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Plasma concentrations of ascorbic acid and C-reactive protein, and risk of future coronary artery disease, in apparently healthy men and women: the EPIC-Norfolk prospective population study

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High plasma concentrations of ascorbic acid, a marker of fruit and vegetable intake, are associated with low risk of coronary artery disease. Whether this relationship is explained by a reduction in systemic inflammation is unclear. We investigated the relationship between ascorbic acid plasma concentration and coronary artery disease risk, and in addition whether this relationship depended on classical risk factors and C-reactive protein (CRP) concentration. We used a prospective nested case–control design. The study consisted of 979 cases and 1794 controls (1767 men and 1006 women). Increasing ascorbic acid quartiles were associated with lower age, BMI, systolic and diastolic blood pressure, and CRP concentration, but with higher HDL-cholesterol concentration. No associations existed between ascorbic acid concentration and total cholesterol concentration or LDL-cholesterol concentration. When data from men and women were pooled, the risk estimates decreased with increasing ascorbic acid quartiles such that people in the highest ascorbic acid quartile had an odds ratio for future coronary artery disease of 0·67 (95 % CI 0·52, 0·87) compared with those in the lowest quartile (*P* for linearity=0·001). This relationship was independent of sex, age, diabetes, smoking, BMI, LDL-cholesterol, HDL-cholesterol, systolic blood pressure and CRP level. These data suggest that the risk reduction associated with higher ascorbic acid plasma concentrations, a marker of fruit and vegetable intake, is independent of classical risk factors and also independent of CRP concentration.

Ascordic acid: Vitamin C: Oxidation: C-reactive protein: Inflammation: Coronary artery disease

The oxidation of LDL particles is a key event in atherosclerosis (Binder *et al.* 2002). L-Ascorbic acid (vitamin C) is a plasma antioxidant capable of scavenging free radicals and is the first-line defence in the control of the redox state, sparing other endogenous antioxidants from consumption (Frei *et al.* 1989; Jialal & Grundy, 1991). High plasma concentrations of ascorbic acid not only correlate with lower concentrations of oxidised LDL (Carr *et al.* 2000), but also protect endothelial cells against the detrimental effects of oxidised LDL once this has formed (Lehr *et al.* 1995; Siow *et al.* 1998, 1999). High concentrations of ascorbic acid are associated with a high intake of fruit and vegetables, whereas low concentrations are associated with cardiovascular risk factors such as smoking (Dietrich *et al.* 2003), diabetes (Sargeant *et al.* 2000), hyperlipidaemia (Ness *et al.* 1996*a*), hypertension (Ness *et al.* 1996*b*) and high plasma concentrations of C-reactive protein (CRP; Langlois *et al.* 2001; Ford *et al.* 2003).

Physiological plasma concentrations of ascorbic acid have been reported to be inversely related to the risk of cardiovascular mortality (Sahyoun *et al.* 1996; Khaw *et al.* 2001), even after adjustment for traditional cardiovascular risk factors. In contrast, randomised trials of supplementation with antioxidants including ascorbic acid showed no effect on systemic inflammation (Bruunsgaard *et al.* 2003) or on the risk of cardiovascular events (Heart Protection Study Collaborative Group, 2002). This discrepancy may reflect differing effects of ascorbic acid at different doses. Alternatively, ascorbic acid levels may not be part of the causal atherogenic pathway but rather a marker of the atherosclerotic process or of other behavioural factors such as a healthy diet or physical activity. Thus, it is unclear whether low ascorbic acid concentrations are a cause or a result of the inflammatory atherosclerotic disease process, but both may be the case. Whether the relationship between high antioxidant protection, of which ascorbic

Abbreviations: CAD, coronary artery disease; CRP, C-reactive protein; EPIC, European Prospective Investigation into Cancer and Nutrition; HDL-c, HDL-cholesterol; LDL-c, LDL-cholesterol; NHANES, National Health and Nutrition Examination; OR, odds ratio.

