

## Low dietary intake of $\beta$ -carotene, $\alpha$ -tocopherol and ascorbic acid is associated with increased inflammatory and oxidative stress status in a Swedish cohort

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Fruit and vegetable consumption has been associated with a reduced risk of several diseases including CVD. A part of these effects seen could be linked to anti-inflammatory and antioxidative effects, although this has not been thoroughly investigated. The present study was designed to investigate the effects of the dietary intake of  $\beta$ -carotene,  $\alpha$ -tocopherol and ascorbic acid on *in vivo* biomarkers of inflammation (PGF<sub>2 $\alpha$</sub> , high-sensitive C-reactive protein (hsCRP) and IL-6 formation) and oxidative stress (F<sub>2</sub>-isoprostane formation), the two important factors associated with accelerated atherosclerosis. The dietary intake of 704 participants in the Uppsala Longitudinal Study of Adult Men (ULSAM) at age 70 years was registered and inflammatory and oxidative stress biomarkers were quantified 7 years later. The registered dietary intakes of ascorbic acid and  $\alpha$ -tocopherol were negatively associated linearly and in quartiles with both PGF<sub>2 $\alpha$</sub> , hsCRP, IL-6 and F<sub>2</sub>-isoprostanes, where ascorbic acid intake generally was more strongly associated. Dietary intake of  $\beta$ -carotene was only significantly negatively associated with F<sub>2</sub>-isoprostanes. In conclusion, the present study is the first to suggest that the intake of food rich in antioxidants is associated with reduced cyclo-oxygenase- and cytokine-mediated inflammation and oxidative stress at 7 years of follow-up. These associations could be linked to the beneficial effects of fruit and vegetables observed on CVD.

### Antioxidants: Prostaglandins: Inflammation: Oxidative stress: Isoprostanes

Fruit and vegetable consumption has been associated with a reduced risk of several diseases including CVD<sup>(1)</sup>. Studies have suggested that chronic low-grade inflammation and free radicals in excess may play a role in the development of atherosclerotic plaques subsequently leading to diseases in the cardiovascular system<sup>(2,3)</sup>. Antioxidants, usually found in fruits and vegetables, may be beneficial by reducing the levels of inflammation and free radicals *in vitro*<sup>(4)</sup> but the role of antioxidants *in vivo* in atherosclerosis and CVD is still largely unknown and under constant debate. Epidemiological studies have shown that humans with either a high dietary intake of or a high blood concentration of  $\alpha$ -tocopherol,  $\beta$ -carotene and ascorbic acid have a lower risk of developing CVD<sup>(5,6)</sup>. On the other hand, large placebo-controlled intervention studies have not shown any beneficial cardiovascular effects of  $\alpha$ -tocopherol,  $\beta$ -carotene or ascorbic acid supplementation<sup>(6–8)</sup>. From these contradictory data it may be concluded that the regulation of antioxidants, inflammation and free radicals in atherosclerosis is a complex process and yet not fully understood. One approach to acquire more knowledge of the role of antioxidants in atherogenesis

may be to study the impact of dietary vitamin intake and to relate these vitamins to the levels of inflammation and free radicals *in vivo*.

Inflammatory processes can be studied by the quantification of cyclo-oxygenase (COX)-induced or cytokine-mediated products<sup>(9)</sup>. Prostaglandins and thromboxanes are derived from arachidonic acid; COX catalyses their formation endogenously. These prostanoids are important mediators of vasoconstrictive and inflammatory processes<sup>(10,11)</sup>. PGF<sub>2 $\alpha$</sub>  is one of the major prostaglandins formed at sites of inflammation<sup>(12,13)</sup> and its formation *in vivo* is preferably quantified by the measurement of 15-keto-dihydro-PGF<sub>2 $\alpha$</sub> <sup>(14)</sup>, which is a major metabolite of PGF<sub>2 $\alpha$</sub>  in plasma. 15-Keto-dihydro-PGF<sub>2 $\alpha$</sub>  has been shown to be a potent indicator of PGF<sub>2 $\alpha$</sub>  formation and COX-mediated inflammatory processes *in vivo*<sup>(15,16)</sup>.

