamin E in humans. *J Lipid Res* 1993;**34**:343–58.
34. Traber MG, Burton GW, Hughes L, et al. Discrimination between forms of vitamin E by humans with and without genetic abnormalities of lipoprotein metabolism. *J Lipid Res* 1992;**33**:1171–82.
35. Traber MG, Olivecrona T, Kayden HJ. Bovine milk lipoprotein lipase transfers tocopherol to human fibroblasts during triglyceride hydrolysis in vitro. *J Clin Invest* 1985;**75**:1729–34.
36. Traber MG, Kayden HJ. Preferential incorporation of alpha-tocopherol vs gamma-tocopherol in human lipoproteins. *Am J Clin Nutr* 1989;**49**:517–26.
37. Arita M, Sato Y, Miyata A, et al. Human alpha-tocopherol transfer protein: CDNA cloning, expression and chromosomal localization. *Biochem J* 1995;**306**:437–43.
38. Hosomi A, Arita M, Sato Y, et al. Affinity for alpha-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. *FEBS Lett* 1997;**409**:105–8.