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acid concentration may be an indicator, and lower risk of coronary artery disease (CAD) is mediated by lower levels of systemic inflammation, as reflected by plasma concentrations of CRP, remains unclear.

We hypothesised that the relationship between high plasma concentrations of ascorbic acid and reduced CAD risk might be mediated through lower plasma concentrations of CRP. We tested this hypothesis using a prospective nested case–control design to study the risk of CAD among apparently healthy men and women in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort study.

Methods

We performed a nested case–control study among participants of the EPIC-Norfolk cohort study, a prospective population study of 25 663 men and women aged between 45 and 79 years, resident in Norfolk, UK, who completed a baseline questionnaire survey and attended a clinic visit (Day *et al.* 1999). EPIC-Norfolk is part of a nine-country collaborative study designed to investigate dietary and other determinants of cancer. Additional data were obtained to enable the assessment of determinants of other diseases. The study cohort was closely similar to UK population samples with respect to many characteristics, including anthropometry, blood pressure and lipids, but with a lower proportion of smokers (Day *et al.* 1999).

Participants were recruited from age–sex registers of general practices. At the baseline survey between 1993 and 1997, participants completed a detailed health and lifestyle questionnaire, and additional data collection was performed by trained nurses at a clinic visit as previously described (Day *et al.* 1999). Participants were identified as having CAD during follow-up if they had a hospital admission and/or died with CAD as the underlying cause. All individuals have been electronically marked for detection of mortality at the UK Office of National Statistics, with vital status ascertained for the entire cohort. Death certificates for all decedents were coded by trained nosologists according to the International Classification of Diseases 9th revision (World Health Organization, 1977). In addition, participants admitted to hospital were identified using their unique National Health Service number by data linkage with the East Norfolk Health Authority database, which identifies all hospital contacts throughout England and Wales for Norfolk residents. Cases were people who had CAD defined as ICD codes 410–414 as their cause of death or cause of hospital admission. We report results with follow-up up to January 2003, an average of about 6 years. The study was approved by the Norwich District Health Authority Ethics Committee, and all participants gave signed informed consent.

Participants

For the present analysis, we identified 979 individuals who did not report a history of heart attack or stroke at the baseline clinic visit but who did develop fatal or non-fatal CAD during follow-up. Controls were study participants who remained free of CAD during follow-up; they were matched to a case by sex, age (within 5 years) and date of visit (within 3 months). For 815 cases, we were able to identify two controls, whereas for the remaining 164 cases, one control could be identified.

Biochemical analyses

Non-fasting blood samples were taken by venepuncture into containers with or without citrate. Samples were stored at 4°C and transported the same day to the central study laboratory. Blood samples were processed for assay at the Department of Clinical Biochemistry, University of Cambridge, or stored at −80°C. Serum concentrations of total cholesterol, HDL-cholesterol (HDL-c) and triacylglycerols were measured in fresh plasma samples with the RA 1000 (Bayer Diagnostics, Basingstoke, UK), and LDL-cholesterol (LDL-c) concentrations were calculated with the Friedewald formula (Friedewald *et al.* 1972).

Plasma concentrations of ascorbic acid were measured from blood taken into citrate bottles; plasma was stabilised in a standardised volume of metaphosphoric acid stored at −70°C. We measured ascorbic acid in duplicate with a fluorometric assay within 1 week of sampling (Vuilleumier & Keck, 1989). The CV was 5·6 % at the lower end of the range (mean 33·2 μmol/l) and 4·6 % at the upper end (102·3 μmol/l). Plasma concentrations of CRP were measured on thawed frozen plasma from cases and controls using a validated assay (Bruins *et al.* 1997). Samples were analysed in random order to avoid systemic bias. Researchers and laboratory personnel had no access to identifiable information and could identify samples only by number.

Statistical analysis

Baseline characteristics were compared between cases and controls using a mixed-effect model or conditional logistic regression where appropriate. Because triacylglycerol concentrations and CRP concentrations had a skewed distribution, values were log-transformed before statistical analysis. Proportions or mean concentrations of traditional cardiovascular risk factors were calculated per sex-specific ascorbic acid quartile. Conditional logistic regression analysis was used to calculate odds ratios (OR) and corresponding 95 % CI as an estimate of the relative risk of CAD. Ascorbic acid concentrations were analysed as categorical variables after division into quartiles based on the distribution in the controls, using the lowest quartile as the reference category.