F<sub>2</sub>-isoprostanes, structural isomers of PGF<sub>2 $\alpha$</sub> , are formed during free radical-catalysed (non-enzymic) peroxidation of arachidonic acid<sup>(17)</sup>. 8-Iso-PGF<sub>2 $\alpha$</sub> , a major F<sub>2</sub>-isoprostane, is a reliable indicator of oxidative stress *in vivo*. Enhanced formation of F<sub>2</sub>-isoprostanes has been associated with coronary artery disease<sup>(18)</sup> and several risk factors for

**Abbreviations:** COX, cyclo-oxygenase; CRP, C-reactive protein.

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atherosclerosis including diabetes, obesity and hypercholesterolaemia, as reviewed earlier<sup>(19)</sup>.

In a recent study we showed that high levels of the antioxidant Se in serum were associated with reduced formation of the inflammatory mediator  $\text{PGF}_{2\alpha}$  and the oxidative stress indicator  $\text{F}_2$ -isoprostanes after a long follow-up<sup>(20)</sup>. The aim of the present study was to study the relationships of registered dietary intake of other antioxidants, namely ascorbic acid,  $\alpha$ -tocopherol and  $\beta$ -carotene, with levels of inflammation ( $\text{PGF}_{2\alpha}$ , high-sensitive C-reactive protein (CRP) and IL-6 formation) and free radicals ( $\text{F}_2$ -isoprostane formation) in a 7-year follow-up study.

## Materials and methods

### Study population

The present study is based on 704 study participants from the re-investigations of the Uppsala Longitudinal Study of Adult Men (ULSAM) in 1990–3 and 1997–2001. This population-based cohort originally started in 1970–3, when all men born 1920–4 and living in Uppsala (Sweden) were invited to participate in a health screening at age 50 years as previously described<sup>(21)</sup>. The cohort was re-investigated after 20 and 27 years. Out of the re-investigated men, 704 were eligible for evaluation of inflammation and oxidative stress at age 77 years and had dietary data from the re-investigation at age 70 years. The Ethics Committee at Uppsala University approved the study and all participants gave informed consent.

### Dietary assessment of vitamins

The intake of dietary ascorbic acid,  $\alpha$ -tocopherol and  $\beta$ -carotene was estimated with the use of a 7 d pre-coded food record as part of the investigation at age 70 years. The validity and design of the dietary assessment has previously been described in detail<sup>(22)</sup>. In brief, a dietitian instructed all subjects to record their dietary intake using a 7 d pre-coded food diary. Daily intakes of the antioxidants were calculated using a computerised program (MATs; Rudans Lättdata, Västerås, Sweden) and the Swedish National Food Administration database.

### Biochemical analysis of indicators of inflammation and oxidative stress

Urine was collected over 24 h. Blood samples were drawn in heparinised tubes in the morning after an overnight fast and plasma was separated. Both urine and plasma were stored at  $-70^\circ\text{C}$  until analysis. Free 8-iso- $\text{PGF}_{2\alpha}$  in urine was analysed by a RIA as previously described by Basu<sup>(23)</sup>. The intra-assay CV was 14.5% at low and 12.2% at high concentrations. Urinary 15-keto-dihydro- $\text{PGF}_{2\alpha}$  was analysed by a RIA developed as previously described by Basu<sup>(15)</sup>. The intra-assay CV was 12.2% at low and 14.0% at high concentrations. Levels of 8-iso- $\text{PGF}_{2\alpha}$  and 15-keto-dihydro- $\text{PGF}_{2\alpha}$  were corrected for urinary creatinine analysed as described previously<sup>(24)</sup>. IL-6 was analysed by a high-sensitivity ELISA kit (IL-6 HS; R&D Systems, Minneapolis, MN, USA). The total CV of the method was 7% and inter-assay CV was 5%. High-sensitive CRP measurements were performed by latex-enhanced

reagent (Dade Behring, Deerfield, IL, USA) with the use of a Behring BN ProSpec analyser (Dade Behring). Intra-assay CV of the high-sensitive CRP method was 1.4% at both 1.23 and 5.49 mg/l. One high-sensitive CRP outlier (116 mg/l) was excluded from the statistical analysis.