OR were calculated taking into account the matching for age and sex, and were adjusted for the following cardiovascular risk factors: smoking (never, previous, current), systolic blood pressure, diabetes, BMI, LDL-c and HDL-c. OR were also calculated after additional adjustment for log-transformed CRP as a continuous variable, and after additional adjustment for use of vitamin supplements (yes/no). Statistical analyses were performed using SPSS software (version 10.1; SPSS Inc., Chicago, IL, USA). Data are presented as means with their standard deviation, percentages (*n*) or medians and interquartile ranges. A value of $P < 0{\cdot}05$ was considered significant.

Results

Matching ensured that age and sex were not significantly different between cases and controls (Table 1). As expected,

Table 1. Baseline characteristics

(Mean values and standard deviations, percentages (*n*) or medians (interquartile range))

	Controls						Cases						
	Mean	SD	Median	Range	*n*	%	Mean	SD	Median	Range	*n*	%	*P*
Men					1138						629		
Age (years)	64·5	8·0					64·5	8·0					Matched
Smoking													
Current					96	8·4					98	15·6	
Past					677	59·5					674	59·5	<0·0001
Never					365	32·1					157	25·0	
BMI (kg/m^2)	26·2	3·0					27·2	3·5					<0·0001
Total cholesterol (mmol/l)	6·0	1·1					6·3	1·1					<0·0001
LDL cholesterol (mmol/l)	3·9	1·0					4·2	1·0					<0·0001
HDL cholesterol (mmol/l)	1·3	0·3					1·2	0·3					<0·0001
Triacylglycerols (mmol/l)			1·7	1·2–2·3					1·9	1·4–2·7			<0·0001
Systolic blood pressure (mmHg)	139	17					144	19					<0·0001
Diastolic blood pressure (mmHg)	85	11					86	12					0·001
History of diabetes					23	2·0					40	6·4	<0·0001
C-reactive protein (mg/l)			1·4	0·7–2·9					2·1	1·0–4·5			<0·0001
Ascorbic acid (μmol/l)	48·5	18·6					43·1	18·7					<0·0001
Supplement users					442	38·8					239	38	0·4
Women					656						350		
Age (years)	66·5	7·1					66·5	7·1					Matched
Smoking													
Current					51	7·8					53	15·1	
Past					240	36·6					137	39·1	<0·0001
Never					365	55·6					160	45·7	
BMI (kg/m^2)	26·1	3·9					27·3	4·5					<0·0001
Total cholesterol (mmol/l)	6·6	1·1					6·9	1·2					0·001
LDL cholesterol (mmol/l)	4·3	1·1					4·5	1·1					<0·0001
HDL cholesterol (mmol/l)	1·6	0·4					1·5	0·4					<0·0001
Triacylglycerols (mmol/l)			1·5	1·1–2·1					1·8	1·3–2·4			<0·0001
Systolic blood pressure (mmHg)	138	19					143	19					0·001
Diastolic blood pressure (mmHg)	82	11					85	12					<0·0001
History of diabetes					6	0·9					19	5·4	<0·0001
C-reactive protein (mg/l)			1·6	0·7–3·4					2·6	1·1–6·0			<0·0001
Ascorbic acid (μmol/l)	59·3	19·7					52	20·3					<0·0001
Supplement users					332	50·6					159	45·4	0·07

Comparisons between cases and matched controls were by conditional logistic regression for categorical variables, and by mixed-effect model for continuous variables. Concentrations of triacylglycerols and C-reactive protein were log-transformed before analysis, but untransformed data are reported here.

individuals who developed CAD during follow-up were more likely than controls to smoke and have diabetes. Concentrations of total cholesterol, LDL-c, triacylglycerols, systolic and diastolic blood pressure, BMI and CRP were significantly higher in cases than controls, whereas HDL-c concentrations were significantly lower. Ascorbic acid concentrations were higher in controls than cases (men: 48·5 (SD 18·6) μmol/l *v.* 43·1(SD 18·7) μmol, $P<0·0001$; women: 59·3 (SD 19·7) μmol/l *v.* 52·0 (SD 20·3) μmol/l, $P<0·0001$).

For each cardiovascular risk factor analysed, the interaction term between sex and ascorbic acid quartiles was not significant (data not shown). Therefore, the relationships between ascorbic acid quartiles and cardiovascular risk factors were not analysed for sexes separately but only for both sexes combined. Increasing ascorbic acid quartiles were associated with lower age, BMI, systolic and diastolic blood pressure, and CRP concentration but with higher HDL-c concentration (Table 2). No association was observed between ascorbic acid concentration and either total cholesterol concentration or LDL-c concentration. With increasing ascorbic acid quartiles, people were less likely to be smokers and more likely to use vitamin supplements. People in the highest ascorbic acid quartile were less likely to have known diabetes.

The interaction term between sex and ascorbic acid quartiles for the risk of future CAD was not significant (data not shown), suggesting that the relationship between ascorbic acid quartiles and CAD risk was no different between men and women. This relationship was therefore only analysed for both sexes combined (Table 3). The risk estimates decreased with increasing ascorbic acid quartile such that people in the highest ascorbic acid quartile had an OR for future CAD of 0·46 (95 % CI 0·36, 0·58) compared with those in the lowest quartile (P for linearity=0·001). This relationship was attenuated slightly by adjustment for diabetes, smoking (current, former, never), BMI, systolic blood pressure, LDL-c and HDL-c, such that people in the highest ascorbic acid quartile had an OR of 0·64 (95 % CI 0·49, 0·82) compared with those in the lowest category

Table 2. Various cardiovascular risk factors by ascorbic acid quartile

(Mean values and standard deviations, or percentages (*n*))

	1				2				3				4			
Ascorbic acid quartile	*n*	%	Mean	SD	*n*	%	Mean	SD	*n*	%	Mean	SD	*n*	%	Mean	SD	*P*
Cases	390				218				205				166				
Controls	480				412				474				428				
Ascorbic acid (μmol/l)			27·6	9·7			47·3	3·2			58·5	3·7			77·1	11·5	<0·0001
Age years			65·5	7·8			65·3	7·7			65	7·9			65·3	7·6	0·7
Smoking																	
Current	157	18·0			51	8·1			54	8·0			36	6·1			
Past	470	54·0			335	53·2			334	49·2			289	48·7			<0·0001
Never	243	27·9			244	38·7			291	42·9			269	45·3			
BMI (kg/m^2)			27	3·7			27	3·8			26·5	3·4			25·6	3·3	<0·0001
Total cholesterol			6·3	1·1			6·3	1·2			6·3	1·1			6·4	1·2	0·6
LDL cholesterol (mmol/l)			4·1	1·0			4·2	1·1			4·1	1·0			4·1	1·1	0·6
HDL cholesterol (mmol/l)			1·3	0·4			1·3	0·4			1·4	0·4			1·5	0·4	<0·0001
Triglycerides (mmol/l)			2·0	0·9			1·9	0·8			1·8	0·9			1·7	0·8	<0·0001
Systolic blood pressure (mmHg)			143	18			142	18			139	18			138	18	<0·0001
Diastolic blood pressure (mmHg)			86	12			85	12			84	11			82	11	<0·0001
History of diabetes	37	4·3			26	4·1			18	2·7			7	1·2			<0·0001
C-reactive protein (mg/l)			4·8	7·5			4·0	6·1			3·1	5·0			2·4	3·6	<0·0001
Supplement users	281	32.3			237	37.6			315	46.4			339	57.1			<0·0002

Relationships between various cardiovascular risk factors and quartiles of ascorbic acid, based on the distribution between controls. *P* values are for linear trend across ascorbic acid quartiles. Concentrations of triacylglycerols and C-reactive protein were log-transformed before analysis, but untransformed data are reported here.