### Statistical analysis

Variables with skewed distribution, according to the Shapiro–Wilk test ( $W < 0.95$ ), were log-transformed to reach normal distribution. Associations between dietary vitamins and eicosanoids and cytokines, respectively, were tested in linear regression models with the respective vitamin as the dependent variable and eicosanoids and cytokines as independent variables. BMI as a continuous variable, diabetes (plasma glucose  $\geq 7.0$  mmol/l or anti-diabetic medication), hyperlipidaemia (serum cholesterol  $> 6.5$  mmol/l or TAG  $> 2.3$  mmol/l or lipid-lowering medication), hypertension (office blood pressure  $> 140/90$  mmHg or anti-hypertensive medication) and smoking at baseline were considered as potential confounders based on associations earlier reported from this cohort or from other studies<sup>(9,19,25)</sup>. Dietary vitamins were entered into the models as continuous variables and in quartiles. *P* values  $< 0.05$  were regarded as statistically significant. Calculations were performed with Stata 8.2 (StataCorp LP, College Station, TX, USA).

## Results

### Baseline characteristics of the study population and dietary vitamin intake

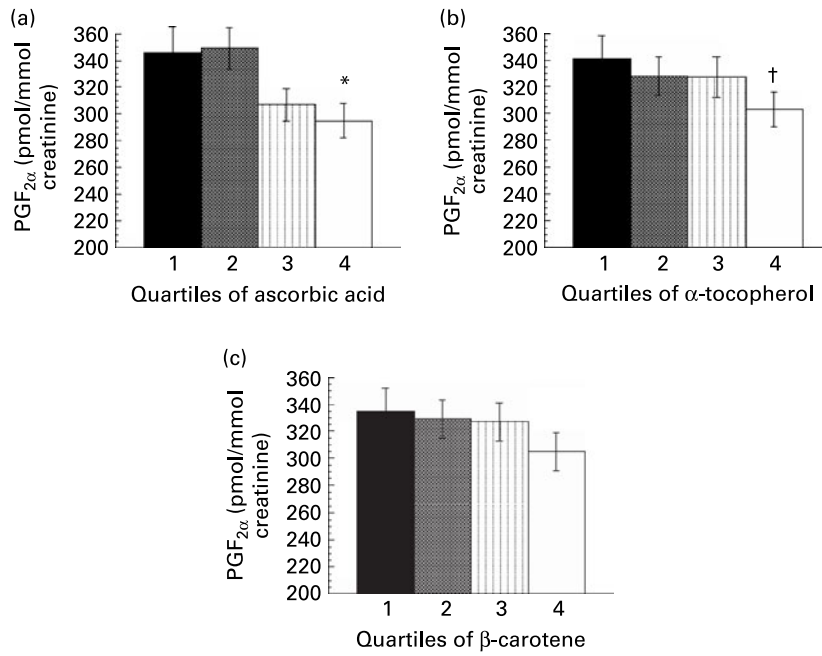
In the cohort of men aged 70 years, 9% had diabetes mellitus, 29% hypertension, 36% hyperlipidaemia and 16% were active smokers. Mean BMI was 26.3 (SD 3.27)  $\text{kg}/\text{m}^2$ . The mean dietary intake of ascorbic acid was 54.2 (SD 35.4) mg/d. Quartile 1 corresponds to 4.73–29.8 mg/d; 2 to 29.9–46.5 mg/d; 3 to 46.6–68.1 mg/d; 4 to 68.2–285 mg/d of ascorbic acid intake. The mean dietary intake of  $\alpha$ -tocopherol was 5.80 (SD 2.03) mg/d. Quartile 1 corresponds to 1.62–4.33 mg/d; 2 to 4.34–5.40 mg/d; 3 to 5.41–6.66 mg/d; 4 to 6.68–34.9 mg/d of  $\alpha$ -tocopherol intake. The mean dietary intake of  $\beta$ -carotene was 1.74 (SD 1.41) mg/d. Quartile 1 corresponds to 0.08–0.79 mg/d; 2 to 0.80–1.38 mg/d; 3 to 1.39–2.20 mg/d; 4 to 2.21–11.9 mg/d of  $\beta$ -carotene intake.

### Dietary vitamins and $\text{PGF}_{2\alpha}$ (indicator of cyclo-oxygenase-mediated inflammation)

The registered intake of dietary ascorbic acid was negatively associated with  $\text{PGF}_{2\alpha}$  formation linearly (regression coefficient (b)  $-46.9$ ;  $P < 0.05$ ) and in quartiles (Fig. 1) at follow-up. The linear association did not substantially change when adjusted for potential confounders at baseline, but the association between the highest and lowest quartile of ascorbic acid intake was attenuated ( $P = 0.085$ ).

Intake of  $\alpha$ -tocopherol was negatively associated with  $\text{PGF}_{2\alpha}$  formation at a borderline significance level linearly (b  $= -35.1$ ;  $P = 0.06$ ) and in quartiles (Fig. 1). These results were substantially attenuated when adjusted for potential confounders ( $P = 0.15$ ).

Dietary antioxidants and inflammation



**Fig. 1.** PGF<sub>2α</sub> formation (pmol/mmol creatinine) in quartiles of dietary intake of ascorbic acid (a), α-tocopherol (b) and β-carotene (c), where quartile 1 is low intake. PGF<sub>2α</sub> formation is estimated as urinary 15-keto-dihydro-PGF<sub>2α</sub>. Note that the y axis is cut at 200 pmol/mmol. Values are means, with standard errors represented by vertical bars. \*Mean value is significantly different from that for quartile 1 ( $P < 0.05$ ). †Mean value is marginally significantly different from that for quartile 1 ( $P = 0.06$ ). For ascorbic acid,  $P$  for trend = 0.007; for α-tocopherol  $P$  for trend = 0.06; for β-carotene  $P$  for trend = 0.21.

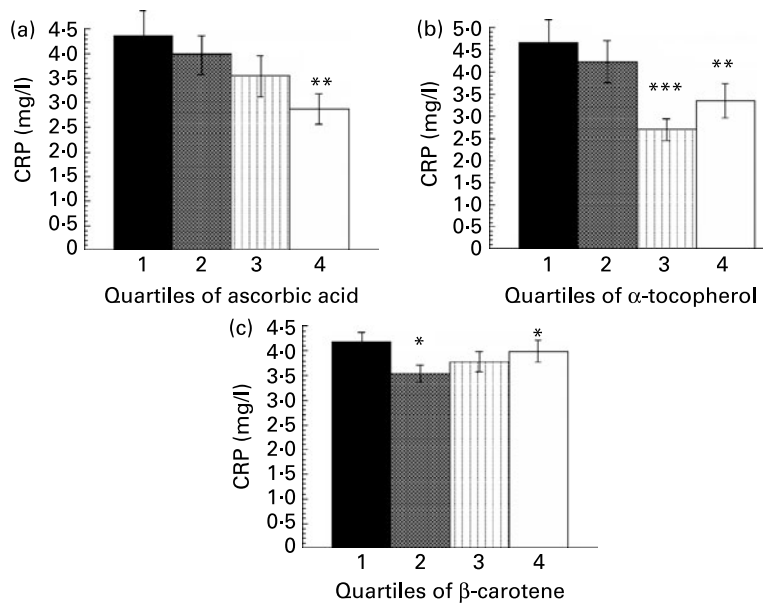
Intake of β-carotene in the diet was not significantly associated with PGF<sub>2α</sub> formation (Fig. 1).

*Dietary vitamins and high-sensitive C-reactive protein (indicator of cytokine-related inflammation)*

The registered intakes of dietary ascorbic acid and α-tocopherol were negatively associated with CRP linearly

( $b = -0.14$ ,  $P < 0.001$  and  $b = -0.15$ ,  $P < 0.001$ , respectively) and in quartiles (Fig. 2) at follow-up. The results did not substantially change when adjusted for potential confounders at baseline.

The estimated intake of β-carotene was not linearly associated with high-sensitive CRP (data not shown), but quartiles with high intake (quartiles 4 and 2) of β-carotene were associated with lower levels of high-sensitive CRP compared with



**Fig. 2.** C-reactive protein (mg/l) in quartiles of dietary intake of ascorbic acid (a), α-tocopherol (b) and β-carotene (c), where quartile 1 is low intake. Values are means, with standard errors represented by vertical bars. Mean value is significantly different from that for quartile 1: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . For ascorbic acid,  $P$  for trend  $< 0.001$ ; for α-tocopherol  $P$  for trend  $< 0.001$ ; for β-carotene  $P$  for trend = 0.14.

the quartile with the lowest intake of  $\beta$ -carotene (Fig. 2). However, adjusting for potential confounders at baseline removed these associations.