($P<0·0001$). Additional adjustment for CRP level had only a very slight impact on this relationship (OR = 0·67; 95 % CI 0·52, 0·87; $P=0·001$). Additional adjustment for use of vitamin supplements (yes/no) did not materially change these results. When the analyses were repeated after excluding all vitamin supplement users, the OR were of similar magnitude, as reported above, but CI were wider owing to the substantial reduction in statistical power (data not shown). Regression analyses by combined ascorbic acid and CRP quartile confirmed the independence of the relationships between both plasma markers and CAD risk (Table 4).

Discussion

In the present large, prospective, nested case–control study, we observed that ascorbic acid concentration had a strong positive correlation with HDL-c concentration and a strong negative correlation with most other cardiovascular risk factors, including age, smoking, BMI, systolic and diastolic blood pressure, and CRP concentration. The fully adjusted risk of incident CAD decreased with increasing ascorbic acid quartile, such that people in the highest quartile had an OR of 0·67 (95 % CI 0·52, 0·87) compared with those in the lowest quartile (P for linearity=0·001). Surprisingly, this association between ascorbic acid concentration and CAD risk was entirely independent of classical cardiovascular risk factors and additionally of CRP concentration.

We have previously reported an inverse relationship between ascorbic acid concentration and mortality in the EPIC-Norfolk cohort (Khaw *et al.* 2001). The risk of cardiovascular mortality decreased with increasing ascorbic acid quintile, an observation consistent with the present results, which are based on the incidence of both fatal and non-fatal CAD. Our findings contrast with those of a 12-year follow-up analysis of the second National Health and Nutrition Examination (NHANES II) in US adults (Loria *et al.* 2000). In that study, there was no decreased risk of cardiovascular mortality in any ascorbic acid quartile in men or women.

Although the overall range and mean concentration of plasma concentrations were similar in the NHANES II and EPIC-Norfolk studies, NHANES II was a multicentre study, so it is possible that measurement variation could account for some of the discrepancies. It is also possible that, in the NHANES II cohort and our EPIC-Norfolk cohort, plasma concentrations of ascorbic acid indicate different dietary patterns of fruit and vegetables, which could have different biological effects. Alternatively, the longer follow-up in NHANES II may have caused a greater misclassification of individuals because they had more time to change their ascorbic acid intake during follow-up. Finally, end points may have been misclassified because of an underestimation of mortality in the NHANES study compared with the present study, in which complete mortality was ascertained.

There are several possible explanations for the inverse relation between plasma ascorbic acid concentration and risk of CAD. Ascorbic acid may play a causal role in protection against atherosclerosis. Ascorbic acid has many biological effects that could plausibly protect against CAD, including scavenging of free radicals, thereby protecting against oxidative damage. Findings from studies in guinea-pigs (which, like man, cannot synthesise ascorbic acid) suggest that

Table 3. Risk of future coronary artery disease by ascorbic acid quartile
(Odds ratios (OR) for risk of future coronary artery disease, and corresponding 95 % CI)

Ascorbic acid quartile	1	2	3	4	*P*
Cases/controls	390/480	236/460	187/426	166/428	
Unadjusted	1·00	0·62	0·54	0·46	<0·0001
95 % CI		(0·50, 0·76)	(0·43, 0·67)	(0·36, 0·58)	
Adjusted 1	1·00	0·67	0·68	0·64	<0·0001
95 % CI		(0·54, 0·84)	(0·54, 0·86)	(0·49, 0·82)	
Adjusted 2	1·00	0·69	0·70	0·67	0·001
95 % CI		(0·55, 0·86)	(0·55, 0·88)	(0·52, 0·87)	
Adjusted 3	1·00	0·69	0·69	0·66	0·001
95 % CI		(0·55, 0·86)	(0·55, 0·87)	(0·51, 0·86)	