#### *Dietary vitamins and interleukin-6 (indicator of cytokine-related inflammation)*

Intakes of dietary ascorbic acid and  $\alpha$ -tocopherol were linearly negatively associated with IL-6 at follow-up ( $b = -0.06$ ,  $P < 0.05$  and  $b = -0.07$ ,  $P < 0.01$ , respectively). The quartile with the estimated highest intake of ascorbic acid and  $\alpha$ -tocopherol had the lowest levels of IL-6 compared with the quartile with the lowest intake (Fig. 3). The results were attenuated when adjusted for potential confounders:  $P = 0.066$  (ascorbic acid) and  $P < 0.05$  ( $\alpha$ -tocopherol).

The estimated intake of  $\beta$ -carotene was not linearly associated with IL-6 (data not shown), but quartiles with high intake (quartiles 3 and 2) of  $\beta$ -carotene were associated with lower levels of IL-6 compared with the quartile with the lowest intake of  $\beta$ -carotene (Fig. 3). However, adjusting for potential confounders at baseline removed these associations.

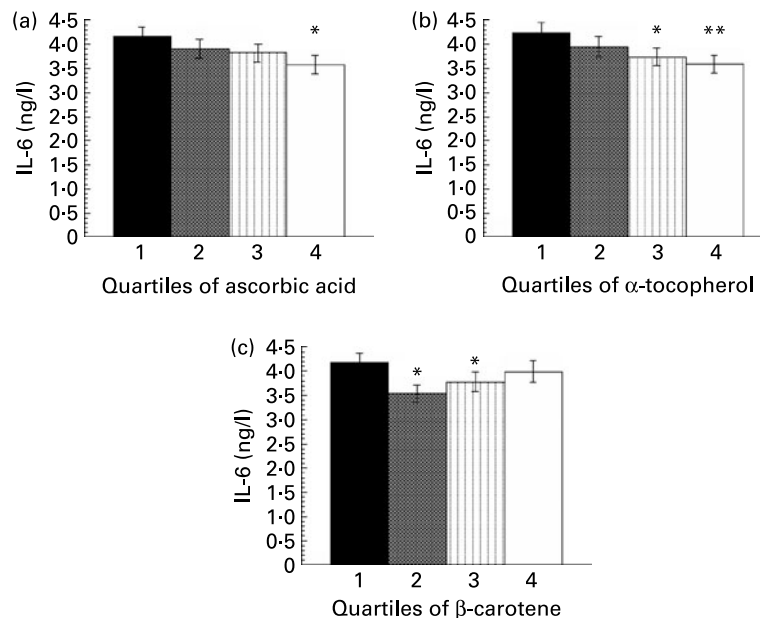
#### *Dietary vitamins and F<sub>2</sub>-isoprostanes (indicator of oxidative stress)*

Intakes of dietary ascorbic acid,  $\alpha$ -tocopherol and  $\beta$ -carotene were linearly negatively associated with F<sub>2</sub>-isoprostane formation at follow-up ( $b = -41.3$ ,  $P < 0.05$ ;  $b = -34.1$ ,  $P < 0.05$ ;  $b = -48.0$ ,  $P < 0.05$ , respectively). The quartile with the estimated highest intake of ascorbic acid as well as the highest intake of  $\beta$ -carotene had the lowest levels of F<sub>2</sub>-isoprostane formation compared with the quartile with the lowest intake (Fig. 4). The results were unchanged (ascorbic acid,  $\alpha$ -tocopherol) or even stronger ( $\beta$ -carotene) when adjusted for potential confounders.