P values are for linear trend across ascorbic acid quartiles. Data are presented for people in each quartile of the distribution among controls, using those in the lowest quartile as the reference category. Odds ratios were calculated by conditional logistic regression, taking into account matching for sex, age and enrolment time. Additional adjustment was for diabetes, smoking (current, former, never), BMI, systolic blood pressure, LDL-cholesterol and HDL-cholesterol (adjusted 1), for the variables mentioned above and in addition (log-transformed) C-reactive protein concentration (adjusted 2), and for the variables mentioned above and in addition use of vitamin supplements (yes/no; adjusted 3).

ascorbic acid deficiency increases the development of atherosclerotic lesions, and that lesion formation is inhibited by high-dose ascorbic acid (Afridi & Keaney, 1996).

In human subjects, ascorbic acid deficiency is also a risk factor for myocardial infarction because it increases the susceptibility of LDL particles to oxidation (Nyyssonen *et al.* 1997). Conversely, inflammatory processes evolving in atherosclerotic lesions produce reactive oxygen species that deplete plasma concentrations of antioxidants (Ross, 1999). It is thus unclear whether low ascorbic acid concentrations could be a cause or an effect of the inflammatory atherosclerotic disease process, but both may be the case. In human subjects, however, long-term supplementation with antioxidants including ascorbic acid had no effect on systemic inflammation (Bruunsgaard *et al.* 2003) or on the risk of cardiovascular events (Heart Protection Study Collaborative Group, 2002). Supplementation with other antioxidants, including β-carotene and vitamin E, also did not reduce fatal cardiovascular end points (Gaziano, 1996; Rexrode & Manson, 1996; Duthie & Bellizzi, 1999; Yusuf *et al.* 2000). Thus, there is a strong discrepancy between the lack of effect of ascorbic acid supplementation on CAD risk and the strong relationship between physiological ascorbic acid concentration and CAD risk.

It is well known that atherosclerosis can cause myocardial ischaemia, which may in turn lead to myocardial neovascularisation. Interestingly, it has recently been shown that oxidative stress is essential in initiating this process and that antioxidant supplementation attenuates it (Zhu *et al.* 2004). Thus, antioxidant intervention suppresses the trigger for compensatory myocardial neovascularisation, which may explain the lack of benefit of antioxidant supplementation. Other explanations include a threshold effect, an interaction with other dietary constituents, too high or too low a dosage, or too long or too short duration of follow-up.

Besides a potentially protective role in atherogenesis, there are several alternative explanations for the observed association between ascorbic acid concentration and CAD risk. First, ascorbic acid concentration is associated with most traditional cardiovascular risk factors (Ness *et al.* 1996*a*,*b*; Sargeant *et al.* 2000). However, the relationship between ascorbic acid concentrations and CAD risk did not change substantially upon adjusting for these risk factors or after excluding people who smoked or had diabetes. The proportion of smokers was low in this cohort compared with national data for the UK. This difference might indicate both the low proportion of smokers living in East Anglia and the fact that people who participated in this study had a healthier lifestyle than the average population. Misreporting of smoking habit is unlikely to be worse in this cohort than in others.

In addition, ascorbic acid concentrations are inversely related to CRP concentrations (Langlois *et al.* 2001).

Table 4. Risk of future coronary artery disease by ascorbic acid and C-reactive protein quartiles
(Odds ratios (OR) for the risk of future coronary artery disease, and corresponding 95 % CI)

Ascorbic acid quartiles	1	2	3	4
C-reactive protein quartiles				
1 95 % CI	1·00	0·51 (0·31, 0·85)	0·75 (0·45, 1·24)	0·51 (0·31, 0·85)
2 95 % CI	0·80 (0·50, 1·29)	0·77 (0·46, 1·28)	0·52 (0·32, 0·87)	0·56 (0·32, 0·97)
3 95 % CI	1·17 (0·74, 1·83)	0·94 (0·59, 1·51)	0·86 (0·52, 1·43)	0·81 (0·48, 1·38)
4 95 % CI	1·53 (0·99, 2·37)	1·27 (0·73, 2·21)	1·00 (0·60, 1·71)	0·82 (0·51, 1·31)

Data are presented for people in each quartile of the ascorbic acid and C-reactive protein distribution among controls, using those in the lowest ascorbic acid quartile and in the lowest C-reactive protein quartile as reference category. Odds ratios were calculated by conditional logistic regression, taking into account matching for sex, age and enrolment time, and adjusting for diabetes, smoking (current, former, never), BMI, systolic blood pressure, LDL-cholesterol and HDL-cholesterol.