## Discussion

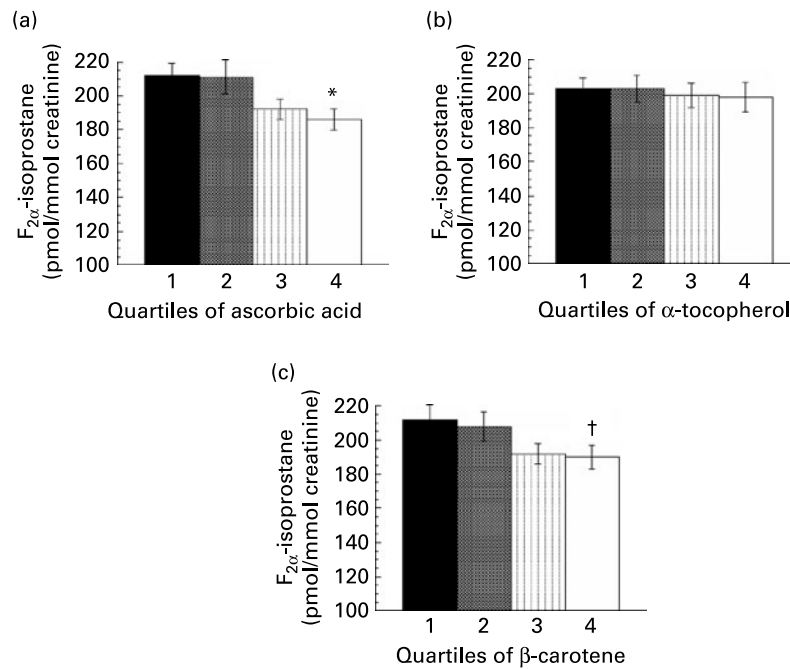
The novel finding in the present study is that the registered dietary intake of ascorbic acid and  $\alpha$ -tocopherol was inversely associated with PGF<sub>2 $\alpha$</sub>  formation, which may be interpreted as the higher the intake of food containing ascorbic acid and  $\alpha$ -tocopherol the lower the level of COX-mediated inflammation. The association with  $\alpha$ -tocopherol was, however, somewhat weaker, especially when adjusting for potential confounders. Studies evaluating the relationship between ascorbic acid and  $\alpha$ -tocopherol, respectively, and PGF<sub>2 $\alpha$</sub>  are very sparse and this may be due to the earlier difficulties in quantification and estimation of PGF<sub>2 $\alpha$</sub>  formation. Serum  $\alpha$ -tocopherol and PGF<sub>2 $\alpha$</sub>  formation was not associated cross-sectionally in this cohort as previously reported<sup>(9)</sup>. On the other hand, there was a positive correlation found between serum  $\gamma$ -tocopherol and PGF<sub>2 $\alpha$</sub>  formation<sup>(26)</sup>, although  $\gamma$ -tocopherol was not studied in the present study. An earlier published study showed that supplementation with  $\alpha$ -tocopherol at relatively low doses (200 mg  $\alpha$ -tocopherol/d) for 2 weeks or gradually increased doses (16–400 IU (10.7–266.7 mg)  $\alpha$ -tocopherol/d) for 4 weeks did not change the level of PGF<sub>2 $\alpha$</sub>  formation<sup>(27,28)</sup>.

A higher intake of food containing ascorbic acid,  $\alpha$ -tocopherol and to some extent also  $\beta$ -carotene was also associated with lower levels of CRP and IL-6, indicating a lower level of cytokine-mediated inflammation related to higher vitamin intake. These associations are supported by a number of cross-sectional studies showing an inverse correlation between ascorbic acid in serum, blood, plasma and diet, and CRP levels<sup>(29–32)</sup>. The relationship between  $\alpha$ -tocopherol and CRP seems to be far more complicated in the literature. Plasma  $\alpha$ -tocopherol has been shown to be inversely correlated with CRP levels in one study<sup>(33)</sup> but serum  $\alpha$ -tocopherol and CRP levels were actually positively correlated in an



**Fig. 3.** IL-6 (ng/l) in quartiles of dietary intake of ascorbic acid (a),  $\alpha$ -tocopherol (b) and  $\beta$ -carotene (c), where quartile 1 is low intake. Values are means, with standard errors represented by vertical bars. Mean value is significantly different from that for quartile 1: \* $P < 0.05$ , \*\* $P < 0.01$ . For ascorbic acid,  $P$  for trend = 0.009; for  $\alpha$ -tocopherol  $P$  for trend = 0.002; for  $\beta$ -carotene  $P$  for trend = 0.18.