Surprisingly, however, we observed that the relationship between ascorbic acid concentration and CAD risk was completely independent of CRP concentration.

Second, although individuals who had suffered symptomatic CVD were excluded from the current analysis, we cannot exclude the possibility that baseline ascorbic acid concentrations correlated with the extent of subclinical atherosclerosis in these apparently healthy individuals.

Third, the observed relationship could be confounded by social class or physical activity. The consistency and strength of the relationship between ascorbic acid concentration and CAD risk is equal to or greater than that seen in other studies for many known factors, including social class and physical activity. This consistent relationship suggests that ascorbic acid status might give a better indication of the CAD risk associated with these other known factors than their direct assessment. Some of the recorded social class variations in health could, however, be mediated through dietary differences, including those in ascorbic acid status.

Finally, plasma ascorbic acid concentrations might be related to other types of behaviour that protect against atherosclerosis. One possibility is that those with high concentrations of ascorbic acid might be taking supplements. These individuals might also take other supplements, including fish oils, which might protect against CVD. Irrespective of the direct effect of supplements, the selection biases in the characteristics of supplement users are well recognised, as is the low mortality of good compliers, even with a placebo, in trials. In the EPIC-Norfolk cohort, a substantial proportion of participants (a third of the men and half the women) reported some sort of supplement use. However, supplement use was not associated with a reduced risk of CAD in this cohort or in the randomised placebo-controlled Heart Protection Study (Heart Protection Study Collaborative Group, 2002). In our earlier report, we indicated that plasma ascorbic acid concentrations were a good indicator of a high dietary intake of fruit and vegetables, which have many nutrients such as dietary fibre, K and folate, which may be potentially cardioprotective.

Limitations

Several aspects of this study warrant comment. Plasma ascorbic acid concentrations were measured once only for each individual, leaving the possibility that individuals changed their ascorbic acid status during follow-up. In addition, plasma ascorbic acid concentrations were measured in non-fasting samples. The intake of vitamin supplements prior to blood sampling might possibly have an important effect on the ascorbic acid concentration measured in the samples. These limitations may have led to a random misclassification of individuals.

Nevertheless, plasma ascorbic acid concentrations are strongly correlated with the intake of fruit and vegetables, as estimated by food-frequency questionnaire, and are not merely a reflection of vitamin supplement use (Bates *et al.* 1991; Khaw *et al.* 2001). This suggests that the true underlying relationship between dietary ascorbic acid intake and risk of future CAD could be stronger than the one observed. In addition, the consistent measurement of plasma ascorbic acid is especially difficult because it is unstable in blood and deteriorates rapidly unless it is stabilised by the addition of other substances, such as metaphosphoric acid, and stored at very low temperatures. Measurement errors might also account for the absence of consistency in studies in which ascorbic acid has been measured. Even though intake and plasma concentration are closely related, other factors such as smoking habit or pre-existing disease could account for differences in plasma ascorbic acid concentration that are not explained by intake. These factors might confound the results.

Conclusion

In this population of apparently healthy men and women, the plasma concentration of ascorbic acid, an indicator of high dietary fruit and vegetable intake, was inversely related to various cardiovascular risk factors. Compared with people in the lowest quartile of the plasma ascorbic acid distribution, those in the highest quartile had a 33 % lower risk of CAD, independent of other known risk factors including age, blood pressure, plasma lipids, cigarette smoking, BMI, diabetes and CRP concentration. These data suggest that the risk reduction associated with fruit and vegetable intake is not mediated by a reduction in CRP concentration.

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