**Fig. 4.** F<sub>2</sub>-isoprostane formation (pmol/mmol creatinine) in quartiles of dietary intake of ascorbic acid (a), α-tocopherol (b) and β-carotene (c), where quartile 1 is low intake. F<sub>2</sub>-formation is estimated as urinary 8-iso-PGF<sub>2α</sub>. Note that the y axis is cut at 100 pmol/mmol. Values are means, with standard errors represented by vertical bars. \*Mean value is significantly different from that for quartile 1 ( $P < 0.05$ ). †Mean value is marginally significantly different from that for quartile 1 ( $P = 0.05$ ). For ascorbic acid,  $P$  for trend = 0.01; for α-tocopherol  $P$  for trend = 0.13; for β-carotene  $P$  for trend = 0.03.

earlier study published from this present cohort<sup>(9)</sup>. Some studies have shown a lack of association between α-tocopherol in plasma and serum and CRP<sup>(29,34)</sup>. However, to our knowledge no study has evaluated the dietary intake of α-tocopherol and CRP or IL-6. Intake of ascorbic acid via supplements has revealed conflicting results; some studies have shown a reduced level of CRP<sup>(35)</sup> while others have failed to show CRP-lowering effects of ascorbic acid supplementation<sup>(36,37)</sup>. The intake of α-tocopherol supplements has been shown to lead to reduced levels of CRP in one study<sup>(36)</sup> while a combination of α-tocopherol and ascorbic acid did not alter CRP<sup>(37,38)</sup> or IL-6<sup>(38)</sup>. A number of earlier published studies of β-carotene estimated in blood, serum and plasma and CRP confirm the weak inverse association between dietary β-carotene and CRP seen in the present study<sup>(30,31,33,34,39)</sup>. There is also a study that shows an inverse association between β-carotene and IL-6<sup>(40)</sup>, a correlation that we could not find in the present study.

A higher intake of food containing ascorbic acid, α-tocopherol and β-carotene was related to reduced levels of F<sub>2</sub>-isoprostanes. This would indicate that the higher the intake of these vitamins in the diet the less oxidative stress *in vivo*. We have previously reported an inverse association between α-tocopherol in serum and F<sub>2</sub>-isoprostane formation<sup>(9)</sup>. A supplementation study with vegetable soup containing ascorbic acid showed that F<sub>2</sub>-isoprostane formation could be decreased by intake of this soup, but a control group was missing in this study<sup>(41)</sup>. There are a number of supplementation studies concerning α-tocopherol and ascorbic acid which evaluated the effects on F<sub>2</sub>-isoprostane formation as reviewed earlier<sup>(19)</sup>. In brief, F<sub>2</sub>-isoprostane formation in healthy humans is generally not affected by α-tocopherol or ascorbic acid supplementation.

In conditions where F<sub>2</sub>-isoprostane formation is increased, such as type 2 diabetes, homocysteinaemia and hypercholesterolaemia, α-tocopherol supplementation may be beneficial in reducing the F<sub>2</sub>-isoprostane level if given in appropriate doses and longer duration<sup>(42)</sup>. Ascorbic acid supplementation may decrease the elevated F<sub>2</sub>-isoprostane formation in smokers<sup>(43,44)</sup>. Supplementation with β-carotene alone did not alter F<sub>2</sub>-isoprostane formation<sup>(45)</sup>.

To summarise, a high intake of food rich in ascorbic acid seems to show the most obvious association with reduced levels of all investigated indicators of inflammation (PGF<sub>2α</sub>, CRP, IL-6) and oxidative stress (F<sub>2</sub>-isoprostanes) in the present study. A high intake of food rich in α-tocopherol was also related to reduced levels of PGF<sub>2α</sub>, CRP, IL-6 and F<sub>2</sub>-isoprostanes, but the associations were weaker and in some case were affected by confounding factors. On the other hand, a high intake of β-carotene from the diet was only related to reduced F<sub>2</sub>-isoprostane formation. It may be that these antioxidants by their different properties and mechanisms affect inflammation and oxidative stress differently. Ascorbic acid is a water-soluble chain-breaking antioxidant, tocopherols and tocotrienols (vitamin E) are lipid-soluble chain-breaking antioxidants and carotenoids (including β-carotene) are lipid-soluble antioxidants that effectively scavenge singlet oxygen. The earlier published inverse associations between Se (which has antioxidative properties mainly through the glutathione peroxidase system), and PGF<sub>2α</sub> and F<sub>2</sub>-isoprostanes, but not CRP, IL-6 or serum amyloid A protein, may support this theory<sup>(20)</sup>. It may also be true that the associations found in the present study merely reflect an association between a large intake of fruits and vegetables in general and a reduced level of inflammation and oxidative stress and

that this association may be mediated through an unknown factor or mechanism not studied here.

A recommended daily intake of vitamins according to the Swedish National Food Administration today is 75 mg ascorbic acid, 10 mg  $\alpha$ -tocopherol and 10.8 mg  $\beta$ -carotene. It may be noted that only those in the highest quartile of the respective vitamin intake achieved these health recommendations. A general well-known problem of food registrations is the constant and sometimes selective underestimation of the dietary intake. However, a possible underestimation of vitamin intake would rather tend to diminish the observed associations in the present study.

It may also be discussed whether these studied vitamins have any direct anti-inflammatory effect or if the reduced levels of inflammation seen related to a high intake of vitamins are secondary to their well-documented antioxidative effect. There are several mechanisms described in the literature by which free radicals (oxidative stress) may be linked to the inflammatory pathway. A recent study showed that intravenous infusion of  $F_2$ -isoprostanes, specifically 8-iso-PGF<sub>2 $\alpha$</sub> , in rabbits causes an increase in COX-catalysed PGF<sub>2 $\alpha$</sub>  formation<sup>(46)</sup>. Phospholipase A<sub>2</sub>, which hydrolyses phospholipids in the membrane allowing arachidonic acid and subsequently prostaglandins to form, can be activated by H<sub>2</sub>O<sub>2</sub> (reactive oxygen species)<sup>(47,48)</sup>. Reactive oxygen species might also up-regulate phospholipase A<sub>2</sub> by the I $\kappa$ B kinase (IKK)/NF- $\kappa$ B pathway<sup>(49)</sup>. NF- $\kappa$ B is a nuclear transcription factor regulating cytokines (IL-6), cell-adhesion molecules, acute-phase proteins (CRP), phospholipase A<sub>2</sub> and COX-2 in a complex series of biochemical processes.

The present study was carried out in a fairly large age-, sex- and ethnicity-matched population, which is an advantage when studying inflammation and free radicals, as these levels may vary due to the above-mentioned factors<sup>(19)</sup>. On the other hand, the results may have limited generalisability to other age and ethnic groups and women. Further, the studied subgroup of the cohort may be healthier than the general population. The studied subgroup may also be healthier than the whole unselected cohort at age 70 years, as can be noted from slightly higher percentages of diabetes mellitus (15%), smokers (21%) and hypertension prevalence (32%) in the whole unselected cohort at age 70 years (compared with the subgroup followed up with biomarkers). Hyperlipidaemia prevalence and BMI were the same in the whole cohort at age 70 years as in the subgroup followed up 7 years later. However, the healthy cohort effect would probably bias the results towards the null hypothesis and not lead to overestimation of the associations. Although the study has a longitudinal design with a follow-up of 7 years, it cannot be excluded that the associations found also would be found in a cross-sectional analysis. A limitation in the study is that we did not have data on vitamin supplements. However, it can be speculated that general Swedish men of age 70 years in 1990–3 did not take vitamin supplements to a large extent.

The dietary intake of vitamins was unfortunately not validated at follow-up. The Swedish National Food Administration concluded based on two food surveys (in 1989 and 1997) that the intake of tocopherols in the diet did not change, while the intake of ascorbic acid in the diet increased slightly and the intake of carotenes decreased slightly in men aged 18–74 years during the

years 1989–97<sup>(50)</sup>. Changes in vitamin intake in the elderly are expected to be smaller. However, it is very unlikely that changes in nutrient intake during the years of follow-up would bias the results towards a type 1 error; rather, the associations would be more difficult to detect. Serum concentrations of  $\alpha$ -tocopherol at baseline and after 7 years of follow-up correlated well ( $R$  0.54;  $P$  < 0.0001), which also supports the assumption of small changes in consumption during follow-up.

In conclusion, the present study is the first to report that a registered large intake of a diet rich in several vitamins is associated with reduced inflammation, mediated by both COX and cytokines, and reduced oxidative stress 7 years later. Whether these effects seen are a consequence of a general effect of a healthy diet or a more direct consequence of intake of these specific vitamins can, however, not be concluded from the results of the present study. Yet, these anti-inflammatory and antioxidative effects of a large intake of food rich in vitamins may be linked to the beneficial effects of fruits and vegetables seen on CVD and cancer.

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None of the authors had any conflict of interest related to the present study.

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